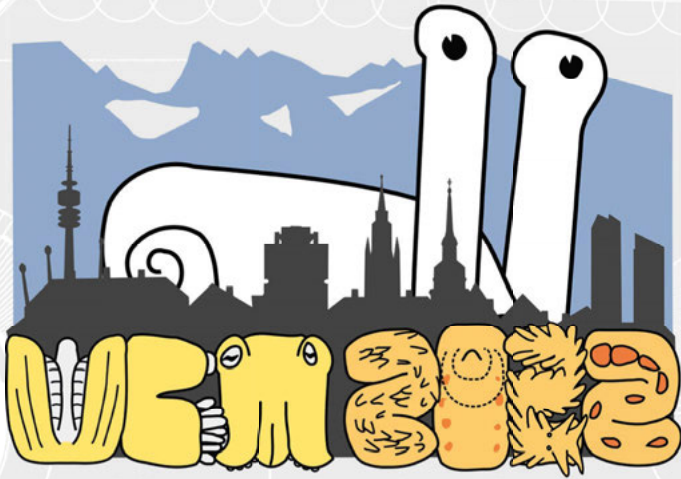


# SPIXIANA

Zeitschrift für Zoologie



**World Congress of Malacology  
Munich 2022**

31<sup>st</sup> July – 5<sup>th</sup> August

# SPIXIANA

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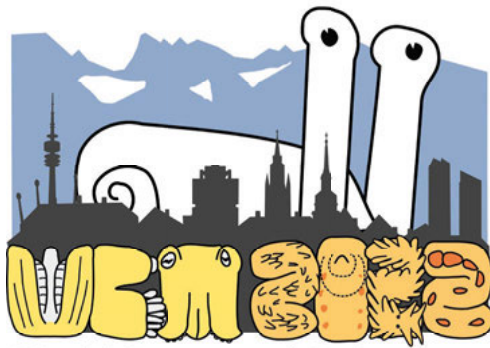
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# SPIXIANA

Zeitschrift für Zoologie

Supplement 30 A



**World Congress of Malacology  
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31<sup>st</sup> July – 5<sup>th</sup> August

Edited by

**Franziska S. Bergmeier, Bastian Brenzinger, Timea P. Neusser**

**LMU Munich, ZSM Munich, Germany**

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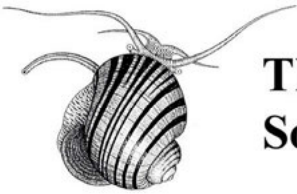
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# Contents

Welcome to the WCM 2022 .....	5
Conference Site: The Biocenter LMU .....	6
Programme Overview .....	8
Symposia of the WCM 2022 .....	10
<b>Abstracts (sorted alphabetically)</b>	
Plenary Talks .....	15
<b>Symposia and Open Sessions</b>	
Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honor of Philippe Bouchet .....	24
Mollusc Evolutionary History .....	75
American Malacological Society President's Symposium: Molluscan Conservation .....	101
Continental Molluscs Facing Environmental Changes .....	126
Systematics and Evolution of (not only Marine) Heterobranchia .....	152
The Ecology of Fossil and Extant Molluscs .....	192
Molluscs as Parasites and Victims of Parasitism: Biodiversity, Ecology, and Evolution .....	223
Volunteers in Malacology .....	232
The EvoDevo Corner .....	240
Insular Ecosystems as Cradles of Mollusc Biodiversity and Evolution .....	248
Open session: Species Delineation .....	265
Open session: Environmental Stress / Adaptation .....	273
Open session: The Malacological Community .....	285
Open session: The Neglected Classes .....	294
Open session: All About Light... ..	304
Open session: Biogeography .....	311
Open session: Diversification and Speciation in Terrestrial Molluscs .....	317
Open session: EvoDevo and Vision .....	330
Open session: Phylogeny .....	336
Open session: Diversity and Distribution .....	343
Open session: Genomics .....	346
Open session: Morphology .....	349

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Quentin **Wackenheim** (Sorbonne University, Paris, France)  
Tim **Wollesen** (University of Vienna, Austria).

# Welcome Remarks by the President of Unitas Malacologica

“Grüß Gott” – our local greeting - to all participants of the World Congress of Malacology 2022, which is the 21<sup>st</sup> international congress of Unitas Malacologica. We feel honored to host WCM 2022 here at the Biocenter of the Ludwig-Maximilians-University in the southwest of Munich, Bavaria. We are pleased to welcome all delegates, more than 320 participants representing close to 40 countries from all over the globe.

Unitas Malacologica was founded at Sept 21<sup>st</sup> 1962, and thus celebrates its 60<sup>th</sup> birthday this year. There is little doubt that malacology as a scientific discipline has never been more important than in our time. Mollusca, in particular the freshwater and terrestrial taxa, is the leading phylum concerning the percentages of endangered organisms. Molluscs play a significant role in all kinds of biomonitoring in order to understand the decline of biodiversity due to human activities or because of the climate crisis. More and more molluscs are important sources for various pharmaceuticals, and the famous conotoxins, which generated new classes of anti-pain medicals, are only the tip of a huge iceberg. Understanding the processes of more than half a billion years of evolution of Mollusca requires a combined approach of larvae and adults, of molecules, morphologies, and behaviors, of geno- and phenotypes of the presence and the past. And – last but not least – the world would be much poorer without the delicious taste of various cephalopods, bivalves or snails. Indeed, I can’t imagine that anybody is not fascinated by the tremendous diversity or simple beauty of molluscs.

It likely has never been so difficult to organize a WCM: we all suffered from the Corona pandemic, and we all are more than happy that the current situation allows us to come together face to face, to chat during breaks, have discussions during lunches and dinners and along icebreakers or excursions. Needless to say the brutal military attack against Ukraine has also affected the registration and preparation process by some unexpectedly significantly raised costs. But to say it clearly: we strongly condemn the invasion, which has had a devastating impact on the people and infrastructure of Ukraine. We offer our support to our scientific colleagues who have been touched by the conflict and our fellow scientific institutions in the Ukraine. While we denounce the actions of the Russian government, we welcome our Russian colleagues here at the WCM 2022. The motto of WCM 2022 is “Meeting of Generations”. We tried hard to make participation manageable and financeable for young scientists and in particular for graduate students. A warm “thank you so much” to all institutions, societies and persons, who supported WCM 2022 in various ways.

This year the conference will honour two outstanding malacologists, Dr. Philippe Bouchet and Dr. Winston F. Ponder, for their extraordinary contributions in the field of molluscan diversity and systematics. Note that both colleagues are curators in museums, thus their legacy will be twofold: all specimens they collected and deposited during decades of scientific activities and their papers: both will be a source of research and will stimulate and educate future malacologists.

I would like to take this opportunity to thank the local organizing committee around Heidi Gensler for their tireless work in preparing WCM 2022, the council and members of Unitas Malacologica for making this important event possible. I am indebted to our university, the Ludwig-Maximilians-Universität (LMU) München: the Congress Center, the Faculty of Biology, and the GeoBio-Center of LMU all provided help in many respects as did the Staatliche Naturwissenschaftliche Sammlungen Bayerns (SNSB) with their various institutions, and the Technische Universität München (TUM). I thank all the sponsors and donors, their contributions have made this congress possible indeed. On behalf of the organizing committee, we would like to thank all participants for your attendance. We all hope that WCM 2022 will be a fruitful and stimulating event, and will provide many opportunities to share your knowledge, expertise, and ideas.

Enjoy Munich, “the largest village on earth” (as it is called by the locals), experience the local dishes, sights, and culture. We wish you a wonderful and rewarding time in Bavaria!

Gerhard Haszprunar – President of Unitas Malacologica 2019-2022



# Welcome to the Biocenter LMU Munich – The conference site of WCM 2022

## About the Biocenter

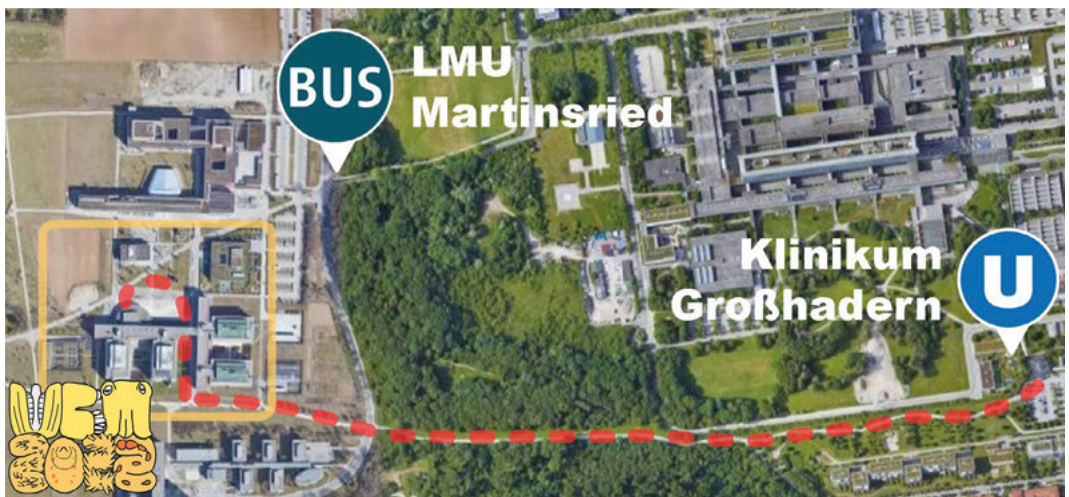
The Biocenter LMU is home to the Faculty of Biology of the Ludwig-Maximilians-Universität München (LMU Munich) and hosts the WCM 2022. The Biocenter lies just outside the Munich city borders, in Planegg-Martinsried. The faculty hosts thirteen divisions, including the Systematic Zoology work-group which is your host here at the Biocenter.

## How to get there

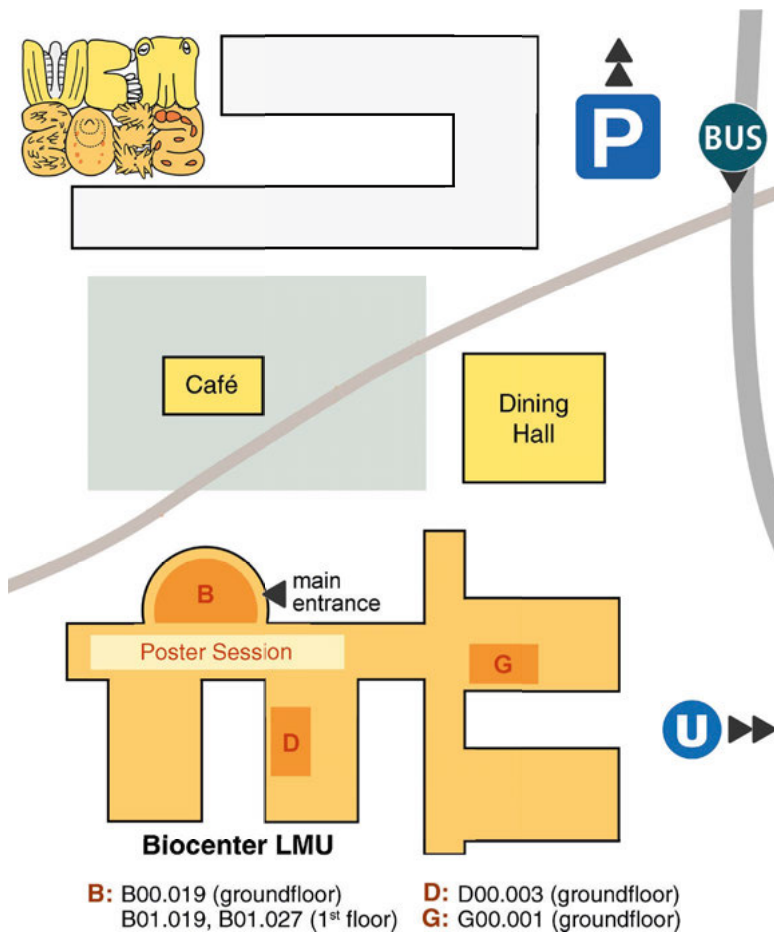
As there are only limited parking lots available, we recommend that you use public transportation to get to the conference site. For your convenience, we will provide you with a ticket valid for all public transportation (selection of trains, suburban train S-Bahn, underground U-Bahn, tram, and bus). You can find detailed instructions on how to get to the Biocenter from MUC airport, central station (“Hauptbahnhof”), and by car on the homepage of the Biocenter.

## By Public Transportation

Take the underground line U6 in the direction of “Klinikum Großhadern” until the last stop “Klinikum Großhadern” (beware: there is an earlier stop called “Großhadern”. Do not get off at this stop). From there on you can either take the bus N° 266 “Planegg Bf” until the stop “LMU Martinsried” (three stops, approx. 4 minutes) from where it is only a short walk to the conference site (see below). Alternatively, you can directly walk to the conference site after you get off the U6 at “Klinikum Großhadern” (ca. 900 m, about 12 minutes, see red dotted line below). Timetables and routes for public transportation in Munich are available here: <https://www.mvg.de/dienste/verbindungen.html> (just switch on the “Automatic Translation” function). Buses and metro generally leave every 10 minutes.







You can also use rental bikes provided by the MVG (Munich Public Transport Corporation) available at the Biocenter and Klinikum Großhadern. Further info is available on the MVG homepage and in the MVGO App (<https://www.mvg.de/services/mvg-rad.html>).

## At the Conference Site

All symposia and open sessions will be held in five different lecture halls at the Biocenter. Posters will be displayed throughout the conference in the main atrium of the Biocenter. Lunch will be available for conference attendees at the Café and the Dining Hall.

If you would like to get food outside of the coffee and lunch breaks, there are several options in walking distance in Martinsried:

- Bakery and Café “Vor Ort”
- Indian restaurant “Moti Mahal”
- Italian restaurant “La Fattoria”
- Groceries and snacks at “Rewe”

## Programme Overview

<b>Monday, 1<sup>st</sup> August 2022</b>					<b>Tuesday, 2<sup>nd</sup> August 2022</b>					<b>Wednesday, 3<sup>rd</sup> August 2022 – FREE DAY</b>					<b>Thursday, 4<sup>th</sup> August 2022</b>					<b>Friday, 5<sup>th</sup> August 2022</b>				
08:40 - 09:00	Hall 1: Welcome & Opening Remarks																							
	Hall 1: Plenary I + II				Hall 1: Plenary III + IV								Hall 1: Plenary V + VI				Hall 1: Plenary VII + VIII							
<b>Morning Session I</b> 09:00 - 10:30																								
10:30 - 10:45	Coffee Break				Coffee Break				Coffee Break				Coffee Break				Coffee Break				Coffee Break			
	Hall 1	Hall 2	Hall 3	Hall 4	Hall 1	Hall 2	Hall 3	Hall 4	Hall 1	Hall 2	Hall 3	Hall 5	Hall 1	Hall 2	Hall 3	Hall 5	Hall 1	Hall 2	Hall 3	Hall 5				
<b>Morning Session II</b> 10:45 - 12:25	EBS	MEH	AMS	SPD	EBS	CON	AMS	MEH	HET	ECO	CON	BIO	ECO	EVO	HET	INS								
12:25 - 14:00	Lunch Break				Lunch Break				Lunch Break				Lunch Break				Lunch Break							
<b>Afternoon Session I</b> 14:00 - 15:40	EBS	MEH	AMS	ENV	EBS	CON	AMS	NEC	HET	ECO	PAR	DIV	ECO	VIS	HET	INS								
15:40 - 15:55	Coffee Break				Coffee Break				Coffee Break				Coffee Break				Coffee Break							
<b>Afternoon Session II</b> 15:55 - 17:15	EBS	MEH	AMS	ENV	EBS	CON	MAL	ALL	HET	ECO	PAR	DIV	VOL	PHY	HET	INS								
Evening	Poster Session				Science Slam/ Auction (B00.019)				Unitas Malacologica General Assembly (B00.019)				Congress Dinner											

Hall 1: B00.019, Hall 2: B01.019, Hall 3: B01.027, Hall 4: G00.001 (Monday, Tuesday), Hall 5: D00.003 (Thursday, Friday).

## Symposia and Open Session Abbreviations

ALL	Open Session: All About Light...
AMS	American Malacological Society President's Symposium: Molluscan Conservation
BIO	Open Session: Biogeography
CON	Continental Molluscs Facing Environmental Changes
DIS	Open Session: Diversity and Distribution (posters only)
DIV	Open Session: Diversification and Speciation in Terrestrial Molluscs
EBS	Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honour of Philippe Bouchet
ECO	The Ecology of Fossil and Extant Molluscs
ENV	Open Session: Environmental Stress/ Adaptation
EVO	The EvoDevo Corner
GEN	Open Session: Genomics (posters only)
HET	Systematics and Evolution of (not only Marine) Heterobranchia
INS	Insular Ecosystems as Cradles of Mollusc Biodiversity and Evolution
MAL	Open Session: The Malacological Community
MEH	Mollusc Evolutionary History
MOR	Open Session: Morphology (posters only)
NEC	Open Session: The Neglected Classes
PAR	Molluscs as Parasites and Victims of Parasitism: Biodiversity, Ecology and Evolution
PHY	Open Session: Phylogeny
SPD	Open Session: Species Delineation
VIS	Open Session: EvoDevo and Vision
VOL	Volunteers in Malacology

# Symposia of the WCM 2022

## Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honour of Philippe Bouchet

Organized by

Nicolas Puillandre (Muséum national d'Histoire naturelle Paris, France)

Ellen Strong (Smithsonian National Museum of Natural History, USA)

Yuri Kantor (Russian Academy of Sciences, Moscow, Russia)

Philippe Bouchet, Senior Professor at the Muséum national d'Histoire naturelle in Paris, will retire in August 2022 after a career spanning almost five decades. Since joining the museum in 1973 as an assistant, he has authored more than 210 original refereed research papers and ca. 160 other publications, and has described over 600 new species of molluscs. Through his leadership, the malacological collection of the MNHN has become the most active in the world, with a remarkable annual growth, especially of type specimens. Philippe's primary interests are in the exploration and description of invertebrate diversity, particularly molluscs. His passion for exploring unknown faunas was the impetus for launching "Our Planet Reviewed", an initiative that has become the gold standard for biodiversity surveys. He is a recognized leader in this field and has cultivated an international network of collaborators, both academics and citizen scientists, to valorize these collections. This symposium organized in his honor will bring together scientists not only to celebrate Philippe's career and accomplishments, but also to present new discoveries made possible by MNHN expeditions.

The organizers of this symposium have offered special support to early career researchers through their travel grant and research award scheme.

All contributions to this symposium are identified by the abbreviation EBS.

## Mollusc Evolutionary History

Organized by

Alexander Nützel (SNSB – Bavarian State Collection for Palaeontology and Geology, Munich, Germany)

Katie Collins (The Natural History Museum, London, United Kingdom)

Mariel Ferrari (Instituto Patagónico de Geología y Paleontología, Puerto Madryn, Argentina)

Molluscs are amongst the most diverse animal phyla and are well-represented in the fossil record. The phylogeny of molluscs has been disputed for a long time, based on morphological, palaeontological and molecular analyses. There are several entirely extinct groups of molluscs, such as Bellerophonitida, Ammonoidea, Hippuritida, or large parts of Nautilida and Trigoniida, to name only a few, which need to be positioned in the mollusc tree of life. The identification of the earliest representatives of clades is a challenging paleontological task. Palaeontologists continuously discover new faunas and taxa during fieldwork or by screening legacy natural history collections.

This symposium welcomes contributions from both palaeontologists and neontologists on the following fields:

- Fossil molluscs in phylogenies: positioning extinct clades, dating of nodes, and evidence for character evolution.
- Discovery of new fossil taxa (taxonomists welcome!).
- Diversity of molluscs including the impact of mass extinction events.

All contributions to this symposium are identified by the abbreviation MEH.

## **American Malacological Society President's Symposium: Molluscan Conservation**

Organized by

Kenneth A. Hayes (Director of the Pacific Center for Molecular Biodiversity,  
Bernice Pauahi Bishop Museum, Hawaii, USA)

The synergistic impacts of centuries of unchecked habitat destruction, invasive species, and climate change continue to exacerbate extinction rates among all taxonomic groups, bringing ecosystems to a tipping point. As we quickly approach the mid-way point of the 21<sup>st</sup> century we find ourselves confronting the most serious impacts of biodiversity loss and climate change. Molluscs, found across marine, terrestrial, and freshwater habitats, have suffered substantial losses, with estimated 1032 species extinctions since 1500 – the year International Union for Conservation of Nature uses as the starting point for counting extinctions. Although this is less than 1.4 % of the total described number of molluscs (~76000), it is probably a dramatic underestimate, with some studies indicating the numbers may be greater than 5000 species.

This symposium on Molluscan Conservation will feature speakers from across habitats and molluscan classes providing an updated picture of molluscan biodiversity in the Anthropocene and highlighting the research efforts to understand and mitigate the decline among molluscs.

All contributions to this symposium are identified by the abbreviation AMS.

## **Continental Molluscs Facing Environmental Changes**

Organized by

Quentin Wackenheim, (Sorbonne University, France)

Salomé Granai, (GéoArchPal, GéoArchÉon, France)

Lucie Juříčková (Charles University Prague, Czech Republic)

Ondřej Korábek (Charles University Prague, Czech Republic)

Over the last million years, continental molluscs survived major climate and environmental changes. Palaeomalacological studies provide valuable insight to understand their modern distribution and ecology. Today, anthropogenic activities and the rapid ongoing climate changes influence the diversity and population dynamics of molluscs and their habitats.

This symposium aims to discuss the response of molluscs to these impacts from the past to the present, from the million years to the year, from the biome to the micro-habitat:

- Ecological tolerance to changes in habitat
- Past distribution vs. modern distribution
- Population and species association changes
- Long-term or short-term variations
- The priority effect in malacology

All contributions to this symposium are identified by the abbreviation CON.

# **Systematics and Evolution of (not only Marine) Heterobranchia**

Organized by

Bastian Brenzinger (SNSB-ZSM, Bavarian State Collection of Zoology  
Munich, Germany),  
Katharina M. Jörger (SNSB-ZSM, Munich, Germany),  
Juan Moles (Faculty of Biology, University of Barcelona, Spain),  
Timea Neusser (LMU Munich, Germany)

Heterobranch snails and slugs contain about half of all extant gastropod species and about one-third of all molluscs. They inhabit aquatic and terrestrial environments all over the world and display a myriad of body shapes and ecological roles with underlying evolutionary processes.

Members include tiny lower heterobranchs, charismatic sea slugs and sea hares, ecologically important pteropods and ubiquitous aquatic and land pulmonates, among others. Traditional classifications have changed dramatically and heterobranch evolution is continuously better-understood thanks to more comprehensive, balanced datasets, and thorough analyses.

Here we invite contributions on any aspects of systematics, evolution and biology of heterobranchs, encouraging broader and more integrative visions across traditional opisthobranch and pulmonate borders, bringing researchers from different taxa, environments and methodologies together.

All contributions to this symposium are identified by the abbreviation HET.

## **The Ecology of Fossil and Extant Molluscs**

Organized by

Simon Schneider (CASP, Cambridge, United Kingdom),  
Andrzej Kaim (Polish Academy of Sciences, Warsaw, Poland),  
Thomas Neubauer (Justus Liebig University Giessen, Germany)

Molluscs have developed a wide range of environmental adaptations, and some of them take an active role as ecosystem engineers. The functional morphology and biomineralisation of mollusc shells record information on their environment. In addition, molluscs have acquired advanced behavioral traits, related to feeding, predation and propagation.

This symposium welcomes presentations on all aspects of the autecology and synecology of fossil and modern molluscs, including:

- Life-life interactions of molluscs, such as parasitism, commensalism, predator-prey or epibiont-substrate relationships.
- The impact of molluscs on their environment, including vegetation, water quality, food chains or community structure.
- Molluscs as (sclero)chronological archives of climate, pollution or other environmental parameters.
- Mollusc-dominated and chemosymbiotic communities, their ecology and evolution, and their environmental significance.

All contributions to this symposium are identified by the abbreviation ECO.



## **Molluscs as Parasites and Victims of Parasitism: Biodiversity, Ecology, and Evolution**

Organized by

Kenneth De Baets (GeoZentrum Nordbayern, Erlangen, Germany),  
Aleksandra Skawina (University of Warsaw, Poland)

Up to half of all animals have been estimated to have a parasitic lifestyle, yet the number of transitions to parasitism and their potential for species diversification and evolution remain largely unresolved. Mollusks are diverse both as parasites and host for parasites. They offer a unique perspective to better understand the impact of parasite-host interactions on the biodiversity, ecology and evolution of both parasites and victims. The symposium brings together researchers working on the co-evolution of mollusks and their parasites or hosts in the broadest sense ranging from the modulation of mollusk immune responses, diversity and distribution in relationship with their parasites as well as the impact of parasitic mollusks on the ecology and evolution of their hosts.

All contributions to this symposium are identified by the abbreviation PAR.

## **Volunteers in Malacology**

Organized by

Anna Holmes (National Museum Wales, Cardiff United Kingdom),  
Ben Rowson (National Museum of Wales, Cardiff, United Kingdom)

Malacological research has always depended on the contributions of unpaid volunteers. Whether such people are called amateurs, enthusiasts, citizen scientists, collectors, data-miners or indeed aspiring professionals, the work of volunteers past and present remains integral to malacology today. Assistance from local communities is often vital to monitoring or recording projects that require many eyes on the ground. Online technologies using image-sharing have revolutionized identification and data collection, and broadened participation. Volunteering at institutes like museums remains a mutually rewarding activity. In some settings, publicly-funded research funding is increasingly tied to community outreach and engagement. Knowing one's audience and how to engage them is as important as an understanding of the limits of volunteer work. Learning from those who have experienced and learned from this practice is invaluable.

This symposium welcomes presentations on any aspect of malacological research involving the contributions of volunteers.

All contributions to this symposium are identified by the abbreviation VOL.

## **The EvoDevo Corner**

Organized by

Tim Wollesen (University of Vienna, Austria)

Mollusks are one of the most speciose metazoan clades exhibiting a stunning diversity of extant and fossil adult body plans. In addition, various modes of direct and indirect development are known with a plethora of fascinating larval types.

In this evolutionary developmental symposium current contributions on molluscan developmental stages and adults will be discussed and approaches comparing their body plans to those of other spiralian or bilaterians are appreciated. Classical EvoDevo topics such as genotype-phenotype relation or life cycle evolution are welcome. Besides morphological and molecular studies, this symposium will also cover paleontological, proteomic, or ethological studies.

All contributions to this symposium are identified by the abbreviation EVO.

## **Insular Ecosystems as Cradles of Mollusc Biodiversity and Evolution**

Organized by

Björn Stelbrink (Museum für Naturkunde Berlin, Germany)

Kostas Triantis (National & Kapodistrian University of Athens, Greece)

Christian Albrecht (Justus Liebig University Giessen, Germany)

In this symposium, we want to bring together malacologists that are interested in both extant and extinct faunas of insular ecosystems. This may not only include true islands and archipelagos but also crater and long-lived lakes, mountain tops ("islands in the sky"), caves and others. We invite contributions from a broad spectrum of topics including biodiversity and conservation, shell morphology and evolution, invasive species as well as phylogenetic and biogeographical reconstructions.

All contributions to this symposium are identified by the abbreviation INS.

# Plenary Talks

## Meeting of generations: how our past efforts in mollusc conservation can steer the young malacologists of the future

Mary Seddon<sup>1</sup>, Manuel Lopes-Lima<sup>1,2</sup> & Monika Böhm<sup>1,3</sup>

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In light of the new Post-2020 Biodiversity Framework and the WCM 2022's motto, "Meeting of Generations", it is timely to reflect on our collective efforts in highlighting the status and trends of the world's molluscs over the past decades. Thanks to the vast amount of work carried out by so many, we are likely to achieve the milestone of 10 000 mollusc assessed on the IUCN Red List of Threatened Species, the world's most authoritative source for extinction risk information of species. Most of the mollusc species assessed (around 80 %) are found in terrestrial or freshwater habitats. Several regional assessment projects, as well as a sampled assessment for freshwater molluscs, have contributed to this milestone. As a result, we now have a large (if still incomplete) dataset to investigate the status of the world's molluscs and the threats impacting them. This in turn allows us to build on previous work on this – often thought to be one of the most imperiled - group of animals, and to allow conservation priorities to be set for future action. The extensive Red List metadata that has been compiled (e. g., distribution, life-span, threats, ecosystem services, etc.) is also being increasingly used as a baseline and important tool for a new generation of malacologists, modellers, macroecologists and conservationists as well as biodiversity activists worldwide!

Here, I will be attempting to summarize the vast amount of work carried out by so many over the past decades: what can we learn from comprehensively assessed regions of the world, like Europe, Africa, and parts of Asia? What is the likely status of non-marine molluscs worldwide? How have recent assessments helped us to understand the major threats impacting these species – and how do threats vary across regions? What are the next steps in "mainstreaming" molluscs into conservation efforts worldwide? And how can we "meet the next generation" of malacologists to carry the torch for mollusc conservation?

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<sup>2</sup> CIBIO/InBIO – Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

<sup>3</sup> Global Center for Species Survival, Indianapolis Zoo, Indianapolis, USA

## Evolutionary crossroads – molluscan evolution during the early Palaeozoic

Jan Ove R. Ebbestad<sup>1</sup>

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Molluscs, present and past, are second only to arthropods in abundance, diversity, morphological disparity from the shell-less to the multi-shelled, and in environmental adaptations. While monophyly of extant molluscan classes is accepted, the relationship within these are unclear and even the origin of the group itself is murky. Possible Ediacaran candidates exist, but not until the early Cambrian, enhanced in part by preservation of hard parts, do we see a radiation of molluscan taxa in the fossil record. Clear crown group molluscs appear later in the Cambrian after an evolutionary bottleneck experienced by several groups. Arguably, the most profound effects on molluscan evolution took place during the unparalleled Ordovician radiation and diversification. A second evolutionary bottleneck came to pass during the intensified cooling of Earth that triggered the second largest mass extinction marking the transition into the Silurian. This period is signified by warming but with perturbed climatic changes and whereas the Ordovician showed a great spread of continents, these are now amalgamated in increasingly larger complexes. Against this backdrop, several key events direct molluscan evolutionary pathways. An early challenge was the Cambrian substrate revolution, whereby burrowing and bioturbation gradually replaced the prevalent early Cambrian algal mat environments. This evolutionary precipice led to extinctions but also new ecological opportunities. In concert, increased predation pressure is evident, which at the transition to the Ordovician may have facilitated the evolution of planktotrophic mollusc larvae. Another effect is the culling of certain gastropod ecomorphotypes, whereby open coiled sedentary forms go extinct. Molluscs also took to the infaunal habitat, seen through elongation (scaphopodization) of their shells and the advent of pedal burrowing in bivalves. Both gastropods and cephalopods display a rich morphological evolution during this time, and endemism is high among the benthic fauna. Centres of radiation, especially in peri-Gondwana areas, may have exerted a significant influence on the dispersal pattern, with speciation taking place in the centres of origins of groups. These are some of the evolutionary pathways of the astounding Molluscan Rhapsody during the lower Palaeozoic, of which we only glimpse so little and have yet to explore more fully.

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## **The contributions of genomics for understanding the phylogeny, ecology and evolution of molluscs**

**Tauana Cunha<sup>1</sup>**

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What processes have shaped the evolution of the diverse, ancient and successful molluscs? In this talk I will highlight some of the latest contributions of genomics to molluscan studies from three major perspectives: evolutionary relationships, molluscan biology, and genome evolution. As the amount of data grows and more taxa are sampled, phylogenies have been better resolved both among and within classes of molluscs. Nonetheless, even the largest available datasets still fail to elucidate a few deep divergences. Focusing on abalones, turban snails and keyhole limpets, I will show how genome-level data is often needed, but not always sufficient, and that accounting for the complexity of large datasets is essential to understand phylogenomic conflict. Genomics is also providing new insights into important biological processes, ecological questions and applications, such as biomineralization, symbiotic relationships, and population dynamics. As an example, I will show how genome-wide data reveals the history of populations of a threatened marine species to inform conservation actions. Finally, as the number and quality of whole mollusk genomes increase, questions about the evolution of genomes themselves could be revisited. I will summarize genomic resources currently available, and their use to explore topics such as the evolution of genome size and structure.

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<sup>1</sup> Smithsonian Tropical Research Institute, Panama City, Panama



## **Here's to the Next Generation Scientists: How to navigate the rocky road towards a scientific career – experiences made and lessons learned from a legend in malacology**

**Andreas Wanninger<sup>1</sup>**

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This invited lecture will build on the WCM 2022 key motto “Meeting of Generations”. Using Gerhard Haszprunar, one of the most influential malacologists of the past decades, as a role model, I will provide a personal view on some key issues and prerequisites when pursuing a scientific career in modern-day academia. I will particularly address questions revolving around important decisions of early stage scientists such as choosing the right lab and supervisor, as well as sacrifices and benefits of early and late career academic life. I will highlight the influence and hence responsibilities PIs, supervisors, and academic teachers have concerning the motivation, scientific, and personal development of their mentees. By specifically addressing Gerhard Haszprunar’s achievements in scientific research, teaching, and mentoring, I will conclude with what, in my view, are key factors when evaluating the success of a scientific career, way beyond impact factors and other seemingly “objective” metrical measures.

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## **Systematics and evolution of (not only marine) Heterobranchia**

**Heike Wägele<sup>1</sup>**

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More than 10 years ago, Opisthobranchia, a group known for two centuries, was literally stabbed down after it became clear that this group was paraphyletic or even polyphyletic. Since then, many molecular analyses have confirmed these results. In my talk, I will briefly discuss the latest genomic results on Heterobranchia, highlighting the groups that are still problematic. I will also use some examples to show how genomic analyses are replacing single gene analyses to explain the evolution of certain ecological traits. Based in part on the phylogenies, I will also summarize the current state of knowledge on some interesting biological phenomena that my research group and I (but not only we) have been working on. These include the incorporation and utilization of photosymbiotic units, as well as defense systems such as cnidocyst sequestration or the incorporation of secondary metabolites. However, I would also like to address biodiversity studies and methods that are of interest for assessing and monitoring marine heterobranch biodiversity. I apologize in advance that I will not be able to cover all the interesting aspects of the truly diverse Heterobranchia in this review and that the focus will be mainly on marine Heterobranchia.

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<sup>1</sup> Leibniz Institute for the Analysis of Biodiversity Change, Museum Koenig Bonn, Germany

## **(Palaeo)ecological aspects to the evolution of shell microstructure and mineralogy**

**Elizabeth M. Harper<sup>1</sup>**

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In recent years shell microstructure and mineralogy has attracted attention from scientists in two separate branches of science, namely those trying to establish characters of phylogenetic significance and material scientists in the search of bio-inspired novel materials. There has been far less recent interest in what might be thought of as the ‘middle ground’ between these endeavours, that is understanding the significance of the wide microstructural and mineralogical diversity.

There seems little doubt that the primitive molluscan shells were wholly aragonitic, and that a combination of prisms and nacre were widespread amongst early taxa. Today this arrangement is uncommon in both bivalves and gastropods, with most taxa employing a wide variety of apparently more derived aragonite and calcite microstructures arranged in different combinations. Where does this diversity come from? Why have some groups of molluscs acquired particularly microstructural/mineralogical arrangements and not others? Does it have to be adaptive? And if so, adaptive to what? Were changes in microstructural arrangements important in determining past (and so possibly future) ecological innovations or challenges?

Demonstrating solid links between the evolution of shell properties and specific ecologies rather than ‘adaptive fairy tales’ is not a trivial exercise. Difficulties arise from problems with demonstrating putative selective advantages, accurately tracing microstructural innovations in fossil material and the lack of analytic power where particular microstructures are autapomorphic. In this talk I will focus on a number of different derived microstructures/mineralogies and associated ecologies and habitats.

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<sup>1</sup> Department of Earth Sciences, University of Cambridge, Cambridge, United Kingdom

## Citizen science: a slug success story

**Imogen Cavadino<sup>1,2</sup>, Hayley Jones<sup>1</sup>, Gordon Port<sup>2</sup>,  
Helen Roy<sup>3</sup> & Gerard Clover<sup>1</sup>**

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Terrestrial slugs and snails are widely detested by gardeners and are frequently the most common pest mentioned in enquiries to the Royal Horticultural Society (RHS) Gardening Advice Service. However, many species of slug and snail play important roles in breaking down decaying material and recycling nutrients into the soil. In domestic gardens the presence of slugs is usually recognized by feeding damage. Despite the impact slugs have on gardens and gardeners, slug species are surprisingly under recorded within this habitat. This talk will explore how the general public were engaged with and empowered to identify species of slug found in their garden following structured survey approaches to create detailed evidence of how the slug fauna is changing within British gardens. More than 794 volunteers have contributed in surveys including the species-specific Cellar Slug survey, and “Slugs Count” which is the first in depth study of the complete British garden slug fauna since the 1940s. Over 22 000 new slug records made by people from across the UK have contributed to our understanding of the slug fauna in Britain and will provide evidence to assess large-scale changes in the distribution of these important invertebrates.

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## **From single cells to complex organs – evolutionary developmental insights from hitherto neglected mollusks**

**Tim Wollesen<sup>1</sup>**

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Mollusks are a highly diverse clade of spiralian protostomes that we all share a fascination for. The majority of current phylogenies suggest a sister group relationship of Conchifera and Aculifera rendering it difficult to polarize character states and to infer how the last common molluscan ancestor looked alike. In contrast to morphological traits, the developmental pathways that give rise to the latter have been studied to a lesser degree, in particular in non-gastropod mollusks.

In order to gain deeper insights into the molecular underpinnings of molluscan development I study neglected taxa that are phylogenetically informative such as polyplacophorans, solenogastres, and scaphopods. In addition, crucial molluscan outgroups such as entoprocts or representatives of the lophotrochozoan sister clade, the Gnathifera, are studied in my lab in order to infer putative apomorphies and synapomorphies of spiralian clades.

Recent advances in RNA-sequencing allow for the identification of cell types based on their gene expression profiles. In my talk, I will focus on the use of state-of-the-art single-cell RNA-sequencing on polyplacophoran developmental stages and explain how these data contribute to our understanding on molluscan body plan development and evolution.

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## **Symposium**

# **Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honor of Philippe Bouchet**

## **Part I**

### **Abstracts of oral presentations**



## The challenges of describing the collapse of marine invertebrate diversity

**Paolo G. Albano<sup>1</sup>**

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Despite only few marine species are known to have gone extinct in historical times and most of these are mammals and birds, marine invertebrates are under increasing pressure by the multiple facets of global change. The description and understanding of the resulting regional-scale collapses, however, have three major impediments. First, the lack of baselines. Due to the shortness of most observational datasets, there is usually limited information on the composition of marine ecosystems before the onset of anthropogenic pressures, hindering the quantification of biodiversity loss. Second, the size bias. The full invertebrate size spectrum is rarely considered in ecological studies, with results biased in favour of large and easily detectable species. Third, the taxonomic impediment. Particularly for tropical taxa, it affects the fine appreciation of which species get lost and delays the reporting of newly settled non-indigenous species.

I will show here how these impediments have been overcome when studying the ongoing biodiversity collapse in the eastern Mediterranean. This basin is warming at two to three times the rate of the global ocean. It is thus a natural laboratory to observe and test hypotheses on how global warming is affecting marine biota because in its warmest sectors temperatures are exceeding the thermal tolerance of native species, resulting in regional scale collapses. At the same time, these new environmental conditions favour the invasion of thermophilic tropical non-indigenous species entering from the Suez Canal.

The lack of baselines was overcome by reconstructing historical taxonomic and functional richness with death assemblages, the accumulations of skeletal parts on the sea bottom. This approach enabled quantifying an up to 90% native diversity loss on the Israeli shelf. The size bias was overcome by using small mesh sizes. The consequent effort to sort time-intense fine fractions resulted in a detailed understanding of the collapse mechanism: young recruits die before they reach reproductive size, making the shallow shelf a demographic sink. The taxonomic impediment is being approached by accurate descriptive taxonomy and by the deployment of integrative taxonomy approaches that, despite still in progress, are already offering unexpected insights into the taxonomic composition of this novel ecosystem.

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<sup>1</sup> Department of Animal Conservation and Public Engagement, Stazione Zoologica Anton Dohrn, Naples, Italy

## On the history of MolluscaBase, and on a modern Linnean apostle

**Ruud A. Bank<sup>1</sup>**

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One of the most fundamental disciplines in biology is taxonomy. It categorizes the diversity of life on Earth, providing scientific names to species. Although the basic unit in taxonomy is the species, there are variety of definitions of what a species is, resulting in disagreements about species boundaries. Apart from that, taxonomic revisions based on newly collected or previously unknown material, and/or by combining a variety of techniques (such as combining morphology data with genetic sequences or proteomics data), results in new insights that often has an effect on species names. Species names are in essence vehicles to organize and store information about taxa, and enables as such communication about the world's biodiversity.

Linnaeus introduced in 1758 a system of nomenclature for animal species, a system that is still in use. The International Code of Zoological Nomenclature established rules for the use of scientific names for animals. What would be extremely helpful, is to establish a global list of molluscan species (marine and continental species, both recent and fossil). MolluscaBase is a taxonomically oriented database which aims to provide an authoritative, permanently updated account of all molluscan species. The project for MolluscaBase began in February 2014, with a meeting at the Flanders Marine Institute (VLIZ) in Oostende, bringing together a group of malacologists (Philippe Bouchet, Gary Rosenberg, Serge Gofas, Simon Schneider, André Sartori, Eike Neubert, Ruud Bank), among whom molluscan editors of World Register of Marine Species (WoRMS) and Fauna Europaea, and members of the data management team at VLIZ. MolluscaBase will contain, apart from the accepted (valid) species name, also data about its classification (presented with a parent/child hierarchy), synonyms, reference of original description and other relevant literature sources, type locality and distribution, stratigraphic range, notes and images. An active editorial team is constantly expanding and updating the database. I will describe in short the road to MolluscaBase (from the perspective of continental molluscs). The father of MolluscaBase is Philippe Bouchet. He also organized numerous expeditions around the globe, resulting in the discovery of thousands of new species. As such he can be considered a modern apostle of Linnaeus.

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## Advancing in the knowledge of Solenogastres (Mollusca Aplacophora) through the expeditions of the Muséum National d'Histoire Naturelle

**M.C. Cobo<sup>1</sup> & K. M. Kocot<sup>1,2</sup>**

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Despite interest due to their phylogenetic importance, Solenogastres (Mollusca, Aplacophora) remain relatively poorly known. This is largely because they usually go unnoticed during sampling due to their small size or are misidentified by non-specialists (e.g., as sea cucumbers, annelids, etc.). Therefore, their presence in scientific collections is rare and valuable. Moreover, their actual diversity is underestimated with nearly all species only known from their type locality (mostly from well-studied areas such as the Antarctic, NW Atlantic and the Mediterranean Sea) and described from one or very few specimens. In addition to the deposited type material (15 species), the Muséum national d'Histoire naturelle (Paris) houses a rich collection of solenogasters with more than 350 specimens. These specimens were collected in 24 expeditions carried out worldwide, including many areas with no or few solenogaster records. Based on habitus and sclerites, at least 143 different morphospecies have been identified in this collection, belonging to three of the four orders of solenogasters. To date, we have prepared descriptions of eight new species from this collection with three from the China Sea and five from the Caribbean Sea. Preliminary results on the remaining 24 specimens collected in the Caribbean point to 16 putative new species. In addition, the 48 specimens collected in the most recent expeditions led by the Muséum national d'Histoire naturelle in Corsica belong to 14 species, all new records for the area. In addition to our discovery of new species in this material, we are working on redescription of known ones, which is valuable to obtain molecular and more detailed anatomical data and will help clarify Solenogasters systematics and improve understanding of morphological character evolution. DNA barcodes generated from these specimens will help future non-experts identify these species easily, and the extracted DNA will be leveraged in our ongoing investigations of aplacophoran phylogeny. These new records of known species, along with the discoveries of new ones from understudied areas (from where 90 % of the collected species are estimated to be new), are crucial to increase our knowledge on the real distribution and diversity of solenogasters.

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## **My time at the MNHN 2002–2022: curation, extinction and Philippe Bouchet**

**Robert H. Cowie<sup>1</sup>**

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I first visited the Muséum national d'Histoire naturelle (MNHN) in 2002. I was working on apple snails (Ampullariidae), and in particular a nomenclatural/type catalogue of the New World species. Philippe (and Virginie Héros, of course) had been very helpful sending copies of literature, but I still needed several obscure publications, and spent two days making copies and taking a quick look at the specimens in the typothèque and zoothèque. The catalogue was published later that year. As a result, at the 2003 American Malacological Society meeting at the University of Michigan, Philippe invited me to curate the MNHN Ampullariidae, which took several years, with a catalogue of the types published in 2012. Meanwhile, I had co-edited Volume 25 (2008) of the Tropical Deep Sea Benthos! During this period I photographed the Succineidae types and photocopied literature for a still unpublished global nomenclatural/type catalogue of succineids. I curated the Hawaiian land snail types and we published a catalogue in 2016. But probably around 2007, Philippe invited me to serve on Claire Régnier's PhD thesis committee, and thus began our collaboration on the global extinction of molluscs. After Claire graduated in 2011, having already published a classic paper on mollusc extinction in 2009, she spent time in Hawaii as a postdoc, which led to a publication on the extinction of all but a handful of the 325 species of the endemic Hawaiian land snail family Amastridae. In the same year, we published part of Claire's thesis, which made catastrophic predictions of the Sixth Mass Extinction, not only of molluscs but of all biodiversity. In 2017, we updated Claire's 2009 paper. And most recently, Philippe and I, with Benoît Fontaine, published a major paper on the Sixth Mass Extinction, suggesting that biodiversity is now going extinct at a rate at least 100 times greater than the background rate. Having spent considerable time at the MNHN over the last 20 years, I have come to love it and the people who work there, but none of it would have happened had it not been for Philippe's initial invitation to curate the apple snails.

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## Chitonidae subclades each have an ancient split between the Americas and the Old World

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Faunal invasions in recent geological epochs have occurred sporadically across northern oceans, but further south along American coasts, those tropical taxa with limited larval dispersal have had less faunal interchange. Such is the case for Chitonidae (Polyplacophora), entirely absent from northern seas and with regional phylogeographic patterns as expected given the brief larval stage typical of chitons. In my own and collaborative molecular studies with help from MNHN, FMNH, and other museums, we have rejected monophyly for multiple chiton genera currently considered to be globally widespread. These cases often revealed morphological convergence for shell features emphasized in chiton taxonomy. Instead, molecular phylogenetic analyses including for Chitonidae support regional geographic affinities. American Chitonidae are found in the Caribbean and throughout the Tropical East Pacific, extending also to Patagonia and west to the Galápagos. In my assembled global data sets, the American Chitonidae genera are well separated their closest relatives from elsewhere, which I have interpreted as reflecting likely ancient tectonic isolation. Dense sampling of mitochondrial 16S + *cox1* for global Chitonidae have led me to hypothesize vicariant or dispersal events with similar rather ancient timing for each of two even more ancient and reciprocally monophyletic Chitonidae subclades: Chitoninae, which lack shell eyes (ocelli), and Acanthopleurinae + Tonicinae, which have shell eyes. Both subclades include genera conventionally thought to be widespread, but mtDNA has revealed a different pattern. Each subclade is further split between the Americas and the Old World, a familiar pattern for terrestrial taxa but quite unusual for marine Mollusca. I propose different interpretations for each Chitonidae geographic split: vicariance possibly due to cooling along southern Gondwana coast for Chitoninae, and for Chitonidae with shell eyes, invasion from the central Pacific followed by impressive radiation. There are a few exceptions, most involving more recent long-distance dispersal via rafting, especially involving species that brood at southern high latitudes. In the tropics, *Acanthopleura* managed to invade the Caribbean from the east. I am revising Chitonidae generic assignment to reflect this lack of overlap, and this extends to additional quite deep regional divergences within both American and Old World Chitonidae radiations.

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## How many species can be hidden under a Chinese hat? The *Calyptraea chinensis* (Linnaeus, 1758) cryptic species complex

**G. Fassio<sup>1</sup>, G. Chiappa<sup>1</sup>, E. Nocella<sup>1,2</sup>, M. V. Modica<sup>2</sup> & M. Oliverio<sup>1</sup>**

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*Calyptraea chinensis* (Linnaeus, 1758) is an eastern Atlantic marine gastropod with a distinctive limpet-like conical shell, resembling a Chinese hat. Its geographic distribution ranges from Angola to Great Britain and Ireland, including the Mediterranean and the Black Sea. Due to its free filter-feeding lifestyle, it can be found on several different kinds of hard substrates (stones, empty valves, living mollusc shells), from shallow to deep waters. Its high phenotypic variability (e.g., shell shape and surface, body and shell coloration) led in the last centuries to the introduction of a number of other species names, currently all considered as synonym of *C. chinensis*. With the aim to define the actual diversity comprised under *C. chinensis*, we produced the first multilocus molecular dataset (COI, 16S, ITS2) of this nominal species, including 160 specimens from the Central and Western Mediterranean Sea, the Atlantic coasts of Spain and France, the English Channel, and the Irish sea. An integrative taxonomy approach revealed that *C. chinensis* is a cryptic species complex composed of 14 species (COI interspecific genetic distances 3–26%). These results are further corroborated by the sympatric occurrence of 8 pairs of species in 12 stations. The species comprised in the *C. chinensis* complex can be divided in two major phylogenetic lineages, both including Atlantic and Mediterranean species. It seems that most of the diversification occurred in the Central-Western Mediterranean, compared with the Atlantic/Alboran Sea (9 vs. 5 species), although alternative factors (e.g. unequal sampling effort) could also explain this pattern. The observation of overlapping morphologies between several different species, with some morphotypes recurring in almost all of them, suggests considering the phenotypic variability within the *C. chinensis* species complex as mainly driven by ecological factors probably acting at the individual level (e.g., different attaching surfaces and habitats), rather than by species or population constraints.

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## Barcoding the diversity of neglected meiofaunal molluscs in the Western Mediterranean (Gastropoda: Heterobranchia)

**Jose Fernández-Simón<sup>1</sup>, Xavier Salvador<sup>2</sup> & Juan Moles<sup>1</sup>**

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Marine interstitial habitats (mesopsammon) have been understudied due to complications of exploring its millimetric and highly adapted organisms. The restrictive ecological conditions of this neglected habitat, including faint light and limited amount of space has forced its fauna, including gastropod molluscs as one of the most common groups, to adapt by reducing body size to minute (often shell-less) vermiform organisms. These usually simplified heterobranch species are hard to collect and problematic to determine, often including cases of cryptic speciation. Here, we explore the interstitial heterobranch diversity alongside the Catalan coast (Western Mediterranean) in order to shed light into the taxonomy of this group. We have collected dozens of different species, providing live photographs, species identification based on the current taxonomic knowledge and species delimitations based on newly generated sequences of the cytochrome *c* oxidase (i.e., COI). Among the species already collected, we have found representatives of “lower heterobranchs” (Omalogyridae, Rhodopidae), Rissoelloidea, Cephalaspidea (Aglajidae, Philinidae, Philinoglossidae), Runcinacea, Acochliidiida (Parhedyliidae, Hedylopsidae) and Nudibranchia (Embletoniidae). Our project aims at describing the interstitial heterobranch diversity of the Western Mediterranean, assessing the phylogenetics of some key groups by providing newly generated sequences. Our preliminary results show first records of scarcely known species and possible new pseudo-cryptic species.

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# Multilocus DNA barcoding and integrative taxonomy of the oysters (Ostreidae and Gryphaeidae) of the Museum National d'Histoire Naturelle (MNHN) from different oceanographic expeditions reveal several MOTUs not assigned to known species

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Oysters play an important role in marine ecosystems and several species are of great economic importance. The families Ostreidae Rafinesque, 1815 (true oysters) and Gryphaeidae Vialov, 1936 (foam oysters) include about 80 and 10 species, respectively, distributed worldwide. Oysters' shells show a high degree of phenotypic plasticity, therefore, morphology alone is often uninformative or misleading for taxonomic classification. In recent years, thanks to molecular studies, several cryptic species have been discovered in different oceans. Nevertheless, we are far from a deep understanding of the taxonomic diversity and systematics of these groups. The Museum national d'Histoire naturelle de Paris (MNHN) hosts one of the most important oysters' collections in the world, built through several oceanographic expeditions in remote oceans. In this study, firstly we assessed a DNA barcoding framework of the MNHN oysters' collection using two mitochondrial markers (COI and 16S). For each marker, we identified the molecular operational taxonomic units (MOTUs) based on sequenced specimens to attempt a preliminary taxonomic identification. We carried out DNA extraction of around 500 specimens and we successfully sequenced 300 DNA samples with at least one marker. Sequenced specimens came from more than 20 oceanographic expeditions from temperate to sub-tropical and tropical seas carried out in the last two decades. Our investigations revealed more than 10 unassigned MOTUs (i.e. MOTUs not assigned to a sequence clusters of a known species) within Ostreidae and at least 2 unassigned MOTUs within Gryphaeidae. Secondly, for unassigned MOTUs, we applied an integrative taxonomic approach to clarify the taxonomy and systematics of each unknown lineage. We carried out different species delimitation analyses using both distance-based and tree-based methods based on single-locus and multilocus approaches (COI, 16S, ITS2 and 28S). When morphology was informative, we compared molecular results with shell characters and in some cases, we complemented these analyses with biogeographic data. In this presentation we show results from one of those cases, for each one of Ostreidae and Gryphaeidae, tackled by an integrative taxonomic assessment. This project will allow a great leap forward in our understanding of oyster systematics and distribution and toward a more complete inventory of marine biodiversity in hotspot regions.

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## Diversity squared: New insights into patterns of heterobranch species richness and diversification of lineages

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Expeditionary field work still remains the most fundamental tool to discover novel species and repetitive sampling in high diversity portions of the Indo-Pacific tropics continues to provide large numbers of previously undocumented taxa. Multidisciplinary collaborative teams and large expeditions are an immense source of novel biodiversity. Micro-scale temporal changes in diverse ecosystems provide a catalyst for new species discovery, as well as insights into the discovery of patterns of trophic and symbiotic divergence. Additionally, phylogenetic analyses of large samples of diverse taxa across geographical gradients have increasingly detected cryptic and pseudo-cryptic species complexes that have dramatically altered our view of species richness. Aposematic and extreme camouflaged colour patterns within the context of fish predatory behaviour provides an evolutionary framework for divergence and convergence of colour patterns. Similarly, recent studies of temperate nudibranch assemblages in temperate waters in Europe, southern Africa and the Pacific coast of North America also demonstrate previously undetected diversity and the presence of colour patterns that likely reflect similarity derived from both common ancestry and convergence. Combining these approaches has documented astonishingly high levels of previously undetected diversity, has huge implications to our knowledge of global biodiversity with a likely 3–5x increase in global species richness, and has developed more appropriate regenerative conservation strategies.

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## Phylogenomics of the Epitoniidae: resolving relationships in a diverse family using target capture

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Approximately 785 epitoniid species are currently recognized from the intertidal to depths of more than 1000 meters, where they are often associated with anemones and corals. The family has a long evolutionary history dating back to at least the early Cretaceous and is well studied, but it has remained difficult to delimit genera and understand evolutionary relationships in this family due to convergence in shell characters. We estimated a molecular phylogeny of the Epitoniidae based on sampling of approximately 800 specimens with a focus on the Indo-West Pacific, including representatives of many genera that have not been previously studied in a molecular phylogenetic framework. A molecular phylogeny based on standard molecular loci (COI, 16S, 28S, 18S, and H3) was informative in delimiting clades and suggested paraphyly of multiple genera, but many deeper nodes of the tree remained poorly resolved. We developed a universal probe set targeting 2896 loci from caenogastropods and used this to recover an average of 1600 ultraconserved elements and exons from epitoniids with target capture. Genomic data from 32 epitoniids representing 18 genera was used to estimate a highly supported backbone phylogeny. Genomic analyses of the genus *Epitonium* confirms that this group is paraphyletic with respect to several other described genera, and in need of taxonomic revision. Species identified as *Gyroscala* are recovered as distinct from *Epitonium* in analyses of both standard molecular loci and genomic data. The genus *Cirsotrema*, characterized by significant variability in shell morphology, is also paraphyletic. The epitoniid *Alexania* is recovered as the most closely related benthic epitoniid to the neustonic *Janthina*. Analyses of the nystielline genera *Ecclisogyra* and *Papuliscala* indicate that some nystielline genera are deeply divergent from epitoniids, but that the subfamily is not monophyletic. The probe set developed for this study has resolved deep phylogenetic relationships in the Epitoniidae with high support and will be used to further explore the systematics of the Epitoniidae and the diversification of other caenogastropods.

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## Do snails from springs and groundwater provide a new perspective on New Caledonian biogeography?

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The question if New Caledonia, a fragment of Gondwana, should be considered a Darwinian island, because it has possibly been entirely submerged in the early Tertiary, or if some land remained subaerial providing refuge for the survival of old Gondwanan elements in situ, is a long standing issue. So far, hard evidence for the latter hypothesis seems to be lacking so that we have to assume that the unique New Caledonian biota arrived and evolved only after Grande Terre has re-emerged about 37 Mya. Also the origin of tateid gastropods, a considerable radiation comprising about 60 extant species mainly dwelling in springs on limestone, has been dated to roughly 28 Mya. However, tateids also occur on ultramafic bed rock supporting a physiologically very specialized flora and fauna. In the course of the Hydrobio expeditions to New Caledonia organized by Philippe Bouchet in 2016 and 2017, about 50 largely very small creno- and stygobiotic morphospecies were discovered. According to preliminary analyses, these snails are not directly related to the limestone radiation and are rooted considerably deeper in the tree suggesting that they could be informative regarding the availability of land and freshwater throughout the Paleogene. As these analyses are based on sequences of only five genes, deeper nodes do not receive strong support. Therefore, we are developing genomic resources for these minute species. Also the morphological descriptions require larger effort including  $\mu$ CT scanning and 3D reconstructions. In this talk, I will report on the progress of our project and share some impressions surrounding the field work with Philippe.

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## Bouchet's theory – vertical migration and surface dispersal of planktotrophic larvae from the deep sea – applies to hydrothermal vent endemics

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Philippe Bouchet made an immediate mark in his early career when, in 1976, he published seminal papers on the larval ecology and dispersal of deep-sea gastropods. His presumption of vertical larval migration from bathyal and abyssal seafloors to productive surface waters, initially based on the presence of multi-spiral protoconchs and actual sampling of veligers in the photic zone, was later confirmed by himself (1981) with the first isotopic data for the protoconch and teleoconch of deep-sea snails. This was further corroborated by Killingley & Rex (1985), who coined the term Bouchet's theory that planktotrophic species in the deep-sea will show a significant difference in isotopic composition between larval and adult shells because larval shells are deposited in shallower, warmer waters.

Larval behaviour should play even more essential roles in determining the evolution and distribution of species endemic to hydrothermal vents – patchily distributed, ephemeral deep-sea habitats. Philippe and his long-time friend Anders Warén hypothesized in 1994 that vent endemics may similarly migrate to the surface for phytoplankton feeding and dispersal. They also postulated that high water temperature in vent environments would hinder the inference from oxygen isotopes alone, and that elemental composition analysis may provide more conclusive results.

We, after decades of Philippe's prophecy, first investigated the oxygen isotope and trace element compositions of molluscan shells from hydrothermal vents. We analysed three species of red-blood limpets of the genus *Shinkailepas* from vent sites at 440–1980 m deep. The oxygen isotope analyses of protoconchs demonstrate that their larvae invariably experience warm temperatures (17–23 °C) that approximate conditions in the surface water above the vent sites. The concentrations of Ba and Mn as possible proxies for hydrothermal fluids were much higher in the teleoconch than in the protoconch. These results show perfect concordance with the larval behaviour and temperature optimum observed in culture experiments and population genetic structures of the study taxa. The vertical migration and surface dispersal are compulsory parts of their life cycle, and they utilize the advantages of high food supply and strong currents. Other vent endemic molluscs await similar integrated studies of analytical chemistry and behavioural observations.

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## Forty three years from *Lyria kuniene* (Bouchet, 1979)

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Neogastropoda is one of the most challenging orders of marine gastropods, comprising around 15000 accepted recent species in 60 families, encompassing some 1145 genera. In terms of global biodiversity of marine molluscs, Neogastropoda constitute nearly 30 % of known species. Not surprisingly, neogastropods attract attention of many researchers – both citizen scientists and professionals in different fields, from alpha-taxonomy to biomedical studies. Philippe Bouchet did not escape the main trend of malacological research and although started with opisthobranchs (first species described in 1975), since 1979 described 285 species of Neogastropoda out of his total 709 valid species. P. Bouchet early recognized the importance of preserving material for molecular studies and since 2004 tens of thousands of specimens have been collected through the efforts of a permanent ‘barcode team’ in every expedition. This material fueled the current molecular-phylogenetic and morphological research of neogastropods by MNHN team and associated international group of scientists. In addition to 5 PhD (in Paris and Moscow) specifically focusing on neogastropod systematics, many other students (master, PhD) and researchers benefited from this collection. 14 new families were described and five re-instated from the synonymy (thus enlarging the number of existing accepted families by 1.5 times), not to mention the description of dozens new genera and hundreds new species associated with the newly obtained molecular sequences. Worth mentioning that the first ever new species of Neogastropoda associated with *cox-1* sequence – *Belloliva dorcas* Kantor and Bouchet, 2007 was based on MNHN-collected material (sequenced by NP). Among published results by extended MNHN team (in chronological order) most important are the molecular and morphology based phylogenies and updated classifications of separate families (Muricidae – 2010; Nassariidae – 2016, Fascioliidae – 2016; Costellariidae – 2017; Mitridae – 2018) and of taxa and informal groups of higher rank (Conoidea – 2011, 2018; mitriform neogastropods – 2015; Olivoidea – 2017; marginelliform neogastropods – 2019; Buccinoidea – 2022). Philippe Bouchet actively participated in every mentioned research. Presently several projects are in progress – Sanger sequencing-based taxonomic revisions of separate genera of neogastropods to exon-capture based phylogenies of families and entire Neogastropoda.

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## **Towards a better assessment of muricid species diversity in the Caribbean Sea (Guadeloupe and Martinique Islands, Lesser Antilles).**

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Over the last years, the Muséum national d'Histoire naturelle (MNHN) has led several field surveys to describe known and overlooked marine benthic diversity in the Caribbean islands of Lesser Antilles, in Guadeloupe and Martinique. During these surveys, several sampling methods have been used to thoroughly characterise the wide range of habitats: brushing, suction samplings and handpicking by scuba diving, intertidal collecting or snorkelling on the shoreline and shallow-water dredging. Specimens have been identified by a network of taxonomist experts during workshops organised by the MNHN.

Such sampling consistency and taxonomic accuracy, deployed by the MNHN at the island scale for both Guadeloupe and Martinique, make these data a gold standard for biodiversity assessment. Qualitative regional comparisons between both sites has revealed a surprising singularity in the marine benthic biodiversity. However, these data have yet to be used at a broader scale in order to better quantify the challenge of biodiversity assessment. A pending question is if this repeated massive sampling effort helps to have a better vision of marine benthic species diversity, in comparison to other – less exhaustive – occurrence datasets.

Here, we focus on muricid species (Family Muricidae Rafinesque, 1815), a well-studied and diverse group of marine molluscs known to be relevant bioindicators of shallow water-marine diversity. We gather occurrence data from both the MNHN and open databases that compiled information from different sources: museum collections, dedicated surveys/programmes, and human observations provided by citizen science. By discriminating MNHN's gold standard from other sampling effort, we aim at i) comparing the sampling intensity performed by the MNHN to other sampling initiatives ii) analysing their level of diversity, by using rarefaction of our gold standard dataset, and iii) assessing the magnitude of diversity that might be overlooked when sampling intensity is not as exhaustive as in MNHN's expedition.

We think that such an approach will raise awareness among scientists and stakeholders about the effort needed to better estimate biodiversity from local to regional scales, and will contribute to ongoing discussions about the implementation strategy of conservation actions in biodiversity hotspot areas.

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## How much diversity do we need to sample to eliminate dark taxa?

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In our ongoing challenge to document biodiversity, especially in light of global change, one might ask, how much do we need to sample to effectively characterize any ecosystem? Sequence-based approaches (e.g. eDNA) are increasingly common to census marine communities. However, in conducting these studies, large proportions of the resulting reads are ‘dark taxa’ – sequences that cannot be confidently assigned to even broad taxonomic groups. The question becomes, “How much of the tree of life must we sequence to assure that future unknowns can be accurately characterized?” To answer this question, we use a comprehensive DNA dataset of cowries to test what level of coverage must be sampled to accurately assign any unknown to the same family: “this sequence is a cowrie”. We first test coverage globally; how many species need sequenced in order to assign all other species confidently to the same family? Then, we focus on recognized marine provinces to examine true-world biogeographic biases. Furthermore, using abundance data, we test both the likelihood of sequencing a species as a representative voucher, as well as the likelihood of sampling that sequence in an environmental library to correct for real-world rarity. We use the mitochondrial COI barcode locus because it is the only locus that provides species-level resolution, and truncate analyses to the standard metabarcoding 313 base pair region. Unsurprisingly, a single representative from the family is not sufficient to confidently assign another unknown species to Cypraeidae, with a misassignment rate between 30 % and 89 % depending on biogeographic region and rarity filter (voucher or sample) employed. We then randomly test 1 %, 5 %, and 10 % of the total diversity within the family to recover identification rates for unknown lineages across marine regions. For the most part, sampling both 5 % and 10 % of the total diversity within cowries is sufficient to accurately identify any other unknown cowrie sequence, with notable exceptions where endemic lineages dominate communities. The underlying diversity and disparity in cowries are similar to other marine groups. Thus, our results suggest that disappearing dark taxa across marine ecosystems will require a focused, yet tractable, endeavour to genetically identify all marine life.

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## Larval shells and poecilogony in gastropods: how has our perception changed since 1970s?

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The role of larval biology on the evolution of marine animals has long been studied, since larval ecology have deep influences on population ecology, genetics, connectivity, micro- and probably macroevolution.

Gastropods and bivalves are very peculiar animals in that they preserve a body part (the embryonic/larval shell) that conveys information on the developmental pathway of embryo and larva, which can be scored in adult specimens, often also in fossils. This makes most gastropods and bivalves ideal models to study the evolutionary implications of larval ecology. The advent of Scanning Electron Microscopy (SEM) in the second half of XX century, provided unprecedented tools to study the morphology of the embryonic/larval shells in both extant and fossil specimens. Partly based on experimental evidence, larval features have long been considered as a ‘conservative’ set of attributes of the species, and systematists worked under the assumption that larval features were optimal phylogenetic markers.

Many marine invertebrate groups show evidence of evolutionary transitions in larval phenotype, such as the loss of planktotrophy that occurred repeatedly in many lineages of marine caenogastropods. Poecilogony is the intraspecific variation in developmental mode, with larvae of different types produced by the same individual, population or species. At the end of the 1980s there seemed to be little or no evidence supporting poecilogony in most marine invertebrates, and particularly in shelled gastropods. Studies in the 1990s and the XXI century, particularly broad range, recent approaches of integrative taxonomy, shed new light on this issue. Caenogastropoda, with their larval shells bearing such clear information of larval ecology, remain poor of confirmed cases of poecilogony, whereas heterobranchs seems to be more prone to larval polymorphisms. However, the few confirmed or suspected cases of poecilogony in caenogastropods (a series of case studies are presented), highlight the need for a modern approach, combining developmental biology with integrative taxonomy.

The presence of larval polymorphism raises a number of questions at the interface between developmental biology, ecology and evolution. How larval polymorphism affects genetic connectivity among the involved populations? How diffused is developmental polymorphism in gastropods, and how is the transition regulated? Is there any phylogenetic constrain to poecilogony?

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## Amongst the tiniest of the small: new discoveries reveal 43 SE Asian luminary species from sediment and scum (Gastropoda: Stylommatophora: Hypselostomatidae)

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Members of the hypselostomatid genus *Angustopila* Jochum, Slapnik & Páll-Gergely, 2014, constitute some of the world's tiniest terrestrial gastropods. So far, only twelve species are known, spanning ranges from Thailand and Vietnam to southern China. In a major taxonomic study based on type material and over 211 newly collected lots from China, Laos, Myanmar, Thailand and Vietnam, a remarkable diversity has been discovered. Altogether, 55 species and one subspecies deriving from 223 sampling sites are recognized, of which 43 and a single subspecies are new to science. Using the "morphospecies" species concept for which only conchological characters are examined to recognize and delimit species, our approach delimits distinct morphotypes based on discrete characters or those which are continuous but with distinct gaps. We reveal that three species have wide distributional areas, indicating that for the description of new angustopilid microsnails, it is requisite that known species from a wide range are examined. Practically all species in this study were found in soil samples collected at the base of limestone rocks and caves. We know little about the ecology of *Angustopila* species. However, they most probably inhabit the moist, deep limestone crevices close to (or on) root systems. The sites with multiple (2–4) *Angustopila* species reveal that this genus is most diverse in northern Vietnam, northern Laos, and the neighbouring Chinese Guangxi Province and Myanmar. The number of *Angustopila* species per site can be as high as 6 and as high as 7 (Qua Vang Cave in the Halong Bay). Both these high incidences derive from northern Vietnam, an indication that this region is the very center of angustopilid diversity. We remark that we examined relatively few samples from Guangxi, where the diversity of *Angustopila* might be richer than we currently know. The majority of *Angustopila* species are single-site or narrow range endemics (5–240 km, but typically less than 60 km). Habitat disturbance can easily lead to the disappearance of *Angustopila* species. The most tangible threats to their preferred habitats are quarrying and modifying caves for tourism and recreational purposes.

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## Cone snail genomics

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Cone snails are marine gastropods with a sophisticated venom system that is used for preying and defense. The venom is mainly a complex cocktail of short peptides called conotoxins, which target neuromuscular receptors. Although knowledge on the venom profile of each cone species is accumulating through transcriptomics and proteomics, very little is known about the genomic basis of venom gene diversity. In this regard, long-read sequencing technologies coupled with chromatin capture methods have recently opened the possibility to assemble the large and complex genomes (with intricate regions such as simple repeats or transposon elements) of many non-model organisms. Here, we used Pacbio long reads and HiC and Chicago libraries to reconstruct a highly contiguous assembly of 3.56 Gb for the Mediterranean cone snail *Lautoconus ventricosus* (Gmelin, 1791). Using transcriptomes of the venom gland for annotation, we report 289 conotoxin transcripts, which were scattered into 35 pseudochromosomes. The ratio gene/transcript was close to one suggesting that toxin diversity is mainly produced through gene duplication and later through post-translational modification of peptides. Moreover, we used the assembly and synteny analyses of orthologous genes, including hox and para-hox genes, to reveal a whole genome duplication, which potentially occurred in the ancestor of the lineage. This high quality assembly will be a value resource in evolutionary genomic studies of gastropods.

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## A life in numbers: Introduction to the symposium in honor of Philippe Bouchet

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Philippe Bouchet, Senior Professor at the Muséum national d'Histoire naturelle in Paris, will retire in August 2022 after a career spanning almost five decades. Since joining the museum in 1973 as an assistant, he has authored more than 210 original refereed research papers and ca. 160 other publications, and has described over 600 new species of molluscs. Through his leadership and thanks to the work of the “Paris malacology team”, the malacological collection of the MNHN has become the most active in the world, with remarkable annual growth, especially of type specimens.

This symposium will be the opportunity to reflect on Philippe’s major impact on malacology and biodiversity exploration. It will bring together scientists not only to celebrate Philippe’s career and accomplishments, but also to present new discoveries made possible by MNHN expeditions. The symposium will start by an introduction that will tentatively summarize his busy and fruitful career and introduce the many topics that will be developed during the symposium. Old timers of Philippe’s expeditions and close collaborators will indeed reminisce on the major findings and the main topics he explored during his career: the renewal of large-scale biodiversity expeditions, with the Tropical Deep-Sea Benthos and La Planète Revisitée programs, the revelation of the importance of the minute and neglected fauna, the reality of the 6<sup>th</sup> extinction and the emergency to describe this threatened biodiversity, the development of an international network of citizen scientists and academics, and of course the many taxonomic discoveries, both at the species and deeper phylogenetic levels, permitted by the MNHN expeditions. The symposium will also give the opportunity to young researchers, recipients of travel grants funded by donors, to present their results, unequivocally illustrating that Philippe’s career will continue to influence future works in malacology and biodiversity exploration.

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## New campaign of land snail research on New Caledonia with first results on Helicinidae

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New Caledonia is a strongly isolated, geologically old southwestern Pacific island group. The land snail fauna consists of almost entirely endemic taxa of currently nearly 200 accepted species. Concerted previous fieldwork took place in the 70<sup>th</sup> and 80<sup>th</sup> of the last century and resulted in conchological-morphological revisions of several groups (e.g. *Andrefrancia*, *Diplomphalus*, *Draparnaudia*, *Placostylus*, *Pseudomphalus*, *Diplommatinidae*, *Helicinidae*).

In 2018, following initial field work in narrowly restricted areas, an international team around Philippe Bouchet, MNHN Paris, resumed the exploration of the terrestrial malacological fauna of New Caledonia. The new approach combines improved sampling methods (e.g. consequent application of leave-litter sampling), the documentation of living animals, and specific tissue preservation for molecular work with the access to yet unexplored sites. So far, four one-month-expeditions with an increasing number of participants sampled about 450 sites across the main island (Grand Terre), the neighbouring islands Belep and Ile des Pins as well as several small islets.

For *Helicinidae*, but also for other families, preliminary molecular results suggest a higher diversity than previously described based on traditional characters. The major split into a northern and a southern radiation as suggested by the morphological revision is so far confirmed. In general, genetic divergence between morphologically similar lineages is relatively high often reflecting a strong geographical pattern. Potential species delimitations based on integrative data will be discussed.

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## **From MUSORTSOM to Our Planet reviewed, the role of Philippe Bouchet in the modern development of naturalistic exploration**

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In scientific communities, naturalistic exploration is generally not considered as a pillar of the scientific activities but rather as a romantic activity rooting in the natural sciences of the 19<sup>th</sup> century. In the 1980s biologists realized that the biological diversity, notably in the marine realm, is essentially unknown, and consequently, exploration becomes again a pillar for building knowledge about biodiversity. In this context, from an opportunistic campaign, conducted in 1976 on the research vessel Vauban, by a small team of researchers of the French Office of Overseas Scientific and Technical Research (ORSTOM) and the Muséum national d'Histoire naturelle (MNHN), emerged the MUSORTOM program. From this starting point, I will redraw the contribution of Philippe Bouchet in the development of the modern exploration of the benthic fauna.

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## New Caledonia, mon amour

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The island of New Caledonia is famous for the extraordinary endemism and micro-endemism of its terrestrial biota at levels exceeded only by those of Madagascar and Hawaii. Despite a land area under 20000 km<sup>2</sup>, it has, e.g., 3380 native plant species, of which 72 % are endemic, and 100 species of reptiles, of which 92 % are endemic. Although the first unambiguous record of a land snail from the island can be traced to the visit of Cook in 1774, it wasn't until the mid-19<sup>th</sup> century that its marine and freshwater mollusks began to attract the attention of naturalists. Several golden ages of discovery ensued and today it ranks as the most intensively sampled South Pacific island. It is this stunning natural laboratory that Philippe Bouchet has claimed as his “scientific homeland”. His lifelong fascination was inspired at a young age in the pages of “Océanie Française” by Pierre Benoit. Philippe’s first mission to New Caledonia came in 1978–1979 when he fulfilled his military service in Nouméa as a marine biologist at ORSTOM, the French Overseas Scientific and Technical Research Office. Over the years, he has returned frequently to continue his exploration of the terrestrial and aquatic malacofauna. Notably, the “Montrouzier” expedition in 1993 to Koumac and Touho was a then unprecedented sampling effort to assess the magnitude of marine diversity in a complex, tropical coastal environment and it transformed our understanding of molluscan species richness and rarity in coral reef habitats. In 2018, under the auspices of “Our Planet Reviewed”, he returned to Koumac investing even greater sampling effort to document how the lagoon has changed in the intervening years. In total, he and his co-authors have described over 150 species from the New Caledonia archipelago since 1979, 50 of them in the remarkable radiation of spring snails. There is still much left to discover and describe, not only of marine diversity, but also of the extraordinary diversity of mollusks in terrestrial habitats and in flowing freshwaters. Now harnessing the power of DNA sequencing technology, another golden age of discovery has already begun.

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## Contributions to the knowledge of New Caledonia deep water cone snail fauna

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For more than 40 years, the Muséum national d'Histoire naturelle, Paris/ORSTOM, then Institut de Recherche pour le Développement, has been carrying out oceanographic expeditions in the deep waters surrounding New Caledonia in the framework of the Tropical Deep Sea Benthos campaigns. These expeditions have surveyed the seamounts of the Norfolk Ridge, dredging and trawling from 80 m to a depth of 3000 m. Other areas covered included the Plateau des Chesterfield and Bellona Reefs in the Coral Sea, as well as the Grand Passage (N New Caledonia) and Loyalty Islands. These research cruises produced large lots of deep-water cone snails, with exact depth and locality data, which have been thoroughly studied. A total of 2377 lots containing 5113 specimens collected in depths between 100 and 1260 m have been examined. About 770 specimens were live collected and allowed for radular and molecular studies. From the examination of these lots, about 20 new species of cone snails have been discovered. Many of them belong to the deep-water genus *Profundiconus*, which seems to constitute one of the main components of the gastropod fauna at depths below 200 m. A total of 76 different species of cone snails were identified among the collected material. Of these, 20 corresponded to typical shallow water species, which were most likely translocated into deeper water, whereas 56 could be considered true components of the deep water (below 100 m) cone snail fauna. Species in *Profundiconus* represent 21 %, *Conasprella* 27 %, and *Conus* 52 %. The most abundant species found (more than 400 specimens) were *Conus* (*Afonsoconus*) *bruuni*, *Conasprella* (*Boucheticonus*) *alisi*, *Conasprella* (*Conasprella*) *boucheti*, and *Profundiconus* *vaubani*. Additionally, these findings may provide access to the discovery of new conotoxins from deep water cone snails.

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## Did humans silently extinguish sessility in freshwater gastropods?

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Earth is currently experiencing a major loss of biodiversity, widely considered to be the beginning of the ‘6<sup>th</sup> mass extinction’. As resources available for conservation action are limited, conservationists are pushing for ecology- and evolution-based conservation vchoices. These prioritization systems typically combine rarity and originality (e. g. phylogenetic distinctiveness and evolutionary innovation).

Here, I report the loss of such unique evolutionary innovation exemplified by the recent eradication of the highly unusual sessile freshwater snail *Helicostoa sinensis* Lamy, 1926. The partly uncoiled and worm-like shells adhere firmly to hard substrates with one of their sides soon after hatching. This makes them truly sessile and allow them to strive in extreme rheophilic environments. *Helicostoa sinensis* was described based on specimens cemented to a limestone rock, deposited at the Muséum national d’Histoire naturelle in Paris. The material was probably collected in the 1910s in the Three Gorges region of the Yangtze River (PR China). Despite intensive search, the species was never found again.

Although it is difficult to prove extinction, especially for ultra-rare species, the lack of records for this conspicuous species in a focal region for biodiversity studies in more than 100 years and the large-scale and massive habitat destruction in the Three Gorges region provide strong evidence for the biological annihilation of this endemic species.

While rapidly rising extinction rates in invertebrates during the Anthropocene are increasingly acknowledged, the loss of originality in these “little things that run the world” has received little attention. Therefore broad surveys of originality in understudied taxonomic groups are urgently needed as a basis for ecology- and evolution-based conservation choices. Such studies are likely to reveal a considerable loss of evolutionary history and innovation in invertebrates in general and molluscs in particular during the Anthropocene. In fact, without a piece of rock in the Muséum national d’Histoire naturelle in Paris and more than a century of curiosity by scientists interested in evolutionary innovations, we would not have recognized the potential loss of sessility in freshwater gastropods – likely caused by a single human construction project conducted in a highly fragile ecosystem.

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## Solariellidae: new snails, new phylogenies, and new understandings of deep-sea evolutionary drivers

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Molluscs comprise almost one quarter of marine taxa, exhibiting rich and varied body morphologies, a plethora of ecologies and trophic habits and inhabiting all realms of the ocean. Such variation is a potential treasure trove for evolutionary biologists, but few scientists have access to wide-ranging, recent, representative collections from the deep sea. Explorations of the deep sea in expeditions around the globe led by Philippe Bouchet and the Muséum national d'Histoire naturelle (MNHN) over the last few decades have resulted in superb collections of molluscan taxa from the intertidal zone down to the deep sea, including many that are totally new to science. Working primarily with MNHN specimens of one such gastropod family, the Solariellidae, we have named 64 new species and five new genera, and together with other colleagues, established the first phylogenetic framework for the family. Most recently, we have investigated the evolutionary loss of shell pigments, colour pattern, and eye structure and show that loss of these traits evolves irreversibly, at different rates, and at different times in this family. Using a Bayesian approach we find support for the existence of evolutionary transition zones, located in the middle of the dysphotic region, where increasing depth correlates with the loss of all three traits. The location and width of the transition zone varies among traits, but all lie within the known region of rapidly diminishing light availability. We propose that in this context, depth can be considered a proxy for light availability, and thus that pigmentation and pattern may be important for camouflage from visual predators in shallow water (<200–300 m) whereas low-resolution vision is useful to solariellid species to depths of about 500 m. Such studies would have been impossible without the incredible MNHN samples so generously made available to the taxonomic community.

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## **Symposium**

# **Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honor of Philippe Bouchet**

## **Part II**

### **Abstracts of poster presentations**

## Southern Ocean molluscan assemblages under the influence of climate change and methane seepage

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Despite over 200 years of scientific research, many areas and habitats of the Southern Ocean remain unsampled. Both the Prince Gustav Channel, the southern end of which had been covered by the Prince Gustav Ice Shelf until its collapse in 1995, and recently discovered South Georgia methane seeps were among these unsampled areas for benthic marine biodiversity. In 2018 R. R. S. James Clark Ross investigated the Prince Gustav Channel and in 2017 the RV Meteor discovered and sampled the methane seeps on the shelf of South Georgia. We investigated the molluscan assemblages in both these areas and compared them to known Southern Ocean shelf assemblages.

The Prince Gustav Channel and neighbouring Duse Bay area are characterised by low species richness (ranging from 7 to 39 species per station) but high abundances (specifically of the Scaphopods with 11331 specimens). The functional traits of the community were dominated by mobile organisms and motile development types. Assemblage analyses of the molluscan species abundances within the Prince Gustav Channel stations showed no pattern by depth or location. However, when bivalve assemblages were analysed with reference to the wider Weddell Gyre region (15 stations from 300 to 2000 m depth), the Prince Gustav Channel sits distinct from the other stations, with a higher dissimilarity with the deeper and more geographically distant areas. The Prince Gustav Channel is believed to be undergoing colonisation following the recent ice shelf collapse.

In comparison the South Georgia methane seepage assemblage also has low species richness, but a very different composition (4 species of bivalve and 5 species of gastropod, no scaphopods or aplousobranchs). There is reduced molluscan diversity and abundance compared to areas not influenced by methane seepage. Climate change is likely to influence both of these types of habitat, with many Antarctic ice shelves threatened, and a likely increase in methane seepage areas, these areas may serve as a case study of benthic faunal succession, with future monitoring.

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## Looks don't matter in *Brotia* – Phylogeny and molecular genetics of freshwater gastropods from Kaek River in Thailand using mtDNA and ddRAD

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Are there really so many species out there, or are we deceived by the outer look, the phenotype as well as the genotype of variable organisms? In the course of studying the alleged adaptive radiation of riverine gastropods, we use the Cerithioidean gastropod *Brotia*, a diverse genus within the Pachychilidae that is typically found in tropical forest rivers and mountain streams in Southeast Asia, from India to Borneo. Previous studies revealed that as a general rule only two conchologically distinct *Brotia* species co-occur in one river throughout the genus' range. However, from an approximately 100 km long stretch of the Kaek River multiple *Brotia* species are known to coexist. While earlier reports described more than ten *Brotia* morphospecies, a later revision distinguished a total of seven species. Those were reported to exhibit distinct shell morphologies and radula types, but in previous studies we demonstrated a lacking genetic diversity of taxa within the Kaek River. Are the different morphospecies of *Brotia* indicating a relatively young species complex that is not distinguishable due to incomplete lineage sorting and/or hybridization, or is this an example of phenotypic plasticity? To adequately address these questions the main focus was set on a genomic approach. With the use of Next-Generation Sequencing methods it is possible to discover, sequence and genotype thousands of SNPs on a genome wide scale. With the use of ddRAD-seq we employed >13 000 loci from 246 specimens of *Brotia* species from Kaek River and adjacent water bodies. Reconciling the genomic results with other lines of evidence implies that the actual number of biological species, i. e. as meaningful evolutionary distinct entities instead of albeit diagnosable, but mere artificially delineated units, might be substantially lower than hitherto proposed.

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## A highly diverse, turritelline-dominated micro-molluscan assemblage from the early Miocene (Burdigalian) of the Dwarka Basin, western India

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The Neogene marine sequence of the Dwarka Basin, western India, harbours a rich haul of diverse molluscan fossils like bivalves and gastropods. The early Miocene succession (i.e., Nandana Member within Gaj Formation) hosts a siliciclastic shell bed and is faunistically dominated in terms of abundance by the turritelline gastropods (constituting ~96 % of the all gastropods) and hence named turritelline-dominated assemblage (TDA) unit. The turritelline gastropods (Family Turritellidae) show exceptionally high abundance (N=7062) and are represented by eight species belonging to four genera, i.e., *Zaria*, *Haustator*, *Turritella*, and *Protoma*. Herein, we report a micro-molluscan assemblage of gastropods and bivalves with size ranging from 2–10 mm associated with this TDA. The associated gastropods show high species richness of 47 species (N=276) belonging to 41 genera with relatively high abundance of families like Nassariidae, Rissoinidae, Potamididae, Pyramillidae, and Scaliolidae. The associated bivalves are represented by 25 species (N=853) belonging to 15 genera with high abundance of families like Corbulidae, Veneridae, Nuculanidae, and Ostreidae.

Ecologically, the dominating feeding habit of the molluscan fauna of the TDA unit is suspension feeders for both gastropods (~96.22 %) and bivalves (89.79 %). Apart from that, herbivore-grazers (1.61 %), scavenger-predators (1.14 %), and carnivores (0.94 %) constitute for the gastropods, whereas, deposit-feeders (9.61 %) and chemosymbionts (0.50 %) constitute the types of bivalve feeding population. Faunistic correlation of this TDA unit of the Dwarka Basin with a seagrass-associated micro-gastropod assemblage of the Quilon Basin (southern India) reveals 57.77 % similarity for gastropods and 33.33 % similarity for bivalves.

Extensive flourishing of turritelline gastropods along with other suspension feeding bivalves suggests a nutrient rich environment, often caused due to ocean upwelling. Although significantly less in abundance, high faunistic similarity between TDA and seagrass associated molluscan fauna explains the occurrence of other opportunist molluscs with different diets dwelling in nutrient rich waters alike seagrass habitats.

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## Sclerites of Solenogastres: their formation and crystal organization

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Solenogastres are small-sized, elongate, cylindrical, worm-like molluscs, which have small calcified sclerites (spicules, spines and scales) embedded in their cuticle. They develop a ventral furrow with a short, rudimentary foot. The latter distinguish them from the Caudofoveata that have a completely reduced foot. Regarding the secretion of the sclerites by the mantle epithelium, two main hypotheses have been proposed: 1) an intracellular origin from a single cell of the hypodermis or 2) from a cell membrane invagination of the epithelial cells. Previous studies indicated that the mineral composition of the sclerites is aragonite.

We have studied by optical and electron microscopy (TEM) the formation of the sclerites in *Rhopalomenia aglaopheniae* (Kowalevsky & Marion, 1887); *Pruvotia sopita* (Pruvot, 1891); *Dorymenia sarsii* (Koren & Danielssen, 1877) and *Anamenia gorgonophila* (Kowalevsky, 1880). In addition, we have analyzed by Electron-Backscatter-Diffraction (EBSD) and Field Emission Scanning Electron Microscopy (FESEM), the crystallographic arrangement of the aragonite of the sclerites of *Dorymenia sarsii*, *Anamenia gorgonophila* and *Simrothiella margaritacea* (Koren & Danielssen, 1877).

Our results point to an intracellular origin of the spicules, which start to form inside a cellular vacuole of the epithelial cells, without any connection to the cellular membrane. Only when the spicules grow and before entering the cuticle, they break the cell membrane. Regarding the crystallographic arrangement of aragonite crystals, the spicules from the studied species are single crystalline, with the aragonite c-axis oriented parallel to the spicule morphological axis.

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## The complex diversity of the carnivorous vetigastropod *Calliostoma* (Gastropoda, Calliostomatidae) in the Mediterranean Sea

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*Calliostoma* (Swainson, 1840) is the most diversified genus of the family Calliostomatidae, with over 250 species from all the seas of the world. Unlike most vetigastropods, these marine snails are almost exclusively carnivorous. The remarkable morphological diversity of this clade in the Mediterranean Sea has led to the description of over a dozen different species based on shell morphology, although the actual value of the external shell characters to define species in this group has long been a matter of debate. Nine recent species of calliostomatids are accepted in the Mediterranean Sea and two more are currently under inquiry (WoRMS Database). In this first attempt to a revision of the genus based on molecular data, species-delimitation (ASAP and bGMYC), and phylogenetic (Maximum likelihood and Bayesian Inference) analyses were carried out on 114 specimens spanning a large part of the morphological diversity. The molecular dataset was based on one nuclear (ITS2) and two mitochondrial fragments (COI, 16S) from specimens belonging to 9 nominal species, sampled at 31 localities in Mediterranean and Atlantic. The results strongly suggest that the number of species of *Calliostoma* in the Mediterranean Sea is overestimated, with several nominal species not supported by the molecular data. Results support the hypothesis that all the assayed specimens, morphologically ascribed to nominal species allegedly endemic to the Mediterranean Sea, actually belong into a single, extremely variable species. Furthermore, the level and patterns of intraspecific variation, both genetic and morphological, of the species with an exclusively Mediterranean distribution, seem to be higher than those of the more homogeneous Atlanto-Mediterranean species. The complex diversity of this genus in the Mediterranean Sea requires further study in order to determine whether 1) the observed abundance of morphotypes has been driven by some adaptive advantage or 2) changes in the trophic and/or reproductive ecology have promoted the diversification among the Mediterranean populations.

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## Revisiting species of the genera *Subulina* and *Striosubulina* (Gastropoda, Achatinidae): Anatomical characterization brings new operational criteria for species delimitation

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Land snail's species formerly ascribed to the family Subulinidae, now recognized as synonym of Achatinidae, constitute a diverse group of pantropical taxa for which there is scarce information on fundamental biological attributes including the anatomy of the soft parts, life history, distribution, and conservation status. Except for a few species that were introduced in non-native areas and are recognized as invasive, most of the species seems to have rather restricted distributions or even to be endemic, confined to some ecoregions and oceanic Islands. The scarcity of taxonomic revisions and the lack of operational criteria for species delimitation are important factors contributing to the taxonomic impediment and the lack of interest in this group. The classification systems proposed for the assembly of genera previously recognized as Stenogyrinae, in the beginning of the 19<sup>th</sup> century and later as Subulinidae, in the 1950s, is entirely based on shell traits and remain nowadays as the main guideline for taxonomists. "Subulinid" shells, however, are relatively plain and uninformative. Besides, plasticity in shell shape has been documented for some genera. Thus, the anatomy of the soft parts, although unknown for most of the representatives of this group, appears as a promising way to solve inconsistencies in species delimitation, as demonstrated for a few genera recently revisited and anatomically characterized. Herein, most of the specimens analysed belong to the malacological collection of the Muséum national d'Histoire naturelle de Paris. Five species were collected in Comoro Islands in the context of the "Inventaire National du Patrimoine Naturelle". Anatomical descriptions for eight species are provided, i.e.: *Striosubulina striatela* from Ivory Coast, *Subulina ferriezi*, *Subulina simplex*, *Subulina cereola*, *Subulina canonica*, and *Subulina normalis* from Comoro Islands, *Subulina angustior* from Cameroon, and *Subulina parana* from Brazil. The anatomy of the soft parts of all the species was compared with *S. octona*, type species of this genus, providing insights on the diagnostic criteria for both genera *Subulina* and *Striosubulina*. Species characterization was expanded, including information on the anatomy of the reproductive and pallial systems which were wanting in their original descriptions. Accordingly, the number of operational criteria for species distinction was increased.

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# **A remarkable outcome of Natura 2000 prospections initiated by Philippe Bouchet: A new subspecies of the Istrian-Dinaridic clausiliid *Cochlodina triloba* (O. Boettger 1877) in France**

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In the frame of the very successful malacological prospections of the Muséum national d'Histoire naturelle Paris (MNHN) for the European network of nature protection areas "Natura 2000" – in collaboration with the Office National des Forêts (ONF) – field work in the Montagne de Lure, Département Alpes-de-Haute-Provence, has been carried out in June 2002. In this part of the calcareous French Prealps as main biotopes for rich snail faunas have proven pristine montane forests dominated by old beeches and silver firs in rather steep slopes reaching to the upper tree line. *Cochlodina triloba* lives there in moist ravines on decaying deadwood, sometimes even xylobiontic. The distance of the newly detected occurrences of *Cochlodina triloba* to the hitherto known area of the species is about 650 km. *Cochlodina triloba* is not the first clausiliid species in the French fauna with a considerable east-west disjunction. As further examples can be named *Cochlodina comensis* (L. Pfeiffer 1850), *Ruthenica filograna* (Rossmässler 1836), and the recently detected *Fusulus interruptus* (C. Pfeiffer 1828). To explain the existence of these isolated populations the most probable hypothesis is to consider them as relicts of an extension of the distribution areas within the Middle Pleistocene during the Holsteinian (Great) Interglacial. A close examination and comparison with Material from Istria has proven, that the French occurrences of *Cochlodina triloba* represent a new and undescribed subspecies. The main characters of this new subspecies from the Montagne de Lure are the following: Shell generally more obese and compact than in the typical form from Istria; less glossy. Aperture more square-cut and parietal callus stronger. Pricipalis only a little bit or not protracting over the upper palatal fold. The clausilium shows the most important characters: Inner lobe markedly reduced and the bight between this and the digitiform middle lobe more shallow; the outer lobe broadly rounded while it is in the typical form narrower and bulbous. In the final publication the new subspecies will be named in honour of Philippe Bouchet.

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## Litthabitellidae: a new family of the Truncatelloidea (Caenogastropoda)

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The genus *Litthabitella* Boeters, 1970, with the type species *L. chilodia* (Westerlund, 1886), inhabits mainly fresh-water springs in the coastal regions of the Balkan Peninsula from Greece to Slovenia, southern Italy, and the Ionian Islands. *Litthabitella* could be found also more far from the sea, sometimes in caves and interstitially. Another species is known from France and Spain. *Litthabitella* is represented by minute snails showing a number of synapomorphic character states: osphradium broadly ovate; pallial oviduct gland complex short; gonoporus deep inside the mantle cavity; the ventral channel not on the ventral side of the capsule gland, but somewhat laterally; thickened oviduct circular; the distal termination of the penis wide and blunt, with sharp and short filament harbouring vas deferens situated laterally, and with two flat, hen-comb-like, four-folded lobes. The characters listed above justifies the family rank of the taxon. Three molecular loci: mitochondrial cytochrome oxidase subunit I, and nuclear 18S and histone H3 also confirm the family-rank distinctness of the Litthabitellidae Falniowski et Hofman, 2022, and support its position as the sister clade of the Hydrobiidae. Molecular data for the ten studied populations, confirmed partly by morphological characters, indicate the presence of four distinct taxa of the species rank, three of them were new for science and were described: *Litthabitella aquaincruce* Jaszczyńska et Osikowski, 2022, *Litthabitella cetinae* Osikowski et Jaszczyńska, 2022 and *Litthabitella levkadiana* Jaszczyńska, 2022.

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## Phylogenetic species delimitation for the mud whelks of the genus *Phrontis* (Gastropoda: Nassariidae)

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In 1989, P. Bouchet refused the stated presence of poecilogony (planktotrophic and non-planktotrophic larval development within a single species) in nassariids, justifying the confusion as a case of unclarified taxonomy. Many years later, during this study, which focuses on the study of the mud whelks in tropical waters, a case of poecilogony was found within the “*Nassarius antillarum*” clade, a lineage with controversial treatment in the literature.

We address the systematics of the mud whelk species previously assigned to “*Nassarius*” in the Tropical West Atlantic and the Tropical East Pacific (Caribbean and Panamic regions). Traditionally, 6 to 12 Caribbean species of “*Nassarius*” – now accepted as *Phrontis* – were recognized as valid. Morphological and/or molecular analyses have now revealed 21 *Phrontis* lineages, many showing extraordinarily similar shells. The tree resulted in recovering several species complexes, such as the “*Nassarius corpulentus*”, “*N. albus*”, and “*N. polygonatus*” aggregates, highlighting our previous underestimation of the specific diversity of Nassariidae.

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## The conservation of the freshwater hydrobiid spring snails versus scarcity and water needs of local populations

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Wells and springs are the main sources of drinking water for people living in semi-arid areas where water scarcity is accentuated. Recent studies carried out on freshwater springs and wells in Morocco have revealed the presence of molluscs adapted to these habitats. The family Hydrobiidae (Mollusca: Gastropoda) show significant radiation and are often endemic and highly localized. We present a list of freshwater species described in springs, wells and caves. The problem of the conservation of these species and their habitats comes up against the high degree of the unawareness of the local populations about the importance of this heritage biodiversity. So, what are the approaches to raising awareness of the concept of conservation among the locals whilst respecting the increasing need for water either for domestic or agricultural use? We provide examples based on the results of two Critical Ecosystem Partnership Fund projects in Morocco on the aquatic biodiversity of Sehb El Masjoune and the Laabid River. The endemic species collected in these two sites from springs and wells were presented during workshops for local residents by explaining their restricted distribution and their high endemism. The correlation between the presence of these species and water quality is explained, emphasizing their value as bioindicators and their heritage value. The wells were protected and covered against pollution and runoff. The springs are rehabilitated and restored to protect these species and attract visitors interested in sustainable ecotourism. These minute molluscs are hence included in the management and development plans of these sites. The data is updated for four species considered as triggers for two key biodiversity areas and two other species are new, one of which is dedicated to the supporting donor (*Giustia cepfi* n. sp.). The work on these tiny molluscs began with the support and supervision of Philippe Bouchet at the National Museum of Natural History of Paris where the types are kept. It is in his honour that we present this work.

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## The Pomatiidae of the Central Canary Islands – just conchological variability or misjudged biodiversity?

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The family Pomatiidae (Gastropoda: Littorinimorpha) is represented in the Canary Islands on each of the major islands by endemic representatives of the genus *Pomatias* Studer, 1798. The Canarian species are sometimes placed in the subgenus *Canaripoma* Starobogatov & Anistratenko, 1991, but this has not been tested by phylogenetic analyses so far. The Central Canary Islands – Tenerife and Gran Canaria – are home to three out of five currently accepted species from the archipelago, namely *P. laevigatus* (Webb & Berthelot, 1833), *P. canariensis* (d'Orbigny, 1840) and *P. raricosta* (Wollaston, 1878). The study of type material housed in the collections of several museums in Europe and North America, as well as the examination of more than 100 documented series of *Pomatias* from both islands in the private collections of the authors and the museums of Santa Cruz de Tenerife, Frankfurt and Hamburg suggests that we have to deal with more than these three species, especially if also shells from Pleistocene deposits are included in the study. Type material of *Cyclostomus adjunctus* Mousson, 1872, *Cyclostoma laevigatum*, *C. canariense*, *C. c.* var.  $\gamma$  *inaequalis* Wollaston, 1878 and *C. c.* var. *praecursor* Boettger, 1908 is documented and lectotypes, if necessary, will be selected. No type material of *C. canariense* var.  $\beta$  *raricosta* Wollaston, 1878 could be found, consequently a neotype ought to be selected from topotypical material. Preliminary investigations of *Pomatias* specimens from both islands by conchological and morphometric methods suggest that we have to deal on Tenerife and Gran Canaria with at least three extant species each and that none of these species is present on both islands. Additionally, forms from the Pleistocene might be recognised as chrono-subspecies. Furthermore, it becomes apparent that the names *P. laevigatum* and *P. canariensis* cannot be applied to any population of the eastern (Fuerteventura and Lanzarote) or western (La Gomera, El Hierro and La Palma) Canary Islands, and the former, as already recognised, is a younger homonym anyway.

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## Biogeographical approach of Truncatelloidea (Caenogastropoda: Littorinimorpha) in Greece

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Greece is considered a biodiversity hotspot for freshwater fauna, including freshwater gastropods. Over 70 % of Greek freshwater snails species belong to the superfamily Truncatelloidea and most of them are found in a single or just a few sites. As a result, many taxa are endemic, or often stenotopic endemic, and due to severe damage of their habitats a lot of them are threatened. Moreover, knowledge about the diversity and distribution of this superfamily in Greece remains limited, which makes its protection difficult. In the present study, we compiled data from 305 scientific papers, concerning the freshwater malacofauna of Greece and adjacent regions. A database with all the data obtained was created and various biogeographical analyses were applied, in order to approach biodiversity patterns and the distribution of Truncatelloidea in Greece. According to our results, 149 valid species of Truncatelloidea are present in Greece, while 16 more references are doubtful as they are considered to be having taxonomic problems. In addition, 38 species are assigned in a threatened category by IUCN. The endemism in Truncatelloidea in the level of genera (11) and species (129) are the highest proportionally recorded for a non-marine faunal group in Greece, highlighting the importance of this superfamily. Although these snails inhabit all types of freshwater ecosystems, springs and ancient oligotrophic lakes (Trichonida, Pamvotida, Megali Prespa, Mikri Prespa) stand out as hotspots of endemism and biodiversity. To sum up, our results highlight the importance of this superfamily, but also the need for further research on this group to tackle numerous taxonomical problems and to design strategies for the protection of endangered species.

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## Mitochondrial genome phylogeny of Cyrenidae (Heterodonta: Bivalvia) confirms parallel evolution of viviparity and multiple radiations into freshwater environments

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The family Cyrenidae (=Corbiculidae) includes more than a hundred species worldwide and represent one of the three major heterodont bivalve groups that have successfully invaded into lentic and/or lotic freshwater environments. They also exhibit a wide spectrum of lineage-specific reproduction modes ranging from oviparity to euviviparity. Recent molecular phylogenetic analysis for mtDNA *cox1* sequences highlighted independent evolution of euviviparity among *Corbicula* species. In order to fully understand evolutionary insights into habitat transitions and reproductive traits across the entire family, further phylogenetic investigation with wider taxon sampling is required. In this study, we performed phylogenetic analysis for 11 complete mitochondrial genome sequences representing major groups of the Cyrenidae including 7 newly determined sequences. The resulting mitochondrial genome phylogeny depicts the South American freshwater species *Neocorbicula limosa* as hierarchically nested between *P. maritima* (the North American marsh clam) and the remaining of Cyrenidae species that forms a dichotomy of two distinct groups: the *Geloina* species group (the brackish water, oviparous species) and the group comprising *Villorita cyprinoides* + *Corbicula* group (a mixture of brackish water and/or freshwater lineages) in which oviparous species (*V. cyprinoides*, *C. japonica*, *C. sandai*) are positioned basal, and stepwise to the remaining freshwater *Corbicula* species with euviviparous reproduction mode. Along with gene arrangement patterns, the mitochondrial genome phylogeny in this study corroborates the previous hypothesis of parallel evolution of viviparity and multiple radiations into freshwater environments within the family Cyrenidae.

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## Exon design for large-scale phylogeny of the Neogastropoda

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The Neogastropoda includes 14960 described species but probably ca. 30 000 to 50 000 species in total. The last classification includes around 60 families, but their phylogenetic relationships, especially the deeper ones, remain mostly unresolved. However, it is crucial to reconstruct the phylogeny of the Neogastropoda to understand the evolutionary processes at the origin of their hyperdiversity. We choose to apply a genome reduce method, the exon capture, which efficiently resolved deep relationships in many other groups. We used the recently published genome of *Conus ventricosus* as a reference and multiple transcriptomes from various lineages of neogastropods to identify the set of exons, first by performing reciprocal blasts and then by applying multiple filters to eliminate exons that are too short, with a non-adequate level of variability, with too extreme GC contents, non-orthologous and absent in at least 30 % of the transcriptomes. For each exon we designed baits from the multiple transcriptomes in order to limit the genetic distance between the baits and their targets to 10 %. To test the correlation between genetic distances between baits and targets and the capture success, we designed two sets of baits, each designed on different lineages of neogastropods (Conoidea/Buccinoidea/Mitroidea vs the rest). Here we present the results obtained with a first batch of 384 specimens, covering the genus-level diversity of the Conoidea, Buccinoidea and Mitroidea, sequenced using a 60 k baits kit designed using 24001 sequences, representing 1125 exon. We obtained an average of 2 million reads per sample. We trimmed reads and assembled them before blasting assembled contigs on exon sequences of the reference (*Conus ventricosus*). We concatenate all the aligned exons to reconstruct a phylogenetic tree.

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# A molecular phylogeny of Donacidae (Bivalvia: Heterodonta): A species-level investigation into the relationships of globally distributed surf clams

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Donacidae is a commercially important family of bivalves and one of the few bivalve lineages that has successfully colonized brackish and fresh waters. However, to date, no phylogenetic hypothesis exists for this widely distributed group of heterodont bivalves. Here we turn to molecular data from the nuclear and mitochondrial genomes and combine them with the extensive fossil record of donacids to propose an evolutionary hypothesis for the family. Our results strongly support the monophyly of Donacidae, including *Galatea*, *Iphigenia* and ‘*Plebidonax*’ *deltoides*, but renders *Donax* paraphyletic. We thus elevate the subgenus *Latona* to genus, to accommodate a clade of Indopacific species, while keeping *Donax* for the clade sister group to *Galatea* and *Iphigenia*, which includes mostly Atlantic and American Pacific species, plus a few Indo-Pacific ones. The diversification of Donacidae seems to be tightly connected with the opening of the North and South Atlantic Oceans in the Cretaceous as well as to the closing of the Tethys Ocean during the Oligocene.

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## Freshwater mussels (Unionida) in the Senckenberg Collection: a globally important resource for research on status of and changes in aquatic biodiversity

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The collection of freshwater mussels (Unionida) of the Senckenberg Research Institute and Natural History Museum in Frankfurt, Germany is among the 10 largest in the world. It comprises >80000 specimens in approx. 20000 series or lots. It contains about 478 primary type series and covers ca. 70 % of the global species diversity of the Unionida. It is a research instrument in great demand internationally and represents a data archive of high scientific value. Accessions date back to the early 19<sup>th</sup> century and they include important collections from outstanding researchers and famous malacologists (E. A. Rossmässler, W. Kobelt, F. Haas, H. G. Bronn, H. v. Ihering, K. L. Pfeiffer, R. A. M. Brandt, H. Schütt). Digitisation of metadata for the entire collection is currently being completed, after an earlier phase of work completed data capture and taxonomic revision for historical and recent material from North Eurasia and North America. Collection data and type-related information, including photographs, will finally be accessible online (via Senckenberg's databases AQUiLA and SeSam). The publication of a revised type catalog will be supplemented by information on the overall holdings (scope, focal points, incorporated collections, additions and corrections to the collection history). The importance of the collection is mainly in understanding biodiversity and species distributions, especially for ongoing revisionary studies of South and Southeast Asian freshwater mussels and for the Unionida fauna from the Mediterranean and Middle East. However, we intend to use this collection to develop approaches to expand access to less traditional specimens. In a pilot project, glochidium larvae specimens will be recorded and documented separately. In particular, we will experiment with different approaches to optimise three-dimensional imaging based on light or scanning electron microscopic methods. Moreover, we are examining protocols to obtain genetic information from dried tissue remains from shells of rare or already extinct species. In total we hope to make as much data as possible publicly available to support all researchers working on the biodiversity and conservation of these important animals.

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## New insights into the diversity of the ‘bug-eating slugs’ Aitengidae (Acochlidimorpha, Panpulmonata)

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Discovered only in 2009, the family Aitengidae belongs to the panpulmonate Acochlidimorpha and comprises amphibious and terrestrial slug species. The type species *Aiteng ater* – selected as mysterious ‘bug-eating slug’ into the Top 10 New Species list 2010 by the International Institute for Species Exploration – lives on the mud in mangrove forests of Thailand. Since then, only two more species were nominally described: *A. mysticus* inhabiting the upper intertidal of coastal caves in Japan and the terrestrial *A. marefugitus* from Palau.

In the present study we give a preliminary overview of the diversity of aitengid species collected during several sampling events in the Indo-Pacific including expeditions in the framework of ‘Our planet reviewed’. Aitengids are now known from coastlines of 9 countries in the tropical Indo-West Pacific, with up to five new species which await formal description. We present new (3D)anatomical and histological data and possible food sources analyzed through gut contents. Our morphological and molecular data, together with direct observations in field and literature data, suggest that Aitengidae are either insectivorous (larvae or pupae) and/or ovivorous (like other freshwater Acochlidimorpha) feeding on snail egg masses. Aspects of aitengid biology are discussed.

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## A molecular approach to the phylogeny and trophic specialization in Ovulidae (Gastropoda: Cypraeoidea)

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Host-parasite relationships, provide very useful models to study adaptive processes. We investigated the interaction between carnivorous marine gastropods, the Ovulidae, and their cnidarian preys. As far as known, most of the species are associated with octocorals, but some feed on hexacorals, whereas the species of the genus *Pedicularia* are known to live on stylasterid hydrocorals. Ovulids inhabit mostly tropical and subtropical waters, with some species occurring in temperate waters. Very little information is available on the phylogenetic relationships and the degree of species specificity of predator-prey relationship of this corallivorous lineage, especially for deep-water taxa. In this work we used a molecular approach to produce a large-scale phylogeny of the family, and to reconstruct the evolution of their trophic ecology. Our dataset included sequences from more than 300 specimens collected in tropical and subtropical areas, representing *c.* 75 species. For the phylogenetic reconstructions we have analysed two mitochondrial (COI and 16S rRNA) and one nuclear (28S rRNA) molecular marker. To identify the coral host of the ovulid species, we have integrated literature data with an empirical approach. For the former, we have associated to the ovulid species present on the tree, the associated cnidarian in literature or internet sources identified at the lowest possible taxonomic level. For the empirical approach, depending on the availability of suitable material, the host corals were identified either morphologically or genetically through the amplification of the 16S molecular marker from tissue fragments. In absence of associated coral samples, host identification was attempted through the amplification of the ITS2 molecular marker from the gastropod stomach content, and subsequent matching against a reference database. In this latter case, amplification success was 10% and the identification of the host was possible at the genus level. The obtained molecular phylogeny of Ovulidae revealed the existence of groups that do not completely correspond to the currently employed subfamilial arrangement. Concerning trophic ecology, our results suggest several ovulid lineages repeatedly shifted between octocorals and hexacorals, while only pediculariines (*Pedicularia* and allied) are associated with hydrozoans (Stylasteridae).

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## Comparative transcriptomics and proteomics of cryptic cone species of the genus *Virroconus*

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The closely related cone snails *Virroconus ebraeus* (Linnaeus, 1758) and *Virroconus judaeus* (Bergh, 1895) live in sympatry in intertidal rocky habitats of the Indo-Pacific region. They are considered cryptic species showing very similar shells, and can only be differentiated using the shape of the radular teeth and DNA sequence data. Phylogenetic inferences based on mitochondrial and nuclear markers render discordant gene trees, which support a past event of mitochondrial genome introgression in *V. judaeus*. The compositions of the venoms of both species have never been compared. Here, the venom duct transcriptomes and proteomes of *V. ebraeus* and *V. judaeus* were assembled and compared. The M, O1, and O2 conotoxin precursor superfamilies were the most abundant in both species. Additionally, three new putative conotoxin precursor superfamilies (Virro01-03) with cysteine pattern types VI/VII and XVI were identified. The most expressed conotoxin precursor superfamilies were SF-mi2 and M in *V. ebraeus*, and Cerm03 and M in *V. judaeus*. Up to 16 conotoxin precursor superfamilies and hormones were differentially expressed between both species, and clustered into two distinct sets using PCA. This difference in venom expression coupled with the ontogenetic change in the radular morphology could represent adaptations of each species to eating different worm species. Finally, we used machine learning algorithms to infer the 3D structures of selected venom proteins, including the differentially expressed Cerm03 and SF-mi2, an insulin type 3, a GVIA-like conotoxin, and an orthologous gene to the well-known *Pionoconus magus*  $\omega$ -conotoxin MVIIA (Ziconotide).

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## Sampling the known and unknown diversity in hyperdiverse groups for molecular phylogeny

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Molecular phylogenies revolutionized our knowledge of the evolutionary relationships among molluscs and deeply impacted our understanding of the diversification of the group. However, some phylogenetic relationships are still resistant to the relentless efforts of molecularists, even when equipped with powerful sequencing tools. Such irreducible taxa include hyperdiverse groups such as the neogastropods, with more than 15 000 described species: though phylogenetic relationships at the deeper taxonomic levels are about to be resolved, only a fraction of the genera, and a fortiori of the species, have been sequenced so far, and no one knows how many new deep lineages remain hidden. In the framework of the ERC “HYPERDIVERSE” project, our goal is to produce a genus-level phylogeny of neogastropods, including ca. 1000 terminal taxa, sequenced using an exon-capture strategy. To maximize the taxonomic representativity, including potential genus-level lineages that are currently not recognized as genera, we are applying the following strategy: 1. Intensive field sampling with a focus on small-sized molluscs (containing most unknown taxa); 2 Sequencing (cox-1 barcode fragment) of all the MNHN neogastropod samples; 3. Identification of all the corresponding vouchers by taxonomists during dedicated workshops; 4. Selection of one sample per described genus (these being the described and sampled genera) and per genus-level lineage revealed by the cox-1 data (these being the undescribed but sampled genera); 5. Search for the missing genera (described but unsampled) in older museum collections. With this strategy, only the undescribed and unsampled genus-level lineages would remain uncovered, still a potentially non-negligible fraction of the total diversity in such a hyperdiverse group. Challenges include the increasing difficulty to sample in the field (in particular due to the need to comply with the ABS principles formalized in the Nagoya protocol), compounded by the difficulty of sampling minute species (constituting the majority of unknown species); the taxonomic impediment, with only a few, if any, taxonomists available for some groups; and the capacity to obtain DNA of sufficient quantity and quality from older museum collections, all challenges by far more problematic than the increasing facility with which large quantity of sequences can be produced with high-throughput sequencing technologies.

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## Studies on the small-sized scaphopod *Cadulus thielei* Plate, 1909 in the Southern Ocean: a not so rare species and evidence genetic diversity

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Prior to this study, *Cadulus thielei* Plate, 1909 was only known from its' type locality in East Antarctica and 3 sites in the Bellingshausen and Ross seas from 157 to 438 m depth, with just 5 specimens known in published literature. They are often present in epibenthic sledge (EBS) samples from the Southern Ocean, spanning from the Amundsen Sea (110°W) to the Lazarev Sea (9°E), and from South Georgia (53°S) to the Filchner Trough (77°S). Our EBS collections hold 11782 scaphopods specimens morphologically assigned to *Cadulus thielei* collected in 161 to 5737 m depth. The aim of this study is to assess the shell morphometrics, to understand distributions, and to undertake an initial COI barcoding assessment of its genetic diversity. To date 360 specimens have been assessed for 10 morphometric parameters, appearing as one species. A negative association between depth and size was discovered. Preliminary COI sequencing of 14 specimens from 4 areas has revealed 10 haplotypes and two genetic clusters (Amundsen Sea vs Lazarev Sea, Shag Rocks & South Georgia). This study has been able to significantly extend the known distribution ranges of *Cadulus thielei* vertically and spatially, confirming it as a circum-Antarctic species. Further investigations will include wider barcoding and multi-locus genetic studies and seek to understand the environmental drivers of this species' distribution and diversity patterns.

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## Deep water cone snail venomics: protein diversity in the venom of *Profundiconus* species from New Caledonia (Gastropoda, Conoidea)

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Conotoxins are bioactive peptide molecules present in the venom secreted by cone snails (Gastropoda, Conidae). Within this group, the species in genus *Profundiconus* are characterized by their deep-water habitat (usually between 100 and 1000 m). Recent studies have disclosed for the first time the transcriptome of the venom ducts of two species of *Profundiconus*, namely *P. neocaledonicus* Castelin & Tenorio, 2016, and *P. vaubani* (Röckel & Moolenbeek, 1995), both from the New Caledonia region. Some of the sequences found are quite divergent with respect to the typical conotoxins found in other Conidae, and have been defined as profunditoxins.

With this background, we have now analysed for the first time the proteome of the venom duct extracts of *P. neocaledonicus* and *P. vaubani* specimens. The extracts were studied by liquid chromatography coupled to mass spectrometry (LC/MS), which allowed the identification of a number of post-translational modified small peptides (less than 4000 Da). Additionally, fractions of the venom extracts were reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin. The resulting digested solutions were analysed by tandem LC-MS/MS (Shotgun proteomics). Comparisons with the corresponding transcriptomes identified a total of 120 sequences in 74 protein groups, including venom-related and metavenom network proteins in addition to conotoxins, with average masses between 1112 and 148299 Dalton. Up to 22 sequences were present exclusively in *P. vaubani*, whereas other 60 were only present in *P. neocaledonicus*. A total of 37 sequences were present in both species. Most of the sequences identified by shotgun proteomics correspond to relatively large peptides, as almost 70 % have molecular weights higher than 7000 Da. Many of these corresponded to proteins (disulfide and prolyl peptidyl isomerases), which are also present in other species of Conidae (*Conus* and *Conasprella*). The 3D structures of the novel profunditoxins have been modelled using AlphaFold2.

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## How many *Kelliella* species in Europe? A reappraisal

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*Kelliella* M. Sars 1870 is a genus of small bivalves inhabiting muddy bottoms on the shelf-edge and slope, commonly in habitats that are oxygen-poor or enriched in organic carbon. We examined populations from Norway, the bay of Biscay, the Ibero-Moroccan gulf, the Alboran Sea and the Gulf of Lion, in a depth range 100–700 m, and also a sample from a Last Glacial Maximum deposit in the Alboran Sea.

Genus-level character states, which diagnose *Kelliella* from the abyssal *Vesicomya* occurring in the same area, were observed in all the populations examined and are 1) the absence of an escutcheon 2) a prodissoconch which comprises a small D-shaped prodissoconch 1 (60–70 µm in maximum length) with a rough pitted sculpture, and a smooth, hourglass shaped prodissoconch 2 with faint commarginal growth lines (190–240 µm in diameter), denoting a planktotrophic development.

Few characters are potentially useful for the discrimination of species; these include the outline (rounded vs. “glossiform” with prominently prosocline umbos) and surface texture of the dissoconch (smooth vs. raised, spaced commarginal lines). There is an overall variation in size (maximum ca. 3 mm in the specimens from Norway and in fossils from the from le LGM in Alboran Sea), consistently less than 1.5 mm in Bay of Biscay, Ibero-Moroccan Gulf, Alboran Sea and Golfe du Lion. In the shallowest population, from 100 depth in Bay of Málaga, the hinge has a markedly thicker cardinal platform with definite, prominent cardinal 4 (left valve) and 3b (right valve).

Notwithstanding this variation, we could not find any particular location in which more than one species of *Kelliella* could be recognized. *Kellyella biscayensis* was distinguished from *K. miliaris* by “the paucity of co-marginal striation, by the more elongate somewhat lozenge-shaped lunule and by the unusually short and characteristic hinge plate”, but these features are all encompassed within the variation observed in *K. miliaris*. Therefore, morphology does not support the existence of more than one common species along the West European margin. This is consistent with the good capacity for dispersal inferred from planktotrophic development.

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## Integrative species delimitation within the genus *Milax* (Gastropoda: Eupulmonata) based on morphology and DNA barcoding

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Milacidae is one of the least known families of terrestrial gastropods grouped into two genera: *Milax* Gray, 1855, and *Tandonia* Lessona & Pollonera, 1882. Some of them are widely distributed, whereas others occur only locally. Some species have economic significance and are therefore important for people; they play a role in gardening and horticulture as they pose a serious threat to cultivated plants, vegetables, and fruits. For this reason, most of the research on these slugs was concerned with the impact of various substances and factors on their population dynamics and the development of methods to reduce their feeding on plants so far.

Studies focusing directly on the taxonomy of slugs are usually more difficult to realize than research on shelled snails because their identification is usually problematic due to a smaller number of taxonomically informative traits which can be extensively tested. As there is a lack of conchological characters, such studies require anatomical dissections, which is often not an easy task. In the presented study we investigated genetically and morphologically two *Milax* species: *M. gagates* (Draparnaud, 1801) and *M. nigricans* (Schultz, 1836) from the collection of the Wrocław Museum of Natural History (Poland). According to data from the literature, these two species should be treated as a single species, as was done in the past by the majority of malacologists. We used two mitochondrial markers (COI, 16S rRNA) and two nuclear markers (histone 3, 28S rRNA) to establish phylogenetic relationships between these two species and other members of the Milacidae family as well as conduct analyses for species delimitation. We detected high genetic variability within the amplified markers in these two species and differences in the appearance of the genitalia, especially a stimulator. These findings suggest that *M. gagates* and *M. nigricans* should be treated as two distinct and valid species.

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**Symposium**

# **Mollusc Evolutionary History**

**Part I**

**Abstracts of oral presentations**

## A fresh look on Late Palaeozoic Rostroconchia, or how we can refine our knowledge of hippocardiid palaeobiology

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Rostroconchia, one of the minor classes of the Mollusca and presumed to become extinct by the end of the Permian Period, are still understudied and often ignored by malacologists. Although the basic rostroconch morphology is fairly well known, several details remain difficult to explain, mostly due to insufficient preservation. Whereas internal characters can be seen after destructive preparation methods or micro-CT analyses, variation in shell shape and ornamentation are mostly unexplored as rostroconchs rarely occur in abundance. Recent studies of about 500 specimens of the Middle Devonian hippocardiid *Bohemocardia hainense* Maurer offered expanding and detailed information on shell architecture and ornamentation important for rostroconch systematics. In addition, specimen size from 3 to 15 mm conch length show different growth stages and, thus, distinctive ontogenetic changes in conch proportions. Specimens embedded in rock matrix allow reconstruction of the hood, a crinoline-shaped shell expansion surrounding the posterior rostral area. Presence or absence of this character combined with general conch shape is regarded as systematically diagnostic in the Rostroconchia and, thus, requires careful attention in future studies.

Further minute specimens of hippocardiid rostroconchs from the Pennsylvanian Finis Shale of north-central Texas confirm our earlier observations on early rostroconch ontogeny. One of the most distinctive automorphic characters, apart from connecting shell layers between left and right halves of the univalved conch, is the univalved cap-shaped larval shell of these taxa. It is always divided into a teardrop-shaped protoconch I and an anteriorly bilobed but still univalved protoconch II. Both larval stages are separated from each other and from the following juvenile dissoconch by more or less distinct growth varices. In contrast to the adult shell, the larval shell covers the soft parts only dorsally and does not envelope the mollusc completely as in later ontogenetic stages.

Our recent observations confirm and precise our ideas on rostroconch palaeobiology collated during the past decades and highlight the peculiar role of the Rostroconchia within the Mollusca.

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## Composite approaches to a time-calibrated family-level phylogeny of the Bivalvia

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Palaeobiological analyses, particularly those aiming to test techniques and metrics, require carefully curated datasets such that conclusions rest more on the assumptions of the methods than those of the data. These analyses increasingly rely on convenient, large databases of palaeobiological data that are variously scraped from online repositories—often without transparency in how the data were aggregated or their underlying uncertainties, assumptions, and taxonomic decisions. This talk centres on vetting of an extensive dataset of extant and selected extinct bivalve families, ultimately used to examine the effects of time calibration of phylogenetic analyses of diversification history. I will explain the process of the compilation of first appearances of 128 bivalve families, starting with the previously-published trees from the Bivalve Tree of Life project, placement of extant families not in those phylogenies, and the extensive literature survey leading to the sister-group placements for the extinct families most directly associated with the extant families, and then discuss the resulting tree topologies, which have 91 % of nodes calibrated to fossil occurrences. Taxonomic, phylogenetic, and stratigraphic decisions in the compilation of this dataset are thoroughly documented, referenced, and available for review alongside the analytically formatted dataset. Interpretation of the literature influences decisions on tree topology, particularly at a few consequential nodes, which can alter the inference of bivalve evolutionary history. For example, with this topology we find that a bifurcating phylogeny yields rate estimates at odds with the fossil record as calibrated with budding originations, including a 4-fold damping of the Cenozoic diversification of the group. ‘Big-data’ analyses are vital tools for our ongoing understanding of the evolution of the natural world through time, and it is important that the raw materials going into those analyses are as rigorously produced and peer-reviewed as the analyses themselves. In taking this approach, we believe that our dataset represents the most up-to-date available synthesis of the state of knowledge on bivalve family phylogeny, and we hope it will serve as a useful resource for the molluscan community going forward.

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## Change in diversity patterns across the Eocene-Oligocene boundary of Kutch Basin, Western India: a molluscan perspective

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The Kutch Basin, western India is well known for its near-continuous Cenozoic marine sedimentary succession which hosts huge haul of marine molluscan fauna, especially gastropods and bivalves. Herein, a preliminary study to document the molluscan diversity of the middle Eocene (Harudi and Fulra Limestone formations) and early Oligocene (Maniyara Fort Formation) marine beds of Kutch Basin is done in terms of both taxonomy and ecology. The data compiled from published literature and our collection reveal a diversity of 105 bivalve species (53 genera and 29 families), and 107 gastropod species (47 genera and 22 families) during the time interval of middle Eocene-early Oligocene. Of the middle Eocene bivalves (represented by 63 species) only seven species cross the Eocene-Oligocene boundary, with a high extinction rate of ~88.8% at species level and ~72.2% at generic level. In case of gastropods, of the 69 species from middle Eocene, ~95.6% of species succumb at the Eocene-Oligocene Boundary, and ~58.06% at generic level. It is noteworthy that no late Eocene marine strata are present in the Kutch Basin.

Ecologically, bivalve populations are mainly dominated by infaunal and epifaunal suspension feeders during the Eocene and Oligocene. However, these feeding guilds substantially decrease in species richness during the transition from the Eocene to Oligocene. In case of gastropods, epifaunal carnivore-predators and semi-infaunal suspension-feeders are most abundant during the Eocene which are gradually replaced by epifaunal carnivores and herbivore-grazers as abundant feeding guilds during the early-Oligocene. This decrease in abundance of suspension-feeders possibly suggests a low supply of nutrients during the transition.

Faunistically, middle Eocene is marked by high species richness of volutids, ampullinids, stromboids, conoids and turbinellid gastropods, all being mainly warm-water dwelling gastropod groups. However, during the early Oligocene species richness of these gastropod groups significantly decreases. Thus overall, the period of Eocene-Oligocene transition records a sharp faunal turnover in molluscan fauna at both generic and specific level which apparently is due to the prevailing major global cooling event during the Eocene-Oligocene transition along with other factors like frequent anoxic condition and paucity of nutrients in the ocean.

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# **Geographic distance matters: diversification process in the deep-sea snail genus *Bathyancistrolepis* (Gastropoda: Buccinidae)**

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Little is known about the speciation of benthic animals in the deep sea, where few obvious barriers exist to prevent admixture of individuals. Intense environmental gradients with depth, rather than geographic separation, would thus play a central role in genetic differentiation and speciation (the depth-differentiation hypothesis). Several empirical studies have corroborated this idea for lineages of bivalves, amphipods and octocorals. However, difficulties in sampling have hampered detailed documentation of existing diversity and hence past speciation process in the deep sea.

Here we show—with among the most extensive datasets for a clade of deep-sea benthos—that geographic distance may play as significant a role in speciation as depth-related factors do, presumably depending on the dispersal ability of individuals. Direct-developing snails of the buccinid genus *Bathyancistrolepis* are endemic to the northwest Pacific (Sea of Okhotsk to Pacific coasts of Japan to southwest of Taiwan) with a depth range from 550 to 5500 m. Specimens from 125 localities encompassing their geographic and vertical distributions were classified into nine biological species, seven of which were new to science, based on the comparison of shell traits and reconstruction of molecular trees. Sister species had either sympatric or allopatric distributions, with or without an overlap of depth ranges. However, genetic distances between conspecific individuals increased with their geographic distances, but not with differences in depth, suggesting that allopatric differentiation prevailed in the evolution of this bathyal–abyssal clade.

How long did it take for the differentiation and speciation in the deep sea? Tectonic uplift in the Japanese archipelago drives on-land exposure of Quaternary deep-water deposits, providing an unrivaled opportunity to access bathyal molluscan fossils. Miocene to Pleistocene fossils of *Bathyancistrolepis* from such deposits were classified into two extant and four extinct species and were used to calibrate the molecular trees for the inference of divergence times. Intraspecific variations in extant taxa seem to have been accumulated by isolation-by-distance since the late Miocene or Pleistocene, presumably reflecting dispersal by crawling on the deep-sea floor, literally at a ‘snail’s pace’.

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## A new hippuritid rudist bivalve from the Caramoan Peninsula, the Philippines, sheds new light on the evolutionary history of the Hippuritidae

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Rudists are bizarre-shaped bivalves comprising the Order Hippuritida. They appeared in the Late Jurassic, and flourished and diversified in the Late Cretaceous carbonate platforms, and became completely extinct at the Cretaceous/Paleogene boundary. The Hippuritidae is one of the representative rudist families in the Late Cretaceous (Turonian–Maastrichtian), but its origin and early evolutionary history remain uncertain. Recently a hippuritid rudist, which represents the first record of the family in the Pacific, was discovered from the allochthonous limestone blocks of possibly the age around Cenomanian/Turonian boundary in the Caramoan Peninsula, southeastern Luzon Island, the Philippines. This taxon is characterized by having seven or eight radial infoldings of the outer shell layer along the whole commissural margin of the right valve. The hippuritids usually have only three radial infoldings (one is a ligamental ridge, and two pillars on posterior flank), and the advanced taxa with more than three infoldings, called multiple-fold hippuritids, diversified in the late Late Cretaceous (Campanian–Maastrichtian). Caramoan hippuritid is distinct from all other multiple-fold hippuritids in the Caribbean and the Mediterranean areas in the viewpoint of morphological characters, such as the shape and the length of infoldings, and the distances between a ligamental ridge and each of two pillars. Furthermore, the comparison of the morphological features of three basic infoldings of Caramoan hippuritid with those of almost all the species of three primitive genera of this family: *Hippurites*, *Hippuritella* and *Vaccinites*, reveals that this taxon is most similar to *Vaccinites rousseli*, one of the earliest (Turonian) species of this genus in the Mediterranean area, and supports the enigmatic age assignment of Caramoan hippuritid. Accordingly, the Caramoan hippuritid should be assigned to a new genus, which is characterized by a unique combination of primitive and “advanced” morphological features, and represents the endemic taxon in the Pacific in the early evolutionary stage of this family. This taxon acquired the multiple-fold system independently from other multiple-fold genera, and much earlier than previously thought. The discovery of the Caramoan hippuritid calls for a complete revision of the evolutionary history of the Hippuritidae by including the Pacific and probably Cenomanian records in future.

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## Phylogeny and evolutionary history of Pleurotomariida (slit shells)

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Gastropoda experienced many major shifts in its evolutionary history including extinction of most of the early groups, survival of a few, and radiation of novel groups. Pleurotomariida (slit shells) is one of the most ancient gastropod groups and has the longest fossil record among living gastropods. It can be traced back to the Cambrian–Ordovician. Our study on Carboniferous Pleurotomariida from the USA supports that they were one of the most diverse and abundant Late Palaeozoic gastropod groups in shallow water environments. A diversity analysis at the Permian-Triassic shows that experienced a major loss at the end-Permian. Although they show a remarkable re-diversification in the Triassic (11 new families) they could not fully recover at the species and genus level. The revision of Upper Triassic St. Cassian Pleurotomariida suggests that the group was represented solely by newly evolved genera by the Late Triassic. As a result of diversity loss during the Late Triassic and end-Triassic, only four families survived into the Jurassic and among them only Pleurotomariidae persisted until today. We present results of the first comprehensive phylogenetic analyses of Pleurotomariida using the Parsimony and Bayesian methods (the latter using fossilized birth death model). Phylogenetic analyses represent also the first implementation of a Bayesian analysis with fossil gastropods. A total of 93 shell characters and 109 taxa ranging from the Ordovician to Recent were used. In the parsimonious analysis, several species that are currently placed in the same genus belong to distant clades and the parsimonious tree is stratigraphically incongruent (i.e., younger groups are reconstructed near the root, older groups are recovered near the tip). Fossilized birth death method incorporates the age information in the analysis, hence the reconstructed tree is more congruent with the stratigraphic information. The Bayesian tree shows that two major clades survived into the Triassic: Wortheniellini and Pleurotomariini. Although Wortheniellini was much more diverse in the Triassic, only Pleurotomariini survived until today with a few forms, suggesting that other factors than diversification played a role in the survival of Pleurotomariida.

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## Seals, whales, and the decline of Cenozoic nautiloid cephalopods

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The Cenozoic distribution patterns of pinnipeds and nautilids from the Oligocene onward show the local extinction of nautiloids in the areas where pinnipeds appeared, eventually resulting in the present-day restriction of *Nautilus* and *Allonautilus* to the central Indo-West Pacific Ocean. In addition, the development of oxygen minimum zones (OMZs) due to enhanced ocean circulation in the Oligocene prevented nautiloids to escape predation by retreating to deeper waters, resulting in their disappearance especially from the west coast of the Americas. The demise of the nautiloid *Aturia* due to predation pressure was less immediate, probably because it avoided predation by fast swimming rather than retreating to greater depths. Ultimately, however, this might have resulted in *Aturia*'s end-Miocene extinction, because its adaptations to fast swimming prevented it from retreating to depths that allowed *Nautilus* to escape the ever-increasing predation pressure. An immediate role of echolocating whales in the demise of shelled cephalopods is not apparent; their long, delicate snouts with numerous teeth likely were ill-suited for handling large shelled nautilids. A possible exception are short-snouted Simocetidae and Agorophiidae in the Oligocene of the North American Atlantic and Pacific coasts, which appeared in this area at the same time as the nautilids disappeared.

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## Taxonomic revision of the genus *Xerosecta*, Monterosato, 1892

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The genus *Xerosecta* Monterosato, 1892 represents a well-known taxon throughout the western Mediterranean area. It is currently represented by 13 species distributed in southwestern Europe. However, its species composition and the distribution area of each species in the south Mediterranean remains poorly known. To confirm the position of recently collected north African *Xerosecta* specimens within the genus, a revision of the genus through an integrative taxonomy approach was conducted. Two mitochondrial markers, the anatomy of the genitalia, as well as the shell morphology was investigated for samples from the whole distribution area of the genus. The results suggest the division of the genus into two subgenera: *Polloneriella* (Alzona & Alzona Bisacchi, 1940) and *Xerosecta*. The subgenus *Polloneriella* includes *P. contermina*, *X. feijaensis*, which is endemic in the northwest of Tunisia, *X. lomelliana*, which is endemic in the region of Jandouba, and a species endemic in the northern mountains of Tunisia. However, the subgenus *Xerosecta* seems to include 18 species. Among them, we could identify two new species from Algeria and one new species from Italy. Within the european *Xerosecta*, our study does not support the separation between *X. cespitum* and *X. arigonis* nor the separation between *X. terverii* and *X. introducta*. However, it supports a separation between the *X. terverii* and a new sister species from Italy. In addition, our results suggest the synonymizing of the italian *X. giustii* with the algerian *X. euphorca*. Regarding the north African radiation of the subgenus *Xerosecta*, we could identify seven species with limited distribution areas: *X. subrostrata* in Morocco, *X. euphorca*, *X. sp1. sp. nov.*, *X. sp2. sp. nov.*, *X. henoniana*, and *X. euphorca* from Algeria and *X. caelestis* from Tunisia. Regarding the insular radiation, on one hand, we could confirm the separation between *X. cfr. cespitum* in Corsica and the *X. cespitum* from western France and on the other hand, we could identify a sister species to the Sardinian *X. dohrni*, which is endemic to the island. The Sardinian radiation is represented by five endemic *Xerosecta* species. Surprisingly, there is no single shared species between the two insular radiation and the mainland radiations.

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## After the catastrophe – gastropods in the Early Triassic: new insights

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Gastropoda suffered a great deal of extinction at the end-Permian. Although the magnitude of extinction was not as devastating as in other invertebrates, this event was a game changer for the evolution of the class Gastropoda and its consequences are effective until today. New gastropod faunas including yet undescribed ones from Oman, Pakistan, Timor, Spitsbergen, and China shed more light on the Early Triassic gastropod diversity and composition. Bellerophonina survived with several genera and have a global distribution but became extinct within the early Triassic, at the Smithian extinction event. A yet undescribed limpet species from Timor with possible patellogastropod relationship is the only known record of this group in the Early Triassic. Vetigastropoda, especially Pleurotomariida, suffered considerably resulting in a low diversity in the Early Triassic. New collections from Timor, the U.S., Oman and China show that more genera than previously known (e.g., *Glabrocingulum*, *Eucochlis*) have survived. Neritimorpha have numerous occurrences in Lower Triassic strata, especially the surviving Palaeozoic genus *Naticopsis*, including newly recovered ones from Oman, Pakistan, and Timor. However, this rich record of neritimorphs is biased by the presence of a calcitic outer shell layer which increases their preservation potential in otherwise poorly preserved faunas. Well-preserved neritimorphs from the Western U.S. and Timor show that not all forms belong to *Naticopsis*. For instance, the earliest member of Neritidae with resorption of the inner shell walls occur in the late Early Triassic. Caenogastropods are present with Palaeozoic holdovers in the Early Triassic (e.g., Soleniscidae, Pseudozygopleuridae) and several new genera evolved during the recovery period. Most caenogastropods with preserved protoconchs indicate planktotrophic larval development. This suggests a sufficient primary production in the seas and argues against previously proposed productivity crisis in the Early Triassic. Heterobranchia flourish with a world-wide occurrence of the newly evolved genus *Sinuarbullina*, which is the oldest acteonoid and probably a descendant of the Palaeozoic Streptacididae. The oldest hyalogyrinid was recently discovered in Lower Triassic rocks of Pakistan. Throughout the Triassic, Caenogastropoda and Heterobranchia diversify much more than the basal clades that fail to fully recover. This has contributed to the clade proportion of living gastropod faunas.

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## Effects of mucin and haemolymph of African giant land snail (*Archachatina marginata*) on cardiac function of toad (*Bufo regularis*)

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Cardiovascular disease is increasing globally and has been associated with many side effects that cause morbidity and mortality in vertebrate. Extract from snail (haemolymph and mucin) of *Archachatina marginata* were reported to contain biological properties that could prevent Cardiovascular disease. The oral administration of the extract is one of the more common ways of managing hypertension among south west of Nigeria. The effects of mucin and haemolymph on cardiac functions have not yet been fully explored. Two hundred and forty (240) Snails were purchased from Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, with an average weight of 180.6 grams. Mucin was extracted with chilled acetone and distilled water while haemolymph was extracted by breaking the last two whorls of the apex. Ten adult (10) toads with mean body weight (102 grams) were obtained from swampy areas in Obantoko, grouped into two with five replicate each for both extracts. Toad hearts were prepared in-situ and the ventricles were connected to the clip of the electronic kymograph at a speed of 2.5 mm/seconds. The toads were injected with 0.3 ml concentration of the treatment, at the hind limb muscle and monitored for 5 minute to ascertain uniform circulation of blood. The heart rate and heart contractility were observed after administering each treatment and compared with their baseline value (control). At all-time intervals recorded, there were significant changes in the heart contractility and the heart rate after infusion of the extracts. Both extracts reduced heart contractility and heart rate of the toads when compare to the control. Mucin and haemolymph showed a potential decrease heart rate and heart contractility thereby enhancing cardiac function in adult common toads.

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## First Cretaceous cephalopod statoliths and the evolution of decabranchians

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We report first cephalopod statoliths from the Lower Cretaceous. These finds fill the gap in the fossil record between Jurassic and Cenozoic forms, and are more similar to the former than to the latter. We compare the morphology of the Mesozoic statoliths coming from unidentified cephalopods with statoliths of Recent decabranchians. This comparison shows that Mesozoic statoliths are morphologically most similar to statoliths of Recent idiosepiids (pygmy squids). Hence, we suggest, that Mesozoic statoliths come from some basal decabrachian cephalopods, a phylogenetic position assumed also for idiosepiids. These finds support also some previous suggestions, that decabrachians diverged from vampyropods earlier than in the Early Jurassic. We discuss the absence of a wing in the Mesozoic statoliths and suggest that a robust spur could have played similar role to the wing in Cenozoic and Recent decabrachian statoliths. We suggest that the fossil statoliths and their morphology might be useful tools in interpreting cephalopod evolution.

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## Bayesian inference of early Palaeozoic cephalopod evolution

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Fossils of cephalopods are ubiquitous in collections and museum exhibitions, with belemnites and ammonoids being particularly well represented. This has led to a strong research interest in these taxa and our knowledge on them has accordingly grown considerably in recent decades. In contrast, the earliest fossil cephalopods – informally called “nautiloids” – are still relatively poorly understood, despite their abundance in early Palaeozoic rocks. Fundamental to a better understanding of this group is a robust phylogenetic framework, which facilitates classification and evolutionary research. Unfortunately, such a framework was missing from previous research and several partly contradicting hypotheses were available, leading to a confusing situation with numerous high-level groups being proposed. For this purpose, we conducted the first ever phylogenetic analysis of this group based on a large, newly compiled morphological character matrix containing 173 species and 141 characters of Cambrian and Ordovician cephalopods. We employed state-of-the-art methods of Bayesian phylogenetic inference using the Fossilized-Birth-Death model to reconstruct a time-tree of early cephalopod evolution. While there are topological uncertainties near the root of the tree, we consistently recovered three major clades, corresponding to the Orthoceratoidea, Endoceratoidea and Multiceratoidea, respectively. Orthoceratoids are mainly characterised by straight conchs and cameral and/or endosiphuncular deposits, while endoceratoids comprise two distinct lineages with endocones within the siphuncle and multiceratoids represent a diverse array of variously curved or coiled forms with predominantly empty siphuncles. In addition, many taxa with endogastrically curved conchs and ventral siphuncles formerly assigned to the Ellesmerocerida and some others represent a paraphyletic assemblage of taxa at the root of the cephalopod tree with uncertain affinities to the major clades. The assignment of any early Palaeozoic cephalopods to either stem or crown group is currently not possible, as the presumable ancestors of coleoids are represented by the Orthoceratoidea, while the living nautiloid lineage may be traced back to either Orthoceratoidea or Multiceratoidea. Regardless, the term “nautiloids” encompasses members of the stem groups of cephalopods, coleoids and nautiloids, as well as crown group cephalopods and nautiloids. In conclusion, we present a robust phylogenetic framework for the classification and future evolutionary studies of “nautiloid” cephalopods.

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## The rise of complex marine ecosystems and the role of mollusks in the Triassic

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Global and local marine biodiversity curves have shown a moderate rise from the Paleozoic to the Cenozoic. Mollusks have contributed to marine biodiversity since the Paleozoic and become a major component of marine assemblages in the Mesozoic and Cenozoic, with a steep rise in mollusk and particularly gastropod proportions in the Cretaceous and Cenozoic. Specifically, the mean proportion of gastropod genera among marine invertebrate genera is between 0.07 and 0.20 throughout most of the Phanerozoic, but increases in the Cretaceous and Cenozoic, reaching values of more than 0.40 in several stages of the Paleogene and Neogene.

The Mesozoic Marine Revolution represents a reorganisation in the structure of marine benthic ecosystems that took place during the Jurassic and Early Cretaceous. It was caused by increased biotic interactions and utilisation of ecospace as well as rising primary productivity and is likely to explain higher biodiversity in the late Mesozoic.

However, there are highly diverse marine ecosystems before the late Mesozoic, such as the Middle to Late Triassic Cassian Formation in the Dolomites (Southern Alps, northern Italy). Total invertebrate species richness is 1421 and the total gamma diversity measured as Shannon entropy is 4.26. 67 % of invertebrates are mollusks, gastropods being the dominant class (39 %), followed by bivalves (21 %).

Several other, even earlier lagerstätten also show high gastropod proportions, such as the well-preserved Paleozoic Buckhorn Asphalt Quarry (Oklahoma, USA; Pennsylvanian), from which 33 % of the invertebrate fauna are gastropods. The Cassian Formation and the Buckhorn Asphalt Quarry are two representatives of a group of lagerstätten that are characterised by an excellent preservation of fossils and a high diversity of molluscs. This type of lagerstätten provide insight into true ecological diversity with little taphonomic bias, yielding a window into past ecosystem structure that is usually obscured by factors such as aragonite dissolution or mechanical destruction of small shells. Uncovering biodiversity patterns through time is critical to understanding principles of community assembly and the rise of complex ecosystems.

High gastropod diversity in late Paleozoic and early Mesozoic assemblages may lead us rethink the timing of the Mesozoic Marine Revolution and the substantial rise of gastropods in the Cenozoic.

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## The fossil record of Mesozoic and Cenozoic freshwater bivalves: a progress report

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With a standing diversity of almost 1400 species, extant freshwater bivalves account for approximately 14 % of modern bivalve biodiversity, occurring from 55°S to beyond the Arctic Circle, on all continents except Antarctica. While molecular phylogenies of freshwater bivalves are rapidly improving, the underlying causes of diversification and the modes of expansion are still poorly understood. The fossil record of freshwater bivalves, albeit much less comprehensive than for marine taxa and biased towards lake environments, helps to explore these issues. We have started databasing fossil freshwater bivalve names in a constrained effort in spring 2020, within the Aphia environment of MolluscaBase ([www.molluscabase.org](http://www.molluscabase.org)), where the data underlying this study are available. As of 9 April 2022, we record 652 genus-group names, as well as 7665 species-group names, of which 1926 are treated as valid; the validity of another 2282 species-group names is currently unassessed. We estimate that we are nearing completeness, with approximately 95 % of all species-group names recorded. Of these, 3443 relate to the Palaeoheterodonta, with 454 names in Trigonioi-doidea, 211 in Pseudocardinioidea and 2778 in Unionida. Of 4222 species-group names in Euheterodonta, 1932 relate to Lymnocardiinae, 573 to Sphaerioidea, 692 to Cyrenoidea, 963 to Dreissenioidea and 62 to freshwater Corbulidae. We recognise at least 18 independent invasions of freshwater, in ten orders within the Bivalvia. At least six of these occurred in the Mesozoic; another nine have no fossil record. Two main centres of diversification are obvious. The large Late Triassic to Late Cretaceous lakes of East and Central Asia hosted the bulk of the Mesozoic freshwater bivalve diversity and potentially are the origin of several major freshwater bivalve clades. Cenozoic freshwater bivalve diversity peaked in the Neogene lakes of the wider Paratethys region, which are the cradle of the freshwater Lymnocardiinae and the Dreissenidae. We currently recognize three main types of bias. Several Mesozoic families and genera, particularly in the Unionida and Sphaerioidea, are poorly defined. Taxonomic over-splitting, commonly at the species-level, is rife. Sampling is likely biased towards the global north. Notwithstanding the above, we predict that the general diversity patterns will stand.

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## ***A Nautilus and the history of its taphonomy***

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*Nautilus* is studied for many decades and various reasons. Living in the Indo-Pacific Ocean along coral reefs in water depths of commonly 300–400 m, after death of the animal shells often float before being deposited eventually. The study of bioerosion may help to reconstruct the course of deposition. From a series of studies, we here present the latest results on bioerosion in *Nautilus* shells recovered from a back-beach shrubbery on Lifou Island, New Caledonia archipelago. Shells in this setting are influenced by marine conditions initially and by terrestrial environments after final deposition. The preservation of the studied shells is of variable quality which, however, did not give any clue on the quantity or quality of borings. Black terrestrial matter inside the shells proves the ultimate deposition in the shrubbery. To study the minute traces inside the conchs the cast-embedding method was used, allowing the investigation of even minute borings with the SEM. The study revealed the presence of more than 20 ichnotaxa and -morphotypes, most of these being found in a single specimen. Most common are *Ichneoreticulina elegans* produced by a chlorophyte and *Scolecia serrata* (? bacterial). Eight trace-morphologies have not been reported before. These might point to terrestrial bioerosion or represent morphotypes of known ichnospecies. Ichnotaxa can be used to determine relative water depths. Since only one of the shells contains a good number of traces only for this one a conclusion can be drawn, and we assume that the water depth of initial deposition represents the photic zone III to deep euphotic zone. Further common are morphotypes of *Fascichnus bellafurcus* (cyanobacterial) possibly indicating brackish conditions. A plausible depositional scenario is: The animal dies, and the shell and corpse float being transported shore wards ending up on the seafloor while bioerosion and taphonomic processes already alter the shell. During a storm event the shell is transported into the shrubbery and marine is replaced by terrestrial bioerosion. Since the two other studied shells show distinctly less bioerosion by marine organisms we conclude that the time before being transported was less than for the extensively bioeroded specimen.

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## Aporrhaidae, their evolution from Rupelian, Oligocene onwards

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The family Aporrhaidae (Gastropoda: Stromboidea) comprises six extant species. In a study of the soft parts of three of them, a deep evolutionary split between “*Aporrhais*” *serresiana* on the one hand and *Aporrhais pespelecani*/*Arrhoges occidentalis* on the other hand was found.

This talk presents an attempt to explain this based on an analysis of the fossil record. Several thousand specimens from Rupelian (Oligocene) to recent faunas were examined and classified regarding their age and geographical origin. A search for morphological patterns reveals some basic characters: the form of rostrum and spines and the existence of a ventral callus. These characters make it possible to initially split the aporrhais into three basic lineages. However, detailed examination of the callus in relation to environmental factors and the sympatric occurrence of two differently callused species point to the existence of a fourth lineage. The species and characters of the lineages are a) the spiky aporrhais with fingers like in “*Aporrhais*” *uttingeriana* or “*Triacontium*” *mirandum*; b) those without a callus, but with a shovel-like rostrum and with a solid wing like in “*Aporrhais*” *speciosa* or *Aporrhais pespelecani*; c) those with a thick, sledge-like ventral callus, like in “*Aporrhais*” *margerini* or “*Strombopugnellus*” *digitolabrum* and d) those with a thin, irregular ventral callus like in “*Cerycium*” *paradoxum* or “*Aporrhais*” *unisinuata*.

The formation of isolated or nearly isolated basins like the Central and Eastern Paratethys, the Mediterranean and the North Sea basin further splits the above mentioned four aporrhaid lineages. It leads to a complex evolutionary lineage pattern, which is at the moment not reflected in taxonomy and nomenclature. Most of the examined specimens fit into this pattern; some, like “*Aporrhais*” *serresiana*, do not. Possible explanations for the existence of these extra-pattern species are hybridization or introgression, extreme environmental conditions, immigration from other areas, and merging evolutionary lineages.

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## Bradybaeninae snails from East Asia are less diverged than other Camaenidae

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Terrestrial snail species from East Asia, typically belong to the sub-family Bradybaeninae, which belongs to the family, Camaenidae (previously belonging to the family, Bradybaenidae). Using phylogenetic analysis performed by RAxML-NG and IQTREE of the large ribosomal subunit (28S), maximum likelihood trees showed Camaenidae genera from East Asia had shorter branch lengths than Camaenidae genera from Southeast Asia. One specific genus, *Satsuma*, was highly diverse and diverged more than other genera within the Bradybaeninae. The genetic distances between subgenera of *Satsuma* (*Satsuma*) and *Satsuma* (*Coniglobus*) are larger than the genetic distances among other Camaenidae genera throughout East Asia. Similarly, a Bayesian inference consensus tree performed by Phylobayes also indicated the same trend, and that East Asian Camaenidae genera had shorter branch lengths than Helicidae genera as well. Furthermore, a maximum parsimony tree (performed by TNT) revealed that East Asian Camaenidae is a monophyletic group with fewer steps needed than the sub-family Camaeninae and the *Satsuma* genus. This may be due to Foster's rule. Some of the challenges I faced so far in my study are: 1) software issues such as MrBayes not being able handle the molecular data matrix; 2) alignment issues caused by using different software (such as MAFFT, Muscle or manually) will give different results, as there is no algorithms or software to evaluate the trimming quality for alignments. 3) depending on what positions were used from different alignments, different topologies were found mainly present within the Camaenidae; 4) the Maximum likelihoods method itself has a problem with repeatability and is unable to have a definitive result.

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## **Symposium**

# **Mollusc Evolutionary History**

## **Part II**

### **Abstracts of poster presentations**

## Purpurinidae: new outlook towards the family composition

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Purpurinidae is an extinct gastropod family, which is considered a possible sister or stem group of neogastropods. The earliest confirmed members of the family are known from the Upper Triassic of the St Cassian Formation (Dolomites, Italy) while the latest became extinct before the end of the Early Cretaceous. Nearly 30 genera were assigned to the family while first described. Most of them vary significantly in the shell shape and morphological characteristics and now they are attributed to other higher gastropod taxa. Our research shows that the following genera should be retained in the family: *Angularia* Koken, 1892, *Cretadmete* Blagovetshenskiy & Shumilkin, 2006, *Khetella* Beisel, 1977, *Pseudoscalites* Kittl, 1892, and *Purpurina* d'Orbigny, 1850. *Globipurpurina* Guzhov, 2004 was proposed as a subgenus of *Purpurina*, however, listed differences in our opinion fall rather into simple intrageneric variability without any need of further subdivisions. The family is characterised by robust, inflated shells with large body whorl and large oval aperture, commonly angulated in adapical part of the lateral flank and sculptured with axial and spiral ribs. Protoconch characters are crucial in determining the family attribution but rarely preserved. The protoconchs of purpurinid species whenever it is known display obtusely conical shape with smooth convex whorls and clearly visible demarcation between protoconch and teleoconch and expressed by thickened apertural margin. These characteristics connect Triassic genus *Angularia* with Jurassic *Purpurina*, whose protoconchs have been documented to date. Triassic purpurinid genera – *Angularia* and *Pseudoscalites* – are characterized by whorls with angulation in adapical portion of the shell, whereas Late Jurassic – Early Cretaceous forms – *Cretadmete* and *Khetella* – have weaker shoulder and larger last whorl with bigger aperture.

Current collection effort in the Upper Triassic of the St Cassian Formation provided a few specimens similar to *Pseudotrionium*—another possible neogastropod ancestor that previously was known from the same locality. Our specimens differ from the type species of *Pseudotrionium* in shell outline, although display somewhat similar ornamentation, which is on the other hand more similar to Jurassic forms of *Purpurina*, than to the members of Purpurinidae from the same formation. It can be assumed that *Angularia* and *Pseudoscalites* became extinct at the end of the Triassic, while *Purpurina* have evolved earlier than it was acknowledged so far, i.e., before the Late Triassic.

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# **Upper Toarcian (Lower Jurassic) marine gastropods from the Cleveland Basin, England: systematics, palaeobiogeography and contribution to biotic recovery from the early Toarcian extinction event**

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A new, diverse late Toarcian (Lower Jurassic) marine gastropod fauna from rocks of the Cleveland Basin exposed on the North Yorkshire coast of England has recently been described. The fossil assemblage comprises sixteen species, of which three are new: *Katosira? bicarinata* sp. nov., *Turritelloidea stepheni* sp. nov. and *Striactaenonina elegans* sp. nov. Four species are described in open nomenclature as *Tricarilda? sp.*, *Jurilda* sp., *Cylindrobullina* sp. and *Cossmannina* sp. The other species have previously been described: *Coelodiscus minutus* (Schübler in Zieten), *Procerithium quadrilineatum* (Römer), *Pseudokatosira undulata* (Benz in von Zieten), *Palaeorissina* aff. *acuminata* (Gründel, 1999b), *Pietteia uncarinata* (Hudleston), *Globularia* cf. *canina* (Hudleston), *Striactaenonina* cf. *richterorum* Schulbert & Nützel, *Striactaenonina* aff. *tenuistriata* (Hudleston) and *Sulcoactaeon sedgwicki* (Phillips). Most of these species are the earliest records of their respective genera and show palaeobiogeographical connections with contemporary gastropod associations from other regions of Europe and South America. The taxonomic composition of the late Toarcian Cleveland Basin gastropod assemblage differs substantially from the faunas of the late Pliensbachian and early Toarcian Tenuicostatum Zone, showing the strong effect of the early Toarcian mass extinction event on the marine gastropod communities in the basin. Only a few gastropod species are shared between the late Toarcian faunas and the much more diverse Aalenian gastropod faunas in the Cleveland Basin, suggesting there was a facies control on gastropod occurrences at that time. This is also a potential explanation for the taxonomic differences between the late Toarcian gastropod faunas in the Cleveland Basin and those in France, and Northern and Southern Germany.

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## Arcoid (Bivalvia) migration into the Late Permian Zechstein Basin of Central Europe

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The Zechstein Basin was an epicontinental inland sea on the Central European mainland in the Late Permian (Lopingian; 258–250 Mya) subdivided into the Northern Permian Basin and the Southern Permian Basin. Due to tropical climate and marine ingression, hypersaline conditions with evaporite successions dominated and fossils are predominantly known from the dolomitic reef complexes of the lower Werra Formation (Z1), lower Zechstein along the basin margins. In general, biodiversity was very low and decreased with increasing salinity. The Zechstein Basin was flooded from the boreal (Arctic) sea, but at least a temporary connection of the Polish Subbasin with the Tethys Ocean is assumed due to similarities of the faunas inside and outside the Zechstein Basin.

Arcoid bivalves, as yet assigned to the dustbin genus *Parallelodon*, entered the Zechstein Basin only with the first ingression (Werra Cycle; Z1). Following our taxonomical revision, only one species of arcoids, *Parallelodon striatus*, adapted to the harsh ecological conditions prevailing in the Zechstein Basin. Due to outcrop conditions, parallelodontids are only known from the western and southern calcareous-dolomitic margin of the Southern Permian Basin (United Kingdom: Northeast England; Germany: Lower Saxony, Hesse, Thuringia). From the Northern Permian Basin, arcoids have as yet not been reported. Based on the currently known global geographic distribution of arcoids in the Permian, it appears most plausible that the ancestors of *Parallelodon striatus* migrated from the Russian (Uralian) shelf along the Arctic Sea into the Zechstein Basin. Comparisons with adjacent arcoid faunas show a high similarity with the Russian platform fauna and confirm a migration of *Parallelodon striatus* out of the Arctic Sea into the Zechstein Basin. Although the Tornquist-Teisseyre corridor may have allowed immigration into the Southern Permian Basin of arcoids from the Southeast, Tethys taxa do not show comparable morphology with *Parallelodon striatus*. In contrast, species of the Russian Platform and boreal faunas are quite similar to *Parallelodon striatus*.

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## Chromosomal-level Whole Genome of the Small Giant Clam *Tridacna maxima* (Subfamily Tridacninae)

**Ruiqi Li<sup>1</sup>, Carlos Leiva<sup>2</sup>, Sarah Lemer<sup>2</sup> & Jingchun Li<sup>1,3</sup>**

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Giant clams are the largest extant bivalves that belong to subfamily Tridacninae. They dwell in shallow and oligotrophic waters across Indo-Pacific Ocean. Besides filter feeding like the majority of bivalves, the giant clams form mutualistic associations with dinoflagellates of the family Symbiodiniaceae. They play important roles in the coral reef ecosystems, such as serving as reef builders, providing shelters and food. However, giant clams are under great pressure from overfishing and calm-bleaching events caused by increasing ocean temperature. Compared to corals, other photosymbiotic organisms such as giant clams are still poorly understudied, especially the genetic mechanism of its mixotrophic lifestyle. In this project, we present the first chromosomal-level whole genome of giant clams. With HiFi PacBio sequencing (60X) and Hi-C (30X) chromatin conformation capture, we sequenced and assembled the whole genome of the small giant clam *Tridacna maxima*, which is not only the first chromosomal-level genome of giant clams, but also the first chromosomal-level genome in the family Cardiidae. Our assembly consists of 1.32 giga base pairs, 18 chromosomes, and 31746 genes. The high-quality genome provides insights of giant clam evolution, interaction with symbionts at molecular level, as well as genomic foundation of their unique traits such as their exceptional size and colourful hypertrophied outer mantles.

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## Early ontogeny of the Trigoniida

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The order Trigoniida is a major clade in the Bivalvia, dating back to the Silurian and surviving with a single relic genus, *Neotrigonia*, in Australian waters today. During the Mesozoic, the Trigoniida were truly cosmopolitan, highly diverse and abundant. Over 90 % of the 2500 species-level and 300 genus-level names assigned to Trigoniida refer to Mesozoic taxa. Considering their remarkable fossil record, surprisingly little is known with regard to the early ontogeny of the Trigoniida. To date, the prodissoconch and early dissoconch are solely documented for a single species of *Neotrigonia*. We present well-preserved larval and post-larval growth stages of seven species of Mesozoic and extant Trigoniida, including representatives from Middle Jurassic (Bajocian–Bathonian), Early (Albian) and Late Cretaceous (Santonian–Campanian) strata in Poland, Madagascar and Antarctica, respectively. The specimens are assigned to five genera (*Trigonia*, *Nototrigonia*, *Neotrigonia*, *Myophorella*, *Oistotrigonia*) in three families (Trigoniidae, Myophorellidae, Pterotrigoniidae) and currently three superfamilies (Trigonioidea, Myophorelloidea, Megatrigonioidea). All species are characterised by rather simple, D-shaped prodissoconchs, which are variable with regard to size, but generally relatively large, and often lack a distinct P-2 stage. This, together with their moderately convex, oval shape, suggests a dominantly lecithotrophic development. Late Cretaceous *Nototrigonia oliveiroi* differs from all other taxa in its prolonged, morphologically distinct nepioconch stage. Early post-larval shells of Mid Jurassic *Trigonia triangularis* are wing-shaped in outline, and thus radically different from adults. All taxa exhibit a brief prosogyrate larval to post-larval growth stage. Transition to opisthogyrate growth likely concurs with the development of a parivincular ligament. Allometric growth is common, with adult shells being more elongate than juveniles. The onset of post-larval shell ornament is probably most revealing with regard to phylogeny. Morphologies within the Trigonioidea (Trigoniidae: *Trigonia*, *Nototrigonia*, *Neotrigonia*) seem more disparate than between Myophorelloidea (Myophorellidae: *Myophorella*) and Megatrigonioidea (Pterotrigoniidae: *Oistotrigonia*). However, broader taxon sampling, particularly within Megatrigonioidea, is needed to decide whether the similarity of juveniles in the latter two genera would suggest that Pterotrigoniidae should be transferred to Myophorelloidea. Moreover, the early ontogeny of Palaeozoic and Triassic Trigoniida is undocumented. Consequently, the plesiomorphic conditions of the prodissoconch, nepioconch and early dissoconch are unestablished.

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## ***Terebralia* – a mudflat and mangrove dwelling gastropod genus of the Cenozoic**

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Mangrove ecosystems are considered important coastal environments that contribute to organic carbon sequestration. The gastropod family Potamididae contains taxa associated with mangrove environments, and taxa living in mudflat environments. In the European fossil record, a multitude of taxa belonging to the family have been identified since the 19<sup>th</sup> century, resulting in the characterisation of numerous sub-species and varieties.

In the present work, we analyse the taxonomic diversity of the potamidid genus *Terebralia* in the European fossil record, so as to assess the history of the genus in the Cenozoic, reconstruct the geographic distribution in the European realm and to review the ecological preferences of each fossil species. Through a literature review and examination of original material, nine species are considered as true members of the genus. Maximal geographical distribution was reached during the early to middle Miocene, covering part of the NE Atlantic, the Proto-Mediterranean and the Paratethys. Species with large dispersion, such as *Terebralia lignitarum* and *T. duboisi*, are commonly found in mudflats, whereas others with more restricted distribution *Terebralia subcorrugata* preferred environments that are rich in organic matter and vegetation such as mangroves.

The origination centre of the genus according to the fossil material is the NE Atlantic (France), although an origination in the Tethyan realm – where modern mangroves also originated – is also likely but could not be confirmed due to the fragmentary preservation of specimens from Turkey that lack some key characters. Finally, the genus disappeared from the Mediterranean in the late Miocene, during the onset of the Messinian Salinity Crisis.

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## Finding reticulated evolution in the fossil record, a case study with putative hybrids in recent and fossil Stromboidea (Gastropoda)

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Morphological information within the fossil record delivers the main criteria when discussing evolutionary lineages. On the other hand: studying recent animals allows digging much deeper into evolution by looking into information from DNA, ecology, behavior, and others. This raises the question of where knowledge of extant animals can improve our understanding of what has been happening in the fossil record.

Speciation via hybridisation may be a mechanism that challenges our view of monohierarchical evolution. While in plants, hybridisation is well-known and leads to reticulated evolution, this mechanism is not evident in animal evolution. Here we try to extrapolate from hybridisation in the extant marine gastropod superfamily Stromboidea and its morphological basis to a related group in the fossil record.

Hybridisation in Stromboidea still lacks molecular research. On the other hand, about 20 different hybrids in Strombidae and Aporrhaidae are postulated by morphological studies. The morphological basis of this claim is discussed here, and resulting mechanisms are applied to the fossil record of Paleogene and Neogene Aporrhaidae. Two instances were discovered: *Aporrhais digitata* (Roth von Telegd, 1915) and the lineage from *Aporrhais peralata* (Sacco, 1893) and secondly: *A. pliorara* (Sacco, 1893) to *A. serresiana* (Michaud, 1828), with the extant species *Aporrhais serresiana* (Michaud, 1828) as the end of the lineage.

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## **Symposium**

# **American Malacological Society President's symposium: Molluscan Conservation**

## **Part I**

### **Abstracts of oral presentations**

## A review of the freshwater mussels (Bivalvia: Unionoidea) of Vietnam

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Vietnam has a high diversity of freshwater mussels in the Indo-Burma area. Fifty-nine freshwater mussel species of Unionoidea have been reported from Vietnam. However, over the past five years there has been a tremendous increase in integrative work on the phylogeny of the freshwater mussels of Asia. Reviewing this work, we have updated the taxonomy of the Unionoid fauna of Vietnam. This has resulted in the recognition of 5 new tribes, 6 new genera, 16 species transferred to different genera, 1 genus synonymized, 1 new species described and another discovered, but not yet described, and 4 species placed into synonymy. The result is a revised list of the Unionoidea of Vietnam recognizing 2 families, 4 subfamilies, 12 tribes, 29 genera, 57 species. Total freshwater mussel species recognized in Vietnam has decreased by two species, but reflects a better understanding of the relationships of the Vietnamese fauna. During the past five years the IUCN Red List of Threatened Species has updated six assessments for Unionoidea from Vietnam. This resulted in two changes from Data Deficient to Least Concern. The taxonomic change of one species changed its conservation status from Not Examined to Endangered. Conservation status and basic biological information on these freshwater mussels is still under researched and information on host fish is lacking. These data are critical to the conservation of these species.

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## How can we can best respond to the biodiversity crisis: Insights from the Areho Natural and Cultural Heritage Seminar

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Cowie, Bouchet & Fontaine (2022) make a timely and compelling argument for recognizing the true severity of the biodiversity crisis, our accelerating trajectory towards a sixth mass extinction and humanity's wholly inadequate response. These realities were also recognized and considered at the regional (Pacific) level by the *Areho, natural and cultural heritage seminar* (French Polynesian Government 2019). This initiative brought together Government, academic and NGO representatives to share and discuss terrestrial mollusc species threats, conservation experience and action needs across the Pacific region. The associated *Statement by the Pacific Countries* highlights the natural and cultural value of the estimated 6000 regionally endemic land snail species, current and emerging threats to these species, and the essential conservation actions needed to optimize survival prospects. Species recovery experience (including the long-running *Partula* conservation breeding and reintroduction programme) provide insight into the challenges of invasive species threat response, ex situ population management, reintroduction and monitoring.

Reflecting on the seminar's discussions and call to action, we suggest some vital engagement needs for the global mollusc and wider conservation community to address if we're to best meet the challenge of Cowie and colleagues. This presentation will summarise the seminar's key messages, lessons learned from species recovery efforts to date and the need for robust iterative threat assessment and policy response. We also highlight the scalability constraints of species-level conservation and call for a global scale effort to ensure all mollusc species are represented in cryopreservation biobanks as an urgent conservation action, and suggest how this might be realized.

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## Terrestrial mollusc conservation in South Africa

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South Africa contains nearly 600 described species of land snail and an estimated 90 % are endemic. 40 % are considered narrow-range endemics clustered within eight hotspots of endemism. Systematic work over the past 25 years has led to augmented distribution data, revisions and the description of 67 new species with several more in the pipeline. Institutions with mollusc collections have participated in focused drives to mobilize existing data. Coupled with improved knowledge of the country's molluscan biodiversity and its accessibility, has been strengthening of legislation to protect invertebrates which were historically neglected partly due to lack of information. For example, the new Species Environmental Assessment Guidelines produced by the South African National Biodiversity Institute contain taxon-specific requirements. A screening tool identifies Species of Conservation Concern in the area under investigation and this triggers the necessity for a specialist study according to stipulated protocols. Species of Conservation Concern are those listed as threatened on the IUCN Red List and range-restricted species, not in decline or exposed to an immediate threat, but nationally listed as very rare. The majority of molluscs and other invertebrates have not yet been assessed and hence a forest spatial layer also triggers the requirement of a specialist study. A potential impediment to implementation of well thought-out legislation is the lack of specialists. Sixteen terrestrial snails had been assessed by 2004 and 14 are listed as threatened. This process has been revived beginning with updating these species and assessing the species in an endemic genus, *Chondrocyclus*, as well as other species which share similar distributions and potential threats. Priorities for molluscan conservation are the continued supply of high quality data from new research and mobilization of existing data, and red-listing of species that qualify for threatened status. The major challenge is capacity with a small handful of people working towards these goals for the whole country. These activities improve legal protection of molluscs and their environments, and inform decision-making such as protected area expansion. The importance of nature reserves, even very small ones, for molluscan conservation is emphasized.

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## Taxonomic investigation of Teredinidae in the Hawaiian Islands: resolving relationships needed to inform conservation

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Teredinidae, marine wood-boring bivalves commonly known as shipworms, are known for their destruction of ships and wooden structures. Despite their historic vilification as termites of the sea, shipworms play a vital role in maintaining ecosystem functions and services by facilitating nutrient cycling from terrestrial systems to marine and creating habitat for other species. Unfortunately, like so many invertebrate groups, an understanding of their ecology, evolution, and conservation is hampered by a poorly resolved and inconsistent taxonomic framework. The first critical step in developing conservation actions is accurate identification and distributions. Shipworms have historically been thought to consists of few species with wide geographic distributions, in part because of their ubiquitous occurrence globally, but also because of the difficulty in delineating and identifying species based on limited morphological characters alone. To better understand the taxonomy, distribution, and potential conservation status of shipworms in Hawai'i, we have undertaken a comprehensive and systematic study of shipworms in the archipelago, combining anatomy, biogeography, ecology, natural history, and multi-locus phylogenetics. Pre-1960s surveys in Hawai'i recorded 16 teredinid species, and since 2015, we have collected over 300 specimens from five Hawai'ian Islands, representing 19 morphologically identified species. Initial phylogenetic analyses of 126 newly generate shipworm sequences, representing 22 species, in combination with previously published sequences and interpretation of anatomical data revealed higher levels of diversity in Hawai'i than previously recognized. Shipworms collected in Hawai'i were recovered in 11 well supported clades, some consisting of only Hawai'ian samples. Biogeographically, the data indicate shipworms in Hawai'i may not be as cosmopolitan as once hypothesized, with some taxa being found only in Hawai'i. These data provide a clearer understanding of evolutionary patterns, indicating that pallets, the primary character in shipworm taxonomy, have a complex history of multiple shifts from segmented to non-segmented. Similarly, life history traits, including brooding and non-brooding evolved multiple times within various lineages. The increased taxonomic resolution and understanding of evolutionary patterns are informing our understanding of shipworm biogeography and ecology, which in turn will help develop marine conservation strategies.

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## Understanding snail symbionts and their role in conservation

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In the current biodiversity crisis, molluscs have the most documented extinctions of any major taxon. Effective conservation of the remaining diversity requires an understanding of the other species that interact with them. These associated organisms include an often-overlooked group, symbionts that live in or on molluscs, including pathogens, parasites and mutualists. Pathogens and parasites may cause decreased fitness, leading to population declines or extinction, whilst certain gut microorganisms are beneficial, increasing fitness and facilitating population growth. The effect symbionts have on their hosts is often modulated by environment, including that created by conservation management such as captive rearing and translocations. Quantifying and managing disease risk in conservation starts with the ability detect and identify symbiotic organisms, which is now more accessible via DNA-based approaches. The once hyperdiverse Hawaiian land snails represent the imperiled of the imperiled. Fewer than 300 of the original ca. 750 species remain, and extinction is imminent for many of these species in the next decade. Captive rearing and *in situ* exclosures have been the main form of active conservation management since the 1980s, supplemented more recently by translocations of captive individuals to these upland forest predator-proof exclosures. However, little is known about the symbionts, both parasitic and mutualistic, of these snails. Disease outbreaks of unknown cause in captivity have highlighted the urgent need to fill this knowledge gap, which we are attempting to do with a metagenomic approach. Here we present our initial method development and some preliminary results; the first look at gut microbiota of invasive gastropods of Hawaii, and the use of molecular techniques to identify potential pathogens and parasites in captive rearing of native snails. The identification of any specific parasites/pathogens that are driving observed mortalities in Hawaiian land snails will enable the development of disease screening tools, and inform rearing protocols. We also seek to characterise the microbiota of wild and captive populations of various host species, since symbiont effects on hosts may be synergistic. An awareness of what the whole community looks like will improve our understanding of ecological factors necessary for long term persistence, translating to improved conservation practice.

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## **Advancing uses of malacological digital data in an era of informatics**

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Collected and curated by thousands of malacologists over centuries, malacology collections comprise an enormous scientific enterprise, critical for analyzing long-term historical trends in molluscan biodiversity. The users of malacology collections were historically dominated by shell collectors and taxonomists who focused on gathering, naming, describing and classifying the rich biodiversity of molluscs, understanding their distributions, and untangling their complex evolutionary relationships. While malacological specimens continue to serve core roles in taxonomy, species identification and evolution, the growing number of reviews on modern uses of natural history data strongly suggest that we have entered a distinctly new era for specimen use, capitalizing on the large amount of digitized data in an informatics context. Although the longstanding uses and continued digitization of malacological specimens remain important, the availability of new tools for data access and analysis has the capacity to expand the use of specimen data beyond conventional research, especially concerning conservation efforts. For example, taxonomy and systematics have always been at the center of collections research, but genomic advances have revolutionized these studies, especially through phylogenetic analyses, which are quickly expanding both in the number of taxa and amount of genetic data (i.e. individual loci to whole genomes). Similarly, recent developments in machine learning have profoundly transformed our ability to extract information from visual data, expediting high-throughput specimen identification and phenotyping. Along with the creation of global data repositories, methodological advances in statistics, computer science, and geography have transformed the representation of species distributions from comparatively coarse occurrence maps to specific predictions of niche suitability. Unfortunately, many of these uses have not been applied to or developed with malacological specimens in mind, despite the millions of available specimens and associated digital information. It is necessary for malacological collections to move into this era of informatics, to expand appreciation for malacological specimens as sources of genotypic, phenotypic and biogeographic data and to inform our ability to steward future biodiversity conservation.

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## **Mangrove molluscs in the Indo-West Pacific in need of assessment for conservation**

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Mangrove forests serve as habitat to a variety of marine and terrestrial species and provide ecosystem services including shoreline protection and carbon sequestration. Despite growing recognition of the value of mangrove forests, they continue to be fragmented and degraded globally by deforestation and pollution. The floral diversity of mangroves is becoming increasingly homogenized, and a large number of mangrove species are listed as threatened or endangered. Similarly, some animals that rely on mangrove habitats are also threatened, including mammal and freshwater fish species. Few species of mangrove invertebrates are currently known to be threatened or endangered, but the proportion of this fauna that have been assessed is small, as low as 8 % of the known diversity of mangrove invertebrates. Molluscs are typically abundant in mangrove forests, and many species that live in these intertidal habitats are large and widely distributed. However, recent surveys of mangrove molluscs indicate that there remain numerous undescribed species in these forests, and still others that remain poorly known. A recent global taxonomic revision of the Onchidiidae led to the description of 25 new species of intertidal slugs, most of them from mangrove forests in Southeast Asia. Several of these species are restricted to high intertidal habitats which are increasingly threatened by habitat loss. Some of these are known only from a protected area in the Strait of Malacca and may be especially vulnerable to local disturbances and habitat loss. In addition, surveys of mangrove gastropods in Southeast Asia have revealed a high diversity of ellobiid snail species, many of which have been recorded only in a small geographic area. Conservation of mangrove invertebrates will depend on protecting the diversity of plants in these forests and improving our understanding of the diversity and distributions of mangrove invertebrates through further surveys and taxonomic studies. Conservation assessments are broadly needed for mangrove invertebrates, particularly in regions where mangrove forests are facing intense pressure from increasing deforestation and pollution, and which are beginning to face the additional stresses of a changing climate and rising sea levels.

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## A genomic and ecological perspective on Windward Islands (Tahiti & Moorea) partulid survival

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The deliberate introduction of *Euglandina rosea* as a biological control agent to the Society Islands had a catastrophic impact on the archipelago's endemic radiation of the genus *Partula*. Of the 18 currently recognized Windward Islands species, 6 are deemed extinct and the 12 survivors persist either in the wild as small remnant populations (7 species) and/or as captive populations (8 species). However, those survival estimates come with a major caveat: study of these species has been plagued by extensive incongruence among taxonomy, morphology, different molecular markers, and degree of reproductive isolation. Using a combination of museum, captive, and remnant wild snails, we obtained the first high-resolution nuclear genomic perspective of the evolutionary relationships and survival of fourteen Windward Island *Partula* species, totaling 93 specimens. We recovered a monophyletic Windward Island clade encompassing five species complexes. Our genomic results largely corroborated previous mitochondrial DNA survival estimates for Moorea and Tahiti, with all five species complexes having members surviving in captivity and/or as remnant wild populations, although the details vary in each case. We were particularly interested in the surviving wild populations: what has enabled them to survive? There is evidence for demographic factors contributing to differential survival of some Tahitian taxa, but the strongest predictor of survival is presence in refuge habitats that the predator either avoids or performs poorly in. The most important of these is the extensive montane cloud forest refuge on Tahiti, where lineages extirpated from Tahiti's valleys still survive. Species-specific valley micro-refuges also exist such as the Moorean Oponuhu Bay mangrove fern habitat for *Partula taeniata* and forest edge solar refuge habitats throughout the valleys of Tahiti for the high-albedo, white-shelled *Partula hyalina*. The survival of a representative subsample of Windward Islands *Partula* lineages (along with their *Samoana* species) is a pleasant, highly encouraging surprise and, assuming continued proactive conservation measures, it is possible to envisage their long-term survival on their home islands.

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## Conservation status of Hawaii's freshwater Lymnaeidae

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Freshwater ecosystems are among the most critically imperiled in the world, with nearly one in three species threatened with extinction. Accurate data regarding species identities, distributions, abundances, and range contractions are critical for developing best practices to manage aquatic resources and stem the tide of extinction. Freshwater snails native to Hawaii belong to two families: Neritidae (3 species) and Lymnaeidae (4 species). Because of their incredibly high diversity, Hawaiian land snails have attracted the most attention among the islands' malacofauna, yet the potential for extinctions among the freshwater snails may be higher. The taxonomic framework for the Hawaiian Lymnaeidae is more than 70 years old. Except for *Erinna newcombi*, which is protected under the US Endangered Species Act, few conservation assessments of lymnaeids in Hawaii have been done in the last century. Hubendick recognized five species of native lymnaeids in a single genus *Lymnaea*, and all were considered widespread on multiple islands, except for *Lymnaea (Erinna) newcombi*. Others subsequently recognized only two species in the genus *Erinna* and two in *Lymnaea*. A fifth species was recognized, but with uncertain affinities for either genus. To update the taxonomic framework necessary for developing effective conservation assessments we are undertaking surveys across the main Hawaiian Islands and analysing historical and recently sampled material in an integrative framework. Phylogenetic analysis of snails from four of the eight main islands indicates that there are at least six, and possibly as many as ten endemic species, including the only two species of sinistral lymnaeids in the world. There are also currently four non-native lymnaeids established in the islands. While the non-natives continue to spread, many of the native species occur in geographically restricted drainages, and in some cases consist of only a handful of individuals in a single population. Hawaii's endemic lymnaeids are found primarily in 'vertical wetlands' associated with waterfalls and seeps, which are highly ephemeral. Negative impacts to the water supply due to habitat modification or climate change, and the spread of invasive species are likely to extirpate these populations, resulting in the extinction of a diverse fauna that remains poorly understood.

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## Conservation on Lord Howe Island and Norfolk Island, Australia's hotspots of land snail diversity

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Lord Howe Island and Norfolk Island are small, isolated islands situated between Australia, New Zealand and New Caledonia, around 900 km apart. They have remarkably rich, narrowly endemic land snail faunas and host Australia's highest land snail diversity, with a combined ~130 unique species. However, these species have suffered considerable impacts from human habitation. Climate change is also an emerging threat to the fauna of both islands.

Unlike many oceanic islands, Lord Howe Island retains much of its original vegetation, and 70 % of the island is protected as a Permanent Park Preserve. Predation by introduced rodents has long been the biggest threat to the endemic land snails and has resulted in several extinctions as well as five species being listed as Endangered or Critically Endangered. The heaviest impact has been to species or subspecies restricted to the cloud forests of the southern mountains, which are currently listed as a Threatened Ecological Community as a result of climate change. After an island-wide rodent eradication in 2019, our surveys have so far indicated a slow trend towards recovery.

Norfolk island has been much more extensively cleared, with most of the endemic snails restricted to the ~15 % of the island protected as a National Park. Rodents and feral chickens are a significant threat and have led to the listing of five species as Critically Endangered. It is likely that three of these species are now extinct. The two remaining species are currently the subject of a conservation programme which includes *ex-situ* breeding, increased pest control, habitat enhancement and trials of exclusion fencing.

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## Effect of low dissolved oxygen on the survival of juvenile *Margaritifera margaritifera*: hypoxia tolerance ex situ

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The siltation of river substrates by fine sediments is one of the main factors for the global decline of the endangered freshwater pearl mussel (FPM) (*Margaritifera margaritifera*). Juvenile FPM detach from the gills of their salmon host and bury themselves to the river substrate where they live for several years. Suitable gravel substrate with functional water flow to replenish oxygen and food supplies for juvenile FPM is assumed to be crucial for their survival. Clogging of the gravel bed prevent the oxygen rich water to enter interstitial spaces. Our study tested the hypoxia tolerance of juvenile FPM in laboratory conditions. 9–10 month old juvenile FPM were exposed to high (control): 8.8–6.2 mgL<sup>-1</sup>, medium: 5.0–0.4 mgL<sup>-1</sup> and low (near anoxic): 1.3–0.04 mgL<sup>-1</sup> dissolved oxygen concentrations for 10 days at 19 °C. Each treatment had 18 juveniles in individual flask with oxygen concentration measurement spot. The survival of juveniles was determined by foot movement and/or valve adduction. The visual inspection was done through the flask wall with a magnifying glass. Juveniles that did not display movement in a 10-minute observation period were taken out of the flask and inspected with microscope on a petri dish. If the juvenile did not move on the petri dish it was determined dead. In high and medium oxygen treatments all juveniles survived and in low oxygen treatment none survived the 10-day experiment. Although some FPM individuals were able to resist hypoxia for several days, our results support the view that low (near anoxic) episodes are fatal to juvenile FPM, and that low substrate quality leading to anoxia may contribute to recruitment failure of FPM. The present results can be used to justify i) substrate restoration in mussel habitats where siltation has lowered substrate permeability and ii) structural restoration (stones and wooden structures) to increase hydrological variability and water pressure to enhance penetration of water into the bottom gravel. Mussel habitat substrate restoration and measuring of oxygen condition in these habitats should be further developed and added to the FPM monitoring programs.

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# **As the smoke cleared: Assessing the impacts of 2019-20 megafires on Australia's land snails**

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The 2019/2020 megafires in Australia were unprecedented in scale, duration, and intensity burning approximately 13 million hectares of forests and woodlands, including many National Parks. Critically, these fires did not only impact fire-adapted ecosystems, such as eucalypt forests, but swathes of habitat that rarely burn, such as rainforests. As the full impacts of these fires may not be fully understood for years to come, a first response aimed at prioritizing those species that were deemed to be particularly vulnerable because large parts of their distributions overlapped with the fire zone. Accordingly, hundreds of species of plants and animals, including 56 species of land snail, had been earmarked as of special concern. We performed post-fire surveys throughout south-eastern Australia to better understand the immediate impacts of the fires on native snails of concern. We found that these land snails suffered substantially in areas that were impacted by fires of high severity. These impacts caused several species to become endangered. However, land snails also revealed some resilience especially in areas affected by low severity fires. As a result, many species have fared better through the Black Summer of 2019/20 than predicted.

In the long term, however, the conservation needs of many species will require updated management strategies that address the challenges arising from an ever-increasing size of fires and an ever-diminishing interval between them. These are the conditions that Australia is predicted to face in a future that is defined by an escalating global trend of warming and drying.

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## Red Listing can protect UK Overseas Territories marine biodiversity

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Antarctic marine organisms are threatened by ocean warming, acidification and deoxygenation. Marine molluscs in particular are susceptible to these predicted climatic changes. They have adapted to this stable, cold environment through slowed metabolism and growth, reproducing through direct development, along with thin shells to expend less energy. With increasing temperatures that may surpass the tolerance of many Antarctic species, these high latitude organisms will be pushed poleward, decreasing their range and eventually leaving them with nowhere to go. It is predicted that Southern Ocean species face species turnover, local extinction, invasion of non-native species, and loss of species richness. To understand how climate change will impact these marine organisms, a set of marine molluscs were assessed for their extinction risk using the International Union for the Conservation of Nature Red List of Threatened Species criteria. The Red List is the most comprehensive and rigorous assessment tool for the extinction risk of species worldwide. To assess these species appropriately considering the threat of climate change, a traits matrix was created. This matrix included the species range and whether it occurs in a specialised habitat, dependence on interspecific interactions likely to be disrupted by climate change, dispersal capacity, and current or potential exposure to: temperatures surpassing tolerance, lower pH causing inability to accrete calcium carbonate, and hypoxic conditions. The matrix was used to conclude whether each individual species had a low or high vulnerability to climate change, depending on their respective traits. This information aided in assigning a threat category to each species. Red List status, whether Endangered or Least Concern, can inform management plans for conserving biodiversity and it is important that molluscs are represented in this effort.

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## Cave and spring snails: providing the evidence for effective conservation of groundwater-obligate snails.

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Even in relatively well-studied regions of the world, cave and spring snails (Hydrobiidae & Cochliopidae) are poorly known. For example, we have recently discovered two new species of subterranean snails in Comal Springs in the karst region of central Texas, a spring where intensive faunal studies have been conducted for decades. Most groundwater-obligate species are believed to have very narrow ranges, however, by recent surveys that include molecular characterization in spring and hyporheic habitats (~150 sites), we extended the known ranges of several snail species by 100 km or more and studies of their diversity and ecology are ongoing. While cave snails are harder to study, snails inhabiting surface springs are more vulnerable as the rapidly drying, permanent springs where these snails occur are often very small, <10 m<sup>2</sup>. In an ongoing study, we are surveying the macro and microinvertebrate fauna for ~100 springs, building a DNA barcoding library for the snails, and using these data to prioritize springs for conservation efforts. During these surveys (~75 springs so far) we have confirmed the continued presence of *Assiminea pecos*, *Pseudotryonia adamantina*, 3 of 5 *Tryonia* species, 3 of 4 nominal *Pyrgulopsis* species, and discovered two new species and 3 new populations of *Pyrgulopsis*.

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## Mollusk diversity patterns in the seven tropical Maar Lakes of San Pablo City, Laguna, Philippines

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Malacofaunal research in the lake ecosystems is limited in the Philippines. Also, prior to this study, there had been no malacofaunal study in the seven tropical maar lakes of San Pablo City, Laguna. To address this information gap, the molluscan diversity survey, and their ecological patterns in the maar lakes was conducted and determined. Across 158 sampling points randomly distributed among seven maar lakes, gastropods, and bivalves were collected by direct handpicking and sieving of sediments. Physico-chemical parameters such as water temperature, pH, conductivity, total dissolved solids (TDS), salinity, dissolved oxygen (DO), and water transparency were also measured. A total of 36 449 individuals belonging to 17 species (15 gastropods and 2 bivalves) from 11 families (Ampullariidae, Bulinidae, Lymnaeidae, Pachychilidae, Physidae, Planorbidae, Stenothyridae, Thiaridae, Viviparidae, Cyrenidae, and Unionidae) were sampled. The most abundant species was *Stenothyra* sp. ( $n=10,532$ ). Three invasive species, *Pomacea canaliculata* ( $n=753$ ), *Sinotaia quadrata* ( $n=1588$ ), and *Sinanodonta woodiana* ( $n=61$ ) were recorded. Species richness and abundance were highest in Lake Sampaloc (17 species,  $n=22076$ ). Diversity index and evenness were highest in Lake Bunot ( $H'=2.10$ ,  $J'=0.54$ ). Species accumulation curves demonstrated an  $\alpha$ -dominated community, and efficient sampling marked by a high completeness ratio ( $CR=1.00$ ). Canonical correspondence analysis demonstrated that TDS and DO were dominant factors influencing species distribution. Generalized linear mixed model revealed that the most significant predictor for species richness was salinity while abundance was temperature. Model averaging indicated water transparency was the most parsimonious model for species richness while the combination of DO and salinity for abundance. This study presented the rich malacofaunal diversity in Philippine maar lakes which requires science-based management and conservation amidst increasing anthropogenic threats.

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## How scientific amnesia threatens biodiversity – a case involving aquatic gastropods

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Measures taken against pests and invasive species are likely to affect non-target species. Therefore, prior to the application of any such measures, possible undesired effects must be evaluated. This generally requires substantial knowledge of the biology of non-target species. Lack of such knowledge may lead to misjudgments with catastrophic consequences for the ecosystems to which the measures are applied. Here I present a case in which pertinent biological knowledge is available but forgotten.

In the late 20<sup>th</sup> century, south-American apple snails (*Pomacea* spp.; Ampullariidae, Caenogastropoda) were introduced intentionally into various tropical and subtropical regions, where they developed into devastating invasive pests especially in rice fields. One reason for the invasive success of these aquatic herbivores is their ability to switch to pedal surface feeding once they have eradicated the natural vegetation of the invaded habitat. Pedal surface feeding, the collection of particles and substances from the water surface by currents generated by the sole of the foot, today is widely considered a specific behaviour that evolved in the Ampullariidae. Maybe unsurprisingly, possibilities of tackling invasive apple snails selectively by delivering molluscicides via the surface currently are being discussed. What has disappeared from view is the fact that pedal surface collection actually is common among aquatic snails. The behaviour, termed ‘plankton fishing’ by malacologists a century ago, will be demonstrated in one of the many species that would be turned into collateral damage if we attempted to attack invasive apple snails ‘selectively’ through the water surface.

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## What digitized natural history collections data can (and can't) tell us about US freshwater mussel diversity, distribution, and decline

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Voucher specimens deposited in natural history collections are a primary data source for many aspects of US freshwater mussel research and conservation. Digitization of natural history collections data has dramatically improved the accessibility and impact of these specimens and their associated records. With the rapid increase in digitized US freshwater mussel records it is difficult to appreciate the full scope of this data resource, let alone its nuanced strengths and weaknesses. We set out to make an inventory of digitized US freshwater mussel collections data and explore the spatial, temporal, and taxonomic distribution of those records. Using over 400 000 specimen records from 46 natural history collections we observed numerous patterns, strengths, and weaknesses. For example, starting in the early aughts, we observed a steady decline in the number of freshwater mussel records per year, which also translated into an overall decrease in the number of geographic areas and species represented each year. These digitized records are unevenly distributed across US freshwater mussel biodiversity and the watersheds in which they inhabit. Identification of these strengths and weakness are important to understanding how to better use and improve natural history collections data.

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## Meeting of generations: how can we use the CBD 2030 targets to mainstream Mollusc Conservation?

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By the WCM 2022 the new Post-2020 Convention of Biological Diversity targets will be better defined. Most governments will sign-up to the CBD framework to meet these targets. At present, there is still a commitment to stop species extinctions, restore ecosystems and start to implement more species conservation plans although finalisation of CBD plans does not occur until October 2022. This paper will reflect on ways in which we can use the new framework to lobby governments to implement conservation actions for Molluscs.

The IUCN Red List is one tool used to monitor the progress towards the CBD target of reducing species extinctions. With over 9000 assessments and some reassessments we can already see that some threats like increased frequency of drought and increasing forest loss will cause an accelerating rate of mollusc extinctions unless timely action is taken to reduce the impact of the threats.

Europe has the comprehensive regional dataset for Molluscs and the new EU project PULSE 2021-2023, will reassess over 2000 species and create the first Red List Index for Molluscs. The last review of the European Freshwater Molluscs showed that this was one of the most imperiled group of animals in Europe with 46% threatened species. This has lead to an increase in field surveys to look at Data Deficient species and establish whether species threatened by increased frequency of droughts are still extant. However, it is becoming clear that legal protection may not be effective. For example, the listing on Annexes for the EU Habitats Directive for species like *Microcondylea bonelli* and *Pseudunio auricularia* has not been effective as the wrong threats were used for original listing in the 1980s.

Moving forward new techniques such as eDNA do provide tools that maybe used to monitor freshwater species living at low densities and establish whether they are still extant in freshwater ecosystems. Refining multispecies action plans may become critical. As scientists and conservationists, we need to look at how we can work with other partners to ensure that information is accessible, habitats are conserved, work with water management agencies to ensure freshwater ecosystems don't suffer from over-abstraction and to reduce pollution of water and also look at the longer term threats posed by climate change and invasive alien species.

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## Land molluscs conservation: what Atlantic islands are teaching us

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Oceanic islands are renowned for their high proportions of endemic fauna and flora resulting from isolation and associated accelerated speciation and adaptive radiation. Unfortunately, these biotas have also suffered most from direct human-induced extinction, a product of habitat destruction and introduced competitors and predators, or even a fast-changing climate.

Among these faunas and floras, land molluscs show the highest levels of endemism and extinction rates. Of all known molluscan extinctions, 70% have occurred on oceanic islands, and in particular cases, extinction rates may be greater than 75%. Moreover, the scale of species listed by the International Union for Conservation of Nature as endangered, extirpated or extinct is undoubtedly underestimated due to incomplete coverage.

In addition, most island endemics species have small geographic ranges, some under 100 square meters, making them highly vulnerable to rapid change and disturbance. Therefore, the continuing human-induced activities introduce a high level of disruption and constitute a significant extinction vector, adding substantial pressure to the species' survival.

The current conservation state of the endangered land mollusc faunas from oceanic islands calls for immediate action to halt the species loss. As a direct answer to this, a number of multistakeholder's conservation projects addressing threatened endemic land molluscs and their habitats in oceanic islands were implemented in the last decade. Case studies from the archipelagos of Madeira, Canaries, Azores, São Tomé e Príncipe and Bermuda will be presented. They cover the four fundamental steps for an effective species conservation program: 1) elaboration of reliable baseline information on species distribution and ecology; 2) managing threats, mainly through eradication and elimination of the key alien invasive species as a vital step; 3) restoring island ecosystems, conservation translocations and managing recovery process, including monitoring success; 4) engaging island communities in conservation efforts through social marketing and information sharing.

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## Hawaiian land snail conservation efforts: from rediscovering species to manning the lifeboats

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From legends to lei (traditional Polynesian garland), and theories of evolution, Hawaiian land snails, known as “Hawaii’s jewels of the forest”, have been important components of Hawaiian culture and lore and instrumental in the development of our understanding of speciation and island biogeography. Unfortunately, habitat destruction, depredation by introduced predators, and now climate change have reduced the highly endemic fauna from at least 759 species to about 300. Of the remaining species, about 100 are estimated to go extinct within the next decade. To prevent the imminent extinction of species facing severe threats, Bishop Museum (BPBM) and the Hawaii State Department of Land and Natural Resources Snail Extinction Prevention Program (SEPP) independently developed captive rearing programs more than 30 years ago, each initially protecting different species. In 2020, BPBM, SEPP and the Honolulu Zoo established a collaborative network of captive propagation facilities to create redundant populations of species with the aim to reduce the risk of extinction, share captive rearing techniques, and increase capacity for land snail conservation. Collectively, these programs currently rear 48 species and at the end of 2020, more than 6000 snails from 10 species from the network were re-introduced into protected areas in the wild. Despite this initial success, captive rearing remains a stop-gap measure and is not a sustainable, long-term solution to the extinction crisis. In concert with the captive rearing programs, multiple integrated actions need to simultaneously occur to ensure the survival of the remaining snails. These include the development of research aimed at answering questions needed to carry out effective conservation, restoring native ecosystems, characterizing life history and ecological requirements for threatened species, and advancing techniques to mitigate and reduce threats (e.g. impacts of invasive species, disease, climate change). Immediately, efforts to survey for persisting species and populations must be scaled up before it is too late, and small habitat segments across islands, must be protected with predator control and exclusion so that snails can both remain and be returned to the landscape. All of these actions require a comprehensive and updated taxonomic framework upon which all other conservation decisions rely.

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## State and fate of Borneo's freshwater mussel diversity

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Biodiversity is being lost faster in tropical freshwater habitats than anywhere else. Effective conservation requires knowledge on the spatial and temporal patterns of biodiversity and threats. However, for freshwater habitats in Southeast Asia – one of the most biodiverse regions of the world – these data are currently not available. Data on freshwater mussels (Bivalvia: Unionida) are particularly scarce. Mussels are one of the most endangered animal groups and provide crucial ecosystem services, from increasing water quality and biodiversity to providing a source of food to humans. The mussel fauna of Borneo is largely unstudied, and most of the 19 species, of which at least 14 are endemic to the island, have not been found for at least 50–100 years. Over the past five years, we surveyed freshwater mussels at almost 200 sites across 30 river basins in northern Borneo, spanning the Malaysian States of Sarawak and Sabah, and Brunei (study area of approximately 150 000 km<sup>2</sup>). Our data suggest that over the past five decades, all but one native mussel species have become very rare and some potentially locally extirpated, largely owing to wide-scale deforestation and land use change. *Rectidens sumatrensis*, also native to Peninsular Malaysia, Sumatra and Java, appears to be the only native mussel species from this region sustaining stable population sizes. Endemic species, including two new species within a new genus (*Khairuloconcha lunbawangorum* and *K. sahanae*), are rare, restricted to pristine forest streams and severely threatened by ongoing habitat destruction. The strong decline of endemic mussels concurred with the spread of the non-native *Sinanodonta* cf. *woodiana*, which is now the most widespread freshwater mussel in northern Borneo. Unfortunately, species distribution models under future climate change and land use scenarios predict a continuation of these trends, as by 2050, habitat suitable for native and invasive mussels will further decrease by 20 % and increase by 30 %, respectively. Other river basins across Borneo should urgently be surveyed to identify any remaining populations of freshwater mussels and put under protection. This should include novel surveying methods, including environmental DNA, which may be particularly useful in surveying rivers that are difficult or too dangerous to sample using traditional hand-sampling.

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## **Symposium**

# **American Malacological Society President's Symposium: Molluscan Conservation**

## **Part II**

### **Abstracts of poster presentations**

## Rescuing the critically endangered land snails species from the Desertas Islands (Madeira: Portugal) from extinction

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Four endemic land snails have been rediscovered in the last 12 years after decades without a live record. They share similar threats, namely restricted location (under 100 m<sup>2</sup>), single populations (<50), predation by house mouse (*Mus musculus*), habitat loss and degradation due to the grazing goats, making them at the very brink of extinction! They all occur in a single location on the same island, Deserta Grande, a steep and dry 10 km<sup>2</sup> island and the largest of three government-owned inhabited islands (Desertas islands, Madeira, Portugal).

Funded by Re:wild, the IUCN Mid-Atlantic Islands Invertebrates Specialist Group launched a species rescuing program in May 2021 to save *Atlantica calathoides*, *Discula lyelliana*, *Geomitra coronula* and *Geomitra grabhami*. A multistep plan was implemented by the Institute of Forests and Nature Conservation (Madeira, Portugal) and international partners (Chester Zoo, Bristol Zoo Gardens and Mossy Earth) for the conservation actions (*in-situ* and *ex-situ*), conservation planning for recovery, local community engagement and social behaviour change.

The rescue of the founder specimens in May and November 2021 was followed by the implementation of a multispecies captive breeding rescue program, supported by a species monitoring scheme and a threat control program in the field, engaging stakeholders and resources on the Deserta Grande. With the support of the IUCN Species Conservation Planning Specialist Group, a species conservation strategy is due by November/22, which will define the actions that provide a sustainable future for the species for the next five years.

The breeding program at Chester Zoo and Bristol Zoo Gardens have already produced significant breakthroughs – successfully breeding three of the four target species of snail (*A. calathoides*, *D. lyelliana* and *G. grabhami*) for the first time in human care. These safety-net populations are fundamental as a precautionary measure to ensure the long-term viability of these species with a high risk of extinction. Furthermore, these species shall reinforce the current populations at Deserta Grande upon completion of the habitat restoration and control of the invasive species that have devastated the islands. Alternatively, they will be reintroduced to the southern island of Bugio, which is free of threats.

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## Production of *Mytilus galloprovincialis* spat from cryopreserved larvae of successive generations.

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Cryopreservation can provide a sustainable and indefinitely source of shellfish larvae to improve aquaculture production and avoid the dependance of collection of natural shellfish spat, which natural recruitment fluctuate throughout seasons and it has been alarmingly low worldwide during last years. It can also enable the inclusion of selective breeding programs and help the conservation effort of endangered aquatic species by enhancing controlled spreading in natural environments. The present work aimed for studying the capacity of cryopreservation of producing *Mytilus galloprovincialis* adults from cryopreserved D-larvae. Moreover, potential long-term effects of cryopreservation through progenies were evaluated. Mediterranean mussel adults were collected from natural environment during natural spawning season and subjected to spawn to collect 72 h-old D-larvae, which were cryopreserved in 10 % Ethylene-Glycol + 0.4 M Trehalose in Filtered Sea Water (FSW), using a cooling rate at -1 °C / min and a water bath at 35 °C for thawing. Then, larvae were cultivated at 18 ± 1 °C into 150 L tanks with constant aeration and feeding, consisting of a mixture of microalgae according with the larval age. After 15–22 days under these conditions, the developed pediveliger larvae were transferred to settlement drums. The resulting mussel juveniles were transported to traditional rafts to study their development cultivated in natural environment. Two years later, mussels were collected for spawning inductions during the natural spawning season and obtain the second generation of larvae, cryopreserved and cultured until settlement following the experimental design described for the first generation of larvae. The cryopreservation of Mediterranean mussel 72 h-old D-larvae yielded 75 % of recovery two days after thawing. At settlement, 5.26 % of cryopreserved F1 larvae survived and over 70 % settled. F2 cryopreservation produced 0.15 % of spat and settlement varied from 35 to 50 %. The delay of shell size showed on cryopreserved larvae declined throughout larval rearing without significant differences with controls from settlement point ( $p > 0.05$ ). The present research showed that it is possible to obtain adult mussels from cryopreserved larvae and cryopreservation does not compromise the quality of following progenies, neither cryopreservation nor post-thawing development of them.

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**Symposium**

**Continental Molluscs Facing  
Environmental Changes**

**Part I**

**Abstracts of oral presentations**



## The effects of soil anthropisation on the composition and organisation of malacological assemblages in oases: the case of the Arabian peninsula

**H. Djerbi<sup>1</sup>, L. Purdue<sup>1</sup>, S. Costa<sup>1</sup> & A. Carré<sup>1</sup>**

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Oases are anthropogenic landscapes which have been occupied and exploited for millennia in the Arabic Peninsula. On the long-term, these landscapes have been subject to both environmental variations and anthropic activities. In order to understand the nature of these changes, malacofauna can provide very useful insights at a temporal and spatial scale. In the framework of recent projects dedicated to the integrated study of oasian landscapes in Arabia (ArcAgr-AU, Al Ula oasis, Saudi Arabia, Dir. L. Purdue and ANROASIWAT, oases of Masafi, Dhayah, UAE and oasis of Rustaq, Oman, Dir. L. Purdue), we developed a systemic field and laboratory approach to study malacofauna assemblages to help reconstruct landscape evolution, past agriculture and measure the impact of water and soil management on malacological biocenoses.

To do so, we created a reference collection in various environments in currently cultivated oases in order to identify specific ecological groups. Key to interpret ancient landscapes, our observations were applied to the study of stratigraphic sequences located in the above-mentioned oases, which have been occupied since the last 4 millennia. Our data were confronted to sedimentary and pedological analyses conducted in parallel.

Our results highlight the impact of topography, sedimentary processes, water salinity and organic matter content on the malacological assemblages and reveal a differential preservation of this proxy in connection with human managements (irrigation, amendment, reorganisation of land parcels, abandonment).

The anthropisation of these ecosystems favours the spatio-temporal dispersion of species, randomly or not, as a result human practices (agriculture, circulation). Moreover, agricultural communities create edaphic conditions, which are suitable to the maintenance and development of malacological populations. The composition of the assemblages is linked to the structuration and organisation of the oases studied. This phenomenon is highlighted by the virtual absence of malacological populations outside of the oases.

Oases have acted as refuge and transit zones for certain species, such as *Vallonia pulchella*, now considered absent from the Arabian Peninsula, but currently known in limithrophic regions (Egypt, Israël, Iran...). Thanks to local humid conditions, insular or very discontinuous distributions have developed in oases.

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## Unravelling the “pulse of rivers” on multidecadal to centennial time scales – A multi-proxy approach for freshwater pearl mussels to reconstruct environmental conditions in stream water

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Non-stationarity as a (new) property of climate causes the detection of shifts in environmental systems to be increasingly challenging. The largely documented acceleration of the hydrologic cycle is a telling example in this respect. Given the short or incomplete, often low-quality monitoring data sets of river stream flow, there is a pressing need for new reliable, high-resolution and long-term proxy archives that can shed new light onto changes in the hydraulic regime of catchments at multi-decadal to centennial time-scales. Such highly innovative sources of information shall eventually contribute to place recent flood events of exceptionally high magnitudes into more robust historical and statistical contexts.

Here we discuss the potential for the long-lived freshwater pearl mussel (*Margaritifera margaritifera*) to serve as a multiproxy archive of environmental conditions in stream water. We present (sub-) seasonally resolved stable isotope ( $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ ) chronologies (over 3000 isotope data) of three shells from the Our River (Luxembourg) extending back to the 1930s. These time-series reveal distinct seasonal and inter-annual/decadal cycles in agreement with the stream water isotope signature. Supported by tank experiments, we have assessed the sensitivity of shell microstructural properties (nacre table thickness) and annual growth rates of the shells to temperature and food availability. Mg, Sr, Ba and Mn-to-Ca values fluctuated synchronously and in tandem with seasonal variations in river discharge, terrestrial influx, primary production and oxygenation of the water body.

As demonstrated by our study, the combined analysis of growth patterns, geochemical and microstructural properties of the *M. margaritifera* shells provides a unique means towards gaining a better understanding of the changing “pulse of rivers” over the last 100 to 200 years.

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# **Paleoenvironmental reconstructions based on molluscan assemblages in archaeological contexts: an overview of possibilities at various scales of time and space.**

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Over the last ten years, in the northern part of France, extensive malacological data have been collected in archaeological sites dating from the last twelve millennia. These paleoenvironmental records come from archaeological features, such as pits, ditches and fountains, but also from stratigraphic sequences with intermittent human influence.

At the micro-local scale, the malacological analysis of an archaeological feature enables to understand its functioning. On a local scale, a malacological analysis in sedimentary sequences provides an insight into the environmental characteristics of a site and their evolution over time. Finally, by multiplying observation points and observing a common environmental trajectory within a common chronological framework, malacology can provide information on the dynamics of palaeoenvironments on a regional scale.

These different scales of palaeoenvironmental reconstruction will be approached through various examples: a Gallo-Roman fountain whose malacological assemblages inform us about the abandonment dynamics of an aristocratic site, contemporary Neolithic sites with different functional vocations, and sites in the same catchment area whose similar environmental trajectories allow us to understand regional trends.

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## Disconcordant patterns resulting from concordant processes: differentiation and speciation of rock-dwelling snail species in the Southern Alps

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In order to better understand the origin of the high diversity and endemism in the Southern Alps in Europe, we have adopted a comparative biogeographical and phylogeographical approach. We examined the phylogeny and population structure of the rock-dwelling snail group *Chilostoma* (*Cingulifera*) in the Southern Alps and compared it with distribution and differentiation patterns of other rock-dwelling snail groups. We generated genomic ddRAD data and mitochondrial sequences of 104 *Cingulifera* specimens from 28 populations and 14 other Ariantinae. Until recently, about 30 *Cingulifera* taxa were classified as subspecies of a single polytypic species. The phylogenetic and population genetic analyses of the ddRAD data and mitochondrial sequences revealed that *Cingulifera* is differentiated into three species in the Southern Alps. Tests that showed that the differentiation of these taxa cannot be explained by isolation by distance corroborated their species status. The distribution and differentiation patterns of the investigated rock-dwelling snail groups strongly differed. In taxa with limited active dispersal abilities like snails, passive dispersal is more important for the colonization of uninhabited areas, the evolution of population structure, and species differentiation than active dispersal. Passive transport of land snails across watersheds is probably mainly by birds. Successful long-distance dispersal of living snails to a suitable habitat is a rare and random event. Long-distance dispersal is the underlying common mechanism that resulted in the observed species specific differentiation and phylogeographic patterns of species with limited active dispersal ability.

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## Forest mollusks – the key to Holocene diversity in Central Europe

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The development of Central European mollusk communities in the Holocene shows local specifics. While forest assemblages have never fully developed in areas inhabited by early farmers, forest fauna has been fully developed in areas immediately adjacent but unsuitable for agricultural use. The rapid development of forest communities started mainly after the cold event 8200 BC. In the Late Holocene, forest assemblages are usually gradually declining. An extreme example is the region of sandstone rock cities, where up to 11 species of clausilids were found synthopically in the Holocene forest optimum. A radical transformation of this temporal ecological equilibrium began in the third millennium BC. Over the next millennia, the species-rich canopy forest mollusc assemblages almost completely disappeared, together with calciphilous rock dwellers. At present, there has been a complete degradation of forest communities due to the decalcification of the landscape. Forest species ranges in Central Europe fluctuated slightly during the Holocene as shows the data from the Carpathians and Alps, reflecting minor climate changes. Overall, forest species are the most important component of the Central European mollusk fauna during the entire Holocene.

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## Locating refugia and dating the arrivals to guess the speed of postglacial expansions

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Fossil land snails are used to reconstruct the development of Holocene ecosystems, and their advantage is that the species present at the excavated site reflect very local conditions. But how quickly are they able to respond to climatic changes by colonizing new areas and how much did their distribution ranges lag behind the available habitats? These questions had in the past inspired impactful research of temperate trees and need to be asked also in the case of land snails. The postglacial colonization of Europe could provide an insight into their ability to shift their distribution ranges and community assembly, but estimating the rates of spread is an enterprise facing multiple obstacles. The location of glacial refugia is usually unknown in widespread species, and fossil data from refugial areas are missing. Tracing the advance of the postglacial expansion in fossil record requires continued integration of datasets between regions, but also extensive revisions of the earliest Holocene records in each region. Correlating genetic data with time is also challenging. Unexplored remain possible effects of interspecific interactions. In this contribution, we will share the experience regarding these topics from our latest efforts to trace glacial refugia and earliest postglacial occurrences in Czechia in three broadly distributed species.

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## Palaeobiodiversity and distribution of Quaternary European continental snails in response to climatic cyclicity

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Reconstructing Quaternary palaeoenvironmental changes from continental mollusc assemblages tells us much about who moves and when. Indeed, climatic cyclicity has strongly influenced the fluctuation of species distribution, particularly in northern Europe, a region severely affected by ice cover movements. In northern territories, the distribution of molluscs shows a dramatic decrease in diversity during Glacial times, leaving only a few tolerant species able to withstand low temperatures. Conversely, Interglacial periods are characterized by diverse faunas, including the development of shade-loving taxa, a category whose occurrence is limited to temperate periods. Interglacials show important differences in diversity due to the occurrence of more forest species during some mild periods of the Middle Pleistocene.

The development of Quaternary malacological studies provide valuable datasets for identifying the location of refugia during pleniglacial periods and recolonization pathways after phases of rising temperature. The malacological series from northwestern European demonstrate that thermophilous colonizers originate mainly from the Iberian Peninsula and central Europe. Mediterranean species play a secondary role and only reach the northern areas during the optimal phases of some Pleistocene Interglacials.

In north-west Europe, Pleistocene forest faunas are richer than the Holocene one. Indeed, the less humid climatic conditions during the Holocene prevented a long-distance malacological recolonization from Central Europe. The lower diversity of western forest fauna compared to central European areas that is recorded since the beginning of the Holocene is a consequence of the position of glacial refuges in the Carpathian Mountains.

Quaternary data also provide an assessment of species extinction rates. They allow a direct comparison between past interglacial periods, where environmental changes were exclusively driven by climatic parameters and modern habitats strongly modified by human impact. In northern France, a well-studied region, 11 species become extinct between 600 000 and 200 000 years ago, all of them being thermophilous mollusc requiring interglacial climatic conditions. Climatic and anthropogenic factors cause range shifts, but over the last two centuries the rate of changes is 18 times greater than during past interglacials and affects mainly xerophilous and aquatic molluscs rather than forest snails.

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## The malacofauna of the degraded cupola spring-fed fen in the Romincka Forest, history and present.

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The contemporary and fossil malacofauna of a cupola spring-fed fen in the Romincka Forest (NE Poland) were analysed. Detailed sediments' analysis documented ca. 11750 years of this fen history with the continuous calcium carbonate precipitation, lasting almost to the nowadays. In the nineteenth century, the economic use of forests in these areas began, and it was accompanied by extensive meliorations of wetlands. Melioration of the PR cupola surroundings took place probably at the turn of the 19<sup>th</sup> and 20<sup>th</sup> centuries, and had a direct impact on the functioning of the fen. The decreased groundwater level terminated supply of the water to the fen surface. Although the recent physicochemical parameters of groundwater are theoretically favourable for the existence of the extremely rich fen ecosystem with no calcium carbonate precipitation, i.e. peat-forming fen, the disturbed hydrological conditions and the desiccated surface of the cupola resulted in the absence of vegetation typical of fens. The cupola is overgrown by eutrophic reed and tall sedge vegetation, typical of degraded spring mires in Poland. The environmental changes had a direct influence on the mollusc assemblage. Among the 25 land snails species occurring at the top of the cupola, only two: *Euconulus alderi* and *Nesovitrea petronella* can be considered typical to the treeless fen. Most species present at this site are common hygrophilous or very hygrophilous species. Unexpectedly, in this open site, few strictly forest species were found. On the contrary, subfossil mollusc fauna comprises highly diversified mollusc assemblages. During the early and middle Holocene aquatic species were common, including *Galba truncatula* and *Radix labiata*, indicating presence of the permanent small water pools on the fen surface. Significant transformation in the ecosystem was observed ca. 4700 cal years BP when concurrently with shifts in geochemical properties of sediments and changes in plant macrofossils, mollusc assemblage became dominated by land species. Despite the low numbers of shells preserved in the sediments, mollusc assemblages occurring in PR sediments are good palaeoenvironmental indicators confirming changes in hydrological conditions on the fen and environmental changes in the area.

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## Native mussels between parasites and invaders under the pressure of water resource management: the case study of Lake Maggiore

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Lake Maggiore and its River Ticino outlet is one of the largest reserves of freshwater and biodiversity, shared between Italy and Switzerland, threatened by user conflicts that make its management complex since the 1950s. The Italy-Switzerland Interreg Cooperation Parchi Verbano Ticino Project aims to assess the threshold limits beyond which anthropic use conflicts with the conservation of biodiversity in order to propose a sustainable water management strategy. The project foresees the use of freshwater mussels as indicators of the impacts of current management based on their extreme susceptibility to habitat changes. As native mussels are undergoing a drastic decline in the last decade, the project includes both the evaluation of the main threats and the use of mussels as potential warning sentinels to indicate the reaching of the conflict thresholds. As a first step we compared the current status of populations with previous data to address the next efforts towards effectively documented threats rather than a wide range of putative threats. Major drivers of native populations decline are invasive bivalves (especially *Corbicula fluminea*) and trematode parasites. Both of these factors are expected to be correlated with changes in the lake's water level. In fact: i) in an unstable habitat *C. fluminea* benefits from its greater capacity for recolonization; ii) the impact of parasites is promoted by all the stressors connected with habitat changes. Since the dominant species, *Unio elongatulus*, is heavily parasitized, we used it as a case study during the "Unionid parasites" training school of the COST Action CONFREMUS. By comparing different study methods, we highlighted that only by histological techniques the parasites' early stages detectability can be improved. Improved detectability meant an increase of the estimated prevalence of the parasite in the target population to over 80%, highlighting a previous underestimation of this impact factor. Hence the need to re-evaluate the role of habitat variations induced by current water management for the development and maintenance of this unusually high parasitosis.

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## Mussel memory: History lessons from freshwater bivalves

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Freshwater mussels are a diverse taxon found across every continent except Antarctica, and act as ecosystem engineers, contributing to water filtration, habitat creation and nutrient cycling. However, these molluscs are increasingly under threat from a suite of anthropogenic pressures including pollution, land-use change, and waterway alteration through damming and canalisation. There is little information on long-term trajectories for mussel populations, and while extinctions and local extirpations may be reported, changes in individual fitness and population stability are largely undocumented. Tracking these changes could provide an early warning of sublethal changes preceding a population crash or extirpation; monitoring is also crucial to improving understanding of the causes and impacts of mussel declines, and informing conservation management and decision-making. My research examines historical data over a range of timescales, from 20<sup>th</sup>-century historical surveys and to Bronze Age archaeological evidence. I compare this evidence with present-day mussel populations to investigate changes in individual size and growth rate, as well as population density, all of which are predictors of the contributions of mussels to ecosystem functioning. In this talk I will discuss my research documenting and investigating decline and changing population dynamics in freshwater mussels over varying timescales of human activity, and discuss the potential causes as well as implications for ecosystem functioning and services.

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## Sophie's choice – which population to preserve under a scenario of global change and extinction

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In response to climate change and expected shifts in temperature and precipitation patterns, some species might change their ecological niches and distribution. However, this can be a challenge for aquatic species with reduced dispersal mechanisms or living in low connectivity environments. The endangered freshwater mussel *Unio tumidiformis* is restricted to temporary streams in the southwestern Iberian Peninsula, a region prone to water scarcity and overall increased temperature due to climate change. This species requires fish belonging to the genus *Squalius* as hosts for their glochidia, to successfully complete its life cycle. Therefore, it is important to understand how climate change might affect this already endangered species. We used Single Nucleotide Polymorphisms (SNPs) to assess the population structure of *U. tumidiformis*. A total of 60 specimens from 15 locations corresponding to 3 river basins (Guadiana, Mira and Sado) were sampled. Additionally, we modelled, for this century, the distribution of *U. tumidiformis* and its potential hosts (genus *Squalius*) using the new socio-economic scenarios SSP245 and SSP585 available from the worldclim datasets climate projections. Our results indicate that genetic diversity is dependent on basin size and show a very strong population structure, with extreme differentiation between populations, as indicated by FST values of 0.27 to 0.71. This suggests that there is no recent gene flux between populations. Even within the same sub-basin or river some structure is already detectable, suggesting that seasonal water restriction is an important factor limiting the dispersion of the mussel. Results from the climate models show a reduction of up to 99 % in adequate habitat for *U. tumidiformis* already visible in the first temporal scenario available (2021–2024), and a concomitant 19 % for *Squalius* sp. The combination of extreme isolation and foreseeable elimination of climatically adequate habitat in its current distribution area poses a serious conservation challenge. The survival of the species may depend on translocations towards outside its current distribution, active *ex-situ* conservation programs and engineered habitats that may hold water during extreme droughts. Even so, it seems evident that not all populations can be tackled through this strategy, leading to difficult decisions on which one should be preserved.

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# **Effects of climate variables on phenotypes, phylogeographic pattern and distribution of the strawberry snail *Trochulus striolatus* (Gastropoda: Hygromiidae)**

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Land snails are perfect to study interactions between climate variables and phenotypic plasticity because they are ectothermal organisms with limited dispersal abilities. Thus, they can be a good model to better understand mechanisms responsible for morphological variation. Genetic evidence suggests that the strawberry snail *Trochulus striolatus* has a monophyletic origin, unlike many other species of this genus. However, its shells show an enormous variation in size and shape, manifested by the recognition of subspecies by some authors. Comprehensive morphological analyses revealed that its ultimate size is mostly a response to prevailing local environmental and/or climate variables, and therefore does not justify the subspecies delimitation. We recognized that the synergetic interactions between seasonality, temperature and moisture availability is responsible for the size variation. We also established the genetic variation of *T. striolatus* based on microsatellite sequences. The analyses showed a clear west-east gradient in the differentiation of microsatellites from Ireland through Great Britain and Western Europe to Central Europe. There is an evident separation between the populations inhabiting the European continent and the British Isles, whose settlement and isolation have led to the genetic diversity of *T. striolatus*. The Canadian specimens were most likely introduced from these islands. However, the isolation of particular populations is not complete, because in some regions there are individuals assigned to genetic clusters typical of other regions. The identified clusters do not coincide with the morphologically distinguished subspecies of *T. striolatus*. Moreover, using bioclimatic data we modelled the present and future distribution of *T. striolatus*. The studies revealed that favourable bioclimatic conditions for this species are present in almost the whole British Isles, north-western part of France and the foreland of the Alps. Assuming future climatic scenario for 2040, we noticed an increasing probability of finding this species in the regions already occupied and an expansion towards the east of Europe. This thermophilous and moisture-loving species with its relative tolerance to habitat composition may benefit in times of environmental change.

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## Freshwater mussels as a flood early warning system

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Future climate scenarios predict an increase of extreme events. Thus, understanding how floods impact freshwater ecosystems and their organisms is important for their conservation. Changes in freshwater mussels' behaviour can be used as an early indicator of environmental disturbances. However, studies that focus on the effect of physical disturbances on freshwater ecosystems related to climate change are lacking. In this sense, the first aim of this work was to perform laboratory experiments in artificial flumes to evaluate the eligibility of mussels' behaviour as a biomonitoring tool used in real-time remote systems. To this end, we used the valvometric technique (that exploits the Hall sensor) to measure the mussels' valve gaping behaviour when subjected to different hydrological and sediment transport conditions mimicking the onset of floods. Freshwater mussels promptly reacted to extreme discharge conditions with sediment transport by increasing valve gaping frequencies, shifting their behaviour. After this validation in laboratory conditions, the second objective was to test their valvometric behaviour in the field to improve the reliability of the data and standardize the use of this methodology for its practical application. For this purpose, experiments were performed in a natural river where we built cages and attached the mussels. The preliminary results of this field work confirmed that mussels sharply increase their valve gaping in response to extreme conditions promoted by natural floods. Both laboratory and field experiments suggest that mussels' behaviour is a suitable indicator of the change of hydrological conditions and can be used as biomonitoring tool in biological early warning system (BEWS).

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## What Quaternary gastropods tell us about the effect of climate change on Canary ecosystems

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Quaternary gastropod faunas on Fuerteventura have been studied in order to derive information about the temporal resolution and genesis of dune sequences on the Eastern Canary Islands as well as information about palaeoclimatic and palaeoenvironmental conditions related to glacial-interglacial cycles for the study area. Our results show significant shifts in species compositions across the past 400 thousand years. We have identified certain species whose occurrence on Fuerteventura was limited to specific periods in time and which can therefore serve as fossil guide species in the sense of biostratigraphic markers. Similar ecological demands of different species communities across time indicate, that the above-described faunal shifts were caused by short-term stress events rather than long-term changes in local climatic conditions. In this context, stable oxygen isotope signals of shells of the genus *Theba* show a first trend that faunal shifts may be linked to sea-level rises (transitions from cold to warm seasons) which in turn may have been associated with indirect environmental changes (e. g., hot winds). Furthermore, stable carbon isotope compositions of the snail shells show that reddish dust layers and volcanic ash layers in the sediment sequences correlate with substrate-related increases in soil moisture and were associated to increases in biodiversity within the gastropods. We suspect that preserving the top layer of dust on Fuerteventura's modern surface could significantly prevent species extinction on the island.

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## Cost of living: Morphometry reveals patterns of clinal variations in US freshwater mussels (family Unionidae)

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Freshwater mussels show high levels of intraspecific shell variation, which for many species has led to a proliferation of synonymous nomina, hampering identification and conservation efforts of the highly diverse and currently endangered North American fauna. Ortmann's (1920) Law of Stream Position posits that some of this variation in shell shape is driven by habitat conditions along rivers: individuals in large rivers produce more inflated shells than conspecifics in smaller tributaries. Our examination of 931 specimens (10 species; three tribes) from the 19<sup>th</sup> to the early 20<sup>th</sup> century in the Smithsonian collection affirms Ortmann's Law. However, we discovered an interplay between inflation and another anchoring trait – shell sculpture. Sculptured and unsculptured species responded differently to the degree of clinal inflation. Smooth-shell species show strongly positive inflation responses, exhibiting a significant increase in inflation from small tributaries to large rivers, in contrast to constitutively sculptured species, which show weakly positive (*Cyprogenia stegaria*) to negative (*Plethobasus cooperianus*) clinal inflation responses. *Cyclonaias pustulosa*, a facultatively sculptured species, shows a moderately positive clinal inflation response while exhibiting more sculpturing in large rivers, suggesting an overlap between the adaptive advantage provided by development of sculptures and inflation, which leads to a potential trade-off.

To investigate the interactions between these two anchoring traits and environmental conditions, we developed a calculation of the volumetric ratios of shell to soft-tissue using structured light 3D-scanning. We measured how resource allocation varies as a function of flow regime, to assess the cost of producing anchoring traits. Sampling contemporaneous specimens from Wesleyan University, CT and the Academy of Natural Sciences, PA, we found that unsculptured and sculptured species displayed differential patterns of shell/soft-tissue allocation. Unsculptured *Fusconaia subrotunda* develops more inflated, thickened shells in large rivers, yielding higher shell/soft-tissue ratios than sculptured *C. pustulosa*. Both anchoring traits are hypothesized adaptations for survival in large rivers, but our results suggest that producing highly inflated shells requires higher resource allocation for shell-building, at the expense of soft tissue growth. Sculpture thus may be a more economical anchoring trait than inflation, which may have caused prevalence of sculptured individuals in large rivers.

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## From Morocco to Spain: How Mid-Holocene land snails shed a new light on extant malacofauna and its response to environmental changes

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‘Conservation Paleobiology’ uses fossil records to analyse past and present biodiversity and its responses to environmental changes and human disturbances, to better guide conservation decisions. In Quaternary studies, fossil land shells are usually studied to investigate past environments and climates across various spatial and temporal scales. Over the last ten years, new molluscan successions from northeastern Morocco and southern Spain have revealed a high diversity of land snail assemblages during Holocene. Nowadays, the highly anthropized Mediterranean Basin is considered one of the most sensitive areas to landscape and biodiversity modification due to climate changes driven by human activities. As one of the most threatened invertebrate groups, many Mediterranean land snail species require major conservation measures to ensure their survival.

On the basis of Moroccan (Aït Said ou Idder; Blirh; Oued Charef) and Spanish (Galera; Baides) sequences, we discuss the significance of Holocene molluscan records from a perspective of paleobiology conservation. Most of the sequences provide records from the Middle Holocene (8200–4200 Before Present) consisting of a rich hygrophile fauna containing *Oxyloma elegans*, *Carychium minimum*, *Vertigo antivertigo*, *Vallonia pulchella*, *Vallonia enniensis*, and *Zonitoides nitidus* as common components. *Vertigo moulinsiana* and *Vertigo angustior*, both protected species under the European Directive, occur regularly. *Vallonia enniensis* is common in several Holocene sites, but now has only a limited distribution in Spain and is unknown living in Morocco. Likewise, the occurrence of the endemic *Orculella aragonica* at Galera and Baides shows that this species also had a much larger populations and distribution during the Holocene supports its endangered species status.

Middle Holocene molluscan successions provide an historical perspective of the occurrence of hygrophilous species before aridification and increased human impact led to the disturbance and fragmentation of wetland habitats. Molluscan fossil records are an ideal temporal tool to better understand the parameters that have shaped today’s biodiversity and to identify the most vulnerable taxa.

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## **CONFREMUS – pan-European COST scientific network to resolve problems of freshwater mussels conservation**

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In second half of 20<sup>th</sup> century, EU has started a project called COST (COoperation in Science and Technology). The project provides funding only for networking, stimulating the transfer of knowledge and technology between different European countries and their neighborhood. In 2019, we launched the CONFREMUS project 'Conservation of freshwater mussels – a pan-European approach' (CA18239), which aims to integrate data on the European species of freshwater mussels, their diversity and abundance in view of planning their conservation. The project analyze the knowledge accumulated so far and scientific horizon of freshwater mussel research and prepare a strategic program for their protection in Europe, both for specific conservation measures and for raise of awareness and involvement of European societies. Despite the Covid epidemic which hampered face-to-face meetings, we have done a lot to prepare pan-European databases, stimulate large international teams to work on the distribution and diversity of bivalve molluscs, on the ecosystem functions and services provided by freshwater mussels, their monitoring and methodological standards of research. Our project is constantly growing, finding new participants from the EU or neighboring countries (over 100 scientists from the 31 countries involved). More about the project on the website: [www.confremus.eu](http://www.confremus.eu), [cost4naiads](https://www.facebook.com/cost4naiads) on facebook, and a new portal [www.e-mussel.eu](http://www.e-mussel.eu).

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**Symposium**

**Continental Molluscs Facing  
Environmental Changes**

**Part II**

**Abstracts of poster presentations**

## Quaternary palaeobiogeography of continental molluscs: a European project

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Quaternary climatic cycles have strongly influenced the diversity and distribution of the terrestrial gastropod fauna. European territories located at the edge of the Pleistocene ice sheets, were particularly affected by this phenomenon. Since the mid part of the 20<sup>th</sup> century, studies on Quaternary malacofauna have been widely developed in Western and Central Europe, providing a huge amount of information on species past distributions. The Quaternary record is relevant to the current geographic extensions of European species. In recent years, many papers have focused on the location of glacial refugia and possible recolonization pathways during interglacial periods using alternatively or both Quaternary and phylogeographic approaches. The Quaternary malacological record can provide insight into the evolution of the group diversity and associated causes of these variations over a long period of time, as well as on the timing of both species retreat/extension and extinction rates during climatic cycles. Therefore mapping the expansion of land snails over time is an important key to understanding the current status and distribution of species and helping to predict potential distributions with accuracy.

However, until now, few attempts to compile data in this field of research have been proposed and none on a European scale. The project of a “European Quaternary Molluscan Database” aims to provide maps of species distribution at different periods and tables of reliable well-dated malacological counts recovered from Quaternary deposits across Europe. Particular attention has been paid to the time division table in order to avoid as much as possible regional discrepancies in the interpretation of chronological attributions.

Originally developed at the LGP (Meudon, France) the project has received strong support in recent years from the Czech Quaternary malacology group. Today, the database includes 609 sites with Pleistocene and Holocene records distributed from Ireland to Slovakia. Further contributions from other countries are now highly desirable to improve the database and make it a useful tool for the European malacological scientific community.

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## Mussel memory: History lessons from freshwater bivalves

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Freshwater mussels are a diverse taxon found across every continent except Antarctica, and act as ecosystem engineers, contributing to water filtration, habitat creation and nutrient cycling. However, these molluscs are increasingly under threat from a suite of anthropogenic pressures including pollution, land-use change, and waterway alteration through damming and canalisation. There is little information on long-term trajectories for mussel populations, and while extinctions and local extirpations may be reported, changes in individual fitness and population stability are largely undocumented. Tracking these changes could provide an early warning of sublethal changes preceding a population crash or extirpation; monitoring is also crucial to improving understanding of the causes and impacts of mussel declines, and informing conservation management and decision-making. My research examines historical data over a range of timescales, from 20<sup>th</sup>-century historical surveys and to Bronze Age archaeological evidence. I compare this evidence with present-day mussel populations to investigate changes in individual size and growth rate, as well as population density, all of which are predictors of the contributions of mussels to ecosystem functioning. In this poster I will present the initial results of this research documenting and investigating decline and changing population dynamics in freshwater mussels over varying timescales of human activity.

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## Snails drifting along – dispersal of land snails in stream corridors

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Snails are embodiment of difficult movement. Fortunately, short-range active dispersal, occurs side by side with long-range passive dispersal. And that is why animals with one clumsy leg are spread all over the world. There are well documented transports on other animals, human machines or by salted water. We were interested in transport by lotic water – stream corridors. In flood deposits forming along waterflows, there is noticeably infinite number of snail shells. Material of flood deposit is picked up from the waterflow vicinity and drifted down until it is cast up somewhere else. This could be a scenario of the snail spreading mechanism, for example in interglacials, when new habitats were forming in the floodplain. Field experiments with floating marked snail shells were conducted to assess how far the snails can travel and more than 50 samples of flood deposits were analysed, to reveal whether the probability of transport depends on shell size or shape or ecological characteristics of the snail species. Field experiments indicate that this transport is going on in small steps, in hundreds of meters, depending on the size of the waterflow. Comparison of flood deposits content and faunistic samples from relevant floodplain and river valley sides shows that flood deposit is a mixture of floodplain and slope species and represent larger area than we expected.

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## New freshwater molluscs from the Pleistocene lignite-bearing Megalopolis Basin (Greece)

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To date, Plio-Pleistocene molluscs from terrestrial and freshwater environments of Greece are poorly known due to a rather low number of described faunas and the lack of taxonomic revisions. The present study deals with mollusc faunas recovered from the Megalopolis Basin in central Peloponnese, extracted from organic-rich sediments (from brown coal to xyloid lignite). The Megalopolis Basin contains fluvial and lacustrine sediments, deposited during the Pliocene to the middle Pleistocene. This basin is one of the few in this area presenting a continuous sedimentary sequence. The sampled material (35 samples from 3 different levels) presented herein originates from the core of an active lignite mine and is of Pleistocene age.

The fossil content includes assemblages that are typical for lacustrine environments, and which suggest fluctuations in oxygen levels. Until now, 20 different species of molluscs (gastropods and bivalves) have been identified including *Gyraulus crista*, *Valvata cristata*, *Planorbis planorbis*, *Hippeutis* sp., *Lymnaea* sp., *Bithynia* sp., *Oxyloma* sp. and *Pisidium personatum*, illustrating the dynamic environments of the Megalopolis palaeo-lake. This is the first systematic record of mollusc faunas from the Pleistocene of southern Greece providing significant data on their taxonomy, palaeoecology and palaeoenvironment. In addition, the reconstruction of the palaeo-environments will shed light on the evolution of the Megalopolis palaeo-lake in relation to climatic fluctuations that occurred during the Pleistocene. The new assemblages provide new insights in the evolution of Pleistocene Greek mollusc faunas from an area, which was missing so far from the European map.

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## Molluscs as bioindicators of paleoclimate and paleoenvironment in sediments of neotropical aquatic ecosystems

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Lake and marine sediments are used to explore past changes in climate and environment. Inferences about the past rely on analyses of physical, chemical and biological indicators such as mineral composition, element chemistry, stable isotopes in carbonates and organic matter, biomarkers, remains of algae (e.g. diatoms) and higher plants (pollen and macrofossils), as well as those of invertebrates such as cladocerans, foraminiferans, ostracodes and molluscs. We used sedimented mollusc remains (gastropods and bivalves) and geochemical data to infer Late Holocene environmental conditions in four aquatic ecosystems (fresh, brackish, marine) in the Neotropics. Study sites included Lake Nahá (Chiapas, Mexico), coastal Ría Lagartos Lagoon (Yucatán, Mexico), and islands Belmonte (São Pedro and São Paulo archipelago, Brazil), and coastal Ilha do Mel (Paranaguá, Brazil). Short sediment cores were collected from Lake Nahá (60 cm) and Ría Lagartos Lagoon (25 cm), whereas multiple coastal surface deposits were retrieved from the Brazilian island sites. Core depth-age relationships were established using <sup>210</sup>Pb and <sup>137</sup>Cs, whereas age determination for the island samples was accomplished by radiocarbon dating. Molluscs in the four records were identified to species level and enumerated. The freshwater gastropod community in Lake Nahá was sensitive to changes in water level and trophic state, whereas the malacological assemblages in the coastal lagoon record show they responded to climate events such as hurricanes and recent human impacts (e.g. highway construction). Vermetid snails (Vermetidae) from the islands are indicators of past sea level changes. This study revealed differential responses of mollusc communities to climate and environmental change across distinct aquatic ecosystems in the Neotropics, but nevertheless illustrates their potential as reliable paleoclimate and paleoenvironmental indicators.

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## Terrestrial and aquatic snails: Insights into climate change in the Sultanate of Oman – from the early bronze age to the present

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During the Early Bronze Age, the social and economic living conditions of people inhabiting the eastern Arabian Peninsula changed, which is particularly visible at some archaeologically significant sites. One of these sites is Al-Khashbah (Sultanate of Oman), where the oldest copper production site of the region has been found. The existence of such an economic hotspot under the hot and dry climatic conditions that prevail in central Oman today is still a matter of debate, because first irrigation systems in this area are only known from the Iron Age onwards. What was found, however, and dated to the Early Bronze Age, are so-called “ditches”. These human-made, U-shaped, up to three metres deep and four metres wide trenches surrounded some building structures (“towers”) are interpreted as a possible early form of an irrigation system, as they had to be filled with water, which is evidenced by the existence of a malacofauna (*Melanoides tuberculata*) at their bottom. The age of these shells was determined to be 4200 uncal yr BC with the use of charcoal residues, which were also found in the ditches. The preservation state of selected specimens was checked by immersion in Feigl solution, before the shells were analysed sclerochronologically for their stable isotope composition. Stable oxygen ( $\delta^{18}\text{O}$ ) and carbon isotope ( $\delta^{13}\text{C}$ ) values were used to reconstruct the prevailing environmental conditions, predominantly temperature. However, not only aquatic but also terrestrial snails (*Zootecus insularis*) were collected, dated and studied, because their stable isotope data, especially the  $\delta^{13}\text{C}$  values, can provide information on consumed vegetation allowing a collection of information on possible agriculture that might have taken place during the Early Bronze Age. The  $\delta^{18}\text{O}$  data obtained from these shells was used to specify palaeoprecipitation regimes. Finally, the Early Bronze Age results were compared with recent representatives of both species to detect possible environmental changes over time.

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## Molluscs of the hypersaline Rio Lagartos coastal lagoon, southern Gulf of México

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Molluscs are a diverse phylum in coastal lagoons. Numerous gastropods and bivalves taxa that inhabit such lagoons display broad ranges of optima and tolerances with respect to temperature and salinity. We present the taxonomic composition and spatial distribution of mollusc assemblages (live-collected with soft parts intact or paired shells for Bivalvia) from surface sediments in the coastal lagoon of the Ría Lagartos Biosphere Reserve, northern Yucatán Peninsula, México. The Ría is hypersaline throughout much of its ~70 km length, with a gradient from the eastern most interior near El Cuyo (>70 psu) to the mouth (33–38 psu). Molluscs were collected in the rainy season (September/October 2017), during the winter “Nortes” season (February 2018) and at the end of the warm, dry season (May 2018). Samples were taken using a Ponar dredge, a cylindrical PVC core barrel, or a beach seine. Sampled molluscs comprised 39 species, 34 genera and 23 families. Nine species were collected during the cool and dry season, 22 species in the rainy season, and 28 species in the warm and dry season. We studied the abiotic characteristics at the sampling sites (proportions of sediment grain-size classes, submersed aquatic vegetation, substrate type) and biotic characteristics (faunal composition, feeding guilds) to explore differences among environments across the salinity gradient. Stenohaline species displayed lower incidence (<20%). We plotted species accumulation curves for samples from euhaline (30–40 psu) and hyperhaline (>40 psu) conditions. Higher richness was encountered in environments characterized by euhaline conditions. Species curves indicate that despite substantial sampling efforts, Ría Lagartos has a greater mollusc diversity and spatio-temporal heterogeneity than reported in this or previous studies, highlighting gaps in knowledge about the mollusc fauna of the region. We encountered a lower number of species in the lagoon than did a similar study conducted >30 years ago. Nevertheless, we interpret these data cautiously, given that different methods (e.g. samplers, season) were used in the two investigations. We propose that stratigraphic analysis of mollusc assemblages in sediment cores that span deposition over the last century can provide a better historical perspective on changing mollusc species diversity in the Ría Lagartos lagoon.

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**Symposium**

**Systematics and Evolution  
of (not only marine)  
Heterobranchia**

**Part I**

**Abstracts of oral presentations**

## Fourier transform infrared spectroscopy as a powerful technique to identify and classify gastropods

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Fourier -transform infrared spectroscopy (FTIR) is a molecular fingerprinting technique well suited towards studying natural materials. It is non-destructive, allows analysis of liquids and solids in real time and helps to identify organic compounds such as proteins or sugars. Here we apply this technique to the study of gastropod mucus and more specifically the mucins within. This extends Skingsley's study 22 years ago in sample collection, level of detail and number of species (to 12: *A. fulica*, *C. aspersum*, *C. nemoralis*, *A. ater*, *A. hortensis*, *L. flavus*, *L. maximus*, *L. haroldi*, *V. sloanei*, *L. stagnalis*, *M. cornuaretis* and *P. diffusa*). Furthermore, we use FTIR not only to characterise but also classify these species. This is demonstrated by comparing a cladogram generated from previous phylogenetic analysis with an ultrametric tree generated from FTIR spectra. Our results confirm that there is a diversity of protein structures present across gastropod mucus and that FTIR is a powerful tool to identify and classify species through their mucus alone. Looking forward, we propose FTIR offers a state-of-the-art window into the study of gastropod mucus which will lead us to a better understand of how these materials exhibit different compositional properties to maintain functionality in different environments.

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## Diversity and phylogeny of the only rock-dweller haminoeid genus *Smaragdinella* (Gastropoda: Cephalaspidea) in the Indo-West Pacific

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*Smaragdinella* A. Adams, 1848, is the only lineage of the Cephalaspidea family Haminoeidae inhabiting rocky-shores. The genus is restricted to tropical latitudes of the Indo-West Pacific and animals are exclusively found under small stones, pebbles, empty shells, or crevices in rocky outcrops on the upper tidal zone. These distinctive features among the haminoeids make *Smaragdinella* of special evolutionary interest, but the processes that have driven the diversification and adaptation of these snails to a unique ecology remain to be understood. According to the World Register of Marine Species (WoRMS) there are four recognised valid species of *Smaragdinella* (*S. seiboldi*, *S. fragilis*, *S. calyculata*, *S. kirsteueri*) among seven nominal names, but the identity of some is questionable and for example our preliminary studies showed that *S. kirsteueri* from Madagascar is a *Phanerophthalmus*.

The goals of this project are to generate a comprehensive review of the diversity of *Smaragdinella* species and a molecular phylogeny for the genus and to use the phylogeny to understand historical processes of speciation and biogeography in the marine realm and ecological adaptation to rocky-shore environments.

Here we present a COI Bayesian tree phylogeny including samples covering the entire geographical span of *Smaragdinella* and an overview of the relevant taxonomic characters resulting from comparative anatomical dissections and scanning electron microscopy of jaws, radulae, gizzard plates, penial papillae, and shells. The results point to at least the presence of 11 species, which is nearly four times higher than previously recognized diversity.

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## What morphology of extant taxa tells us about the first 100 Million years of heterobranch evolution

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Heterobranchia constitute 40 to 50 percent of extant mollusc species. Large-scale phylogenetic and phylogenomic approaches finally disentangle how the “Heterobranchia Tree” contains a grade of mostly low-diversity groups of aquatic snails with multispiral shells (“lower” Heterobranchia) and a large monophylum of snails and slugs with diverse modified morphologies and ecologies (Tetratentaculata, Euthyneura). According to the fossil record, successful Euthyneura and at least some extant lower heterobranch subgroups radiated in the early Triassic, but “lower” heterobranchs principally originated much further back in the Paleozoic.

We here discuss previous ideas of early heterobranch evolution in light of the “New Heterobranchia Tree” analyzed from both transcriptomes (see abstract No. 270 by Knutson et al.), our own Sanger sequencing data of two carefully curated “Standard” markers (COI+18s) covering all families, and of our own micro-anatomical analyses of all the “lower” heterobranch families.

Based on those extant survivors of ancient heterobranch lineages, we discuss the modification of shells, mantles, head sensory areas, reproductive tracts, and inferences of ecology for the time window between the oldest unequivocal fossils of crown group heterobranchs (~300 Mya) and the common occurrence of bubble-shelled Euthyneura around 200 Million years ago.

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## 50 Shades of Red: the red-reticulate group of *Goniobranchus* (Heterobranchia: Nudibranchia: Chromodorididae)

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The Chromodorididae family tree has been refined in recent years via molecular phylogenetic analyses which have clarified many relationships between taxa. The genus *Goniobranchus* is one clade within Chromodorididae that was previously included within the genus *Chromodoris*. However, based on recent molecular phylogenetic results, *Chromodoris* was determined to be non-monophyletic and *Goniobranchus* was resurrected. In this study, we performed molecular and morphological analyses to resolve the internal relationships among *Goniobranchus* species, specifically the red-reticulate species complex of three previously described species, *Goniobranchus tinctorius*, *G. reticulatus*, and *G. alderi*, which display a red network of lines over a white mantle and are widely distributed across the Indo-Pacific Ocean. We sequenced two mitochondrial genes (COI and 16S) and one nuclear gene (H3) for 339 *Goniobranchus* specimens, and in our phylogenetic analyses the red-reticulate species group emerged as a monophyly. This current work has indicated there are at least eleven distinct species within this species complex, including the only three previously described species and another described species, *G. splendidus*, was added to this clade. The molecular data and the morphological differences among species will be discussed, and we present a possible way forward to clarify the taxonomy of the red-reticulate species complex.

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## Biodiversity exploration and the program ‘La Planete Revisitee’: marine heterobranch gastropods from Corsica Island

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Marine molluscs from Corsica (France), in the Mediterranean Sea, have been in study since the beginning of the nomenclatural system. Despite this fact no recent project has tackled a comprehensive approach to the fauna of the island as a whole until Philippe Bouchet, under the umbrella of the Program ‘La Planete Revisitee’ at the ‘Museum national d’Histoire naturelle’, organized the first of the CORSICABENTHOS fieldtrips.

From 2019 to 2021, even during the worst of the pandemic, 3 fieldtrips were completed based in 3 different parts of Corsica: Natural marine parc ‘Cap Corse et de l’Agiate’ (2019), Bonifacio National Park (2020), and Natural parc Scandola (2021). Samples were taken in 598 stations by direct pick, brushing, vacuum sampling or dredging, covering almost all the coast of the island, from the surface to 616 m deep.

After this effort more than 2000 specimens of at least 120 species of sea slugs were collected alive and preserved in Ethanol 96%, precisely establishing their distribution in Corsica and their abundance and providing valuable information for a better management of the marine environment in the island.

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## The *de novo* genome of the “Spanish” slug *Arion vulgaris* Moquin-Tandon, 1855, a perspective of genetic forces driving water-to-land transition

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Stylommatophoran pulmonate land slugs and snails successfully completed the water-to-land transition from an aquatic ancestor and flourish on land. Of the 30000 estimated species, so far very few land snail genomes were published, and none of any terrestrial slug. Here, we characterized a chromosome level genome of the “Spanish” slug, *Arion vulgaris* Moquin-Tandon, 1855, a notorious pest in gardens and agriculture and invasive into large part of northern and eastern Europe. Using this reference genome, we conclude on a whole genome duplication event which occurred approximately 93–109 Mya at the base of Stylommatophora and might have promoted land invasion and terrestrial adaptive radiation. Compared with other related aquatic and marine species, we found that genes related to the development of kidney, blood vessels, muscle and nervous systems had been expanded already in the last common ancestor of land pulmonates, likely to cope with the challenge of gravity and water loss. Compared with two stylommatophoran snails, the “naked” *A. vulgaris* showed tandem or proximal duplication derived expansion genes enriched in the function of immune response, synthesis of pigments, and excretion, and positive selection of a group of anti-inflammatory genes. We conclude the slug evolved a stronger ability to counteract the greater threaten of external damage, solar radiation, and water loss lacking a protective shell. Furthermore, a recent burst of long interspersed elements in the genome of *A. vulgaris* might affect gene regulation and contribute to rapid phenotype changes of *A. vulgaris*, which could help *A. vulgaris* to quickly adapt to new environments and contribute to its invasiveness and competitiveness.

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## A sticky situation: Investigating the contradictory nature of gastropod mucus

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Gastropod mucus has two contradictory properties, adhesion and locomotion, which gastropods are able to seamlessly move between whenever the functional need arises. The mechanisms behind this transition have not been identified as the two types of mucus are typically studied independently, despite both having similar components and a range of unique viscoelastic, non-linear rheological properties. Furthermore, scientometric analysis of the field of gastropod mucus research revealed a divided approach in investigating these contradictory material properties. Highlighting that gastropod mucus is often studied in isolation from the animal, where biological function can only be extrapolated from research undertaken in a purely engineering context.

In an attempt to bridge this divide, a gastropod locomotion translation device to incite a need for adhesion was created, which enabled simultaneous visualisation of gastropod movement and sample collection. This provided a more holistic view of mucus structure and function within the context of gastropod behaviour. Our study utilised a combination of Fourier transform infrared spectroscopy, video analysis, and microscopy to identify the mechanisms used by gastropods to manipulate their mucus' structural composition to meet a specific functional demand. A biocrystallisation effect was observed across a range of gastropod species; indicating a potential class-wide fast-acting mechanism. We propose this is activated and moderated through the use of metal ions to rapidly alter the viscoelastic properties of mucus between locomotion and adhesion. By using *Cornu aspersum* as a model species, we sought to explore the impact both monovalent and divalent ions have on the structure and function of pedal mucus in real time using rheology. Results revealed an unexpected relationship between the adhesive properties of mucus and ion valence, which may help explain the opposing properties of gastropod mucus.

This study indicates a potential fast-acting mechanism based on both divalent and monovalent metal ions, though further research is needed to understand the specific mechanisms behind the observed functional changes. Moreover, linking the various observable crystal structures and compositions to specific metal ions may shed light on the exact mucin structures and molecular motifs used by gastropods to control their mucus to meet a range of seemingly contradictory needs.

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## Shining disco: shedding light into the systematics of the family Discodorididae (Gastropoda: Nudibranchia)

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The Discodorididae are a family of Sea slugs hard to identify. Phylogenetic relationships within the family are contentious and poorly understood. *Discodoris* is the most species-rich genus within Discodorididae since it has been traditionally used as a dumping ground for species of ‘discodoridid’ morphology that could not be placed in more narrowly defined genera. The need for a full taxonomic revision of the genus has been recognized by several authors. In this study, we aim to provide a working hypothesis of the phylogenetic structure of the family and uncover overlooked species based on newly generated molecular data for a large amount of Discodorididae species not sequenced before. We shed light on the conflicting taxonomy of the family using a Sanger target gene approach, based on the concatenation of the mitochondrial genes

cytochrome c oxidase subunit I and 16S rRNA, and the nuclear genes 28S and histone H3. We generated 59 new sequences, including eleven type species of genera from the Mediterranean Sea, Eastern Atlantic Ocean, and Central and South America. Phylogenetic analyses supported the monophyly of the family Discodorididae, and species delimitation methods further unveiled previously overlooked diversity in genera such as *Taringa*, *Tayuva*, *Paradoris*, and *Geitodoris*. Our results did not recover the monophyly of some genera such as *Discodoris* and *Diaulula*, which warrant a full morphological revision and some species may need to be transferred.

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# Jumping through the hoops: Unveiling functional mitogenomic structural rearrangements in the genus *Dendrodoris*

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Since the advent of molecular phylogenetics, relationships among heterobranch sea slugs have been mostly tackled by target gene approaches, using few mitochondrial and nuclear markers. However, the genus *Dendrodoris* has defied phylogenetic resolution using classical markers. On one hand, mitochondrial sequences have been challenging to align, and when resolved, bizarre topologies consisting in clades split by long branches are recovered. The sum of these patterns is presumed to hint the existence of pseudogenes. In the present study, we aim at overcoming former molecular limitations by sequencing at 30x depth the genome of *Dendrodoris temarana* to “de-novo” assemble it and combine it with six additional Dendrodorididae species at 7x depth. We successfully circularized the mitochondrial genomes of all the species with an average coverage of 200x, and generated the first genome drafts for the family, with a completeness degree based on universal single copy orthologs ranging from 10 % to 60 %. Our results reveal a novel mitochondrial structural variant in the smooth dorsum *Dendrodoris* species, and the presence of several non-synonymous mutations in the same species. We modelled the secondary structure of all mitochondrial genes of a representative species of each major clade, showing conservation of protein structure among all three main clades, confirming that the previously suspected pseudogenes are actually functional genes. Finally, mitogenomic phylogeny supports three major clades in Dendrodorididae: *Doriopsilla*, tuberculated *Dendrodoris*, and smooth *Dendrodoris*. Our study represents a starting point in the analysis of the origin of these particular structural jumps and its implications in the evolution of nudibranchs.

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# Gene novelty in understudied groups: using the chromosome-level genome of the nudibranch gastropod *Berghia stephanieae* as a case study

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Evolutionarily novel (or *de novo*) genes are considered an important way for new phenotypes to emerge during evolution. In Mollusca, a speciose and functionally diverse metazoan phylum, novel genes are thought to be recruited for processes such as biomineralization and some characteristic molluscan features, such as the radula. But what does novelty mean in these contexts? Researchers are often unable to identify homologs to their putative genes using common databases due to the lack of functionally annotated genomic resources for many molluscs, which leads to overestimates of “novel” genes. Gastropods, the most diverse class of molluscs, are particularly underrepresented when it comes to high quality genomic data. Here we present the genome for the nudibranch gastropod *Berghia stephanieae*, an emerging model species for neuroscience. This species also falls within a clade of nudibranchs (called Aeolidioidea) that has the unusual ability to sequester cnidarian nematocysts, an ability that likely uses both novel and co-opted genes. Post filtration with purgedups and blobtoolkit, the *B. stephanieae* genome is in 18 scaffolds (~1.1 Gb) with an N50 of 86 Mb. We found 93.3 % complete and 95.9 % complete + fragmented BUSCO core genes represented from the Metazoa BUSCO database in the final genome. To investigate the distribution of novel genes in *Berghia*, we compared the predicted proteome from the genome to 49 predicted proteomes obtained from MolluscDB, ENSEMBL, and transcriptome data obtained from the NCBI Sequence Read Archive, including other nudibranchs, gastropods, and molluscs, and other metazoans. We found that over 2000 *Berghia* genes are clade-specific to Mollusca, Gastropoda, Nudibranchia, or Aeolidioidea, and over 2000 genes appear to be *Berghia*-specific. We find that many of these genes also appear to be expressed only in certain tissues, such as the brain (564 clade-specific, tissue-specific genes). These analyses provide short lists of potential targets for future functional assays, and will be especially helpful for future work in *Berghia* that seeks to understand the value of “novel” genes in the context of character evolution. Ultimately, this investigation into gene novelty in *Berghia* will also provide important insights into the impacts of novel genes on mollusc biodiversity and evolution.

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# Integrative taxonomy and phylogeography *Eubbranchus rupium* – *exiguus* (Gastropoda: Nudibranchia) species complex

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Nudibranch molluscs of the *Eubbranchus rupium* – *exiguus* species complex have similar external morphology. Based on the published data, it is impossible to conclude whether they are separate species or should be reduced to synonyms. The representatives of this complex show an amphiboreal distribution. Species with this type of distribution often represent complexes of cryptic or pseudo-cryptic species. It makes them an appropriate model to study modes of speciation in boreal and Arctic regions.

A total of 199 samples, collected from the White, Barents and Japanese Seas, and Norway. Integrative taxonomy methods were used to investigate species identity of studied groups and phylogenetic relationships between them, including molecular genetic methods (phylogenetic analysis using sequence COI, 16S and 18S as markers), haplotype networks and morphological analysis (specimen dissection, light and scanning electron microscopy).

Our analysis recovered *Eubbranchus rupium* and *Eubbranchus exiguus* as separate distinct species. They can be distinguished by coloration, radular and reproductive system morphology. Both are recovered as supported monophyletic clades in our phylogeny reconstruction. Additionally we found two pseudocryptic species – one is sister similar to *E. rupium* and another – to *E. exiguus*.

The *Eubbranchus rupium* has a wide distribution and a fragmented range. In the Sea of Japan a similar species was found. This new species is 4.7 % different from *E. rupium* in COI marker and also shows a specific coloration.

The second pseudocryptic species, similar to *Eubbranchus exiguus*, is found sympatrically with true *E. exiguus*. This new species is 11 % different from *E. exiguus* in COI marker. Morphological differences are found in the coloration of individuals and the structure of the digestive gland enclosed in cerates. *Eubbranchus* taxonomy should be revised to accommodate these new findings, and two new species should be described.

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## What would an ancestor for the Sacoglossa have looked like?

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Phylogenetic relationships of higher gastropods, especially the Heterobranchia, have undergone major changes during the last 20 years. Molecular analyses have placed Ringiculidae at the base of the Nudipleura branch, Newnesiidae at the base of the Cephalaspidea branch, and even the “Lower Heterobranchia” have been at least partly resolved into a few separate branches. The Sacoglossa were removed from Euopisthobranchia to the base of a Panpulmonata branch, but since then the relationship with other panpulmonate groups has remained unclear. Integrative phylogenetic analyses have shown rampant cryptic speciation within many genera, and several new genera have been described. It has also been deducted that the split between shelled Oxynoacea and non-shelled Plakobranchacea happened very early in the evolution of the Sacoglossa, but which families and genera are actually at the base of each of these two branches differs among analyses depending on included taxa as well as outgroups. Here the most important morphological synapomorphies for the Sacoglossa, Oxynoacea and Plakobranchacea will be described. Most of these are associated with suctorial feeding and the function of the uniseriate radula. Synapomorphies of the reproductive system are present, but the function of many parts remain unclear. The ancestral sacoglossan would have had a muscular pharynx for suctorial feeding, a uniseriate radula with interlocking, articulating teeth arranged in two sections, a lower limb of used teeth and an upper limb of newly formed teeth. The teeth would probably be short with ill-defined cusps but strong bases. Preradular teeth would be present for use in newly settled larvae. The odontophore would have two sets of muscles, one for moving the radula and one for depressing the odontophore in connection with suction. The dorsal wall of the pharynx would have been muscular, but septation of muscles might have been weakly developed. Additional muscular structures for enhancing suctorial power have developed in many higher sacoglossan taxa, but would probably have been weakly developed in the ancestral form. The penis was cephalic, internal, with an internal, closed vas deferens connected to the visceral reproductive system. Presence of penial armature remains unclear. Shell morphology is also unclear.

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# Phylogenomic methods provide robust resolution of heterobranch gastropod phylogeny: implications for our understanding of evolution in the clade

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Heterobranchia is one of the most diverse, speciose and charismatic clades among molluscs including groups known to many such as garden snails/slugs, nudibranch sea slugs, marine bubble snails, sea hares, pelagic pteropods, sundial snails and several others. Resolving long-standing questions of heterobranch phylogeny will enable the study of many intriguing aspects of gastropod evolution, including extensive convergence, the evolution of shell-loss, transitions between marine, freshwater and terrestrial habitats, toxin production and sequestration, gill evolution, and kleptoplasty – to name a few. Historically, phylogenetic resolution among these taxa was hindered by extensive morphological convergence in the clade, however, thanks in large part to molecular phylogenetics and now phylogenomic methods, our knowledge of heterobranch phylogeny has greatly improved over previous decades. Here we present a phylogenetic backbone for the entire Heterobranchia clade reconstructed using more than 120 transcriptomes generated and assembled *de novo* in combination with sampling across all major lineages. In particular, we include the first transcriptomes for several lineages of the paraphyletic grouping known as ‘lower’ Heterobranchia and previously unassigned lineages of micromolluscs such as muchisonellids and omalogyrids. We identify a clade comprising Architectonicoidea (sundials and their kin) and microscopic *Ammonicera* as the sister group to the rest of Heterobranchia – not Valvatoidea as previously hypothesized on the basis of anatomy. To test the robustness of the phylogeny, we explore the effects of different phylogenomic reconstruction methods on topology. Additionally, we discuss some pitfalls and challenges of incorporating the heterobranch fossil record into our analyses. For the first time in heterobranch systematics, our phylogeny shows robust resolution of the relationships among the non-euthyneuran ‘lower’ heterobranchs, finally enabling us to better understand and reconstruct the early evolution of Heterobranchia, and ultimately the conditions that may have led to the speciose and diverse clade Euthyneura.

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## Toward a deeper understanding of nudibranch colour

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Animal colouration is fascinating and plays an important role in predator avoidance, mate recognition and communication. Nudibranchs are some of the most brightly coloured organisms in our oceans with both aposematic (warning) and cryptic (camouflage) colouration among and within species. Despite the clear adaptive importance of these colour phenotypes, we know very little about their origins. Here, I investigate colour variation in the cryptic sea lemons and aposematic chromodorids- two nudibranch groups with multiple polychromatic species. I discuss the role of diet and environment in mediating colour pattern in the sea lemons, and the role of introgression in generating colour patterns in mimetic *Chromodoris* and *Felimida*. I also discuss future work which will integrate a multi-omics approach to identify the genetic basis of colour variation in these animals.

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## Molecular insights into the global phylogeny of *Elysia* Risso, 1818 (Gastropoda, Heterobranchia) with a focus on the European seas

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The genus *Elysia* Risso, 1818 comprises about one-third of the richness species in Sacoglossa. However the species diversity in the genus needs an extensive phylogenetic characterization in some areas like the eastern Atlantic and Mediterranean European waters. In order to clarify the systematics of this sacoglossan genus as well as characterize the species diversity in such regions, we performed through an integrative approach based on a thorough literature review, molecular analyses and species delimitation approaches (e.g. ABGD, GMYC, bPTP). We used phylogenetic methods such as Bayesian inference (BI) and Maximum Likelihood (ML) based on partial sequences of two mitochondrial genes (COI and 16S) and two nuclear genes (H3, 28S), which confirmed the presence of five of the recognized European *Elysia* species: *Elysia viridis* (Montagu, 1804), *Elysia timida* Risso, 1818, *Elysia flava* Verrill, 1901, *Elysia margaritae* Fez, 1962 and *Elysia rubeni* Martín-Hervás, Carmona, Jensen, Licchelli, Vitale and Cervera, 2019. Moreover, a new *Elysia* species was identified in the Azores, and *Elysia gordanae* Thompson and Jaklin, 1988, currently considered a junior synonym of *Elysia margaritae*, was recovered as a distinct species. Otherwise, we consider *Elysia hetta* Perrone, 1990 as junior synonym of *Elysia gordanae*, and *Elysia translucens* Pruvot-Fol, 1957 as a *taxon inquirendum*. Finally, the tropical *Elysia evelinae* Er. Marcus, 1957 is recorded in European coasts for the first time. Two molecular phylogenies were constructed, including i) all the sequenced European elysiids to discriminate among distinct species; and ii) all available sequences of worldwide *Elysia* species, to evaluate the evolutionary relationships of the European species with congeneric taxa in a global phylogenetic framework. The phylogenetic reconstructions did not recover the Mediterranean and Atlantic *Elysia* species as a monophyletic group. Nevertheless, one pair of Mediterranean sister species indicated some speciation within the basin, while three other pairs included one Mediterranean and one amphiatlantic or eastern Atlantic species. This fact suggests peripheral isolation in the Mediterranean as a recurring driver of allopatric speciation.

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## The nudibranch *Berghia stephanieae* (Váldes, 2005): a transitional form towards a stable photosymbiosis

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Photosymbiosis is an association between heterotrophic and phototrophic organisms found in many animal phyla. The onset and subsequent maintenance of this relation is complex. The basis is the interplay of the innate immune system of the animal host, an inhibition of the phagosomal maturation including the symbiosome establishment involving a nutrient exchange between the partners, and the activation of mechanisms to keep photobiont induced oxidative stress in the host cells at bay. Cnidarians are certainly the best understood photosymbiotic system, but in other animals, like Cladobranchia (Gastropoda, Nudibranchia), such molecular and cellular mechanisms are poorly understood. Cladobranchs acquire their algal photobionts (Symbiodiniacea) from their cnidarian prey and incorporate them into epithelial cells of the digestive gland system. Within Cladobranchia, different levels of the photosymbiosis are distinguished, including unstable (a few days) and stable symbioses (several weeks or months). Hence, cladobranchs are an ideal model to study the evolution of photosymbiosis in taxonomically diverse groups. To shed light on the molecular mechanisms leading to the successful evolution of photosymbiosis in Cladobranchia, we analysed the gene expression of *Berghia stephanieae*. This stenophagous species obtains its photobionts from the cnidarian model organism *Exaiptasia diaphana* (Rapp, 1829). *B. stephanieae* is not able to maintain the photobionts for longer than a couple of days and is thus considered to be in an unstable photosymbiosis. Further, *B. stephanieae* can be cultured in an aposymbiotic state (symbiont-free), which allows comparing the host gene expression in the presence and absence of the algae. Our results show that the recognition of the photobiont is mediated by innate immune receptors similar to the ones present in its food prey. In the presence of the algae, the slugs are able to cope with elevated levels of oxidative stress. However, the slugs are still digesting and/or expelling the algae, and do not regulate long-term maintenance innate immune signalling pathways such as Transforming Growth Factor- $\beta$ , Toll-like receptor, NF- $\kappa$ B, or complement. Hence, one major step towards the evolution of a stable photosymbiosis in Cladobranchia is based on the inhibition of the photobiont digestion/expulsion and the immunosuppression response of the host against the photobiont.

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# All teeth blazing!

## Ancestral state reconstruction of buccal armature in Doridina (Nudibranchia, Gastropoda)

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Nudibranchia is a large monophyletic order of gastropod molluscs, consisting of two suborders: Doridina and Cladobranhia. The dorid nudibranchs have remarkably wide range of feeding mechanisms (from rasping to drilling), feeding objects (e.g., sponges, bryozoans, polychaetes and gastropods) and feeding apparatus morphology. The evolution of the feeding apparatus is closely related to the diet preferences that follow the phylogeny of dorids. Despite a large amount of data on the feeding apparatus morphology in Doridina, there is no clear idea on the ways of adaptation of dorid nudibranchs to various food objects and the ancestral state of the feeding apparatus.

Using a comparative approach by combining phylogenetic analyses with morphological method (SEM), we compiled a comprehensive dataset to reconstruct the evolution of the buccal armature in Doridina. We utilized data on 115 species of dorid nudibranchs. Twenty-eight characters were used in the analysis regarding radula (27 characters) and labial cuticle (one character). The states were coded based on our own morphological investigations and literature data. Ancestral states of the separate characters of the buccal armature were reconstructed using Mesquite v.3.70 software. We also reviewed the features of feeding biology, which often determines the morphology of the feeding apparatus.

As a result, we have determined the characters of the buccal armature, which show a strong correlation with the current phylogenetic hypothesis and feeding mode, and identified the key evolutionary events. Also, here we suggest a revised terminology for buccal armature structures in dorid nudibranchs.

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## A thousand species, “all”-family phylogeny of the largest gastropod subclass (Heterobranchia)

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A pivotal strategy for biodiversity conservation relies on promoting phylogenetic diversity, an endeavour still in its infancy for many large groups. Gastropod molluscs inhabit (almost) all habitats on Earth and represent vital sources for food, cosmetics, and pharmaceutical applications, as well as playing a critical role as pests, snail-borne human diseases, and biological indicators of ecosystem wellbeing and climate change. Although snails and slugs worldwide impact our economy and ecosystems, still scarce studies have tackled their evolutionary history and diversity using genomic data. In this talk, I will present a new methodology, preliminary data, and the key objectives of the newly-funded Spanish National Project. This project aims to delve into the phylogenetic relationships of a major clade of gastropods, heterobranchs (ca. 44 000 species, including opisthobranchs, pulmonates, and other ecologically diverse groups), by applying a genomic, museomics, and all-family approach with 1000 targeted species. We will apply target enrichment of ultra-conserved elements (UCEs) as a paragon genomic technique for the effective assembling of up to 2259 genes using high throughput data of museum material, outperforming any other method currently available. I developed and successfully tested this novel genomic approach for a small selection of heterobranchs, thus possibly leading to ground-breaking changes in our understanding of snail and slug evolution and diversity. Using a comprehensive taxon sampling, five specific objectives will shed light on known controversies: 1) understanding the rise of Heterobranchia the early lineages or ‘lower heterobranchs’; 2) to ascertain the enigmatic position of Acteonimorpha, with implications in our understanding of body detorsion and the euthyneurous nervous system, with implications on Doridina and Cladobranchia nudibranchs; 3) investigating the relationships within Euopisthobranchia particularly regarding the benthic Cephalaspidea and the pelagic Pteropoda; 4) the examination of morphological and anatomical novelties towards the adaptation and transition to and from marine, limnic, and terrestrial habitats in Panpulmonata, providing a first genomic framework for Stylommatophora snails and slugs and the marine, solar-powered Sacoglossa; and 5) to test the universality of the UCE probe set against all six Gastropoda subclasses and the remaining seven classes of Mollusca, highlighting the use of museomic approaches. My overall scope aims at understanding the ecological and morphological adaptations of Heterobranchia through systematics. Overall, reconstructing the evolutionary history of heterobranch gastropods with advanced genomic data, benefitting from a thorough taxon sampling, will illuminate our understanding of the triggers of biodiversity thus facilitating ecosystem protection.

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## Connective tissue patterns in dorid nudibranch mantle

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There has been increased interest in characterizing the full molluscan scleritome, particularly the arrangements beyond traditional “shells.” Dorid nudibranchs often possess innumerable calcareous spicules. These spicules are unusual in that, rather than forming part of the outside surface, they are buried in the mesoderm. In non-molluscan taxa, the interaction between internal hard elements and fibrous connective tissue matrix is important for their function. However, comparatively little is known about this connective tissue layer in molluscs. To address this question, we used brightfield light microscopy and staining to investigate connective tissue arrangement and composition in the mantles of multiple dorid species.

All spicule networks were wrapped in connective tissue fibres. These sheaths were typically made of collagen, though stain affinities suggest that more than one fibre type may be used. Species differed in the amount of spicule (16.7–21.9%) and connective tissue (15.5–58.8%) in cross sectional area. These amounts varied somewhat independently of each other; i.e. there was no clear positive or negative relationship between the two traits. Arrangement of connective tissue varied across taxa. In some taxa (e.g. *Cadlina*) connective tissue is restricted to the area immediately next to the spicules. In others (e.g. *Platydoris*), the areas between spicule network layers were largely filled with diffuse collagen fibres mixed with ground substance and the mix of spicules and connective tissue nearly filled the mantle cross section. Extent and location of haemal channels also varied. Overall, differences among connective tissue patterns matched differences in spicule organization, and these groupings were similar to those shown in recent molecular phylogenies. Therefore, further investigation of skeletal networks may help clarify functional differences that contributed to dorid diversification.

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## Diversity and comparative taxonomy of Antarctophilinidae gastropods (Heterobranchia: Cephalaspidea)

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Recent studies have shown greater hidden diversity and high incidence of cryptic speciation in Antarctic marine gastropods. Originally, philinoid cephalaspideans in the Southern Ocean belonged to the genus *Philine*, but molecular and morphological studies have shown that three recently erected genera encompass all known diversity instead. These are *Antarctophiline*, *Waagelea*, and *Spiraphiline*, the first two belonging to the recently erected family Antarctophilinidae. In this study, 55 specimens were collected from the South Shetland Islands across the South-Atlantic Antarctic Ridge to Bouvet Island, and from South Sandwich Islands and Bransfield Strait, between 134–4548 m depth. Considering our current understanding of their diversity, we provide morpho-anatomical and phylogenetic analyses to describe two new *Antarctophiline* species. Moreover, we evaluate the morphological affinities of the most common Antarctic species, the *A. alata* species complex. A comparative morphological framework of key characters provides enough evidence to formally describe both species. In accordance, the molecular results support the validity of the two different species, matching with the morpho-anatomical differences observed in the digestive system (i.e., shape of gizzard plates and salivary glands), shell shape, and further external characters. Overall biogeographical distributions are discussed in a systematic context. Our study is yet another example of how Antarctica keeps revealing itself as a cornerstone of gastropod diversity.

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## Joining the dots: Punctoidea systematics and biogeography

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The superfamily Punctoidea traditionally included seven families of minute snails distributed worldwide, to which a possible origin in North America had been assigned. Its classification, however, was rather unstable and given the difficulty in acquiring and preserving specimens, a molecular phylogeny had never been attempted. We have started to build a phylogenetic framework for this superfamily, with a published phylogeny and further ongoing work on several fronts. The first results showed that Punctoidea was polyphyletic, comprising two unrelated groups: the Laurasian Discoidea (containing Oreohelicidae and Discidae) and the Gondwanan Punctoidea sensu stricto (Endodontidae, Cystopeltidae, Charopidae and Punctidae). The position of Laurasian Helicodiscidae could not be ascertained, but they do not belong in either of those groups, being more closely related to Arionoidea. The Discoidea originated in North America, with the earliest records being from the Late Cretaceous of Canada, and had expanded into Europe by the Late Palaeocene (records from France). In Punctoidea, the most basal branch is the Endodontidae, today restricted to Oceania and eastern Asia and with a poor fossil record. The next branch is a newly recognized family arrangement, the Cystopeltidae; it contains two subfamily-level clades, one Australian and one South American. Charopidae is paraphyletic (it contains Punctidae as its crown group); and ongoing work has showed that the earliest branch is a southern African clade, the Trachycystinae, followed by the New Zealand-endemic Flammoconchinae. By the Eocene, Cystopeltidae, Charopidae and Punctidae all occurred together in South America. Punctidae became extinct there, but one branch eventually reached North America and Europe. The early fossil record of these families elsewhere is poor and of difficult classification. Ongoing work is increasing the coverage of the first phylogeny, focusing on branches and taxa of particular interest. The main topics of investigation now are: 1) the monophyly of Trachycystinae and its phylogenetic position; 2) the relationship between New Zealand, Tasmania and mainland Australia “Charopidae”; 3) the validity of “Charopidae” subfamilies; 4) the number of dispersion events of “Charopidae” to South America; 5) the diagnosis of South American Cystopeltidae and “Charopidae”, including fossils; 6) the phylogenetic position of other supposed African Discoidea/Punctoidea.

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## Diversity of Symbiodiniaceae in Cladobranchia

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The vast majority of the 400 known cladobranch species (Heterobranchia, Gastropoda) establish a symbiosis (photosymbiosis) with dinoflagellates of the family Symbiodiniaceae. Cladobranchia obtain their photobionts through horizontal transmission, meaning that the symbionts must be acquired anew in each generation via their food source, which are photosymbiotic cnidarians. Most cladobranch species are considered to have an unstable photosymbiosis and the symbionts are usually digested or expelled after a couple of days. Yet, some species, such as *Melibe engeli* Risbec, 1937 and *Phyllodesmium briareum* (Bergh, 1896), are known to form a stable symbiosis with their algal symbionts and these photobionts can fully support the host for several months during starvation periods. The mechanisms involved in the formation of a stable photosymbiosis are well described in cnidarians, however marine slugs have been neglected in this regard. For example, the stability of the photosymbiosis, growth rate and temperature tolerance are related to the different clades of the photobiont present in cnidarians. In Cladobranchia, the Symbiodiniaceae clades and subclades involved in this symbiosis are still not characterised. Thus, determining the Symbiodiniaceae found in different cladobranch species can contribute to the understanding of this photosymbiotic system. Here, we give new insights into cladobranch associations with Symbiodiniaceae by sequencing regions of the Symbiodiniaceae ITS rDNA for several cladobranch species using Next Generation Sequencing. Our analyses revealed that cladobranchs with a stable photosymbiosis exclusively host the Symbiodiniaceae clade C that is known to have a high carbon fixation and to translocate high amounts of carbon to the host. Species with an unstable photosymbiosis mainly incorporate representatives of clade A. Clade A is considered to be potentially parasitic since cnidarians associated with this clade show a lower overall fitness due to its decrease in the release of fixed carbon products. Therefore, a key to a successful evolution of a stable photosymbiosis in Cladobranchia might be based on the association with certain Symbiodiniaceae clades such as clade C.

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## Diversity and biogeography of the deep-sea *Scaphander* gastropods

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The Cephalaspidea gastropod family Scaphandridae was recently redefined, and three genera of complex taxonomic history are currently considered valid, namely *Scaphander* Montfort, 1810, *Sabatia* Bellardi, 1876 and *Nipponoscapander* Kuroda & Habe, 1971. The genus *Scaphander* is the most diverse of the family and these snails are soft-bottom, mostly deep-sea dwellers characterized by their strong external shell and distinctive calcified gizzard plates. Their taxonomy has been traditionally based on empty shells and in this work, we built on previous systematics efforts and used an integrative taxonomic approach combining morphological characters, molecular phylogenetics, and molecular species delimitation methods to revise the systematics of the genus. Our results recognized 28 species of *Scaphander* worldwide and using fossil data, geographical distribution of species and Bayesian ancestral state reconstruction, we provide the first insights into the speciation and biogeographic history of the genus.

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## Alternative reproductive strategies (viviparity/oviparity) in the phylogeny of a hyperdiversified group of land snails

**Anna Sulikowska-Drozd<sup>1</sup>, Tomasz Mamos<sup>1</sup> & Katarzyna Janiszewska<sup>2</sup>**

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Phaedusinae constitute the most species-rich clausiliid subfamily, inhabiting eastern and southeastern Asia and the Pontic region. With funding from National Science Centre (2016/21/B/NZ8/03086), we reconstructed the phylogeny of the subfamily with a special focus on the evolution of various reproductive strategies within the group. Phylogenetic analysis that employed multiple mitochondrial markers (COI, 16S) and nuclear markers (28S, ITS1, ITS2, H3, H4) supported the monophyletic origin of the Phaedusini + Serulinini clade and the subsequent divergence of Phaedusini into several major lineages. Almost all of these lineages include both oviparous and viviparous taxa, suggesting the relative lability of the reproductive mode during Phaedusini divergence. Reconstruction of ancestral states of the reproductive mode revealed that transitions from oviparous to viviparous reproduction occurred independently and repeatedly. The high reproductive success of the intermediate strategy of embryo retention, detected in several species, may explain frequent changes in the strategy. In the shells of viviparous Phaedusinae, we identified adaptations that facilitate the passage of the rigid and inflexible embryonic shell of the offspring through the parental shell canal, among others: 1) reduction of the clausiliar apparatus including the clausilium, 2) widening of the shell canal and the clausilium plate, 3) decrease in the width of the embryonic shell. It appeared that clausiliid shells are shaped by opposing selection pressures: on the one hand, a tendency to produce large neonates, which requires widening the shell canal, and on the other hand, a need of protection of the parent body with strong apertural barriers, which provide protection, i.e., against attacks from malacophagous insects. As the fourth adaptation in viviparous taxa, we discovered the unusual protein-mineral structure of embryonic shells, which remains flexible and malleable until birth. In laboratory breeding colonies of 70 Phaedusinae species, we obtained unique data on their reproductive mode and other life history traits. In addition to direct observations of living snails, using  $\mu$ CT we identified many species that retained embryo in museum collections, allowing us to determine their reproductive strategy. We cautiously estimate that oviparous reproduction predominates in Phaedusinae, while even 20–30 % of taxa could be viviparous.

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# Diversity and phylogeny of the genus *Haminoea* Turton & Kingston, 1830 (Gastropoda, Heterobranchia, Cephalaspidea) in the Atlantic and eastern Pacific

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The marine herbivorous gastropods of the genus *Haminoea* have been long considered a group with a worldwide distribution dwelling along temperate and tropical shorelines, yet, recent studies found “true” *Haminoea* to be a radiation geographically restricted to the Atlantic Ocean (including the Mediterranean Sea) and the eastern Pacific Ocean, with one putative species occurring in temperate stretches of the coast of South Africa in the Indian Ocean. In total, 14 species of *Haminoea* are currently accepted as valid in recent scientific literature and field guides. However, we could identify 48 nominal names, most of uncertain taxonomic status. In the current work we generate the first comprehensive phylogeny of the genus *Haminoea sensu stricto* based on multilocus molecular characters using a taxon set accumulated over a period of 15 years, which we believe to cover the entire diversity of the genus and include a good geographical coverage of the distribution of species. Our main goals are to define the number of valid species in *Haminoea* and give insights on their relationships and geographical distributions, while generating a framework for future detailed systematic reviews of the genus and to study the processes underlying speciation and historical biogeography.

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## Between sea angels and butterflies: a comprehensive phylogeny of pteropod molluscs

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Pteropods are a group of holoplanktonic gastropods that are of special interest because of their role in food webs and carbon cycling in marine ecosystems. In addition to their relevance for ecosystem functioning, some organisms of this group (Thecosomata and Pseudothecosomata) are indicators of ocean acidification due to the high sensitivity of their shells to calcification processes. Because of global change, conserving the biodiversity of pteropods is key to maintaining the balance in these ecosystems and therefore studying their taxonomy and genetic diversity would be relevant for a better understanding of the possible effects of rising temperatures, deoxygenation, or acidification in the oceans.

In this study, we covered the most comprehensive phylogenetic study of the order Pteropoda to date. Our taxon sampling includes ca. 80 species (up to 800 specimens) from nearly all oceans, gathered from GenBank and ZSM collections. Moreover, freshly collected species from the Mediterranean Sea (ca. 15 specimens and 6 genera; *Creseis*, *Pneumodermopsis*, *Hyalocilis*, *Cavolinia*, *Cymbulia* and *Clio*), a poorly known area, are included in this worldwide phylogenetic framework for the first time. We compared three already sequenced molecular markers that show differences at genus and species level, i.e., 28S rRNA, histone H3 and cytochrome *c* oxidase I. Both Bayesian inference and maximum likelihood show strong support at higher taxonomic rankings and has allowed us to definitively close several open questions in the phylogeny of Pteropoda. Moreover, we identify complexes of cryptic species thus providing the broadest view yet of the diversity of this group.

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## **Symposium**

# **Systematics and Evolution of (not only marine) Heterobranchia**

## **Part II**

### **Abstracts of poster presentations**

## Formalin-fixed specimens, not a fruitless endeavour? – method based discussion on formalin-fixed extractions and sequencing

**Lynn J. Bonomo<sup>1,2</sup>, Athena W. Lam<sup>1</sup>,  
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Formalin-fixed specimens were a standard preservation method for museum specimens, necessitated by the need for high-quality morphological preservation. This preservation method was especially common for soft-bodied marine invertebrates, particularly nudibranchs, into the 1990s. Unfortunately, formalin causes many issues for molecular work as it causes cross-linking, fragmenting, and modifying bases. These older specimens are often the only available specimen for a species, and obtaining molecular data from them is essential for resolving phylogenetic questions. Using a formalin extraction method developed by ichthyologists, we extracted DNA from two formalin-fixed nudibranchs and did low-coverage whole-genome sequencing. The low-coverage whole-genome sequencing was done with both double-stranded and single-stranded library kits. This poster presents new methods and complications of this protocol and aids in discussing new techniques or methods to improve results.

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# **One colour, many faces: Integrative taxonomy reveals the occurrence of a complex of species in the reddish runcinid *Runcina ferruginea* Kress, 1977 (Heterobranchia, Runcinida)**

**Juan Lucas Cervera<sup>1,2</sup>, Ana Karla Araujo<sup>1</sup>, Marta Pola<sup>3</sup>,  
Manuel A. E. Malaquias<sup>4</sup> & Fabio Vitale<sup>5,6</sup>**

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The species *Runcina ferruginea* Kress, 1977, first described from Plymouth (England), belongs to one of the less known groups of sea slugs, the order Runcinida, and is a paradigm of the challenges facing the taxonomy of this small group of heterobranch sea slugs. This species is characterized by a plain reddish-brown colour with fine dark brown spots spread over an elongated body. A broad molecular study of European Runcinida revealed the possible occurrence of multiple lineages under the name *Runcina ferruginea*. The aim of this study is to investigate the taxonomic status of this species combining multi-locus molecular phylogenetics and morpho-anatomical characters. To aid in species delimitation, the ABGD and COI *p*-distances analyses were employed. Our results revealed the existence of a complex of four candidate species: *Runcina ferruginea*, *Runcina zavodniki* and two undescribed species, one belonging to the genus *Runcina* and the other to an undescribed genus.

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## Choose your Ocean: speciation and evolution of boreal and Arctic *Coryphella* (Heterobranchia: Nudibranchia)

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The species delineation is particularly acute in boreal seas, which are often inhabited by species with broad geographic ranges and high degrees of intraspecific morphological and molecular diversity. Environmental conditions in boreal and Arctic regions significantly changed multiply times in recent past, promoting the formation of geographic barriers and leading to allopatric speciation events. Unlike with sympatric species, no separation in ecology happens, and truly cryptic species can be formed with overlapping morphological variation, but significantly distant genetically.

In this study we studied the systematics and phylogenetic relationships within wide spread genus *Coryphella* (Heterobranchia: Nudibranchia: Cladobranchia). For this purpose, we used a set of 5 standard mitochondrial and nuclear markers: COI, 16S, H3, 28S and 18S and a large variety of species delimitation (ABGD, GMYC, bPTP), phylogenetic and phylogeographic methods (i.e., population analysis; ancestral area reconstruction). The morphological analysis included standard morpho-anatomical examination using the light microscopy and scanning electron microscopy.

Based on our integrative results we identified cases of true amphiboreal species, but also cases of cryptic species being formed allopatrically following Pliocene-Pleistocene Climate Change. Ancestral area reconstruction (AAR) provides evidence for a Pacific origin of the genus *Coryphella*. Different trans-Arctic lineages of the genus demonstrate different levels of genetic differentiation and, according to our molecular clocks calibration, different divergence times. This indicates that the invasion of the Arctic and Atlantic regions occurred multiply times starting from the first opening of the Bering Strait in late Miocene. In the North Atlantic we detected several species evolved in sympatry during the Pleistocene and suggest ecological-driven speciation events in this case.

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# **Teasing apart cryptic species: A revision of the *Melampus bidentatus* (Panpulmonata; Ellobiidae) species complex**

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The coffee bean snail *Melampus bidentatus* is a medium sized (shell height: 0.6–1.5 mm) snail that occurs in high densities (>1000 individuals/m<sup>2</sup>) in salt marshes along the North American Atlantic- and Gulf coasts, and in the Caribbean. Recent genetic work has shown that *M. bidentatus* is a complex of three apparently cryptic species (provisionally named North, South & Gulf) with disjunct and occasionally overlapping distribution ranges. In order to assign proper scientific names to the three cryptic species, we checked the type localities of the various synonyms of *M. bidentatus* sensu lato and sequenced specimens from localities where the specific identity couldn't be inferred from already existing data. In order to try to separate the cryptic species, we calculated a Principal Component Analysis and a Canonical Variance analysis using a) a set of 11 landmarks and b) the outline of the shell, simplified to 500 landmarks. These morphological comparisons did not separate the three species, suggesting that their genetic divergence has not been accompanied by changes in outward appearance.

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## New data on taxonomy of phyllidiid nudibranchs (Heterobranchia: Phyllidiidae) and biodiversity in Southern Vietnam

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Nudibranchia is a group of shell-less and strikingly colored marine molluscs, encountering more than 4000 species. They are distributed worldwide with the highest diversity in the Indo-West Pacific region. Family Phyllidiidae is the less typical of all families included in Nudibranchia. Their unique traits are the lack of radula and partly external digestion. Phyllidiidae has always been a complicated group for taxonomical studies. Due to lack of the radula the species identification is much more difficult than in other nudibranch families. Despite some several recent works using molecular methods have been published, the taxonomy of the family is a challenge due to a great number of true cryptic species. In the present study, we observed diversity of Phyllidiidae in Southern Vietnam using an integrative approach included traditional morphological (anatomical research, SEM) and modern molecular (phylogenetic analysis, species delimitation) methods. The material was collected in 2016-2021 from Nha Trang Bay and Spratly Islands in Vietnam waters using SCUBA diving and snorkeling. A cryptic diversity was shown within several genera, e. g., the species *Phyllidiella pustulosa* (Cuvier, 1804) is represented by nine highly divergent clades.

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## Evolutionary processes leading to terrestrial invasions by ellobiid snails

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Invasion of the land by aquatic animals is one of the most significant events in Earth's history of life. Gastropods have been remarkably successful in this habitat shift with more than ten independent clades of terrestrial taxa. We have been investigating the evolutionary processes of terrestrial invasion in the eupulmonate snail family Ellobiidae; many ellobiids inhabit intertidal to supratidal zones of salt marshes, mangrove swamps and rocky shores worldwide, whereas all species of the subfamily Carychiinae and a few in the genus *Pythia* of the subfamily Pythiinae are fully terrestrial and are supposed to have independently colonized the land.

Our phylogenetic reconstruction for the superfamily Ellobioidea revealed that previous studies were hampered by significantly different evolutionary rates of mitochondrial genes. Nuclear-gene phylogeny as well as mitogenomic trees without long-branched taxa recovered genera with multispiral protoconchs (e.g. *Melampus*, *Pedipes* and *Microtralia*) paraphyletic to a robust clade with paucispiral protoconchs (Carychiinae + Ellobiinae + Pythiinae). New observation on egg masses, rearing of hatched larvae and population genetic analyses suggest that most marine species of the latter clade develop as short-lived pelagic larvae with or without phytoplankton feeding. These findings imply successive transition from ancestral, long-lived planktotrophs to short-lived lecithotrophs, and finally to benthic development that paved the way for the colonization of inland habitats.

A separate phylogenetic reconstruction for the species of *Pythia* revealed the acquisition of benthic development and succeeding terrestrial invasion in two independent lineages within the genus. We are currently focusing on one of the terrestrialization events that occurred on young oceanic islands in Okinawa, Japan. The *P. nana* species complex, which is sister to *P. pachyodon* with a pelagic larval phase, provides us a unique opportunity to elucidate the evolutionary process of terrestrialization from ontogenetic, ecological and physiological perspectives at a short evolutionary time scale in the Quaternary.

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## Phaedusinae versus Clausiliinae and Alopinae: similarities and differences in the structure of the reproductive system

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The widely distributed and species-rich Clausiliidae family that includes snails differing in reproductive modes was the focus of the National Science Centre project (2016/21/B/NZ8/03086) conducted in the years 2017–2022. Among others, we investigated the anatomical, histological and ultrastructural structure of the genital system in oviparous and embryo-retaining species classified mostly in the east and southeast Asian subfamily Phaedusinae and compared the results with data on members of the European subfamilies Clausiliinae and Alopinae. Histological examinations of selected species of Phaedusinae showed that in oviparous and embryo-retaining species the structure of the spermoviduct and the free oviduct is very similar. In the spermoviduct, the prostate is clearly visible with epithelial cells and subepithelial secretory cells containing numerous granules (serous cells). Next to the prostate, there is the autospermiduct, a partly open duct whose wall is made up of a layer of epithelial cells. Another partially open duct visible in the spermoviduct is the oviduct, composed of a layer of epithelial cells and a subepithelial layer of mucous cells. In any examined Phaedusinae member, we did not record a typical allospermiduct, which is observed in Clausiliinae and Alopinae as the third, partly open duct, parallel to the autospermiduct, composed of the epithelial layer and serous subepithelial cells. Ultrastructural studies (TEM) allowed us to identify a new additional structure located opposite the autospermiduct. The epithelial cells that form the wall of the newly observed channel are an extension of the epithelial layer of the autospermiduct. In addition to typical ciliated epithelial cells, it includes mucus cells and cells that accumulate homogeneous secretion. These cells proliferate and form a pool of cells that produce or accumulate numerous vesicles, then they are transformed into giant cells filled with a clear fluid. The observed structure seems to function as a secondary allospermiduct.

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## Seasonal differences in the distribution, size, and population density of the nudibranch *Nanuca occidentalis*

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The distribution, seasonality, and life history of many heterobranch sea slugs remains unexplored. *Nanuca occidentalis* is a carnivorous Aeolid nudibranch from the Caribbean that feeds on the polyps of hydroids and sequesters juvenile nematocysts. Individuals have been frequently observed throughout Tampa Bay, Florida, most often on the hydroid *Eudendrium carneum*. Although *N. occidentalis* is relatively common, little is known about the potential relationship between their life cycle and population density. A study was launched to document the fluctuations in seasonal abundance of *N. occidentalis* on the hydroid *E. carneum* over the course of a year. Every month four hydroid colonies of *E. carneum* were collected from Tampa Bay, FL. These colonies were transported to the lab and through the use of dissection microscopes were searched for sea slugs. All observed sea slugs were identified to species and measured. Sea slug species richness was highest in June 2021, but a total of 16 different species of heterobranch sea slugs were identified during this study. Most species were other Aeolid nudibranchs; however, sacoglossans, headshield slugs, and sea hares were also found. The highest abundance of *N. occidentalis* was found in July of 2021 and the lowest occurred in January of 2022. Higher *N. occidentalis* densities were found during the summer months, which is also when slugs were the smallest in size; this suggests that spawning events are primarily occurring in the spring or early summer. Patterns observed in *N. occidentalis* seasonal distribution, size, and population density may all be directly related to their life cycle, but further research is necessary to confirm these findings. This study provides valuable baseline data on the seasonal population fluctuations of a common, but poorly studied nudibranch.

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## Oh my God! Really? No way! Psychedelic sea slugs: Observations on colour ontogeny on nudibranchs from the genus *Nembrotha* (Polyceridae, Nembrothinae)

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Nudibranchs are sea slugs known for their extravagant colouration and diversity of shapes. In recent decades, thanks to the use of integrated taxonomy, the traditional recognition of a species based on observation and identification of the colors of these animals, has become increasingly difficult, mainly due to the presence of cryptic species complexes. Thus, species with the same color pattern can be genetically differentiated species. But things can get even more complicated. What happens when the same species radically changes its color pattern as it ages? Here we present two extraordinary examples in species of the genus *Nembrotha*.

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## Does colour count? – Morphological investigations and an overarching morphological phylogeny of the genus *Phyllidia* Cuvier, 1797

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The genus *Phyllidia* Cuvier, 1797 (Mollusca, Gastropoda, Nudibranchia) comprises 29 accepted species and is the largest and most widespread genus within the Phyllidiidae Rafinesque, 1814. While these colourful and conspicuous nudibranchs have been studied for 225 years, the taxonomic order within this genus is still a matter of debate. As several species of the genus *Phyllidia* are suspect of pharmaceutical studies, evaluating their toxins for pharmacological usability, knowledge of the evolutionary relationships within this genus and a coherent taxonomy of its species is crucial. Here we show that colour patterns – while being of historical focus and immediate prominence – need to be accompanied by thorough morphological and anatomical information in species descriptions and phylogenetic studies regarding this genus. Especially the anatomy of the genital apparatus, the rhinophoral morphology and overall patterns and morphology of the notal tubercles seem to be important characters in phylogenetic analyses. The ventral position of the anus, used to distinguish between the genera *Phyllidia* and *Fryeria* Gray, 1853 (synonymized with *Phyllidia*), is seemingly paraphyletic as previously shown in molecular investigations.

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# **First occurrence of the genus *Pleurobranchaea* Leue, 1813 (Pleurobranchida, Nudipleura, Heterobranchia) in British waters, with the description of a new species**

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In the North-eastern Atlantic and Mediterranean the pleurobranchid genus *Pleurobranchaea* is represented by two species, *Pleurobranchaea meckeli* (Blainville, 1825) and *Pleurobranchaea morosa* (Bergh, 1892). The former is a well-known species distributed from northern Spain to Senegal (including the Macaronesian archipelagos) and the Mediterranean, while the second is a poorly described species erected on the basis of a single specimen that has never been seen following the original description. In this contribution, species delimitation analyses (ABGD and COI/16S *p*-distances) have identified a third undescribed *Pleurobranchaea* species from samples collected in south-western UK waters and the Gulf of Cadiz (SW Spain). This species is characterized by an oval body with a large rough mantle forming irregular polygons delimited by shallow grooves. The ground colour is translucent with a minute cream to brown pigmentation, which may not always be present, as well as opaque white specks irregularly spread all over the mantle. The oral veil has few digitations on its anterior edge and the posterior region of foot is rounded and without a caudal spur. The British specimens constitute the first occurrence of the genus *Pleurobranchaea* in UK waters.

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## A new illustrated fauna for the Swedish species of Eupulmonata, Hygrophila and Pylopulmonata

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A new Swedish Fauna for the Eupulmonata (126 species), Hygrophila (34 species) and Pylopulmonata (33 species) is in production. It will be published in 2022 as a volume in the series *The Encyclopedia of the Swedish Flora and Fauna* (Nationalnyckeln), produced by the SLU Swedish Species Information Centre (Artdatabanken). This will be the first complete fauna for land and freshwater snails covering Sweden, that has been published for more than 100 years. The chapter on species of marine pylopulmonates will be the first ever published for the country.

The volume contains detailed descriptions of the morphology, variation, and ecology of all species as well as distribution maps (for the limnic and freshwater species), and photos of all species from several angles. Beside photos of shells, more than 90 % of the species in Eupulmonata and Hygrophila are covered with live photographs. For Pylopulmonata that figure is 40 %. In appropriate cases also anatomical details are described and illustrated. In the introductory sections the systematics, anatomy, ecology, and reproduction etc. are treated. Determination keys for adult specimens are presented in Swedish and English. Limnic and terrestrial species of the Neritimorpha and Caenogastropoda (9 species in total) are included in an appendix. Hot-house species and temporary introductions are briefly mentioned. The Valvatidae (4 species) has been treated in a former volume).

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**Symposium**

**The Ecology of Fossil  
and Extant Molluscs**

**Part I**

**Abstracts of oral presentations**

## Belemnite growth rates and trace elemental composition across the Pliensbachian-Toarcian boundary event

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The Pliensbachian-Toarcian boundary event has a marked impact among nektonic, benthic, and planktonic communities seen by extinction of some taxa, changes in their diversity and in their species richness. This event resulted in belemnite body-size reduction in assemblages and dominant species at the Toarcian GSSP, but it remains unclear to what degree this lilliput effect reflects changes in growth and reproductive strategies. The concentric low-Mg calcitic increments on the rostrum reflects the belemnites growth, that have been secreted in daily monthly chronological rhythms. Combining sclerochronological analysis and thigh-resolution elemental records from the rostrum yields valuable insights into the influence of the Pli-Toa warming event on the organisms' physiological and morphological features. Six specimens of two belemnite species (*Catateuthis longiforma* and *Passaloteuthis bisculcata*) were analysed from three different stratigraphic levels (before, during and after the Pliensbachian-Toarcian boundary) in the Peniche section of the Lusitanian Basin in Portugal. The current work revolves around understanding: a) whether there are any differences in the environmental preferences seen within the species and ontogeny across the Pliensbachian-Toarcian boundary, and b) whether the trace elemental records are investigating the physiological and ontogenetic effects, or major changes in the ambient conditions. The limitation on belemnites increments visualisation has been resolved via applying three different methods for the increments' width measurements and growth lines counting; polished sections, thin-sections under translucent light, and Mutvei's solution-stained sections. Between the species there was a clear environmental preference shown by a high number of the secreted growth lines before the Pli-Toa crisis in *Passaloteuthis bisculcata*, and during the event in *Catateuthis longiforma* which was also marked by a significant body-size reduction, this is reflecting the different environmental preferences and thermal niches between the two species. The proposed daily and monthly chronological origin behind the secreted growth layers would reduce the impact of the Pli-Toa warming event on the trace element records. The trace elemental records were indicating physiological and ontogenetic effects.

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## High-resolution multi-proxy reconstruction of marine primary production using the short-lived bivalve *Pecten maximus*

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Marine phytoplankton contributes significantly to ecosystem functioning as it forms the basis of marine food webs influencing higher trophic levels. Moreover, photoautotrophic organisms are a key element in global oxygen production and carbon fixation. Climate change, increased anthropogenic inputs into marine ecosystems of artificial fertilizers and pollutants can have dramatic consequences on phytoplankton community structures leading to eutrophication events and harmful algal blooms, which in turn affect commercial fisheries as well as marine biodiversity. Understanding the temporal and spatial dynamics of phytoplankton of the past is an essential task to identify long-term trends and anthropogenically induced impacts.

As demonstrate here, barium, molybdenum and lithium in shells of the fast-growing bivalve *Pecten maximus* (Bay of Brest, France) serve as proxies for phytoplankton dynamics. Highly-resolved trace element time-series of barium-to-calcium ( $Ba/Ca_{shell}$ ), molybdenum-to-calcium ( $Mo/Ca_{shell}$ ) and lithium-to-calcium ratios ( $Li/Ca_{shell}$ ) show remarkably synchronous peaks among contemporaneous specimens. Sclerochronological analyses of modern shells from 2011, 2012 and 2019 combined with detailed phytoplankton monitoring data revealed that the timing and magnitude of such trace element peaks are linked to short-term phytoplankton blooms.  $Ba/Ca_{shell}$  patterns agree with the occurrence of various phytoplankton species, while peaks in  $Mo/Ca_{shell}$  and  $Li/Ca_{shell}$  time-series seem to be indicative of specific dinoflagellate and diatom species as well as periods of nutrient limitation. A short time lag of one to two weeks exists between the environmental signal and the geochemical response in the shell calcite. Accordingly, shells of *P. maximus* record important information about the temporal dynamics of phytoplankton.

Our results refine the applicability of a multi-proxy approach for the reconstruction of past phytoplankton dynamics using different trace element chronologies obtained from shells of *P. maximus*.

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# The latitudinal gradient of functional diversity of miocene mollusks from Chile

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Understanding latitudinal variations in diversity is central for biogeography. Along the coasts of the South-east Pacific, several taxa show inverse latitudinal patterns of diversity, that is, increasing species numbers from lower to higher latitudes. A plausible explanation for these patterns is that fjords, formed during the Pleistocene glaciations, generated niche opportunities that allow for higher diversity in high latitudes. Testing this hypothesis requires to analyze functional diversity (which is intimately related to niche use) and latitudinal patterns of biodiversity in the absence of fjords, that is, earlier than their formation. Here we test if the fossil record before the generation of fjords shows higher functional diversity at lower than at higher latitudes (a “regular” diversity gradient).

We analyzed four components of functional diversity (functional richness, functional divergence, functional dispersion, and functional evenness) for a fossil marine mollusk fauna from the lower Miocene (~18 million years ago) across four regions spanning more than 10 latitudinal degrees of the Chilean coast (between ~34°S and 45°S).

According to our prediction, functional richness of gastropods and bivalves decreased from low to high latitudes. Contrarily, functional evenness of gastropods remained relatively constant across the region and that of bivalves peaked at 45°S. Multivariate analysis uncovered a latitudinal pattern in the abundance of gastropods, but not that of bivalves.

These results suggest that niche breadth might have decreased with latitude in the lower Miocene. In general, functional richness decreased towards higher latitudes, contrasting today's inverse latitudinal patterns. However, the latitudinal patterns of functional diversity were far from linear, especially when species abundances are considered. This work can shed light into the mechanisms underlying the latitudinal variation of diversity observed today by offering a better understanding how these patterns changed over time.

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## Within-host ecological shift does not mean within-burrow coexistence for two bivalves commensal with mantis shrimp

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A classic question in marine biology is the nature of within-ocean basin diversification for species with high-dispersal larval development. Symbiotic relationships have been shown to not only be a key mechanism in the sympatric diversification of closely related marine species in benthic systems but also have significant ecological implications. The bivalve superfamily Galeommatoidea has served as a model system for investigating mechanisms of marine diversification in symbiotic benthic species, and commensal galeommatoideans are assumed to speciate through dynamic intra- and interphylum host switching. In contrast, *Divariscintilla*, a galeommatoidean genus of mantis shrimp commensals, shows evidence of within-host speciation. Seven congeners are commensal with a single mantis shrimp host in eastern Florida. Two of the seven form a cryptic sister species pair consisting of a burrow wall commensal, *D. octotentaculata*, and an ectocommensal, *Parabornia squillina*. *P. squillina* may have speciated through a within-host ecological shift from an ancestral commensal lifestyle on the host burrow wall to a derived ectocommensal lifestyle on the host body, emphasizing the importance of ecology and phylogeny in understanding within-host speciation and the contemporary coexistence of symbiotic marine species. This study examines the ecology of this genus by investigating the relative distribution of congeners across available host burrows in the Indian River Lagoon as well as their trophic ecologies and their micro-spatial distributions in a lab experiment. We found clustered colonization patterns among burrow wall commensals and differential colonization of sister species *D. octotentaculata* and *P. squillina* where *D. octotentaculata* is not found to co-occupy burrows with ectocommensal *P. squillina* and vice versa. Analyses of trophic niches showed neither significant trophic niche differentiation among commensal species nor evidence of sharing resources with their host. Artificial burrow observations showed no evidence for micro-spatial separation but found that the host-commensal relationship can breakdown resulting in commensal mortality through predation. These distributional, trophic, and behavioral data give new insights to the ecology of this genus and lay the groundwork for investigating possible control mechanisms of coexistence, such as differential larval recruitment or juvenile survival, in these closely related commensal bivalves.

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# **Mesh-size effect as bias in palaeoecological interpretations of fossil mollusc-dominated assemblages collected by different sampling methods**

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The choice of the used sampling method for palaeoecological estimations of fossil assemblages can introduce a major bias and hence, lead to wrong interpretations. This study has focused on the Late Triassic Cassian Formation because the fine-marly sediments and very good preservation allow to conduct both major sampling techniques, bulk sampling with subsequent wet-sieving and surface sampling. Therefore, a comparison of palaeoecological estimations regarding sampling method is possible. Various Cassian collections have been analysed comprising different fossil mollusc-dominated assemblages from a range of different palaeohabitats. Regression analyses indicate that species-abundances vary significantly across bulk and corresponding surface samples. In addition, the size of fossils differs with very small specimens and species dominating in the bulk samples and larger fossils characterising the surface samples. Rank conservation, i. e. the abundance rank space in an assemblage occupied by a certain species, is also different in many cases, because very small species and specimens which dominate in bulk samples are missing in the corresponding surface samples. These patterns can be recognised regardless of the palaeoenvironment looked at. This bias concerning palaeoecological interpretations can be called a mesh-size effect, since fossils smaller than about 3 mm (the average eye resolution for picking fossils in the field by the naked eye) cannot be found and hence, collected via surface sampling; however, for wet-sieving of bulk samples every desired mesh size can be used in the laboratory, which was 0.5 mm as lower limit in the study presented here. Therefore, the mesh-size effect bias has to be kept in mind when using surface samples for diversity and palaeoecological characterisations of fossil assemblages.

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## Environmental forcing on bivalve shell microstructure – a case study of *Arctica islandica*

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Bivalve shells come with a great variety of microstructures. Traditionally, microstructural properties of shells were predominantly studied to assess phylogenetic relationships and evolutionary processes. Recently, however, image analysis techniques have evolved greatly and enabled fast quantitative microstructural analyses of scanning electron microscopy images. Based on such data it was demonstrated that changes of the shell microstructure are partly controlled by environmental variables. Morphometric analysis of the microstructural units may thus be used to infer environmental information and increase the understanding of biomineralization processes. Here, we present results of quantitative microstructural analyses performed on *Arctica islandica* shells collected at different sites of the northern European shelf and specimens raised in the laboratory under controlled conditions. We observe that the size of individual biomineral units of *A. islandica* shells gradually increased with water temperature. This relationship can potentially be used to infer paleotemperature variability. Unfavourable growth conditions such as low salinity and/or low dissolved oxygen content, however, led to the formation of smaller, acute and elongated biomineral units overprinting any temperature signals potentially recorded in the shells.

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## **Paleocene molluscan macrofauna from an Outer Carpathian deep-sea hydrocarbon seep deposit and the Paleogene history of seep faunas**

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We report an ancient seep deposit and associated molluscan fauna from Paleocene deep-sea environments of Bystrice, Czech Republic (Outer Western Carpathians). The studied rocks belong to the Frýdlat Formation of the Subsilesian nappe. Dinoflagellate cysts *Cerodinium speciosum*, *Palaeocystodinium golzowense*, *Palaeoperridinium pyrophorum* indicate Seladian age of the sediments. The deposit comprised three metre-scale carbonate boulders, which are interpreted to have formed at ancient hydrocarbon seep based on carbonate textures and presence of  $\delta^{13}\text{C}$ -depleted biomarkers typical for methanotrophic archaea. Benthic macrofauna found within the carbonates is of low diversity (3 species) and composed exclusively of molluscs normally associated with seep environments. The most common in the assemblage is a small (ca. 1 cm long) thyasirid bivalve, forming mass accumulation of thousands of clustered individuals. It is accompanied by large abyssochrysoid gastropod (possibly a species of *Abyssomelania*) which is nearly as abundant as the thyasirid. The third mollusc in the assemblage is a yet unidentified species of lucinid bivalve. The composition of the molluscan fauna, with the abundance of thyasirid bivalves and abyssochrysoid gastropods, is similar to that of some Paleogene and many Late Cretaceous seep faunas, where both groups occur and are often especially common. This find and its palaeontological context illustrates that during large part of the Paleogene seep faunas retained their Cretaceous composition and that Paleogene emergence of bathymodiolin and vesicomyid bivalve-based seep faunas was a step-wise proces.

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# A novel trophic archive: High-resolution compound-specific amino acid (CSIA) $\delta^{15}\text{N}$ analysis of bivalve shells (*Arctica islandica*)

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Stable nitrogen isotope analysis ( $\delta^{15}\text{N}$ ) of bulk tissues is widely used for reconstructing the diet and trophic position of organisms. However, bulk  $\delta^{15}\text{N}$  analysis could be influenced by the metabolism of the animal and the environmental nitrogen baseline ( $\delta^{15}\text{N}$  baseline). Compound-specific stable isotope analysis of amino acids (CSIA-AA), specifically nitrogen ( $\delta^{15}\text{N}_{\text{AA}}$ ), overcomes such challenges by refining the trophic position of organisms and determining the ecosystem  $\delta^{15}\text{N}$  baseline from any organism of the food web. Applying CSIA-AA to bivalve shell carbonate-bound organics in combination with growth pattern analysis (bivalve sclerochronology) could extend this information back in time and reconstruct past ecosystem baselines and changes of the TP of bivalves. However, due to the low content of AA in bivalve shells, the measurement of  $\delta^{15}\text{N}_{\text{AA}}$  values is extremely challenging. Here, we present a refined analytical technique for calibrating  $\delta^{15}\text{N}_{\text{AA}}$  data obtained by gas chromatography-combustion-isotope ratio mass spectrometry. We also optimized the demineralization method by comparing three demineralization methods. Furthermore, we used the refined analytical technique and sample pre-treatment procedure to generate the first high-resolution (temporal resolution of 1 to 3 years per sample, subject to further improvement) sclerochronological  $\delta^{15}\text{N}_{\text{AA}}$  series of an *Arctica islandica* specimen. The  $\delta^{15}\text{N}_{\text{AA}}$  data from the inner portion of the outer shell layer show a gradual TP decline with increasing ontogenetic age, suggesting a change of food preferences through lifetime from organic detritus at the seafloor to pelagic phytoplankton. Our findings propose a novel, high-resolution proxy (i. e.,  $\delta^{15}\text{N}$  of phenylalanine [source AA],  $\delta^{15}\text{N}_{\text{Phe}}$  in shell carbonate-bound organics of ontogenetic older shell portions) for the reconstruction of the nitrogen isotopic baseline that supports the ecosystem. In addition, the new technique opens promising potentials to reconstruct finer-scale historical changes of the origin and fate of nitrogen in aquatic food webs.

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## Mating behaviour and genital anatomy in three species of *Ambigolimax* slug

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We have made numerous video-recordings of the matings of three slug species in the genus *Ambigolimax* (earlier considered part of *Lehmanna*). Some pairs were immobilised rapidly with freezer spray, then dissected. One aim was to relate the structures in retracted genitalia with their configuration and function when everted during copulation. In all three species, one slug follows the other before the leader doubles back, so that they assume a yin-yang configuration. Within seconds the animals twist together and each partially everts its penis. After some seconds in this state, penis-to-penis contact triggers almost immediately (e. g. within 0.06 s) explosive eversion of the rest of the penis, thereby swapping sperm reciprocally. Rapid penis retraction is followed by the slugs writhing together for up to about a minute. The smallest and simplest penis is that of *A. parvipenis* (name here disclaimed for nomenclatural purposes). Initial eversion of the penis forms a round knob. Penis-to-penis contact triggers explosive further eversion of the penis from the anterior half of this knob into a slight hollow on the posterior half of the partner's knob (corresponding in the retracted state to two flaps within the penis). Here the sperm is deposited. Within 0.5 s of the start of the explosive phase the penes have already separated. In *A. valentianus*, the flaps inside the penis are protruded to form a sucker-like structure initially. Penis-to-penis contact triggers further penial eversion: one part punches into the partner's penis (complete within 0.16 s); from this the innermost part of the penis everts, depositing sperm, followed directly by the penial appendage everting around the partner's penis. This explosive phase takes only 0.3 s. Retraction completes within 3.8 s. In *A. melitensis* the explosive phase is slower and takes longer. In a disjunct phase subsequent to sperm exchange, the penial appendage everts over the partner's head. Both *A. valentianus* and *A. parvipenis* may remate repeatedly the same evening, in one instance just 30 min after a successful copulation. *Ambigolimax valentianus* and *A. parvipenis* attempt to copulate with each other; *A. valentianus* often fully everts its penis unilaterally, but the sperm is lost.

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## Comparison of the effectiveness of barrier control measures for invasive slugs

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*Arion vulgaris* (Moquin-Tandon, 1855) is a common pest slug capable of causing high damage to agricultural and horticultural plants. The species was included in the list of invasive species in Lithuania in 2016. Many different measures were recommended to control them, but the task of this research was to determine and compare the effectiveness of environmentally friendly barrier measures. In addition to previously recommended measures such as copper strips and zinc tin-sheet metal barriers, a new barrier control for slugs, invented in Germany and available since 2016 – Schnexagon paint – has been included. Experiments were provided in the field – in Vilnius University Botanical Garden during Bachelor study in 2020 from August until September. Three barrier's surfaces were used: zinc, unplanned wood and plastic. The height of the barriers was at least 20 cm. Objects in contact with the barriers were removed and the barriers were inspected regularly to ensure that the plants growing nearby did not allow the slugs to move to the other side of the barrier with their leaves or other parts. The aim of the experiment was to determine how vegetables will be damaged in 10 days in enclosures madden from different barrier's surfaces and protected by different measures. Each study variant was performed 3 times. The results showed different efficiencies of the applied measures. Schnexagon was the most efficient for the first twenty days (by applying the paint once on a smooth surface). Later efficiency declined and finally reached 72 %. Copper strip was less effective (58 %), and the efficiency of zinc tin-sheet was not confirmed at all. Much less efficiency was achieved when Schnexagon was applied to an uneven surface – on unplanned wooden boards it reached only 43%. It was found that after rain, the continuity of the copper strip on unplanned wooden boards was always damaged (it simply bounced off the uneven surface). In conclusion, Schnexagon was the most effective barrier for the first 20 days when applied on a flat surface. There are two ways to extend its effectiveness – to apply additional paint and to protect Schnexagon paint from direct weather factors.

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# Molluscs from the Late Cretaceous hydrothermal vent deposits from Cyprus: A glimpse into Mesozoic hydrothermal vent ecosystems

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The Troodos Ophiolite from Cyprus is a 91 million-year-old (Upper Cretaceous; Turonian) piece of Tethyan ocean seafloor that contains numerous ancient hydrothermal vent deposits. Six of these (Kambia, Kinousa, Memi, Peristerka, Kapedhes and Sha) contain relatively well-preserved pyritized fossils: worm tubes (in all six sites), gastropods (in three sites: Kambia, Kinousa, Memi), and single gaudryceratid ammonite (Kambia only). Surprisingly, there are no bivalve taxa recovered from the Cypriot vent deposits so far. All gastropods belong to Abyssochrysoidea with three species of *Desbruyeresia*, two species of *Paskentana*, and one species of *Hokkaidoconcha*, *Ascheria*, and *Cypriiconcha*. The gastropod fauna contains the first representatives of the genera *Desbruyeresia*, *Hokkaidoconcha*, *Ascheria*, and *Paskentana* from hydrothermal vents, and also the youngest representative of *Paskentana* in any environment. The intriguing feature of the Cypriot vent gastropod fauna is a total lack of any vetigastropods and neomphalids – either limpet-shaped or spiral shaped – and any other gastropod groups, which are common in modern hydrothermal vents. This suggests either preferential preservation of abyssochrysoids (provannids, paskentanids, hokkaidoconchids) in the Cypriot vent deposits, or, more likely, a lack of other gastropod groups associated with worm tube clusters in Upper Cretaceous Neotethyan hydrothermal vents.

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## Holocene environmental evolution of the Bangkok Clay, Samut Sakorn, Central Thailand: Molluscs as indicators of sea-level change in Northern Gulf of Thailand

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The Chao Phraya and the Tha Chin delta plains, located in Samut Sakorn Province, Northern Gulf of Thailand, preserve very thick and wide Holocene marine sediments, which are collectively known as Bangkok Clay. Three sediment cores, 20–40 m depth, were retrieved from Samut Sakorn area for sedimentological analysis, taxonomic molluscan identification, quantitative analysis on molluscan assemblages, geochemical analysis and <sup>14</sup>C absolute age determinations. The molluscan assemblages, comprising 69 species in total, are dominated by gastropods (46 species), followed by bivalves (19 species) and scaphopods (4 species). The presence of species shows that most of mollusc species are marine inhabiting mainly intertidal zone, and some are brackish water mollusks and a freshwater gastropod. The <sup>14</sup>C dating, together with shells, sedimentological and geochemical analysis allowed separation of sedimentary facies into sedimentary units I, II, and III in ascending order. Unit I (37930 ± 390 yr BP) at the basement strata consists of tan sand with quartz, kaolinite, muscovite, and feldspar. The oyster shells (*Crassostrea* sp.) are abundant in Unit I representing the Late Pleistocene shallow marine environments. Unit II, unconformably overlies Unit I, consists of dark gray clay and silty clay with quartz, montmorillonite, aragonite, and feldspar with several marine faunas. The abundance of molluscan shells, such as *Ringicula* sp., *Polemicella* sp., *Anadara* sp., *Timoclea* sp. and *Paratapes* sp., scattered throughout the sediments. Their ages (2250 ± 30 and 4760 ± 30 yr BP) suggesting that this unit formed during the Holocene in intertidal and shallow subtidal environments. Unit III, which is the uppermost unit, contains dark reddish brown fine-grained sediments with quartz, pyrite, halite, muscovite, calcite, and aragonite together with coal seam, plant debris and a freshwater snail (*Brotia* sp.). These evidences indicate that fluvial system has developed at the latest. In addition, rarefaction analyses, diversity indices and taxonomic composition also suggest that diversity and structure of molluscan assemblages of three units are different, in which the diversity of Unit II is distinctly higher than that of the others. The present study also shows that molluscs have great potential for paleoenvironmental investigations.

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## The molluscs' periodic table: Elemental analysis reveals distinct mineralisation patterns in radular teeth

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Molluscs are capable of foraging on a variety of food sources, which is, in most taxa, enabled by the radula, one important autapomorphy of this phylum. The radula can consist of a thin, chitinous membrane with rows of embedded teeth, which vary in shape, mechanical, and chemical properties between taxa. Since the teeth serve as actual interfaces between the organism and its food and thus act as load transmitting regions, all parameters mentioned can be directly related to the food preferred. With regard to the mechanical properties and their structural and chemical origin, some taxa (Polyplacophora and Patellogastropoda) have been extensively studied in the past decades, due to their specificity to incorporate high proportions of iron, calcium, and/or silicon in their teeth. There is, however, a huge lack of knowledge about radular chemical composition with regard to incorporated minerals and its potential contribution to the teeth biomechanics for most molluscan taxa.

The here presented work aims at shedding some light on the radular chemistry by performing energy-dispersive X-ray spectroscopy (EDX) analyses on mature teeth from overall 24 molluscan species. By EDX, the presence of elements and their proportions can be measured, which allows for the formulation of hypotheses about the minerals present in teeth. Two polyplacophoran taxa with high proportions of iron and calcium were studied, but the majority of examined species, thereof two Cephalopoda and 20 Gastropoda, possess rather chitinous teeth with low proportions of embedded elements. The found disparity in the elements detected, in their distribution and proportions highlights the diversity of evolutionary solutions, as it depicts multiple biomineralisation types to be present within Mollusca.

For selected species, we additionally examined the teeth mechanical properties by nanoindentation. The identified large-scaled gradients within the teeth are related, to some extent, to the chemical composition, but seem to have their origin also in the microstructure of the tooth material and the degree of chitin tanning.

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## Host-symbiont cross talk in photosymbiotic bivalves

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Photosymbiotic associations between heterotrophic hosts and photosynthetic algae play crucial roles in maintaining the trophic and structural integrity of marine ecosystems. This relationship has independently evolved in diverse eukaryotic lineages, such as sponges, corals, and bivalves. However, basic ecology of many photosymbiotic systems and their evolutionary implications are not well-understood. In this study, we used the bivalve subfamily Fraginae to investigate the ecological and molecular interactions between the hosts and their algal symbionts. Three Fraginae host species from shallow to deep habitats were subjected to light gradient treatments. By comparing gene expression patterns of the hosts and symbionts in normal light, reduced light, and total dark, we identified a set of genes that are important for host-symbiont metabolic crosstalk. These genes are involved in nitrogen assimilation, carbon concentration, and carbohydrate metabolism in the photosymbiosis. In addition, we revealed that different bivalve host species harbor closely related but divergent algal symbiont lineages. Some symbiont lineages are associated with multiple hosts, while others are specialized in one. These divergent symbionts also exhibit differential gene expression patterns and certain symbiosis-related genes show signatures of selection. Overall, we demonstrated that host-symbiont interactions are important drivers of bivalve and algal adaptations at the ecological and molecular level.

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## Increasing temperature shifts food choice of a terrestrial snail, *Cryptozona siamensis* (Pfeiffer, 1856), from herbivory to scavenging

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Land snails are predominantly herbivores which occasionally exhibit opportunistic feeding behaviors as scavengers. In this study, we determined the effect of varying temperatures on feeding preference of *C. siamensis*, a widely distributed gastropod in Indochina. Cafeteria experiment was done using different food items (fresh leaves, dead plant material, dead snail, and dead slug). A total of 30 individuals were observed per temperature level (15°C, 20°C, 25°C, 30°C, and 35°C) and the humidity was relatively kept constant (80% ± 20). In general, the snails preferred leaf litter most regardless of the temperature with 25°C as the optimum temperature for foraging. However, significant preference differences were obtained between plant and animal food materials indicating a shift from herbivory to scavenging with 0.06370 unit-interval of temperature increase ( $p=0.0144$ ). This was further supported by logistic regression showing a sudden increase in dead snail consumption beyond 25°C (est=0.0171,  $p=0.0014$ ). This shift of food choice could be due to the demand for high nitrogen content food when subjected to stress which is essential for tissue repair. Selection of high-quality food may likewise support the optimal foraging theory. Further studies varying other parameters such as humidity, light, etc., and incorporation of different land snails are needed to elucidate if the pattern is common throughout the taxon. Nonetheless, this small-scale study shows the potential effects of temperature increase on the feeding dynamics of land snails and their possible responses to a changing environment.

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## Boreal Ocean Jurassic-Cretaceous methane seep molluscs

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Four areas of Late Jurassic to Early Cretaceous aged methane seepage are known from present-day high-latitude sites in the Arctic region: 1) NE Greenland at Kuhnpasset, Wollaston Foreland (Barremian) and Leitch Bjerg, Geographical Society Island (Campanian); 2) Spitsbergen (Tithonian-Berriasian); 3) Novaya Zemlya (three ages: Oxfordian/Kimmeridgian and Tithonian, and latest Berriasian-Valanginian); and 4) Canadian Arctic on Prince Patrick and Ellef Ringnes Islands (both Albian). In the Mesozoic the latter three areas were located in sub-basins of the Boreal Ocean, which, like the present-day Arctic Ocean, was a relatively isolated marine basin with limited connections to the Tethys and ancient Pacific (Panthalassic) Oceans at various times. The East Greenland seeps occurred in a more southerly position in the narrow Norwegian-Greenland Seaway that linked the Boreal Ocean to the Tethys, through NW Europe. The previously studied Spitsbergen and Kuhnpasset seep communities show an intriguing mixture of mollusc species: the Spitsbergen seeps have a large percentage of background taxa and few restricted to seeps, whilst the Kuhnpasset seeps contain a large percentage of seep obligates (e.g. the Jurassic-Cretaceous seep bivalve genus *Caspiconcha*) and few background species. Some fossils common in the Panthalassic and Tethyan Mesozoic seep sites are completely absent from the Spitsbergen and Kuhnpasset seeps (e.g. the Jurassic-Cretaceous seep gastropod genus *Paskentana*). This suggests that in the Mesozoic there was a seep fauna in the Arctic area distinct from that of contemporary lower latitude seeps and those from the southern high latitudes. We will present new fossil mollusc data from an expedition to the Kuhnpasset seeps in 2019, and preliminary taxonomic work on the mollusc fauna from the Novaya Zemlya and Canadian Arctic seeps that explores the concept of distinct Boreal palaeobiogeographic seep fauna during the Jurassic and Cretaceous.

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## Impacts of extreme water level rise on sublittoral gastropods in Lake Kinneret

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In lakes and reservoirs water levels fluctuate in response to natural, anthropogenic, and climatic factors. We present the first observation of the responses of sublittoral gastropod populations to extreme water level rise in Lake Kinneret (the biblical Sea of Galilee). Quantitative samples of soft surface sediments were collected by SCUBA diving from 5 to 16 m depth with typical time intervals of 2–4 weeks. The following gastropod species were investigated: *Falsipyrghula barroisi*, *Melanoides tuberculata*, *Melanopsis costata*, and *Bithynia phialensis*. The first two species were more dominant than other species on soft-bottom habitats. The depth of gastropod distribution was restricted by 14-m isobath during winter-spring holomixis and early stage of summer stratification when the lake bottom was well oxygenated. From late June to December no mollusks were found below 12–13 m isobath. This depth limit matches with the shallowest position to which the anoxic metalimnion can be elevated due internal seiches. The maximal abundances of *M. tuberculata* and *F. barroisi* were on sandy-silt substrate at 5 to 8 m. These snails are well adapted to the typical annual water level variations of 1.5–2 m in Lake Kinneret. An extreme four-meter elevation of the water level in winter-spring 1992 caused a large decrease in gastropod abundance during the next few months. Then, a large increase in gastropod reproduction took place, such that the density of the dominant species reached exceptionally high values by the middle of 1993. Such an increase in the abundance was followed by reduction of gastropod reproduction and growth, which is characteristic for the negative density-dependent population regulation. We discuss the role of the climate-associated extreme events (droughts and floods) and anthropogenic impact (increasing demand for water) on water level fluctuations in inland aquatic ecosystems and, as a result, on the ecology of benthic gastropods. Further we stress the importance of deep understanding of the influence of extreme events on freshwater gastropods, development of adequate monitoring programs, and mitigation measures to rapid changes of aquatic ecosystems.

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## Predation on Miocene marine Mollusca along a latitudinal gradient in the southeastern Pacific

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Predation traces in the fossil record have been the subject of several studies over the years because they are evidence of predator-prey interactions in the past. Through the analysis of drillings and repair scars in mollusk shells, information can be obtained to infer the macroevolutionary impact of predators on the structure of communities, and evaluate the effect of latitude on these interactions, considering that globally there is a gradient of increasing species diversity from high to low latitudes. Today, Chile has an inverse latitudinal gradient of species diversity (which increases from low latitudes to high latitudes). This gradient originated during the Quaternary, while before this period the latitudinal gradient was conventional (with increasing diversity from high to low latitudes), which would influence a higher frequency of predation interactions at low latitudes. We analyzed 15627 gastropod, bivalve and scaphopod shells from the Navidad Formation, Ranquil Formation, Lacui Formation and Ipún beds. Our study shows that a latitudinal gradient exists in predator-prey interactions in the lower Miocene of Chile as inferred from the frequency of predation traces (drillings and repair scars). This increase in predation frequency from higher towards lower latitudes, which is influenced by latitudinal factors and species richness, is evidence that predator-prey interactions are important for the configuration of these populations of marine mollusks.

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## Ecological and Environmental attributes of shell morphologies of the western Indian Jurassic gastropods

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Gastropods are morphologically very diverse, which vary spatio-temporally in response to variation in different biotic (e.g., predation pressure, population density) and abiotic (e.g., temperature, salinity, substrate) factors. The Jurassic western Indian (Kutch and Jaisalmer basins) gastropods, represented by 170 species, inhabited different depositional environments (i.e., coastal, subtidal, and shallow shelf) and ecological (i.e. predation pressure) conditions. Here, we have quantified the morphologies of these gastropods, using four Raupian parameters – rate of whorl expansion (W), rate of whorl translation (T), position of the generating curve in relation to the coiling axis (D), and shape of the generating curve (S) – to analyze the role of depositional environments on gastropod morphological variation across different Jurassic time intervals as well as their relationship with the ecological conditions (here, gastropod drilling predation pressure). Neither the Non-metric Multidimensional Scaling (nMDS) nor the Principal Component Analysis (PCA) as ordination metrics reveal any specific clustering of gastropod morphs with respect to any specific depositional environments. Predation pressure also did not affect western Indian gastropod morphologies during the Indian Jurassic records (from Bajocian to Tithonian), showing overlapping morphology between drilled and non-drilled gastropod families. Since gastropod shells are very simple in terms of coiling geometry, high morphological convergence is very common in gastropods. Moreover, the Jurassic sediments of Kutch and Jaisalmer basins have been deposited under near-coastal to shelf conditions, therefore have similar facies and ecological conditions, further explaining why we do not get any facies or ecology-related morphological clustering in the western Indian gastropod species.

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## Carbon and nitrogen stable isotopes as a screening tool to determine the food sources of land snail *Ena montana* (Draparnaud, 1801)

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*Ena montana* (Draparnaud, 1801) is a common species in most parts of the Europe, although in some countries like in Sweden species is quiet rare. The similar situation is in Lithuania. This species was found only in a small area in Biržai forest. Several studies have been made in order to understand the main factors of its prevalence. The main aim of this study is better understand dietary habits of species, specifying its food sources using stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in the body tissues. Carbon stable isotope ratio reflect the dietary source(s) with  $\sim 0,5\text{--}1\text{‰}$  between diet and consumer; meanwhile nitrogen stable isotope ratios vary according to the trophic level and the difference of two levels are within the range of  $\sim 2,5\text{--}3,6\text{‰}$ . In this research 30 individuals of *E. montana* have been collected together with 17 samples of the potential food sources. Carbon and nitrogen stable isotope ratios were measured in muscle and gastrointestinal tissues of snails, as well as of bulk material of food sources. The differences in carbon isotope ratios between the mollusk tissues and the potential food source were found to be less than expected. Differences in nitrogen isotope ratios reflect potential food sources: the most probable are lichen *Parmelia sulcata* (trophic enrichment – 3.7) and elm, hazel, and ash bark (trophic enrichment – 3.1). However, using a mixing modeling, a combination of food sources is most likely when the ratio of isotopes of an individual falls within the triangle of food sources: lichens (*Lecanora pulicaris*, *P. sulcata*) and soil. These food sources are easily explained by the ecology of the snails: they overwintered on the ground and fed on the trunks of the trees, feeding on suitable lichens (two of six species were chosen by snails). The low  $\delta^{13}\text{C}$  values ( $-32\text{‰}$ ) of moss and tree bark reveal that the habitat is relatively depleted in terms of carbon. It is likely that stable isotope ratios can be influenced by seasonal differences (change of hibernation and active feeding periods), thus further research is needed to better understand the trophic ecology of this species.

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## Host exploitation of eulimid gastropods: in situ observation, molecular identification and stable isotope analysis

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Gastropods of the family Eulimidae (Caenogastropoda: Vanikoroidea) are parasites of the Echinodermata of all five classes: Asteroidea (sea stars), Crinoidea (sea lilies and feather stars), Echinoidea (sea urchins), Holothuroidea (sea cucumbers) and Ophiuroidea (brittle stars). Eulimidae is an unrivaled lineage to investigate evolutionary aspects of parasitism in molluscs by exhibiting a large variety of parasitic modes, sexual strategies and shell shapes. The authors have been investigating their evolutionary history, which is however a huge challenge due to the general lack of information on eulimid parasitic ecology. Many species have a long autonomous period and therefore been collected only as free-living specimens. Moreover, deep-sea eulimids tend to be detached from the host when they are collected in trawl and dredge hauls with bottom sediment and other animals.

In this presentation, we introduce our attempts to uncover the host exploitation of eulimid gastropods. First, successive field surveys have provided opportunities for direct observations of hosts and infection sites of eulimids. This allowed us, for example, the first host identification for the deep-sea genus *Haliella*. Second, we have developed a molecular method for host identification, which involves DNA extraction from the snail's digestive tract and PCR with echinoderm-specific primers. Sequences of echinoderm origins have been obtained from seven out of nine eulimid specimens collected as free-living (five were direct-sequenced while two required a cloning process). Lastly, stable isotope analysis has proven a powerful tool to elucidate host use in eulimids. We have obtained  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the muscle and epidermal tissue from 15 species and their echinoderm hosts. Most of the eulimids exhibited trophic levels similar to or higher than those of their hosts, suggesting nourishment from the body fluid or the epidermal tissue, respectively. Such niche segregation was found in two congeneric species of *Parvioris* on the same individual of the sea star *Archaster typicus*. Mapping these ecological traits on a robust phylogeny would greatly advance our understanding of the diversification processes of the Eulimidae.

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## Linking microevolutionary and macroevolutionary dynamics in freshwater molluscs of the East African Rift System

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Understanding the interplay of microevolutionary and macroevolutionary drivers and dynamics of diversification remains a critical challenge for model and non-model organisms alike. This challenge ensues from both conceptual and methodological limitations, in part because microevolution and macroevolution are studied at different timescales and with different approaches. I develop a conceptual model that is rooted in community ecology to link microevolutionary and macroevolutionary dynamics. This model consists of the dynamic interplay of species selection, ecological drift, speciation and dispersal in the context of habitat heterogeneity and ecosystem stability, and, therewith, allows to integrate biotic and abiotic factors in a holistic approach. Using this model, I explain how Afrotropical freshwater mollusc communities arise in space and time, notably in the East African Rift System. Understanding how key community assembly processes interact in the environmental context allows to predict changes in morphological disparity, genetic diversity and demographic history at a general level throughout diversification, which I align with previous studies and observations in extant and extinct focal freshwater mollusc taxa. Subsequently, in light of the abovementioned methodological limitations, I introduce a novel workflow to generate genomic data in non-model organisms without proximate well-assembled and annotated genomes to undertake integrative studies of microevolutionary and macroevolutionary dynamics in African freshwater bivalves of the tribe Coelaturini. This workflow consists of target enrichment of open reading frames established from ingroup transcriptomes combined with ultraconserved elements defined through comparative genomics using distant mollusc genomes. I combine the obtained genomic data on Coelaturini with geometric morphometric and environmental data to test predictions of the abovementioned conceptual model.

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## Sulfur detoxification via biomineralization in the hadal chemosynthetic bivalve “*Axinulus*” *hadalis* (Thyasiridae)

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Animals harboring intracellular chemosynthetic bacteria take in hydrogen sulfide and other compounds from seawater for their symbionts, and the detoxification and discharging of excessive hydrogen sulfide and sulfur metabolites produced by the bacteria is a key issue for the host. The hadal chemosynthetic bivalve “*Axinulus*” *hadalis* (Veneroida: Thyasiridae) inhabiting over 7000 m depth in Japan Trench is the only thyasirid known to exhibit a novel spherulitic-like structure with a low-crystalline aragonite phase containing a high concentration of sulfur (average 16.2 at%). This shell crystallographic feature is not found in shallow-water thyasirids, which also host sulfur-oxidizing bacteria but extracellularly, or closely-related lucinids with intracellular symbionts. In order to determine whether the sulfur contents in the shells of “*A.*” *hadalis* were derived from symbiotic bacteria or the environment, <sup>34</sup>S/<sup>32</sup>S sulfur isotope ratio analysis was carried out using high lateral resolution secondary ion mass spectrometry (NanoSIMS). The δ<sup>34</sup>S values of the shells were significantly lower than those of shell surface attachments, soft parts (gills, mantles, muscles, etc.) and calcium sulfate biominerals in the statoliths of moon jelly *Aurelia aurita*. This suggests the shells of “*A.*” *hadalis* is a site of potential detox function where the host disposes sulfite metabolites from their thioautotrophic endosymbiotic bacteria. Furthermore, we attempted to reproduce the shell microstructure *in vitro* to consider the significance of sulfur secretion into shells. Our results show that the amorphous shell crystal phase containing sulfate ions is more stable than pure aragonite crystals. This points to the possibility that this is also an adaptation to maintaining a relatively thick shell the Carbonate Compensation Depth (CCD: about 3000 m in the western Pacific). Elucidating new functions of biomineralization in hosts of chemosynthetic bacteria sheds light on the disparate adaptive strategies of mollusks in ‘extreme’ marine environments.

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## **Symposium**

# **The Ecology of Fossil and Extant Molluscs**

## **Part II**

### **Abstracts of poster presentations**

## Multi-proxy records of Late Triassic aragonitic megalodontoid bivalve shells: implications for western Tethys paleoclimate

**Najat Al Fudhaili<sup>1</sup>, Matthias López Correa<sup>1,2</sup>, Martin Ziegler<sup>3</sup>, Alexander Nützel<sup>4</sup>, Pim Kaskes<sup>5</sup>, Philippe Claeys<sup>5</sup> & Niels de Winter<sup>3,5</sup>**

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Bivalves are sensitive to changes in their environment, which are recorded by their incremental shell accretion, growth rate changes, variations in trace element patterns and stable isotope ratios. Three megalodontoid bivalve specimens, two of *Cornucardia hornigi* and one of an undefined species, have been collected from the Late Triassic St. Cassian Formation (Dolomites, Northern Italy), that examines one of the best warm tropical faunal records for the Early Mesozoic. The exceptional preservation of their aragonitic crossed lamellar layer suggests that these specimens are reliable archives for paleoenvironmental reconstructions using a combined sclerochronological and geochemical approach. The aim of this study is to shed light on the effective environmental factors on the bivalve chemical composition, and the absolute temperatures of the ambient conditions during the Late Triassic. We do this by performing a multi-proxy approach using trace element records, clumped isotope ( $\Delta_{47}$ ) thermometry and stable isotopes. Each specimen reveals a set of alternating fine bands that reach >200 growth increments of regular width (with an average of ~35  $\mu\text{m}$ ) developed within major dark and light bundles. High-resolution  $\delta^{18}\text{O}$  records reveal strong seasonality, with an inferred temperature amplitude of 8°C. Seasonal cycle records of one to two years were used to assign the spatial resolution of the clumped isotope measurements intended to capture the maximum and the minimum temperature. Non-destructive micro-X-ray fluorescence ( $\mu\text{XRF}$ ) analysis has been used to obtain reproducible high-resolution trace element profiles and element abundance 25  $\mu\text{m}$  maps (e.g. Mg, Sr, Fe and Mn). The aragonitic shells' preservation has been evaluated using the micro X-ray fluorescence ( $\mu\text{XRF}$ ) heatmap. Variations on the multi-proxy reflect the influence of different environmental factors, e.g. salinity or monsoonal circulation with seasonal river inputs on seawater  $\delta^{18}\text{O}$  and its temperature. Which both control the  $\delta^{18}\text{O}$  fluctuations in the shell aragonite, while metabolism and physiology are presumed to be reflected in the Sr/Ca-signal. A crucial outcome will be absolute temperature values from the clumped isotopes, that would allow the  $\delta^{18}\text{O}_{\text{seawater}}$  of the western Tethys Late Triassic tropical shallow waters to be reconstructed.

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## Coupling between biomineral morphology and Sr/Ca of *Arctica islandica* (Bivalvia) – Implications for shell Sr/Ca-based temperature estimates

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High-resolution paleoclimate archives are required to understand past and future climate dynamics. Shells of marine bivalves offer outstanding potential for environmental reconstructions. However, despite significant advances in sclerochronological research, the number of well-accepted and reliable environmental proxies in shells is limited. Specifically, the reconstruction of temperature based on shell Sr/Ca data remains challenging, although this method is routinely used in other biogenic aragonites. In marine bivalves, the incorporation of strontium into the shell is strongly regulated by vital and kinetic effects and tightly linked to the prevailing microstructure (or underlying processes controlling it). Therefore, we have tested the hypothesis that temperature can be reconstructed from detrended shell Sr/Ca ratios, i. e., once microstructure and/or growth rate-related bias has been mathematically eliminated from shell Sr/Ca data. For this purpose, the relationship between Sr/Ca and increment width as well as morphological parameters of the building blocks (biomineral units) of shell microstructure has been assessed in three different shell portions of field-grown *Arctica islandica* specimens. Subsequently, microstructure and/or growth rate-related variation was mathematically removed from the shell Sr/Ca data and residuals compared to seasonal seawater temperature. After detrending, shell Sr/Ca was positively correlated to seawater temperature, which contradicts thermodynamic expectations and findings from inorganic aragonite. Although temperature may be recorded by shell Sr/Ca, this signal seems to be overprinted by other external forcings. Unless respective variables are identified, it will remain impossible to infer seasonal temperature variations from Sr/Ca. Given the strong coupling of shell Sr/Ca with biomineral unit size, a detailed characterization of the shell microstructure will most likely remain an integral part of subsequent attempts to reconstruct temperature from shell Sr/Ca.

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## Palaeoecological analysis of two Late Pleistocene continental mollusc assemblages from Uruguay

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Extant continental (freshwater and terrestrial) molluscs are very informative from an environmental point of view. The specific environmental requirements of different taxa can be extrapolated to the fossil assemblages in order to reconstruct past environments. This is especially true for the Quaternary, when fossil assemblages are mostly composed by extant species. We analysed two associations of continental molluscs from the Late Pleistocene of Uruguay, with the goal of reconstruct the depositional environments for each assemblage. For this analysis, 11 localities from the Sopas Formation and 10 from the Dolores Formation were selected. The statistical analyses aimed to understand the diversity of each locality and to make comparisons among them, using traditional diversity indices (Shannon, Simpson, Equitability). To estimate the diversity Rarefaction and Chao1 were applied. Lastly, the species occurrence in all localities were compared, using several multivariate analyses: NMDS with axes rotation by Principal Components Analysis, Correspondence Analysis, and Cluster Analysis. The multivariate analyses show that all local assemblages are distributed in two main associations: one composed mostly by Sopas Formation localities (Sopas Association) and the other composed mostly by Dolores Formation localities (Dolores Association). Only one locality from each Formation was interchanged. The Sopas Association records mostly the families Cyrenidae (36.1 %), Tateidae (32 %), Cochliopidae (26.1 %), and the only record of the family Chilinidae. Also, the large, massive species *Diplodon charruanus*, *D. wymanii* and *D. peraeformis* are present. Meanwhile, the Dolores Association records mostly representatives of Cochliopidae (54.4 %), Planorbinae (20.2 %), Sphaeridae (16 %), and has the only records of Physidae and Succineidae. Cochliopidae (*Heleobia*) are present in both associations, which is logical since currently they are quite ubiquitous in most lotic and lentic environments. Ampullariidae and the subfamily Ancylinae are also present in both associations, along with the delicate species *Diplodon rhuacoicus*, which is the only *Diplodon* from the Dolores Association. Presently, the communities that include Sopas-like assemblages are mostly found in high to moderate current lotic environments, with rocky to coarse bottoms. Meanwhile, the communities that include the taxa typical of the Dolores Association are common in lentic or very calm lotic environments, with fine sediments and abundant aquatic vegetation.

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## Calcium availability and its effects on *Oreohelix* land snail gut-associated microbes

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Land snails in the genus *Oreohelix* are known to be associated with calcium-rich environments and their shell ornamentation has been found to be tied to proximity to calcareous rock. One hypothesis is that the microbial communities associated with the digestive system in these snails have facilitated this association. This project aims to test this hypothesis by characterizing the taxa and function of the gut microbiomes in *Oreohelix* snails that naturally occur on and off calcareous rock. To do so, we characterized the gut microbiomes of snails sampled at 9 sites varying in their calcareous content. More specifically, we used 16S rRNA amplicon sequencing and performed deep shotgun sequencing on a subset of the samples to look for genes that provide functional insight into the mechanisms behind microbiome variation between individuals and populations. We consider a variety of factors that may be driving snail gut microbiome community assembly and we test whether the variable of CaCO<sub>3</sub> availability and geography can help predict the presence of certain microbes, the microbial species diversity and abundance distribution, and their functional capacity within *Oreohelix* land snails. Because microbes can affect many host health outcomes and ecosystem processes, it is important to find environmental signals that allow public health and/or conservation practitioners to predict the microbial communities of a host of interest. Being able to predict host microbiomes, perhaps through pH monitoring or geographic/geological information, would allow researchers to understand an important factor regarding the health of a species while being minimally invasive.

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# *Pachyoliva semistriata* (Olividae) escapes predation by *Agaronia propatula* (Olividae) by ‘freezing’ in response to olfactory stimuli

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*Pachyoliva* (formerly *Olivella*) *semistriata* is an intertidal, suspension-feeding gastropod on central American sandy beaches. The predatory *Agaronia propatula* forages on the same beaches, preying mainly on *P. semistriata*. The defensive repertoire of *P. semistriata* comprises a variety of specific responses, including rapid flight when a predator is approaching. However, when *P. semistriata* encounters a recent track left by *A. propatula* or a resting predator, it ‘freezes’: the animals suddenly stop all movement and remain completely motionless for prolonged periods. Here I characterize the ‘freeze response’ by kinematic analyses of locomotion, and report field experiments which demonstrate that the response is triggered by predator-specific olfactory stimuli. The ‘freeze response’ represents a successful defensive strategy in the face of predators that detect the prey’s presence mainly through its mechano-sensory capabilities.

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## Fossilize organic periostracum from the Dinosaur era: new insights into phylogeny and palaeoenvironmental reconstructions in palaeoheterodontids

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Biopolymers in terrestrial environments are easily degraded and replaced during the fossilization process, and examples of pre-Cretaceous animals retaining their original chemical state are extremely limited. We found the periostracum fossils in five freshwater palaeoheterodontids, including *Trigonioides*, whose phylogenetic position is uncertain, from the Cretaceous Balemian (125 million years ago) Kitadani Formation of the Tedor Group, the largest dinosaur fossil site in Japan. In this study, microstructural observations and chemical analysis of this periostracum will be carried out to discuss its phylogenetic relationships by comparing it with recent seven families (Trigoniida: Trigoniidae; Unionida: Etheriidae, Hyriidae, Iridinidae, Margaritiferidae, Mycetopodidae, Unionidae) 23 species, and to examine whether this organic fossil retains a chemical state that allows paleoenvironmental reconstruction by geochemical analysis. The results showed that the original microstructure of the almost complete periostracum was observed electron microscopically from all the fossil species, consistent with the characterization of the recent unionid species. Paleoheterodontids that expanded into freshwater were found to have acquired a synapomorphy, the honeycomb structure of the inner layer of the periostracum. And the fossilized periostracum has been identified as  $\beta$ -chitin molecules, which updates the world's oldest record in the terrestrial water area. The taphonomic factor that prevented the original chitinous material from undergoing metamorphism may have been the rapid coating of a thin film of silicon dioxide on the shell surface after the death of the individual during fossilization in the lake, which was presumably alkaline at the time of deposition. As the chitinous and protein-complex periostracum is a relatively poorly degraded and replaced part of the organic material of mollusks, depending on the depositional environment, the fossil is expected to provide a valuable sample for reconstructing the paleoecology, paleoenvironment and phylogeny.

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## **Symposium**

# **Molluscs as Parasites and Victims of Parasitism: Biodiversity, Ecology, and Evolution**

## **Part I**

### **Abstracts of oral presentations**

## Variation in natural selection on gene expression across the immune system in a freshwater snail

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A strong immune defence should evolve as a response to parasitism, which, however, can be constrained by associated trade-offs with other fitness-related traits. The form and strength of natural selection on immune activity are generally poorly understood because of difficulties in examining the fitness consequences of complex immune systems under natural conditions. Here, we investigated natural selection on the gene expression of several components of the immune system of the freshwater snail *Lymnaea stagnalis* in a field experiment. We individually caged more than 200 snails in a pond for six weeks. We quantified snail fitness (i.e., total reproductive output) and immune activity at the gene expression level. We show that multiple components of the snail immune system were subject to natural selection. Positive directional selection predominated, especially for the components of non-self-recognition. However, also stabilizing and even negative directional selection (mainly genes involved in the production of reactive oxygen species) were observed. Additionally, we found variation in selection on the expression of some genes with similar functions (e.g., within laccases and fibrinogen-related proteins). Our findings highlight the diversity of natural selection operating on different components of the complex immune system and the power of multi-gene expression analysis to examine it.

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## Escalation in parasite-host bivalve interactions throughout the Phanerozoic

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Parasites are widespread in modern ecosystems, occupy one of the most (if not the most) successful life modes, promote ecosystem stability, and, despite their typically small size and lack of a mineralized skeleton, are often identified in the fossil record. Bivalve molluscs have occupied marine aquatic environments since the Cambrian, comprise an excellent fossil record, and commonly preserve traces of interactions with their parasites. Here we review and analyse parasite-host interactions of living bivalves and the record of parasitism of bivalves that reaches as far back as the Silurian. Generalized linear models (GLMs) of parasite occurrences and period-level shareholder quorum sub-sampled (SQS) diversity measures support the dilution hypothesis for bivalves, while GLMs of prevalence and SQS diversity measures support the amplification hypothesis for molluscs. Irrespective, escalation in parasite-host bivalve interactions seems to have occurred in both the middle Palaeozoic and the late Mesozoic to Cenozoic, similar to trends documented in other antagonistic interactions.

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## Fish infestation with glochidia of *Unio crassus* Philipsson, 1788 in the wild

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To complete its ontogenic development, large, bodied freshwater mussels (naiads) have to pass through an obligatory, parasitic phase of larva, which attach to the skin or gills of freshwater fish. This ontogenic transition influences population perspectives and is very important in active conservation projects. The relations between mussel and fish varies in specificity: some species use single species; some have large spectrum of potential hosts. The possibility of successful larvae development is usually verified during artificial infestation of fish in laboratory conditions, whereas the actual (in situ) infestation intensity is rarely studied. We studied the infestation of wild fish by endangered thick-shelled river mussel *Unio crassus*, in natural conditions in the river Warkocz (Świętokrzyskie Mts.) during two breeding seasons. The three sampling plots were selected, where fish were caught and presence of glochidia on their fins was verified. The results demonstrated, that two species were infested with the greatest intensity: common minnow (*Phoxinus phoxinus*) and gudgeon (*Gobio gobio*). Glochidia were attached mostly to fins which are responsible for locomotive functions (pectoral fins and caudal fin). The selection of host species and infestation intensity were different between the study plots. The availability of potential hosts or the mussels abundance did not influence the number of locally infected fish, the most plausible explanation for the infestation intensity is the local composition of functional habitats.

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# **Aggressive mimicry mantle lure polymorphisms in *Lampsilis fasciola* model fish or leech host prey and differ in morphology and pigmentation, but not in display behaviour.**

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Unionoida are free-living apart from a brief, obligately parasitic, larval stage that infects fish hosts and female mussels have evolved a spectrum of strategies to infect hosts with their larvae. In many North American species this involves displaying a mantle lure: a pigmented fleshy extension that acts as an aggressive mimic of a host fish prey, thereby eliciting a feeding response that results in host infection. The mantle lure of *Lampsilis fasciola* is of particular interest because it is apparently polymorphic, with two distinct primary lure phenotypes. One has “eyespot”, a mottled body coloration, prominent marginal extensions, and a distinct “tail”, whereas the other lacks these features and has an orange coloration. We investigated this phenomenon to 1) confirm that it is a true polymorphism; 2) investigate its ecological persistence; 3) identify the respective models targeted in this mimicry system; 4) determine if it includes a behavioural component. Within-population phylogenomic (ddRAD-seq) analyses of females bearing different lures, and detection of within-brood lure variation, confirmed that this phenomenon is a true polymorphism. It appears stable over ecological timeframes: the ratio of the two lure phenotypes in a River Raisin (MI) population in 2017 was consistent with that of museum samples collected at the same site in 1962. Within the River Raisin, the “eyespot” lure visually approximate co-occurring darter species, and the “orange” lure resembles a widespread common leech, *Macrobdella decora*. Darters and leeches are typical prey of *Micropterus dolomieu* (smallmouth bass), the primary fish host of *L. fasciola*. *In situ* field recordings were made of the *L. fasciola* “darter” and “leech” lure display behaviours, in addition to the non-polymorphic lure display of co-occurring *L. cardium*. Although pronounced inter-specific differences in lure display behaviours were observed, the *L. fasciola* “darter” and “leech” lure displays did not differ significantly. We conclude that the *L. fasciola* mantle lure polymorphism does not include a behavioural component. Our discovery of discrete within-brood inheritance of the lure polymorphism implies potential control by a single genetic locus and identifies *L. fasciola* as a promising study system to identify regulatory genes controlling a key adaptive trait of freshwater mussels.

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## The gastropod hosts of schistosomes: patterns, processes and mechanisms

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Members of three major lineages of molluscs (gastropods, bivalves and tusk shells) support the asexual development of larval digenetic trematodes, a prominent group of platyhelminth parasites, members of which are responsible for significant human and animal diseases. As one of the best-known groups of digeneans because of their role in causing human schistosomiasis, the digenean family Schistosomatidae can offer unique insights with respect to processes underlying diversification of parasite lineages. Our aims are to gain a more detailed overview of gastropod lineages exploited by schistosomes, to infer what processes might lie behind the patterns observed, and to suggest underlying mechanisms amenable to testing. Our own concerted search for schistosome infections among gastropods from multiple continents coupled with provision of sequence data for both schistosomes and infected gastropods along with examination of literature with comparable schistosome-gastropod sequence data provide the database from which our results were obtained. As far as known, all schistosome use either coenogastropod or heterobranch gastropod intermediate hosts. More basal gastropod lineages are not found infected with schistosomes. Marine, freshwater and amphibious schistosome life cycles are known. Experimental infection studies indicate relative specificity at the snail host level, yet paradoxically, the present-day record implies host switching has been pervasive, both within and between gastropod families. Schistosomes have colonized several gastropod families but are conspicuously absent from others. Schistosome host switches may be facilitated by co-infections involving immunosuppressive parasites, altered temperature regimes or other conditions stressful to hosts, hybridization among diverging schistosomes, or new ecological circumstances placing schistosomes in constant contact with new host snail species, favoring rare infectious variants.

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## Vampire tales: adaptations to hematophagy in the marine snail *Cumia reticulata*

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Despite being a quite uncommon trophic habit in molluscs, blood-feeding has convergently evolved in at least three families of Neogastropoda: Cancellariidae, Marginellidae, and Colubrariidae. The buccinoidean family Colubrariidae includes about 30 marine species inhabiting mostly shallow-water hard bottoms in tropical, subtropical and temperate seas. The entirety of the species included in the family are considered hematophagous and feed during the nighttime on benthic fishes, which are contacted by extending an extremely long proboscis to gain access to the blood vessels. In the last years, we have actively investigated the molecular adaptations involved in the evolution of this peculiar feeding habit in the Mediterranean colubrariid vampire snail *Cumia reticulata*. Using a transcriptomic approach, we have identified several protein families that play key roles in hematophagous feeding, and we have carried out an in-depth investigation of their molecular diversity and evolution, which is often characterized by recurrent domain and gene duplication events, as reported for other blood-feeding and venomous organisms. We have further focused on the characterization of a novel protein family with a peculiar architecture, comprising exclusively vWA1 domains, for which we were able to experimentally confirm an antiplatelet activity of great adaptive value, but also of remarkable biotechnological interest. Our results shed a new light on the molecular adaptations underlying the evolution of hematophagy in marine gastropods, which seem to be characterized by a high level of tissue and lineage-specificity, in some cases accompanied by a remarkable intraspecific variability, and by instances of structural and functional convergence with respect to other lineages of blood-feeding Metazoa.

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## **Gastropods as parasites and carnivorous grazers – a major guild in marine ecosystems and its fossil record**

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Parasitism and similar life styles such as carnivorous grazing or mucus feeding without killing the prey or host are important traits in marine gastropods. Some highly diverse living gastropod families have this feeding behavior. Taxonomic uniformitarianism, fossil taxa had the same or a similar feeding behaviour as their closest living relatives, is the most important tool to infer parasitism or similar life styles in fossil gastropods. The extant family groups in question (Eulimidae, Epitoniidae, Pyramidellidae, Architectonicidae, Coralliophilinae, Ovulidae, Cerithiopsidae and Triphoridae) originate mostly in the Late Cretaceous (Cerithiopsidae in the Middle Jurassic) and Paleocene. They are performing an ongoing adaptive radiation and some of the mentioned families belong to the most diverse gastropod groups forming a considerable part of marine ecosystems regarding species richness and relative abundance. At the same time, origination and radiation of the carnivorous, commonly predatory Neogastropoda took place. This points to a trophic revolution in Gastropoda that forms an important aspect of the Mesozoic Marine Revolution. Most modern parasitic gastropods are small, high-spired, show high diversity and low disparity within families and belong to Apogastropoda. By analogy, some extinct gastropod families which show the same properties might have lived parasitic too (e. g., Pseudozygopleuridae, Zygopleuridae, Meekospiridae, Donaldinidae). However, this will remain speculative to a large degree until direct host associations are found. Direct evidence for parasitism is exceptional with the Palaeozoic platyceratid/crinoid interaction being one of the best studied examples. In Gastropoda, functional shell morphology may help to identify parasitism in the fossil record but this field is scarcely studied.

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## Early evolution of parasitism and gill anatomy in Unionida bivalves

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Bivalves are known to associate with a variety of organisms; however, the true parasites are today known only from the marine Galeommatoidea and freshwater Unionida. Both required establishing a close association with the host's body, resulting in being enclosed within its tissues. All the Recent Unionida have a tiny parasitic larva (most commonly the glochidia) that must infect a fish – this is widely accepted as an adaptation to dispersion in freshwater. Additionally, today females protect their larvae with maternal care via brooding them in their modified eulamellibranch gills and some of them even act to facilitate contact with the host for their offspring. Here I discuss the early evolution of these adaptations.

Members of the Unionida are known from freshwater since the Triassic, thus before the Pangea split. Because unionoid parasitic larvae and brooding in eulamellibranch gills are together crucial for the unionoid life cycle and present in all Recent lineages, on every continent, one may expect they already existed in the last common ancestor of advanced lineages of Unionida – thus probably before the Middle Jurassic, when advanced unionoids already lived (and the Pangea was already broke). Unfortunately, the direct fossil record of both of these characters is scarce and young. The oldest fossils of glochidia are known from the deposits as old as Miocene, oldest eulamellibranch gills – are from the Late Cretaceous. Nevertheless, in the Late Triassic deposits of Poland three examples of phosphatized gills in early unionoids were found. Oldest – Carnian – is of filibranch anatomy, similarly to the gills of their accepted marine ancestors, Trigoniida. This finding does not exclude establishing parasitic relations with co-occurring fish then but is a strong indication of possible difficulties in introducing maternal care to larvae. The new findings of the preserved gills from Norian and Rhaetian deposits suggest a lack of evidence for eulamellibranch anatomy, thus they may complete the picture of what we know about the evolution of eulamellibranchy in Unionida. They are narrowing a time interval for obtaining eulamellibranchy – and possibly the brooding behavior – to ca. end of the Triassic-Middle Jurassic.

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**Symposium**

**Volunteers in Malacology**

**Part I**

**Abstracts of oral presentations**

## The search for hitchhiking Caribbean Molluscs in Wales – a citizen science project

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In the 1980s a speedboat washed ashore in Cornwall, England with Caribbean bivalves attached. Decades later winter storms have sporadically thrown bait pots, crates, buckets, crab traps and other plastic items ashore with over 20 species of tropical West Atlantic rafting molluscs. The debris, originating in the warm waters of the Caribbean and SE coast of USA, is transported by the Gulf Stream to the northeast Atlantic however, most of the species attached currently don't stand a chance of thriving in our chilly waters. Few of the molluscs survive the journey and those that do cannot reproduce as they need sea temperatures of over 20°C. However, the Florida rock snail (*Stramonita floridana*) has been observed alive in the south of England and even producing eggs, although they were non-viable. Additionally, the Bicolor purse oyster (*Isognomon bicolor*), considered an invasive species in Brazil, has been found several times in the southwest of England. With records of Caribbean molluscs hitchhiking on plastics in Ireland and England, one would suspect that they are also in Wales, but there are no confirmed records yet. Are they here?

This potential threat to our shores remains unmonitored and could provide a way for non-native invaders to become invasive species. To aid recording of these species I have produced an identification guide for use by citizen scientists and have begun to run workshops with organisations whose staff and volunteers are regularly out on the beaches of the Welsh coast. This has all been facilitated by connecting with wildlife trusts, universities, government and non-government organizations in the search area and by following through on the enthusiasm of those involved. Through social media I am engaging families and citizen scientists to look for plastics on our shores and check them for hitchhiking molluscs. It is only with many eyes on the ground that we will be able to spot any potential threats to our wildlife.

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## Description of a healthy population of the freshwater mussel *Anodonta cygnea* in a pond in Wuppertal, Germany

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An approximately 5300 m<sup>2</sup> nature reserve with former private fish breeding ponds near Wuppertal, Germany, is the focus of our study. The site is now maintained by volunteers and serves as a nature reserve primarily for habitat conservation, youth education, and training of prospective teachers. During a students' survey of the pond's fauna in 2019, first evidence of freshwater mussels of the species *Anodonta cygnea* (Linnaeus, 1758) was provided. Further work during preservation measures in 2020 and 2021 delivered data of a healthy population of more than 200 individuals of various sizes and ages. Shell length, width, height and weight were measured to learn about the age and size distribution. Furthermore the European bitterling (*Rhodeus amarus* (Bloch, 1782)), which is listed as an endangered species in Germany and is important for the development of larvae of *Anodonta cygnea*, since it serves as host for the glochidia, was discovered in the pond. We are facing an increasingly severe biodiversity crisis and rapid species extinctions, while especially in urban areas alienation from nature and decrease in species knowledge can be observed. In this study, species conservation, nature protection and education are sustainably linked.

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## Freshwater molluscs of India: Challenges and opportunities for research and conservation

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India is home to four internationally recognised biodiversity hotspots of the world. The varied climatic regimes, topography, vegetation and the geological history of landmass have resulted in a highly diverse biota. India also has varied freshwater ecosystems, from high altitude saline and freshwater lakes to coastal wetlands and several major rivers. India has more than 200 species of freshwater molluscs with nearly 50 % being endemic. The freshwater molluscs of India are unique and form biographically important lineages. To assess the challenges and opportunities in the conservation of freshwater molluscs in the Indian region, a review of the status of knowledge was carried out. Research on natural history, biology and ecology for most species is lacking. Though the taxonomy of some of the taxa, especially from the Western Ghats, has been resolved, it is yet to be determined for other regions. There is an inherent lack of understanding of the species distribution for many taxa, which is known as the “Wallacean shortfall”. With rapid changes in land use, the populations of a large proportion of the restricted-range species have reduced considerably. Rapid urbanization, loss of habitat, pollution, sand mining and unregulated use of pesticides pose a serious challenge to the conservation of freshwater molluscs. Climate change is another threat, especially in the Himalayas. Less than 5 % of India’s geographic area is covered under a protected area network (PA) and the current extent of these PAs is not effective in conserving freshwater molluscs. Wildlife Protection Act (1972) does not list any freshwater molluscs under any schedule. On the policy front, there is a plethora of freshwater related policies at the state and federal levels, but there are a lot of contradictions between them. This has implications for the conservation of these imperilled groups. A comprehensive action plan needs to be developed and citizen science initiatives that address the “Wallacean Shortfall” need to be adopted. There is a need to create awareness among different stakeholders, and extend the PA network. Changes in policy, increased funding for research, and the involvement of different stakeholders in the conservation of freshwater molluscs are essential. An interdisciplinary approach is needed for better conservation and management of freshwater molluscs in the Indian region.

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## Non-marine mollusc distribution mapping by the Conchological Society of Great Britain & Ireland: a citizen science project in the 19<sup>th</sup>, 20<sup>th</sup> and 21<sup>st</sup> centuries

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Since its formation in 1876, the Conchological Society of Great Britain & Ireland has undertaken voluntary distribution recording of non-marine molluscs. The dataset remains the primary source of information on their distribution in both the United Kingdom and the Republic of Ireland. To date, around 1000 volunteers have contributed over 300 000 records mapped at 10 km<sup>2</sup> resolution or better, comprising terrestrial snails (150 species), slugs (45 species), freshwater gastropods (40 species) and freshwater bivalves (30 species). The data are publicly available via the UK's National Biodiversity Network (NBN), Ireland's National Biodiversity Data Centre (NBDC), and through the Global Biodiversity Information Facility (GBIF).

The mapping dataset is remarkably complete. Importantly, it includes and distinguishes subfossil records from the Late Glacial (15000–10000 years BP) to the Roman invasion (43 AD). This shows long-term distributional change and, given that the data concern post-glacial islands, allows a strict definition of which species are native and where. A continued flow of records reflects major changes in land use and pollution, and indicates how newly-introduced species have accumulated and spread, while a few natives have dwindled or become extinct. The data remain fundamental in species red-listing and protection. Since 2017, the data have been downloaded nearly 7000 times and used many more by naturalists, conservation bodies, planners, archaeologists, and in major reports to Government.

This talk will outline the history and development of this endeavour, a classic taxon-based “recording scheme”. The 19<sup>th</sup> century witnessed the ad hoc development of a basic “Census” for reference publications. The 20<sup>th</sup> century saw increasing centralization as state support for conservation grew. The dedication of a broad community of enthusiasts, mostly volunteers, culminated in the “Atlas” compiled by Michael Kerney in 1999. Digitization has transformed the accessibility of the data in the 21<sup>st</sup> century and aided new identification guides to stimulate further recording. In the smartphone era, electronic participation has flourished, including via Facebook, the UK's iRecord platform and increasingly via iNaturalist and image recognition technology. While the technical challenges have varied, the basic logic of recording remains the same, in an excellent example of the contribution made by volunteers to malacology.

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## **Symposium**

# **Volunteers in Malacology**

## **Part I**

### **Abstracts of poster presentations**

# Using citizen science in the biodiversity research of Nudibranchia

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Traditional research methods can often provide only limited insight into the biodiversity of marine animals, as extensive habitat analyses require the collection of very large amounts of global-scale data. For instance, biodiversity research on nudibranch sea slugs is often impeded due to time and resource consuming diving trips. To facilitate scientific work by identifying habitats in which target-species for scientists occur, citizen science could become a major improvement for the research on nudibranchs.

Here, we investigated how amateur divers could contribute to our knowledge about the population size, distribution, diet, and adaptive strategies in Nudibranchia. For this, we sourced available photos and videos from two social media platforms, Instagram and YouTube.

Our results show that there are numerous people around the world who are interested in nudibranchs and share their images and videos on the Internet, particularly on Instagram. Generally, the quality and quantity of media deposited on Instagram was significantly better than on YouTube. Overall, we were able to collect data on 42 species and to provide new and confirm previous results on species distribution. Moreover, to a limited extent, information on the diet and on feeding was obtained but an estimation of the population size was not possible using the current data. Future studies with an optimized methodological set-up could process data on a larger scale and thus lead to more detailed results. In summary, with guidance for the implementation of subsequent projects, citizen science has great potential for the study of nudibranchs and their biodiversity.

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## The Tomlin archive... bridging science and history

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John Read le Brockton Tomlin (1864–1954) was one of the most highly respected British malacologists of his time and in 1955 his extensive shell collection was bequeathed to Amgueddfa Cymru-National Museum Wales as the Melvill-Tomlin collection. Alongside the collection and library was a unique archive of documents curated by Tomlin to represent the breadth of his correspondents, but also to record handwriting examples and signatures of other important malacologists. Tomlin described it as his malacological autograph collection and it contains nearly 1500 documents dating from 1762 to 1953.

The documents mostly concern the everyday aspects of shell collecting and identification, but in addition to scientific history it is an archive of personal and social history. It has brought into focus aspects of the lives of the people that contributed to it, recounting collecting expeditions and voyages, personal illness and hardship, war, and even dinner invitations. Archives such as these form a bridge between history and science, which is a different route into engagement with our natural science collections, and it offers the opportunity to investigate the colonial history of collecting and the communication of women in science during this period.

To make the content accessible the archive has been at the centre of a volunteer transcription project since 2015 with 3 dedicated volunteers between 2015–2018 and a further 3 from 2019–2022: transcribing each of the letters, exploring the characters involved, interpreting the content, and highlighting stories of historical and social interest. The volunteers came from a mixture of science, history and language backgrounds which have all brought invaluable input to the project. The linguistic skills have been a particular asset with a quarter of the archive being in non-English languages and both transcription and translation have been undertaken on many of these. The work has influenced career decisions for some of the volunteers and supported others living alone through the Covid-19 pandemic whilst working remotely.

The Tomlin archive will be published in 2022 on the ‘Mollusca Types in Britain & Ireland’ online platform (<https://gbmolluscatypes.ac.uk/>) where it will spearhead the beginning of the first digital British and Irish malacological archive repository.

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**Symposium**

**The EvoDevo Corner**

**Part I**

**Abstracts of oral presentations**

## Patterning the cephalopod body plan

**Caroline B. Albertin<sup>1</sup>, Hannah Schmidbaur<sup>2</sup>, Karen Crawford<sup>3</sup>, Emily Garcia<sup>1</sup>,  
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Cephalopods have a highly derived body plan and a suite of morphological innovations with no obvious correlates in other animals. How this highly derived body plan relates to those of other animals has been obscure. Here, we describe the genomic arrangement and expression of the HOX genes in *Octopus bimaculoides*, the California two-spot octopus, and *Doryteuthis pealeii*, the longfin inshore squid. Chromosome-scale genome assemblies for these species recover a single, intact, but massively expanded, HOX cluster in each of these species. Despite the dramatic increase in the size of the cluster, we find that cephalopod HOX expression exhibits the canonical nested domains of expression. Moreover, the early expression of the HOX genes reflects the derived arrangement of cephalopod development: expression domains radiate out from the center of the embryo, forming the nested, bilaterally symmetrical sectors expected for a polar coordinate system. These data support an ancestral role for HOX genes in patterning the cephalopod body plan despite the dramatic increase in the size of the genomic cluster and the radial geometry of the early embryo. We also observe major differences in both the HOX gene complements and expression patterns between squid and octopus that may point to a key role for HOX genes in the evolution of their body plans. This work was funded by NSF grant IOS-1354898, the Grass Foundation, and the Hibbitt Early Career Fellows Program at the Marine Biological Laboratory.

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## Surprising effects of pharmaceutical 5-alpha-reductase inhibitors on freshwater gastropod embryonic development

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In the field of environmental toxicology, molluscs were thought to have similar endocrinology to vertebrates, like fish, both using steroid sex hormones such as testosterone. However, more recent findings suggest that vertebrate-type steroid androgens are not utilised in molluscan reproductive development, genomic searches have revealed that molluscs do not possess a nuclear androgen receptor and many of the steroidogenic enzymes required to make testosterone are not present in mollusc genomes. Nevertheless, homologs of some “vertebrate” steroidogenic enzymes have been identified in molluscs, including 5 $\alpha$ -reductase. In vertebrates, testosterone is converted to the more potent androgen 5 $\alpha$ -dihydrotestosterone by 5 $\alpha$ -reductase enzymes, and is key in male reproductive development. Whereas, in some plants (e.g. *Arabidopsis thaliana*) the 5 $\alpha$ -reductase homolog DET2 is vital in the biosynthesis of brassinolide, a plant steroid involved in growth. Therefore, the role of 5 $\alpha$ -reductase in molluscs is intriguing.

Dutasteride is a highly specific pharmaceutical inhibitor of 5 $\alpha$ -reductase, developed to limit the conversion of testosterone to dihydrotestosterone in patients with prostate cancer. We have employed dutasteride as tool to explore 5 $\alpha$ -reductase’s function in the freshwater gastropod *Biomphalaria glabrata*, during embryo development.

*B. glabrata* embryos were exposed to dutasteride from blastula stage. These initial experiments unexpectedly found that exposure to dutasteride (or similar pharmaceutical 5 $\alpha$ -reductase inhibitor finasteride) during early embryonic development caused a strong, highly reproducible, phenotypic response to shell morphology. Dutasteride exposed embryos are elongated with open-coiled “banana-shaped” shells. Similar phenotypic results are also seen in *Physella acuta* and *Lymnaea stagnalis*.

Our recent investigations suggest that the critical window of dutasteride exposure for inducing the “banana-shaped” shell phenotype in *B. glabrata* is the trochophore stage, which coincides with early shell-field development. Preliminary RNA CEL-seq2 of embryos at trochophore and early veliger stages, reveal patterns of differentially expressed genes between control and dutasteride exposed groups. On-going studies will also determine 5 $\alpha$ -reductase expression levels during *B. glabrata* embryo development, to identify if 5 $\alpha$ -reductase expression correlate with the sensitive critical window of disruption.

The role of 5 $\alpha$ -reductase in molluscs is still largely unknown. But, ongoing work aims to uncover links to possible endocrinological control of embryo development, shell growth and body patterning.

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## Modern tools for Molluscan Evo-Devo

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Recent advances in modern technologies make it an exciting time for molluscan evo-devo. Tools that only five years ago were available exclusively in traditional model animals such as mouse, fish, frog and fly, are now accessible in a wide range of our favourite molluscs. In this talk I will outline the mollusc-specific modifications we have made to the ACME (ACetic-MEthanol) protocol in order to generate a developmental cell-type atlas for *Crepidula fornicata* organogenesis. I will highlight the cell-types of interest that we have captured and outline a genome-free analysis approach. The combination of analysis pipeline and multiple “pause points” in the ACME protocol make this approach widely accessible for molluscan evo-devo questions.

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## Connecting pattern to process: Growth of spiral shell sculpture in the gastropod *Nucella ostrina* (Muricidae: Ocenebrinae)

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Shell morphology is a well-suited and underused system to examine the development of novel forms. The three-dimensional structure produced (the shell) is separate from the largely two-dimensional tissue that secretes it (the mantle), allowing us to disentangle the pattern from the process. Despite knowing a great deal about the mechanics of shell secretion (process), and the variety of shell shapes that exist (pattern), little effort has been made to understand how the mantle changes to produce different shell shapes. I investigated this question in the dimorphic snail *Nucella ostrina*, which exhibits both smooth and ribbed shells to determine how ribs are formed by the mantle. Rib thickenings are produced only in the outer calcitic shell layer and secreted by the distal Outer Mantle Epithelium (OME). The evenly thick inner aragonitic layers are secreted by the proximal OME. Here we show that locally thicker ribs in *N. ostrina* are produced by increasing the amount of outer (calcitic) layers of shell produced, through two changes in the dimensions of the distal OME: elongation in the direction of growth and increase of OME cell height. This should increase the amount of shell material secreted, producing locally thicker shell (ribs). I have just started as an assistant professor at the University of Saskatchewan, and my research programme will look deeper into how the mantle controls shell growth. As a first step I aim to test the neurosecretory hypothesis that the nervous system in the well-innervated mantle directly controls shell secretion using morphology, bio-informatics, and evo-devo approaches. I am recruiting graduate students who are interested these types of questions to join my lab.

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**Symposium**

# **The EvoDevo Corner**

**Part II**

**Abstracts of poster presentations**

## Quantitative gene expression analysis sheds light on the role of 5-alpha-reductase in the development of a freshwater gastropod

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In vertebrates, steroidogenic enzymes 5-alpha reductases (5αR) convert testosterone to its more potent 5α-dihydrotestosterone. Despite a growing body of evidence indicating the absence of a nuclear androgen receptor and the inability of molluscs to biosynthesise testosterone de novo, homologues of 5-alpha reductase genes (5αR1 & 5αR2) have been identified in mollusc genomes. Consequently, the function of 5αR in mollusc tissues remains unknown. Pharmaceutical disruption of 5αR enzymes in the freshwater gastropod *Biomphalaria glabrata* was shown to disrupt normal embryo development causing the emergence of a “banana-shaped” shell. Expression of 5αR transcripts in the mantle tissues of *Biomphalaria glabrata* have also suggested a potential link with molluscan shell formation. This study is using quantitative gene expression analysis to measure the expression levels of 5αR1 and 5αR2 in *Biomphalaria glabrata* embryos at different stages of development (day 3–5 post oviposition). Total RNA was isolated from pooled embryo tissues at different developmental stages (day 3, 4 and 5 post-oviposition) and albumen gland tissues from *Biomphalaria glabrata* snails of the same age/condition. Total RNA was purified using an extra step of DNase-I treatment prior to cDNA synthesis. Real-time qPCR was performed using all available embryo samples and the albumen gland as a control. Preliminary quantification data has confirmed the expression of both 5αR1 and 5αR2 transcripts in day 3–5 post oviposition embryos and the albumen gland. RNA isolation from day 1 and day 2 post oviposition embryos is currently being optimised to confirm 5αR1 and 5αR2 expression in earlier stages of development. The data will be normalised against a set of endogenous control genes which are currently being tested, in the hopes of correcting for sample variation so that accurate comparison between expression levels and developmental stages can be achieved. Investigating those novel patterns of expression will shed some light on the role of 5α-reductases in gastropod snails and expand our understanding of molluscan endocrinology. Consequently, gene expression data will help to elucidate the link between molluscan endocrine system and shell formation. The optimisation of this assay will thus allow further investigation into the role of other gene targets involved in early gastropod development.

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## Critical windows of dutasteride exposure in *B. glabrata* to elicit “banana-shape” shell

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Exposing *Biomphalaria glabrata* to pharmaceutical 5 $\alpha$ -reductase inhibitors, dutasteride and finasteride, during early embryonic development causes a strong, highly reproducible phenotypic response – exposed embryos are elongated with open-coiled shells. Here we investigate if there is a critical window of development, within which dutasteride exposure (5 $\alpha$ -reductase inhibition) elicits the “banana-shape” shell phenotype.

In our lab, at 27 °C, *B. glabrata* hatch around 5 days post oviposition (dpo). For each experiment, egg masses were collected shortly after oviposition and exposed to control waters or 100  $\mu$ g/L dutasteride starting from day 0 (standard exposure) or from 1, 2, 3, or 4 dpo. The resulting phenotypes were recorded on day 6.

The timing of exposure did alter the response to dutasteride, indicating a critical window of development for inducing the “banana-shaped” phenotype. Embryos exposed to dutasteride from day 4 (veliger or hippo stage) did not develop the “banana-shaped” phenotype. A few embryos exposed from day 3 (mainly early veliger stage), developed the “banana-shaped” shell, however these results were not statistically significant when compared to the solvent control group. Both day 2 (trochophore stage) and day 1 (blastula and gastrula stage) exposure groups resulted in a significant induction of the “banana” phenotype compared to solvent control. With those exposed from day 0 and day 1 having most of the embryos develop the “banana-shaped” shell (76 % and 75 %, respectively). From this investigation, the trochophore stage is likely to be the critical window of dutasteride exposure for inducing the “banana-shaped” shell phenotype in *B. glabrata*.

Exact mechanisms behind the “banana-shaped” phenotype have yet to be eluded. Further research is needed to determine how dutasteride (5 $\alpha$ -reductase inhibition) disrupts normal shell development of *B. glabrata* embryos. However, this research highlighting the critical window of disruption is likely to be during the trochophore stage, provides links to early body patterning stages and shell field development, and provides a time window to focus future molecular investigations for 5 $\alpha$ -reductase gene expression and downstream effects of dutasteride exposure. From this investigation, the trochophore stage is the critical window of exposure for inducing the “banana-shaped” shell phenotype in *B. glabrata* embryos.

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## **Symposium**

# **Insular Ecosystems as Cradles of Mollusc Biodiversity and Evolution**

## **Part I**

### **Abstracts of oral presentations**

## Island Africa – insights into evolution and biogeography from freshwater molluscs in insular systems

**Christian Albrecht<sup>1,2</sup>, C. Clewing<sup>1</sup>, F. D. Chibwana<sup>3</sup>, M. C. Dusabe<sup>1</sup>, A. Mahulu<sup>4</sup>,  
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Insular systems such as oceanic islands, crater and long-lived lakes and mountain tops ('islands in the sky') are ideal settings for studying evolutionary and biogeographical patterns and processes. We use phylogenetic and phylogeographic datasets of various freshwater mollusc groups spanning from continental to local scales to study the origination, the colonization history as well as subsequent *in situ* diversification. The significant role of drainages and insular systems in shaping diversity and distribution patterns on the continental scale has been demonstrated for *Lanistes* (Ampullariidae). In various groups, the African large rift lakes are often characterized by spectacular gastropod radiations, and we show for Lake Tanganyika (Paludomidae) and Lake Victoria (Viviparidae), the important roles of their drainage systems as sources and sinks for mollusc diversity in the actual lakes. In contrast, the mollusc fauna of the dozens of crater lakes in western Uganda are used to demonstrate archipelago dynamics. Moreover, examples of Sphaeriidae, Lymnaeidae, Bulinidae and Burnupiidae from highlands in eastern, central and southern Africa demonstrate how the endemic diversity evolved in such extreme (high-altitude) environments. For all these studies, we show the often complex patterns that are involved in different time windows from the Oligocene to the Holocene. Moreover, we discuss dispersal routes, diversification events, species decline and loss. Lastly, we highlight and promote freshwater insular systems in Africa, which deserve greater attention and should become model systems for mollusc research and conservation.

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## Threat and origin of the unique freshwater fauna on the western Indian Ocean islands

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The islands of the Indian Ocean including Madagascar and smaller island systems such as the Mascarene Islands, the Seychelles and the Comoros are considered a biodiversity hotspot given their large share of endemic species. The geological and palaeogeographical history of these islands is well studied and has been used by biologists to explain affinities of its biota in several biogeographical studies. However, most of the research with a phylogenetic or biogeographical background is focused on few selected taxonomic, mainly non-freshwater, groups. We here use a comparative approach by analysing several generic-level freshwater mollusc phylogenies to test whether diversity is a function of island parameters and whether biogeographical patterns (biogeographical origins and colonization routes) differ between native and non-native taxa due to different dispersal mechanisms. Remarkably, our study shows that large share of the mollusc fauna on the smaller islands (e.g. Mauritius and Mahé) consists of non-native species, some of which are newly recorded for these regions. Moreover, the current phylogenies indicate that many taxa are of Oriental origin. We discuss the geological history of the archipelago as well as different dispersal strategies/types in order to explain the observed patterns. This study also contributes to the general understanding of biodiversity of this island system in the western Indian Ocean, which is critical for further conservation efforts, particularly in freshwater habitats that face an increased anthropogenic pressure.

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## LIFE SNAILS – Azores: Mending the pieces to give them a chance

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The Azorean terrestrial malacofauna (freshwater and halophilic pulmonates excluded), comprises 111 species, of which 45 % are endemic. This richness has been dwindling over the past three decades, such that, in Santa Maria Island, in spite of intense surveys, some species collected alive in the 90s have not been found for 20 years and many have become extremely rare.

Following an awareness campaign during a IUCN workshop in Santa Maria, a LIFE project was designed for 3 endangered species: *Leptaxis minor*, *Oxychilus agostinhoi* and *Plutonia angulosa*.

The project (LIFE20 NAT/PT/001377, under the coordination of the Regional Secretariat for the Environment and Climatic Change) seeks to increase habitat suitability and to reduce fragmentation on historical areas of distribution, through an inter-connected “green infrastructure”, and to improve its quality by controlling invasive plants, restricting cattle access and ensuring nature-based solutions favoring humidity and moisture in soil and ground cover.

The area of intervention is centered on the Nature Reserve of Pico Alto, but it is intended to expand. Habitat increase will be achieved through (re)naturalization of forests by diversification of trees and shrubs. In private land, farmers will be rewarded if adhering to a results-based framework targeting (re)naturalization of hedgerows and fences along the margins of pastureland, thus helping to install an integrated mosaic of ecological corridors that interconnect with waterlines and remaining spots of high quality habitat.

Special effort will be dedicated to awareness of the population, the privileged guardians of their own biodiversity and natural heritage.

Although only three species are targeted by name, due to the small size of the island and the present fragmentation of the habitats the project will benefit most of the native rich malacofauna as well as other endemic biota.

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## The Pomatiidae of the Central Canary Islands – just conchological variability or misjudged biodiversity?

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The family Pomatiidae (Gastropoda: Littorinimorpha) is represented in the Canary Islands on each of the major islands by endemic representatives of the genus *Pomatias* Studer, 1798. The Canarian species are sometimes placed in the subgenus *Canaripoma* Starobogatov & Anistratenko, 1991, but this has not been tested by phylogenetic analyses so far. The Central Canary Islands – Tenerife and Gran Canaria – are home to three out of five currently accepted species from the archipelago, namely *P. laevigatus* (Webb & Berthelot, 1833), *P. canariensis* (d'Orbigny, 1840) and *P. raricosta* (Wollaston, 1878). The study of type material housed in the collections of several museums in Europe and North America, as well as the examination of more than 100 documented series of *Pomatias* from both islands in the private collections of the authors and the museums of Santa Cruz de Tenerife, Frankfurt and Hamburg suggests that we have to deal with more than these three species, especially if also shells from Pleistocene deposits are included in the study. Type material of *Cyclostomus adjunctus* Mousson, 1872, *Cyclostoma laevigatum*, *C. canariense*, *C. c.* var. *γ inaequalis* Wollaston, 1878 and *C. c.* var. *praecursor* Boettger, 1908 is documented and lectotypes, if necessary, will be selected. No type material of *C. canariense* var. *β raricosta* Wollaston, 1878 could be found, consequently a neotype ought to be selected from topotypical material. Preliminary investigations of *Pomatias* specimens from both islands by conchological and morphometric methods suggest that we have to deal on Tenerife and Gran Canaria with at least three extant species each and that none of these species is present on both islands. Additionally, forms from the Pleistocene might be recognised as chrono-subspecies. Furthermore, it becomes apparent that the names *P. laevigatum* and *P. canariensis* cannot be applied to any population of the eastern (Fuerteventura and Lanzarote) or western (La Gomera, El Hierro and La Palma) Canary Islands, and the former, as already recognised, is a younger homonym anyway.

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## Phylogenomic insights into the diversification of Partulidae across Oceania

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Partulid tree snails are restricted to Pacific high islands and, although predominantly composed of single-island endemics, they have achieved a collective range that extends across ~10000 km of Oceania. Many aspects of their extraordinary biogeography remain enigmatic. For instance, we have yet to identify a convincing sister lineage for Partulidae; 3 of the 5 currently recognized genera have restricted distributions and the other 2 have widespread, but highly disjunct, ranges; and approximately half of the species diversity is restricted to a single, remote, hot spot archipelago. These attributes have elicited much discussion and speculation by generations of partulid workers, however, insights have been severely restricted by the unavailability of a robust, range-wide phylogeny of the group. Using a combination of museum, captive, and remnant wild snails, we have obtained a high-resolution nuclear genomic phylogeny of the Family. It incorporates 43 of the 105 recognized species, including the type species of all 5 genera, as well as many extinct or extirpated (surviving-only-in-captivity) taxa, from a total of 14 archipelagoes spanning the familial range. These new data undermine the taxonomic validity of two Palauan endemic genera, lead to a significantly expanded concept of the genus *Samoana* and provide a refined perspective of the evolutionary relationships within the genus *Partula* in both Western and Eastern parts of its range.

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## Spatial distribution and connectivity of new vent related species in *Anatoma* (Gastropoda) from hydrothermal vent fields in the Indian Ocean

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Biodiversity inventories are part of feasibility studies for the exploration of polymetallic sulphides at hydrothermal vent fields in the Indian Ocean conducted by the German Federal Institute for Geosciences and Natural Resources. To date, more than 30 gastropod species live in the vent fields along the Central and Southeast Indian Ridge and all of them are assumed to be vent related. During the INDEX project, 270 representatives of the genus *Anatoma* (Anatomidae, Vetigastropoda) were sampled in six abyssal hydrothermal vent areas. For the first time, traditional morphology and molecular analyses were combined to study their diversity and connectivity. Mitochondrial DNA COI and 2b-RAD sequencing were used to complete the morphological species identification. Four species were described on the basis of their morphological characters (shells, anterior anatomy, radulae) by using CLSM and SEM, while two singletons were left undescribed. The sampled taxa are solely known from a trajectory of about 800 kilometres along the plate margins. Besides, a high genetic similarity was detected between *A. paucisculpta* and an undescribed anatomid, which was sampled from a hydrothermal vent field in the Lau Basin in the southern Pacific Ocean. Therefore, we conclude our species to be vent related. Studies on Indian Ocean vent fauna proved that the Rodriguez Triple Junction is a dispersal conduit for the vent related gastropods *Alviniconcha marisindica* and the “scaly-foot snail” *Chrysomallon squamiferum* along the Central and north part of Southwest Indian Ridge. To verify this theory also for Anatomidae along the Central and Southeast Indian Ridge, our three most abundant species were compared according to their connectivity between the six sampled vent fields by analysing the whole genome with 2b-RAD sequencing. The results of this connectivity analysis are discussed in terms of dispersal, biotic and abiotic factors. With regard to possible future mining, it is important to understand the population dynamics and the human influence on this deep-sea communities, therefore this study provides the first connectivity analyses for Macrofauna along the Central and Southeast Indian Ridge.

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## Material properties of radular teeth reflect adaptations to distinct substrates, a functional morphology study on the paludomid gastropods from Lake Tanganyika

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The paludomid gastropods from Lake Tanganyika and surrounding river systems form a species flock of high interest, because of questions of its diversity origin and evolution. Since these gastropods show distinct radular tooth morphologies, hypotheses about potential trophic specializations are at hand, because being actual interfaces between the organism and its ingesta (i. e. food, feeding substrate) the teeth are closely related to the food preferred. To identify adaptations in their radulae we examined the mechanical properties (hardness, elasticity) of the radular teeth by the nanoindentation technique. We detected the large-scaled gradients along each type of tooth. These heterogeneities in material enable the teeth from one row to rely on the teeth from adjacent rows, redistributing the stresses and preventing structural failure. The degree of failure prevention, i. e. the capability of teeth to interlock, was found to reflect trophic preferences, as well as functional specialisations of the distinct tooth types. In contrast, the tooth morphologies do not necessarily reflect trophic specialisations, they seem to be more phylogenetically fixed. Finally, we aimed at shedding light on the structural origin of these functional gradients. The elemental composition of teeth, determined by elemental dispersive X-ray spectroscopy (EDX), was identified and compared to the mechanical properties. Even though teeth showed regional differences in composition, we could not always relate the gradients with the elemental proportions. By applying confocal laser scanning microscopy (CLSM), we found that the mechanical properties rather correlate with the degree of tanning. The latter technique is commonly used in studies on the mechanical properties of arthropod cuticle, but was never applied in this context on radular teeth before. In general, we detected that both nanoindentation and CLSM techniques can complement one another, leading to a better understanding of structure-function relationships and to the allocation of trophic adaptations.

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# How many goodly creatures are there here! Origin, radiation and diversification of *Hemicycla* land snails in the Canary Islands (Gastropoda: Stylommatophora: Helicidae)

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The genus *Hemicycla* represents with over 40 (sub-)species the second largest radiation of land snails in the Canary Islands. In a first step, we carried out phylogenetic analyses and palaeogeography-informed time-stratified ancestral range estimation based on mitochondrial and nuclear sequence data to confirm the monophyly and to elucidate the biogeographic history of the tribe Allognathini (Helicidae). The six genera in Allognathini (*Cepaea*, *Iberus*, *Allognathus*, *Hemicycla*, *Idiomela* and *Lampadia*) originated in Western to South-western Europe according to our ancestral area estimation. The disjunct distribution of the Balearic Islands (*Allognathus*) and Macaronesian (*Hemicycla* + *Idiomela* + *Lampadia*) sister clades and the mainly Iberian *Iberus* clade can be explained by the separation of the Betic-Rif System from the Iberian Peninsula during the late Oligocene to early Miocene, along with independent Miocene dispersals to the Balearic Islands and Macaronesia from the Iberian Peninsula, where the ancestral lineage leading to the Macaronesian–Balearic clade became extinct. Preliminary analyses of mitochondrial sequences data of nearly all extant *Hemicycla* species suggest a complex pattern of inter-island colonisations and intra-island diversification, but reveal also cases where morphology-based classifications are in conflict with the mitochondrial phylogeny. In a first case study, we investigated the case of *Hemicycla mascaensis* and *H. diegoi*, two short-range endemic taxa that occur allopatrically in western Tenerife, and which were not recovered as two reciprocally monophyletic clades in the mitochondrial tree. Using multilocus AFLP and double-digest restriction site-associated sequencing data, as well as data on distribution and morphology in an integrative approach, we assessed the status of these two taxa. Our analyses suggest that although multilocus data support the monophyly of the two taxa, species delimitation methods tend to recognise all investigated populations as distinct species, albeit neither lending unambiguous support to any of the species hypotheses. This highlights the need for a balanced weighting of arguments from different lines of evidence to determine species status and calls for cautious interpretations of the results of molecular species delimitation analyses, especially in organisms with low active dispersal capacities and expected distinct population structuring such as land snails.

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## Lakes as islands of evolution and the fossil record of freshwater gastropods

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Lakes, and particularly long-lived (ancient) lakes, are widely perceived as islands of evolution. The geographic isolation paired with an extensive geological duration sparked radiations in numerous lakes world-wide, both in the fossil record and today. A group that is widely represented in freshwater lakes are gastropods. They have achieved stunning diversity levels in many lakes and, due to their high fossilization potential, they qualify as a prime model group to investigate patterns and processes of diversification across space and time. Here, I provide an overview of the diversity developments of freshwater gastropods in Europe and North America, accounting for a total diversity of more than 3700 species. I present latest data on the diversification histories on both continents and potential underlying abiotic and biotic controls, including the impact of large-scale geological and climatic events. The time frame covers the Mesozoic to Pleistocene, but specific focus is given on the rich Neogene (Miocene–Pliocene) lacustrine faunas and ecosystems of central to south-eastern Europe. Furthermore, using a combined dataset of fossil and recent European faunas, I discuss the impact of lake properties as well as the snails' ecological strategies on their extinction risks.

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## Shelled treasures: discovering and preserving the endemic-rich malacofauna of the Gulf of Guinea oceanic islands (central Africa)

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The malacofauna of the oceanic islands of the Gulf of Guinea is rich in endemic species but has long remained understudied. Since 2016, the number of the terrestrial mollusc species for the islands has increased, and it is now at 96. These include 62 endemic species, 92 % of which are single island endemics, and 14 endemic genera. Land-use change and biological invasions are key threats to the native malacofauna, but not knowing the exact consequences of human activities makes it hard to establish conservation priorities for this group. On the largest island, São Tomé, forest degradation is creating novel niches that favour biological invasion, while the native mollusc assemblage prefers wetter and cooler forests with intermediate levels of forest degradation. The presence of introduced plants might facilitate the invasiveness of introduced malacofauna, which tend to be more abundant in anthropogenic habitats. The West African giant land snail *Archachatina marginata* is an introduced species that is distinctly relevant for its nutritional and economical value, highlighting the critical need to integrate the perspective of human populations in the management of exotic species for conservation. At the same time, its endemic congener, the threatened and culturally important Obô giant snail *Archachatina bicarinata*, has suffered a rapid decline in the last decade and has become a flagship for the conservation of the endemic-rich malacofauna and native ecosystems.

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## Re-approaching land snail fauna in three Cyclades islands 45 years later: preliminary biogeographical and ecological results

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The Aegean archipelago, comprising numerous islands with great heterogeneity in topographic, geological, historical and environmental properties, offers an ideal natural laboratory for ecological and evolutionary research, and has been the stage for a very long interaction between human civilizations and local ecosystems. Following more than 10000 years of continuous human presence, many islands of the Aegean have witnessed major landscape changes in the last few decades, predominantly through arising as major touristic attractions and intense urban-centric economic growth. Land snails of the Aegean islands have long attracted scientific attention and their biogeography is particularly well-studied, an asset which along with the group's intrinsic traits of low dispersal ability, presence in a variety of habitats and high number of species, renders them an effective and highly amenable group to study human-nature interactions. In this context we resurveyed three Aegean islands at the same localities that were surveyed 45 years earlier by one of the authors, Moisis Mylonas. Each island has witnessed the effects of humans to a varying degree in the last 45 years: Anafi, the smallest of the three islands (38.6 km<sup>2</sup>), has minorly changed, Kea (148.9 km<sup>2</sup>) has been moderately affected, mainly through land use transformation, whereas Santorini (90.7 km<sup>2</sup>) has been the stage of major touristic and residential development. We herein report changes of species composition at the island level, and compare the species richness of each of the three islands with the species richness of other Cyclades islands in order to address whether any changes recorded through the study period have altered previously reported biodiversity patterns. Contrary to expectations, heavily affected islands are found to have enhanced species richness (ranging between 3–10), mainly through the addition of anthropophilous and/or invasive species, such as *Theba pisana* and *Xerotracha conspurcata* which prevail in both abundance and extended presence across sampling sites. Our results highlight the Aegean archipelago as an appropriate island system to address biotic assemblage changes driven by anthropogenic activities, overall calling attention to the ways such activities may have led to an increased role of biotic homogenization in observed patterns of species richness in the rapidly progressing era of the Anthropocene.

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## Lake Tanganyika's gastropod superflock – after 160 years what do we know and what would we like to know?

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It is now over 160 years since the first species of Lake Tanganyika's remarkable endemic paludomid (Cerithioidea) gastropod radiation were described and over 140 years since the malacologist Bourguignat of "Nouvelle École" infamy introduced 128 species and varieties alone, based almost entirely on beach-worn dead shells. Study of this fauna can be grouped into three chronological phases with differing foci: 1) 1859– circa 1910: discovery, taxonomic outlining/profiling and descriptions (conchological and anatomical), speculation/debate on relationships and origins; 2) 1912–1953: large-scale sampling (dredging), taxonomic revision, distributional synthesis; 3) 1980s–ongoing: widescale SCUBA sampling of living animals, detailed regional sampling, taxon discovery, distributions, synthetic analyses, taxonomy, phylogenetics, phylogeography and ecology.

Despite this long history of research, the molluscan fauna remains understudied. Barriers remain due to logistical challenges in adequately sampling a very large (675×70 km) and species-rich lake that is structurally and geomorphologically complex, and as a result ecologically diverse. These difficulties are compounded by a shoreline extending through four countries, frequently limited infrastructure, and poor road access. As a result, some persistent questions remain. We will briefly touch upon the following questions with respect to the gastropods through examination with new data.

- What is the taxonomic diversity of the gastropod fauna? How do we best establish this?
- Species recognition – is CO1 enough to delimit species?
- Phylogenetic relationships: interpreting origins – did the entire monophyletic radiation evolve in situ? What are the relationships of lacustrine taxa with those in inflowing and outflowing rivers and palaeo-Lake Rukwa?
- What are the lacustrine biogeographic patterns? What does phylogeography tell us?
- What is the 'deep water' fauna and does it show a response to anthropogenic climate change?

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## **Symposium**

# **Insular Ecosystems as Cradles of Mollusc Biodiversity and Evolution**

## **Part II**

### **Abstracts of poster presentations**

## 3D X-ray microscopy reveals *Zospeum troglobalcanicum* Absolon, 1916 and new allied species in caves of the Western Balkans (Eupulmonata: Carychiidae, *Zospeum*)

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Caves of *Zospeum*'s Eastern Alpine and Dinaride range have historically been unevenly sampled such that the northern half of the distribution is richly represented by shells in certain European museum collections while that of the southernmost, Croatian and Western Balkan extension is hardly known. Up to now, only one southern Dinaride species has been described from a cave in Herzegovina. Due to insufficient original description and lack of type material, *Z. troglobalcanicum* Absolon 1916, has remained a taxonomic ghost. Recent investigations at the Natural History Museum in Vienna (NHMW) recovered a syntype shell, now designated lectotype for this species. New finds and other southern Balkan *Zospeum* shells from museum collections are now taxonomically assessable. Since *Zospeum* are tiny, fragile, and rare, 3D X-ray microscopy provides the best technology for accessing the few diagnostic characters sequestered in their shells. Our analysis revealed a remarkable spectrum of morphological variability including: the notch at the junction of the peristome and the upper point of the parietal shield, parietal shield shape and degree of callus deposition on the left side of the peristome, internal columellar configuration, with or without a lamella, and columellar axial alignment as well as its relationship to the base of the shell via the degree of depth and presence of an umbilical depression. Additional diagnosis includes the coiling of the body whorl (ventral perspective) in respect to its alignment with the umbilical depression such that it is either "to the right and oblique of", "directly parallel to" or "on top of the umbilicus". In turn, the last quarter of the body whorl (ventral perspective) presents a compact or non-compact coiling dynamic. By investigating the spectrum of morphological variability in just 15 *Zospeum* populations of this very dense cavernous region, we reveal a species complex in the genus' southernmost Balkan distribution. We expect future molecular investigations to corroborate these findings and hypothesize that these populations encompass 10 species of single-site or narrow range endemics. This rich degree of endemism reflects the highly differentiated geology and subterranean drainage boundaries associated with the isolated island-like landscape of southern Dinaric karst.

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## Snails and slugs in the caves of Greece

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Greece is characterized by its complicate and rich geological structure, counting more than 10000 caves. The fauna of these caves is not well documented, especially for molluscs, but the last years there has been a notable attempt to study the biodiversity of these unique ecosystems. Here, we present for the first time a list of the gastropod fauna from 125 Greek caves based on the collections of the Natural History Museum of Crete. In total, the collection contains 250 specimens coming from the studied caves, which are distributed in various areas of insular and mainland Greece. The specimens were collected between 1984 and 2003, mainly by K. Paragamian (co-author) as well as other Greek speleobiologists. Overall, more than 50 species, belonging to 30 genera and 18 families, were found inhabiting the caves. In most caves only one species was found, and less than 15 caves had four or more species. A large number of the species, 40 %, are Greek endemics with some of them living only in one cave. Given that the present data correspond to approximately 1 % of the Greek caves, it is safe to say that diversity and endemism will increase if we continue to study the thousands of the remaining caves of Greece.

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## The land snails of Paros island (Aegean Sea, Greece) with faunistic and taxonomic remarks

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The Aegean islands, are numerous, with high environmental variability and a long presence of humans, thus provide a ready-made stage for the study of biodiversity patterns and biogeography. Having long attracted the interest of malacologists, they emerge as a top-tier biodiversity hotspot, hosting an impressive land snail fauna of more than 400 species. In a continuous effort to accumulate basic faunistic data on insular snail biotas and to gain insights into the processes influencing insular communities we have surveyed the terrestrial malacofauna of Paros island. Paros, with an area of 196.3 km<sup>2</sup> is the third largest island of Cyclades and stands out as a rapidly developing touristic destination, thus is heavily impacted by human activities in the last few decades. We compiled the complete land snail species list of the island, with the presence of numerous anthropophilous species to be distinctive. Furthermore, we approach taxonomical misconceptions in several genera, based primarily on anatomical comparisons of their reproductive systems. Our results highlight taxonomical uncertainties mainly deriving by highly plastic conchological characters, overall once again pointing out that land snail taxonomy not based on reproductive characters can be usually incomplete and as a matter of fact misleading. Inferentially, sampling live specimens and not only empty shells is of paramount importance to correctly approach land snails' biodiversity on the Mediterranean islands.

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**Open session**

# **Species Delineation**

**Part I**

**Abstracts of oral presentations**

## Whole-genome analyses of *Candidula* (Gastropoda: Geomitridae) reveals the speciation and population structure of the genus

**Luis J. Chueca<sup>1,2</sup> & Markus Pfenninger<sup>2,3</sup>**

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Genomics is significantly enhancing our understanding of biodiversity and speciation relative to traditional genetic approaches and thus has important implications for species delineation and conservation. Due to their low dispersal abilities, land snails have been employed long since in many evolutionary and population genetics studies. While these studies are mainly based on few loci, transcriptomes or mitochondrial genomes, studies at whole genomic level are still scarce. The Geomitridae are one of the most diverse families of molluscs in the Western-Palearctic region. The family is composed of small to medium-sized species, characterized by presenting several (reproductive) adaptations to xeric habitats. For this work, we employed the recent published draft genome of *Candidula unifasciata* and a comprehensive whole-genome analysis of 62 specimens representing almost all species currently recognized within the genus (*C. cavannae*, *C. conglomeratica*, *C. rugosiuscula* and *C. unifasciata*). The phylogenomic and population structure analyses based on around 5000000 single nucleotide polymorphism sites, support four well defined species, corresponding to the described taxa. However, we found a strong genetic structure within *C. unifasciata*, the species with the widely distribution range. In general, our results indicated limit gene flow and admixture between the well-defined species, but also between the widely distributed *C. unifasciata* populations. However, we found a high level of admixture in *C. unifasciata* populations from Western-Alps. This work provides a baseline expectation for future studies on speciation and evolutionary studies at genomic level as well as providing a comparator for similar land snails species.

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## Five slug species confused under the name *Limax nyctelius*

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In 1861 Bourguignat named an Algerian slug *Limax nyctelius*. He both commented on and illustrated the forward position of the pneumostome, convincing us that he was describing the arionid currently known as *Letourneuxia numidica*. Surviving syntypes of *L. nyctelius* include a specimen of *Ambigolimax melitensis*, but our decision to designate the specimen illustrated as the lectotype of *L. nyctelius* means that *L. numidica* takes the name *Letourneuxia nyctelia*. Meanwhile the name *nyctelius* has been applied to four other species, all lacking a penial appendage. 1) A North African species illustrated by Simroth that lacks a rectal caecum, so potentially a *Malacolimax* species. 2) A *Lehmannia* species, usually with a long twisted penis that is swollen at its tip. It occurs, mostly at high altitudes, along the Carpathian Mountains and from northern Albania to eastern Bulgaria. We have named this *L. carpatica*. 3) An *Ambigolimax* species, also usually with a long convoluted penis, probably native in Algeria, but known from Elba and established in South Africa, Australia and New Zealand. We have named this *A. waterstoni* after A. R. Waterston who discovered it in the Royal Botanic Garden Edinburgh. One distinctive character is two prominent flaps running inside the penis, joining together at its posterior end, and between which the penis wall has a honeycomb appearance. 4) An *Ambigolimax* species with a very short penis. It is spreading rapidly in the British Isles and is recorded also from France, Greece, Spain, California and Arizona. We have named this *A. parvipenis*. Besides the differences in genitalia, the length of the rectal caecum proved a useful identification character; it extends to, or beyond, the tip of the digestive gland in *Lehmannia* but stops well anterior to the tip in *Ambigolimax*, except that in *A. valentianus* it reaches almost to the tip.

(All names and nomenclatural acts in this abstract are disclaimed for nomenclatural purposes.)

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# How many species? Limits of species concepts in the genus *Trochulus* (Gastropoda, Eupulmonata, Hygromiidae)

**Jeannette Kneubühler<sup>1,2</sup>, Małgorzata Proćków<sup>3</sup>, Eva A. Bischof<sup>1</sup>, Marco T. Neiber<sup>4</sup>, Simon Bober<sup>4</sup>, Olivier Gargominy<sup>5</sup>, Ted von Proschwitz<sup>6,7</sup> & Eike Neubert<sup>1,2</sup>**

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Most of the species' descriptions of the hairy snail genus *Trochulus* Chemnitz, 1786 are based on morphological and anatomical characters. Unfortunately, these characters fail to address the diversity of *Trochulus* by leaving cryptic species unrevealed. Therefore, it is crucial to include molecular data, which could – at least for cryptic species – serve as the backbone of a taxonomic decision. In this integrative study, we included 581 specimens from 393 populations. In order to cover the entire distribution range of species affiliated to this genus, additional sequences from databases of 131 specimens were added. We used two mitochondrial (COI and 16S) and one nuclear marker (5.8S rRNA + ITS2) for the Bayesian Inference, Maximum Likelihood and IQtree analysis of these 712 specimens. In these three analysis methods, we found 22 stable lineages, but their relationships differed between the methods. In order to stabilise the phylogeny, additional ddRAD sequencing was carried out with 100 of the 581 specimens.

For the first time, the morphology in *Trochulus* was analysed using landmark and semi landmark-based geometric morphometric methods (GMM) instead of traditional, linear measurements. The advantage of using landmarks and semi landmarks is that shape variations of morphologies are covered. In order to morphologically define the lineages found, a 2D landmark-based geometric morphometric analysis was carried out on photos of 204 adult specimens. For this, we used the frontal and ventral photo view and combined these two data sets in the analyses. A Principal Component Analysis (PCA) was run to visualize the multivariate data. Some lineages could be well separated morphologically, but some cryptic species could not be well separated even with shape-based morphometric analyses.

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## Diversity of the land snail genus *Cyclophorus* (Caenogastropoda, Cyclophoridae) in Southeast and East Asia: spatial patterns and underlying mechanisms

**Parm Viktor von Oheimb<sup>1</sup> & Katharina C. M. von Oheimb<sup>1</sup>**

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Operculate land snails of the genus *Cyclophorus* (Caenogastropoda, Cyclophoridae) are widely distributed in South, Southeast and East Asia. During the last years, we have studied the diversity and distribution of this group in Vietnam and Japan using material from museum collections and recent field work. Analyses of community structure based on characters from molecular phylogenetics and shell morphology were applied to *Cyclophorus* communities from limestone karst areas in Vietnam. The predominant pattern of phylogenetic and morphological overdispersion indicated a major role of interspecific competition in shaping the communities. While co-occurring *Cyclophorus* taxa were found to differ considerably in shell morphology, molecular phylogenetics revealed that various morphologically highly similar representatives of the genus occur in allopatry. Processes of convergent evolution were probably involved in their origin. In Japan, many islands are inhabited by not more than one *Cyclophorus* lineage. While oversea dispersal of the snails must have taken place in the past, limited ecological niche space might have prevented the establishment of multiple species on individual islands.

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## Diversification of *Littorina horikawai* endemic in the western coast of Kyushu, Japan

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The genus *Littorina* is an abundant and common marine snail species distributed in the temperate and sub-arctic regions of the northern hemisphere. Due to its high ecological, morphological and genetic diversity, *Littorina* has long been treated as a model taxon for ecological and evolutionary studies. Here, we focused on the East Asian *Littorina* species, *L. horikawai*. The distribution area of *L. horikawai* is a narrow range compared with other *Littorina* species and is limited to the western coastal area and the adjacent islands around Kyushu, Japan. Our previous molecular study based on mitochondrial DNA demonstrated that *L. horikawai* exhibited a significant genetic population differentiation consistent with the geographical structure. Besides, its shell morphology was reported to vary among islands. However, the details of morphological diversity and phylogenetic relationships are unknown. In the present study, we conducted field surveys and sampling covering most of the distribution range of *Littorina horikawai* (Tsushima Island–Yakushima Island). We performed the morphological evaluation based on shell contour and high-resolution genetic analysis using an SNPs dataset obtained by ddRAD-seq. The shell morphology was broadly classified as ribbed and smooth, with each island differing in size and shape. Also, the level of genetic differentiation among islands is high and most island population is monophyletic. The above morphological and genetic evidence confirmed that remarkable diversification of *L. horikawai* occurred within a narrow geographic area in the western coastal area of Kyushu. It is suggested that this diversification of *L. horikawai* was driven by its low dispersal ability, discontinuous island inhabitation, and ecological opportunities.

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**Open session**

# **Species Delineation**

**Part II**

**Abstracts of poster presentations**

## Assessing species boundaries in the freshwater snails' family Physidae using coalescent-based delimitation methods

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A common issue for several freshwater snails' families is the fact that, for some genera, the criteria for species delimitation are scarce, subjected to intraspecific and ontogenetic variation, or insufficiently discrete to allow a clear distinction between species. For these genera, the use of molecular approaches is decisive in recognizing and validating species. Herein, we applied coalescent methods to delimit evolutionary significant units (ESUs) in Physidae. We used 496 sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene available in GenBank and ascribed to six genera and 25 nominal species; and the algorithms GMYC (General Mixed Yule-Coalescent) with two criteria (single-threshold and multiple-threshold), PTP (Poisson Tree Process) and mPTP (multi-rate Tree Poisson Process). From the analysis of these four delimitation methods, we found out that the mPTP represent a more accurate alternative to solve inconsistencies in recognizing and delimiting significant evolutionary units in Physidae. The majority of ESUs inferences using mPTP showed high bootstrap support. The matrices of genetic distance generated for the mPTP showed that most of the pairwise combinations of ESUs presented interspecific distance higher than 5 %, considered as the threshold of interspecific distance for Physidae. The mPTP presented the higher frequency of match profiles and showed the smaller number of splitter cases. Some ESUs delimited by the mPTP with high support values were split in different ESUs by the other methods, with genetic distances that fall into the intraspecific limits. These results demonstrate the strong support for most of the ESUs delimited by mPTP and the corresponding taxonomic outcomes concerning species limits. Herein we applied coalescent delimitation methods to Physidae, a family of freshwater snails that present several issues related to the recognition of species boundaries. This approach allowed us to address important taxonomic questions, showing new avenues for future taxonomic research. Our finds showed that the morphological operational criteria used to delimit several valid species fall into the limits of intraspecific variability, evidencing the presence of taxonomic inflation within this family. Finally, our results have also evidenced the presence of cryptic diversity under the nominal species *Physella acuta*, *Beringophysa jennessi*, *P. pomilia* and *P. gyrina*.

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**Open session**

**Environmental  
Stress/Adaptation**

**Part I**

**Abstracts of oral presentations**

## Genomic relatedness matrix-based heritability of immune function in the pond snail *Lymnaea stagnalis*

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Complex immune defences have evolved to counter the serious threat parasites and pathogens pose on organisms. Here, we asked how heritable are the non-specific immunological traits of the immune system? We used high-marker density genotyping to estimate the genetic relatedness of self-compatible hermaphroditic snails, *Lymnaea stagnalis*, from a natural population to assess quantitative genetic parameters for immune function under field conditions. We estimated additive genetic variance and covariance of three phenotypic immune traits: haemolymph phenoloxidase, laccase and antibacterial activity while controlling for variation in immune activity arising from the season and snail resource level. We also controlled for trematode infection status of individuals as *Lymnaea stagnalis* is a common host for several trematodes that castrate the snails. We calculated genomic relatedness between 600 field-collected snails using around 19000 independent Single Nucleotide Polymorphism markers. Heritability of immune traits ranged from 0.12 to 0.22. We found positive genetic correlation between phenoloxidase and laccase activity and signs of negative genetic correlation between laccase and antibacterial activity. Our results suggest that potential genetic trade-offs among immune traits may maintain within-population genetic variation in immune activity.

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## Genetic limits of phenotypic plasticity – genome comparison of two ecotypes of *Theodoxus fluviatilis*

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In the light of the current climate change and its consequences to species with limited dispersal mechanisms, the ability to respond to novel and changing environmental conditions, either by phenotypic plasticity or by genetic adaptation, is pivotal to long-term survival. *Theodoxus fluviatilis* (Gastropoda: Neritidae) is a euryhaline snail that, due to the lack of a free-swimming larva, has limited dispersal ability. It forms two fairly isolated ecotypes in central Europe, the limnic/freshwater and the brackish water ecotypes. In laboratory experiments, individuals from the different ecotypes differ in their responses to salinity changes and show different survival rates in extreme salinities that cannot be matched even upon slow and stepwise acclimation. Under hyperosmotic stress *Theodoxus fluviatilis* uses organic osmolytes (amino acids, urea) for maintaining tissue volume. However, the mode of osmolyte generation seems to be different in animals of the two ecotypes. Other factors (inorganic ions, ion transporters, skin water permeability) supposedly contribute to the physiological and survival differences. Our hypothesis is that these differences between the two ecotypes are due to genetic differences. We performed whole genome sequencing (Illumina) with individuals from either ecotype and compared these individual genomes within populations and between ecotypes and identified genetic differences that may be related to local adaptations. Preliminary results of the bioinformatic analysis pipeline will be shown.

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## Combined effects of heatwave and toxic algal bloom on the pacific oysters, *Crassostrea gigas*

**Ellen Grace Funesto<sup>1,2</sup>, Thomas Cameron<sup>1</sup>, Andrew Turner<sup>3</sup>,  
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Increasing ocean temperature has facilitated the intensification of toxic algal blooms. With the global climate change projections, these two stressors can co-occur more frequently in the future. Despite this, limited studies have investigated the effects of both stressors on the marine environment and organisms, specifically in sedentary bivalves, which cannot escape a heatwave occurrence. These bivalves are also constantly exposed to toxic algal bloom in the water as they filter-feed. Our study investigated the direct and delayed effects on the physiology (oxygen consumption and body condition) and toxin accumulation of *Crassostrea gigas* (pacific oysters) that were exposed to a simulated heatwave and bloom of *Prorocentrum lima* (a diarrhetic shellfish toxin-producer). Our study provides evidence that exposure to the individual stressors and their combination modifies the metabolic response of the oysters. Specifically, the oxygen consumption rate of the oysters increased significantly when exposed to heatwave alone. However, in the combined treatment where both heatwave and *Prorocentrum lima* bloom were employed, the oysters did not have the same response to a heatwave that in normal conditions leads to a particular increase in metabolic activity. Body condition was not affected by individual stressors and their combination. Toxin accumulation was higher in bivalves exposed to the heatwave, and *P. lima* bloom than those exposed to *P. lima* bloom alone. The findings of this study will have an implication on the ability of the oysters to survive a stressful environment that requires an immediate response and may have an impact on the future of shellfish production.

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## Ecoimmunology of *Physella acuta* (Gastropoda, Panpulmonata, Hygrophila)

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(A)biotic stressors challenge distribution and survival of molluscs in natural environments. Fitness at both individual and population level is influenced by genetically determined immune capabilities, potentially driving speciation. Traditional immunological studies, usually performed with inbred laboratory strains under artificial conditions have characterized molluscan immune mechanisms. This so-called mechanistic immunology, however, cannot reveal all immune capabilities, effective immune alleles, nor individual nuances in immune responses that shape fitness among and within populations of molluscs. This study of interactions between fitness and immunity in different environments uses the freshwater snail *Physella acuta*. In the native range (North America), the species *P. acuta* includes two genetically distinct populations with differential fitness. Mitohaplotype A distinguishes the invasive *P. acuta* population that achieved global distribution from snails with mitohaplotype B (10 % sequence difference), restricted to North America. Uniparental offspring was generated by selfing to establish genetically-defined lab-maintained populations A and B. Adult *P. acuta* (shell length  $\geq 5$  mm) of these two populations are compared for fitness and immunobiology, under lab conditions and in the field. For the latter, snails are “rewilded”: lab-reared *P. acuta* are placed in natural conditions in flow-through cages at a field site (1–2 weeks). Lab-maintained populations A and B showed similar growth rate and size to maturity. Compared to the lab, rewilded *P. acuta* from population A and B similarly increase reproductive output (number of egg masses). Differential fitness was evident from B snail populations suffering higher mortality in the field than A snails (3/7 replicates). Illumina RNAseq (screened against parasite field infections; rDNA, mitochondrial sequences removed) indicates different gene expression between lab-maintained and rewilded snails, within and between populations, based on different GC-content and differential presence of select immune transcripts among experimental groups. Results will be presented from ongoing differential expression analyses that are applied to characterize the transcriptomic profiles that underlie the differential fitness of populations A and B of the species *P. acuta*, with special focus on immune factors.

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## Plasticity in energy budgeting as an adaptive response to environmental stress in larval and juvenile clams (*Mercenaria mercenaria*)

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Larval stages of bivalves are particularly susceptible to acidification, as changes in seawater chemistry increase the energetic cost of building a shell and maintaining homeostasis. Consequently, energy used to cope with acidification reduces larval survival, decreases growth, and development time. The cumulative negative effects of combination of stressors on individuals may magnify to differences in population persistence, and subsequently risk of extinction with continued climate change. This study focuses on clam (*Mercenaria mercenaria*) populations located in the coastal bays of Long Island, New York (USA) where clam spawning annually co-occurs with acute summer acidification, hypoxia, and thermal stress, and where large-scale restoration efforts are on-going. This study evaluates whether, in the absence of adaptation, larval exposure to stressors can increase population tolerance by leveraging existing variation in the energy metabolism. Experimental cohorts were reared from embryo through early juvenile life stages under combinations of stressors (high/low pH x high/low dissolved oxygen), using standard metrics of tolerance (shell growth and survival rates) and the energy metabolism (respiration rates, total carbohydrate, lipid, protein and % organic content). Plasticity in allocation (energy storage: growth: maintenance) were significantly associated with the larval environment an individual experienced, suggesting that naivety to stressors and lasting energy deficits from larval stages are key factors influencing post-metamorphic (juvenile) performance.

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## Reproduction in the giant clam *Tridacna gigas* is compromised during bleaching stress

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Giant clam bleaching which is defined as the breakdown of symbiosis between giant clams and their zooxanthellae caused by elevated seawater temperatures was observed to cause mortalities in giant clams. After bleaching disturbance, the recovery of their population relies on reproduction and recruitment success. However, how bleaching will directly affect the reproductive performance of giant clams is not yet known. Here, we examined the direct impacts of bleaching on the reproduction of the giant clam *Tridacna gigas* between 2020 and 2021 in the Philippines. A continuous six month in-situ monitoring of egg concentration, egg stage, egg size, and survival in the prolonged bleached (moderate or severe) and non-bleached *T. gigas* was conducted. The probability of producing eggs and the proportion of intact eggs (developing and mature) were higher in healthy giant clams, followed by moderately bleached giant clams, and lowest in severely bleached giant clams in which >50 % individuals had empty gonads. The oocyte mean diameter was similar among giant clam type. Only five severely bleached giant clams showed partial color recovery, and two healthy and 12 severely bleached giant clams died. This study shows that bleaching can reduce egg production, disrupt gametogenesis, and cause mortality, particularly in the severely bleached giant clams *T. gigas*. These results suggest that the intensification of bleaching due to climate change can lower the reproductive potential of giant clams and likely other zooxanthellate organisms resulting in reduced recruitment and decline in their population.

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## Ecotoxicological assays employing the freshwater gastropod *Biomphalaria glabrata* (Say, 1818)

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Ecotoxicological assays establishment with native organisms sensitive to different classes of pollutants and greater ecological significance is increasingly encouraged in all countries. In this sense, gastropods are considered innovative, responsive and of extreme environmental importance. However, standardised tests with molluscs are still scarce, and more information is needed about the biology of promising organisms as bioindicators, as well as their interactions with different chemical substances are required. Among the most studied species for such analyses in Europe, stands out the freshwater gastropod *Lymnaea stagnalis* (Linnaeus, 1758), regulated by the Organization for Economic Co-operation and Development. In Latin America, some species are more representative, with wide geographic distribution and presents a large volume of data available (most of them developed in Brazil), mainly with the freshwater gastropod *Biomphalaria glabrata* (Say, 1818). In addition to the most common ecotoxicological assays, the search for techniques with lower disposal of substances generated in laboratory analysis, and that evaluate the sub-lethal risks of pollutants at low concentrations, as well as the development and reproduction analysis are relevant and suggested by environmental monitoring programs. In this work, reference substances (copper sulphate and sodium dodecyl sulphate) were used to establish the acute, chronic toxicity and sublethal effects assays with *B. glabrata*. Such compounds are proposed as a positive control for the tests and need to have their toxicity ranges defined for each of the endpoints evaluated. In this way, this work aimed to carry out the intercalibration between laboratories in different regions of Brazil and define the conditions of the test carried out in parallel by three Laboratories (Butantan Institute, Embrapa and University of Caxias do Sul) for later regulation at ABNT (Brazilian Association of Technical Standards), agency for the elaboration of standards for tests in Brazil, with the aim of adapting tests to regional Brazilian needs, with native species and greater ecological significance. The proposed protocols for developmental and toxicity assays were reproducible between laboratories. The realisation of different approaches to studies allows the establishment of permissible limits and to evaluate the impact of compounds in the ecosystems with environmental and ecological relevance for one country.

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## Studies on the Microbiome of *Crassostrea gigas*

**J. Wink<sup>1</sup> & H. Pira<sup>1</sup>**

The Pacific oyster (*Magallana gigas*, Syn.: *Crassostrea gigas*), also known as the Pacific rock oyster, is the commercially most important type of oyster with a world market share of 93.7 % (2003). It is called Huitre creuse (du Pacifique) in French and Pacific (cupped) oyster in English. This species of oyster is extremely hardy and disease resistant, and it grows very quickly. This species of oyster is native to the coastal waters of the western Pacific. In the meantime, however, it has been spread over large parts of the world as farmed oysters. According to genetic analyses, the breeding animals came from the coast of Miyagi Prefecture in the northeast of the Japanese main island of Honshu. It has spread further from the oyster cultures in Europe. In 1964 the species was introduced into the Oosterschelde (Netherlands), from where it subsequently spread northwest and reached the Wadden Sea near Texel around 1980. In the area of the German North Sea coast, it was first discovered in 1986 near the first German oyster farm west of Norddeich. Until 2002 there were only a few Pacific oysters in the Wadden Sea of Lower Saxony but today it is one of the most common shells in this region.

One of our approaches to isolate and cultivated uncommon microorganisms for the evaluation of the capacity to produce bioactive compounds is the usage of oyster bank material. In the oyster banks we have the living oyster on one hand but also the dead animals and the lumen between the shells that is filled with sediment containing many different microorganisms. In the talk an overview of our first results will be given:

- Description of novel bacterial species from oyster with the example of *Pacificimonas pallium*
- Novel species description and detection of new prodigiosin derivatives active against oyster pathogenic bacteria
- Identification of a *Photobacterium* species originally only known from Korean beach
- Antimicrobial potential of culturable actinobacteria isolated from the pacific oyster

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## Genomic adaptation shapes holobiont metabolism in deep-sea hydrothermal vent mussels

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Evolutionary adaptations to extreme environments are often associated with drastic phenotypic modifications, yet, the genomic basis of such adaptations is still largely unknown. Deep-sea hydrothermal vents offer unique opportunities to study how life adapts to extreme environments. However, limited availability of deep-sea species' genomes has hitherto hindered progress in this area. To investigate potential deep-sea mussel adaptations, we sequenced two mussel genomes, *Bathymodiolus thermophilus* (a hydrothermal vent mussel with a sulfur-oxidizing bacterial symbiont, SOB) and its shallow-water relative *Modiolus nipponicus*, and compared them with coastal mussel species and a deep-sea mussel with a methane-oxidizing symbiont (MOB). Comparative genomic analyses revealed that deep-sea species show significant expansion of gene families involved in innate immunity, apoptosis, and hydrolysis of bacterial cell walls, key to maintaining symbiosis. Additionally, *B. thermophilus* features a duplicated 3'-phosphoadenosine 5'-phosphosulfate synthase (PAPSS) gene, which likely enhances the mussel's ability to detoxify symbiont-derived sulfate, while simultaneously providing valuable sulfate donors for pivotal sulfation reactions. This integration of host and symbiont metabolic routes into a holobiont metabolism might present a hitherto unknown niche-specific adaptation to sulfide-rich hydrothermal vent environments.

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**Open session**

**Environmental  
Stress/Adaptation**

**Part II**

**Abstracts of poster presentations**

## Freshwater bivalves – accumulation and retention of micro and nanoplastics

**Aleksandra Skawina<sup>1</sup>, Agata Bonk<sup>2</sup>, Bohdan Paterczyk<sup>3</sup> & Agnieszka Dąbrowska<sup>4</sup>**

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Plastics of a variety of compositions, shapes, and sizes are currently widespread in the soils, freshwaters, and oceans. Although freshwater is considered an important source of plastics to the oceans, little is known about the role and patches of the plastics in the freshwater ecosystems.

Here we discuss the early results of the exposition of the freshwater bivalves (three species) to the three possible plastic stressors: polystyrene nanoplastics (15, 100 nm), polystyrene and poly(methyl methacrylate) microplastics (1, 20 µm), and polyethylene gathered from the cosmetics (50–400 µm). We tested the stress parameters in the exposed bivalves' tissues and analyzed the physics-chemical parameters of the plastics. We found that freshwater bivalves can gather the selected fractions of plastics' suspension; they can accumulate and deposit in their droppings the collected particles into the sediments. Thus, they become a crucial element in freshwater ecosystems to slow down the downstream flow of plastic suspension to the oceans and likely may introduce plastic particles to the freshwater food webs.

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**Open session**

# **The Malacological Community**

**Part I**

**Abstracts of oral presentations**

## Equity, diversity, inclusion and accessibility in UK polar science and malacology

**Madeline Anderson<sup>1</sup>, Lucy Stephenson<sup>1</sup>, Donna Frater<sup>2</sup>, Huw Griffiths<sup>1</sup>, Katrin Linse<sup>1</sup>,  
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The polar community has been historically dominated by White men, dating back to the “heroic age of Antarctic exploration”. After the colonial discovery of the continent some 200 years ago, it wasn’t until the 1980s that British female scientists were allowed to do fieldwork in Antarctica. The diversity of the UK Polar Science community, including that of malacological science, still does not reflect that of the wider UK Society. This underrepresented talent includes women, Black, Asian and minority ethnic people, the LGBTQ+ community, people with disabilities, and those from a lower socio-economic background. The continuing exclusion of these groups through systemic bias and underrepresentation (and the subsequent loss of diversity and innovation) confirms the need for targeted actions to improve equity and inclusion within the workplace. Whilst this is not an issue unique to polar or malacological science, it is one that we need to tackle for our research to remain relevant in society today.

The desire for meaningful, proactive change has resulted in the 2019 establishment of the Diversity in UK Polar Science Initiative (DiPSI). The Initiative celebrates existing diversity, as well as enhancing further polar science opportunities for underrepresented groups. Since DiPSI’s formation, Equality, Diversity, Inclusion and Accessibility (EDIA) has increasingly grown in presence in UK Polar Science, with organisations and groups systematically including an EDIA voice in much of our work. We will introduce the recent work from committees and organisations, including the 2021/22 Natural Environment Research Council (NERC)-funded EDI internships. These are timely, with the Covid-19 pandemic disproportionately affecting underrepresented groups. The internships are an opportunity for such Early Career Researchers to address their experience and network gaps by providing lab-time and in-person working to help advance their careers. Two individuals undertaking malacological projects benefited, resulting in publications and furthering malacological research interests.

With alternative technologies and ways of working, malacological and polar science roles are evolving. It is imperative that there is a continued commitment to EDIA through processes, investment, listening and leadership to work towards a more inclusive and respectful culture across all areas of STEM and research.

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## Laudatio for Dr. Winston F. Ponder – a life of malacology

**D. Lindberg<sup>1</sup> & G. Haszprunar<sup>2</sup>**

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For well over half a century Winston Ponder has been at the forefront of molluscan systematics, functional morphology, evolution, and conservation. As a boy Winston tramped over the steep hills behind his home in Lower Hutt, New Zealand collecting land snails. But the main place he experienced molluscs was Wai-kanae Beach on the Kapiti Coast, northwest of Wellington. There he wandered the tideline and estuary looking for shells. Winston attended Victoria University in Wellington in 1959 but moved to Auckland University in 1960 to study under the newly appointed Professor John E. Morton, with whom he completed his B.Sc. in 1963 (Galeommatoidea), his M.Sc. with 1<sup>st</sup> Class Honors in 1965 (Rissoidea), and his PhD in 1968 (Neogastropoda). A DSc would later follow in 1991. Winston's first curatorial position began before he had completed his doctorate when he was appointed Curator of Marine Invertebrates at the Dominion Museum in 1967. However, this position was short lived and he moved across the Tasman Sea to Australia and took the position of Curator of Molluscs at the Australian Museum in 1968. He would spend the next 37 years there. Winston has published in almost 50 different scientific journals and numerous edited books and volumes. He has collaborated with a diverse array of co-authors, and like the journals he published in they also came from around the world; as of now he has published with almost 100 different co-authors. Winston's research program has addressed multiple taxa and questions across the phylum. He has been a strong advocate for Australasian malacology, bringing both the history and diversity of this fauna to the attention of a discipline with deep roots in European and North American faunas and processes. His advocacy has also included a robust outreach to students to ensure continued investigation and documentation of this important molluscan fauna. However, his advancement of Australasian malacology was not done to the exclusion of the global role and needs of the discipline. As one of the primary promoters for a World Congress of Malacology, Winston was instrumental in the political discussions and strategies for its creation.

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## **Stewarding Harvard University's malacology collection into the future**

**J. Trimble<sup>1</sup>**

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Natural history museums are facing increasing challenges in the face of decreasing biodiversity due to habitat loss and climate change, financial woes, and more stringent international collecting policies. At the same time, unparalleled genetic sequence capabilities of museum specimens and other innovative techniques allow for novel and exciting avenues of research for our specimens. These combining factors call for careful stewardship and honoring of past efforts while, at the same time, embracing advancements such as targeted digitization efforts, imaging initiatives, and genetic and chemical techniques to position museum repositories as first tier resources for scientific use. The Malacology Department at Harvard's Museum of Comparative Zoology is spearheading such digitization and genetic preservation efforts to ensure the collections relevance and utility into the future. With a collection dating back to 1860, starting with the purchase of land and freshwater snail specimens from J. G. Anthony, onward to the enthusiastic oversight of its past curators including, W. J. Clench, R. D. Turner and K. J. Boss, the Department of Malacology at the Museum of Comparative Zoology has developed one the largest and most diverse mollusk collections in the world. With our newest curator and museum director, Gonzalo Giribet, the department continues to grow and integrate new methodologies of specimen curation. Currently, the Museum of Comparative Zoology's Malacology Department's efforts to modernize and increase accessibility of the collections for the current research community include grants focusing on database innovations, specimen data capture, imaging techniques and improved storage facilities. Through these strategic efforts the museum will continue to embrace malacological research and preservation.

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**Open session**

# **The Malacological Community**

**Part II**

**Abstracts of poster presentations**

## An advertisement for the journal *Archiv für Molluskenkunde*

**J. M. C. Hutchinson<sup>1</sup>**

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*Archiv für Molluskenkunde* is the oldest malacological journal still publishing (founded 1868). Here are some reasons for you to submit your manuscripts to us:

- SCIE ranked: impact factor = 0.704. CiteScore = 1.4.
- No page charges, even for colour plates.
- Articles become free to download after 4 years behind a modest paywall (€ 19 per article).
- Alternatively authors may opt to pay for immediate open access (€ 1500 for ≤30 printed pages).
- 25 free reprints per article + pdf to distribute privately.
- Published twice per year.
- All content is in English.

We have mostly published about systematics, taxonomy, phylogeny, and morphology of molluscs, including Cenozoic fossils. But we would like more submissions about molluscan ecology, biogeography, and behaviour. We consider manuscripts dealing with historical issues (e.g. biographies, bibliographies) if these have relevance for taxonomic research.

Also, consider subscribing. This costs € 68 per year. Alternatively, by joining the German Malacological Society (Deutsche Malakozoologische Gesellschaft: <http://www.dmg.mollusca.de/index.php>) you can receive *Archiv* at a reduced rate and also receive their *Mitteilungen* (another high-quality journal); there are discounts for students.

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## Extended mollusk specimen network at the University of Michigan Museum of Zoology (UMMZ)

**T. Lee<sup>1</sup>, T. F. Duda Jr.<sup>1</sup> & D. Ó Foighil<sup>1</sup>**

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The UMMZ Mollusk collections have served as a premier resource for scientific research and education for over a century. To enhance its research potential, we have led an effort to digitize the collection and to link specimen records with associated extended data. Since the turn of the century, data of more than 200 000 lots of mainly freshwater and terrestrial specimens have been entered to the UMMZ *Specify* database, a process facilitated by the support of five National Science Foundation (NSF) awards, including four Thematic Collections Networks (TCN) grants: ‘Great Lakes Invasives,’ ‘InvertEBase,’ ‘Pacific Island Land Snails Biodiversity Repository’ and ‘Mobilizing Millions of Marine Mollusks of the Eastern Seaboard’. This effort has involved the training and active participation of 106 UM graduate- and undergraduate students. Networking of georeferenced locality data have formed a solid foundation for distribution mapping. Over 50 000 specimen images and 10 000 label/catalogue scans have been added to verify and enhance specimen data. Digitized data and images are available through Symbiota web portals (invertebase.org and pilsbry.org), national resources (idigbio.org and gbif.org) and the University of Michigan Digital Library. Consequently, we now have a much more accessible and integrated body of information that has been generating over 60 000 downloads and 50 million views per year since 2019. In addition, during the past decade, the entire collection was relocated to a newly renovated Research Museums Center (RMC) with environmentally controlled collection spaces and new archival specimen cabinets and drawers. Our future endeavors will continue to reinforce the UMMZ Mollusk collection’s extended specimen database. Data entry foci will shift to include not only marine, wet and tissue specimens but also derivative products such as gene sequences and metagenomes. Efforts will be made to identify and complete records lacking critical data fields to maximize their value. All the associated digital data residing in disparate databases will be linked directly to the specimens.

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## DORA: Digitize – Organized – Reliable – Automated

**Nora Lentge-Maaß<sup>1</sup>, Erik Trostmann<sup>2</sup>, Margot Belot<sup>1</sup>, Michael Schiller<sup>2</sup>,  
Silvio Sperling<sup>2</sup>, Andreas Herzog<sup>2</sup> & Frederik Berger<sup>1</sup>**

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Natural History collections are undergoing a vast digitization process in recent years. This digital transformation addresses mainly digital cataloguing of metadata related to collection objects in central databases. Since most collections struggle to inventorize these objects within a decent amount of time, new ways of fast and effective inventory, as well as semi-automated pictorial digitization need to be brought up in natural history collections to reach the goal within the next decade.

The Museum für Naturkunde Berlin started an action plan in 2020 regarding the full inventory of the approx. 30 million collection objects, creating a complete digital catalogue of its holdings within 10 years. The biggest challenges here are the object rich collections like Mollusca, fossil Invertebrates or insect collections. Therefore, the managers of digitization and collection staff team up with partners from other institutions as well as, industries to invent and create ways of digitization using new techniques.

One of these new techniques and next generation digitization stations is DORA, jointly created by the Museum für Naturkunde Berlin (Dr. Berger, Dr. Lentge-Maaß, Ms. Belot) and the Fraunhofer Institute for Factory Operation and Automation IFF (Mr. Trostmann, Mr. Schiller, Dr. Herzog, Mr. Sperling). DORA is a Scanner with four industry cameras. Three cameras are used for object digitization and are able to produce sharp pictures with focus stacking. While the fourth camera captures the labels and is able to read QR-codes. Those QR-codes have been recently introduced into the collection enabling automated connection between photos and metadata. Prior to specimen scanning, the object metadata can be catalogued and bond to a QR-code via the new DORA-inventory-tool. This tool is designed to produce Json-files storing a minimal-dataset consisting of taxonomic information, collection number, location as well as, the type's status, and connects this with a machine readable QR-code. Once an inventorized object is scanned with DORA, pictures will be automatically connected with its metadata from DORA's library. This way a time frame below 5 minutes per lot in the malacological collection can be achieved, making the complete inventory and digitization by 2030 a reachable goal.

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## 20 years of “Weichtier des Jahres” (German Mollusc of the Year)

Vollrath Wiese<sup>1</sup>

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The “Weichtiere des Jahres” (German Mollusc of the Year) project presents interesting molluscs to the public since 2003. The project was founded by Karl-Heinz Beckmann (1948–2007), at that time member of the board of the German Malacological Society (“Deutsche Malakozoologische Gesellschaft” = DMG). The campaign was intended to publish information about the elected “Mollusc of the Year” and to promote molluscs in public. Especially popular media such as newspapers, journals and broadcasting institutions should be encouraged and enabled to present the “Weichtiere des Jahres” by texts and photos. The “Weichtiere des Jahres” is elected by a board of trustees (“Kuratorium”), comprising the malacological societies and institutions in Germany (in 2022 fifteen institutions are involved). A leaflet about the particular “Weichtiere des Jahres” is published annually in print and online on the webpage of the German Malacological Society. The DMG is now responsible for the project.

“Weichtiere des Jahres” – German Molluscs of the Year:

- 2003 Bauchige Windelschnecke *Vertigo moulinsiana* (Dupuy 1849)
- 2004 Gemeine Kahnschnecke *Theodoxus fluviatilis* (Linnaeus 1758)
- 2005 Tigerschneigel *Limax maximus* Linnaeus 1758
- 2006 Gemeine Flussmuschel *Unio crassus* Philipsson 1788
- 2007 Maskenschnecke *Isognomostoma isognomostomos* (Schröter 1784)
- 2008 Mäuseöhrchen *Myosotella myosotis* (Draparnaud 1801)
- 2009 Husmanns Brunnenschnecke *Bythiospeum husmanni* (C. Boettger 1963)
- 2010 Gemeine Schließmundschnecke *Alinda biplicata* (Montagu 1803)
- 2011 Zierliche Tellerschnecke *Anisus vorticulus* (Troschel 1834)
- 2012 Schlanke Bernsteinschnecke *Oxyloma elegans* (Risso 1826)
- 2013 Europäische Auster *Ostrea edulis* Linnaeus 1758
- 2014 Knoblauch-Glanzschnecke *Oxychilus alliarius* (Miller 1822)
- 2015 Mantelschnecke *Myxas glutinosa* (O. F. Müller 1774)
- 2016 Große Erbsenmuschel *Pisidium amnicum* (O. F. Müller 1774)
- 2017 Schöne Landdeckelschnecke *Pomatias elegans* (O. F. Müller 1774)
- 2018 Neptunschnecke *Neptunea antiqua* (Linnaeus 1758)
- 2019 Heideschnecke *Helicella itala* (Linnaeus 1758)
- 2020 Gefleckte Schüsselschnecke *Discus rotundatus* (O. F. Müller 1774)
- 2021 Gewöhnlicher Tintenfisch *Sepia officinalis* (Linnaeus 1758)
- 2022 Bayerische Zwergdeckelschnecke *Sadleriana bavarica* Boeters 1989)

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**Open session**

# **The Neglected Classes**

**Part I**

**Abstracts of oral presentations**



## Life's a beach: Diversity and ecology of shallow water interstitial Solenogastres (Mollusca, Aplacophora)

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Most species of Solenogastres (Aplacophora, Mollusca) have been described from the lower continental shelf and the upper bathyal zone of our oceans. Recent investigations into abyssal and hadal solenogaster fauna have revealed surprising species richness in the deepest parts of our oceans. Remarkably, only few solenogaster species are known from the most accessible depth zone: the intertidal and shallow subtidal.

Among this handful of species are some of the largest and most colorful Solenogastres, but also representatives from the meiofaunal size class which inhabit the pore spaces among sand grains as part of the specialized interstitial fauna. Knowledge on the diversity and distribution on these shallow-water interstitial Solenogastres has so far mostly been restricted to the respective type localities of the few known species in the temperate Northern Atlantic and tropical Atlantic. To investigate whether the low number of shallow-water species from the interstitial habitat indicates actual rarity, we searched for Solenogastres within the mesopsammon in different areas of the Atlantic (e. g., Brazil, Azores) and Pacific (e. g., Japan, Hawai'i, New Caledonia). Based on an integrative taxonomic approach combining morphological and molecular data, we identified several new lineages of interstitial Solenogastres. While some of those candidate species can be assigned to families with known meiofaunal representatives (e. g. Dondersiidae Simroth, 1893, Lepidomeniidae Pruvot, 1902, Meiomeniidae Salvini-Plawen, 1985), others challenge traditional classification and highlight the need to revise current solenogaster taxonomy. We indirectly sequenced gut contents of these worm-molluscs, revealing anthozoan and hydrozoan cnidarians as the main food source across different lineages of interstitial Solenogastres. Overall, while solenogaster diversity seems to be less speciose in the shallow-water interstitial habitat than in the deeper parts of the oceans, the current low species numbers and occurrence records in the upper zones mostly reflect lack of exploration. Further collaborative sampling efforts are needed to shed light on the diversity of Solenogastres in the mesopsammon and elucidate their patterns of diversity and distribution.

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## Contributions to softpart morphology of monoplacophorans by microCT

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Monoplacophora are the least known group of molluscs, because of their low number of species and low abundance, and their occurrence nearly exclusively in the deep sea. Up to now, the internal morphology of only five species has been investigated in detail (by section series). In recent years, micro X-ray tomography (MicroCT) has emerged as another option – alternative to the most commonly practised serial section method – for soft tissue examination of (small) invertebrates. X-ray (-CT) is known for the examination of hard parts such as bones and calcareous shells, but soft tissues can also be visualized after immersion in contrasting agents. We have applied this, combined with subsequent 3D-analysis, to two specimens of two presumably undescribed species collected in the Pacific deep sea. As is typical for most monoplacophorans, the two specimens studied here exhibit eight pairs of dorsoventral muscles, five pairs of ctenidia, two pairs of far posteriorly located atria and two sets of foregut pouches. On the other hand, the investigated animals also show peculiarities. For example, the larger specimen (male, shell length ca. 12 mm) has 7 intestinal loops, which represents the largest number in Monoplacophorans so far, and there is also a prominent jaw. One set of the foregut pouches contains internal musculature suggesting pumping function anteriorly and large posterior lappets. The smaller specimen (female, shell length ca. 2.9 mm) shows a remarkable organization of the musculature: The (anterior) dorsoventral muscles are composite with distinct portions and the oblique muscle system is very prominent with larger strands than the dorsoventral muscles in the posterior region. The pedal nervous connectives lie at the same dorsoventral level like the lateral connectives. The present study reveals that different organs systems are not equally suited for this type of analysis. CT data work very well for the muscular and digestive system, while it is difficult to discern the nervous system from the surrounding tissue. Nevertheless, we recommend this method for wider application. Because of the relative low effort involved, it may allow resolving many morphological questions also in malacology.

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# The scaphopod mess

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Scaphopoda with their roughly 600 extant species are the least studied major group of conchiferan molluscs, except for tryblidian monoplacophorans. This lack of scientific interest may be due to their apparently limited variation in ecology and anatomy and exacerbated by the difficulty to collect live specimens of most taxa. Their evolutionary origin is enigmatic and internal phylogenetic relationships are unresolved. Diagnoses for genus and family-rank taxa are almost entirely shell-based, often vague and compromised by homoplasy. Consequently, species identifications and allocations to higher taxa are often ambiguous and present superb challenges for molecular phylogenetic analyses. We present trees from nuclear and mitochondrial genes and mitochondrial genomes of the largest scaphopod taxon sample to date. About 90% of the genus and family-level taxa represented by two or more species are non-monophyletic, e.g., *Dentalium*, *Antalis*, *Gadila*, and *Siphonodentalium*, Gadiliniidae and Laevidentaliidae. There is support for the monotypic Calliodentaliidae being the sister lineage to Dentaliidae. Remarkably, this group is sister to a moderately supported and hitherto unrecognized clade comprising all scaphopods with smooth and shiny shells, such as *Fustiaria*, *Rhabdus*, *Episiphon*, and *Laevidentalium*. We hope that further expansion of the taxon set will help disentangling the systematic mess and improve taxon definitions and diagnoses. A robust and comprehensive phylogeny will shed light on many questions on scaphopod evolution and may even help to locate their position within the Conchifera.

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## Genomic insights from the enigmatic Aplacophora

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The great diversity and success of molluscs can, at least in part, be attributed to their ability to secrete diverse biomineralized structures. Aplacophora is a group of vermiform molluscs that notably lack a shell. This group, along with chitons (Polyplacophora), form a clade sister to all other molluscs called Aculifera. Both aplacophorans and chitons produce calcareous scales or spines referred to as sclerites. Aculiferan sclerites are composed of calcium carbonate polymorphs much like conchiferan shells, however it is unknown if the same gene regulatory networks underlie their production. Transcriptomic and proteomic approaches have provided insight into the genes and proteins responsible for patterning the shells of conchiferan molluscs (e.g., gastropods and bivalves). These studies have shown the expression of highly conserved genes along with a high level of sequence novelty in mantle transcriptomes between distantly related mollusc species, but investigation into the biomineralization toolkit of Aculifera is needed. Here we present the progress of an ongoing project aimed at uncovering the genetic toolkit underpinning mineralization within the genomes of the aplacophoran molluscs *Neomenia megalatrapezata* and *Epimenia babai*. Using an assembly strategy leveraging PacBio HiFi reads and Phase Genomics Hi-C, we have produced highly contiguous genomes for these aplacophorans (e.g., the ~700 Mbp *E. babai* genome is near chromosome-level with 190 contigs and BUSCO score of 91.8%). Using these genomes, we investigated 'biomineralization genes' known from conchiferans and their gain and loss over evolutionary time by comparing gene models from our new aplacophoran genomes to published genomes from most major groups of Mollusca using genomic phylostratigraphy. Coupled with functional annotation of these genes, this exploration into retention and loss of genes will aid in untangling the evolutionary history of biomineralization and other innovations within this incredibly morphologically disparate phylum.

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**Open session**

# **The Neglected Classes**

**Part II**

**Abstracts of poster presentations**

## Solenogastres enter the stage of mitogenomics: comparative characterizations of the first solenogaster mitochondrial genomes

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With the advances in high-throughput sequencing and bioinformatic pipelines, mitochondrial genomes become more and more popular in phylogenetic analyses across different clades of molluscs. Despite the vast increase in available mitogenomic datasets on molluscs, only one partial mitochondrial genome of the small molluscan class Solenogastres was publicly available prior to this study. We present six new mitochondrial genomes of Solenogastres (including the first complete mitogenomes) and comparatively analyse mitochondrial gene arrangements from six different solenogaster families representing three of the four traditional orders of these worm-shaped molluscs. In contrast to other molluscan mitogenomes which are characterized by a remarkable variation in size, radical gene (re)arrangements, and frequent duplications or losses of genes, solenogaster mitogenomes are comparably small with a length of approx. 12500 bp and present a highly conserved order of protein-coding genes (PCGs). No duplications or losses of PCGs are present, apart from a duplication of *cox3* putatively unique to the family Amphimeniidae. The order of protein coding genes resembles the hypothesized ancestral aculiferan gene order, but the arrangement of the typically 22 transfer RNAs (tRNAs) is variable among the different solenogaster families. Based on our data, the positions of tRNAs T and P between *atp6* and *nad5* is considered apomorphic for Solenogastres. The 'KARNI'-tRNA cluster present in Polyplacophora is variable across the investigated Solenogastres and Caudofoveata, while the 'MCYWQGE' tRNA cluster is conserved in Solenogastres and also Polyplacophora, but expanded by tRNA L1. Mitogenomics might provide a useful tool to reconstruct the evolutionary history of Solenogastres and thereby also helps to re-evaluate hypotheses on the ancestral molluscan mitochondrial gene arrangement.

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## Solenogastres (Mollusca Aplacophora) biodiversity in the Clarion-Clipperton Zone (CCZ)

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Before the extent of the potential impact of anthropogenic activities such as deep-sea polymetallic nodule mining can be assessed, determining the biodiversity of these habitats is crucial. Solenogastres (Mollusca, Aplacophora) is a small group of marine molluscs considered essential to understanding the evolution of the phylum. The scarce knowledge on the species that inhabit the deep sea is exemplified in Solenogastres with only 31 species (out of 298 described to date) known from depths below 3500 m. However, they are commonly found by expeditions sampling in the deep-sea and many ‘known unknown’ species are waiting to be formally named. The study of these worm-shaped molluscs requires use of time-consuming techniques to describe the small and fragile sclerites that cover their body and their internal anatomy. The use of DNA has been helpful to help accelerate the identification process, but traditional taxonomic expertise is still needed to fully characterize this fauna. Here, we present the results of an integrative taxonomic study on a collection of 59 solenogasters from the Clarion-Clipperton Fracture Zone (CCZ; SO 205 MANGAN and DeepGreen Campaign 05a,d). Specimens were first classified into morphospecies based on their habitus and mantle sclerites. At least one specimen of each morphospecies was selected for detailed study. These were cut in three parts with the middle region imaged under a low-vacuum, low-accelerating voltage SEM, which makes it possible to obtain ultrastructure-level data on the mantle sclerites and high-quality DNA (for 16S and COI barcodes) from the same sample. The anterior and posterior regions, which contain the taxonomically informative internal anatomical structures, were then histologically sectioned. Morphological and molecular results indicate that this relatively small collection is surprisingly diverse as we identified at least 24 distinct species representing eight different families and two orders. As is typical for solenogasters collected from anywhere an expert has not already intensively worked, most of the species appear to be new to science. Thus, studies of solenogasters are fundamental to understanding the rich biodiversity of polymetallic nodule areas, and in turn the study of these areas leads to a better understanding of the richness of these interesting molluscs.

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## Aloha! First shallow-water Solenogastres from Hawaii

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Solenogastres exhibit the highest species diversity in the deep sea, but ongoing sampling events have revealed a considerable undiscovered diversity also in the interstitial pore spaces of fine to coarse sands in shallow-waters. Five years ago, during the MarineGEO workshop on Coconut island, Hawai'i, we discovered 20 specimens of meiofaunal Solenogastres. These were assigned to three different species new to science belonging to two different families (Lepidomeniidae and Meiomeniidae), based on scleritome and anatomical data, as well as phylogenetic analyses. One species of the Hawaiian Lepidomeniidae is quite exceptional and challenges our current classificatory system by a unique mix of characters: In contrast to the otherwise adpressed scleritome composed of different types of imbricated scales, which gives lepidomeniids a smooth and shiny appearance, it bears elongated radially projecting lanceolate scales. Other taxonomic characters include a distichous radula and foregut glands of Type A, numerous large, turgescient mesenchymal cells, which fill the entire body cavity, an unusual muscular spawning duct and the presence of a dorso-terminal sensory organ. Based on this morphological character matrix, the species cannot be reliably assigned to any existing family, but in molecular phylogenetic analyses it clearly clusters among Lepidomeniidae, sister to a still undescribed species from the shallow interstitial of the Western Atlantic, thus requiring an expansion of the family diagnosis. Lepidomeniidae and other meiofaunal Solenogastres can be considered as poor dispersers with low reproductive output. Unfortunately, biogeographic hypotheses towards understanding on how meiofaunal Solenogastres colonized oceanic archipelagos are currently still hampered by the lack of primary data and sketchy sampling. Local species inventories like in Hawai'i typically result in the discovery of novel species, which however are difficult to place within the currently existing classificatory system. A community effort is needed to carefully revise the current taxonomy based on integrative datasets, as our findings – and several other examples – show that hypotheses derived from molecular phylogenetic approaches are indispensable to work round to a classificatory system which corresponds to the evolutionary history of the class.

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## Computed microtomography (micro-CT) in the anatomical study and identification of Solenogastres (Mollusca)

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Solenogastres are vermiform marine molluscs characterised by an aculiferous mantle, a longitudinal ventral pedal groove and a terminal or subterminal pallial cavity. Their classification is based in part on the type of mantle sclerites, but identification to even the family level generally requires the study of internal anatomical characters. Taxonomically important internal characters include those related to radular structure, the type of ventrolateral glandular organs of the pharynx and the reproductive system, among others. In order to study their internal anatomical organisation, according to the classical reconstruction method, serial histological sections of specimens are made, from which the 2-dimensional internal anatomy of the specimen can be reconstructed manually. However, this is a time-consuming technique that results in destruction of the specimen. Computed microtomography is a non-destructive technique based on the measurement of the attenuation of X-rays as they pass through a specimen. Computed microtomography is faster than histology for studying internal anatomy and it is non-destructive, meaning that specimens may be used for e.g., DNA extraction or retained as intact vouchers. In this paper, the utility of computed microtomography for studying taxonomically important internal anatomical structures was assessed. Results of the 3D anatomical study of the soft parts of four specimens of three species using computed microtomography are presented: *Pronomenia sluiteri*, *Dorymenia menchuescribanae* and *Anamenia gorgonophila*. Computed microtomography enabled detailed study of most taxonomically important anatomical characters, precise measurements of structures, and observation of the relative position of organs from a variety of angles. However, it was not possible to observe the radula and some details of the ventral foregut organs could not be discerned. Despite these limitations, results of this study highlight computed microtomography as a valuable tool to compliment histology in the study of solenogaster anatomy and in non-destructively identifying animals to the family and even genus-level.

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**Open session**

# **All About Light...**

**Part I**

**Abstracts of oral presentations**

## ***Latia neritoides* – studying the gland system of a glowing mucus producing freshwater snail**

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Endemic to the freshwater streams of New Zealand's North Island, *Latia neritoides* evolved a unique defence system: the sudden release of a huge amount of greenish glowing, sticky mucus when attacked by a predator. Although chemically this defence mucus is basically characterised, the origin and morphology of the gland system producing it still remain unclear. Two candidate glandular areas of mucus origin are: the lateral foot region and the mantle cavity.

This study focuses on the morphological and histochemical characterisation of these areas and their gland cell types. Histochemical and lectin stainings were performed on tissue sections and on the two different mucus types (defence/trail).

In the lateral foot area there are four subepithelial gland cell types, in contrast to only two subepithelial gland cell types in the ventral foot epithelium. The differences in glycostaining (reactivity with single lectins) of defence and trail mucus could not be traced to the gland cell types. Nevertheless, the large size and high density of the two gland cell types in the lateral area suggest a participation in the defence system. Similar gland cell types could not be identified in the freshwater snail *Neritina sp.*, which was used for comparison. In *Latia*, the roof of the mantle cavity comprises two epithelial gland cell types of interest with different ultrastructure and histochemistry. Their high density, together with massive defence mucus appearance near the pneumostome, indicates a possible role in this unique defence system. At this point it remains open whether the glowing mucus has a single source or results from interactions of secretions from different gland areas in the lateral foot epithelium and the mantle cavity. Additional information is expected from ongoing HREM stacking and  $\mu$ -CT scans, not only in regard to the origin of the glowing mucus but also in view of the general morphology of *Latia*.

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## Solar-powered sea slugs – the ups and downs of heading toward the light

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Certain members of the clade Sacoglossa are capable of functional kleptoplasty, the ability to steal and retain functional chloroplasts from their algal food. Kleptoplasty can benefit these slugs by providing camouflage and photosynthetically-derived sugars but it also involves a tradeoff. While light is required to power photosynthesis, high light intensities can damage the photosynthetic machinery and lead to the formation of Reactive Oxygen Species (ROS). To combat these consequences, plants and algae have evolved a multitude of mechanisms that limit photodamage and repair damaged photosystems, but these mechanisms are limited in sacoglossans since they lack algal nuclear gene transcription. Furthermore, sacoglossans are regularly exposed to irradiances that exceed the amount needed for optimal photosynthesis under natural conditions meaning they must cope with the effects high light has on their kleptoplasts. To investigate this tradeoff, we examined four species of sacoglossan slugs that have varying kleptoplastic abilities and their algal food sources. We measured their light exposure in the field and the optimal irradiance for maximum photosynthetic yield, finding that all species are exposed to light intensities that exceed their optima. We also conducted behavioral trials to examine which light intensity each slug species preferred, uncovering that the long-term kleptoplastic species *Elysia crispata* prefers moderate light while short-term kleptoplastic species *Elysia ornata* and *Elysia velutinus* and non-kleptoplastic *Elysia subornata* strongly prefer very low light conditions. *E. crispata* also reacts to acute high-light exposure by closing its parapodia, possibly to shade its kleptoplasts. Its white parapodial margins shield its chloroplasts, reflecting wavelengths below 500nm and decreasing the light intensity its chloroplasts receive by an average 191  $\mu\text{mol m}^{-2}\text{s}^{-1}$  due to both protein structure and pigments. Hydrogen peroxide, a prominent ROS produced via photosynthesis, was only observed accumulating in *E. ornata* digestive tubules (where the chloroplasts are located), suggesting that the other species may utilize cellular mechanisms to prevent ROS formation or they may effectively neutralize ROS that are produced. Overall, this research highlights certain adaptations kleptoplastic slugs may utilize to combat the tradeoff between the excess light to which they are exposed and the benefits of kleptoplasty.

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## Dietary preferences and performance of the photosynthetic sea slug *Elysia papillosa*

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Most sacoglossan sea slugs are specialized herbivores who feed suctorially on algae. Host algae is utilized by many of these slugs as both a food source and a substrate on which the slugs live. Remarkably, some sacoglossans are also store chloroplasts taken their food algae inside of their own cells in a process termed kleptoplasty. Kleptoplastic slugs can maintain these chloroplasts and become photosynthetic animals gaining substantial nutrition. The duration of photosynthesis varies greatly depending on both the species of sacoglossan and the algae which they have consumed. *Elysia papillosa* is a short term kleptoplastic sacoglossan which feeds and lives on algae in the genus *Penicillus*. Previous studies have shown that *E. papillosa* is more likely to be found on the algae *P. capitatus* than *P. lamourouxii* in mixed algal beds where both species are common. Surprisingly, however, slugs fed *P. lamourouxii* in the laboratory grew up to twice as large as slugs fed *P. capitatus*. Here we tested the duration of photosynthesis of *E. papillosa* fed on different algae diets and conducted behavioral experiments to see if slugs preferentially oriented towards chemical cues from either algal species. *Elysia papillosa* was able to photosynthesize for twice as long after feeding on *P. lamourouxii* compared to slugs which ate *P. capitatus*. Slugs collected from *P. capitatus* were also significantly more likely to orient and travel towards chemical signals from *P. capitatus* than they were from signals from *P. lamourouxii*. This is unusual as slugs could grow larger and photosynthesize longer if they choose *P. lamourouxii*. The apparent preference for *P. capitatus* may be due to other factors such as value as a refuge from predators or environmental threats.

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## Light conditions and acclimation of kleptoplasts modulate the abundance and preference of *Elysia crispata*

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The long-term retention of chloroplasts in animal cells (kleptoplasty) is an uncommon and peculiar phenomenon that occurs exclusively in Sacoglossa sea slugs. Information on kleptoplast-bearing sea slugs in their natural habitats and host behaviour in relation to light is fundamental to understand the process of kleptoplasty. *Elysia crispata* is an exceptional ecological model for this purpose because it is a large species that occurs in a wide range of habitat depths and keeps functional chloroplasts for several weeks and from different algal sources. To understand the response of *E. crispata* to different light conditions, we performed field surveys and laboratory experiments to: i) describe and compare changes in abundance and size of *E. crispata* as a function of water depth and time of day; ii) examine slug preference for different light intensities and spectra; and iii) assess whether *E. crispata* modifies its light preference depending on the light acclimation of the algae they consume. Field observations using SCUBA diving were performed to count and measure all individuals in each of ~8 quadrats (25 m<sup>2</sup>) within each of ~8 transects (40 m) at different times of day (sunrise, zenith, sunset, night) and two depths (0–4 m and 8–12 m). Preference experiments consisted in placing slugs at the centre of a cross maze with the following contrasting light conditions accessible simultaneously for 30 min: 1) four light colour choices, 2) four light intensity choices; and 3) two light intensity choices offered to slugs that had previously been fed with high light (HL) acclimated algae. All preference experiments used a no-light condition to control for passive selection. The frequency, duration and sequence of light choices were recorded and considered as dependent variables. Field observations, comprising a total of 375 organisms, indicate that *E. crispata* is more abundant at shallow depths, particularly at night, whereas sea slug size did not vary statistically between times of day. Laboratory experiments show that slugs actively reject both red light and HL intensities (~425–1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), but modify their light intensity preferences when fed with HL acclimated algae.

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**Open session**

# **All About Light...**

**Part II**

**Abstracts of poster presentations**

## The phototactic behavior of *Berghia stephanieae* (Váldes, 2005) is based on an active search for food

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The sea slug *Berghia stephanieae* is a subtropical species endemic to the Gulf of Mexico and serves as a pest control for *Exaiptasia diaphana* (Rapp, 1829) for many aquarists. Most research on *B. stephanieae* focuses on neurology and the evolution of the ability to steal cnidocysts and algae from *Exaiptasia diaphana*. The slugs use the cnidocysts for self-defense to compensate the loss of a protective shell. The algae serve as a symbiont (photobiont) in a non-mutualistic association that can last a couple of days when the slugs are starving (unstable photosymbiosis). Generally, photosymbiotic species behave in a positive phototactic way to increase the photosynthetic efficiency of their photobionts. Yet, photosymbiotic species also avoid radiation exceeding natural light intensities to limit light-induced damage to the photobiont. Although preying on a photosymbiotic cnidarian, and incorporating the algae into their own cells, *B. stephanieae* is considered a nocturnal species. As a potential nocturnal species, we would expect that *B. stephanieae* does not show a behavior related to the photosynthetic performance of the photobiont. To shed light on the phototactic behavior of *B. stephanieae*, we analyzed freshly fed slugs in a light vs. dark preference set-up and recorded the behavior of the slugs for one hour. In order to further understand if the behavior is also influenced by the other factors, we repeated the experiment with animals starved for one week. Freshly fed *B. stephanieae* tend to avoid light, whereas starved slugs, which do not bear photobionts, spent significantly more time in the light. Hence, the phototactic behavior of *B. stephanieae* is not related to optimize the photosynthetic activity of the ingested algae, but might be more linked to an active search for food, which inhabits the photic zone.

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**Open session**

# **Biogeography**

**Abstracts of oral presentations**

# Phylogenetic systematics and distribution of *Pomacea sordida* (Swainson, 1823) and *Pomacea intermedia* (Férussac in Quoy & Gaimard, 1825) (Caenogastropoda: Ampullariidae)

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In contrast to their relative ecological and economic importance, many species of Ampullariidae remain poorly studied, and lack robust data needed to clearly circumscribe the species or understand their biogeographic distributions. A few, like *Pomacea maculata* and *Pomacea canaliculata*, have been well studied, in part because of their notorious reputations as invasive pests. Unfortunately, beyond broad phylogenetic studies, we know little about the relationships and distributions of the dozen or so other species with which these two well-known ampullariids have and continue to be confused. The ability to delimit such species not only has implications for invasion biology, but also for conservation of native species. The ampullariid *Pomacea sordida* is endemic to the state of Rio de Janeiro, Brazil. Recent molecular analyses revealed that several populations previously referred to as *Pomacea sordida*, belong to another genetically distinct species, *Pomacea intermedia*, currently a junior synonym of *Pomacea sordida*. To evaluate the validity of this previous study, we examined all the material identified as *Pomacea sordida* from the Mollusc Collection of the Instituto Oswaldo Cruz and carried out additional surveys across the known distribution of these species. Phylogenetic analyses of COI sequences from 96 snails sequenced for this study along with additional sequences from GenBank recovered *Pomacea sordida* and *P. intermedia* as sister taxa in a clade sister to *Pomacea flagellata* and *Pomacea patula*. Surveys of aquatic habitats in the state of Rio de Janeiro recovered both species in allopatric populations with *Pomacea sordida* primarily restricted to seven sites in the southwestern part of the state, while *P. intermedia* occurred in six sites in the central part of the state, northeast of *Pomacea sordida* populations. Although cursory examination of male reproductive anatomy of these two species does not provide taxonomically informative characters that allow delineation of these two species, other traits such as juvenile shell characters, egg morphology and kidney shape and arrangement appear to be informative and provided necessary data to rescue *Pomacea intermedia* from synonym with *Pomacea sordida*.

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## Deriving new insights into South Africa's marine biogeography through analysis of offshore benthic molluscan distributions

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Whilst South Africa is a traditional hot-spot of marine biogeography research, its currently recognised offshore biogeographic breaks are based on abiotic variables or specialist opinion rather than data-driven approaches. Here we investigate biogeographic breaks based on the distribution of offshore marine benthic molluscs collected during the Natal Museum Dredging Programme (1981–1997) along the coast of South Africa, between the south-eastern Atlantic and south-western Indian Oceans. Spatially constrained (depth, latitude and longitude) cluster analyses were applied to three resemblance measures; the Jaccard coefficient, popular in biogeographic studies, and gamma+ and theta+ coefficients, where taxonomic relatedness is considered when comparing sites. We used these resemblances to 1) find the most consistent biogeographic breaks and 2) compare the results of different measures to better understand the patterns and possible processes involved in the biogeographical classification. Although there were differences in the overall results from the three resemblance measures, they produced comparable findings at depths shallower than 80 m. Our data-driven approach indicated that the biogeographic break between the warm-temperate and sub-tropical assemblages lies 140 km north of the current Ecoregion-based inshore break. The break between the sub-tropical and tropical mollusc assemblages agreed more closely with the present Ecoregions. Compared to Jaccard, where relatedness is not considered, gamma+ and theta+ results revealed a more substantial depth effect, and samples collected in the same depth range were more closely related and shared a more recent evolutionary history than geographically close samples from different depths. Furthermore, assemblages found deeper than ~80 m demonstrated greater diversity in terms of relatedness, an essential consideration in marine spatial planning and consequent designation of protected areas. This study reveals the importance of using in-situ data in future marine spatial planning but will benefit from more comprehensive datasets, especially from the tropical south-western Indian Ocean area. Cruises coordinated by Philippe Bouchet of the Muséum national d'Histoire naturelle have sampled molluscs widely across this area. Incorporating species distribution data from this broader region would doubtless enhance our understanding of biogeographic patterns and processes in the south-western Indian Ocean, for which little data-driven biogeographic research exists.

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## Diversity and endemism of bathyal gastropods on the South Azorean Seamounts

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The diversity of molluscs living on the summits and bathyal slopes of the seamounts south of the Azores has been investigated since the late 19<sup>th</sup> century. Particularly the results gathered from the cruises made by the research vessels *Hirondelle* and *Princesse Alice* showed a high biodiversity and a high degree of endemism. A second large step change in the assessment of the biodiversity was enabled by the Seamount 2 expedition that was coordinated by the Muséum national d'Histoire naturelle (Paris) in 1993. Several publications on bivalve and gastropod genera and families have been published based on Seamount 2 material. More recently, molluscs collected during the Poseidon 397 and Meteor 151 cruises that were funded by the Deutsche Forschungsgemeinschaft have been investigated in conjunction with those from Seamount 2 and a new set of genera and families were reviewed since 2019 yielding many new species and confirming a consistent picture of endemism in a 1000 by 500 km seamount area between the Azores and the Meteor Seamounts. To date, more than 400 known benthic species have been identified based on shell morphology; about 84 % of this set are gastropods. Approximately 120 species are solely known from the seamount area; the great majority are gastropods with protoconch characters that suggest a non-planktonic development. A significant number of endemics are only known from a single seamount; an abyssal gap with a depth in excess of 2000 m and a width larger than 100 km between seamounts seems adequate to limit their distribution. A larger group of molluscs live either on the southern or on the northern cluster of seamounts; these gastropods have been capable to cross the smaller distances between seamounts but not the 300 km gap between the southern and northern clusters. A third group is found in the full seamount area; these species have been capable to cross the abyssal gaps within the area but they have not managed to distribute to for example the Rockall and Hatton Banks, Madeira, the Canary Islands, and the continental slopes of western Europe, Africa and eastern America.

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# Cantharidinae (Vetigastropoda: Trochoidea) phylogenomics unveils the origin, evolution, and potential biogeographic events of the western temperate clades

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The subfamily Cantharidinae comprises more than 200 known living species of marine snails with a wide distribution, from Central and Western Indo-Pacific regions to the Eastern Atlantic Ocean, including the Mediterranean Sea and both African coasts. Previous phylogenetic studies based on multi-locus sequence data suggested that the North-East Atlantic and Mediterranean groups (genera *Clelandella*, *Gibbula*, *Jujubinus*, *Phorcus*, and *Steromphala*) belong to one natural lineage that originated upon the closure of the Tethys Ocean, about 14 million years ago. These phylogenetic analyses also detected a high degree of shell convergence, which seriously compromised previous morphological classifications of the subfamily and necessitated changes to the taxonomy. For instance, the genus *Gibbula* from temperate waters of North-East Atlantic, Mediterranean Sea, and Africa, is highly diversified with about 40 living species, and requires urgent revision. Here, we sequenced a genomic dataset (combining nuclear and mitochondrial sequences) of 30 species covering a wide distribution in order to reconstruct a robust phylogenomic framework for the Cantharidinae and thus to assess the origin, evolution, and potential biogeographic events that could have driven this radiation. Preliminary results suggest the non-monophyly of the Mediterranean and North-East Atlantic clades and indicate three plausible biogeographic hypotheses on their origin and diversification. Furthermore, our results demonstrate that species currently ascribed to *Gibbula* should be classified into at least five different genera. Finally, we incorporated previous multi-locus information from an extended taxon sampling into the genomic-based phylogeny in order to obtain the most complete taxonomic hypothesis of the subfamily to date.

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## Anti-tropical distribution and larval ecology of *Plesiothyreus cinnamomeus* Neritimorpha: Phenacolepadidae)

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Anti-tropical distribution is an intriguing biogeographic pattern of marine fauna; a single species or closely related taxa are found in both hemispheres, but absent near the equator. Temperature is regarded as a primary regulator of the anti-tropical distribution. For example, the Pleistocene glacial periods apparently allowed the dispersal of some temperate taxa across equatorial regions, while interglacial warming might have resulted in their tropical extirpation. However, anti-tropical taxa have seldom been studied for their population connectivity across the equator and thermal preference or tolerance, particularly with their pelagic larval period in mind.

In estuaries along the temperate coasts of Japan and eastern Australia, phenacolepadid limpets with a brownish shell are often ubiquitous and being called *Phenacolepas pulchella* or *Cinnalepeta cinnamomea* in the respective regions. Our molecular and morphological examination revealed that these belong to a single species, *Plesiothyreus cinnamomeus*, which rarely occurs in Papua New Guinea, Vanuatu, and New Caledonia, but apparently not in the centre of the Coral Triangle. Analyses of mitochondrial COI sequences recovered three major groups with 4–5 % genetic distances from each other. The three genetic groups largely corresponded to the respective populations of Japan, PNG and South Pacific, but some individuals from Japan and PNG belonged to the South Pacific group. Nuclear ITS loci showed similar correlation but again with an exception, supporting their conspecific nature and limited genetic exchange.

What mechanism might have caused the observed disjunct distribution and population differentiation? In our 60-d experiments, fed veligers of *P. cinnamomeus* showed differential survival rates at 30 °C (70 %), 25 °C (50 %), 20 °C (20 %), and 15 °C (0 %). Growth rates also increased with increasing temperature and no growth observed at 15 °C, which corresponds to the winter sea-surface temperature at the northern and southern limits of the species' distribution. Under the optimal condition at 30 °C, larvae were estimated to spend approximately four months for the attainment of shell size at metamorphosis, which would enable connectivity across the equator. The anti-tropical distribution of *P. cinnamomeus* may instead be attributable to biological constraints after metamorphosis, including habitat preference, niche competition and/or predation.

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**Open session**

# **Diversification and Speciation in Terrestrial Molluscs**

**Part I**

**Abstracts of oral presentations**

## Phylogeography and population differentiation of *Clausilia dubia* across Europe

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The door snail species *Clausilia dubia* is widespread across Europe from the Pyrenees to the Carpathian Mountains and from the Alps to Scandinavia. It is usually found at moist shaded rocks or on trees. The morphological variability is low in most of its distribution range, whereas several morphologically differentiated groups of populations can be distinguished in the eastern Alps. These forms differ by shell characteristics (e.g., shell dimensions, sculpture, features of the closing apparatus) and were considered narrow range endemic subspecies with a potential higher degree of environmental adaptation (e.g., to exposed calcareous rocks) compared to the widespread mesophilic populations. However, these taxa proved to be not monophyletic in mitochondrial gene trees. In the presented study, we assessed the phylogeography and evolutionary history of *Clausilia dubia* across Europe with a special focus on the population differentiation in the eastern Alps based on ddRADseq data of 170 individuals from 37 populations.

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## Molecular phylogeny of the genus *Metafruticicola* von Ihering 1892 (Gastropoda, Hygromiidae) reveals the polyphyly of the genus

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The land snail genus *Metafruticicola* von Ihering, 1892 is distributed in NE Mediterranean from the Aegean through Turkey and Cyprus to Israel. Currently, 24 species are morphologically recognized (two polytypic), half of them present in the Aegean region. It is one of the most common land snail genera in the Aegean, found in the majority of islands and islets from seaside to the subalpine zone (e. g., in the island of Crete). The taxonomy within the genus (subgenera, species, subspecies) is based primarily on shell morphology and the micro-sculpture of the shell in particular, where in some cases the anatomy of the genitalia is also used, while no phylogenetic relationships among the species have ever been studied. In this study, a well resolved molecular phylogeny is presented, based on genetic [mitochondrial (16S rRNA & COI) and nuclear (ITS1, 5,8S-ITS2-28S rRNA, 60SL17, 60SL13, 60SL7, RPL14) loci] and genomic data [thousands of single nucleotide polymorphisms (SNPs) based on double-digest restriction-site-associated DNA sequencing (ddRADseq)], through Bayesian Inference and Maximum Likelihood approaches and using the sister genera *Cyrtortheba* and *Hiltrudia* as outgroups. The phylogenetic/phylogenomic analyses supported the non-monophyly of *Metafruticicola*, revealing the presence of four different clades. Moreover, the current taxonomic subdivision of the genus in five subgenera is not supported. Although most of the present species are monophyletic (e.g. *M. naxiana*, *M. pellita*), there are few that are polyphyletic (e.g. *M. noverca*, *M. nicosiana*).

This study was funded by the General Secretariat for Research and Innovation (GSRI) & the Hellenic Foundation for Research and Innovation (HFRI).

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## Hidden biodiversity hotspot: A survey of groundwater hydrobiid microsnails from Western Transcaucasia

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Among the great diversity of hydrobiid snails, the taxa that have adapted to live in groundwaters and cave reservoirs are of particular interest. Though this family is extensively studied, most information on its taxonomy, diversity, and ecology is based on material from European countries, whereas other regions remain relatively understudied. Here, we give an outline of the current knowledge of the hydrobiid fauna of Western Transcaucasia. This territory likely represents a hidden hotspot of biodiversity of the Hydrobiidae, with many endemic genera and species still pending for a formal taxonomical description.

Initial steps in the study of subterranean malacofauna of Caucasus and Transcaucasia were made more than a century ago. No more than 10 species of hydrobiids were known from Transcaucasia. Traditionally, all endemic species of hydrobiid snails from this area were assigned to genera described from the other territories, such as Balkan Peninsula, based on a similar shell shape. However, as it is clear now, this approach is untenable. Our research has questioned the validity of some previously described species and demonstrated substantial differences between the Caucasian and Balkan hydrobiid genera. According to our data, no representatives of the Balkan genera occur in Transcaucasia; all 12 genera and 42 species of stygobiont Hydrobiidae, known in the region to the date, are endemics. Most of them were described since 2014. A total of 12 species were discovered for the first time, and 8 more were redescribed and included in new genera. According to the relative conchological uniformity of the Transcaucasian hydrobiids known to date, these molluscs demonstrate a high diversity in their reproductive anatomy, which indicates presence of several distinct genera in this area. Results of recent molecular studies also support the presence of endemic genera in Caucasus. Each isolated karst massif tends to have its own endemic fauna of the Hydrobiidae. Possibly, the real value of hydrobiid diversity in Transcaucasia is much higher, approaching 80–100 species and several still undiscovered genera.

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# Isolation and endemism in subterranean aquatic snails: *Belgrandiella* A. J. Wagner, 1927, *Montenegrospeum* Pešić et Glöer, 2013 and *Kerkia* Radoman, 1978

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The subterranean aquatic snails may serve as a model of endemism and isolation vs. migration in subterranean habitats. Of the approximately 20 000 worldwide described species of subterranean animals there are over 350 described species of stygobiont (obligate subterranean-dwelling aquatic) gastropods, with 97% of them representing the superfamily Truncatelloidea. The Western Balkans are the worldwide hot spot of this fauna, represented by more than two hundred species. Collection of stygobiont Truncatelloidea is difficult, especially in adequate numbers for any study of population genetic structure – subterranean populations are usually not rich in individuals, and their habitats are hardly accessible and explorable. Often nothing but shell, sometimes incomplete, is known for a species. With such data, coupled with the widespread belief among many taxonomists in geographic isolation and unavoidable, immediate speciation of the cave animals has resulted in descriptions of new species (nearly) in each cave or other subterranean habitat. Our molecular studies on interpopulation genetic diversity and gene flow tested this approach. The interpopulation differentiation pattern of *Montenegrospeum bogici* (Pešić et Glöer, 2012) collected at eleven localities (cave and interstitial ones) followed, in general, neither the infinite-island model of interpopulation differentiation, expected for isolated populations, nor the stepping-stone one, but rather the isolation-by-distance model. With the same haplotype found within the range of more than 200 km. Our results suggest that interstitial habitats provide ways of migration for the stygobiont *M. bogici*. *Belgrandiella kuscerei* (A. J. Wagner, 1914), studied on 23 cave and interstitial localities, presented similar pattern to the one found for *Montenegrospeum*, with interstitial habitats as the ways of migration, although the observed geographic range was much less wide – only about 42 km. The pattern found in 18 populations of *Kerkia* was different. Morphostatic evolution resulted in eight cryptic but molecularly evidently distinct species, five of them new for the science. The species of *Kerkia* are geographically restricted to small geographic areas. Molecularly the species are grouped in three clades, also reflecting geography. The endemism in *Kerkia* is high, thus interstitial habitats not exploited for expansion, perhaps as a result of planorboid shell, hardly suitable for infaunal mode of life.

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# Micro-spatial behaviour of two syntopic rock-dwelling *Albinaria* species: aggregation, substrate preference and trail following

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The spatial behaviour of animals affects their fitness and is of particular interest for land snails due to their low mobility, which makes them ideal for such studies both in the laboratory and in the wild. We investigated the spatial behaviour of individuals of two land snail species of the genus *Albinaria* (*A. arcadica* and *A. grisea*). These species inhabit vertical limestone rocks and are sometimes found syntopic in Peloponnese (southern Greece). Specifically, we investigated: 1) whether these species tend to aggregate, if the degree of aggregation differs between them, and whether this aggregation is altered when these two species coexist, i. e. when they are syntopic, 2) the choice of substrate/microhabitat and the degree of crypticity of the two species, i. e. whether a preference for smooth substrate or substrate with crevices/holes for resting occurs and if this preference changes when the two species coexist, and 3) whether trail following occurs, i. e., if there is a chemical/behavioural mechanism for recognition, attraction or avoidance, among individuals, and within or between species.

To address questions 1 and 2, laboratory experiments were conducted using climate-controlled chambers, where we placed rectangular boxes, with marble frames (squared and numbered), either with holes to act as potential shelters, or without holes. The positions of marked individuals inside each box were recorded every 24 hours for 14 days. A total of 15 combinations and 78 replicates of these experiments were performed.

To address the 3<sup>rd</sup> question, arenas were used where trail overlap between individuals acting as markers and trackers was tested. The path of the individuals was recorded with a camera and captured using Ethovision XT software (Noldus). Six different marker/tracker combinations were tested and a total of 22 replicates were performed on each combination.

Statistical analysis showed that *A. arcadica* exhibits a high aggregation and cryptic behaviour, while *A. grisea* displays a lower aggregation and no substrate preference. This completely different spatial behaviour between the two species probably acts as a pre-copulatory isolation mechanism between them and may also explain their syntopic occurrence. No trail following behaviour was noted in either species, suggesting weak recognition mechanisms.

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# De novo assembly and genome skims reveal LTRs dominate the genome of limestone endemic Mountainsnails (*Oreohelix*)

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Calcareous outcrops, rocky areas predominantly composed of calcium carbonate (CaCO<sub>3</sub>), often host a diverse, specialized, and threatened biomineralizing fauna. Despite the repeated evolution of physiological and morphological adaptations to the high amount of mineral supply in these environments, we still do not understand the genomic mechanisms underlying calcareous rock specialization in land snail species. In this study, we show that the expansion of long terminal repeat retrotransposons (LTRs) is associated with calcareous rock specialization in *Oreohelix* Mountainsnails through comparative genomics of whole assemblies and genome skims of ornamented limestone endemics and smooth non-limestone species. One of the sequenced genomes, the *O. idahoensis* genome assembly, is the largest mollusc genome published to date (~5.4 Gb) and has the greatest proportion of repetitive elements of any mollusc genome assembly sequenced thus far (85.64 %). The repetitive landscape of ornamented species was dominated by an expansion of long terminal repeat (LTR) transposable elements which have shaped the evolution of genome size, gene composition through retrotransposition of host genes, and ectopic recombination. These findings shed light on the genomic architecture underlying calcareous rock specialization in land snails and advances our understanding of how repeat content changes across molluscs.

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## Elucidating the factors influencing processes of community assembly in Galápagos Islands land snails

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Elucidating the processes that drive community assembly and species coexistence remains one of the biggest challenges in the study of evolution. Recent scientific development has identified important ecological and evolutionary factors influencing the patterns of species coexistence. Despite these advances, the mechanisms that underlie how, and in what sequence, species come to coexist over time and across space remains relatively unknown. The Galápagos Islands land snails (genus *Naesiotus*) is the ideal group to address this question because of the varying degree of geographic overlap among species and presence of many island replicates where each island forms a unique phylogenetic clade. In this study, I utilize species range data and the reconstructed phylogenetic relationships among Galápagos *Naesiotus* species to examine if patterns of diversification in this remarkable group are linked to their patterns of coexistence. Furthermore, these data will be used to assess how local communities are distributed on the phylogenetic tree, identify which community assembly processes result in the observed patterns, and how these processes vary between Islands. These results provide a dataset from which I can evaluate community assembly patterns across geographic space and evolutionary time.

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# Diversification and speciation in an endemic radiation of Galápagos Land Snails (*Naesiotus*: Bulimulidae)

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Adaptive radiations occur in the presence of ecological opportunity, but underlying mechanisms that promote diversification are often poorly understood. To test for influential components of an adaptive landscape, we analyze the impact of extrinsic characters on diversification of an endemic radiation of land snails in the Galápagos Archipelago. We tested ecological (microhabitat and vegetation zone) and geological (island age, size, isolation) factors. All analyses accounted for phylogenetic signal and state-dependent speciation extinction (SSE) models were implemented to measure shifts in diversification rates across niche and physical space. We calculated branch specific diversification rates for analysis as they changed over the ontogenetic lifespan of the volcanic islands. Our findings suggest that rates of speciation and diversification are 1) higher in humid than arid zones, 2) higher in terrestrial than arboreal species, and 3) higher on geologically young than old volcanic islands in the Galápagos Archipelago.

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## Dispersal is a driver for the diversification of *Gyraulus* spp. on both continental and oceanic islands

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Dispersal is an essential mechanism for maintaining biodiversity and, in particular, is a principal foundation of the biodiversity on oceanic islands, which have never been connected to any other land since their formation. On the other hand, the contribution of dispersal to diversification on a continuous region, such as continental islands, and inside the islands and the continents, is not clear enough, and the attention to the dispersal process is increasing. Non-marine molluscs generally have limited mobility, and so the importance of vicariance or geographic isolation has traditionally been focused on as a major factor in the species and lineage diversification. On the other hand, some recent studies emphasized that the dispersal contributed to the formation of diversity even on the non-marine molluscs with limited mobility. However, most studies are often conducted on narrow geographic scales due to limited distributions of non-marine molluscs, and comprehensive examination of the role of dispersal at broad geographic scales is limited. Here, we focused on *Gyraulus* spp. (Hydrophila: Planorbidae), freshwater snails having wide distribution in eastern Eurasia. These snails inhabit many islands, including oceanic islands, to the continent and are suitable material to reveal the impact on the diversification via dispersal comprehensively. We utilized genome-wide information obtained from next-generation sequencers to conduct high-resolution phylogeography at broad geographic scale at first. As a result, our phylogeny showed that geographical boundaries, such as oceans, were not significant boundaries of phylogenetic structure of these snails, and divergence age estimation suggested that the geographic structure was formed after the landmass became an island. Next, to test the role of this dispersal on a narrower geographic scale, we focused on *Gyraulus* sp. on oceanic Chichijima Island. Our genome-wide information suggested the distinct population structure existed even on small islands of about 20 km<sup>2</sup>, and there is little gene flow between populations. Furthermore, landscape ecological analysis estimated that this structure was maintained by watershed boundaries. In summary, dispersal over the geographic boundaries may played a major role in the genetic diversification of *Gyraulus* spp. both continental and oceanic islands.

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**Open session**

# **Diversification and Speciation in Terrestrial Molluscs**

**Part II**

**Abstracts of poster presentations**

## Diversity of land snail in RAPELD plots in the State Park of Ilha Grande, Angra dos Reis, Rio de Janeiro, Brazil

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The RAPELD (Rapid surveys in long-term ecological research) methodology is important for consolidation of long-term ecological research in Brazil, as a standard sample design allowing comparisons of different areas and processes that shape biodiversity. We investigated the distribution of terrestrial molluscs occurring in eight permanent plots of RAPELD at East Module located in the State Park of Ilha Grande. Ten collecting points were established each 25 meters in each plot. Snails were searched by direct collecting for 1 hour/point, totalling 10 hours/search. Also, in each point litter samples were collected using 25x75 cm quadrats, which was screened for micro-molluscs. We calculate the richness and abundance of species in each plot to assess community structure. For the diversity analysis we used Simpson index, nonparametric estimators Jackknife, Chao1 and curve of rarefaction. To analyse the environmental factors that most influenced the composition and abundance of species and the separation of plots we used PCA and NMDS. We found 373 specimens distributed in 42 species, 30 genera and 11 families. Scolodontidae was the richest family (28.5% of the sample), followed by Bulimulidae (14.5%) and, Charopidae and Subulinidae (11%). Scolodontidae was also the most abundant family (41 % of specimens). *Beckianum beckianum* (Pfeifer, 1846) showed the greatest abundance, with 48 specimens (13 %) distributed in only two plots. Five species were recorded for the first time to Ilha Grande Island. The PCA for the eight plots indicated that soil temperatures and environmental temperature average, litter moisture and environmental moisture average contributed to the greater percentages in the two first axes, discriminating the plots according to habitat characterization. A simple regression between the single axis of NMDS and the second axis of the PCA was significant ( $r=0.698$ ;  $p=0.05$ ), indicating that molluscs communities are structured according to a humidity gradient. This work is pioneer in using the RAPELD methodology to study the distribution of terrestrial molluscs in Brazil and through integration with data from different areas, mainly botany and soil sciences, it will be possible to obtain new information on the distribution of malacofauna in the Atlantic Forest.

Grants: Faperj, UERJ, Capes

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## Introduced slug species in Hungary

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The Hungarian molluscan fauna is thoroughly explored. Slugs, however, usually ignored in ecological and faunistic studies, due to two main reasons. Firstly, their storage in ethanol is more complicated compared to dry snail shells, and secondly, their identification is difficult because of the few reliable identification guides and the fact, that dissection is essential for a number of species groups.

Our aim is to write an identification book of Hungarian slugs, including photographs of living slugs and the anatomical characters. For this project, during the last four years we have been collecting slugs intensively all over Hungary. These investigations resulted in the discovery of *Tandonia kusceri* (Wagner, 1931), *Krynitzkillus melanocephalus* Kaleniczenko, 1851 and *Limacus maculatus* (Kaleniczenko, 1851). The former two are already widespread in the country and can be abundant locally, as revealed by means of citizen science using social media platforms. One sub-project focussed on horticultures in Budapest. This resulted in the discovery of three additional non-indigenous slug species, *Deroceras invadens* Reise, Hutchinson, Schunack & Schlitt, 2011, *Ambigolimax valentianus* (A. Férussac, 1821) and *Milax nigricans* (Philippi, 1836), previously not reported from Hungary. Contrary to *T. kusceri* and *K. melanocephalus*, their distributions are so far only limited to horticultures, botanical gardens and other urbanised sites in Budapest. With these species, the Hungarian slug fauna exceeded 30 species, and the number will probably keep increasing.

The increasing number of introduced molluscs is traditionally explained by the climate change and the increased international transportation of goods, mostly agricultural and horticultural products. Since most species are still restricted to horticultural areas, it seems that the latter is the key factor. Slugs can easily hide in the pots of horticultural plants and tolerate long distance journeys lingering in the soil.

In the future we will monitor the distribution of the non-native slug species, collect data on the horticultural damage they make. However, there are no realistic ways to stop or slow down their spread within Hungary and between countries.

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**Open session**

# **EvoDevo and Vision**

**Abstracts of oral presentations**

# Why so many? Variation in eye abundance among scallops (Bivalvia: Pectinidae) reveals ontogenetic and evolutionary convergence associated with life habits

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Eyes are remarkable systems to investigate the complex interaction between ecological drivers and phenotypic outcomes. Scallops are bivalves with tens to hundreds of eyes along with their bodies, forming a remarkable distributed visual system. However, the evolution of multiple-eye systems remains obscure. For instance, it is unclear whether eye number changes over a lifetime or varies among scallop species. We tested whether eye abundance scales with body size among individuals and species and whether it varies with life habits (i.e., cementing, recessing, byssal-attaching, free-living, and gliding). Our hypotheses predicts that larger and more mobile species will have more eyes. We performed comparative analyses, including a phylogenetic ANCOVA and evolutionary model comparisons, based on eye count and shell height (as a proxy of body size) across 31 scallop species. Our analyses reveal that patterns of positive static allometry are not concordant among taxa and suggest ontogenetic convergence caused by similar ecologies. Accordingly, selective optima in eye numbers are associated with shifts in life habits. For instance, gliding scallops (long-distance swimming) and free-living scallops, which represent the most mobile life habits, have significantly more eyes than species with low mobility (recessing) or no mobility at all (cementing). The convergent evolution of greater eye abundance in more mobile scallops likely indicates a visual improvement based on oversampling the surrounding environment.

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## Gametogenic cycle and gonadal apoptotic cell detection in the semelparous side-gilled sea slug *Pleurobranchaea maculata* (Quoy & Gaimard, 1832)

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Most species belonging to the Superorder Nudipleura (Heterobranchia) have been described as semelparous reproductive strategists, mainly based on changes observed in population density. However, their gametogenic cycles have rarely been studied. This work describes the gametogenic cycle of the simultaneous hermaphrodite sea slug *Pleurobranchaea maculata* (Pleurobranchaeidae) in Patagonia, Argentina. The gametogenic cycle was studied by classical histology. Additionally, apoptosis during the gametogenic cycle was observed using the immunohistochemical TUNEL technique. *P. maculata* exhibited sperm and oocytes development occurring asynchronously in separate gametogenic acini. Sperm development began in February while oocyte development began in April. The highest spermatogenic and oogenic activity was observed from July to January characterized by long periods of partial evacuations. In February and March, a high percentage of individuals with deteriorated acinar structure (post-reproductive) were found. Maturity calculated from oocyte size and presence of mature sperm showed that immature and post-reproductive individuals coexisted during these months, while mature individuals were rarely observed. This finding suggests that *P. maculata* is a semelparous species with an annual life cycle, with a single spawning season extending from July to January (winter to summer southern hemisphere). The use of TUNEL to study apoptosis during the gametogenic cycle, as a novel approach for heterobranchs, evidenced that during the different reproductive stages apoptotic processes occur in different cell types. At the beginning of the reproductive cycle (mature spermatogenic acini and immature oogenic acini), positive apoptotic signals are located mainly in spermatogonia, probably to maintain homeostasis in reproductive cells. Reproductively active individuals showed a lack of apoptotic nuclei in all types of gonadal cells. However, in post-reproductive individuals, apoptotic nuclei were visualized in the structural cells of the basal membrane of both gametogenic acini, compromising gonadal viability. This outcome evidences that apoptotic processes are involved in the gonadal deterioration and could be responsible for the mechanisms underlying death in this species.

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## Evolution of high-resolution spatial vision in the conch snail group Stromboidea

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Members of the gastropod superfamily Stromboidea have camera-type eyes that range widely in size, with some species having the largest eyes of any gastropod, thus providing an excellent opportunity for eye evolution studies. Estimates of visual acuity from anatomical and behavioural data suggest that the large eyes of Rostellariidae, Seraphsidae, and conch snail family Strombidae, provide surprisingly fine resolution vision. In contrast, estimates from the small eyes of Aporrhaidae, Struthiolariidae and Xenophoridae indicate much coarser resolution. Three-dimensional reconstructions of histological data reveal further differences in eye structure, including variations in length of photoreceptor cells in the retina.

To better understand the evolution of eyes in Stromboidea, we used Sanger sequencing data for three mitochondrial genes (COI, 12S and 16S) and one nuclear gene (28S) to build a robust phylogenetic framework of the superfamily. We obtained morphological traits (including eye size, aperture size, and lens diameter) from rapid mCT-scanning and light microscopy measurements and mapped these onto the phylogeny, along with ecological aspects related to vision, such as depth and habitat. This enabled us to identify key transition points in the evolution of large, camera-type eyes and further explore how visual structures vary across the group. Finally, the excellent stromboid fossil record allowed for the use of fossil calibrations in phylogenetic analyses, to determine when these complex eyes originated.

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# Opening a can of snails: Predicting developmental gene regulatory networks in the slipper snail, *Crepidula fornicata*

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Computational predictions of gene regulatory networks show great promise for emerging and non-traditional model systems. These methods provide a starting place for building developmental GRNs in new systems. While sequencing costs are continually declining, older datasets from emerging models often lack sufficient sample numbers, while some species may be biologically constrained in ways that reduce the efficiency of algorithms designed for model systems such as yeast (e. g. high heterozygosity, low reproductive output, poorly annotated genomes and transcriptomes). A common approach to differential expression analyses is to use multiple methods to find common up- and down-regulated expression patterns. Similarly, leveraging multiple predictive models should provide stronger support for suggesting key developmental nodes. Here, we use multiple predictive methods to identify key nodes in a previously generated dataset to predict developmental gene regulatory networks in the Atlantic slipper snail, *Crepidula fornicata*. Our dataset includes a time series of early development (11.5–40 hours post fertilization) in addition to pharmacological perturbation data. We tested multiple network-predicting methods including fuzzy c-means clustering, correlation networks (i. e., WCGNA), and machine learning algorithms (i. e., dynGENIE3, PEAK). We also used transcriptome-level annotation data to compare network properties, including Gene Ontology term enrichment. Combined with in situ hybridization expression patterns to validate our findings, these analyses will be used to suggest future targets for functional validation experiments.

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# Molluscan genomes reveal distinct patterns of opsin evolution across the phylum

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Morphologically diverse eye types have evolved numerous times within the Phylum Mollusca. Yet, we know very little about how these different eye types evolved, including the sets of genes recruited to support their development and function. Opsins are the protein component of photopigments that mediate the visual transduction pathway and are fundamental to the genetic architecture of vision. Broadly, we know that molluscs have three groups of opsins: r-opsins, xenopsins, and tetraopsins, the latter including neuropsin, G<sub>o</sub>-opsin, and retinochrome. However, variation in opsin composition has not been characterized across molluscan lineages due to the previous paucity of assembled molluscan genomes. With technological advances in sequencing chemistry and bioinformatic tools, molluscan genomes are increasingly available in public databases allowing for more comprehensive phylogenomic comparisons within this diverse phylum. We collected opsin sequences from 80 molluscan genomes to characterize their opsin repertoires and analyze opsin family evolution across the phylum. We found extensive gene family expansion and contraction, particularly in bivalve xenopsins and gastropod G<sub>o</sub>-opsins. In contrast, r-opsin, neuropsin, and retinochrome diversification is much rarer in molluscs. The fluctuations in the opsin repertoire appears to be limited to bivalve and gastropod lineages, with cephalopods maintaining relatively few opsins and having lost at least two major opsin clades: neuropsins and G<sub>o</sub>-opsins. We also found complete loss of opsins in multiple deep-sea species. These results help set the stage for connecting genomic changes, including opsin family expansion and contraction, to patterns of evolution in eye type and vision in molluscs.

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**Open session**

# **Phylogeny**

**Part I**

**Abstracts of oral presentations**

# Could hyperdiversification of neogastropods be related to a whole genome duplication?

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Understanding why some groups of organisms are more diverse than others is a central question in evolutionary biology. Neogastropods, a group of marine predators including almost 30 % of the known gastropod diversity, evolved a wide range of feeding strategies, including the production of molecular compounds to subdue their preys. However, whether the diversity of these compounds is at the origin of the hyperdiversification of the group and how genome evolution may correlate with both the compounds and species diversities remain understudied. In this context, gastropod genomes are increasingly being sequenced (~45 genomes), among which eight of neogastropods, but their quality is uneven. As sequencing cost is decreasing and sequencing quality is improving, it becomes easier to obtain chromosome-level assemblies in under-explored taxa. Here, we generated chromosome-level assemblies of two species belonging to the Cymatiidae and Muricidae families (*Monoplex corrugatus* and *Stramonita haemastoma*). These are large genomes (3 and 2.2 Gb, respectively), with 35 chromosomes each (N50 > 50 Mb), similar to what was recently reported in the Conidae *Conus ventricosus*. We predicted 36 924 and 34 865 protein-coding genes with a Busco score of 82 % and 78 %, respectively. A comparison with the genome of *Pomacea canaliculata* (the most closely-related to the neogastropod chromosome-level genomes) revealed the occurrence of syntenic blocks found in a single chromosome of *P. canaliculata* in multiple chromosomes of both *M. corrugatus* and *S. haemastoma*. In addition, manually annotated hox genes (involved in body plan differentiation of Bilateria and known to be conserved throughout evolution) were found as duplicated clusters in both neogastropod genomes and in *C. ventricosus*. We extended the comparison to all publicly available genomes so far described in gastropods in order to estimate the timing of the duplication using synonymous divergence between orthologous genes and by inferring a phylogeny on which the evolution of chromosome numbers was mapped. Our analyses confirmed that a whole genome duplication event occurred in neogastropods (and potentially in other related groups). Indeed, whole genome duplication was observed across a wide variety of eukaryotes lineages, like plants or fishes, and contributed to their diversification – a hypothesis that can also be proposed for neogastropods.

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## Exon-capture-based phylogeny of the Neogastropoda

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The order Neogastropoda encompasses over 18 000 Recent species, or about  $1/4$  of the entire molluscan species diversity. Almost exclusively marine carnivores, neogastropods inhabit entire diapason of latitudes and depths, and play important roles in benthic communities, and in economics of coastal countries. However, phylogenetic relationships of the Neogastropoda remain poorly understood. Despite recent studies established compositions of their major lineages, uncertainty persists in i) branching order of major lineages, and ii) relationships at the base of the neogastropod radiation. Here we present a nearly resolved phylogeny of the order based on analyses of exon capture data. Since WCM 2019, we further increased taxon sampling, and improved data occupancy resulting in a dataset of 145 taxa, representing 51 neogastropod families, and 2050 loci totaling 481 kb, with only 33% of missing data. Of the seven extant currently recognized neogastropod superfamilies, we consistently recovered monophyly for Conoidea, Mitroidea, Muricoidea, and Olivoidea. Buccinoidea as currently construed appeared paraphyletic. Traditional Turbinelloidea would comprise at least five unrelated lineages. Members of Volutoidea fell into two separate clades: monophyletic Cancellariidae, and a Volutidae-Cystiscidae-Marginellidae-Granulinidae clade.

Aminoacid and nucleotide data analyses generated two alternative topologies at the base of Neogastropoda: i) Tonnoidea + Ficoidea as sister to the monophyletic Neogastropoda (nt), or ii) Tonnoidea + Ficoidea as sister to Cancellariidae (aa, not supported), with the resulting clade as sister to the remaining Neogastropoda. Both, the support values of alternative topologies, and morphology were strongly in favor of the monophyletic Neogastropoda (i). Within it, three first consecutive offshoots corresponded to Cancellariidae, true Volutoidea (Volutidae-Cystiscidae-Marginellidae-Granulinidae clade), and Volutomitridae. The remaining lineages formed a crown radiation, where most nodes were also resolved. Our results provide a robust phylogenetic framework to guide revisions of the neogastropod taxonomy. We recognize thirteen neogastropod lineages worthy of a superfamily rank, of which four defined here for the first time: Cancellariidae, Volutoidea (redefined), Volutomitridae (new), Ptychatractidae + Costellariidae (new), Columbariidae (new), Vasidae-Dolicholatiridae-Belomitridae (new), Olivoidea, Harpidae (new), Muricoidea, Buccinoidea (redefined), Turbinelloidea (redefined), Mitroidea, Conoidea. Our results will foster further studies on the diversification dynamics of the taxa in the order, and will prompt re-interpretation of the fossil record of the Neogastropoda.

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# Characterization and Phylogenetic Analysis of Eleven Apple Snail (Ampullariidae) Complete Mitochondrial Genomes

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Ampullariidae, known as apple snails, are a globally distributed family of freshwater snails within Caenogastropoda. Ampullariid operculum fossils date to the Cretaceous of China, but the family is thought to have diversified in Gondwana and subsequently the New World after the breakup of the supercontinent. The nine recognized genera include at least 150 species, with the highest diversity in the New World genus *Pomacea*. Species belonging to the latter are well known for the invasive potential, with at least two species, *Pomacea maculata* and *Pomacea canaliculata*, being among some of the most invasive freshwater snails in the world. Ecologically, ampullariids play important roles as ecosystem engineers in critical wetlands globally. As such, understanding their diversity and distributions has implications for conservation of these imperiled systems. Previous multilocus phylogenetic analyses has provided substantial resolution at the species level for the family, but some deeper relationships and affinities among some of the major clades remain poorly supported. To help further resolve some of these relationships we have produced twelve new mitochondrial genomes (mtGenomes) from species representing three Old World (*Afropomus*, *Lanistes*, and *Pila*) and three New World (*Asolene*, *Felipponea*, and *Pomacea*) genera concurrently with representatives from families Campanilidae, Cyclophoridae, and Viviparidae as potential sister taxa. All new mtGenomes were concordant with previously published ampullariid genomes with 37 genes encoding 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs, all in the same order. Initial phylogenetic analyses of protein coding genes and rRNAs, as well as the complete mtGenomes are consistent with previous relationships among genera and provide increased resolution at deeper nodes, with only minor differences that may be the result of taxon sampling. Additional analyses from the complete dataset will be used to better assess the sister taxon of ampullariids and provide insights into their evolution. The broader understanding of their relationships combined with additional data on their distributions and ecology will help inform conservation management strategies for these species and others.

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## First comprehensive molecular phylogeny of the genus *Deroceras* (Gastropoda: Eupulmonata: Agriolimacidae)

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*Deroceras* Rafinesque, 1820, is the most speciose slug genus within the family Agriolimacidae. It is estimated that the genus comprises more than 100 species, which constitutes about 90% of all Agriolimacidae taxa. Some of them occur locally, e. g. on islands or in particular geographical regions, whereas others are widely distributed and may have economic significance for people. The GenBank database contains sequences only for some species of the genus *Deroceras*, constituting only a small fraction of the all taxa currently recognized within this group. Therefore detailed phylogenetic relationships between *Deroceras* species are still poorly known.

The main objective of the presented research was to resolve *Deroceras* phylogeny based on two mitochondrial (COI, 16S rRNA) and two nuclear markers (28S rRNA, histone 3). During the study we investigated samples from natural history collection deposited in German and Polish museum collections in i) Görlitz, ii) Dresden, iii) Frankfurt am Main, iv) Stuttgart, and v) Wrocław (Poland). In total 105 *Deroceras* species were examined. Along with elucidating relationships between taxa within the investigated genus, this study also greatly contributes to the species identification, thanks to the newly created DNA library of the examined taxa.

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**Open session**

# **Phylogeny**

**Part II**

**Abstracts of poster presentations**

## The phylogeny of freshwater mollusc genus *Brotia* H. Adams 1866 (Gastropoda: Pachychilidae) from northeast India

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The Pachychilid genus *Brotia* H. Adams 1866 is distributed across south and Southeast Asia, and comprises of 46 species. This genus is relatively poorly studied in terms of systematics and phylogeny as well as its generic and familial affinities remains unclear. In India, the members of the genus *Brotia* are distributed in the Ganges-Brahmaputra river basin and the extreme northeastern regions of India. Although *Brotia* has recently been revised, the taxonomic issues for the Indian species still need to be addressed. In the present study, we have tried to resolve the phylogenetic relationships and decipher the divergence time of this genus, using molecular data. Fieldwork was carried out in different northeastern states of India, such as Assam, Meghalaya, Manipur, Mizoram, Nagaland and Tripura. Specimens were collected from lentic and lotic habitats and preserved in absolute ethanol for further taxonomic and molecular studies. Using already published primers and protocols, we amplified two mitochondrial markers – Cytochrome oxidase I and 16S ribosomal RNA. Obtained sequences were used to reconstruct Maximum Likelihood and Bayesian Inference trees, delimit species and carry out divergence time analysis using BEAST. The genus *Brotia* was considered to have one species in India, but our initial delimitation results suggest five distinct species. All the Indian lineages are nested within the Southeast Asian clade, with moderate to strong support, suggesting into-India dispersal. The molecular dating results show two major dispersal events. The first dispersal event seems to have occurred around 20 million years ago (Miocene) for the putative species consisting of the *Brotia* Manipur\_2 clade. The second dispersal event seems to have occurred broadly around 15 million years ago (mid-Miocene) for remaining four species. This is the first study highlighting the phylogenetic analysis of *Brotia* from India, and makes a significant addition to the limited knowledge on the systematics of Indian freshwater gastropod fauna. Since *Brotia* species are consumed on a large scale in northeast India, resolving the taxonomic status is crucial to assign conservation status and develop effective management strategies to conserve freshwater molluscan biodiversity and habitats.

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**Open session**

# **Diversity and Distribution**

**Abstracts of poster presentations**

## Global hotspots (and coldspots) of marine gastropod diversity and DNA barcode availability

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Records of species occurrences are useful in inferring spatial patterns of recent biodiversity, especially for cosmopolitan and diverse taxa such as marine gastropods. With the on-going efforts in digitising museum collections, increased participation of citizen scientists, and greater accessibility to research data, an accurate determination of global patterns of diversity has become more achievable. In this study, we used curated occurrence records from the Global Biodiversity Information Facility (GBIF), with annotation of authoritative taxonomy from the World Register of Marine Species, to evaluate the taxonomic richness of marine gastropods and to identify putative global species hotspots for this group. Additionally, the availability of mitochondrial genomes and cytochrome c oxidase I (COI) barcodes of species within hotspots was reviewed to detect potentially important regions where genetic resources for identifying these organisms are wanting (i.e., “coldspots”). Across 201 countries and territories, we find 32512 unique and valid species under 3291 genera belonging to 371 gastropod families that have been recorded from 1670–2022. Globally, only 11.7 % of reported species are linked to a COI barcode in the Barcode of Life Data System, whilst 23.7 % of the gastropod families are represented with complete mitogenomes in GenBank. Georeferenced occurrence records show the wide geographic coverage of the dataset as well as the disparate distribution of observations and species counts. The compiled data, published by 269 organisations that are restricted to just 49 countries, reveal a seeming lack of submissions by local institutions. Nevertheless, it also demonstrates the growing contribution of citizen science platforms in the past decade. This study provides the first baseline information of taxonomic richness for marine gastropods based on GBIF data. We present several putative gastropod species hotspots in 28 marine provinces across the globe and call for further work and stronger involvement, particularly within COI coldspots, to address the observed barcoding resource inequity. Lastly, we highlight the important and relevant role of open and inclusive science to biodiversity monitoring and research, as has been exhibited in this dataset of hyperdiverse and ecologically important marine gastropods.

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## Urban cemeteries as habitat for terrestrial gastropods in Berlin

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While cemeteries represent potentially suitable habitat for many species of terrestrial gastropods within urban environments, this land use type is considerably understudied compared to other urban green spaces. In the German capital Berlin, around 200 cemeteries exist, which cover a total area of more than 1000 hectares. Due to their high number and scatteredness in the city, these cemeteries provide a unique system for studying the diversity and distribution of terrestrial gastropods in the urban environment. On this poster, we introduce the study area and provide first results from our surveys on selected cemeteries. The cemeteries in Berlin differ considerably in size, age, habitat heterogeneity, management and degree of isolation. Species records are examined in respect to relevant parameters. Structures that are characteristic for cemeteries (e.g., tombstones, dry stone walls, certain types of buildings) are a particular focus, and their role as microhabitat for terrestrial gastropods is discussed.

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**Open session**

**Genomics**

**Abstracts of poster presentations**

# How many single-copy orthologous genes from whole genomes reveal deep gastropod relationships?

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The Gastropoda contains 80 % of existing mollusks and is the most diverse animal class second only to the Insecta. However, the deep phylogeny of gastropods has been controversial for a long time. Especially the position of Patellogastropoda is a major uncertainty. Morphology and some mitochondria studies concluded that Patellogastropoda is likely to be sister to all other gastropods (Orthogastropoda hypothesis), while transcriptomic and some other mitogenomic studies indicated that Patellogastropoda and Vetigastropoda are sister taxa (Psilogastropoda). With the release of high-quality genomes, orthologous genes can be better identified and serve as powerful candidates for phylogenetic analysis. The question is, given the current limitations on the taxon sampling side, how many markers are needed to provide robust results. Here, we identified single-copy orthologous genes (SOGs) from 14 gastropods species with whole genomes available which cover 5 main gastropod subclasses. We generated different datasets from 395 up to 1610 SOGs by allowing species missing in different levels. We constructed gene trees of each SOG, and inferred species trees from different collections of gene trees. We found as the number of SOGs increases, the inferred topology changed from Patellogastropoda being sister to all other gastropods to Patellogastropoda sister to Vetigastropoda plus Neomphalina (Psilogastropoda s.l.), with considerable support. Our study thus rejects the Orthogastropoda concept showing that the selection of the representative species and use of sufficient informative sites greatly influence the analysis of deep gastropod phylogeny.

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## Comparisons among genome assemblies and the importance of complete reference genomes. The pending task in malacology

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Genomic resources allow the comparison across taxa at a resolution never reached before. While most malacological research was traditionally focused on taxonomic and phylogenetics using few markers, the burgeoning field of genomics opens a wide range of new possibilities such as the study of gene gain/loss, structural comparisons, phylogenomics and phenotype-genotype associations, among others. However, a compromise must be sought between cost, effort, and quality of the genomes that better fits the goals of each study. Here, we compare the quality and performance of eight new genome assemblies. Six belonging to Dendrodoiridae species (*Dendrodoiris limbata*, *Dendrodoiris elongata*, *Dendrodoiris tuberculosa*, *Dendrodoiris krusensternii*, *Doriopsilla areolata* and *Doriopsilla spaldingi*) were sequenced using Illumina low-coverage whole genome sequencing (WGS). Additionally, *Dendrodoiris temarana* was obtained with Illumina high-coverage WGS. Finally, the tunicate *Styela plicata* reference genome was obtained from a combination of long reads, high-coverage WGS and Omni-C data. We will discuss the main outcomes derived from each sequencing strategy and identify their strengths and weaknesses, and define what studies can be performed by each one. Our results demonstrate low-coverage sequencing is an excellent tool when recovering high quality mitogenomes. Nevertheless, the assembled nuclear genomes become incomplete and fragmented, although a variable number of markers can be obtained to be used for phylogenetic approaches. Genome size estimations could not be carried out with low-coverage data. However, *Dendrodoiris temarana* high-coverage data overcame this limitation opening a door to genome size evolution research. Finally, the hybrid assembly of *Styela plicata* provided the best results, with contiguity at chromosome-level, allowing the annotation and location of all its repetitive elements and protein coding genes. This reference genome assembly represents a significant improvement in research, since it allows the performance of evolutionary studies on chromosomes, transposable elements, and identification of structural variants, among many others. We conclude that, although low-coverage approaches may suffice for some studies, efforts towards construction of high-quality mollusc reference genomes will pay off, as they will allow to tackle new challenges in malacology. We foresee continuous advances in laboratory protocols and sequencing technologies which will make this strategy a feasible and affordable one in non-model species.

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**Open session**

# **Morphology**

**Abstracts of poster presentations**

## 20 years of 3D microanatomical research by the Munich malacologists around Prof. Haszprunar – a synopsis.

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The method of computer-assisted 3D reconstruction based on complete series of semi-thin sections (0.5–2 µm) has opened up a very fruitful field of research in zoomorphology, also for the study of small mollusc species. Before this approach, in many cases only external structural data were available (shell structure, SEM of soft body surface) and partly radula structures. Now it is possible to investigate the histology and the 3D microanatomy of all organ systems (e. g. digestive tract, nervous system, reproductive organs, circulatory and excretory systems etc.) in the soft body of complete specimens with justifiable effort, to display them in any perspective and organ constellation, and to compare species and developmental stages.

This means that even mollusk species in the millimeter range can be described or re-described in unprecedented morphological detail, drawing conclusions about the functional architecture of the organ systems and about adaptations to the habitat and way of life. The method thus provides a solid and modern basis for taxonomy and reconstruction of evolutionary history.

From 2003 to 2022 several generations of researchers from LMU, SNSB-ZSM, and coworkers examined representatives of various mollusk groups (Polyplacophora, Solenogastres, Monoplacophora, Cephalopoda, Bivalvia, Scaphopoda, and many Gastropod groups, i. e. Patellogastropoda, various Vetigastropoda, Cocculinida, Neomphalida, Caenogastropoda, and Heterobranchia like Rhodopemorpha, Hyalogyrinidae, Valvatoidea, Acochlidimorpha, Sacoglossa, Cephalaspidea, Pteropoda, and Nudibranchia) creating a large collection of section series and digital reconstructions equally suitable for research and teaching.

In 2022, the potential of the method is by no means exhausted, hence many species are waiting to be examined in order to expand the data situation and create more detailed systematic trees (also in combination with µCT, TEM and genetic data).

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## A Shape analysis of three commercial estuarine bivalve species from western Portugal

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Bivalves, such as oysters, mussels, cockles, and clams are some of the most consumed seafood products worldwide. The cockle (*Cerastoderma edule*), manilla clam (*R. philippinarum*) and grooved carpet shell clam (*R. decussatus*) fisheries account for 73 % of the bivalve fishery sector in continental Portugal totalling 6 thousand tons of landed product a year. The industry supports hundreds of families that live around the water bodies from where they are harvested, and the growth of bivalve consumption and international markets has led to the occurrence of illegal harvesting from restricted areas and has made apparent the need for seafood traceability tools in order to ensure food safety, fisheries control and combat fraud. This work aims to explore whether morphological differences modelled using geometric morphometric tools, could be used to discriminate species origin from nearby estuaries or bays (from 20 km to 300 km apart). The samples were collected from 5 systems on the west coast of Portugal (The Ria de Aveiro, Óbidos and Albufeira coastal lagoons and the estuaries of the river Tagus and Sado ) and the shells were compared both using traditional morphometric approaches (shell length, width, and height) and geometric morphometric methods (using landmark analysis – based on homologous points on the shell interior). Geometric morphometric methods allow the partitioning of shape and size components, preserving the main geometric properties of the specimens and generating a visual representation and shape variables than can be statistically analysed. Due to their hard un-deformable shells, bivalves are an excellent group to apply GM methods. After the digitisation of the shells, landmark positions were rotated, scaled and translated through Generalized Procrustes Analysis. The respective partial warps were then estimated, and the shape variables compared between locations and species using multivariate analysis to assess the use of this technique to discriminate specimens geographical origin.

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## Histological study of the dorsal mantle and warts of *Graneledone pacifica* and *Pareledone charcoti*

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The skin of shallow-water cephalopods is renowned for its colour and texture change abilities, unlike the skin of deep-sea octopods which is limited in these regards. In some deep-sea octopods, notably the genus *Graneledone*, the dorsal mantle, head and some arms are covered with subdermal warts that appear to be permanent, fixed structures. Although the warts are generally termed cartilaginous, their composition has been unknown. To expand our knowledge of the skin and the composition of the warts in *Graneledone pacifica*, we investigated them using brightfield and scanning electron microscopy. A layer of reticulated collagen fibre lies under the epidermis; beneath it, chromatophore organs form multiple, poorly organized layers. Chromatophore organs in shallow-water taxa, in contrast, form one layer immediately under the epidermis. The warts of *Graneledone* underlie the chromatophore organs and appear to be formed from extensive ground substance (amorphous gelatinous material usually composed of water and large organic molecules). Scattered muscle fibres run throughout the ground tissue but do not penetrate the collagen fibres near the epidermis. The wart's overall anatomy appears clearly comparable to and potentially homologous with that of the extensible dermal papillae of shallow-water octopods reported by other authors. The ground substance amid fibres has parallels with dermal structures of several other deep sea cephalopods, as do the strongly reduced mantle musculature and considerable connective tissue superficial to the mantle muscles. We hypothesize the dorsal dermal structures and reduced mantle muscles offer a functional benefit to these deep-sea animals.

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