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World Congress of Malacology
Munich 2022

31st July – 5th August

Posters

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Münchhausenstraße 21, 81247 München, Germany
Tel. +49 (0)89 8107-0 – Fax +49 (0)89 8107-300
E-mail: SPIXIANA@snsb.de
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Edited by

Martin Heß

LMU Munich, Germany

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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 21st 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 21st 1962, and thus celebrates its 60th 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

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 Bellerophontida, 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 21st 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 (~76 000), 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 Juřičková (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 (sclerochronological) 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 spiralians 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.

Posters

Ice Shelf Collapse

Background

The Prince Gustav Channel, located at the tip of the Eastern Antarctic Peninsula, has undergone dramatic changes in ice-cover and environmental conditions over the past 200 years.

The Prince Gustav Ice Shelf collapsed in 1995. Until 2018 no benthic biological sampling of the marine benthos, including molluscs, of this area had occurred.

Methods

Samples were collected with an epibenthic sledge, fixed in 96% ethanol and identified to species level.

Macrobenthic molluscan biodiversity of the Prince Gustav Channel was assessed with uni- and multivariate methods, including species richness, abundance and diversity indices.

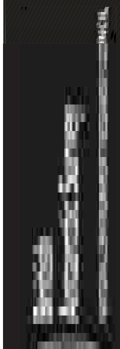
Bivalve assemblages were compared with Weddell Gyre data.

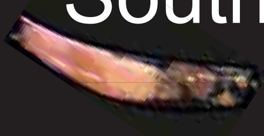
Results & Discussion

1. The Prince Gustav Channel molluscan assemblage is distinct and species poor. It comprises gastropods (24 spp), bivalves (18 spp), scaphopods (2 spp) and solenogastres (6 spp).
2. Some of these species occur in high abundances. Scaphopods make up 0.9-8% abundance in published Southern Ocean assemblages, while in the Prince Gustav Channel they make up 72% of the overall abundance.

The Prince Gustav Channel is geographically isolated from the Weddell Gyre and the dominant species show planktonic dispersal strategies, with direct developing species being rare.

The Prince Gustav Channel is recolonising after the recent changes in ice cover.
(Anderson et al., 2021, Frontiers in Marine Science)





Southern Ocean Molluscs & Climate Change

Madeline P. B. C. Anderson*, Huw J. Griffiths, Katrin Linse
✉ *mason@bas.ac.uk 🐦 @DiverMads

Methane Seeps

Background

Methane is a climate change gas more potent than carbon dioxide. Southern Ocean marine methane is estimated to comprise ¼ global marine methane. Increased marine methane seepage may be caused by warming of the Antarctic continental shelf waters.

Marine methane in the Southern Ocean could play an important role in 1) climate change and 2) impact benthic biodiversity and ecosystem function.

Methods & Preliminary Results

Macrobenthic fauna was collected on the continental shelf around South Georgia by multicorer. 26 tubes from 14 sites were fixed in 96% ethanol.

Fauna was identified to morphospecies for the macrobenthic diversity assessment.

Of the total 3000 individuals collected, 180 were molluscs. 2 mollusc classes were present: gastropods (n=8, 6 spp) and bivalves (n=172, 6 spp).

Next Steps - for my PhD

1. Use of environmental parameters for macrobenthic community pattern assessments and their drivers.
2. Investigation of the community composition, functional traits and trophodynamics of benthic fauna, including molluscan communities, from hydrocarbon and carbon enriched habitats in the Southern Ocean which are known or hypothesised to be influenced by methane seeps.
3. Analysis of the role of bivalves as potential indicator species for hydrocarbon and carbon enriched habitats in the Southern Ocean, leading to identification of potential further hydrocarbon enriched sites. Bivalves will be analysed for isotope composition of soft parts and shells.

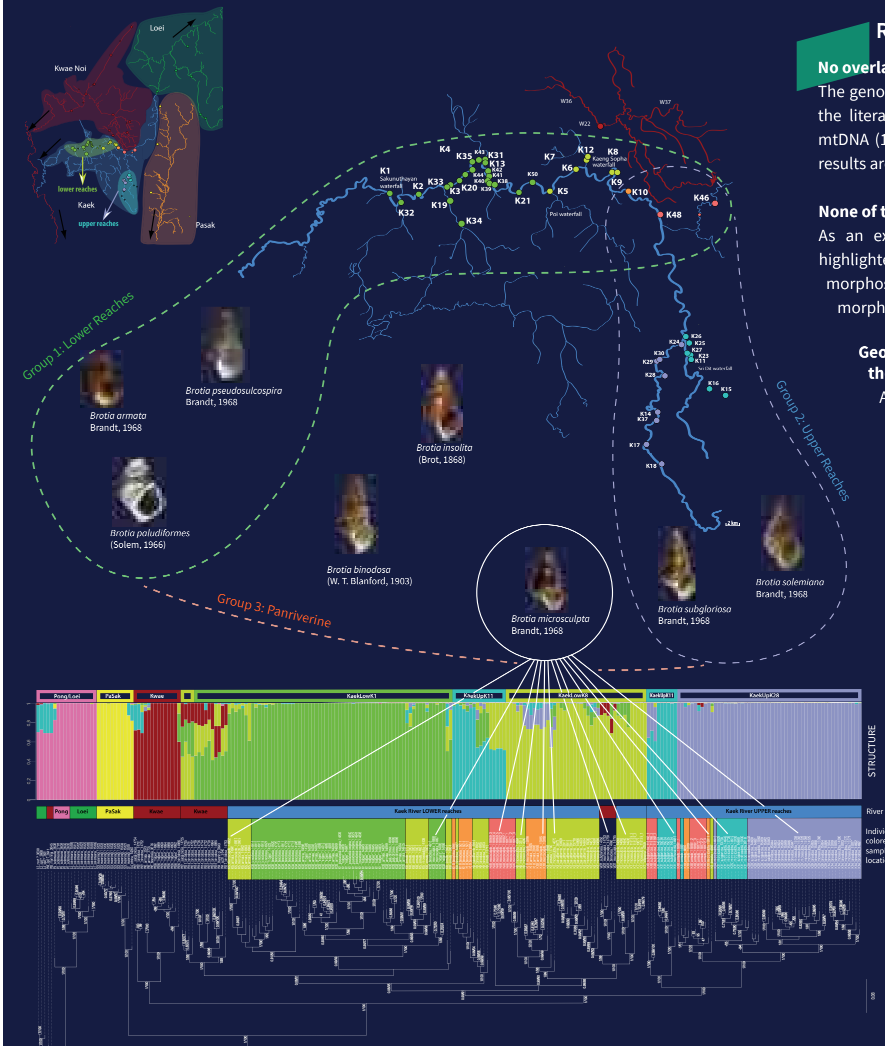
Looks don’t matter in *Brotia* – Phylogeny and molecular genetics of freshwater gastropods from the Kaek River in Thailand using mtDNA and ddRAD

Simon Bober[1], **Nora Lentge-Maaß**[2], **Marco T. Neiber**[1], **Matthias Glaubrecht**[1]
[1]Department Biodiversity of Animals, Universität Hamburg, and Leibniz Institute for the Analysis of Biodiversity Change (LIB), Hamburg, Germany
[2]Museum of Natural History, Leibniz Institute of Evolution and Biodiversity, Berlin, Germany

Introduction

Species of *Brotia* are typically found in tropical forest rivers and mountain streams of Southeast Asia, from India to Borneo. In the middle of the last century, Brandt (1968, 1974) described ten *Brotia* morphospecies (including subspecies) from an approximately 100 km long part of the Kaek River. This is unique since previous studies suggest that *Brotia* appears to occur in predictable combinations along rivers with usually one more globular and one more turreted shell form (Davis 1982, Köhler et al., 2010).

It is the aim of this study to investigate the genetical diversity of *Brotia* species along the Kaek River in concert with specimens from adjacent river systems. Assuming a relatively young species flock with potentially little variation within common marker genes, additionally the whole genome was sampled using ddRAD sequencing. The herein presented results are only a fraction of a larger project and solely focusing on the genetics part of this comprehensive study.



Map of the Kaek River with all sampling locations and previously reported morphospecies. The distribution of each morphospecies is indicated by groups (dashed lines). *B. microsculpta* Brandt, 1968 was used as an example to show the wide appearance of one morphospecies within the ddRAD tree. Below the map the results of the STRUCTURE analysis were plotted according to the topology of the tree.
It is a ML-tree based on ddRAD data from 246 individuals, the tips are labled based on morphological determination and colorcoded by sampling locality. The ML tree was calculated using IQ-Tree 2 (Bui et al., 2020) with the evolutionary model (GTR+G) as suggested by PartitionFinder. Support values were calculated by the UFBoot algorithm set to 1,000 replicates (Hoang et al., 2018).

Methodological details

Analyses of the dataset were performed with the program STACKS version 2.0b (Catchen et al., 2011). The final settings used were m3 M2 n2 N4 -X “populations: -r 0.9 -min maf 0.013” resulting in 2002 loci of which 1683 are polymorphic with 4690 SNPs. The loci within this dataset seem to be rather unique since increasing -M (distance allowed between stacks to form a locus) does increase the number of formed loci and decrease the number of polymorphic loci. The reason for this is the formation of rare alleles in the population, which are filtered by the minor allele frequency filter (--min-maf) in STACKS. This filter was set to a comparatively low value of 1.3% (-- min-maf 0.013), meaning one allele must be present in at least three individuals for being processed.
For the STUCTRUE analyses one random SNP was selected per locus which reduced the amount of SNPs from 4690 to 1784. Groups were defined based on the analysis of the dataset using STRUCTURE, which found K = 7 as the most probable number of populations.

Results

No overlap between morphological and genetical data
The genomic sampling failed to recover entities described as species in the literature. Morphospecies were found spread across the tree for mtDNA (16S, COI) and ddRAD loci. (For visual reasons only the ddRAD results are shown here)

None of the formally decribed species was found to be monophyletic
As an example *B. microsculpta*, a panriverine morphospecies was highlighted here to show the almost random appearance of morphospecies across the tree, giving no overlap between morphological and genetical characters.

Geographical structure suggests two distinct clusters within the lower and upper reaches
A geographical structure was found within mtDNA and ddRAD loci, which divides the upper reaches from the lower reaches. A zone of admixture is found where the upper reaches meet the lower reaches (K10, K46, K48), which genetically neither belongs to the upper nor exactly to the lower reaches.

Morphological differences support the hypothesized clusters
The two clusters per river reach were found to be significantly different in shell size and shape, suggesting the postulated occurrence of globular and turreted *Brotia* species within the Kaek River. Athough statistically detectable, hard to identify by eye due to a high degree of apparent phenotypic plasticity.

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simon.bober@uni-hamburg.de

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

Kanishka Bose[1], Shiladri Sekhar Das[1], Subhronil Mondal[2]

[1]Geological Studies Unit, Indian Statistical Institute, 203, B.T. Road, Kolkata, India.

[2]Department of Earth Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, West Bengal, India.

Background and Objectives:

- Turritelline dominated assemblages (TDA) are globally very common in both siliciclastic and carbonate sedimentary successions through ages (see Allmon and Knight, 1993).
- Only a handful of research works exists on the Miocene TDAs of the Kutch Basin, western India (see Halder and Singha, 2016 and Goswami et al., 2020). Jain (2014) first mentioned the occurrence of a 'turritella' bed from the adjacent Dwarka Basin, western India although did not carry out any detailed study on it. **Herein, an extensive spatial collection is done on this particular bed to reveal its true diversity picture of the macro as well as micro gastropods and bivalves.**
- Miocene marine sequences are terra-incognita for the micro-molluscan faunas and only two notable works are present on sea-grass associated micro molluscs assemblages of the early Miocene (Burdigalian) of Quilon, southern India (Harzhauser, 2014) and Banyunganti, south-central Java, Indonesia (Reich et al., 2014). **In the present endeavour, we report a considerable number of early Miocene (Burdigalian) micro-molluscs found from a TDA in the Dwarka Basin, western India.**

Materials and Methods:

- **Collection of the specimens**: The gastropod and bivalve specimens collected from three different fossil bearing localities (Loc. 1, 2 and, 3) of the TDA (Nmb) Unit of the Gaj Formation of the Dwarka Basin.
- **Taxonomic Diversity Analysis:** Pie charts and bar plots are used at family, genus and species level. The adequacy of the specimens and species richness are obtained using rarefied curves.
- **Ecological Diversity Analysis:** Pie charts are used based on their diet.

❖ **The gastropod diets are**: Predatory carnivores (CP); Browsing carnivores (CB); Omnivores-grazers (HO); Herbivore-grazers (HG); Suspension feeders (SU); Scavenger-predators (SP).

❖ **The bivalve life habits diets:** Micro-carnivores (MC); Deposit feeders (DF); Suspension feeders (SU); Chemo symbiotic feeders (C).

- **Comparison of the molluscan faunal composition** of our TDA is done with a seagrass associated micro and macro molluscan fauna of another contemporary basin of Indian subcontinent i.e., Quilon Basin, southern India based on Simpson's Similarity Index.

Results:

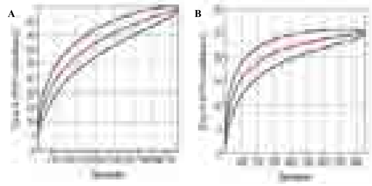
- The <u>turritelline gastropods</u> (Family Turritellidae) show exceptionally high abundance (**N=7062**) (constituting ~96% of the all gastropods) and hence named turritelline-dominated assemblage (TDA) unit and are represented by **eight species** belonging to four genera, i.e., *Zaria*, *Haustator*, *Turritella*, and *Protoma*.
- The <u>associated gastropods</u> show high species richness of **49 species** (**N=271**) belonging to 41 genera with relatively high abundance of families like Nassariidae, Rissoinidae, Potamididae, Pyramillidae, and Scaliolidae.
- The <u>associated bivalves</u> 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 <u>gastropods</u> (**~96.22%**) and <u>bivalves</u> (**89.79%**). Apart from that, **herbivore-grazers (1.61%)**, **scavenger-predators (1.14%)**, and **carnivores (0.94%)** constitute for the <u>gastropods</u>, whereas, **deposit-feeders (9.61%)** and **chemosymbionts (0.50%)** constitute the types of <u>bivalve</u> 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**.

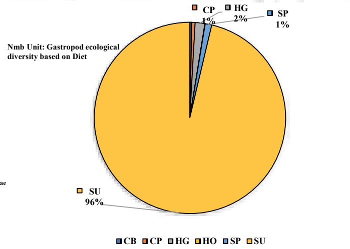
Geological Setting and Stratigraphy:

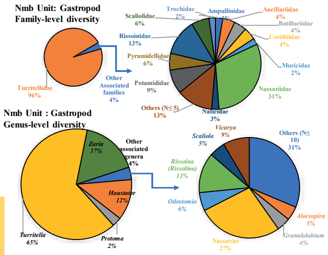
- The Dwarka Basin, situated at the western fringe of the Kathiawar Peninsula, is a peri-cratonic shelf basin and filled with an extensive succession of the Neogene marine sediments (Jain, 2014).
- The succession is subdivided into three formations, namely the Gaj, Dwarka, and Miliolite Limestone formations in ascending stratigraphic order ranging from the early Miocene (Burdigalian) to Holocene and overlying unconformably on the Deccan Traps and laterite (Jain, 2014).
- The basal Nandana Member of the Gaj Formation is a ~10 m thick and is further subdivided into four units: Nma, Nmb, Nmc and Nmd units.
- The **Nmb (TDA) Unit** is characterized by a highly fossiliferous **brown shale** hosting a near consistent turritelline dominated assemblage along with bivalves, echinoids, crabs and fish teeth.

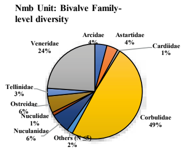
Geological map of the Dwarka Basin (modified after Jain, 2014) showing the TDA fossil localities.

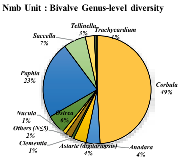
Overall rarefied curve showing sample adequacy and species richness of the Nmb Unit for (A) Gastropods and (B) Bivalves.

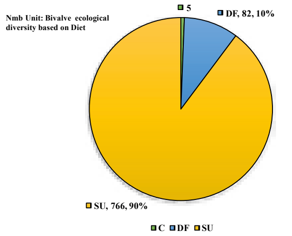


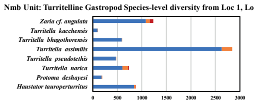












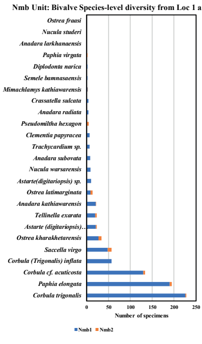


Plate 1: (1-2) *Zaria angulata* Sowerby 1840; (3-4) *Turritella narica* Vredenburg, 1928; (5-6) *Turritella assimilis* Sowerby, 1840; (7-8) *Haustator tauroperturritus* Sacco, 1895. Scale bars = 10.0 mm.

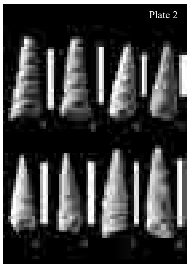


Plate 2: (1-2) *Turritella bhagothorensis* Vredenburg, 1928; (3-4) *Protoma deshayesi* d' Archaic, 1850; (5-6) *Turritella pseudotethis* Vredenburg 1928; (7-8) *Turritella kachhensis* Vredenburg, 1928. Scale bars = 10.0 mm

Inferences and Conclusion:

- Extensive flourishment of turritelline gastropods along with other suspension feeding bivalves suggests a nutrient rich environment, often caused due to ocean upwelling (see Allmon and Knight, 1993; Allmon, 2007).
- Although significantly less in abundance, high faunistic similarity between TDA and seagrass associated molluscan fauna explains the occurrence of the other opportunist molluscs with different diets dwelling in nutrient rich waters alike seagrass habitats.

Plate 3: (1-2) *Nassarius* cf. *beui* Jain, 2014; Scale bar = 10.0 mm. (3-4) *Rissoina* (*Rissolina*) cf. *costulata* (Dunker, 1860); (5-6) *Scaliola subbela* Harzhauser, 2014; (7-8) *Finella bruchae* Harzhauser, 2014; (9-10) *Granulolabium plicatum* Bruguière, 1792; Scale bars = 2.0 mm.
(11) *Vicarya verneuili* d'Archiac, 1850 Scale bar = 10.0 mm.
; (12-13) *Alocospira* sp.; (14) *Mitromorpha* sp.; (15-16) *Neverita* sp.; (17-18) *Retusa ashtamudiensis* Harzhauser, 2014; (19) *Nassaria harzhauseri* Jain, 2014; (20-21) *Odostomia* sp. Scale bars = 2.0 mm.

Plate 4: (1-2) *Corbula trigonalis* Sowerby, 1840; (3-4) *Paphia elngata* Jain, 2014; (5-6) *Corbula* cf. *acuticosta* Martin, 1885; (7-8) *Corbula (Trigonalis) inflata* Jain, 2014; (9) *Saccella virgo* (Martin, 1879); (10) *Ostrea kharakhetarensis* Jain, 2014; (11) *Astarte* (*Digitariopsis*) *trigonoequilatera* Jain, 2014; (12-13) *Anadara kathiawarensis* Jain, 2014; Scale bars = 5.0 mm.

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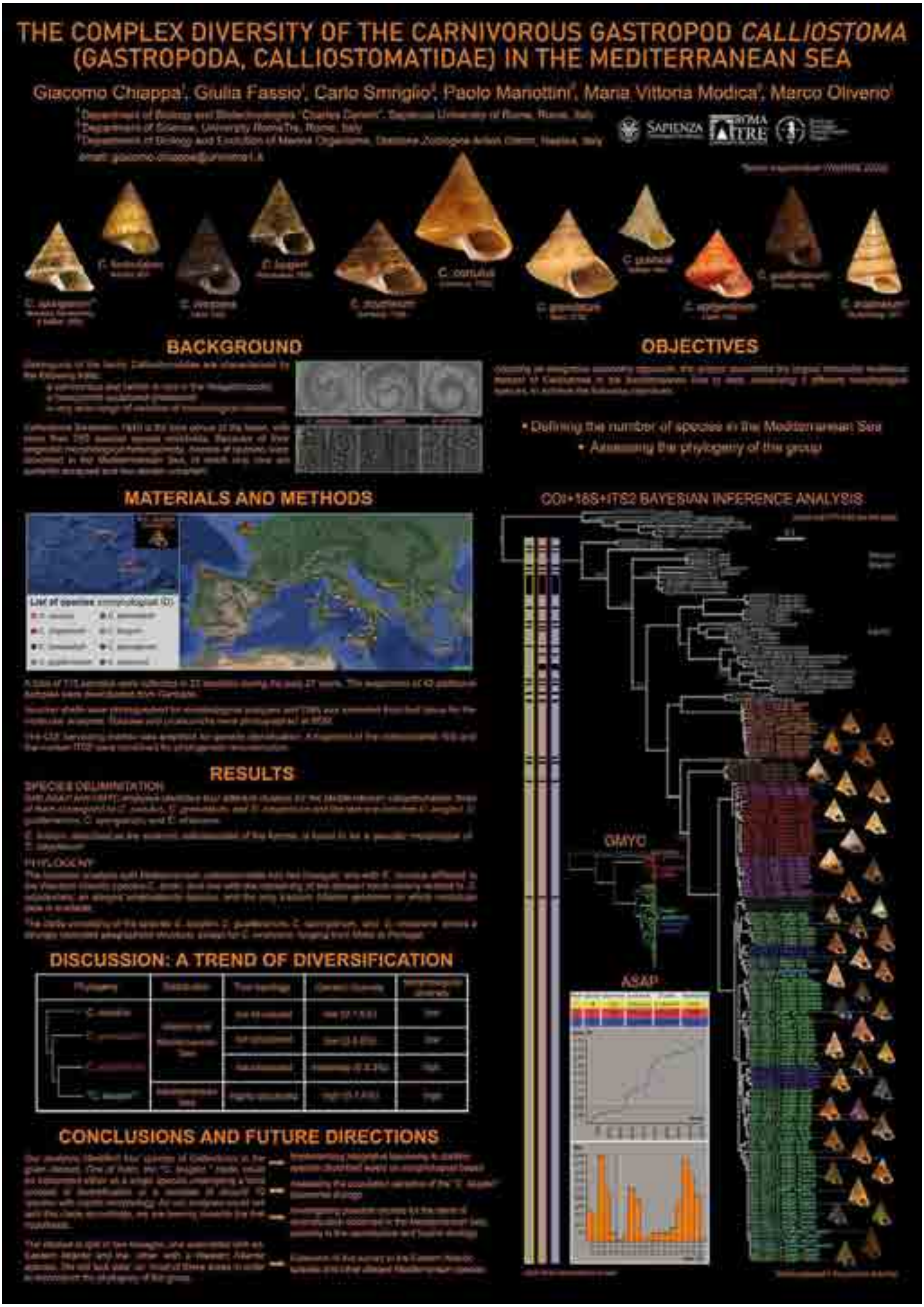


THE COMPLEX DIVERSITY OF THE CARNIVOROUS GASTROPOD *CALLIOSTOMA* (GASTROPODA, CALLIOSTOMATIDAE) IN THE MEDITERRANEAN SEA

Giacomo Chiappa[1], Giulia Fassio[1], Carlo Smriglio[2], Paolo Mariottini[2], Maria Vittoria Modica[3], Marco Oliverio[1]

[1] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy
[2] Department of Science, University RomaTre, Rome, Italy
[3] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy
email: giacomo.chiappa@uniroma1.it

BACKGROUND

OBJECTIVES

- Defining the number of species in the Mediterranean Sea
- Assessing the phylogeny of the group

MATERIALS AND METHODS

COI+16S+ITS2 BAYESIAN INFERENCE ANALYSIS

RESULTS

GMYC

ASAP

DISCUSSION: A TREND OF DIVERSIFICATION

CONCLUSIONS AND FUTURE DIRECTIONS

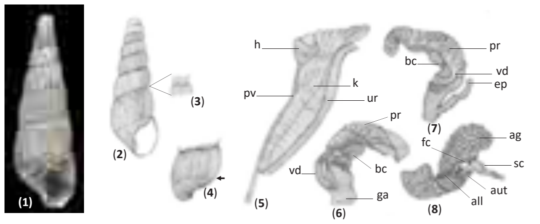
Revisiting species of the genera *Subulina* and *Striosubulina* (Gastropoda, Achatinidae): Anatomical characterization brings new operational criteria for species delimitation

Sthefane D'ávila
Museu de Malacologia Prof. Maury Pinto de Oliveira – MMPMPO, Universidade Federal de Juiz de Fora, Minas Gerais, Brazil.
Email: sthefanedavila@hotmail.com

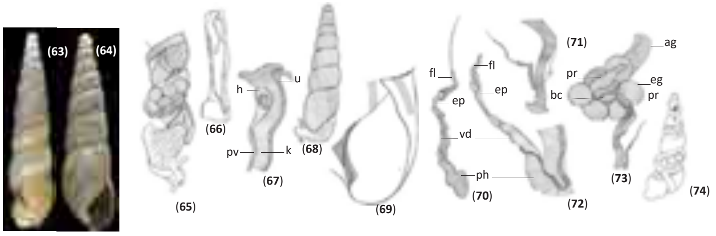
Land snail's species formerly ascribed to the family Subulinidae, now recognized as synonym of Achatinidae, constitute a diverse group of pantropical taxa for which there is scarce information on fundamental biological attributes including the anatomy of the soft parts, life history, distribution, and 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 19th 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 analyzed 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, ie.: *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.

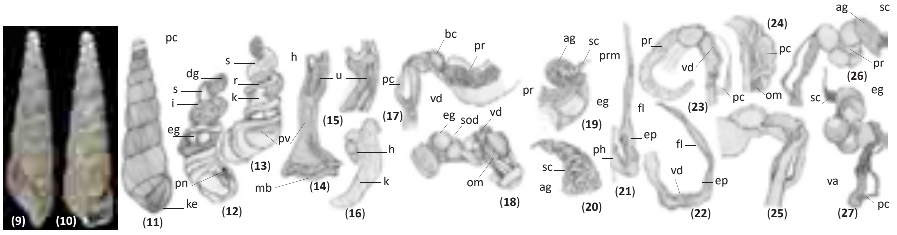
Acknowledgements. To Philippe Maestrati and Barbara Buge for all the assistance during the analysis of specimens in the MNHN, Paris, France. PM also provided the images of the shells.



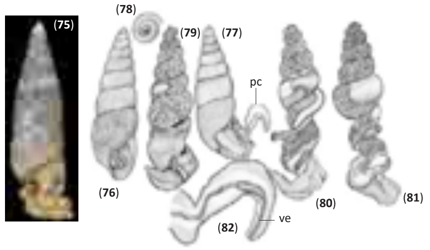
***Subulina angustior* (Dohrn, 1866).** Original label: "Kamerun, Nonge 1891. y Jjöstadt". (**1**) Shell, 14 mm. (**2**) Shell. (**3**) detail of the suture. (**4**) Dorsal face of the shell body whorl showing a well marked keel (black arrow). (**5**) pallial organs. (**6-8**) reproductive system.



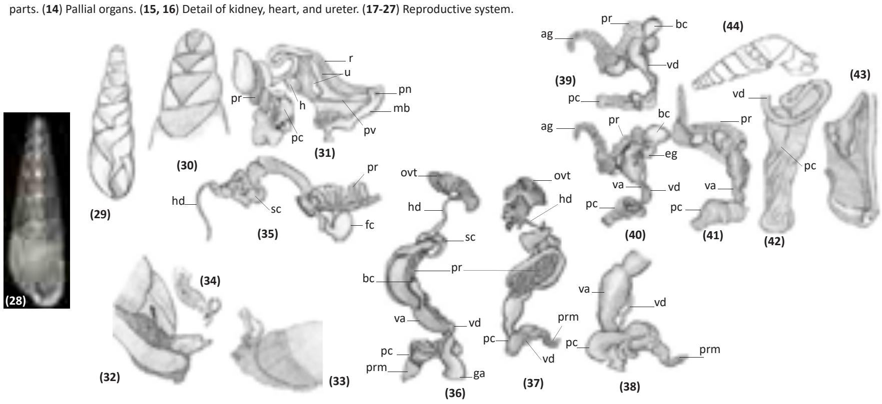
***Subulina cereola* (Morelet, 1860).** Original label: "Mayotte, Presq' ile de Boueni Boungoudranavi, forêt naturelle au pied de falaise, alt. 180 m Réc. P. Bouchet & A. Abdou, 3 Mar 2001". (**63, 64**). Shell, 23.7 mm and 20.2 mm. (**65**) Soft parts. (**66**) mantle and pallial organs. (**67**) Detail of the Kidney, ureter, heart. And pulmonary vein. (**68**) Shell and cephalopodal mass. (**69**) Shell aperture. (**70-74**) Reproductive system.



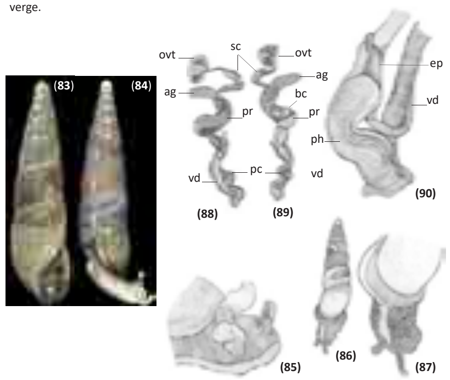
***Striosubulina striatella* (Rang, 1831).** Original label: "Stenogyra; Cote d'Ivoire; M. Ehoié 1901". (**9, 10**) Shell, 17 mm. (**11**) Shell showing a well marked keel. (**12,13**) Soft parts. (**14**) Pallial organs. (**15, 16**) Detail of kidney, heart, and ureter. (**17-27**) Reproductive system.



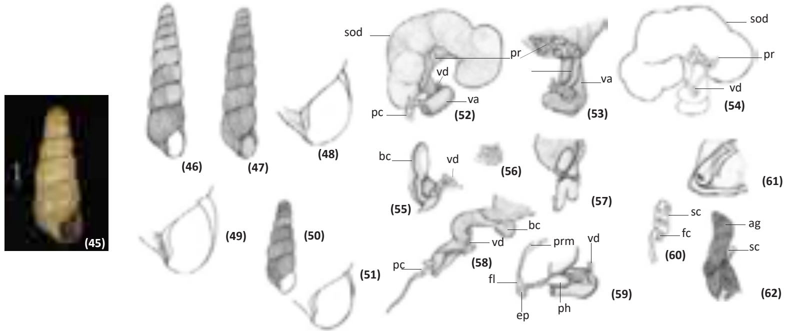
***Subulina canonica* (Morelet, 1886).** Mayotte, Bondrelé, Valée de la Mro ona Patsé, Forêt de la Tha lueg, Alt. 70-150 m., 25 Mar 2001". (**75**) Shell, 17.2 mm. (**76, 77**) Shell. (**78**) Protoconch. (**79-81**) Soft parts. (**82**) Everted phallus showing the verge.



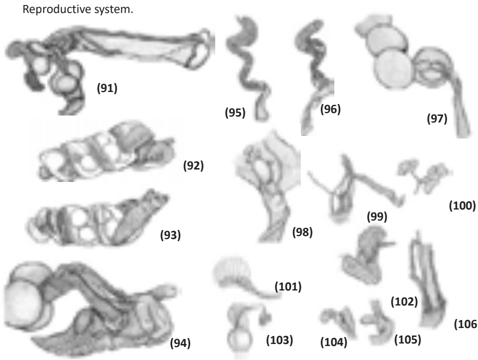
***Subulina simplex* (Morelet, 1882).** Original label: "Mayotte, massif de Sondai Chounoui sur Eboulisi, dans forêt sèche alt. 80-150 m, 24 Mar 2001". (**28**) Shell, 8mm. (**29,30**) Shell showing detail of the columella. (**31**) Soft parts partially dissected. (**32, 33**) Snail with everted phallus. (**34**) Everted phallus in detail. (**35 - 42**) Reproductive system. (**43**) Pallial organs. (**44**) position of the eggs seen through the transparent shell.



***Subulina ferriezi* (Morelet, 1882).** Original label: "*Subulina ferriezi*, Mayotte, Hachirangou, forêt secondaire, bois humide avec mousses. Alt. 200m". (**83, 84**). Shells, 18.2 mm and 16.5 mm. (**85**) Cephalopodal mass and everted phallus. (**86, 87**) Cephalopodal mass and soft parts seen trough the transparent shell. (**88-90**) Reproductive system.



***Subulina parana* Pilsbry, 1906.** Original label "MZUSP 15408, Subulina, Aracajú, Sergipe, col. Menezes, 13 Jul. 1962. (**45**) ANSP 91127, Lectotype, 12 Feb. 1906, Para, Brasil (available from http://clade.ansp.org/malacology/collections/details.php?mode=details&catalognumber=91127). (**46-49**) Adult shells and details of shell aperture. (**50,51**) Juvenile shell. (**52- 62**) Reproductive system.



***Subulina octona* (Bruguière, 1789).** Original label: "MZUSP 92655, *Leptinaria* sp., Praia Grande SP, Brasil, Boqueirão (Terrenos Morros), col. LR Simone, det. LR Simone".

World Congress of Malacology 2022 LMU München: ***Exploration, Biodiversity and Systematics of Molluscs: A Symposium in Honour of Philippe Bouchet***

France
Montagne de Lure

Slovenia
Lokev east of Triest

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

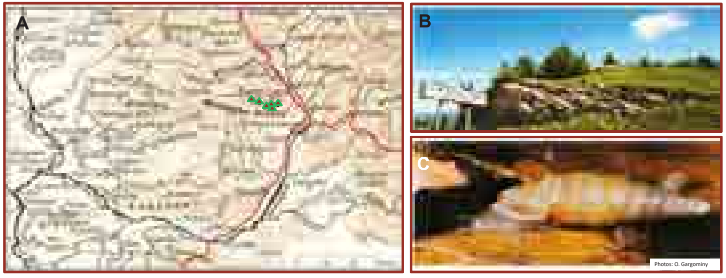
Gerhard Falkner & Margrit Falkner[1]

[1]Staatliches Museum für Naturkunde Stuttgart, Rosenstein 1, DE-70191 Stuttgart (Germany) Email: falkner.ehrenamt@smns-bw.de

Abstract:

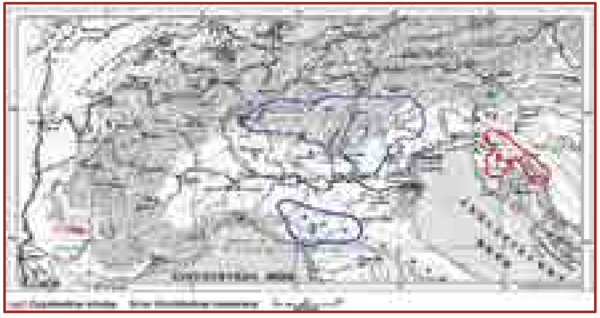
In the frame of the very successful malacological prospections of the Muséum national d'Histoire naturelle Paris (MNHN) for the Eurpean network of nature protection areas "Natura 2000" – in collaboration with the Office National des Forêts (ONF) – field work in the Montagne de Lure, Département Alpes-de-Haute-Provence, has been carried out in June 2002. In the 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 kms. *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.

Fig. 1: Finding sites (△) of *Chochlodina triloba*



(A) At Col du Pas de la Graille, *Cochlodina triloba* was found for the first time during prospecting work. In total, the species was found at 7 sites. **(B)** A special feature of the Montagne de Lure are near-natural beech forests, which are partly intermixed with extensive stands of silver fir. These primary forests, which are not or only extensively managed, are rich in deadwood and harbour the biotopes of *Cochlodina triloba* in shady areas, which extend into the high-altitude combat zone of the beech trees. The biotpes of the Montagne de Lure are aptly described in the fact sheet for the ZNIEFF (= Zones naturels d'interet écologique faunistique et floristique) by Villaret & al. 2020. By an Arrêté from 02.06.2010 large parts of the Montagne de Lure have meanwhile been protected as "Zone Spéciale de Conservation" under the "Directive Habitat". **(C)** *Cochlodina triloba* is a characteristic soil dweller. During our investigations, we repeatedly noted a preference for rotting wood biotopes in permanently moist environments and even found transitions to a purely xylobiont way of life.

Fig. 2: Distribution of *Cochlodina triloba* compared with *Cochlodina comensis*



The red dots in the eastern area of *C. triloba* indicate the occurences of a remarkable dwarf form (3 series in collection). Subspecies of *Cochlodina comensis* (distribution according to Giusti & Mazzini 1971): 1 *C. comensis* (L. Pfeiffer 1850), 2 *C. trilamellata* (A. Schmidt 1868), only loc. typ., 3 *C. lucensis* (Gentiluomo 1868).
Cochlodina comensis: was discovered by B. Rensch near Digne (Jaeckel sen. 1963); next locality at Lake Lugano.

Fig. 3: *C. triloba* in France as a new subspecies

1 - *Cochlodina triloba triloba*, Slovenia, Corgnale (= Lokev), entrance of the cave Vilenica; leg. Erjavec (SMF 145038); lectotype preselected by A. Zilch (unpubl.). with dorsal view.

2 - *Cochlodina triloba* n. ssp., Alpes-de-Haute-Provence, Montagne de Lure, Combe de la Sapée, 1480 m NN; leg. G. u. M. Falkner, O. Gargominy, B. Fontaine & M. Klein 12.06.2002 (MNHN); intended holotype of a new subspecies to be described; with dorsal view.

3-4 - *Cochlodina triloba triloba*, Slovenia, Lokev near Sežana, leg. H. Nordsieck 11.08.1968 (SMNS-Nords 04235)

5 - *Cochlodina triloba triloba* (large form), Croatia, Buzet; leg. H. Nordsieck 15.04.1969 (SMNS-Nords 02888)

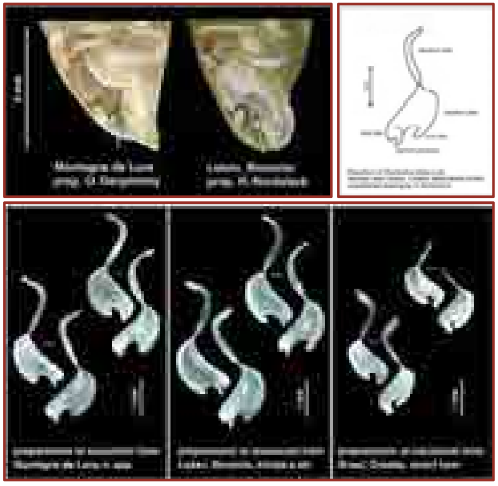
6-7 - *Cochlodina triloba* ? ssp. (dwarf form) Brseč, Mošćenička Draga; leg. H. Nordsieck 17.04.1968 (SMNS-Nords 02888).

8-9 - *Cochlodina triloba* n. ssp., Alpes-de-Haute-Provence, Montagne de Lure, Combe de la Sapée, 1480 m NN; leg. G. u. M. Falkner, O. Gargominy, B. Fontaine & M. Klein 12.06.2002 (MNHN)

10 - *Cochlodina triloba* n. ssp., Alpes-de-Haute-Provence, Montagne de Lure, Col du Pas de la Graille, 1600 m NN; leg. G. u. M. Falkner, O. Gargominy, B. Fontaine & M. Klein 10.06.2002 (MNHN)

Photographs by P. Glöer; SMF = Senckenberg-Museum, Frankfurt a. M.; SMNS = Staatliches Museum für Naturkunde Stuttgart; MNHN = Musèum national d'Histoire naturelle, Paris

Fig. 4: Features of the Clausilium



Characteristics of the new subspecies:

- *C. triloba* from the Montagne de Lure is generally more obese and compact than the typical form from Istria and Slovenia. It is also less glossy.
- The apertural edges are more strongly connected and the aperture as a whole is more angular. The angle of the aperture is more distinctly raised.
- Palatal folds: the principalis is not or only slightly extended above the upper palatal fold.
- Important features are found on the clausilium (Fig. 4).

Discussion:

Between 2000 and 2002 Philippe Bouchet has initiated and organised the funding for several missions of malacological prospection of the Muséum national d'Histoire naturelle Paris (MNHN) for the Eurpean network of nature protection areas "Natura 2000" in the region Provence-Alps-French Riviera (overview in Gargominy 2002). In the frame of these very successful missions field work in the Montagne de Lure has been carried out in June 2002. Among many nice findings there was the detection of a new clausiliid for the French fauna. The largely isolated occurrence of the Istrian-Dinaridic *Cochlodina triloba* is not only new for France but represents also an undescribed subspecies.

The distance of the newly detected occurrences of *Cochlodina triloba* to the hitherto known area of the species is about 650 kms. *Cochlodina triloba* is not the first clausiliid species in the French fauna with a considerable east-west disjunction. As further exemples can be named *Cochlodina comensis* (L. Pfeiffer 1850) (Jaeckel sen. 1963: 79), *Ruthenica filograna* (Rossmässler 1836) (Gittenberger 1978: 112-114), and the recently detected *Fusulus interruptus* (C. Pfeiffer 1828) (Combrisson 2020).

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.

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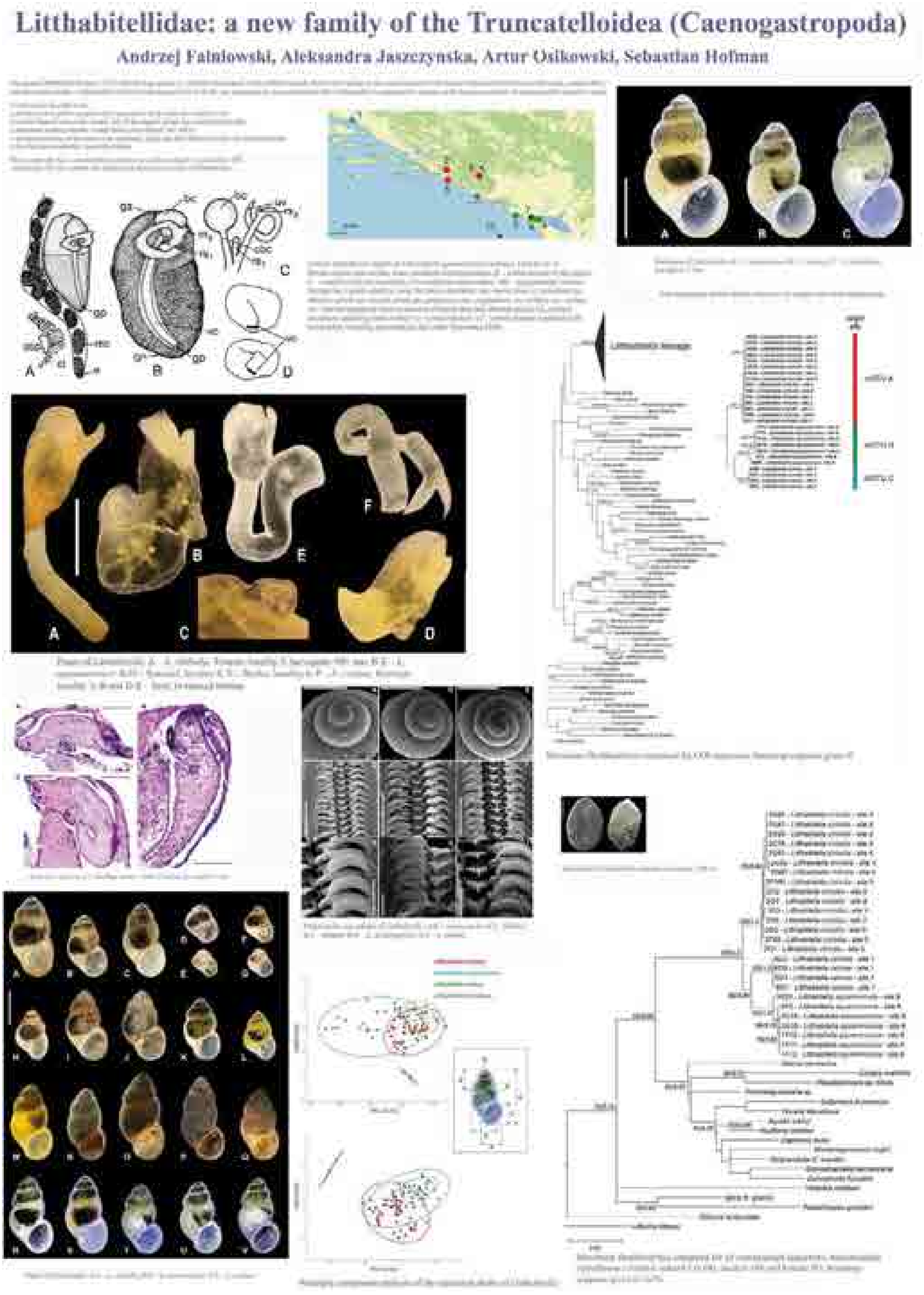


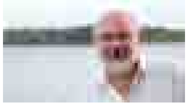
Litthabitellidae: a new family of the Truncatelloidea (Caenogastropoda)

Andrzej Falniowski, Aleksandra Jaszczyńska, Artur Osikowski, Sebastian Hofman

UNIVERSITE CADI AYYAD

Muséum d'Histoire Naturelle de Marrakech

Homage to Philippe Bouchet



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

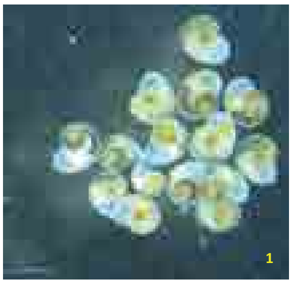
Mohamed Ghamizi, Khadija Boulaassafer, Abderrazak Boudellah and Sanae El Ghali

mohamed.ghamizi@gmail.com

Muséum d'Histoire Naturelle de Marrakech, Département de Biologie, Laboratoire EauBiodiCC, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco.

Abstract

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. 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.



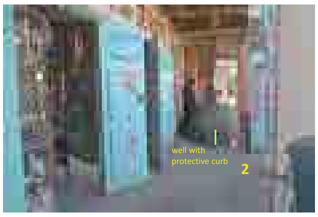
Conflicts of interest / Proposals

How to combine the conservation of underground micromolluscs (as photo 1) living in wells (photo 2) or in springs (photo 3) while preserving the water needs of local populations? In the presence of those concerned, we introduced a pedagogy to raise awareness of the importance of these species as bioindicators of water quality and as a natural heritage that can enhance their localities on an international scale.

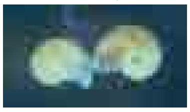
Convincing users for the conservation of species involves offering them alternative solutions to use and to preserve water quality:

For wells: use well covers and install well protection copings

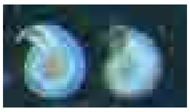
For sources: set up domestic washhouses outside the spring while improving the good working conditions of the women most concerned by outdoor washing



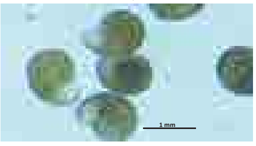
CEPF 111540 / AESVT



CEPF 110217 / RESING



CEPF 110212 / CDRT



New Hydrobiid species being analyzed and described:
the generic level is provisionally assigned to the genus Giustia

The species of CEPF 111540 / AESVT is dedicated to Pr. Touargui of AESVT who has just passed away after our mission in the field (*Giustia touarguii* n.sp.)

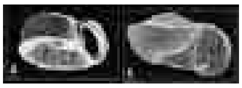
The CEPF species 110217 / RESING is dedicated to the type locality (source Tamda Bzou): *Giustia bzouensis* n.sp.

The species of CEPF 110212 / CDRT is dedicated to the project funder (CEPF): *Giustia cepfi* n.sp.

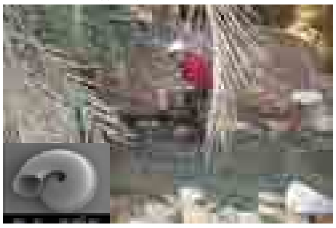
Methodology

Sampling is carried out in the wells using the phreatobiological net and the sediment from the springs is cleaned in nets to collect the micromolluscs. Local populations often attend our investigations. We explain to them the objectives of our work and the interest of studying this underground biodiversity. Socio-economic surveys reveal the importance of water for populations, especially in arid environments, such as the case of the site of Sehb El Masjoune, province of Rehamna. Field missions within the framework of CEPF-funded projects have made it possible to collect new stygobiont and endemic freshwater species.

PhD students engaged for the study of subterranean freshwater biodiversity under the interested observation of local residents. Phreatobiological net used for the collection of species from wells and aquifer analyzed by the piezometric probe



These species are considered as triggers of the KBA (Key Biodiversity Area) of Sehb El Masjoune (A: *Giustia saidai* and B: *Giustia costulata*) on which the CEPF ecosystem profile was based to launch aquatic biodiversity conservation projects for this KBA in Morocco. These species are deposited at the MNHN of Paris



The sediment from the source is cleaned in nets to collect the micromolluscs.

Results:

The users of the wells have accepted the actions of protection of the wells to preserve the quality of groundwater and the conservation of endemic species (case of Hydrobiid snails) by associating the objective of protecting children and livestock who risk falling into unprotected wells (see the example of a well next to a school presenting a risk for children, photo A).

The visiting CEPF leadership team (photo B) endorsing this action also convinced local people of the international importance of the conservation of these endemic freshwater species.

Conclusion*:*

The researcher who studies endemic aquatic species, often threatened because of their limited location, must involve local populations in his investigations. It is necessary and advantageous to explain to them the interest of these aquatic species on a scientific, heritage and utility level as bioindicators of water quality. Water scarcity combined with habitat degradation and pollution are threats both to the domestic water needs of local populations and to the endemic species living in these groundwaters.

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

Klaus Groh[1] & Marco T. Neiber[2]

[1] Hinterbergstr. 15, Bad Dürkheim, Germany; e-mail: klaus.groh@conchbooks.de

[2] Dept. Biodiversity Research, University of Hamburg, Hamburg, Germany; e-mail: marco-thomas.neiber@uni-hamburg.de

Introduction

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, which is supported by preliminary phylogenetic analyses (Lehmann, 2022). Till 2001, only two *Pomatias* species were accepted for the Canary Islands (Ibáñez et al., 2001; Starobogatov & Anistratenko, 1991), the smooth *P. canariensis* (= *Cyclostoma laevigatum* Webb & Berthelot, 1833) (Fig. 1) and the ribbed *P. adjunctus* (Mousson, 1872). Bank et al. (2002) accepted three additional species and indicated indirectly the occurrence of further species taxa by not mentioning members of the genus from several islands despite the fact that *Pomatias* was previously recorded there. Subsequently, this was misinterpreted and consequently several 'new records' were reported.

According to Bank et al. (2002), the Central Canary Islands – Tenerife and Gran Canaria – are home to three out of five currently accepted species known from the archipelago, namely *P. laevigatus*, *P. canariensis* (d'Orbigny, 1840) and *P. raricosta* (Wollaston, 1878), but additional taxa have been proposed. Since there is considerable uncertainty as to the actual number of valid taxa in Tenerife and Gran Canaria, we reviewed the available material from major museum and private collections with the aim to revise the species-group systematics of *Pomatias* in these islands.

Figure 1. *Pomatias canariensis* (= *Cyclostoma laevigatum* Webb & Berthelot, 1833), Tenerife, Ladera de Güimar, below Mirador de Don Martín.

Material and methods

We investigated, aside from the material in the authors' collections, material, especially type material, in the following museum collections: Senckenberg Museum, Frankfurt/Main, Germany (SMF), Zoological Museum, Hamburg, Germany (ZMH), Natural History Museum, Santa Cruz de Tenerife, Spain (TFMCM), Natural History Museum, London, UK (NHMUK), National Museum Wales, Cardiff, UK (NMW), Zoological Museum, Zurich, Switzerland (ZMZ), Academy of Natural Sciences, Philadelphia, USA (ANSP).

Standard shell measurements (shell height, shell width, height of body whorl, aperture height, aperture width) were taken with a digital calliper. Additionally, the number of spiral ribs, if present, were counted on the body whorl.

Results

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/Main and Hamburg (Fig. 2) suggests that we have to deal with more than three species in Tenerife and Gran Canaria, especially if also shells from Pleistocene deposits are included in the study. Type material of *Cyclostomus adjunctus* Mousson, 1872 (Figs 2J, 4C, D), *Cyclostoma laevigatum* Webb & Berthelot, 1833 (Figs 2H, 4A), *C. canariense* d'Orbigny, 1840 (Fig. 2H, see also Fig. 4B), *C. canariense* var. γ *inaequalis* Wollaston, 1878 (Fig. 2F) and *C. canariense* var. *praecursor* Boettger, 1908 (Fig. 2A) 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.

Shell sculpture and standard shell measurements (Figs 3, 5) suggest the presence of seven different taxa on Tenerife (5 taxa) and Gran Canaria (3 taxa), with *P. adjunctus* being the only species that is reported from both islands (Figs 6-7).

Figure 3. Apex and sculpture of body whorl of distinctly ribbed *Pomatias* species from Tenerife and Gran Canaria. **A.** *P. praecursor*. **B.** *P. raricosta*. **C.** *P. inaequalis*. **D.** *P. adjunctus*. **E.** *Pomatias* sp. 1.

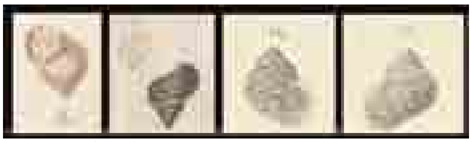


Figure 4. Original figures of Canarian *Pomatias*. **A.** *Cyclostoma laevigatum* Webb & Berthelot, 1833 (see d'Orbigny, 1836). **B.** *Cyclostoma canariense* d'Orbigny, 1840 (see d'Orbigny, 1842). **C, D.** *Cyclostomus adjunctus* Mousson, 1872 (see Mousson, 1872).

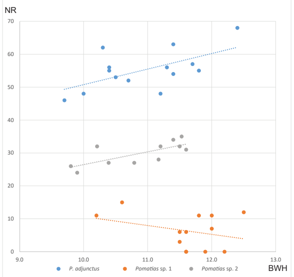


Figure 5. Plot of body whorl height (BWH) vs. number of ribs on body whorl (NR) for the species from Gran Canaria.

Figure 2. Shells of *Pomatias* species from Tenerife and Gran Canaria. **A.** Holotype of *Cyclostoma canariense* var. *praecursor* Boettger, 1908, SMF 262215, Tenerife, Tejina, Quaternary. **B.** *P. praecursor*, TFMCM, Tenerife, Tejina, Quaternary. **C.** *P. adjunctus*, KG, Tenerife, San Andrés., Playa de las Teresitas **D.** *P. raricosta*, KG, Tenerife, Costa El Draguillo near Taganana. **E.** *P. raricosta*, TFMCM, Tenerife, Anaga Mountains. **F.** Syntype of *Cyclostoma canariense* var. γ *inaequalis* Wollaston, 1878, ANSP 97320, Tenerife. **G.** *P. inaequalis*, TFMCM, Tenerife, Las Canteras. **H.** Syntype of *Cyclostoma laevigatum* Webb & Berthelot, 1833, NHMUK 1854.9.28.71, Tenerife, Santa Cruz de Tenerife (also a syntype of *Cyclostoma canariense* d'Orbigny, 1840). **I.** *P. canariensis*, KG, Tenerife, Tejina, Quaternary. **J.** Syntype of *Cyclostomus adjunctus* Mousson, 1872, ZMZ, Tenerife (doubtful, see Wollaston, 1878). **K.** *P. adjunctus*, TFMCM, Gran Canaria, Llanos de Botija. **L.** *Pomatias* sp. 1, KG, Gran Canaria, Barranco de Azuaje W Firgas. **M.** *Pomatias* sp. 1, TFMCM, Gran Canaria, Barranco de Azuaje W Firgas. **N.** *Pomatias* sp. 1, TFMCM, Gran Canaria, Barranco de Apacador. **O.** *Pomatias* sp. 2, KG, Gran Canaria, Barranco Hondo. **P.** *Pomatias* sp. 2, KG, Gran Canaria, Puerto de las Nieves. **Q.** *Pomatias* sp. 2, KG, Gran Canaria, Punta de Arena, Quaternary. Scale bar: 1 cm.

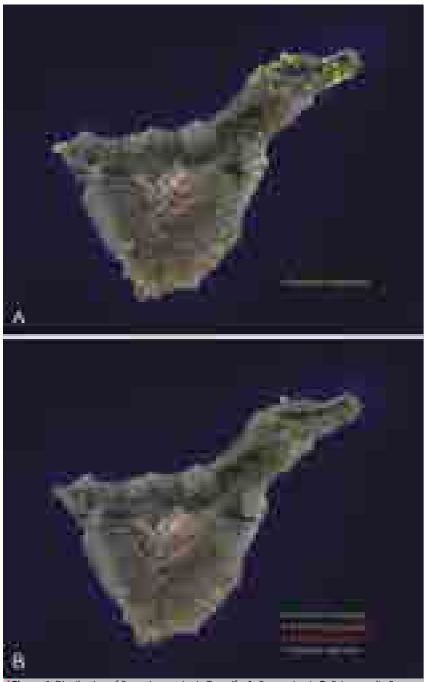


Figure 6. Distribution of *Pomatias* species in Tenerife. **A.** *P. canariensis*. **B.** *P. inaequalis*, *P. praecursor*, *P. raricosta* and *P. adjunctus*.

Figure 7. Distribution of *Pomatias adjunctus, Pomatias* sp. 1 and *Pomatias* sp. 2 in Gran Canaria.

Conclusions

Preliminary investigations of *Pomatias* specimens from Tenerife and Gran Canaria 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 possibly only one 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. laevigatus* 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 and has to be replaced by *P. canariensis*.

Acknowledgements

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Biogeographical approach of Truncatelloidea (Caenogastropoda: Littorinimorpha) in Greece

Nefeli Kassari[1,2], Katerina Vardinoyannis[2], Moysis Mylonas[1,2]

[1]Biology Department, University of Crete, Irakleio, Greece
[2]Invertebrate Department, National History Museum of Crete-University of Crete, Irakleio, Greece
Email: ne.kassari@gmail.com

INTRODUCTION

Truncatelloidea is the richest superfamily of freshwater gastropods, accounting 3210 species belonging to 360 genera and 31 families (MolluscaBase, 2022). They are found in all freshwater habitats in every continent except Antarctica (Strong et al., 2008). According to Radea (2020) more that 70% of the Greek freshwater gastropods belong to this superfamily and most of them are found in a single or just a few sites. Greece is considered a biodiversity hotspot for freshwater fauna, including freshwater gastropods (Strong et al., 2008).

In this study we present all the available data for Greece, we bring out certain taxonomical problems, and analyze their diversity and distribution.

MATERIALS & METHODS

Data were retrieved from 305 papers concerning the freshwater gastropods of Greece from 1832 to 2022. Additionally, we analyzed papers from the Balkans and other Mediterranean countries in order to have an overall view for the distribution of the taxa. More than half of the analyzed papers were published the last 20 years. For each species we recorded, in Microsoft Access database, the exact site it was found, the habitat it was found, the water basin, the geographic region, ecological and taxonomical notes, the Natura 2000 site and their status in the IUCN Red List. The names of the taxa follow the latest taxonomical work or MolluscaBase (2022). Data were analyzed with PAST 4.05 (Hammer et al 2001), while maps were produced in ArcGIS.

RESULTS-DISCUSSION

According to our results, 149 species of Truncatelloidea are present in Greece, belonging to 36 genera and 7 families. It is worth noting that the last 5 years (after the work of Bank & Neubert, 2017 who recorded 6 families, 31 genera and 91 species) **54 species have been added** to the Greek list, most of them being newly described species (e.g Glöer & Hirschfelder, 2019) and few were due to resolved taxonomical problems (e.g. Delicado & Hauffe, 2022). Even though our attempts, 16 taxa remain doubtful as we couldn't assign them to any known species and further research is needed.

The richest family is Hydrobiidae both in genera (27 out of 36) and species (78 out of 149) (**Figure 1**), which is also the case in Bulgaria (Georgiev & Hubenov, 2013), but not for Serbia (Marković et al., 2021). Most species belong to *Bythinella* (35 species) and nearly all of them are endemic (**Figure 1**), which is also the case in Bulgaria (Georgiev & Hubenov, 2013) and Serbia (Marković et al., 2021). Moreover, 11 out of 36 genera are endemic to Greece (**Figure 1**), which is among the highest endemism (30.6%) recorded for a non-marine faunal group in Greece. All but one endemic genera belong to Hydrobiidae (**Figure 1**), and only *Clameia* to Moitessieriidae. Furthermore, 7 non-endemic genera are represented in Greece only by endemic species (**Figure 1**), a case found also in terrestrial Gastropods (Vardinoyannis et al., 2018) and terrestrial Isopods (Sfenthourakis & Schmalfuss, 2018).

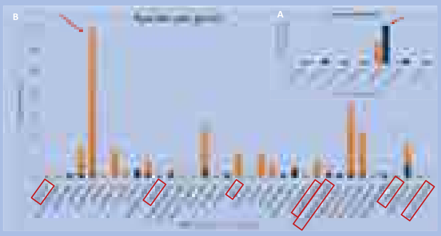

Figure 1. Endemic and non endemic genera per family (A) and endemic and non endemic species per genus (B). Genera in red squares are non-endemic represented only by endemic species. The red arrows mark the most diverse family (A) (Hydrobiidae) and the most diverse genus (B) (*Bythinella*).

Species endemism is the highest recorded in Greece, 86.6%, both among freshwater (25% for freshwater Fish - Leonardos, 2020; 26% for freshwater Decapods - Koutrakis et al., 2020) and among terrestrial animals, (67% Isopods - Sfenthourakis & Schmalfuss, 2018; 59% terrestrial Gastropods - Vardinoyannis et al., 2018). Additionally, most of the endemic species are narrowly endemic, reported from a single or just a few sites, which has been similarly observed in Bulgaria (Georgiev & Hubenov, 2013). The remaining species are distributed and in the nearby countries. High endemicity is the result of the geomorphology, past and present, of Greece, the limited dispersal ability of these organisms and the high isolation of the aquatic ecosystems.

Higher diversity is found in springs (37%) and lakes (18%), while most endemic species inhabit springs (41%) and streams (14%) (**Figure 2**). Also, the ancient oligotrophic lakes (Trichonida, Pamvotida, Megali Prespa, Mikri Prespa) stand out as hotspots of endemism as already argued by Albrecht et al. (2012).

Figure 2. Number of species per ecosystem. Combination symbolizes that a species inhabits more than one ecosystem.

Most species have been recorded from Peloponnese (60 species), certain Aegean islands (43) and Central Greece (41), while Crete and Thrace are the least studied, with 8 and 3 species respectively. The same pattern appears in the Greek water basins, and it is worth mentioning that in 3 catchment areas only one species has been recorded, while 3 more have not been surveyed (**Figure 3**). Interestingly, there is scarce data for big rivers (e.g. Aliakmonas, Strymonas) or lakes (e.g. Kerkini, Kastoria) and in areas higher than 900m of altitude. In the clustering analysis most basins were grouped randomly (**Figure 4**), with the exception of the two Prespa lakes (GR1, GR46), the water basins of Crete (GR39, GR40, GR41) and certain Ionian islands (GR12, GR13, GR34, GR44, GR45). This is in contrast to the division of Greece by Zogaris et al (2009) based on freshwater fish. The random clustering of water basins is due to the small number of species per basin, the many stenoendemic species but mainly to the uneven survey of Greece.

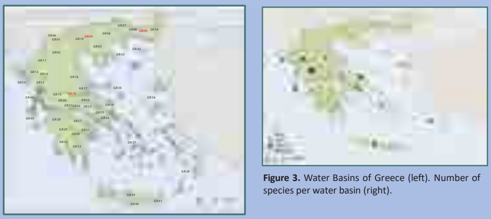

Figure 3. Water Basins of Greece (left). Number of species per water basin (right).

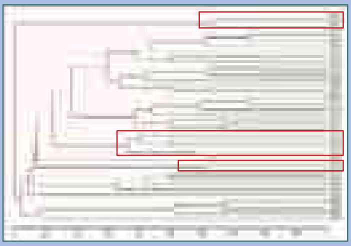

Figure 4. Clustering of data for faunal similarities among water basins using UPGMA and Simpson index.
The three red squares depict the basins that are grouped together (from top to bottom: the water basins of Crete, certain Ionian islands and the two Prespa lakes).

Despite the very high richness and endemism of Truncatelloidea in Greece and even though 50% of species assessed on the IUCN Red List belong to one of the three risk categories (CR, EN, VU) (IUCN, 2022), most of them are not included in the NATURA 2000 protected areas (**Figure 5**), mostly due to the original delimitation of the areas based mainly on terrestrial vertebrates and plants. Additionally, 30% of the assessed species in the IUCN Red List are classified as Data Deficient (DD) (IUCN, 2022) which equals limited knowledge of their ecological data, and even if they are threatened we are unable to organize action plans for their protection.

Figure 5. Distribution of all Truncatelloidea species.

However, the above results may be subject to change and must be carefully taken into consideration. First of all, many species have been described using non-informational characters (e.g. shell characters in Glöer & Reuselaars, 2020), while little work has been done with unraveling molecular relationships, usually using only COI subunit (e.g. Falniowski & Szarowska, 2011). Moreover, the fact that not all Greek regions and catchment areas are equally surveyed, provokes concerns about the richness and endemism patterns. The same applies for diversity in areas above 900 m of altitude, as well as for big lakes and rivers, some of which are not studied at all. More targeted sampling and clarification of the taxonomy through a combined study of morphology and phylogeny are expected to give better insights and provide a better understanding of this group.

CONCLUSIONS

- **Greece is a hotspot of endemism and diversity for Truncatelloidea**
- **Truncatelloidea present highest endemism for a non-marine group in Greece**
- **Springs are hotspots of endemism and richness, while lakes are hotspots of diversity**
- **Hydrobiidae is the most species and genera rich family.**
- **Neither regional units nor catchment areas are equally studied in Greece**
- **Hardly no clear pattern concerning water basins' faunal composition**
- **50% of species assessed on the IUCN Red List are considered CR, EN or VU**

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Exon design for large-scale phylogeny of the Neogastropoda

Thomas Lemarcis[1], Jawad Abdelkrim[2], Alessandro Derzelle[1], Paul Zaharias[1], Yuri I. Kantor[1,3], Alexander E. Fedosov[1,3], Nicolas Puillandre[1]

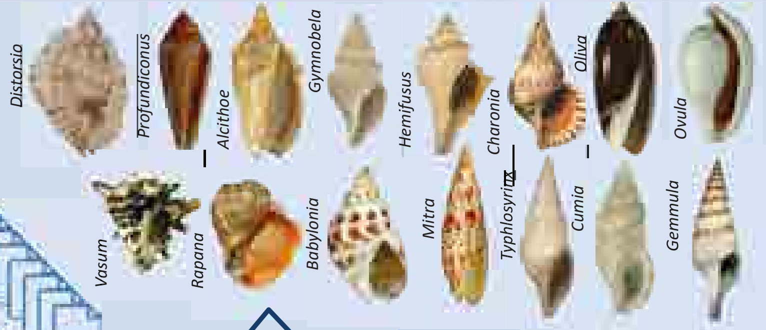
[1]Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France. thomas.lermacis@mnhn.fr
[2]UAR 2700 'Acquisition et Analyse de Données pour l'Histoire naturelle', Service d'Analyse de Données, CNRS,
Muséum National d'Histoire Naturelle, Sorbonne Universités, CP26, 43 rue Cuvier, 75231 PARIS CEDEX 05
[3]A.N. Severtzov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia.

CONTEXT

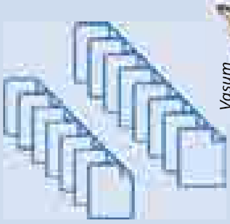
- The published neogastropod phylogenies are mostly unresolved and largely incomplete
- Goal: to produce an, as complete as possible, phylogeny using an exon-capture approach
- However, previously used set of baits are not able to capture all specimens/all exons (lots of missing data), and many nodes remain unresolved (Abdelkrim *et al.* 2018): design of a new set of baits.

Reference:

DATA



METHODS



15 transcriptomes

1 complete annotated proteome


Conus ventricosus

1. BLASTp e-value 10e-10

2. Keeping *Conus* proteins found in :
- Unique BLAST match
- >75% of the transcriptomes
- with <100 transcripts

3. Alignment between *Conus* exons and contigs from transcriptomes

4. Keeping sequences with indels <35% and PID >50%

5. 2nd alignment between *Conus* exons and contigs from transcriptomes

6. Trimming transcript sequences at *Conus* exon boundaries
Removing exons with :
- <4 transcripts aligned
- >5% indels
- GC content <30% or >70%
- genetic diversity <2%

Pipeline Legend
BLAST
Filtering
Alignments

Reconstruction of ancestral sequences

We reconstructed an ancestral sequence for 6 pairs of the most divergent taxa.
1st batch : *Conus* / *Gemmula*, *Conus* / *Mitra* and *Cumia* / *Hemifusus*
2nd batch : *Charonia* / *Babylonia*, *Charonia* / *Alcithoe* and *Charonia* / *Rapana*

1st batch : 2,221 ancestral sequences
2nd batch : 1,896 ancestral sequences

7. Splitting dataset in 2 batches to reduce genetic distance

1st batch:
CONOIDEA
MITROIDEA
BUCCINOIDEA

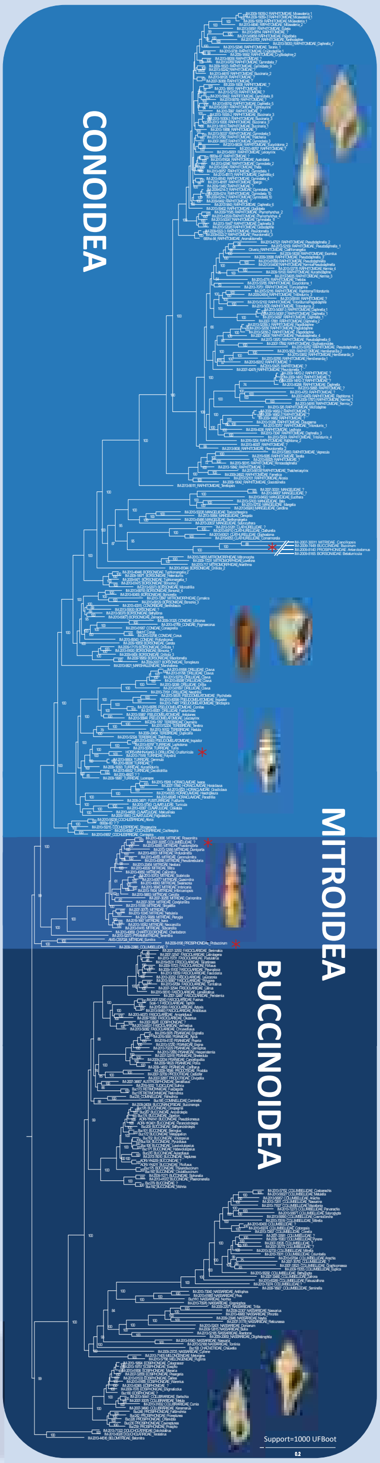
2nd batch:
OTHER NEOGASTROPODA
+ TONNOIDEA/FICOIDEA

RESULTS

FINAL DATASET
1,125 exons
Probe design:
7,208 original sequences + 2,221 ancestral sequences for 1st batch
6,476 original sequences + 1,896 ancestral sequences for 2nd batch

1st batch:
- 384 samples (5 samples removed during assembly (no reads))
- 1,124 exons captured
- An average of 1,006 exons per sample
- An average of 326 samples per exon

Phylogenetic Tree:
- 371 correctly placed samples
- 8 samples with doubtful placement *



This action has received funding from the European Union's Horizon 2020 research and innovation program & European Research Council under grant agreement ERC HYPERDIVERSE (GRANT AGREEMENT 865101)

SENCKENBERG
world of biodiversity

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

Karl-Otto Nagel*, Ronald Janssen, Sigrid Hof, Julia Sigwart Marine Zoology – Malacology, Senckenberg Research Institute, Senckenberganlage 25, 60325 Frankfurt am Main, Germany *Karl-Otto.Nagel@senckenberg.de

1. Collection basics

Freshwater mussels (Unionida) of the Senckenberg Research Institute and Natural History Museum in Frankfurt represent one of the most important international collections. With approximately **20,000 series** and **> 80,000 specimens**, it is among the 10 largest in the world[1]. Its special value is that it formed the basis for the last monograph on this group[2]. It contains about **478 primary type series** (holo-, lecto-, neo- and syntypes) and covers ca. **70 % of the global species diversity of the Unionida**. The focus of the collection is the western Palaearctic and especially Europe. Nevertheless, about 197 primary types originate from other areas, which explains the worldwide importance of the collection. It is a research instrument in great demand internationally and represents a data archive of high scientific value.

Collection inventory (as of 15.3.2022)

Family	N lots*	species		primary type series
		SMF	total[3]	
Margaritiferidae	867	12	15	13
Unionidae	16.866	537†	779	391
Hyriidae	858	70†	92	37
Mycetopodidae	496	41†	53	30
Iridinidae	257	26†	39	4
Etheriidae	69	4	4	3
total	19.413	690	982	478

* dry + alcohol † revision partially or fully pending

2. History

Accessions date back to the early 19th century and comprise among others, the collections of Emil Adolph **Rossmässler** (1806 – 1867), Wilhelm **Kobelt** (1840 – 1916) and Fritz **Haas** (1886 – 1969). More recently, material from Heinrich Georg **Bronn** (1800-1862), Hermann **von Ihering** (1850-1930), Karl Ludwig **Pfeiffer** (1874-1952), Rolf A.M. **Brandt** (1917-1989) and Hartwig **Schütt** (1923-2009) added significantly to the Senckenberg Unionida collection.

Founding fathers of Senckenberg's Unionida collection and some of its most important researchers

Emil Adolph **Rossmässler**

Wilhelm **Kobelt**

Hermann **von Ihering**

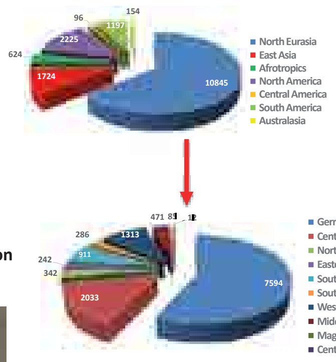
Fritz **Haas** collecting freshwater mussels in the Albufera de Valencia in 1917, supported by local fisherwomen and fishermen

3. What we do

Digitisation of the old collection and recent additions is currently being completed. Material from North Eurasia and North America has already been revised according to current systematics. Revision of the Palearctic material showed a rate of 4-6% of incorrectly determined. This value is likely to be even higher for specimens from other regions. It is therefore essential to look at each individual shell again, even from large series, to avoid erroneous conclusions about the distribution and ecology of species.

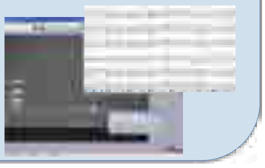
Senckenberg's collection is essential in ongoing revisionary studies of South and Southeast Asian freshwater mussels[4] and for the Unionida fauna from the Mediterranean and Middle East[5,6,7].

Senckenberg Unionida collection lots per region[3]



Dried remains of the mussels' soft body (adductor muscles, mantle tissue) are a potential source of genetic information. We are testing protocols to obtain this information from shells of rare or already extinct species.

Anodonta exulcerata Porro, 1838
Paralectotype (formerly syntype) of a recently redescribed species from Southern Europe.[6]



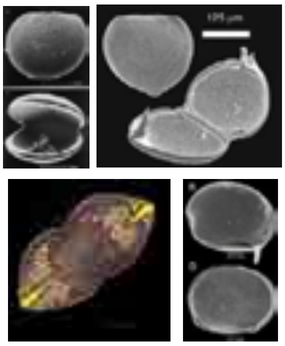
4. What you can expect

We will shortly publish a revised type catalog, supplemented by information on the overall holdings (scope, focal points, incorporated collections, additions and corrections to the collection history). Collection data and type-related information including pictures will be accessible on the internet (e.g. via Senckenberg's databases AQUiLA and SeSam).

Indonaia gratiosa (Philippi, 1843) comb. nov.
Specimen used in a recent revision of the freshwater mussels of the Indian subcontinent.[4]

Margaritana (= *Margaritifera*) *margaritifera parvula* Haas, 1908
Holotype of a genetically distinct ecotype of the freshwater pearl mussel that recently became extinct.

The larval forms of Unionida (glochidium, lasidium) provide important information for the systematics and taxonomy of the group.[8,9,10] So far, these data are usually not available in research museums and collections. In particular, the possibility of a three-dimensional representation based on light or scanning electron microscopic images is to be tested.



Glochidium larvae of (from left corner clockwise)
Pseudunio auricularius (Spengler, 1793)[11]
Velesunio ambiguus (Philippi, 1847)[12]
Solenaia khwaenoiensis Panha & Deein, 2004[13]
(= *Solenaia emarginata* (Lea, 1860))
Sinanodonta woodiana (Lea, 1834)
Image of *S. woodiana* using confocal laser scanning microscopy, a promising technique to visualize details of the larvae's soft body.[14]

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

Timea P. Neusser[1], Adam J. Bourke[2], Katharina M. Jörger[3], Yasunori Kano[4], Michael Schrödl[1,3] & Bastian Brenzinger[3]

[1]Biocenter of the Ludwig-Maximilians-University Munich, Germany.
[2]College of Engineering, Information Technology and the Environment, Charles Darwin University, Australia
[3]SNSB - Bavarian State Collection of Zoology, Munich, Germany.
[4]Atmosphere and Ocean Research Institute, University of Tokyo, Japan
E-mail: neusser@bio.lmu.de

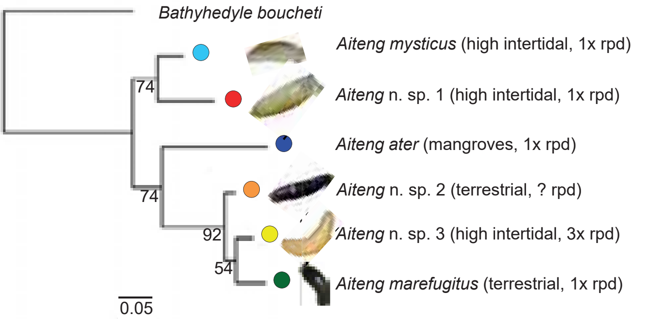
INTRODUCTION

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 (Okinawa) and the terrestrial *A. marefugitus* from Palau. In the present study we give a preliminary overview of the diversity of these nocturnal slugs collected during several sampling events in the Indo-Pacific, including three 'Our planet reviewed' expeditions organized by MNHN Paris.

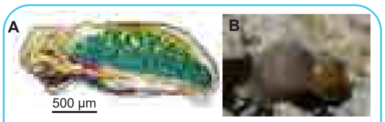
Habitats and living specimens of Aitengidae. *Aiteng mysticus* (5 mm) from Japan (**A**), Vanuatu (4-5 mm) (**B**), New Caledonia (**C**). *Aiteng* n. sp. 1 (10 mm) from Papua New Guinea (**D**) - A similar species was documented from the Philippines[1]. *Aiteng* n. sp. 2 from Yap Island (**E**). *Aiteng* n. sp. 3 (10 mm) from northeastern New Caledonia (**F**). *Aiteng marefugitus* (5 mm) from Palau Island. Specimen found under a calcareous stone together with an ant nest with eggs and pupae (**G**). *Aiteng* cf. *ater* (4 mm) from Australia (**H**). *Aiteng* cf. *ater* (6 mm) from southeastern New Caledonia (**I**). H and I not included in COI phylogeny.

Credit: Esri, USGS | Esri, HERE, Garmin, FAO, NOAA, USGS | Earthstar Geographics

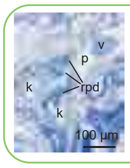
Distribution of Aitengidae. ● Australia; ● Japan; ● ● ◍ New Caledonia; ● ⊕ Papua New Guinea; ● Palau Island; ● Philippines; ● Thailand; ⊗ Vanuatu; ● Yap Island. Type localities without stripes.



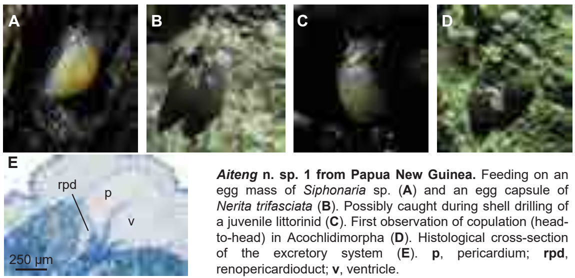
Phylogeny of Aitengidae based on COI sequences and informations on the habitat and number of rpd. Tree reconstruction was made in RAxML with GTR+G model, no partitioning to codons and 500 replicates for Felsenstein's bootstrap support values. **rpd**, renopericardioduct.



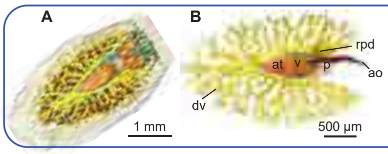
***Aiteng mysticus* (Japan).** 3D-reconstruction (left view) (**A**). Possibly caught during shell drilling of an assimineid snail (**B**).



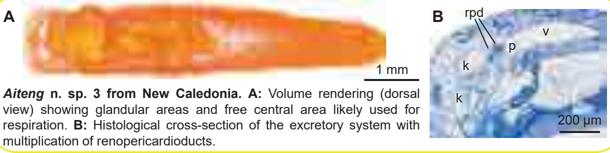
***Aiteng* n. sp. from Australia.** Histological cross-section of the excretory system. **k**, kidney; **p**, pericardium; **rpd**, renopericardioduct; **v**, ventricle.



***Aiteng* n. sp. 1 from Papua New Guinea.** Feeding on an egg mass of *Siphonaria* sp. (**A**) and an egg capsule of *Nerita trifasciata* (**B**). Possibly caught during shell drilling of a juvenile littorinid (**C**). First observation of copulation (head-to-head) in Acochlidimorpha (**D**). Histological cross-section of the excretory system (**E**). **p**, pericardium; **rpd**, renopericardioduct; **v**, ventricle.



***Aiteng ater* from Thailand.** 3D-reconstructions of the animal (dorsal view) (**A**) and the excretory and circulatory systems (ventral view) (**B**). **ao**, aorta; **at**, atrium; **dv**, dorsal vessel; **p**, pericardium; **rpd**, renopericardioduct; **v**, ventricle.



***Aiteng* n. sp. 3 from New Caledonia. A:** Volume rendering (dorsal view) showing glandular areas and free central area likely used for respiration. **B:** Histological cross-section of the excretory system with multiplication of renopericardioducts.

***Aiteng marefugitus* from Palau Island.** 3D-reconstructions of the animal (right view) (**A**) and the excretory and circulatory systems (ventral view) (**B**). Histological cross-section of the digestive gland with food residue (arrow) of cuticular appearance (**C**). **ao**, aorta; **dv**, dorsal vessel; **h**, heart; **k**, kidney; **nd**, nephroduct; **p**, pericardium; **rpd**, renopericardioduct.

CONCLUSIONS

- Aitengidae are now known from coastlines of 9 countries in the tropical Indo-West Pacific, with up to five new species which await formal description.
- Aitengidae are either insectivorous (larvae or pupae), ovivorous (like other freshwater Acochlidimorpha) feeding on snail egg masses or, to be confirmed, feeding on other molluscs by shell drilling.
- Multiplication of renopericardioducts are known from limnic *Acochlidium*; the convergent multiplication in *Aiteng* can be an adaptation to humid (semi-) terrestrial habitats[2,3].
- Sperm transfer occurs via copulation ('head-to-head').

ACKNOWLEDGEMENTS

We thank Ellen Strong, Nico Puillandre and Yuri Kantor for organizing the symposium „Exploration, biodiversity and systematics of molluscs – a symposium in honor of Philippe Bouchet“ at the WCM 2022 in Munich. Sincere thanks are given to Philippe Bouchet for the generous opportunity to join the 'Our planet reviewed' expeditions and to work on these fantastic slugs. Valentin Dutertre, Hiroaki Fukumori, Takuma Haga, Tsuyoshi Takano and Jocelyn Senia helped in field work and collection of specimens. Isabella Stöger and Robert Fernandez Vilert helped in the molecular lab. Heidi Gensler kindly prepared the histological sections of Aiteng n. sp. 3 from New Caledonia. 3D-reconstruction was supported by the GeoBioCenter/LMU. The Leibniz Supercomputing Centre/Munich provided access to the remote visualization. Studies on Aitengidae were financed by grants from the German Research Foundation (DFG SCHR 667 to MS, DFG BR5727/1-1 to BB) and partly funded by the 'Award for equal opportunities for women in research' granted by the LMU (to TPN).

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Pellasimnia brunneiterma (C. N. Cate, 1969)

A MOLECULAR APPROACH TO THE PHYLOGENY AND TROPHIC SPECIALIZATION IN OVULIDAE (GASTROPODA: CYPRAEOIDEA)

E. Nocella[1-2], S.S. Zvonareva[3], G. Fassio[1], L. Leotta[1], S. Schiaparelli[4], M.V. Modica[2], M. Oliverio[1]

[1]Department of Biology and Biotechnologies "Charles Darwin", Sapienza University, Rome, Italy
[2]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy
[3]Laboratory of Ecology and Morphology of Marine Invertebrates, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia
[4]Department for the Study of Territory and its Resources, University of Genoa, Genoa, Italy

BACKGROUND

- Cnidarians are consumed as food by multiple gastropod lineages.
- Ovulidae feed on Anthozoa (Octocorallia and Hexacorallia) and on Hydrozoa (Stylasteridae).
- Over 260 currently Ovulidae accepted species.
- Predominantly tropical family, with only few species inhabiting temperate waters.
- A broad mantle covers the shells and, in most cases, camouflages the ovulids on the host coral.
- Pediculariines are sometimes regarded as a distinct family.
- Phylogenies and host specificity information are mostly available for shallow water species.


Prosimnia semperi on *Melithaea* sp., Papua New Guinea, 13 m depth.
Photo: Laurent Charles


Pedicularia sp. on *Stylaster* sp., Indonesia, 15 m depth.
Photo: Daniela Pica

AIM 1

Reconstructing the evolutionary history of the Ovulidae to identify major lineages and depict a phylogenetic framework to study ovulid

METHODOLOGY

Phylogenetic analyses were performed using Maximum Likelihood and Bayesian approach, on a dataset including sequences from one nuclear (28S rDNA) and two mitochondrial (COI and 16S rDNA) molecular markers. Sequences from a total of 342 specimens were analyzed (150 of which obtained in this study) representing all the currently recognized subfamilies of Ovulidae

Our dataset included sequences from 342 specimens collected in tropical and subtropical areas

AIM 2

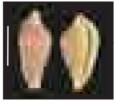
Reconstructing trophic ecology of the Ovulidae at the genus level of the coral host

METHODOLOGY

Literature data were integrated with an empirical approach: host corals collected with ovulids samples were identified either morphologically or genetically, applying a species delimitation approach on the sequences of the 16S rDNA molecular marker. Sequences from a total of 54 coral samples were analyzed

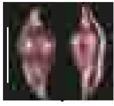
RESULTS

Dentiovula doruosa
(Hinds, 1844)

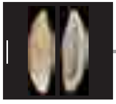


Contrasimnia xanthochila
(Kuroda, 1928)

Dentiovula eizoi
(C. N. Cate & Azuma, 1973)

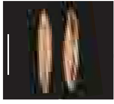


Naviculavolva deflexa
(G. B. Sowerby II, 1849)



Prosimnia semperi
(Weinkauff, 1881)

Aclyvolva coarcata
(G. B. Sowerby II, 1849)



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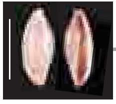
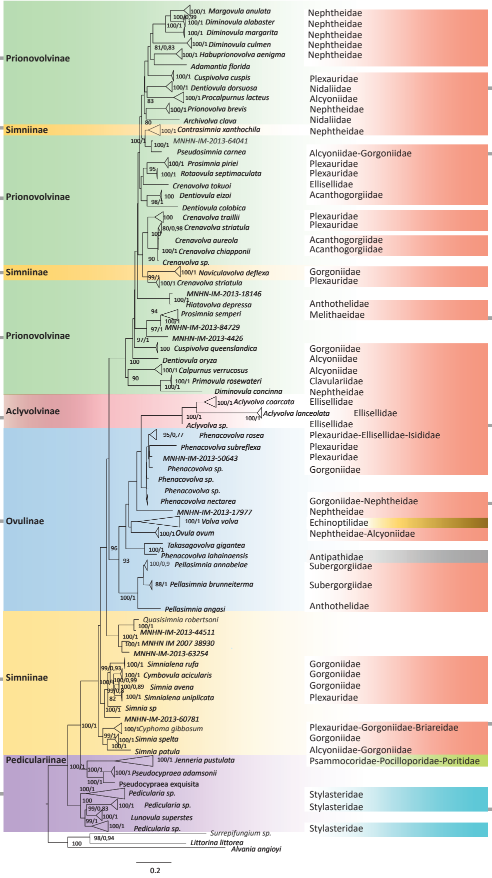
Volva volva
(Linnaeus, 1758)

Simnialena uniplicata
(G. B. Sowerby II, 1849)



Pedicularia sp.
(Swainson 1840)

Scale bar length: 6 mm



Dentiovula dorsuosa on *Siphonogorgia* sp., Papua New Guinea, 15 m depth


Photo: Laurent Charles

Pseudosimnia carnea on *Alcyonium* sp., Corse, France, 15 m depth


Photo: Gilles Devauchelle

Prosimnia semperi on *Melithaea* sp., Papua New Guinea, 15 m depth


Photo: Laurent Charles

Aclyvolva sp. on Ellisellidae, Papua New Guinea, 15 m depth


Photo: Laurent Charles

Phenacovolva nectarea on Nephtheidae, Papua New Guinea, 13 m depth


Photo: Laurent Charles

Cyphoma gibbosum on *Pseudoplexaura* sp., Curaçao, 9 m depth


© Reijnen and Van der Meij 2016

Pedicularia sp. on *Distichopora* sp., Indonesia, 5 m depth

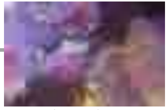


Photo: Daniela Pica

ASSOCIATED CORAL

🟥 Alcyonacea
🟫 Pennatulacea
⬜ Antipatharia
🟩 Scleractinia
🟦 Anthoathecata

Maximum likelihood phylogenetic reconstruction of Ovulidae based on COI, 16S and 28S molecular markers, obtained for 342 specimens.
Numbers at nodes correspond to ultrafast bootstrap support for ML, and posterior probability for BI, respectively (only UfB ≥80 and PP ≥0.8 are reported)

CONCLUSION…

- Subfamilies as currently recognized are not monophyletic.
- Pediculariines, whatever their rank, do not include *Jenneria* and *Pseudocypraea,* which may represent a distinct lineage.
- Most of Ovulidae species are associated with Alcyonacea (Octocorallia), only two species are associated with Hexacorallia and the single *Pedicularia* genus feeds on Stylasteridae.

…AND FUTURE PERSPECTIVES

- Improving phylogenetic analysis by increasing number of outgroups.
- Reconstructing Ovulidae ancestral trophic ecology.

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SEM photo of a *Cadulus sp.* specimen

Studies on the small-sized scaphopod *Cadulus thielei* Plate, 1909 in the Southern Ocean: A not so rare species and assessing morphometric diversity

Lucy Stephenson[1], Huw Griffiths[1], Katrin Linse[1]
[1]British Antarctic Survey, High Cross, Madingley Road, Cambridge, England, CB3 OET, UK
lustep@bas.ac.uk

Background

- Southern Ocean contains highly endemic species and is globally important in understanding how biodiversity responds to environmental change, as Antarctica's unique oceanographic and climate history lead to species evolution and diversification happening in isolation.
- Scaphopoda are a low diversity class (around 600 listed worldwide and 13 in the S Ocean).
- Previously only 5 specimens of the scaphopod species *Cadulus thielei* (Plate, 1909) were reported in the literature, - its' type locality in E Antarctica and 3 sites in the Bellingshausen and Ross seas from 157-438m depth.
- High local abundances were found in epibenthic sledge samples on cruises in the Southern Ocean[1].

Objectives

① Comprehensive assessment to review bathymetric distribution and range of specimens collected.

② To conduct shell morphometric analysis and assess whether there is morphometric diversity between different geographic locations.

Methods

- Epibenthic sledge samples collected on cruises in the in the Southern Ocean have yielded 11782 new specimens, morphologically assigned to *Cadulus thielei*.

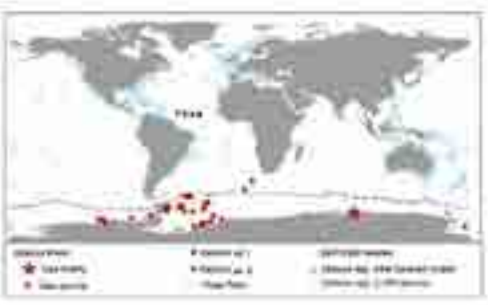

Figure 1: Map of samples used in the study, currently morphologically assigned to *Cadulus thielei*.

- Images taken of scaphopod shells using a Stemi SV 6 stereomircroscope and camera attachment

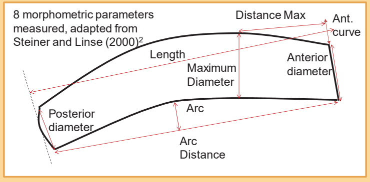

Image analysis to measure morphometrics conducted on **360** specimens using **ImageJ 1.53k** software.

- Additionally, volume was calculated using the following equation, using the volume of two cylinders:

$$\boldsymbol{Volume} = \pi\, DistanceMax\, \frac{\left(\frac{Maximum\ Diameter\ +\ Anterior\ Diameter}{2}\right)^2}{2} + \pi(Length - DistanceMax)\, \frac{\left(\frac{Maximum\ Diameter + Posterior\ Diameter}{2}\right)^2}{2}$$

- Only adult specimens (those whose anterior diameter was less than their maximum diameter) were included in analyses.
- R Studio 7.1 was used to carry out regression analysis and principal components analysis.

Results

- The vertical and spatial range of *Cadulus thielei* is extended by these new records, confirming it to be **a circum-Antarctic species.**
- It has been found in Southern Ocean samples, 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**). They were collected in **161-5737m** depth.

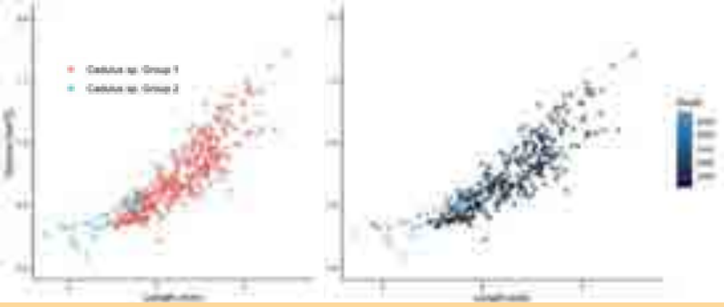

Figure 2: A graph showing a relationship between length and volume of *Cadulus sp.*. Sp. Group 2 overall smaller and this relationship seems to correspond to depth.

- Linear models showed a negative association between depth and volume ($F_{1,379}$=46.28, R^2=0.79, p=0.004091).

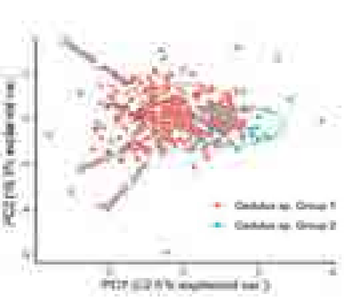

Figure 3: Canonical variate analyses based on the principal components (PC1 and PC2) of the morphological variation recorded among the 2 species groups based on location. **Groups not entirely separated along either PC axes.** 90% mean confidence ellipses.

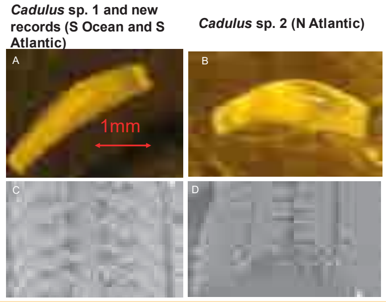

Figure 4: Light images (A and B) and SEM photos of radulae (C and D) of *Cadulus* specimens from the two groups, **showing some morphological diversity.**

Conclusions and Ongoing Studies

- The vertical and spatial range of *Cadulus thielei* is extended by these new records, confirming it to be **a circum-Antarctic species.**
- **Negative association of depth with shell size**, related to decreasing resource availability with depth. *Cadulus* sp. *2* were from shallower locations in the N Atlantic vs deeper locations in the S Ocean for *Cadulus* sp. 1.
- A priori hypotheses that specimens from *Cadulus sp.* 1 and 2 would be morphologically distinct **were not supported by PCA analyses or regression**. However, morphological distinctions observed in radulae teeth and stereomicrosope photos.
- **Ongoing COI barcoding is being used to investigate whether genetic diversity exists between the specimens.** There is currently a scarcity in the literature of molecular studies on Scaphopoda.
- Ongoing studies into how environmental conditions have played a role in the diversity within this group using GBIF/OBIS data.

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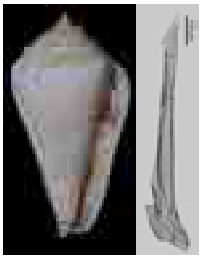
Deep water cone snail venomics: protein diversity in the venom of *Profundiconus* species from New Caledonia (Gastropoda, Conoidea)

Manuel Jiménez Tenorio[1], Juan Carlos G. Galindo[2], Rafael Zardoya[3] and Nicolas Puillandre[4]

[1] Departamento de CMIM y Química Inorgánica-INBIO, Facultad de Ciencias, Universidad de Cádiz, Puerto Real, Cádiz, SPAIN. E-mail: manuel.tenorio@uca.es
[2] Departamento de Química Orgánica-INBIO, Facultad de Ciencias, Universidad de Cádiz, Puerto Real, Cádiz, SPAIN.
[3] Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, SPAIN
[4] Muséum National d'Histoire Naturelle, Paris, FRANCE

Conotoxins are bioactive peptide molecules present in the venom secreted by cone snails (Gastropoda, Conidae). In this group, the species within genus *Profundiconus* are characterized by their deep-water habitat (usually between 100 and 1000 m). A recent study (Fassio et al. 2019) have disclosed for the first time the transcriptome of the venom ducts of two species of *Profundiconus*, namely *P. neocaledonicus* Tenorio & Castelin, 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**. We have now analysed the proteome of the venom duct extracts of these species.

Profundiconus neocaledonicus
Tenorio & Castelin, 2016



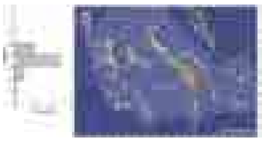
Specimen MNHN-IM2013-68152
SW Ile des Pins, stn DW4703, 22º46'10.8012''S, 167º19'18.0156'', 348 – 350 m
Expedition KANACONO, 2016-08-12
S_L 69 mm. Radular tooth shown.

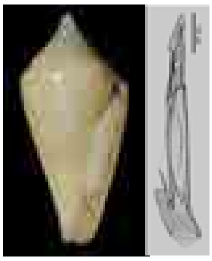
Species distribution and bathymetric range

Profundiconus vaubani
(Röckel & Moolenbeek, 1995)

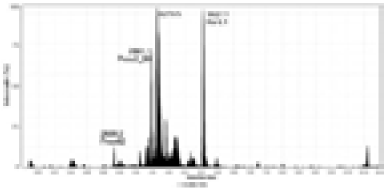
Specimen MNHN-IM2013-66005
SW Ile des Pins, stn DW4666, 22º53'7.8''S, 167º16'53.9832'', 530 – 545 m
Expedition KANACONO, 2016-08-12
S_L 22 mm. Radular tooth shown.

Species distribution and bathymetric range

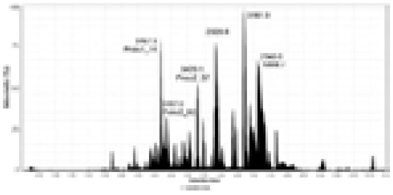




LC/MS ANALYSIS OF VENOM DUCT EXTRACTS

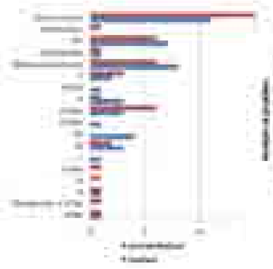


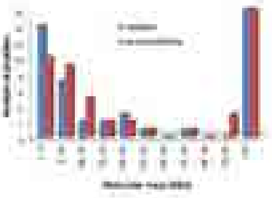
UHPLC/MS analysis of the extracts of the venom ducts of *P. neocaledonicus* (left) and *P. vaubani* (right) yielded around 26 and 55 monoisotopic mass peaks respectively, corresponding to peptides in the mass range 1000-7000 Da. Tandem LC-MS/MS analysis of the trypsin-digested extracts identified 181 proteins in 48 groups for *P. neocaledonicus*, and 170 proteins in 42 groups for *P. vaubani*. 20 top proteins were found in both species. The main components of the venom are venom proteins with molecular mass greater than 10000 Da. Conotoxins represent 41-45 %.



P. neocaledonicus ← *Venom composition and distribution in superfamilies of conotoxin precursors* → ***P. vaubani***

The molecular mass distributions are similar for both species. There is an overexpression of conotoxins of the **O3-like** superfamily in *P. neocale*donicus, and of the **O1** and **O2-like** superfamilies in of *P. vaubani*. **Profunditoxin Pvau1_23-PFC01** was identified in the latter, whereas conotoxin **Ne6.1** of the **A-like** superfamily was present in both samples .





MOLECULAR MODELING WITH ALPHAFOLD2

Superposition of the structures of conotoxin **Neo6.1** (A-like) (brown) and toxin **OmTx3** from the scorpion *Opisthacanthus madagascariensis* (blue), a member of the K⁺ channel inhibitor kappa-KTx family.

Superposition of the structures of conotoxin **Pvau1_23-PFC01** (brown) and conotoxin Cal14.7 from *Californiconus californicus* (blue).

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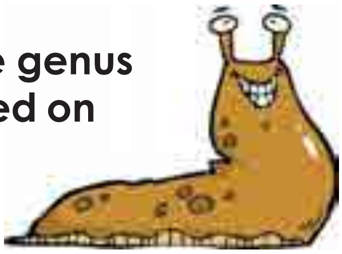


Integrative species delimitation within the genus *Milax* (Gastropoda: Eupulmonata) based on morphology and DNA barcoding

Kamila S. Zając[1], Paulina Gąsienica[2], Dorota Lachowska-Cierlik[3]

[1] - Nature Education Centre, Jagiellonian University, Kraków, Poland; [2] - Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; [3] - Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland

INTRODUCTION

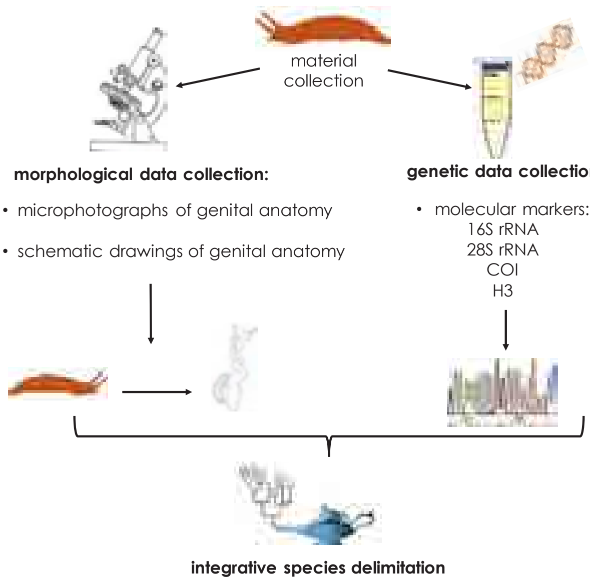
Milacidae is one of the least known families of terrestrial gastropods grouped into two genera: *Milax* Gray, 1855, and *Tandonia* Lessona & Pollonera, 1882. Some of them are widely distributed, whereas others occur only locally. Some species have economic significance and are therefore important for 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.

Fig. 1. Slug collection in MNHW.

In Poland, in the Museum of Natural History in Wrocław (MNHW) there is the richest collection of slugs, thanks to the hard and excellent work of prof. Andrzej Wiktor – a malacologist, a world class specialist in the field of gastropods taxonomy. Prof. Andrzej Wiktor described many species new to science during his carrier. In total he described about 60 new species for science.

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. 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.

METHODOLOGY



material collection

morphological data collection:

- microphotographs of genital anatomy
- schematic drawings of genital anatomy

genetic data collection:

- molecular markers:
  16S rRNA
  28S rRNA
  COI
  H3

integrative species delimitation

Fig. 2. Schematic overview of the workflow.

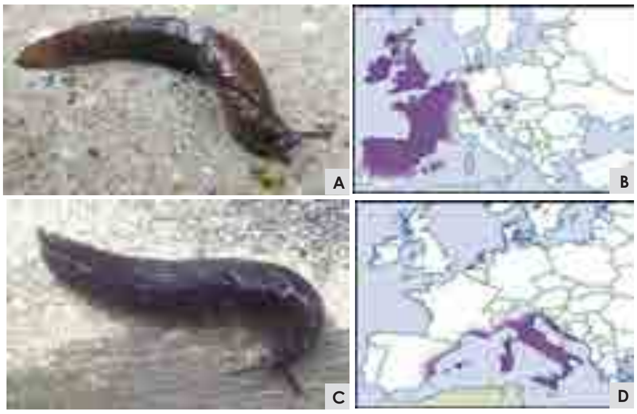
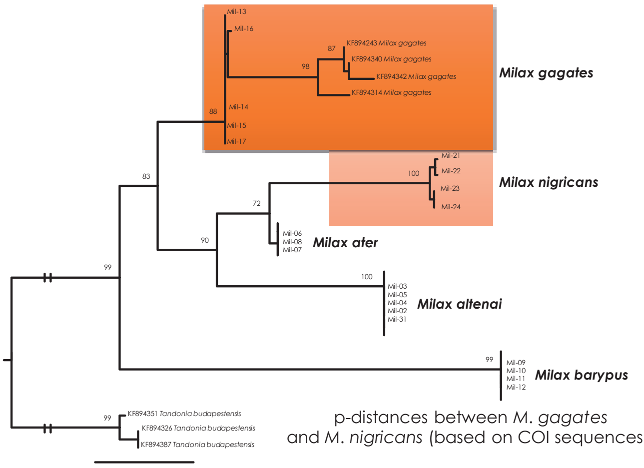


Fig. 3. External appearance and distribution of *M. gagates* (A–B) and *M. nigricans* (C–D) (source: Wikipedia; Animalbase).

RESULTS

Fig. 4. Schematic drawings of genitalia of *M. gagates* (A) and *M. nigricans* (B) after Wiktor (1987). Scale bars = 1 mm.



p-distances between *M. gagates* and *M. nigricans* (based on COI sequences):

from **5.35%** to **7.49%** (6.95% on average)

Fig. 5. Maximum-Likelihood phylogeny of *Milax* species based on multiple molecular markers.

CONCLUSIONS

- 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.

ACKNOWLEDGEMENTS

We would like to thank Jolanta Jurkowska and the Museum of Natural History, Wrocław University (Poland) for making the collection of Prof. Andrzej Wiktor available for this study. This work was supported by a grant from the Jagiellonian University (DS/D/WB/INoŚ/8/2019).

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

Julia C. Friedel & Michael R.W. Amler

University of Cologne, Institute of Geology and Mineralogy, Zülpicher Str. 49a, 50674 Cologne, Germany, julia.friedel@uni-koeln.de, michael.amler@uni-koeln.de

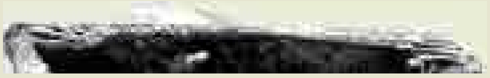
Introduction

The Zechstein Basin was an epicontinental sea on the Central European mainland in the Late Permian (Lopingian; 258-250 Mya) subdivided into the Northern Permian Basin (NPB) and the Southern Permian Basin (SPB). It stretched from present-day England to the Baltic, from southwestern Germany to the North Sea. Due to arid climate and limited seawater influx, hypersaline conditions with evaporites dominated. Fossils are mostly known from the dolomitic reef complexes of the lower Werra Formation (Z1 Cycle), lower Zechstein[1, 2]. It is assumed that the Zechstein Basin was flooded mainly from a northerly direction, from the Arctic Sea via the Central and Viking rifts, but water influx from the Tethys into the Polish Subbasin is also recorded.

Late Permian arcoid bivalves had a distribution in fully marine environments and immigrated into the Zechstein Basin with the first ingression of seawater. Therefore, the conditions were fully marine and successively became higher saline during the Werra Cycle (Z1), until finally unacceptable environmental conditions prevailed. Later, during the Staßfurt (Z2) and Leine (Z3) cycles, different species re-immigrated from the Arctic Sea into the Central European Basin, but arcoids are only known from the first marine ingression.

Arcoid Morphology

Arcoidea, a superfamily of bivalves, has an elongated, rounded rectangular shape with variable ornamentation. Systematically important is the long hinge plate with two groups of dentition and the organic duplivincular ligament. Within the superfamily Arcoidea, the Parallelodontidae is the only family that occurred in the Permian.



left and above: *Parallelodon meridionalis* (De Koninck, 1885), Carboniferous, in lateral view (above) and hinge line with dentition (left). Length: 3 cm.

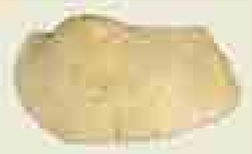
Ecology

Due to their numerous life habits, bivalves are good indicators for the reconstructing past ecological conditions. From Recent arcoids it is known that this group can produce a byssus, i.e. organic filaments for attachment to rocks or shells, a structure better known in mussels[3].

With a planktic larval stage, a widespread distribution in the whole basin was possible and the adaptation to slightly increasing hypersaline conditions restricted the bivalves (and other biota) of the Zechstein Basin to an endemic fauna.

Zechstein Basin

Until now, *Parallelodon striatus*, the only arcoid species of the Zechstein Basin, is only known from the SPB. Sedimentary rocks occur abundantly in many outcrops in Central Germany, Poland and East England. However, the NPB is poorly accessible and only known from boreholes. Due to the marine connection to the Arctic Sea, it seems very likely that *P. striatus* also occurred in the NPB, but as yet occurrences of *P. striatus* are not recorded.



above: *Parallelodon striatus* (Schlotheim, 1816)[4] (in lateral view; length 10 mm) has compact and rounded shells with dominating radial ribs. The taxon is well documented based on ample material from many outcrops along the southern margin of the basin.

above: Overview of the Permian Zechstein Basin in Central Europe[1]. Stars indicate arcoid occurrences. Rose: deeper water (sedimentation of salt), light blue: shelf and platform (sedimentation of carbonate & anhydrite), brown: continental sediments, blue: open marine environment.

In the Permian, many parallelodontids were described from the Ural strait and the northern Tethys. With the closing of the Ural during the Permian and the warm climate, the Uralian species also migrated to the Arctic Sea. One of which, *Parallelodon suzukii* occurring in Spitsbergen is assumed link and ancestor of *P. striatus*. *P. suzukii* shows great morphological similarity to *P. striatus* and lived in the Central and Viking rifts, the direct connection to the Zechstein Basin.

below: *Parallelodon suzukii* (Nakazawa, 1999)[5] (in lateral view; length 41 mm) has a similar, although more elongated shape than *P. striatus* and shows more numerous radial ribs.

Conclusions

- Arcoids are found in the Zechstein Basin only within the Werra Formation (Z1), unlike other bivalves and further biota which also occur in the following formations.
- Each of the Zechstein formations represents a marine ingression into the Zechstein Basin through the northern Central and Viking rifts, with the organisms entering the basin accordingly.
- Climatic conditions caused increasing salinity and evaporation, which lead to the repeated extinction of organisms during each cycle.
- Due to matching morphology of *P. striatus* to the Permian species from Spitsbergen (*P. suzukii*), at that time located at the northern Central Rift, and more distant morphological similarity to Uralian species an immigration from the Ural across the Arctic Sea into the NPB is assumed. This assumption matches well with observations from other biota.
- Connection to the Tethys via the Polish Subbasin is also mentioned by authors. Therefore, it cannot be completely excluded that Uralian and northern Tethyan species could have immigrated into the SPB through a possible Tornquist-Teisseyre corridor, even if this is not supported by bivalves[6].

World Congress of Malacology – August 2022

Acknowledgements

We would like to thank F. Hrouda, Museum für Naturkunde Gera, Dr. E. Mönnig, Naturkunde-Museum Coburg and Dr. S. Brandt for providing well-preserved Permian material.

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

Ruiqi Li[1*], Carlos Leiva[2], Sarah Lemer[2], Jingchun Li[1,3]
[1]Ecology & Evolutionary Biology, University of Colorado Boulder, Boulder, USA
[2]Marine Laboratory, University of Guam, Guam, USA
[3]Museum of Natural History, University of Colorado Boulder, Boulder, USA

Overview

Giant clams (subfamily Tridacninae) are the largest extant bivalves. Besides filter feeding like most bivalves, giant clams form mutualistic associations with dinoflagellates of the family Symbiodiniaceae. They play important roles in coral reef ecosystems, such as serving as reef builders and providing shelters and food. In this project, we present the first chromosomal-level whole genome assembly of a giant clam individual. With HiFi PacBio sequencing (60X) and Hi-C (30X) chromatine conformation capture, we sequenced and assembled the whole genome of the small giant clam *Tridacana maxima*, which is not only the first chromosomal-level genome of giant clams, but also the first chromosomal-level genome in the family Cardiidae.

Figure 1. Photo of the the small giant clam *Tridacna maxima*. Note that the colors of mantles can vary from yellow to blue. Photo from Wikipedia

Genome Statistics

Table 1. Whole genome statistics. Data summarized from genome assembly report from Dovetail Genomics, QUAST (Quality Assessment Tool for Genome Assemblies) results, and BUSCO results.

Sequencing Company	**Dovetail Genomics**
Sequencing Methods	PacBio +Hi-C
Sequencing Depth	60X + 30X
Contigs	13908
Genome Size	1.32 Gbp
N50	64 Mbp
L50	9
Number of Chromosomes	18 (1.14 Gbp, 86%)
Missing Bases	0.12%
Total Number of Genes	31,746
GC content	36.35%
K-mer-based completeness	99.86%
BUSCO completeness (eukaryota)	84%

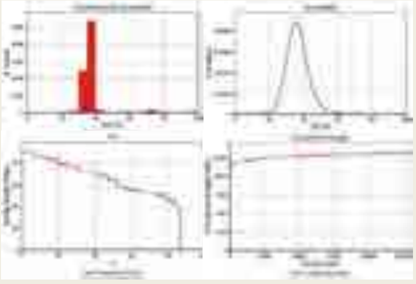


Figure 2. Genome QC results showing contig GC contents and contig lengths, summarized from QUAST output.

Karyotype Representation

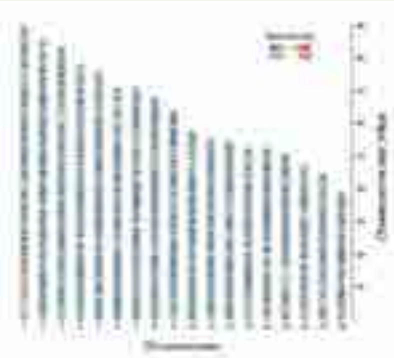


Figure 3. Karyotype representation of The *T. maxima* genome with gene density (250 Kbp windows). The total size of 18 chromosomes is 1.14 Gbp, which is 86% of the whole assembly.

PSMC

Figure 5. Pairwise Sequentially Markovian Coalescent (PSMC) results using 10 years per generation and a mutation rate of 0.1x10-8 (Liu et al., 2020). Time is shown in million years ago, more recent on the left. Effective population size with 100 bootstraps is represented in red, number of global reef sites is shown in green (adapted from Kiessling, 2009).

Phylogenetic position

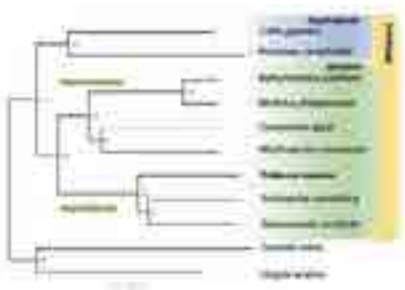


Figure 4. Phylogenetic position of *Tridacna maxima*. The maximum likelihood tree was constructed from 629 single copy orthogroups which have at least 73% (8/11 species) representation (bootstrap=1000). An annelid genome and a brachiopod genome were used as outgroups. Q.yeast+F+R4 was used as the substitution model.

Comparative Analyses

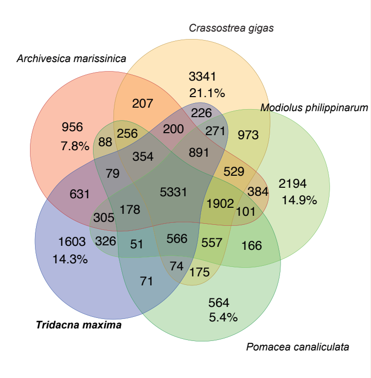


Figure 6. Comparative analyses of 4 bivalve and 1 gastropod genomes. The Venn diagram shows the unique and shared gene families among the 5 genomes. Results are summarized from the OrthoFinder results.

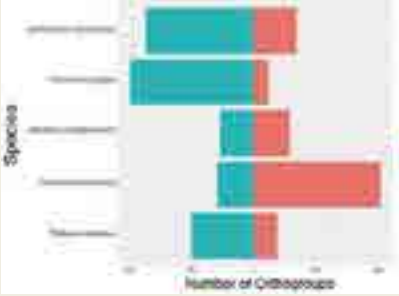


Figure 7. Number of gene families that are expanded (red) and extraction (green) of 5 Mollusk genomes. Results were adapted from CAFÉ (Computational Analysis of gene Family Evolution) results.

Table 2.1 Functions of selected gene families that are expanded in the *Tridacna maxima* genome. Results were summarized from CAFÉ output and eggnog mapper annotations. Bold: possible symbiosis-related genes; *: possible growth regulation related genes.

Function
Ammonium Transporter Family
Reverse transcriptase*
Ribonuclease H protein
regulation of transcription*
chromatin organization*
steroid hormone mediated signaling pathway*
mannose metabolic process
collagen*
positive regulation of TOR signaling
DDE superfamily endonuclease
proton channel activity

Table 2.2 Functions of selected gene families that are expanded in both *Tridacna maxima* and *Archivesica marissinica* (deep sea symbiotic bivalve) genomes.

Function
Reverse transcriptase*
Ribonuclease H protein
proton channel activity
DNA polymerase type B*
DDE superfamily endonuclease

Conclusion

- Global population size shifts of small giant clam correspond to the expansion and decline of modern coral reefs.
- The high-quality chromosome assembly of the small giant clam (*Tridacna maxima*) provide an opportunity to reveal the genomic basis and evolution of bivalve-Symbiodiniaceae symbiosis.
- The regulation of symbiosis may involve expanded gene families related to nutrient transportation (e.g., ammonium transporter), transcription regulation, and growth regulation.

Email: Ruiqi.Li@Colorado.edu

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

Danae Thivaiou[1], Mathias Harzhauser[2], Efterpi Koskeridou[1]
[1] National and Kapodistrian University of Athens, Zografou University Campus, Greece
[2] Natural History Museum Vienna, Austria

Terebralia is a genus present in **Europe** from the Eocene to the Miocene. Its robust shells have a good preservation potential and are often found fossilised in large numbers.

We analyse the taxonomic composition of the gastropod genus *Terebralia* (Potamididae: Caenogastropoda) of the European fossil record. Studied throughout the 19th century, its fossil representatives are present in many marine and brackish environments of the European realm. Molecular studies of the last decade have shed light on the phylogenetic relationships of Potamididae. Here, we aim at the clarification of the fossil taxa of *Terebralia* in Europe, where many species and subspecies have been defined.

(2) How many species in Europe?

Nine fossil species in Europe.
Eight taxa excluded from the genus.
Two possilbe middle-Eocene species.

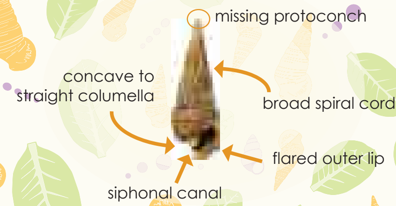
No more than three species co-occur in the fossil and recent records.

Occurrences of fossil *Terebralia* in the European realm.

(1) What does it take to be a *Terebralia*?

Redescription of the genus: medium-sized shells, direct larval development, larval shell bulbous with two keels for the type species *Terebralia palustris*, teleoconch comprises broad spiral cords separated by narrow interspaces and densely-spaced axial ribs; in some species beads can form in intersections of spiral cords and ribs; varices often form in all the length of the teleoconch. The last whorl has a convex base and inflated lip with denticles. A siphonal canal is present, which accommodates the olfactory organs.

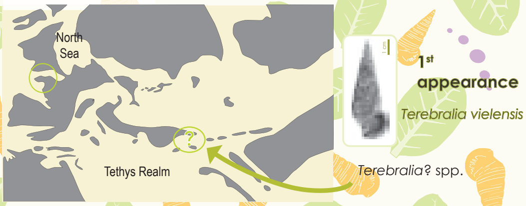
Protoconches are rarely preserved - even for living specimens - because of the high-energy environments in which the species live.



(3) Where and when to find them?

Eocene	NW France (certain) Turkey (uncertain)
Oligocene	NE Atlantic, Western Tethys, Paratethys, Tethyan Seaway
Miocene	NE Atlantic, Paratethys, Proto-Mediterranean

MIDDLE EOCENE



EARLY MIOCENE

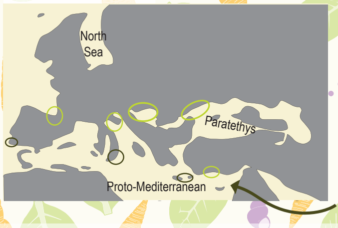
Terebralia lignitarum

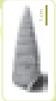
Terebralia duboisi and *Terebralia lignitarum* are the most common species in the Miocene.

OLIGOCENE

Terebralia subcorrugata is found in the Western Tethys and the NE Atlantic in the Oligocene and Early Miocene.

LATE MIOCENE





Terebralia duboisi

Last occurrences in the Messinian of southern Europe (Portugal, Italy, Greece).

- Species of *Terebralia* live in coastal environments and are found in sands or sandy marl sediments.
- They co-occur with mangroves in parts of all the basins and the Tethyan Seaway.
- A maximum of three species co-occur geographically and temporally (also true for modern species).
- They are extirpated from Europe with the onset of the Messinian Salinity Crisis.

Part of the research was financed by the Ernst Mach Worldwide scholarship of the Austrian Agency for International Cooperation in Education and Research (OeAD). Thanks to Unitas Malacologica for the financial support to attend the World Congress of Malacology - WCM22 in Munich.

dthivaiou@geol.uoa.gr

Finding reticulated evolution in the fossil record,

a case study with putative hybrids in recent and fossil Stromboidea (Gastropoda)

Ulrich Wieneke*

Citizen Scientist, Murnau, Germany
Email: stromboidea@gmail.com

Introduction

Morphological information within the fossil record delivers the main criteria when discovering evolutionary lineages. Yet, studying recent animals allows digging much deeper into evolution by looking into information from DNA, zoology, anatomy, behaviour etc. This raises the question of how knowledge of extant animals can improve our understanding of what has been happening in the fossil record.

Speciation via hybridization may be a mechanism that challenges our view of [illegible] evolution. While in plants hybridization is well-known and may lead to reticulated evolution (Goulet et al. 2017), this mechanism is rarely known in animal evolution (Abbott et al., 2013). This study tries to extrapolate from (putative) hybridisations in the extant marine gastropod superfamily Stromboidea and its morphological basis to a related group in the fossil record.

Fig. 1. Stromboidean hybrids. [illegible]

Methods

A simple hybrid-index, similar to that one Anderson had developed in 1949 for plants, is used to study several stromboideans. Some of these have already been classified as hybrids, whereas others as species. Figure 1 shows those that are considered here as putative hybrids. Further studies (molecular, ecological, anatomical) need to be made to verify this result. Analyzing the set of hybrids provides the rapid information from the hybrid-index (has character states from both parent species and has intermediate character states of the parent species character states, see Fig. 2). They also meet two further criteria for the identification of hybrids: [illegible] and sympatric. The prerequisite for all these considerations is a good knowledge about the morphology of the parent species, including the variability of the parents, and also knowledge about their stratigraphical [illegible]. With this set of criteria, the fossil record of the Aporrhaidae from Oligocene and younger epochs was studied.

Fig. 2. Comparison *Aporrhais* [illegible] [illegible]

Results and Discussion (1)

A hybrid-index analysis resulted in the identification of 25 stromboidean hybrids. There might be more because several stromboidean groups were not examined. In the past, four hybrids had been described as species (see Fig. 1: 4, 5, 7 & 9). Since no evidence confirms their species status (like „Are they fertile?" and „Are there existing populations?"), the status of being a species is rejected here. A few of the hybrids presented in Fig. 1 are illustrated for the first time: Fig. 1: 3, 6, 10, 17 & 24.

The large number of hybrids leads to the conclusion that hybridization in Stromboidea is not rare, and further investigation for specimens of this superfamily within the fossil record is warranted.

For better comparison with fossil Aporrhaidae, the case of the *Aporrhais* [illegible] x [illegible] hybrid was chosen. Figure 2 shows that the hybrid has intermediate character states, and some states are the same as those of one of its parents. A similar case is the upper Oligocene *Aporrhais callosa* x „*speciosa*" (see Fig. 3).

Fig. 3. Comparison *Aporrhais callosa* (A), hybrid *A. callosa* x „*speciosa*" [illegible]

Results and Discussion (2)

The *A. callosa* x „*speciosa*" hybrid is found, together with its parents, in a shallow sublittoral fauna in Eger, Hungary, in the [illegible] (Báldi, 1973). Contrary to its parents, the hybrid seems to have no ancestor and no descendant. This hybridisation event seems to have not influenced further evolution.

No ancestor is found in the *Aporrhais* [illegible] case. In the Messinian, before the Messinian Salinity Crisis (MSC), there were two different species found in the Mediterranean: *Aporrhais uttingeriana* and *A. pespelecani*. Repopulation of the Mediterranean after MSC from the Atlantic is the most favoured hypothesis, which brought back *A. uttingeriana* and *A. pespelecani*. But a third species occurs: *Aporrhais* [illegible], here interpreted as a hybrid of the other two. Descendants of *A.* [illegible] are fossil: *A.* [illegible] and *A. serresiana*. Therefore *A.* [illegible] is considered a hybrid species (Fig. 4).

Fig. 4. *Aporrhais* [illegible]

Conclusion and Outlook

Based on morphological considerations, 25 extant and 2 fossil putative hybrids are recognized. One fossil hybrid had a temporarily limited occurrence, the other is the beginning of an evolutionary lineage. Further studies are needed to support this model: molecular, anatomical, and ecological studies to validate the status of the extant hybrids, and morphometric and fine stratigraphical studies to support the status of the fossil hybrids.

[illegible] 2003 found that *Aporrhais serresiana* and *A. pespelecani* have very different soft parts, a view that supports the model of this contribution, but also might have its origin in a different evolutionary development. So especially the position of *Aporrhais serresiana* relative to *Aporrhais pespelecani*, *A. uttingeriana* and [illegible] needs further investigation.

References

[illegible]

Acknowledgements

The author would like to thank all contributors to www.stromboidea.de for making their material accessible and for further support, especially:

[illegible]

Thanks also to the organizing team of the World Congress of Malacology for the opportunity to present this study.

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

Pablo Heres[1], Jesús Troncoso[1*], Estefania Paredes[1]

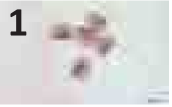
[1]Centro de Investigación Mariña, Departamento de Ecoloxía e Bioloxía Animal, Laboratorio de Ecoloxía Costeira (ECOCOST), Universidade de Vigo, Vigo, Spain.

INTRODUCTION. The shellfish aquaculture is reliable to catches of wild seed → conditioned by **environmental variations**, the **climate change**, **pathogens**, **competitors, predators,** the **pollution** and limitations imposed by the **seasonal spawning**.

AIM. 1. To study **potential long-term effects of cryopreservation** on larvae of successive generations. 2. To study the capacity of the larval cryopreservation protocol described in Paredes et al., 2021 and Heres et al., under review of producing **competent mussel spat.**

IN ORDER TO: improve aquaculture production; **diminish the reliance of seasonal supply** of natural mussel spat; **avoid hazards** of natural populations; capacity to **implement other techniques** on aquaculture (**selective breeding**).

EXPERIMENTAL DESIGN.



Selected 72 h-old D larva (**Fig. 1**). Cryopreserved in **10% Ethylene-Glycol + 0.4 M Trehalose in Sea Water (Paredes et al., 2021; Heres et al., under review)**: cooling rate: -1°C/min / thawing by immersion into a water bath at 35°C.

First generation of larvae Cultured into 150 L tanks (**Fig. 2**) for 22 days post-fertilization, with aeration and feeding. Sampling periodically for survival counts and shell measurements.

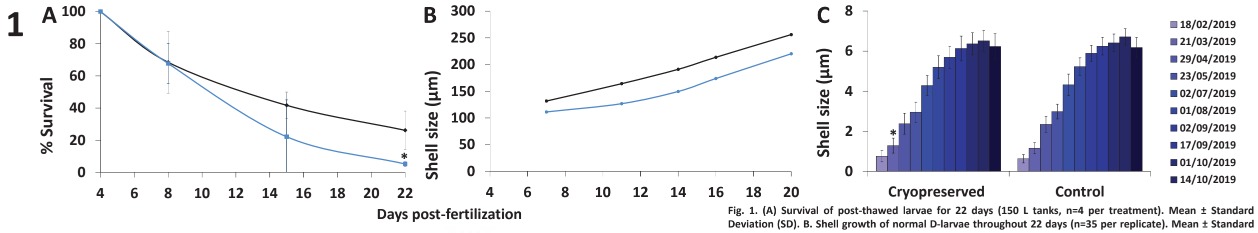
Developed pediveliger larvae cultured into settlement drums (**Fig. 3**) for 33 days. At the end, mussel juveniles collected for total counts.

Spat cultured for 27 days to allow the settlement on ropes (**Fig. 4**) and transported to rafts to study its development (**Fig. 5**).

Second generation of larvae. Obtained two years after. Cultured into 150 L tanks (**Fig. 2**) for 22 days and transferred to settlement drums (**Fig. 3**) to study settlement success.

RESULTS.

First generation of larvae



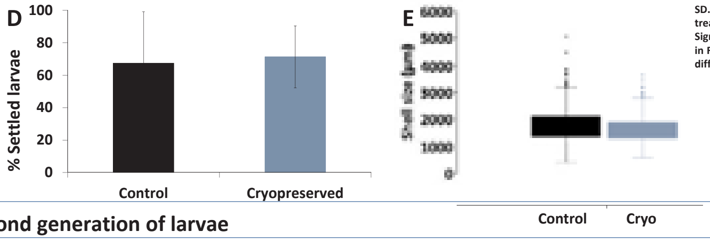


Fig. 1. (A) Survival of post-thawed larvae for 22 days (150 L tanks, n=4 per treatment). Mean ± Standard Deviation (SD). B. Shell growth of normal D-larvae throughout 22 days (n=35 per replicate). Mean ± Standard SD. (C) Percentage of settled larvae developed (150 µm mesh) (10,000 larvae/drum, n=8 50 cm^3 drums per treatment). Mean ± SD. D. Box plots for the distribution of shell sizes in microns (µm) of settled larvae. Signification was considered a $p<0.05$. C. Shell size (cm) of mussels developed from larvae (F1) cultured in rafts in Ria de Vigo, NW Spain. Mean ± SD, n=10 per culture rope, 4 ropes per treatment. Asterisk shows significant differences between treatments ($p<0.05$).

Survival at settlement point (Fig. 1.A).	
Control: : 26.19 ± 11.95%.	Cryopreserved: 5.24 ± 1.44%.
Settlement success (Fig. 1.D).	
Control: 67.45 ± 31.65%.	Cryopreserved: 71.27 ± 19.10%.
No significant differences in growth from settlement (Fig. 1B,C,E).	

Second generation of larvae

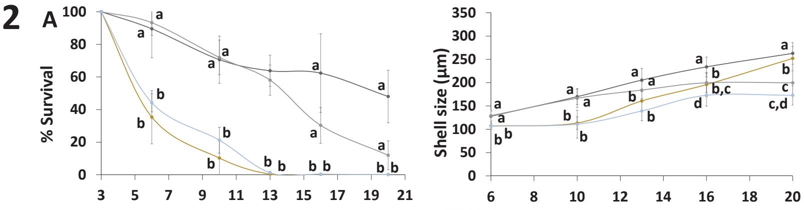
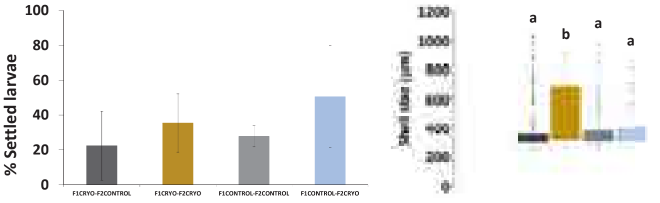


Fig. 2. A. Survival percentages of F2 larvae cultured over 20 days. B. Shell lengths of developed larvae for 20 days. Mean ± Standard Deviation (SD), n=3 tanks per treatment (150 L). C. Percentages of settled larvae .Mean ± Standard Deviation (SD), n=3 tanks per treatment (50 cm3). D. Box plots of shell sizes in microns (µm) of settled juveniles (n=35, 3 settlement drums per treatment (50 cm3)). Legend: F1CRYO-F2CT: control larvae obtained from adults developed from cryopreserved larvae. F1CRYO-F2CRYO: cryopreserved larvae obtained from adults developed from cryopreserved larvae. F1CT-F2CT: control larvae obtained from adults developed from control larvae. F1CT-F2CRYO: cryopreserved larvae obtained from adults developed from control larvae. Statistical signification was considered when $p<0.05$.



Survival at settlement point (Fig. 2.A).	
F1CRYO-F2CONTROL: 48 %	F1CONTROL-F2CONTROL: 11.92%
F1CRYO-F2CRYO: 0.15%	F1CONTROL-F2CRYO: 0.12%
Settlement success (Fig. C).	
F1CRYO-F2CONTROL: 22.37%	F1CONTROL-F2CONTROL: 27.76%.
F1CRYO-F2CRYO: 35.41%	F1CONTROL-F2CRYO: 50.54%
No significant differences in growth at settlement (Fig. 2.D).	

CONCLUSIONS.

Cryopreservation yields a decrease on larval survival and a delay on larval development, but from settlement onwards, the resulting juveniles are able to develop as fast as control individuals. Cryopreservation does not compromise gamete quality and viability of the following generations.

Acknowledgements. This research was funded by the Consellería de Educación, Universidad y Formación Profesional da Xunta de Galicia (Spain) (P.H. PhD programme grant) and Assemble+, grant from the European Union's Horizon 2020 research and innovation programme (No. 730984). Authors would like to acknowledge the staff of Centro de Investigación Mariña—Estación de Ciencias Mariñas de Toralla (CIM-ECIMAT) for their skilled assistance.

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The modern distribution of *Hendersonia occulta* (Say, 1831) (Gastropoda: Helicinidae) in Illinois, U.S.A.

Jochen Gerber
Gantz Family Collections Center, Field Museum of Natural History, Chicago, U.S.A

The cherrystone drop, *Hendersonia occulta* (Say, 1831), is one of only four species of the operculate land snail family Helicinidae occurring in North America (north of Mexico). It has currently a disjunct distribution with known populations clustering in the midwestern states of Iowa, Wisconsin, Minnesota and Michigan in the West, and the Appalachian mountains from Pennsylvania to Tennessee in the East (Hubricht 1985, Lynum et al. 2013). Outlying, isolated populations have been reported from central Missouri (Oesch et al. 2013), east-central Kansas (van der Schalie 1939), northeastern Oklahoma (Branson 1963) and central Illinois (see below).

Modern (blackened counties) and fossil (x) distribution of *H. occulta*. Based on Hubricht 1985, with additions .

In Pleistocene deposits, *Hendersonia occulta* is known from a much larger area: Toronto, Ontario, Canada (Clarke 1966) south to southern Mississippi, west to south-central Nebraska and north-central Kansas, east to northeast Ohio (Hubricht 1985).

Previous reports of modern *Hendersonia occulta* in Illinois

Pilsbry (1897: 46) lists "Athens, Ill." among the modern occurrences of *H. occulta*. Pilsbry (1948: 1088) again lists Athens, Menard Co., as a Recent record and gives E. Hall (= Elihu Hall) as the source for the record. All subsequent mentions of modern *H. occulta* in Illinois are based on Pilsbry.

Elihu Hall (1822–1882) was an early Illinois malacologist who resided in Athens, Menard Co., Illinois. Hall's collection is deposited at the Field Museum. It contains exactly one specimen (FMNH 15376) of *H. occulta* which consequently is the voucher for Pilsbry's (loc. Cit.) Illinois record and all its subsequent citations. Its label reads, "Lick Branch nr. Athens, Menard Co., Ill." The specimen is devoid of a periostracum, chalky white, and filled with fine, silty sediment. Its condition is consistent with that of a Pleistocene fossil. *H. occulta* has been recorded from Pleistocene deposits in Menard County (Leonard & Frye 1960, Leonard, Frye & Johnson 1971).

In conclusion, previous modern records of *H. occulta* in Illinois are erroneous and actually refer to a fossil specimen.

H. occulta from the E. Hall collection at the Field Museum (FMNH 15376)

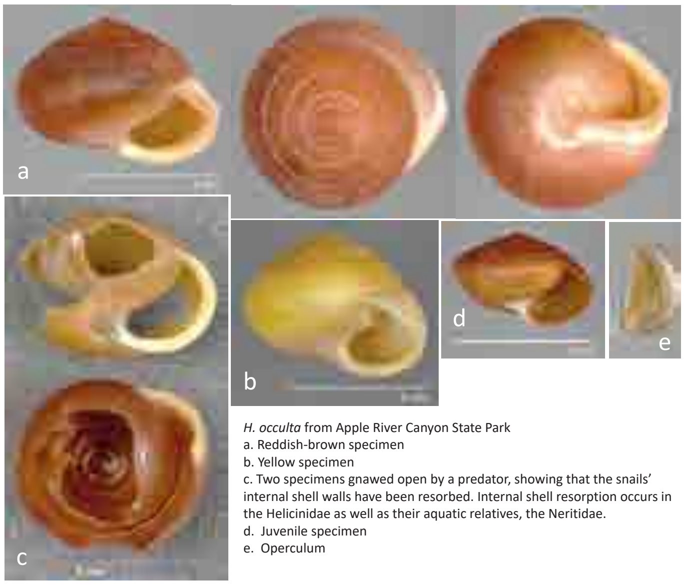
Hendersonia occulta in Jo Daviess County, Illinois

During a terrestrial gastropod survey in 2019 in Apple River Canyon State Park in northwestern Jo Daviess County, Illinois, live *H. occulta* were collected at two sites.

Both stations are well shaded by tree cover, characterized by an ample layer of leaf litter and the presence of dolomite outcroppings. At one station, the presence of cool-air vents, crevices in the rock from which cool air escapes, is worth mentioning.

Habitat of *H. occulta* at Apple River Canyon State Park, Jo Daviess Co.

Living *H. occulta from the site on the left*



H. occulta from Apple River Canyon State Park
a. Reddish-brown specimen
b. Yellow specimen
c. Two specimens gnawed open by a predator, showing that the snails' internal shell walls have been resorbed. Internal shell resorption occurs in the Helicinidae as well as their aquatic relatives, the Neritidae.
d. Juvenile specimen
e. Operculum

Apple River Canyon State Park is located in the North-East corner of Jo Daviess County. The County itself is the northwestern-most county in Illinois, bordering Iowa to the west and Wisconsin to the north. Jo Daviess County marks the southeastern edge of the "Driftless Area" in southwestern Wisconsin, southeastern Minnesota, northeastern Iowa, and northwestern Illinois. The Driftless Area remained unglaciated during the last (Wisconsin) ice age. In addition, the Area's unusual topography provides habitats suitable for cold-adapted species, such as deep, moist ravines, shaded dolomite cliffs and algific talus slopes. As a consequence, the Driftless Area serves as a refugium for plant and animal species that were extirpated from large parts of North America by either the advancing ice shields or by post-glacial warming and the habitat changes in its wake.

The modern distribution of *H. occulta* is disjunct with populations in the Upper Midwest of the United States in the West and the Appalachian Mountains in the East. Considering the numerous fossil occurrences in between, these areas can be interpreted as a relict distribution. *H. occulta* has long been known to live in the Driftless Area of Iowa, Minnesota and Wisconsin (Hubricht 1985, Lynum et al. 2013, and references therein). The discovery in Apple River Canyon is the first record of the species in the Illinois portion of the Driftless Area and currently the only modern record in that state.

Modern distribution of *H. occulta* in Illinois and adjacent states.
Open circles: counties with *Hendersonia* populations
Solid circle: Jo Daviess Co., Illinois, with a living *Hendersonia* population
x : Menard Co., Illinois, erroneously presumed to have living *Hendersonia*

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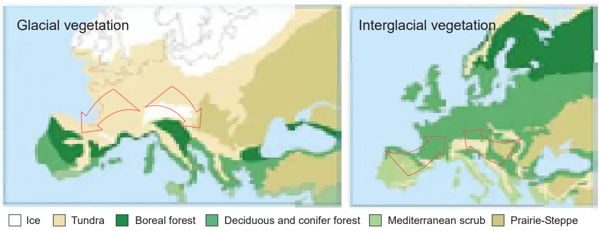
Quaternary palaeobiogeography of continental molluscs: a European project

N. Limondin-Lozouet[1], C. Legentil[1], S. Granai[1], O. Moine[1],
J. Horácková[2], L. Juricková[3], M. Horsák[4], I. Baly[5], V. Fichet[1]

1. Laboratoire de Géographie Physique, UMR 8591 CNRS, Université Paris 1, UPEC, Thiais, France.
2. Department of Landscape and Urban Planning, Faculty of Environmental Sciences, Czech University of Life Sciences, Czech Republic.
3. Department of Zoology, Faculty of Science, Charles University in Prague, Czech Republic.
4. Department of Botany and Zoology, Masaryk University, Brno, Czech Republic
5. UMS 3468 CNRS-MNHN, BBEES Paris, France

nicole.lozouet@lgp.cnrs.fr

Quaternary climatic cyclicity strongly influenced diversity and distribution of land snails. European territories located at the edge of the Pleistocene ice sheets, were particularly affected by climatic changes.



Vegetation distribution within a climatic cycle

During glacial times most species retreated to southern refugia, conversely during interglacial periods many snails distribution areas re-extended towards northern regions.

Number of land snails (non slugs) in NW Europe today

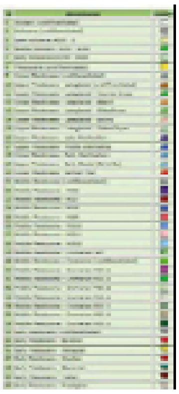
Current distribution of continental molluscs is directly inherited from repeated climatic oscillations during the Quaternary.

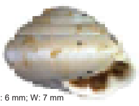
The Quaternary malacological record can provide insight into the evolution of the group diversity and associated causes of these variations over a long period of time, as well as on the timing of both species retreat/extension and extinction rates during climatic cycles. Therefore mapping the expansion 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.

The “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.

Choice for map or table can combine “and” “or”: species/chronology/climate/geography

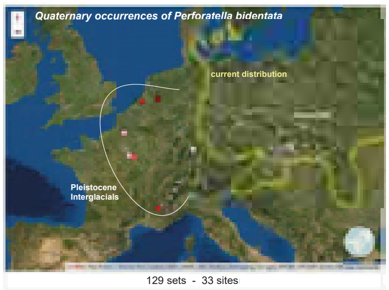
Time color code





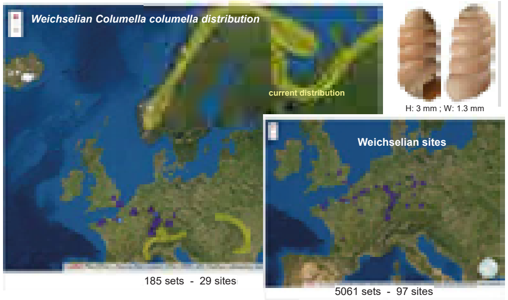
Request per species

H: 6 mm; W: 7 mm



129 sets - 33 sites

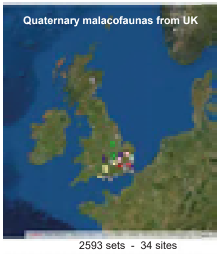
Request per period



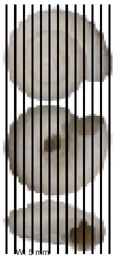
185 sets - 29 sites

5061 sets - 97 sites

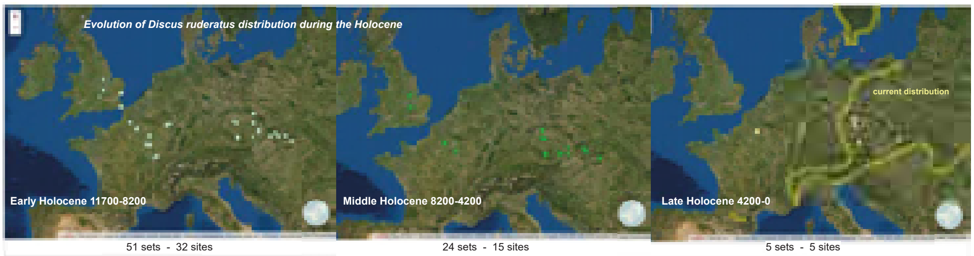
Request per territory



2593 sets - 34 sites



Request per age



51 sets - 32 sites

24 sets - 15 sites

5 sets - 5 sites

Current status : 66054 sets - 385 sites

Interested in contributing, information ... contact me : nicole.lozouet@lgp.cnrs.fr

Mussel memory: History lessons from freshwater bivalves

Isobel S. Ollard[1], David C. Aldridge

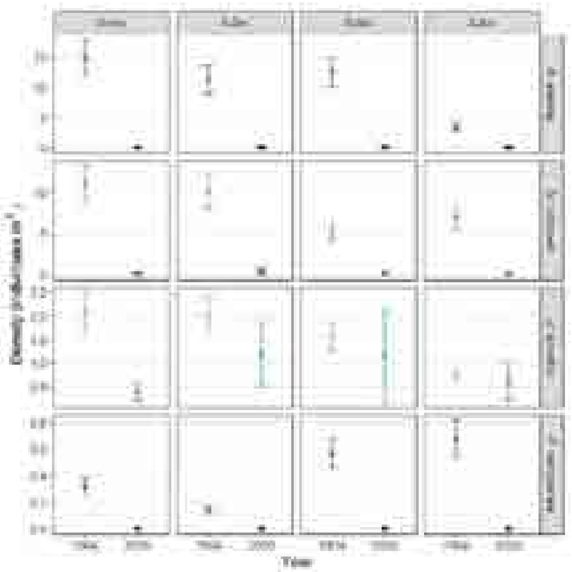
[1]Department of Zoology, University of Cambridge, Cambridge, United Kingdom
Email: iso21@cam.ac.uk

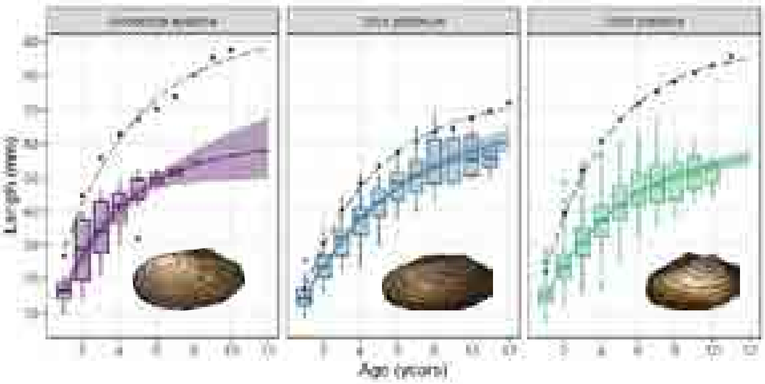
Background

- Freshwater mussels (Unionida) are a globally widespread taxon with key impacts on river and lake ecosystems, including water filtration, habitat creation and nutrient cycling[1].
- Mussels are declining globally and face a suite of threats including pollution, habitat alteration and invasive species[2].
- To track this decline, information is needed on long-term trajectories of mussel populations. This requires baseline data to reconstruct historical population characteristics.
- A 1964 survey of mussels in the River Thames at Reading, UK[3], is one of the earliest quantitative surveys of freshwater mussel population parameters. We resurveyed the site in 2020 and compared mussel species composition, population density, growth rates and production to assess change in the past half century in this major river.

Methods

- We replicated surveys conducted in 1964, using quadrat hand-searching and dredging. Surveys were split between four depth zones, giving population density estimates for each.
- We measured growth rates for individual mussels using external shell annuli, which are formed on an annual basis, to calculate Von Bertalanffy growth curves.
- We obtained abiotic data for the period 1970-2020 from the Environment Agency[4].





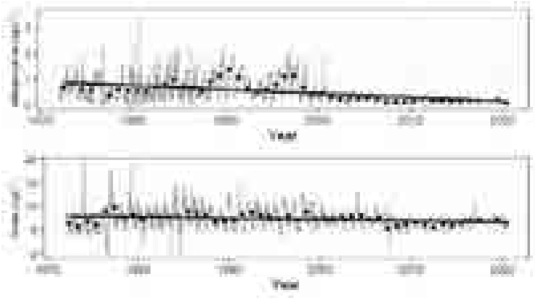
▲ **Figure 1:** Growth rates for mussels in 1964 (black line and points) and 2020 (coloured line and boxplots). Data for 1964 is available as averages only. For all species, growth rates are significantly lower in 2020 than in 1964.

◀ **Figure 2:** Population density, separated by species and by depth zone. Population density has declined significantly for all species except *U. tumidus*, with no live individuals of *P. complanata* found in 2020.

▼ **Figure 3:** Changes in orthophosphate and nitrate concentrations (measured at Caversham Weir, River Thames) 1970-2020. Significant declines have occurred in concentrations of both nutrients.

Results and conclusions

- **Densities were significantly lower** in 2020 for all species except *Unio tumidus. Anodonta anatina* and *Unio pictorum* declined to 1.1% and 3.2% of 1964 density respectively.
- **Species composition has changed**: no live *Pseudanodonta complanata* were found in 2020, and *U. tumidus* now dominates. Invasive *Dreissena polymorpha* and *Corbicula fluminea* are now present.
- **Individual growth rates were reduced** by 10-35% in 2020 in all extant unionid species.
- **Overall estimated annual biomass production declined** by 92.5%.
- **Concentrations of nitrate and phosphate have declined** steadily at the site over the study period.
- Declines in unionids may be due to both pressure from invasive bivalves and from declining nutrient levels and food availability.



Future directions

- We documented change at a single site. Integrating data on changes in mussel populations and environmental conditions across multiple sites would provide a broader picture of the scale and extent of decline, and enable us to identify common drivers.
- We used baseline data from 1966. A more complete picture of anthropogenic declines requires pre-anthropogenic baseline data on mussels, from sources such as sub-fossil shells.
- Examine relationship between individual growth rate and fitness / health.
- Extend monitoring of 'common' species to improve ability to detect declines.

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

E. Rentoumi[1*], M. Harzhauser[2], G. Iliopoulos[3], E. Koskeridou[1]

[1] National and Kapodistrian University of Athens, Greece; [2] Natural History Museum Vienna, Austria; [3] University of Patras, Rio Patras, Greece.
* evrentoumi@geol.uoa.gr

Introduction

The present study deals with mollusc faunas recovered from the Megalopolis Basin in central Peloponnese (Fig. 1), extracted from organic-rich sediments (from brown coal to xyloid lignite). Most specifically the sampled sequence came from Choremi mine (Fig. 2). The fossiliferous layers consists fluvial and lacustrine sediments, deposited during the Pliocene to middle Pleistocene. This basin is one of the few in this area presenting a continuous sedimentary sequence.

The first systematic record of mollusc faunas from the Pleistocene of southern Greece providing significant data on their taxonomy, palaeoecology and palaeoenvironment is presented herein. In addition, the reconstruction of the palaeo-environments will sheds 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.

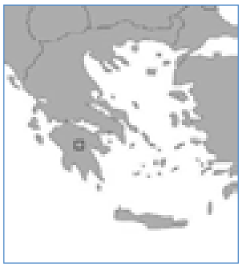


Figure 1: Location of the sampling area in Megalopolis Basin, Central Peloponnese, Southern Greece; Figure 2: Location of Choremi mine in Megalopolis Basin, Peloponnese, Southern Greece.

Sampled material

Middle Pleistocene: coarse and fine sediments representing glacial and interglacial periods respectively are recorded by the lake.

The sampled material (35 samples from 3 different levels, E13 (Fig. 4), E15 (Fig. 5), E16) presented herein originates from the core of an active open-cast lignite mine and is of Middle Pleistocene (Fig. 3) age.

Due to the hard nature of the lignite material samples were:

a) Disintegrated with H_2O_2 solution (25%).
b) Wet sieved (1mm, 500μm, 250 μm, 125 μm) under tap water multiple times each, until dissolved. About 37% of the studied samples contained fossil molluscs.

Figures 4, 5: Photographs of the Choremi section in the centre of the Megalopolis Basin lignite mine.

Aknowledgment

Thanks to Unitas Malacologica for the financial support to attend the World Congress of Malacology, 2022.

Results

The studied material includes assemblages that are typical for lacustrine environments, and which suggest fluctuations in oxygen levels. Until now, twenty different species of molluscs (gastropods and bivalves) have been identified (Plate 1) including Lymnaeidae sp. (1a, b), *Acroloxus lacustris* (2a-c), *Gyraulus* sp. (3a-c), *Bithynia* sp. (4a, b), *Gyraulus crista* (5a, b), *Gyraulus* cf. *albus* (6a-c), *Valvata* cf. *macrostoma* (7a-c), *Valvata cristata*, *Planorbis sp.*, *Segmentina nitida*, *Lymnaea* sp. (8a, b), *Oxyloma* sp. and *Pisidium personatum*, illustrating the dynamic environments of the Megalopolis palaeo-lake.

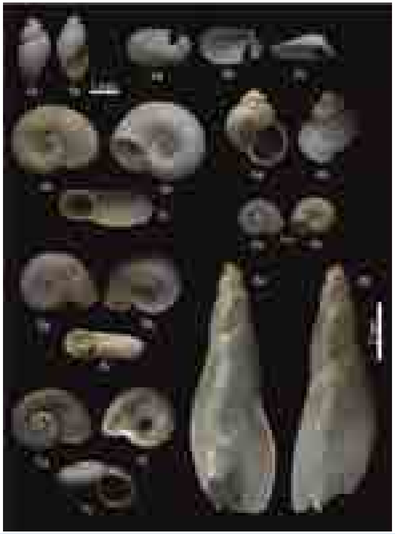
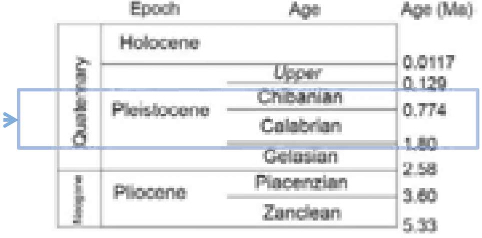


Plate 1: Most common species found in the recovered fossil material, a, b, c represents a different side of each specimen.



	Epoch	Age	Age (Ma)
Quaternary	Holocene		0.0117
	Pleistocene	Upper	0.129
		Chibanian	0.774
		Calabrian	1.80
		Gelasian	2.58
Neogene	Pliocene	Piacenzian	3.60
		Zanclean	5.33

Figure 3: Geochronological time scale of Pliocene – Pleistocene. The blue box highlights the age of Choremi sequence (van Vugt et al., 2001).

Conclusions – next steps

- The Middle Pleistocene depositional environment of Choremi section is characterized by freshwater taxa.
- Environmental fluctuations shows alternations in the area of the Megalopolis palaeo-lake.
  Freshwater lake with rich vegetation →Marshland
- Fossils were found in organic-rich sediments indicating deposition during warm-humid time period (interglacial).
- New information for the Middle Pleistocene includes new occurrences of freshwater molluscs for the first time in this biogeographic area.
- Study of Tripotamo location outside of the Megalopolis lignite basin.
- Study of Ptolemais lignite-bearing basin, in order to have a complete record of the molluscan faunas (Pliocene-Pleistocene) of the great palaeo-lakes of Greece. Differences and similarities.
- Investigate the potential role of Greece as a refugium during glacial time periods.

Reference

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Molluscs as Bioindicators of Paleoclimate and Paleoenvironment in Sediments of Neotropical Aquatic Ecosystems

Karla Rubio-Sandoval, Nancy Yolimar Suárez-Mozo, Augusto Luiz Ferreira-Júnior, Alexander Correa-Metrio, Nuno Simões, Paula Spotorno-Oliveira, Susete Wambier Christo, Maria Cristina Souza, Rodolfo José Angulo, Mark Brenner, Alessio Rovere, Liseth Pérez

INTRODUCTION

- 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.
- Molluscs are one of the most abundant groups in aquatic ecosystems and can be used as environmental indicators since their abundances vary depending on fluctuations in the environmental conditions.
- We used sedimented mollusc remains (gastropods and bivalves) to infer Late Holocene environmental conditions.

STUDY AREA

The study area includes four aquatic ecosystems (fresh, brackish, marine) in the Neotropics: Nahá Lake (Chiapas, Mexico), coastal Ría Lagartos Lagoon (Yucatán, Mexico), Ilha do Mel and Ilha de Currais islands (Paranaguá, Brazil).

Figure 1. Study area in the neotropics, Nahá Lake and Ria Lagartos Lagoon in southern Mexico and the Ilha do Mel and Ilha de Currais Islands in southern Brazil.

METHODOLOGY

LAKE & LAGOON

The methodological process consisted in: 1) Recovering the sedimentary sequence, 2) Build the age model, 3) Analysis of biological indicators.

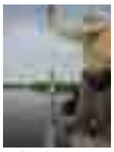


Figure 2. Ria Lagartos core

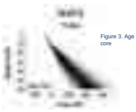


Figure 3. Age model of Nahá core

Figure 4. Age model of Ria Lagartos core

ISLANDS

The methodological process consisted in: 1) Identification of the vermetid reef, 2) Survey of the elevation data, 3) Sampling and Chronology, 4) Analysis of biological indicators.

Figure 4. Bioestromes of living vermetids and calcareous algae. The upper level (with arrows) corresponds to the mean low tide level. A) Vermetid reef sample from Ilha do Mel, B) Vermetid reef sample from Ilha de Currais (both scales are 2 cm). Modified from Angulo et al., 2013.

RESULTS & DISCUSSION

LAKE

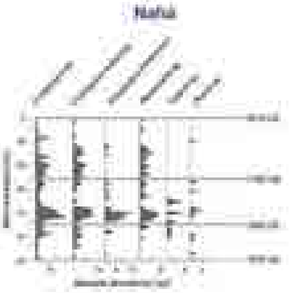
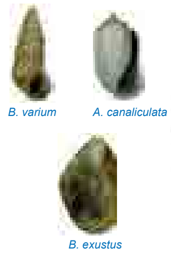


Figure 5. Absolute abundances of gastropods identified in the Nahá Lake sedimentary sequence.

Figure 6. Nahá Lake sedimentary sequence.

LAGOON



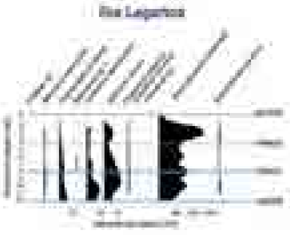


Figure 7. Absolute abundances of gastropods identified in the Ria Lagartos Lagoon sedimentary sequence.

Figure 8. Ria Lagartos Lagoon sedimentary sequence.

ISLANDS

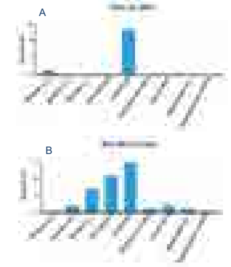


Figure 9. Absolute abundances of mollucs identified in the Islands´ vermetid reefs. A) Abundances of Ilha do Mel, B) Abundances of Ilha de Currais.

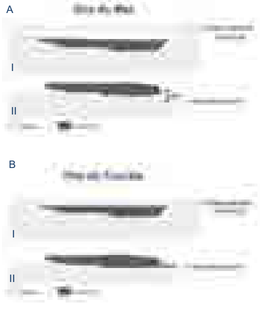


Figure 10. Sketch of paleo sea level levels. A) Ilha do Mel, B) Ihla de Currais, I) Depositional conditions during the Holocene sea-level highstands, II) Present position. Modified from Angulo et al., 2013.

CONCLUSIONS

- The freshwater gastropod community in Lake Nahá was sensitive to changes in water level and trophic state.
- The malacological assemblages in the coastal lagoon record show they responded to climate events such as hurricanes and recent human impacts.
- Vermetid reefs are indicators of past sea level changes.

ACKNOWLEDGMENTS

The first author KRS thanks Unitas Malacologica for its support through the travel grant to attend this conference. The authors thanks to Posgrado de Ciencias Biológicas and Posgrado de Ciencias del Mar y Limnología UNAM, as well as the Consejo Nacional de Ciencia y Tecnología CONACyT for their institutional support during the development of this project. We thank all those who participated in the different field campaigns involved in this research. This research was developed under the framework of the projects: IA101515, entitled " Nuevas técnicas paleoambientales a partir de análisis combinado de quironómidos, ostrácodos e isótopos estables en la Selva Lacandona, Chiapas, México; IA100317 "Evaluación neo y paleoambiental en lagos kársticos de la Selva Lacandona y Quintana Roo; CONACYT 252148: " Impacto humano en lagos de media altura de la Selva Lacandona, norte de los Neotrópicos and project FSE20140406249795 funded by CONACYT SENER Sustentabilidad Energética. Finally, the authors express their sincere gratitude to the Comisión Nacional de áreas Naturales Protegidas de México for their support during the field campaigns in the Lacandon Rainforest and in Ria Lagartos.

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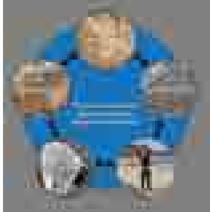
Contacts: krubiosandoval@marum.de
@KarlaRubioSand



TERRESTRIAL AND AQUATIC SNAILS: INSIGHTS INTO CLIMATE CHANGE IN OMAN FROM THE EARLY BRONZE AGE TO THE PRESENT

Katharina Schmidt[1], Eric O. Walliser[1], Bernd R. Schöne[1], Christoph J. Gey[2], Conrad Schmidt[3]

[1]Institute of Geosciences, University of Mainz, Mainz, Germany, [illegible]
[2]Museum Wiesbaden, Wiesbaden, Germany
[3]Institute for Ancient Near Eastern Studies (IANES), University of Tübingen, Tübingen, Germany

Introduction

Al Khashbah in the Sultanate of Oman (Fig. 1A) is an important (archaeological) site as it contains a number of well preserved monumental buildings ('towers') with associated (manmade) pits (excavations ('ditches')) and wells from the Early Bronze Age, plus a modern palm oasis belonging to the Late Islamic period. Till today it is poorly understood how such an economic hotspot could cope with water demands under hot and arid conditions, as the 'falaj', an irrigation system that allows all year-round water supply, could only be archaeologically proven from the Iron Age.

Stable isotope data of oxygen ($\delta^{18}O_{shell}$) and carbon ($\delta^{13}C_{shell}$) of two different gastropod species, namely the fully aquatic snail, *Melanoides tuberculata* (Fig. 1B) and the terrestrial taxon, *Zootecus insularis* (Fig. 1C) were used to reconstruct prevailing environmental conditions. In total, 13 shells were selected after being macroscopically examined for the absence of abrasion, fractures and other conspicuous features. Diagenesis has been excluded by the usage of Feigl's solution. Micromilling of the specimens was performed from the aperture to the 3rd–4th whorl of the shell.

The snails originate from three different locations: the first site (Fig. 1D, KSB18B-q0450) is a trench (UTM: 605055, 2506411) close to the surface, which is dated to the Early Bronze Age. The second site (Fig. 1E, KSB18B-q0565), a 3 m deep and 4 m wide ditch (UTM 605069, 2506421) was dated (^{14}C analysis of charcoal) to the Early Bronze Age. The third site (Fig. 1F, KSR19C-q0514) is a deep cut in a palm oasis at Al Khashbah (UTM 606880, 2506140), dated with previous archaeological surveys to the 18th–20th century CE.

Fig. 1: [illegible]

Results

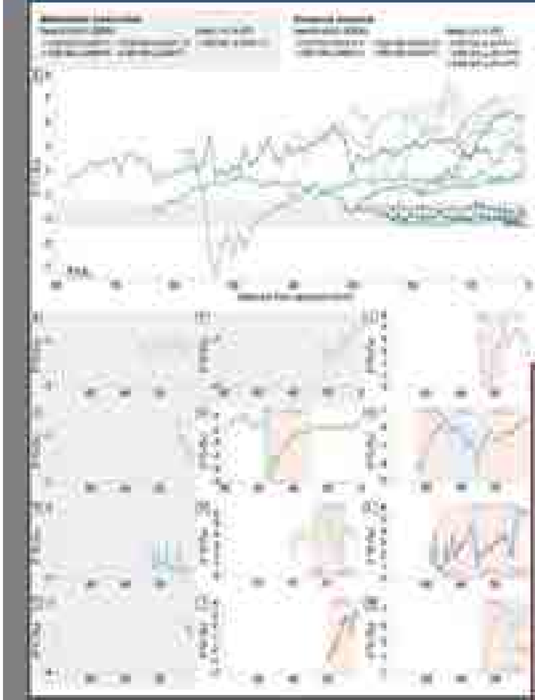


Fig. 2: [illegible]

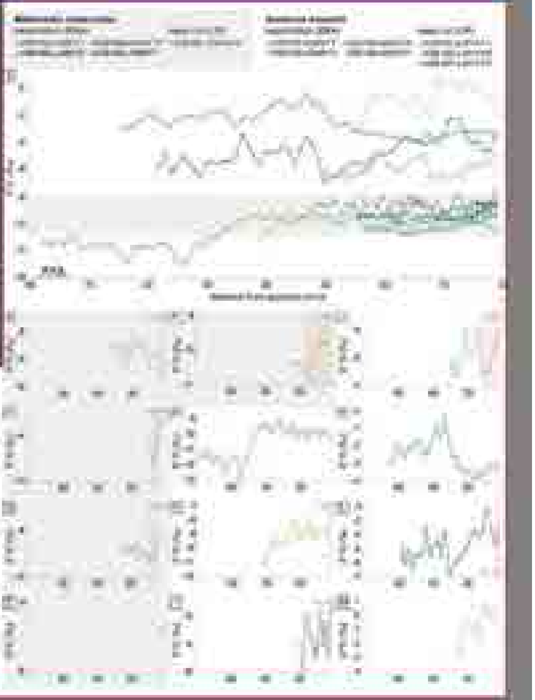
M. tuberculata (Fig. 2 B – F):
- Oxygen isotope data within a similar range (~1.0‰) without a distinct cyclicity
- Fossil shells are, on average, up to 1.4‰ higher than the modern snails

Z. insularis (Fig. 2 G – M):
- Very large variations both within and between specimens
- Similar pattern in all shells: sharp decrease to a minimum, followed by a steep rise until a 'pre-peak' is reached, after which the oxygen values drop slightly to rise again to the maximum value

M. tuberculata (Fig. 3 B – F):
- Similar isotopic range
- Fossil shells are up to 1.5‰ more positive
- No cyclicity but all snails show an increase in the carbon isotope data towards the aperture

Z. insularis (Fig. 3 G – M):
- Early Bronze Age: more positive values than those dating to the Late Islamic period
- Most carbon isotope values increase towards the aperture

Fig. 3: [illegible]



Interpretation

(a) The occurrence of the fully aquatic snail **M. tuberculata** in the ditches of Al Khashbah requires permanent, non-stagnant, shallow water. The attenuated seasonal $\delta^{18}O_{shell}$ range of max. 1.0‰ could imply a temperature change of only 4 °C if the inhabited water bodies had a strong environmental buffering effect. Therefore, a continuous recharge of the ditches with freshwater (e.g., groundwater) having a constant temperature and/or $\delta^{18}O_{water}$ signal seems highly probable. Additionally, the isotope values did not indicate a sudden rain event filling the ditches with water followed by subsequent evaporation.

(b) Using the $\delta^{18}O_{shell}$ values of the terrestrial snail *Z. insularis*, a highly variable paleoprecipitation regime in the Al Khashbah region was reconstructed. One shell recorded a cyclonic precipitation pattern (sudden drop in the $\delta^{18}O_{shell}$ values followed by a gradual increase; Fig. 2G), deriving from the south (the Indian Ocean), while all the other shells recorded a precipitation signature of air masses arriving from the north (the Mediterranean). The $\delta^{13}C_{shell}$ values suggest C4 plants (e.g., grasses or maize) dominated the region during the Early Bronze Age precluding the presence of large-scale agriculture. However, as indicated by the $\delta^{13}C_{shell}$ values, this changed since the Late Islamic period, as the snails primarily fed on C3 plants (e.g., oil trees, rice, wheat, soy), which could have only grown if farming existed.

Fig. 4: [illegible]

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

Lynn J. Bonomo*[1,2], Athena W. Lam[1,3], Matthew H. Van Dam[1,3], James B. Henderson[1], and Terrence M. Gosliner[2]
***Presenting author, [1]Center for Comparative Genomics, [2]Department of Invertebrate Zoology and Geology, [3]Department of Entomology | California Academy of Sciences**

Abstract

Formalin fixation was 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 of DNA. 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 performed low-coverage whole-genome sequencing. Library construction was performed using both standard (double-stranded) and single-stranded DNA methods. This poster presents new methods and complications of this protocol and aids in discussing new techniques or methods to improve results.

Original Description of *Goniobranchus tinctorius* (Rüppell & Leukhart 1830)

Original German:
Doris tinctoria -
Der milchweifse Körper ist, besonders auf der Mitte des gewölbten Rückens, mit sehr seinen blutrothen Adernetzen, in denen sich ein Paar Reihen eben so gefärbter Puncte befinden, gezeichnet. Der Rand des Mantels ist schwefelgelb gesäumt. Es ragt derselbe nur wenig über den Fuss vor. Auch die obere nach hinten freie Fläche des Fufses ist mit unregelmäfsigen rothen Flecken colorirt. Die 19 pyramidenförmigen, gefiederten Kiemen können völlig in den Mantel zurückgezogen und von demselben verdeckt werden. – Die Individuen dieser Dorisart haben die Eigenheit, selbst noch nach einem zehnmaligen Wechsel des Weingeistes, diesen braunschwarz zu färben.
Länge 2 Zoll. – Von den in Weingeist befindlichen Exemplaren ist das gröfste 8, das kleinste 5 Linien lang. - Bei Tor im März gefunden.

English translation:
The milk-white body, especially on the middle of the arched back, is marked with its blood-red vein nets (?), in which there are a couple of rows of dots just as colored. The edge of the coat (mantel) is hemmed in sulfur-yellow. It protrudes little over the foot. The upper surface of the foot, which is free to the rear, is also colored with irregular red spots. The 19 pyramid-shaped, feathered Gills can be completely withdrawn into the coat (mantel) and covered by it. The individuals of this Doris species have the peculiarity of coloring this brown-black even after changing the spirit of the wine ten times.
Length 2 inches. - The largest of the specimens in wine spirits is 8, the smallest 5 lines long. - Found at Tor in March.

Importance

- *Goniobranchus tinctorius* is the first described red-reticulate
  - Important to clarifying the red-reticulate species complex
- Has not been collected in years from the Red Sea
  - Closest specimen at CASIZ was collected by John L. Earle from Arabian Sea, Oman from Nov. 1993
- Red Sea usually has distinct species of nudibranchs
  - Supported by molecular and morphological work
  - Examples are seen in genera, such as *Chromodoris, Halgerda, Asteronotus*

Figure 1. Pictures of live specimen of *G. tinctorius* collected in Oman CASIZ 097438

Figure 2. Drawing of *Goniobranchus tinctorius* from Rüppell & Leukhart (1830)

Methods

Extractions:
- Protocol from Gould et al. 2021 (modified from Hykin et al., 2015 and Ruane & Austin, 2017)
- Up to a 72-hour buffer soak using GTE buffer, ATL buffer, Ethanol, and proteinase K. Combine with a shaking heat block.
- Used the Qiagen QIAamp DNA Micro Extraction

Sequencing:
- Tested both dsDNA (NEBnext Ultra II DNA, with slight modifications to adapt for ancient DNA) & ssDNA (xGen ssDNA & Low-Input DNA Library Preparation) library prep methods
- Libraries were sequenced at 10x coverage on a NovaSeq System, Paired-end 150
- Sequencing done through NovoSeq partial lane at MedGenome (San Francisco, CA)

Analyses:
- Python coding
- Geneious map to reference function – reference sequence was cytochrome oxidase subunit I (COI) for *Goniobranchus* cf. *reticulatus* (CASIZ 191022A)

Results & Conclusion

The extraction methods produced 169-698.5 ng of DNA total (ng/µl x total µl) from a quarter of the nudibranch. Both library prep methods yielded libraries that were able to be sequenced with good bioanalyzer traces, average fragment lengths that were smaller (<100 bp), which is expected for such starting material. The expected number of reads were produced, but a high percentage matches bacterial sequences. We were unable to align the filtered reads to the *Aplysia* or mitochondrial genomes or COI sequences produced from the same or closely related species.

Conclusion: We successfully identified extraction and DNA library preparation methods that might be appropriate for formalin preserved samples. However, bacterial contamination during the preservation and/or storage of samples overwhelmed the data produced. Further work will be done, focusing on decontamination methods both physically and bioinformatically.

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Rüppell E. & Leuckart F.S. (1828–1830). Mollusca [in] Atlas zu des Reise im Nordlichen Afrika von Eduard Rüppell. 1. Abth. Zoologie. 5. Neue wirbellose Thiere des Rothen Meers. Frankfurt, H.L. Brönner pp. 1–22, pl. 1–12 [1828], pp. 23–47 [probably 1830], page 32, Plate 9, Figure 4

CHOOSE YOUR OCEAN: SPECIATION AND EVOLUTION OF BOREAL AND ARCTIC *CORYPHELLA* (HETEROBRANCHIA: NUDIBRANCHIA)

IRINA EKIMOVA[1], ÁNGEL VALDÉS[2], MANUEL MALAQUIAS , CESSA RAUCH[3], ANTON CHICHVARKHIN[4]
ANNA MIKHLINA[1], TATIANA ANTOKHINA[5] AND DIMITRY SCHEPETOV[1]

[1]Lomonosov Moscow State University,
[2]California State Polytechnic University,
[3]University Museum of Bergen,
[4]National Scientific Center of Marine Biology,
[5]A.N. Severtsov Institute of Ecology and Evolution

Introduction

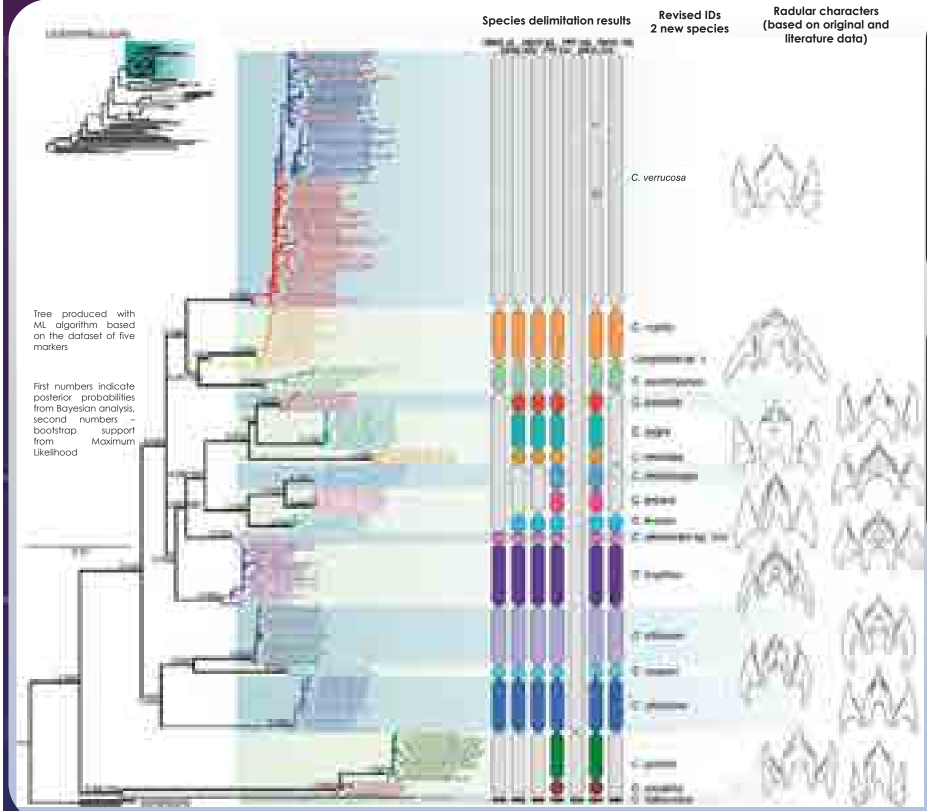
The species delineation is particularly acute in boreal seas, which are often inhabited by species with broad geographic ranges and high degrees of intraspecific morphological and molecular diversity. Environmental conditions in boreal and Arctic regions significantly changed multiple times in recent 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.

Material & Methods

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.

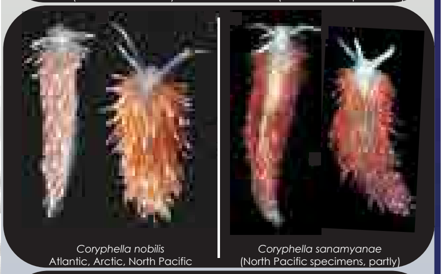
Results & Conclusions

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, different divergence times. The invasion in the Arctic and Atlantic occurred multiply times starting from the first opening of the Bering Strait in late Miocene. In the North Atlantic several species evolved in sympatry during the Pleistocene.

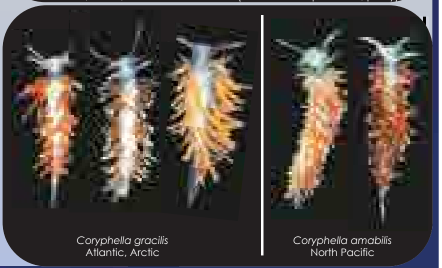



Coryphella verrucosa
(Arctic and Atlantic)

"*Coryphella pseudoverrucosa*"
(North Pacific specimens)


Coryphella nobilis
Atlantic, Arctic, North Pacific

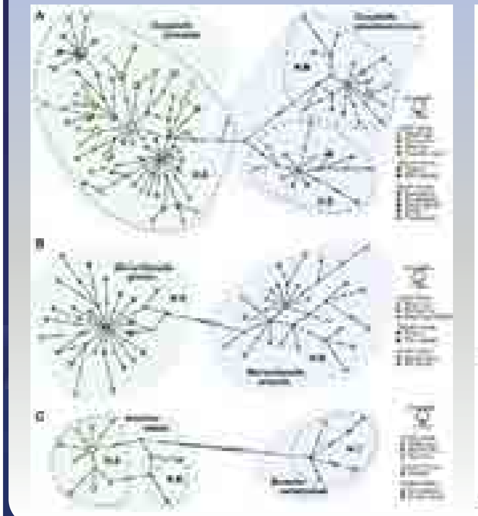
Coryphella sanamyanae
(North Pacific specimens, partly)


Coryphella gracilis
Atlantic, Arctic

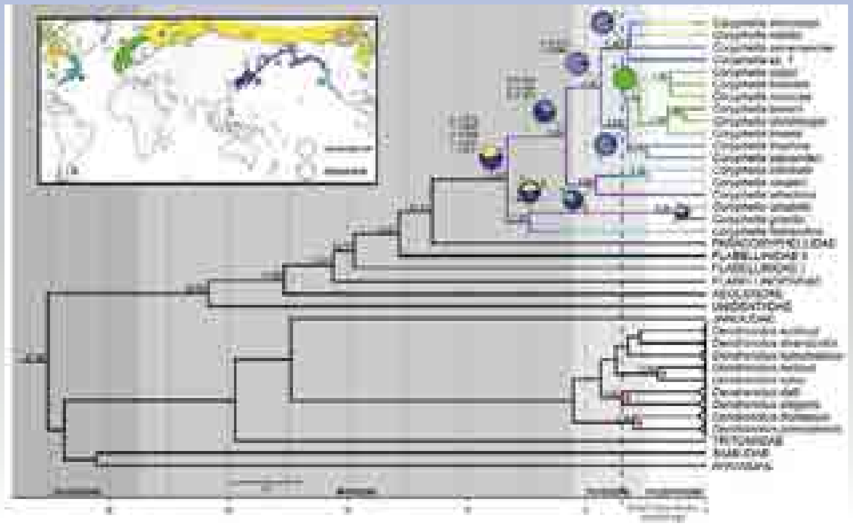
Coryphella amabilis
North Pacific

Phylogeography

Population structure of trans-Arctic clades



Ancestral area reconstruction and divergence times estimation



This study was supported by Russian Science Foundation grant #20-74-10012.

Corresponding author email: irenekimova@gmail.com

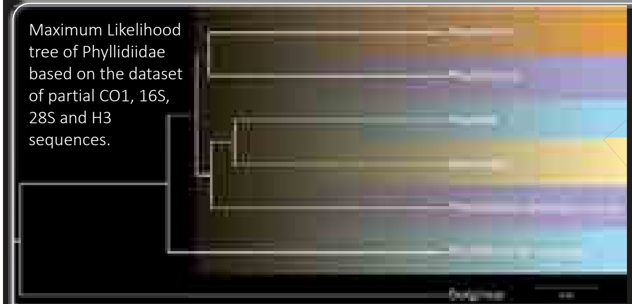
New data on taxonomy of phyllidiid nudibranchs (Heterobranchia: Phyllidiidae) and biodiversity in Southern Vietnam

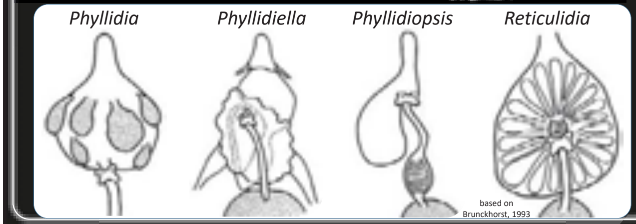
Jana V. Indriksone[1], Yury V. Deart [2], Tatiana I. Antokhina[2], Dimitry M. Shepetov [1], Irina A. Ekimova [1]
[1] Lomonosov Moscow State University, Moscow, Russia. [2]A.N. Severtzov Institute of Ecology and Evolution RAS, Moscow, Russia.

Nudibranchs of the family Phyllidiidae stand apart from all other nudibranch families as they demonstrate several unique traits: lack of radula and labial cuticle, lack of gills, the presence of adaptive gills 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 work 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 the diversity of Phyllidiidae in Southern Vietnam using an integrative approach included traditional morphological (anatomical research, SEM) and modern molecular phylogenetic methods. In the latter case two mitochondrial (COI and 16S rRNA) and two nuclear (histone H3 and 28S rRNA) markers were studied. The material was collected in 2016-2021 from Nha Trang Bay and Spratly Islands in Vietnam waters using SCUBA diving and snorkeling.

Maximum Likelihood tree of Phyllidiidae based on the dataset of partial CO1, 16S, 28S and H3 sequences.

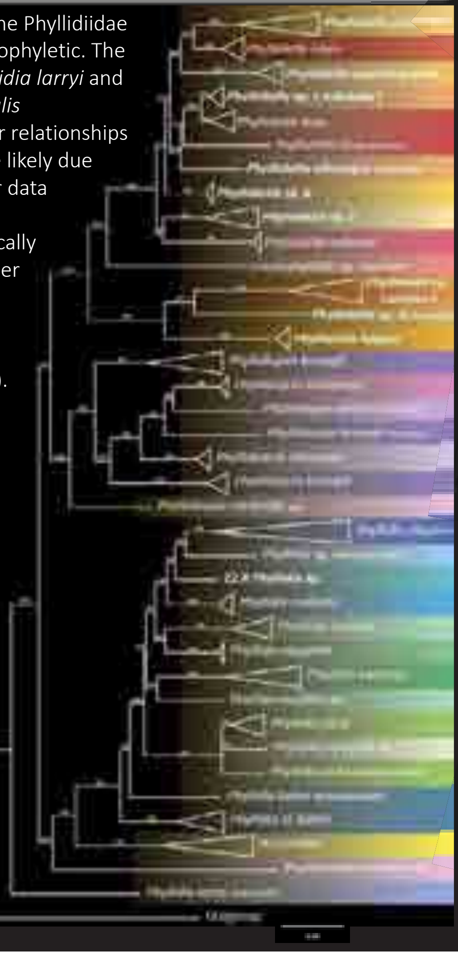


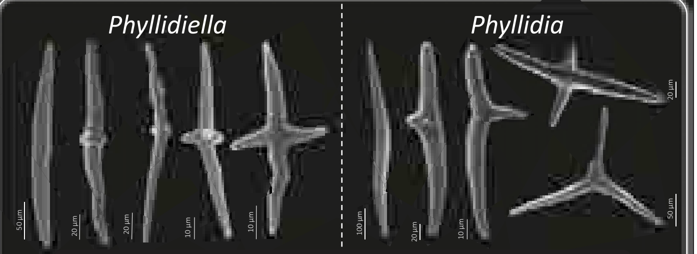


Each genus within the Phyllidiidae was recovered monophyletic. The exceptions are *Phyllidia larryi* and *Phyllidiopsis cardinalis* demonstrating sister relationships to other Phyllidiidae likely due to limited molecular data for these species. Genera morphologically differ from each other in the anatomy of digestive system (digestive gland and pharynx specifically).

As Phyllidiidae are still pourly studied, this is the first time nuclear markers were included in molecular analysis.

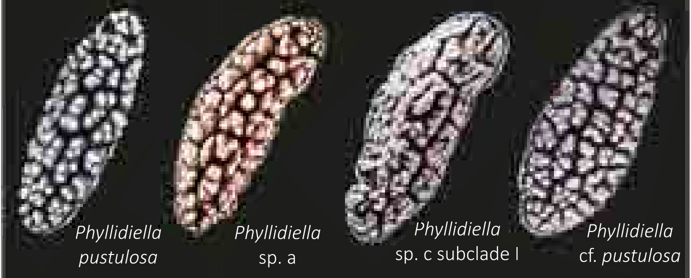
Maximum Likelihood tree of Phyllidiidae based on the dataset of partial CO1, 16S, 28S and H3 sequences.





Some differences in the spicule morphology can be seen between the genera, but this character did not vary between species.

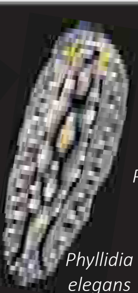
Phyllidiella pustulosa species complex



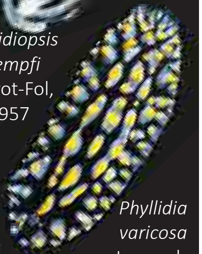
The *Phyllidiella pustulosa* species complex currently appears to be splitted into 9 clades (bold on the tree). In our material 4 of them are present. They all are highly divergent in coloration. Anatomically they show no differences. They vary only in secondary metabolites, what was shown in Papu et al. (2022).


Phyllidiella lizae Brunckhorst, 1993


Phyllidiella rudmani Brunckhorst, 1993


Phyllidia elegans Bergh, 1869


Phyllidiopsis krempfi Pruvot-Fol, 1957


Phyllidia varicosa Lamarck, 1801

22.4 – *Phyllidia* sp. nov.

Our specimen differs from others both in color and molecular data. Unfortunately, only a piece for molecular studies was collected, so its anatomy cannot be studied.


Phyllidia coelestis Bergh, 1905


Phyllidia ocellata Cuvier, 1804

correspondence: indriksonne@gmail.com

Phaedusinae versus Clausiliinae and Alopiinae - similarities and differences in the structure of the reproductive system

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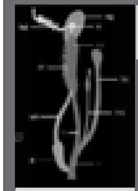
Tomasz K. Maltz[1], Izabela Jędrzejowska[2], Anna Sulikowska-Drozd[3]
[1]Museum of Natural History, University of Wrocław, Poland
[2]Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wrocław, Poland
[3]Department of Invertebrate Zoology and Hydrobiology, University of Łódź, Poland

ALLOPIINAE & CLAUSILIINAE

PHAEDUSINAE

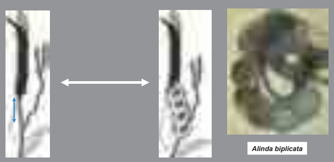
MORPHOLOGY OF REPRODUCTIVE SYSTEM



reproductive system:
- hermaphroditic
- syntremic

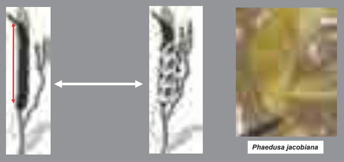
abbreviations:
ag – albumen gland, bc – bursa copulatrix, c – carrefour, fo – free oviduct, hd – hermaphroditic duct, mg – mucus gland, p – penis, pr – prostate, so – spermoviduct, v - vagina

species with embryo retention: embryons retained in free oviduct

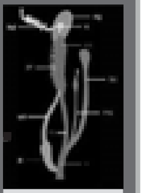

Alinda biplicata

schematic drawing vs. photo

species with embryo retention: embryons retained in spermoviduct


Phaedusa jacobiana

reproductive system:
- hermaphroditic
- syntremic

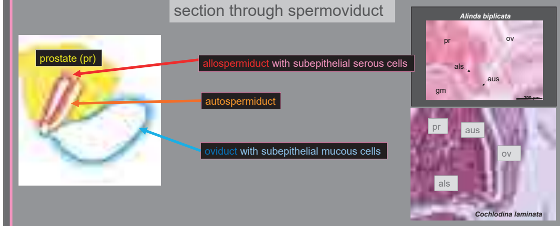


abbreviations:
ag – albumen gland, bc – bursa copulatrix, c – carrefour, fo – free oviduct, hd – hermaphroditic duct, mg – mucus gland, p – penis, pr – prostate, so – spermoviduct, v - vagina

HISTOLOGY OF REPRODUCTIVE SYSTEM

semitriaulic reproductive system built of:
- oviduct (ov)
- autospermiduct (aus)
- allospermiduct (als)

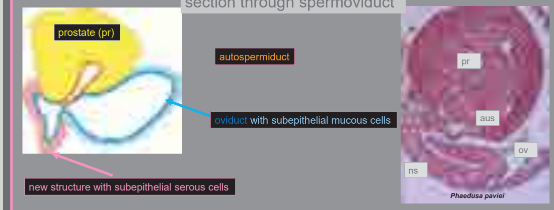
section through spermoviduct


prostate (pr)
allospermiduct with subepithelial serous cells
autospermiduct
oviduct with subepithelial mucous cells

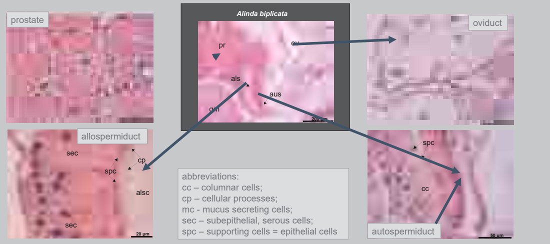
schematic drawing vs. photo

semitriaulic (?) reproductive system built of:
- oviduct (ov)
- autospermiduct (aus)
- (?) new structure (ns)

section through spermoviduct


prostate (pr)
autospermiduct
oviduct with subepithelial mucous cells
new structure with subepithelial serous cells

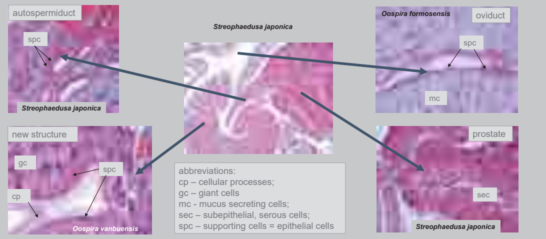
histological structure of channels



abbreviations:
cc – columnar cells;
cp – cellular processes;
mc - mucus secreting cells;
sec – subepithelial, serous cells;
spc – supporting cells = epithelial cells

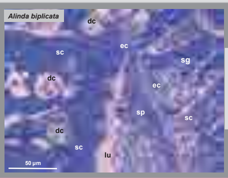
paraffin section (7µm); **haematoxylin** and **eosin** staining

histological structure of channels



abbreviations:
cp – cellular processes;
gc – giant cells
mc - mucus secreting cells;
sec – subepithelial, serous cells;
spc – supporting cells = epithelial cells

histological structure of allospermiduct with subepithelial, serous cells

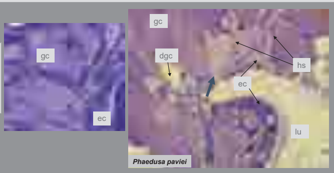


abbreviations:
dc - degenerating serous cells;
ec - epithelial cells;
lu – lumen;
sc – subepithelial, serous cells;
sg - secretory granules;
sp – secretory products

epon semithin section (0.7-1 µm); **methylene blue** staining

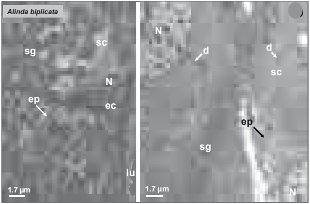
histological structure of new structure with subepithelial, giant cells containing homogeneous secretion

abbreviations:
dgc - degenerating giant cells;
ec - epithelial cells;
gc – giant cells;
hs - homogeneous secretion;
lu – lumen;



ULTRASTRUCTURE OF REPRODUCTIVE SYSTEM

allospermiduct: serous cells bordering epithelial cells

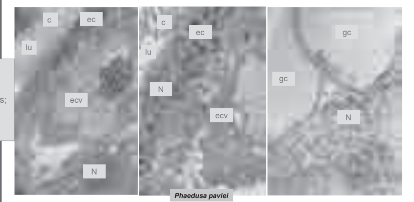


abbreviations:
d - dictyosomes;
ec - epithelial cells;
ep - epithelial cell projections;
lu – lumen;
N – nucleus;
sc – subepithelial, serous cells;
sg - secretory granules

epon ultrathin section (70-90 nm); TEM

allospermiduct: giant cells with homogeneous secretion bordering epithelial cells

abbreviations:
c - cilia;
ec - epithelial cells;
ecv – epithelial cells with vesicles;
gc – subepithelial giant cells;
lu – lumen;
N – nucleus



Seasonal Differences in the Distribution, Size, and Population Density of the Nudibranch *Nanuca occidentalis*

Julia R. Piper, Erika N. Molina, Samantha S. Schlegel, Louis J. Ambrosio, Daniella Gutierrez-Andrade, and Michael L. Middlebrooks

Department of Biology, The University of Tampa, Tampa, United States

Introduction

- Despite their fascinating biology heterobranch sea slugs are understudied organisms and for most species' basic information regarding life cycle and history as well as seasonal and geographic distribution and are not documented[1].
- Aeolid nudibranchs are carnivorous sea slugs that feed on a variety of prey organisms including many Cnidarians. Often their prey provide not only food, but habitat and shelter for the nudibranch as well[2].
- *Nanuca occidentalis*, previously *Dondice occidentalis*[3] (Figure 1), is an aeolid nudibranch native to the Gulf of Mexico and Caribbean Sea [4,5].
- *Nanuca occidentalis* feeds on hydroids in the genus *Eudendrium*, which form large extensive colonies. These colonies serve as habitat for a variety of small organisms, causing them to be local biodiversity hotspots[6].
- By studying colonies of *Eudendrium carneum* and recording sea slug species presence, this study aims to provide data on the seasonal abundance of sea slugs as well as additional information on the seasonal variation of size and density in *N. occidentalis.*

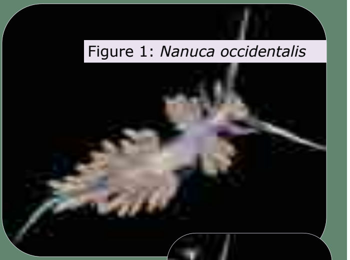


Figure 1: *Nanuca occidentalis*

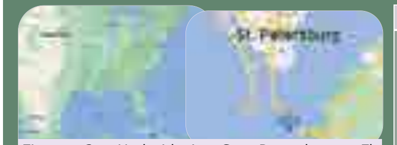


Figure 2: Hydroid in St. Petersburg, Fl. collection sight located near the Skyway Bridge

Methods

- Within the first week of each month from June 2021 to May 2022, four colonies of the hydroid *E. carneum* were collected by snorkel from the Skyway Bridge in St. Petersburg, Fl (Figure 2).
- Dissection microscopes were used to thoroughly search each colony for sea slugs. Discovered sea slugs were identified to species and measured for length by photographing them in an elongated position next to a metric ruler. These photos were then digitally measured using ImageJ software.
- Once all sea slugs had been removed, the hydroid colony was dried in a drying oven. This dry weight was then used to calculate population density for *N. occidentalis.*

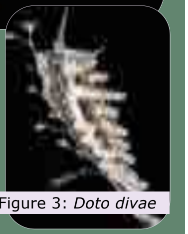


Figure 3: *Doto divae*

Results

- Throughout the year-long study a total of 638 sea slugs were found across 15 different species; 420 of these slugs were *N. occidentalis.*
- During the study, individuals from the following taxonomic groups were found: Cladobranch Nudibranchs, Sacoglossa, Cephalaspidea, and Aplysiida (Table 1). The highest species diversity, with ten documented species, was seen in June 2021. *Nanuca occidentalis* were present every month and *Doto divae* (Figure 3) were present in ten of the months.
- The lowest species diversity, with two documented species, was seen in the months of September 2021, October 2021, February 2022, and April 2022 (Figure 4).
- The largest quantity and highest density of *N. occidentalis* was found in July 2021 with 2.919 individuals/g dry wt. hydroid, and the smallest/lowest found in February 2022 with 0.126 individuals/g dry wt. hydroid (Figure 5).
- The largest average size of *N. occidentalis* was recorded in February 2022 (16.68mm) and the smallest in July 2021 (4.22mm)(Figure 6).

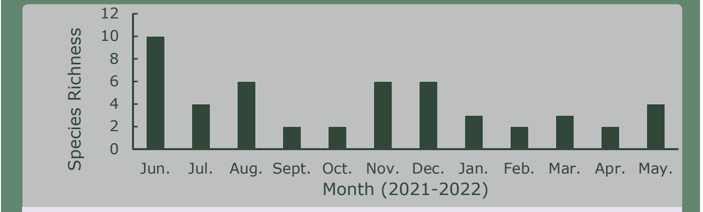


Figure 4: Number of different species found each month from June 2021 to May 2022.

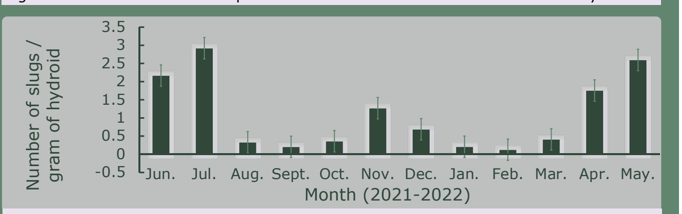


Figure 5: Number of *Nanuca occidentalis* individuals found each month per gram of dry weight of *Eudendrium carneum*. Bars are representative of standard error.

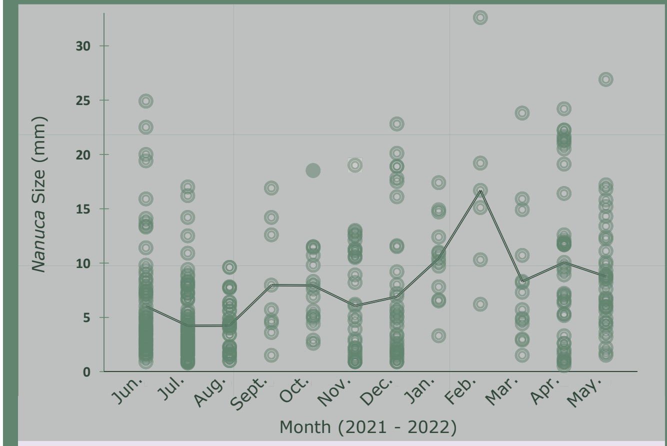


Figure 6: Scatter plot of the size of each individual *Nanuca occidentalis* in millimeters per month ranging from June 2021 to May 2022. The connected line shows mean size per month.

Table 1: Heterobranch sea slugs found on *Eudendrium carneum* and the number of individuals found, (#).

Taxonomic Group	Species
Cladobranch Nudibranchs	*Nanuca occidentalis* (420), *Doto divae* (125), *Doto chica* (27), *Cuthona perca* (3), *Learchis poica* (2), *Spurilla braziliana* (2), *Eubranchus conicla* (1), *Cratena pilata* (1), *Flabellina dushia* (1), *Tenellia adspersa* (1), *Calmella bandeli* (1)
Sacoglossa	*Placida kingstoni* (2), *Stiliger fiscovittatus* (38)
Cephalaspidea	*Haminoea antillarum* (6)
Aplysiida	*Bursatella leachii* (1)

Discussion

- This study has showcased the dynamic, variable habitat provided by *E. carneum*. This colonial hydroid hosted a variety of heterobranch sea slugs by acting as shelter; and additionally served as a food source to some nudibranchs.
- Resident species, *N. occidentalis* and *D. divae,* were found each month feeding on the host hydroid. Other, more transient species found on *E. carneum*, were suspected to have been feeding on other organisms growing on the colonial hydroids; such as epiphytic filamentous or microalgae.
- The fluctuations in species richness may be due to each sea slug's life cycle or food availability. These two factors, a combination of them, or unknown factors are responsible for these fluctuations.
- In the summer months, the density of *N. occidentalis* was the highest; however, these individuals also had the lowest average size. This could be due to recent spawning or recruitment events that led to an abundance of juvenile slugs. The opposite trend was observed in late winter, suggesting that few slugs survive into late adulthood. This corresponds to a type III survival curve common in mollusks. The spring months show an increase in the density of *N. occidentalis* possibly indicating the early stages of a spawning season.
- The observed sea slug decline seen seasonally could be due to senescence, predation, seasonal water temperature changes, or a variety of unexplored factors. Certainty for the causation of this experiments observed seasonal pattern requires further research.

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Does Colour Count?

Morphological analysis and phylogeny of the genus *Phyllidia* Cuvier, 1797

Lina M. Raubold[1], Adelfia Papu[2], Heike Wägele[1]
[1] Centre of Molecular Biodiversity, Zoological Research Museum Alexander Koenig, 53113 Bonn, Germany
[2] Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, 95115 Manado, Indonesia
Email: s6liraub@uni-bonn.de

Introduction

The genus *Phyllidia* Cuvier, 1797 (Mollusca, Gastropoda, Nudibranchia) 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 to 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.

Material & Methods

Dissections, histological examinations and an extensive literature review of the genus were conducted resulting in an extensive set of putatively parsimony-informative characters, first phylogenetic analyses (using TNT) and a taxonomic revision.

Conclusion

- Colour patterns need to be accompanied **by thorough morphological and anatomical information** in species descriptions and phylogenetic analyses.
- The ventral position of the anus, used to distinguish between the genera *Phyllidia* and ***Fryeria*** Gray, 1853 (synonymized with *Phyllidia*) is seemingly **polyphyletic**.
- **Müllerian mimicry rings** might be present within this genus. One example might be the **'ocellata-clade'** including *Phyllidia ocellata*, *Phyllidia undula*, *Phyllidia multituberculata*, *Phyllidia* cf. *babai* and the synonymized *Phyllidia ocellata* japonica form.
- This **morphological analysis** still awaits a similar molecular analysis of phylogenetic relationships within this genus.

Spot the difference

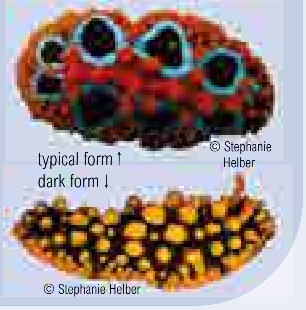
The species depicted here are very often identified or published as *Phyllidia ocellata* Cuvier, 1804. However, validity of *P.* cf. *babai* as a separate species has been confirmed by molecular analyses by Papu *et al*., 2022. We cannot exclude that the **other species** here are also **still valid** and they should therefore not be synonymized (see Brunckhorst, 1993 or Gosliner *et al*., 2015). They would exhibit a nice example of Müllerian Mimicry.

Phyllidia ocellata Cuvier, 1804

Phyllidia undula Yonow, 1986

© Avivit Fischler

Phyllidia multituberculata Boettger, 1918



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Phyllidia ocellata japonica form

Synonymized species like *Phyllidia japonica* Baba, 1937 should be re-evaluated taking mimicry into account.

Beware of Mimicry

Phyllidia cf. *babai* sensu Papu *et al*., 2022

First phylogenies of the genus *Phyllidia*

… based on morphological data give us a first glance of possible evolutionary relationships within this genus:

- *Phyllidia larryi* and *Phyllidia flava* may represent the most ancestral state within this genus.
- A relatively clear distinction is present between forms with minute or large notal tubercles.
- Possible mimicry rings are resolved as either monophyletic or polyphyletic based on the inclusion or exclusion of colour-based characters.

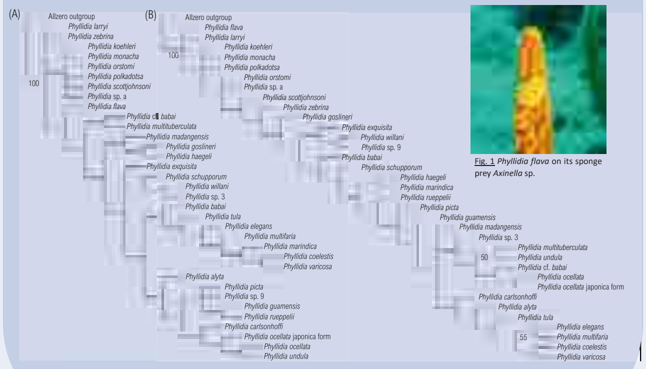

Fig. 1 *Phyllidia flava* on its sponge prey *Axinella* sp.

Fig. 1 (A) Phylogeny of the genus *Phyllidia* based on a dataset excluding characters based on colour and colour patterns. Majority rule consensus (cut 50) of 75 most parsimonious trees (length: 98 steps, CI: 0.39, RI: 0.71) under implied weighting. (B) Phylogeny of the genus *Phyllidia* based on a dataset including characters based on colour and colour patterns. Majority rule consensus (cut 50) of 12 most parsimonious trees (length: 329 steps, CI: 0.32, RI: 0.62) under implied weighting. Values underneath branches indicate bootstrap support.

Parsimony-informative characters

Ventral colouration

- Oral tentacles
- Colouration of foot and gills
- Black ventral markings

Fig. 2 Comparative colour patterns of the head region of *Phyllidia* cf. *babai* (left) and *Phyllidia coelestis* (right).

Fig. 3 Comparative ventral colour patterns of the foot and gills of *Phyllidia* sp. a, *Phyllidia* cf. *babai*, *Phyllidia ocellata* and *Phyllidia elegans* (from left to right).

Rhinophores

- Number of rhinophoral lamellae

Tubercle morphology

- Size
- Arrangement
- Shape

Anatomy

- Morphology of the pharyngeal bulb
- Morphology of the genital apparatus
- Morphology of the penial spines

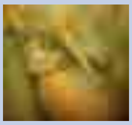

Fig. 4 Dorsolateral view of the anterior part of the digestive system of *Phyllidia varicosa*.

? Too little information is available for reliable results.

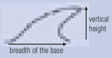

Fig. 5 Scheme of a penial spine of *Phyllidia varicosa* with putatively parsimony-informative measurements.

Colour of large notal tubercles

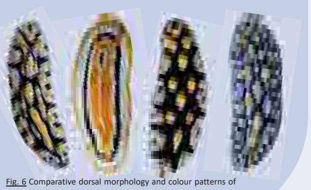

Fig. 6 Comparative dorsal morphology and colour patterns of *Phyllidia varicosa*, *Phyllidia* sp. a, *Phyllidia picta* and *Phyllidia elegans* (from left to right).

We are grateful for the generous financial support of Unitas Malacologica. Thank you to Dr. Stephanie Helber and Avivit Fischler for the provision of photographs for this poster.

References: Papu, A., Bogdanov, A., Bara, R., Kehraus, S., König, G. M., Yonow, N., & Wägele, H. (2022). Phyllidiidae (Nudibranchia, Heterobranchia, Gastropoda): an integrative taxonomic approach including chemical analyses. *Organisms Diversity & Evolution*, 1-45.
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Krynickillus melanocephalus.

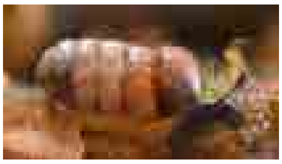
A new illustrated fauna for the Swedish species of Eupulmonata, Hygrophila and Pylopulmonata

Ted von Proschwitz[1, 2], Kennet Lundin[1, 2], Jonas Roth[3] & Ulf Bjelke[4]

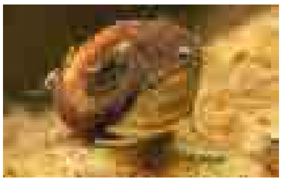
A Swedish fauna for the Eupulmonata (126 species), Hygrophila (34 species) and Pylopulmonata (33 species) is in production. It will soon be published as a volume in the series The Encyclopedia of the Swedish Flora and Fauna (Nationalnyckeln), produced by the SLU Swedish Species Information Centre (SLU Artdatabanken).

This will be the first complete fauna for Swedish land and freshwater gastropoda for over a hundred years. The chapter on marine pylopulmonates will be the first presentation of this group ever published for the country.

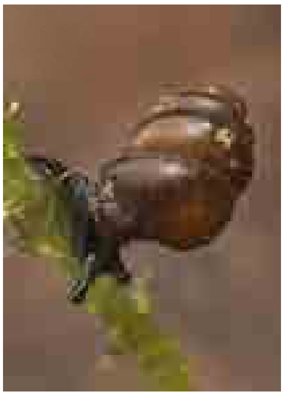
The volume contains detailed descriptions of the morphology, variation, and ecology of all species as well as distribution maps (for the terrestrial and freshwater species), and photos of all species from several angels. Beside photos of shells, more than 90% of the species in Eupulmonata and Hygrophila 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 (13 species in total) are included in an appendix. Hothouse species and temporary introductions are briefly mentioned.



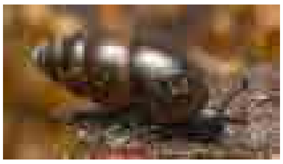
Columella columella. A fastiduous species restricted to calcareous habitats in the high mountain area in the north.



Gyraulus acronicus. A common species in streams, rivers and lakes in the northern parts of the country.

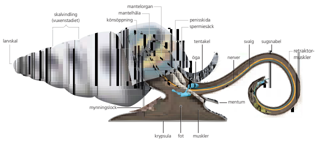


Vertigo lilljeborgi. A hygrophilous species, common in oligo-mesotrophic fen and shore habitats.

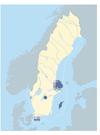


Cochlicopa nitens. The most endangered species in the Swedish landsnail fauna.

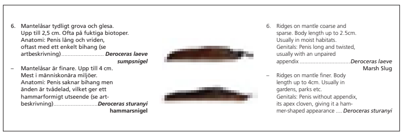
Arianta arbustorum, scalarid form.



Detailed descriptions of morphology. Pylopulmonata. Illustration: Robert Back



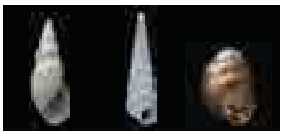
Example of the distribution maps. *Cochlicopa nitens.*



6. Mantelåsar tydligt grova och glesa. Upp till 2,5 cm. Ofta på fuktiga biotoper. Anatomi: Penis lång och vriden, oftast med ett enkelt bihang (se artbeskrivning) .... ***Deroceras laeve*** **sumpsnigel**
– Mantelåsar är finare. Upp till 4 cm. Mest i människonära miljöer. Anatomi: Penis saknar bihang men änden är tvådelad, vilket ger ett hammarformigt utseende (se artbeskrivning) .... ***Deroceras sturanyi*** **hammarsnigel**

6. Ridges on mantle coarse and sparse. Body length up to 2.5cm. Usually in moist habitats. Genitals: Penis long and twisted, usually with an unpaired appendix .... ***Deroceras laeve*** Marsh Slug
– Ridges on mantle finer. Body length up to 4cm. Usually in gardens, parks etc. Genitals: Penis without appendix, its apex cloven, giving it a hammer-shaped appearance .... ***Deroceras sturanyi***

Determination keys for adult specimens are presented in Swedish and English.



Examples of shell photographs: *Ondina obliqua, Pyrgiscus crenatus, Vertigo lilljeborgi.*

Photos this poster: Jonas Roth.

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[1] Gothenburg Natural History Museum, Box 7283, SE-40235 Gothenburg, Sweden
Email: ted.v.proschwitz@vgregion.se

[2] Gothenburg Global Biodiversity Centre, University of Gothenburg, P. O. Box 461, SE-40530 Gothenburg, Sweden

[3] Edsberga, Solliden, SE-58593, Linköping, Sweden

[4] Swedish Species Information Centre, Swedish University of Agricultural Sciences, P. O. Box 7007, SE-75007 Uppsala, Sweden

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

Cornélia Brosset[1], Nils Höche[1], Kotaro Shirai[2], Kozue Nishida[3], Regina Mertz-Kraus[1], Bernd R. Schöne[1]

[1]Institute of Geosciences, University of Mainz, Mainz, Germany
[2]Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba, Japan
[3]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
Email: cornelia.brosset@uni-mainz.de

INTRODUCTION

Shells of marine bivalves as high-resolution paleoclimate archives offer outstanding potential for environmental reconstruction to understand past and future climate dynamics [1-3]. Yet, well-accepted and reliable proxies are limited (e.g., $\delta^{18}O$ [4], growth rate [5]). Temperature reconstruction based on marine bivalve shell Sr/Ca remains challenging, although this method is routinely used for other biogenic carbonates (e.g., corals [6]). Specifically, the incorporation of strontium in the shell of *Arctica islandica* is regulated by vital and kinetic effects, and linked, directly or indirectly, to the shell microstructure [7].

Hypothesis: Sea surface temperature (SST) from NE Iceland (Fig. 1) can be reconstructed from shell Sr/Ca ratios of *A. islandica* after correction for microstructure and growth rate-related effects.

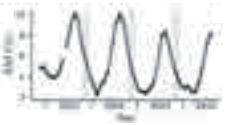

Fig. 1. Weekly sea surface temperature data at Grímsey station [8] between December 2003 and August 2006.

MATERIAL & METHODS

- Cross-sections of juvenile *A. islandica* shells: Outer and inner shell layers (OSL, ISL) are separated by the myostracum. OSL is subdivided into an outer and inner portion (oOSL, iOSL). Homogenous microstructure (HOM) dominates in the oOSL and crossed-acicular microstructure (CA) in the iOSL and hinge.
- Chemical analysis: Laser Ablation - Inductively Coupled Plasma - Mass Spectrometry (LA-ICP-MS).
- Microstructural analysis: Scanning Electron Microscopy (SEM) images analysis and morphometry of each biomineral unit (BMU) from both microstructure types.
- Shell growth and microstructural bias analyzed and mathematically removed from the Sr/Ca signal: Assessment of the Sr/Ca to SST relationship.

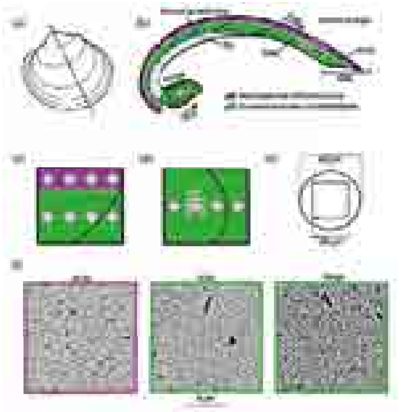

Fig. 2. Element chemical and microstructural analyses overview in shells of *A. islandica*. (a) Left valve cutting axis (black dotted line). (b) Cross-sectioned shell slab with shell layers and dominant microstructures. Magenta = HOM, green = CA. Direction of growth (dog) = arrows. (c) Enlargement of (b) showing the sampling positions in the ventral margin. (d) Enlargement of (b) showing the sampling positions in the hinge. (e) Schematic laser spot (diameter = 60 µm) and SEM image (square of 35 µm). (f) Microstructure examples: HOM and CA in the ventral margin, and CA in the hinge.

RESULTS

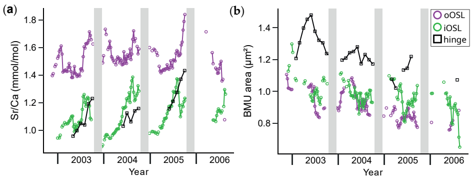

Fig. 3. Average shell Sr/Ca and BMU area chronologies of studied *A. islandica* specimens. Magenta circles = weekly data from the oOSL (HOM) of the ventral margin; green circles = weekly data from the iOSL (CA) of the ventral margin; black squares = monthly data from the hinge (CA). Vertical gray bars = annual growth lines. (a) Average Sr/Ca chronologies of oOSL, iOSL and hinge. (b) Average BMU area chronologies of oOSL, iOSL and hinge.

The seasonality of Sr/Ca and BMU area suggested an external forcing of these parameters (Fig. 3). In the ventral margin, Sr/Ca levels were different in the oOSL (HOM) than in the iOSL (CA) whereas the respective BMU sizes were similar. In addition, a BMU area dissimilarity in the same microstructure type (CA) for different shell portions (ventral margin and hinge) suggested that additional processes were responsible for the apparent Sr/Ca signal.

The shell layer-specific model of the relationship between Sr/Ca and the shell growth rate provided a better fit than the general model for the ventral margin (Fig. 4a). A similar result was obtained for the Sr/Ca vs BMU area: A negative correlation is observed for both oOSL and iOSL with the layer-specific model, where the general models (ventral margin and hinge) did not provide strong correlations (Fig. 4b).

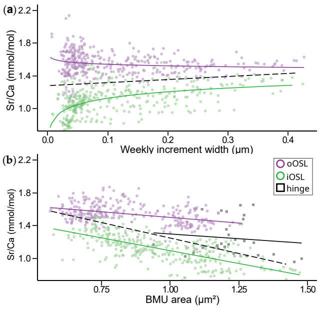

Fig. 4. Relationship of Sr/Ca with growth rate and BMU area of studied *A. islandica* specimens. Color coding as in Fig. 3. Regression curves are depicted in same color as data of respective shell portion. Black dashed lines represent linear regression model of entire OSL (oOSL + iOSL) dataset. (a) Sr/Ca and growth rate relationship in the ventral margin. (b) Sr/Ca and BMU area relationship in the oOSL, iOSL and hinge.

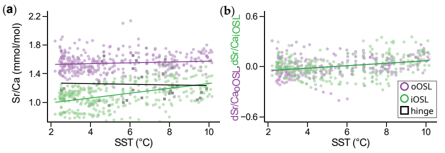

Fig. 5. Relationships between shell Sr/Ca data of *A. islandica* and SST. Color coding as in Fig. 4. (a) Undetrended shell Sr/Ca of the oOSL, iOSL and hinge. (b) Double-detrended Sr/Ca chronologies of the ventral margin (shell layer-specific growth rate and BMU area detrending; 'd' prefix = detrended).

Sr/Ca is weakly positively correlated to SST in both sublayers of the OSL, with a stronger correlation in the iOSL, whereas the linear regression was not significant in the hinge (Fig. 5a). The bias introduced by growth rate and shell microstructure was mathematically removed from the Sr/Ca signals, i.e., detrending, using layer-specific models for oOSL and iOSL of the ventral margin separately. The residuals, i.e., detrended Sr/Ca ratios (dSr/Ca_{oOSL} and dSr/Ca_{iOSL}), were more strongly correlated to SST. Additionally, the level difference between the sublayers of the OSL was removed (Fig. 5b).

DISCUSSION

I. The influence of shell growth rate on Sr/Ca

Sr/Ca ratios of the OSL sublayers were weakly linked to the shell growth rate. However, this relationship differed between the iOSL and oOSL. This suggests that the direct relationship between Sr/Ca and the shell growth rate is ambiguous, or that other factors had a larger impact.

II. Relationship between Sr/Ca and shell microstructure

The robustness of the negative correlation between Sr/Ca and BMU size differed between the two sublayers of the OSL of the ventral margin, suggesting an indirect link between the two parameters.

The incorporation of strontium into the shell is under strong biological control [9], and the energetic cost of building BMUs potentially differs between different types of microstructure. Simpler blocks (e.g., at the annual growth lines, or in HOM) would be less demanding and the different BMU shape, and its idiomorphy, could facilitate the incorporation of Sr^{2+} ions resulting in higher strontium content in the BMUs more affected by crystal lattice defects (e.g., in CA) [10].

Organic components could, indirectly, play a role in these microstructural differences in Sr/Ca by promoting the crystal nucleation and regulating the presence of crystal defects [11]. Organic matrices could also have a direct impact on the Sr/Ca by enveloping BMUs in strontium-rich organic components [12].

III. Temperature control of shell Sr/Ca?

Sr/Ca was positively correlated to the temperature, even after detrending for microstructure and growth rate-related effects. Other studies have found similar [13], and opposite [14] correlations between SST and Sr/Ca.

This positive correlation, reinforced by the detrending process, is opposite to the results of inorganic aragonite precipitation experiments [15] (but in agreement with thermodynamics expectations). This suggests that the result obtained in this study could be representative of a causal relationship between the SST and the Sr/Ca signal, but is also linked to strong vital effects.

CONCLUSIONS

Currently, Sr/Ca values of *A. islandica* measured between annual growth lines cannot be used to reconstruct water temperature. Shell Sr/Ca was positively correlated to temperature, which agrees with thermodynamics predictions, but contradicts findings in some other bivalve species, scleratinians corals and inorganic aragonite (which all show a negative correlation). A detailed characterization of the shell microstructure (e.g., shape, habits), will most likely remain an integral part of subsequent attempts to reconstruct temperature from shell Sr/Ca. An ultra-high-resolution characterization of shell organics may help to understand the Sr distribution patterns in the shells.

ACKNOWLEDGEMENTS

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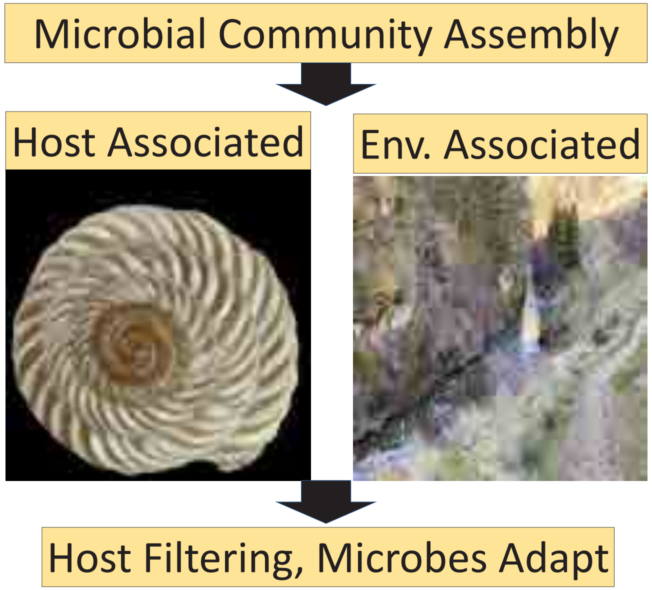
Drivers of Gut Microbiome Community Assembly in *Oreohelix* Land Snails

Authors: Ian Oiler, Mason Linscott, and Christine Parent

Figure 1. Examples of variation in shell ornamentation in genus *Oreohelix*. From left to right, ornamentation increases with calcium-rich geology. Photo: Mason Linscott



Microbial Community Assembly

Host Associated

Env. Associated

Host Filtering, Microbes Adapt

Results

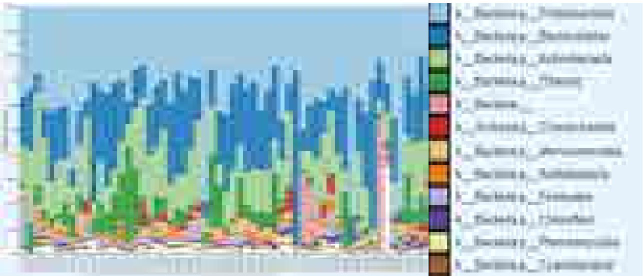


Figure 2. Relative abundance of bacterial phyla in snail gut tissue samples. Organized by site.

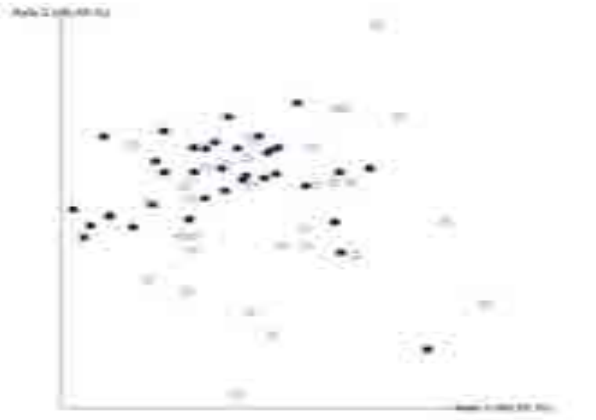


Figure 3. Principle coordinates analysis of microbiome dissimilarity (Robust Aitchison Distance). Dark points are smooth snails and light points are ornamented snails. PERMANOVA p-value = 0.019

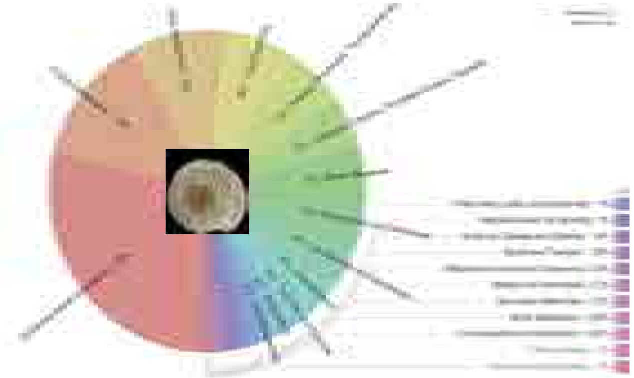
- PERMANOVA significant by shell form
- Partial Mantel Test for shell form x microbiome dissimilarity – geographic distance also significant.

Conclusions

- Small, but significant difference between shell forms
- Hypothesized mechanism of soil influence not clearly supported
- Differences between shell forms likely caused by another factor

Future Directions

- Shotgun metagenomics
  - Diet differences
  - novel parasite loads
  - Functional gene annotation



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- Funding Sources: Conchologists of America, Graduate and Professional Student Association, College of Graduate Studies, National Science Foundation

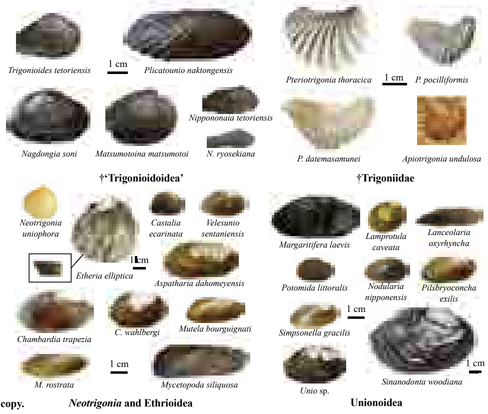
Fossilized organic periostracum from the Dinosaur era:
New insights into phylogeny and palaeoenvironmental reconstructions in Palaeoheterodonta

Taro YOSHIMURA[1*], Takenori SASAKI[1], Kentaro NAKAYAMA[2], Kaito ASATO[1], Yoshikazu NODA[2]
[1]The University Museum, The University of Tokyo [2]Fukui Prefectural Dinosaur Museum E-mail: * yoshimura@um.u-tokyo.ac.jp

ECO 29

1. Background

The extinct bivalve 'Trigonioidoidea' (Palaeoheterodonta), which emerged in the Middle Jurassic, possess combined features of both orders marine Trigoniida and freshwater Unioinida. Due to frequent convergent evolution of shell macro-morphology, the taxonomic position of 'Trigonioidoidea' has been contentious in past studies. In the current systematics, the superfamily is classified in the order Trigoniida, based on similarities in rib morphology and the position of shell adductive scars[1], but its placement should be reconsidered with microstructural characters.



2. Methods and materials

In this study, the periostracal microstructure of 20 species from all seven families of the Recent Palaeoheterodonta (Unionidae, Margeritiferidae, Etheriidae, Hyriidae, Iridinidae and Mycetopodidae) and six species from all five extinct 'trigonioidoidid' families (Trigonioididae, 'Nakamuranaiadidae', 'Nippononaiidae', 'Plicatounionidae' and 'Pseudohyriidae'), as well as four Cretaceous trigonids were compared with FE-SEM and CSLM.

Fig. 1. Molecular phylogenetic tree of Palaeoheterodonta[2].

Fig. 2. Quantification of the honeycomb-like periostracum using confocal scanning laser microscopy.

3. Results and discussion

3.1. Process of fossilization

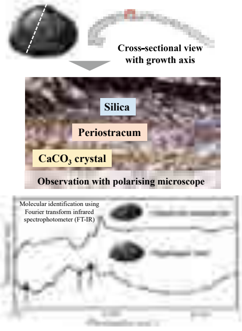


Fig. 3. Preservation of organic fossils by silica coating.

Peak of **β-chitin** $(C_8H_{13}O_5N)_n$, which is more resistant to degradation, coincides with that of the Recent species.

Preservation of chitin molecules in Cretaceous fossil periostracum

3.2. Periostracal microstructure: extant vs. extinct taxa

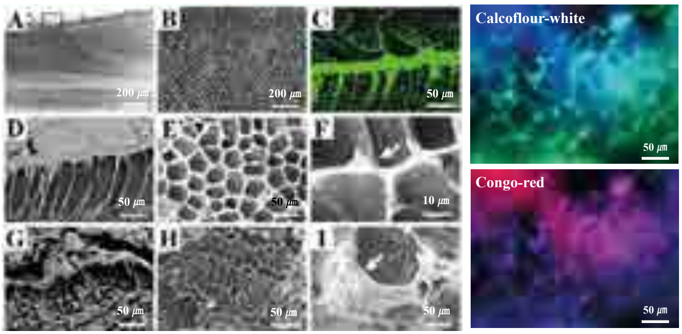
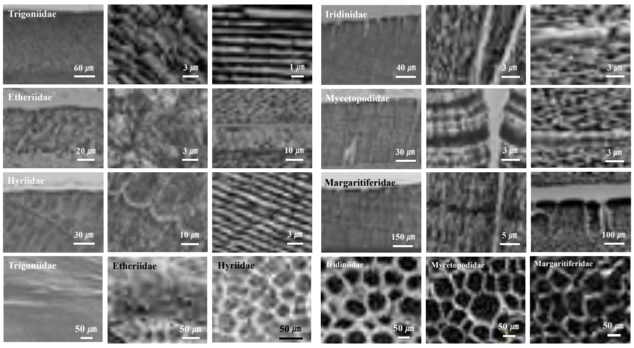


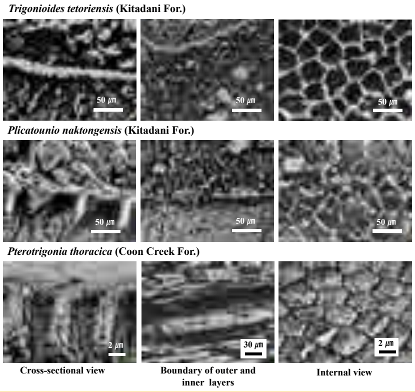
Fig. 4. Field emission scanning electron microscopy (FE-SEM) and fluorescence microscopy images.
A-F. *Nodularia douglasiae* (Unionidae), **G-I.** *Nagdongia soni* ('Nakamuranaiadidae'), **A, C, D, G.** Cross-sectional view in growth axis, **B, E, F, H, I.** Internal view of decalcificated periostraca, **C.** Nitrogen distribution (frame A), **F, I.** Arrows indicate organic mass.

3.3. Recent Palaeoheterodonta



Simple to more **three-dimensionally complex honeycomb-like periostraca**

3.4. 'Trigonioidoidea'



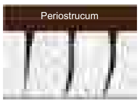
Trigonioides tetoriensis (Kitadani For.)

Plicatounio naktongensis (Kitadani For.)

Pterotrigonia thoracica (Coon Creek For.)

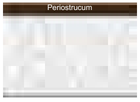
Cross-sectional view | Boundary of outer and inner layers | Internal view

'Trigonioidoidids'



- The thick periostracum
- **Large honeycomb**
- **Three-dimentional**

Trigonids



- The thin periostracum
- **Fine honeycomb**
- **Flat**

These results are consistent with previous studies in the Recent trigonids[3].

4. Conclusion

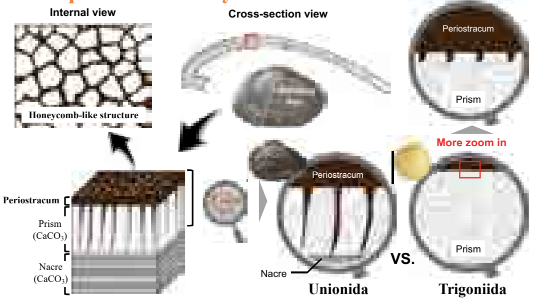
1. All families of 'Trigonioidoidea' have a **honeycomb-like periostracum** as one of **synapomorphies of the order Unionida**.
2. Based on the macro- and micro-morphology of the shell, all five families should be **placed under the superfamily Unionoidea**.
3. Imaging in periostracal microstructures may provide **novel taxonomic traits** to decipher the higher phylogeny of molluscs.

Fig. 5. Size comparison of the honeycomb structures

Table. Family-specific shell morphological comparison in the Recent Palaeoheterodonta and superfamily 'Trigonioidoidea'

Abbreviation in crystal microstructure: Simple (P) and complex (Pc) prism, columnar (Nc) and layered (Nl) nacre

5. Graphical summary



The honeycomb-like periostracum may be an evolutionarily conserved trait in the **Unionida**. This microstructure probably function to **prevent the detachment of the periostracum in rapidly shell dissolving freshwater environment**.

6. References

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2 Graf & Cummings (2006). *Zoo. Jour. Lin. Soc.*, **148**(3), 343-394.
3 Checa *et al.* (2014) *PLoS One*, **9**(2), e90033.

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Comparison of the effectiveness of barrier control measures for invasive slugs

A. Jucevičiūtė[1] & G. Skujienė[1]

[1] Department of Zoology, Institute of Bioscience, Life Science Center, Vilnius University, Vilnius, Lithuania

Introduction

- *Arion vulgaris* (Moquin-Tandon, 1855) is a common pest slug capable of causing high damage to agricultural and horticultural plants.
- Conventional chemical control agents can pose risks to humans, animals and the environment due to their inherent properties and associated uses

The aim

The task of this research was to determine and compare the effectiveness of environmentally friendly barrier measures (copper strips, zinc tin-sheet metal and „Schnexagon" paint)

Materials and methods

Slugs were counted and collected in 10 fields using J. Valovirta (1996) the checkerboard linear transect method (100 m^2) (Fig. 1).

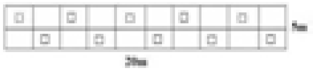

Fig. 1. Scheme of a checkerboard linear transect 100 m^2 (according to J. Valovirta, 1996)

Experiments (Fig. 2):

I. Zinc tin-plate enclosures + anti-slugs coating (Schnexagon) or copper tape or control fence without application. 5 enclosures for each group x 20 slugs.
II. Plastic pot + anti-slugs coating or copper tape or control pot without application. 10 pots with lettuce plant and slice of squash for each group.
III. Wooden enclosure + anti-slugs coating or copper tape or control without application. 2 enclosures with lettuce plant and slice of squash for each group.

Fig. 2. Design of research: I – left; II – center; III – right. Photo by A. Jucevičiūtė

Results

Abundance in VU Botanical Garden

~12 times more slugs were found in the Vingis section; more smaller slugs.

Table 1. The results of the abundance of *Arion vulgaris* (Moquin-Tandon, 1855) found in the Vingis and Kairenai sections of VU Botanic Garden.

Zinc tin-plate enclosures effectiveness

Effectiveness: 73,3% Effectiveness: 40,3%

Effectiveness: 18,6%

Fig. 3. Decrease in the average number of slugs on different test days: (A) - SXG material in coated enclosures; (B) - in enclosures covered with copper tape; (C) - in galvanized tin enclosures.

Efficiency of Shnexagon and copper tape

On plastic barriers

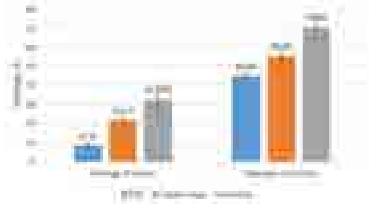

Fig. 4. Comparison of the average lettuce leaves and zucchini damage in different plastic pot test (± SE)

On wooden barriers

Fig. 5. Comparison of the average lettuce leaves and zucchini damage in different wooden barrieer tests (± SE)

Conclusions

1. Without the use of special measures to control invasive slugs, it is impossible to control the amount of slugs by simply picking and destroying visible slugs. 12 times more slugs were found in VU Botanical Garden in Vingis than in VU Botanical Garden in Kairenai, where chemical plant protection measures are additionally applied.
2. In the Lithuanian climate, the effectiveness of SXG lasts not 8 weeks (as stated in the instructions), but 3 weeks, so after that the application needs to be renewed again.
3. Of the three different barrier control measures tested, it was confirmed that the most effective barrier protection measure is SXG (72.065%), slightly less effective - copper strip (57.741%), and the effectiveness of galvanized sheet was not confirmed at all (32.263%).

6 pav. *Arion vulgaris* (Moquin-Tandon, 1855). Photo by A. Jucevičiūtė

Abstract

The aim of the work is to determine and compare the effectiveness of barrier measures for slug control (SXG, copper strip and galvanized sheet metal) in Lithuania, in field conditions. The analysis of the abundance of invasive slugs *Arion vulgaris* (Moquin-Tandon, 1855) in the Kairenai and Vingis sections of the Vilnius University Botanical Garden shows that there are 12 times more of these slugs in the study area in Vingis section than in Kairenai section. The Analysis of the different barriers showed different efficiencies. SXG was the most efficient (72,065%), copper strip was less effective (57,741%), and the efficiency of galvanized sheet metal was not confirmed at all (32,263%).

Literature

Balčiauskas L., Butkus R., Dagys M., Gudžinskas Z., Šidagytė E., Vaitonis G., Virbickas T., Žalneravičius E. 2017. Invazinės rūšys Lietuvoje.

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Luzitaninis arionas (*Arion lusitanicus* auct., non j. Mabille) https://am.lrv.lt/lt/veiklos-sritys-1/gamtos-apsauga/invazines-rusys/porubrikes-informacija-apie-invazines-rusis/luzitaninis-arionas-arion-lusitanicus-auct-non-j-mabille (žiūrėta: 2022.01.07, 21:35)

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The Tomlin archive... bridging science and history

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 - 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 Contents

SCIENCE & EXPLORATION

- Shell collecting & expeditions
- Identifying specimens & discussing differences of opinion
- Finding & describing new species
- Describing mollusc habitats
- Discussing, writing & requesting malacological publications
- Sending hand-drawn illustrations of shells & anatomy

COMMUNITY

- Arranging shell meetings & collection viewings
- Swapping, donating & selling specimens
- Discussing collections, collectors & type material
- Sharing portraits, handwriting & autograph examples

Archives such as these form a bridge between social history and science, which is a different route into engagement with our natural science collections.

It offers the opportunity to investigate the colonial history of collecting and the communication of women in science during this period.

The Insights

FRIENDSHIP

- Gestures of kindness, support & condolence
- Sending festive greetings
- Invitations to homes
- Sending thanks & appreciation

Tomlin is a prince

SHARING

- Stories from holidays, voyages & expeditions
- Portraits & photographs of personal experiences
- Personal & family illnesses
- Hardships through war & adversity
- Sharing opinions of others in the field

CULTURE

- Sharing differences in cultures, language, environment & geography
- Highlighting women malacologists
- Insights into colonial Britain

Our Volunteers

In 2015 we devised a volunteer transcription programme to make the archive accessible. We worked with 3 dedicated volunteers between 2015-2018 and a further 3 from 2019-2022.

They transcribed each of the letters, explored the characters involved, interpreted the contents, and highlighted 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 Tomlin archive will be published 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.

THEIR SKILLS

Languages
History
Science

THE WORK

Data Entry
Transcription
Translation
Article writing

THE DEVELOPMENT

Handwriting interpretation
Researching people, scientific names & historical references
Working in a museum environment
Article & blog writing

MUSEUM BENEFITS

Much faster progress with the project
A more dynamic & diverse department
Staff gained management experience

VOLUNTEER BENEFITS

Skills development
Influencing career decisions & directions
Support & purpose during the Covid-19 pandemic

Harriet Wood, Jennifer Gallichan & James Turner
Volunteers 2015-2018: Cal James, Elin Sutton, Talia Brown
Volunteers 2019-2022: Susan Jones, Megan Wilkes, Kajal Davies

AMGUEDDFA CYMRU

Department of Natural Sciences
Amgueddfa Cymru - Museum Wales
Cardiff, UK, CF10 3NP
Email: harriet.wood@museumwales.ac.uk

Quantitative gene expression analysis **to shed light** on the role of **5-alpha-reductase** in the **development** of a freshwater gastropod.

Konstantinos Panagiotidis[1], Giang Huong Duong[1], Alice Baynes[1]

[1]Department of Life Sciences, Brunel University London, Uxbridge, United Kingdom. Email: konstantinos.panagiotidis@brunel.ac.uk

1. Introduction

In vertebrates, steroidogenic enzymes 5-alpha reductases (**5aR**) convert **testosterone** to 5a-dihydrotestosterone. However, the function of **5aR1 & 5aR2** in molluscs remains **unknown**.

Pharmaceutical disruption of 5aR in ***Biomphalaria glabrata*** was shown to disrupt normal embryo development causing the emergence of a **"banana-shaped" shell**[1].

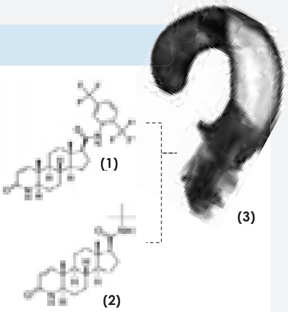


Figure 1: Pharmaceutical exposure to Dutasteride (1) and Finasteride (2) causes the emergence of "banana-shaped" shell in *B. glabrata* embryo (3).

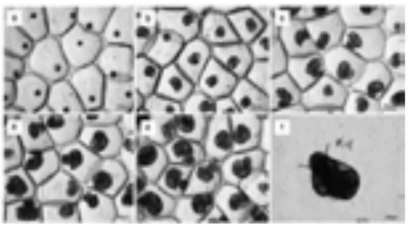
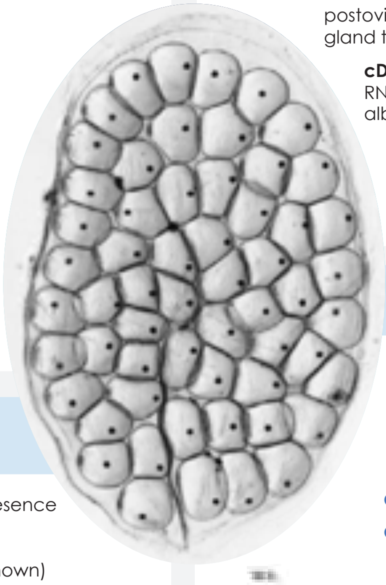


Figure 2: Early developmental stages of *Biomphalaria glabrata* embryos; **(a)** day 1 postoviposition, **(b)** day 2 postoviposition, **(c)** day 3 postoviposition, **(d)** day 4 postoviposition, **(e)** day 5 postoviposition, **(f)** early juvenile.



3. 5aR expressed in embryos and albumen gland tissues

Gene expression analysis confirmed the presence of **5aR1** and **5aR2** in:

- day 2 postoviposition embryos (data not shown)
- day 3-5 postoviposition embryos and albumen gland tissues (Figure 4).

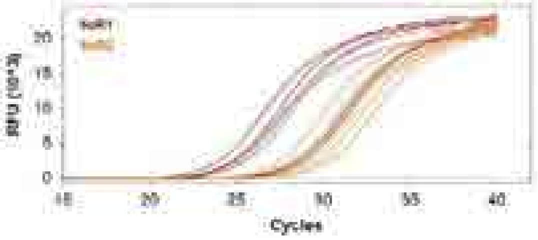


Figure 4: Quantification of 5aR1 and 5aR2 in day 3-5 postoviposition embryos and albumen gland tissues by RT-qPCR.

2. Methodology

Aims:

1. Identify **patterns of expression** of 5aR.
2. Identify **endogenous control genes** in ***B. glabrata*** **embryos.**
3. Understand the **role of 5aR** in early development of ***B. glabrata***.

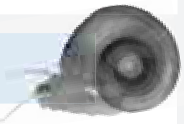
Figure 3: Embryo collection and RNA isolation from *Biomphalaria glabrata*: ***(1)*** *dissection of embryos from egg capsules,* ***(2)*** *preservation of embryos in RNA-later,* ***(3)*** *removal of RNA-later,* ***(4)*** *homogenization of tissue,* ***(5)*** *RNA extraction using RNA isolation kit.*

Total RNA was extracted from day 1 - day 5 postoviposition embryos (Figure 2 & 3) and albumen gland tissue.

cDNA synthesis was carried out using 200ng total RNA from each embryo stage (Figure 2), and albumen gland (i.e. adult) tissues respectively.

RT-qPCR was used to confirm the presence and measure the "quantity" of **5aR1 & 5aR2** in all samples.

4. Preliminary normalisation data



Candidate endogenous control genes **Lhis2a, Lywhaz** (previously validated in *Lymnaea stagnalis*[2]), **Myoblogin** and **a-Tubulin** are being validated for stability.

Group 1: day 2-5 embryos + Albumen gland

Group 2: day 2-5 embryos

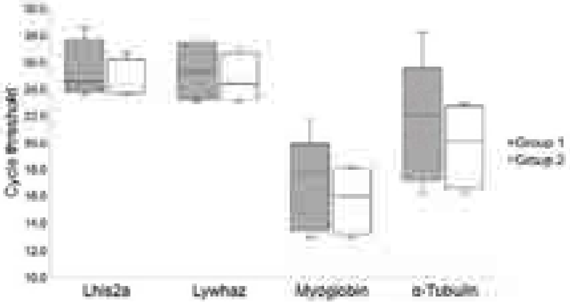
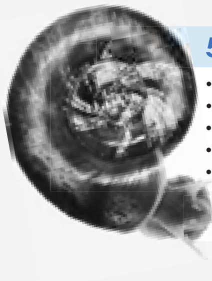


Figure 5: Cycle threshold (Ct) values (n=3) for candidate endogenous control genes in group 1 (day 2-5 embryos & albumen gland) and group 2 (day 2-5 embryos).



5. Limitations and future approaches

- **Lhis2a** and **Lywhaz** normal expression levels were altered by NRT amplification.
- Validation of endogenous control genes in *B. glabrata* is a work in progress.
- Plans to test and validate **>10 extra candidates** that were identified through literature.
- Stability normalisation software will be implemented to find the most suitable pair.
- **5aR1** and **5aR2** will then be normalised against the most suitable pair of endogenous control genes.

References: [1]Baynes, A., Montagut Pino, G., Duong, G. H., Lockyer, A. E., McDougall, C., Jobling, S., & Routledge, E. J. (2019). Early embryonic exposure of freshwater gastropods to pharmaceutical 5-alpha-reductase inhibitors results in a surprising open-coiled "banana-shaped" shell. Scientific Reports, 9(1), 1–12. https://doi.org/10.1038/s41598-019-52850-x

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Picture credits: Dr Alice Baynes, Tom Kennedy and Coen Adema/UNM

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

A. Jochum[1], P. Michalik[2] & B. Ruthensteiner[3]

[1] Naturhistorisches Museum Bern (NMBE) and University of Bern
[2] Zoologisches Institut und Museum, University of Greifswald
[3] SNSB–Zoologische Staatssammlung München

Introduction:

The character poor shells (< 1.5 mm) of the subterranean genus *Zospeum* (Bourguignat, 1856) are difficult to morphologically access for taxonomic assessment. Due to their small size and fragility and the associated difficulty of finding them alive for molecular and ecological studies, accessing information from rare shells is a taxonomic priority (Jochum et al. 2015). Though we know more about *Zospeum*'s widely sampled northern distribution in caves of the Eastern Alpine and Dinaride mountain ranges (Inäbnit et al 2019), its southernmost range in southern Croatia, Bosnia-Herzegovina and Montenegro is still largely underexplored and under sampled. In this study, we use 3D X-ray microscopy to access significant characters in *Zospeum*'s only known southern Balkan member, *Z. troglobalcanicum* Absolon, 1916. Up to now, this species has lacked known voucher material and is documented via a single image of 13 shells piled together from the early 1900's. Recent research at the Natural History Museum Vienna (NHMW), Vienna, Austria has recovered a singular syntype shell deriving from Absolon's collection. By comparing 3D X-ray images of this shell to those from shells deriving from 15 different southern Balkan cave populations culled from museum collections, we identify significant internal character states for interpreting and proposing species hypotheses based on these morphological data.

Southern Balkan Cave populations (15) assessed in our study.

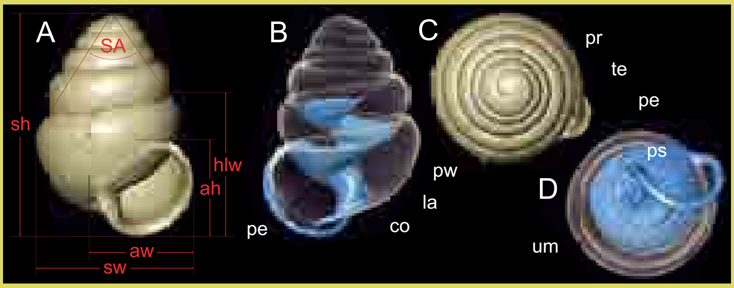
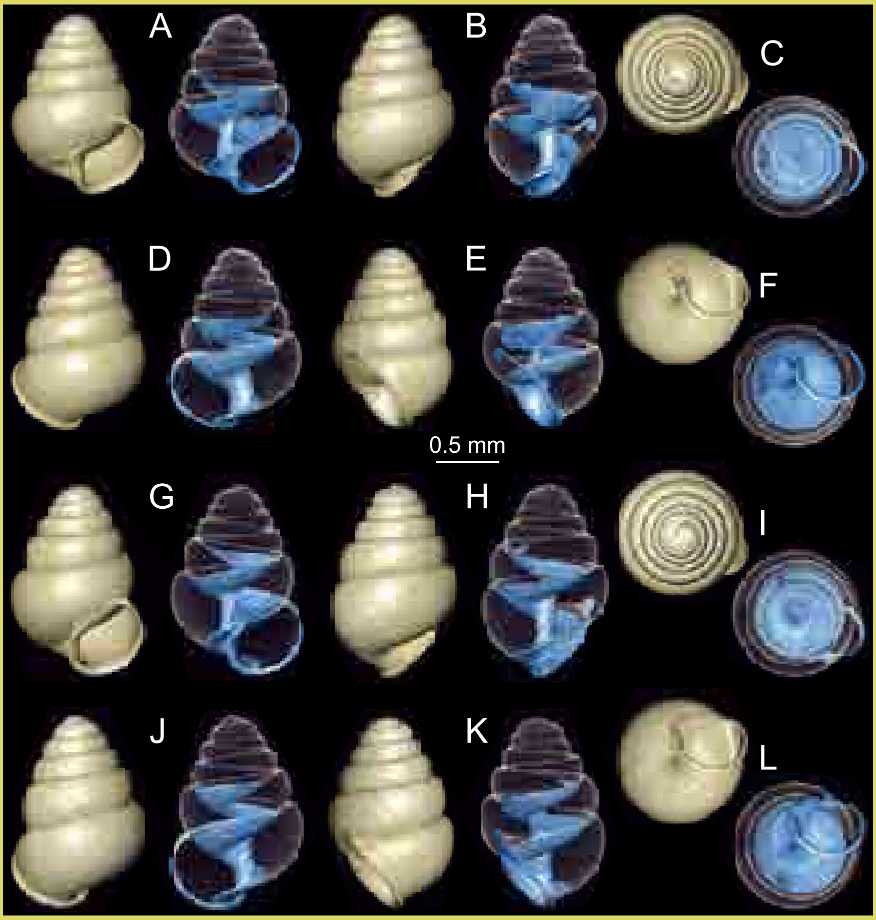


Image of proposed lectotype of *Zospeum troglobalcanicum* Absolon, 1916 from Benetina pećina cave, Herzegovina (NHMW Moll.Coll.Edlauer 32.749) showing assessed morphological character states. A. Measurements include shell height (sh), shell width (sw), aperture height (ah), aperture width (aw), height of last whorl (hlw) and spire angle (SA). B–D. Morphological character states include columella configuration (co), Lamella form and position on the columella (la), peristome configuration (pe), whorl number and form (wh), protoconch form (pr), teleoconch sculpture (te), parietal shield shape and size (ps) and the configuration of the umbilical zone (um).



3D images of X-ray CT scanned shells from Montenegro. Shells from two different populations aligned showing character states differentiating two different species: A–F, paralectotype of *Z. troglobalcanicum* Absolon, 1916 (MCSMNH-PMSL-Mol.-FVelkovrh 29606) Lipska pećina and G–L, *Z. orisellipticum* Jochum & Ruthensteiner sp. n. (MCSMNH-PMSL-Mol.-FVelkovrh 30360) Duboki do cave.

Methods:

A Phoenix Nanotom m (mostly 1600 projection images, 360° rotation for ca. 80 mins, voxel size ca. 1 µm, voltage 80 kV, current 325 µA) and an XRadia MicroXCT-200 (mostly 1600 projection images, 360° rotation for ca. 90 mins, voxel size ca. 2 µm, voltage 40 kV, current 200 µA) X-ray micro-computed tomography system were used for the micro-CT acquisition. 3D graphical procedures were carried out with Amira 6.4 software applying manual segmentation for discrimination of external and internal shell structures. Final visualization was conducted using the Volume Rendering module.
Shells were measured for height (sh), shell width (sw), aperture height (ah), aperture width (aw), height of last whorl (hlw) and spire angle (SA). Diagnostic and measurement tables were constructed to compare significant morphological features (ie. character states) recognized in each of the 3D-scanned perspectives. Species were assigned based on these criteria.

Results & Discussion:

Our study integrated both internal shell and external morphological data, providing taxonomic support for the species, *Zospeum troglobalcanicum* and *Z. simplex* Inäbnit, Jochum & Neubert 2021 as well as strong evidence for 10 undescribed species deriving from 15 populations. A lectotype is proposed for *Z. troglobalcanicum*. Overall, we found that 3D X-ray microscopy significantly aided in the determination of characters and the interpretation of morphological analyses. Moreover, since the tiny shells are devoid of any residual animal tissue (ie. mummies), precluding eventual molecular study using this option, 3D X-ray microscopy is the best means for nondestructively accessing and assessing such material. Our results not only demonstrate the relatively high morphological diversity in zospeid snails of this region but are in sync with other studies concerning single site, narrow-range endemics in subterranean fauna of the southern Western Balkans. Characters such as the internal configuration of the columella, its terminal position in the alignment of the umbilical notch, and the presence of a weak and incomplete lamella reflect locally isolated speciation processes. Until new exploration and fresh material allows for additional molecular investigations of populations from the same caves in an integrative taxonomic approach, this comprehensive study especially provides a solid basis of morphological data for all future studies concerning congeners at *Zospeum*'s southernmost Dinaric range.

References:

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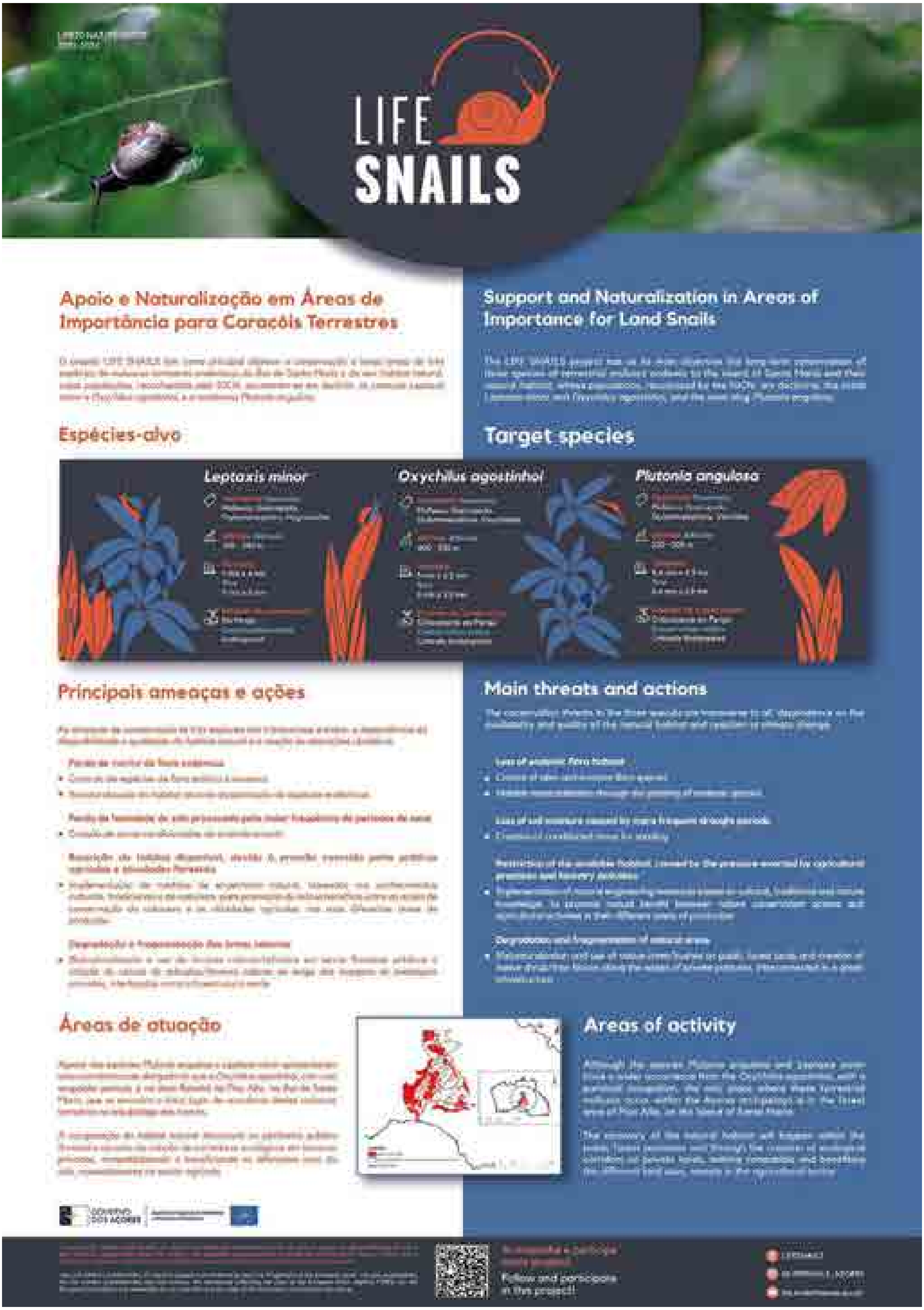
Jochum A, Slapnik R, Klussmann-Kolb A, Páll-Gergely B, Kampschulte M, Martels G, Vrabec M, Nesselhauf C, Weigand AM (2015) Groping through the black box of variability: An integrative taxonomic and nomenclatural re-evaluation of *Zospeum isselianum* Pollonera, 1887 and allied species using new imaging technology (Nano-CT, SEM), conchological, histological and molecular data (Ellobioidea, Carychiidae). Subterranean Biology 16: 123–165.

Acknowledgements:

We thank Marko Vrabec for compiling the map and Anita Eschner (NHMW) and Rajko Slapnik for kindly loaning us shells housed in the collections in Vienna and Ljubljana.



LIFE SNAILS

Apoio e Naturalização em Áreas de Importância para Caracóis Terrestres

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Support and Naturalization in Areas of Importance for Land Snails

[illegible]

Espécies-alvo

Target species

Leptaxis minor

Oxychilus agostinhoi

Plutonia angulosa

Principais ameaças e ações

[illegible]

Main threats and actions

[illegible]

Áreas de atuação

[illegible]

Areas of activity

[illegible]

Acompanhe e participe neste projeto!

Follow and participate in the project!

b-tu
Brandenburgische
Technische Universität
Cottbus - Senftenberg

The world in a snail shell

by Claudia Tluste, Klaus Birkhofer and Udo Bröring

Tluste, C., & Birkhofer, K. (2021). Shells of the Roman snail are important microhabitats for soil invertebrates. Soil Organisms, 93(3), 141-152.

INTRODUCTION

Shells of molluscs from snail farms are used as food additives or construction material but can also function as a microhabitat for wild bees or other arthropods (Müller et al, 2008; Potts et al., 2005). Especially shells of *Helix pomatia* (Linnaeus, 1758) are more often used by shell adopters, which may be due to the relatively large shell size (Bogusch et al., 2019; Heneberg et al., 2020).

With this study, we aim to understand how **shell size**, the **local dominant vegetation** and **climatic differences between sample periods** affect utilization patterns by shell adopters.

METHOD

1408 empty shells of H. pomatia were placed in eight subpopulations. 704 in Feb. 2019 till Nov. 2019 and 704 in Feb. 2020 till June/July 2020. Within those subpopulations, we choose 16 study plots based on two dominant vegetation types: herbaceous vegetation or deciduous trees without herbaceous vegetation.

LITERATURE

Bogusch, P., Roháček, J., Baňař, P., Astapenková, A., Kouklík, O., Pech, P., Janšta, P., Heller, K., Hlaváčková, L. & Heneberg, P. (2019): The presence of high numbers of empty shells in anthropogenic habitats is insufficient to attract shell adopters among the insects. - In Insect Conservation and Diversity 12: 193–205, DOI: 10.1111/icad.12335
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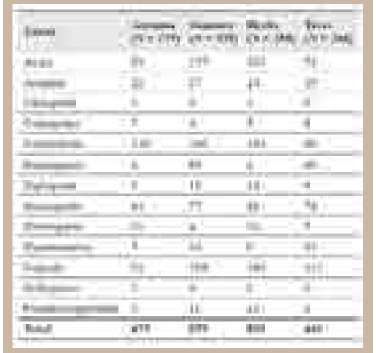
Müller, A., Praz, C. & Dorchin, A. (2018): Biology of Palaearctic Wainia bees of the subgenus Caposmia including a short review on snail shell nesting in osmiine bees (Hymenoptera, Megachilidae). - In Journal of Hymenoptera Research 65: 61–89, DOI: 10.3897/jhr.65.27704
Potts, S.G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'Eman, G. & Willmer, P. (2005): Role of nesting resources in organising diverse bee communities in a Mediterranean landscape – In Ecological Entomology 30: 78–85, DOI: 10.1111/j.0307-6946.2005.00662.x

RESULTS & DISCUSSION

91.4 % of all collected shells were occupied and the average number of shell adopters was 1.5 time higher in shells collected in summer compared to autumn. The number of shell adopters per shell was 1.5 times higher in study areas dominated by herbaceous vegetation compared to trees. Shell width significantly affected the composition of shell adopter communities. Shells of *H. pomatia* provide important multipurpose benefits for a wide range of soil organisms. The use of empty shells from heliciculture in local restoration projects of open, tree-free areas, holds the potential to support a diverse invertebrate fauna with additional refuge habitats.



SPIXIANA	Supplement 30 B	WCM 2022	SPD	06	München, September 2022	ISSN 0177-7424

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

Bianca Sartini do Espirito Santo[1,2,] Mariana Fonseca Rossi[1,2,3], Ximena Maria Constanza Ovando[1,2] & Sthefane D'ávila[2]
1Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil.
2Museu de Malacologia Prof. Maury Pinto de Oliveira, Universidade Federal de Juiz de Fora, MG, Brazil.
3Laboratório de Protozoologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil.
Email: sthefanedavila@hotmail.com

Email: sthefanedavila@hotmail.com

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*.

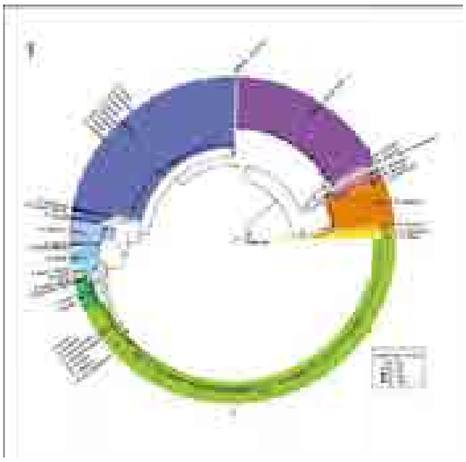


Figure 1. Phylogenetic tree inferred by maximum likelihood based on 657 bp of the mitochondrial cytochrome oxidase subunit I (COI) gene of Physidae species. *Pseudosuccinea collumela* (Say, 1817) was used as outgroup. The circles close to the nodes represent the bootstrap values. The evolutionary significant units (ESUs) delimited by the *multi-rate Poisson Tree Processes* (mPTP) are highlighted in the colors frames.

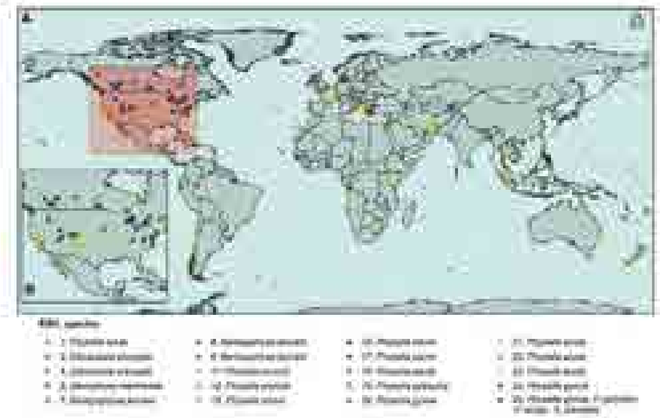


Figure 2. Geographic distribution of the significant evolutionary units (ESUs) recovered using the coalescent delimitation method *multi-rate Tree Poisson Process* – mPTP.

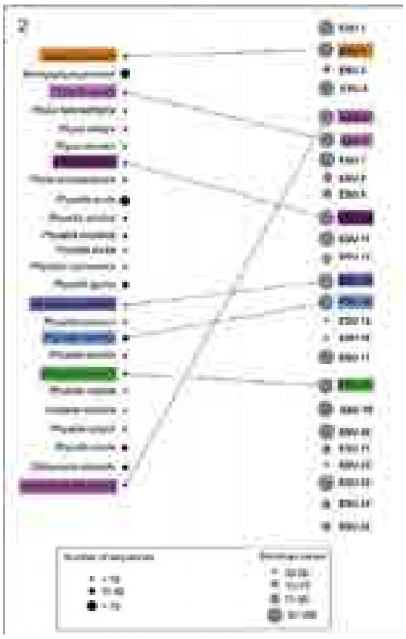


Figure 3. Nominal species recovered as significant evolutionary units (ESUs) by the coalescent delimitation method *multi-rate Tree Poisson Process* − mPTP.

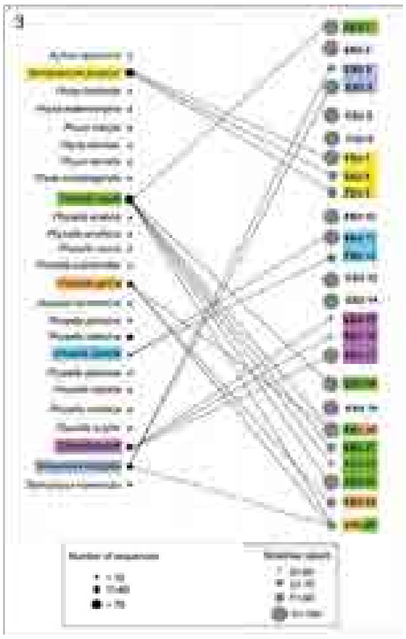


Figure 4. Splitter cases in Physidae using the coalescent delimitation method *multi-rate Tree Poisson Process* − mPTP. ESU: significant evolutionary unit.

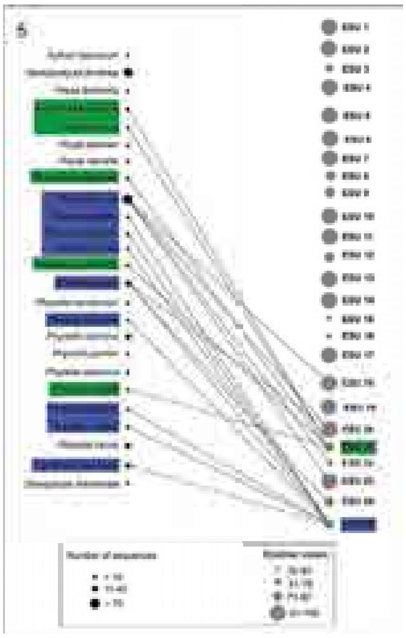


Figure 5. Lumper cases in Physidae using the coalescent delimitation method *multi-rate Tree Poisson Process* − mPTP. ESU: significant evolutionary unit.

EXTENDED MOLLUSK SPECIMEN NETWORK AT THE UNIVERSITY OF MICHIGAN MUSEUM OF ZOOLOGY (UMMZ)

Taehwan Lee, Thomas F. Duda, Jr., and Diarmaid Ó Foighil
University of Michigan, Department of Ecology and Evolutionary Biology and Museum of Zoology
3600 Varsity Dr., Ann Arbor, MI 48108 USA

Introduction

The UMMZ Mollusk collection has served as a premier resource for scientific research and education for over a century. To enhance its utility, we have led a long-term effort to digitize the collection and to link specimen records with associated extended data. This process has transformed the collection into a globally-accessible, integrated body of information that has consistently generated > 60,000 downloads and > 50 million views annually since 2019. Our future endeavors will focus on the continued development of the collection's extended specimen database.

UMMZ Mollusk Collection

The University of Michigan began building a collection of mollusks in the first half of the 19th century. With current holdings of approximately five million specimens, the Mollusk Division's collection has long ranked as one of the most important freshwater and land snail collections in North America.

- Houses ~ 251,000 cataloged lots including > 400 holotypes and > 1600 paratype lots
- Holds excellent taxonomic and geographic coverage of non-marine mollusks
- Includes several significant subcollections:
  - *Bryant Walker Collection*: one of the world's largest private mollusk collections with >100,000 lots and ~2 million specimens
  - *Royal Ontario Museum Collection*: North American, mainly Canadian, freshwater/land mollusks
  - *Stelfox Sphaeriid Collection*: important reference collection of cosmopolitan freshwater bivalve
  - *Lyophilized Tahitian Partulids*: ~1600 freeze-dried tissues of a largely extinct snail family

A New Century for the UMMZ (Research Museums Center)

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.

Fig. 1. Newly renovated Research Museums Center (RMC)

Digitizing Molluscan Biodiversity at UMMZ

Since the turn of the century, data for > 200,000 lots of mainly freshwater and terrestrial mollusks have been entered into the UMMZ *Specify* database, a process facilitated by the support of five National Science Foundation (NSF) awards, including four Thematic Collections Networks (TCN) grants.

I. Computerization of the University of Michigan Museum of Zoology's Mollusk Collection (2005-2010)

- Implementation of *Specify* as the UMMZ Mollusk Division Database
- Development of a searchable online mollusk collection database on the UMMZ website

II. Great Lakes Invasives TCN:

Documenting the Occurrence through Space & Time of Aquatic Non-indigenous Fish, Mollusks, Algae & Plants Threatening North America's Great Lakes (2014-2018)

- Digitization of non-indigenous species, and their congeneric indigenous taxa, present in the Great Lakes Basin.
- Participation of > 25 institutions from Canada and eight U.S. states

Fig. 2. Student workers image specimens and enter associated data into the local *Specify* database.

III. InvertEBase TCN:

Reaching Back to See the Future: Species-Rich Invertebrate Faunas Document Causes and Consequences of Biodiversity Shifts (2014 - 2019)

- Rapid data entry of georeferenced locality data to form a solid foundation for distribution mapping and to examine change over time due to large scale perturbations
- Inclusion of 18 arthropod/mollusk collections with special focus on the US fauna

IV. PILSBRy TCN:

Enhancing Access to Taxonomic and Biogeographical Data to Stem the Tide of Extinction of the Highly Imperiled Pacific Island Land Snails (2019 - 2023)

- Digitization and enhancement of biological information of land snails from Polynesia-Micronesia + East Melanesia biodiversity hotspots
- Development of a centralized online online database - PILSBRy.

V. ESB TCN:

Mobilizing Millions of Marine Mollusks of the Eastern Seaboard (2020 - 2024)

- Assembly of data from roughly 4.5 million mollusk specimens from 14 institutions
- Improved access to data that can be utilized to establish patterns of genetic/morphological variation of mollusks on the Gulf and Atlantic coasts of the U.S.

VI. Achievement:

- > 50,000 specimen images and > 10,000 label/catalogue scans have been added to verify and enhance specimen data
- Networking of georeferenced locality data have formed a solid foundation for distribution mapping
- Digitized data and images are available through Symbiota web portals (greatlakesinvasives.org, invertebase.org and pilsbry.org), national resources (idigbio.org and gbif.org) and the University of Michigan Digital Library.
- A total of 106 students (UM/non-UM graduates/undergraduates and high school volunteers) have received training and have collectively participated in this effort so far.

Fig. 3. Generated images and extracted data are uploaded to Symbiota, iDigBio, GBIF portals and the UM Digital Library where they can be accessed by the general public.

Future Directions

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.

Fig. 4. Some of the 106 students and volunteers who have participated to-date in digitizing molluscan biodiversity at the UMMZ.

Acknowledgments: These awards are made as part of the National Resource for Digitization of Biological Collections through the Advancing Digitization of Biological Collections program (EF 14-05302 and EF 14-04964) and all data resulting from this award will be available through the national resource (iDigBio.org).

Museum für Naturkunde Berlin

Fraunhofer IFF

DORA

DIGITIZE-ORGANIZED-RELIABLE-AUTOMATED

Dr. Nora Lentge-Maaß [1]
Collection Transformation Manager for Invertebrates and Paleobotany

Erik Trostmann [2]
Optical Metrology

Margot Belot [1]
Scan Operator

Michael Schiller [2]
Software Development

Silvio Sperling [2]
Design and Electrical Engineering

Dr.-Ing. Andreas Herzog [2]
Cognitive Processes and Systems

Dr. Frederik Berger [1]
Collection Digitization

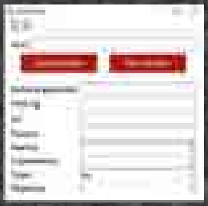
[1] Museum für Naturkunde (MfN)
Leibniz Institute for Evolution and Biodiversity Science
Department for Collection Future
Invalidenstrasse 43
10115 Berlin – Germany

contact: **DORA@mfn.berlin**

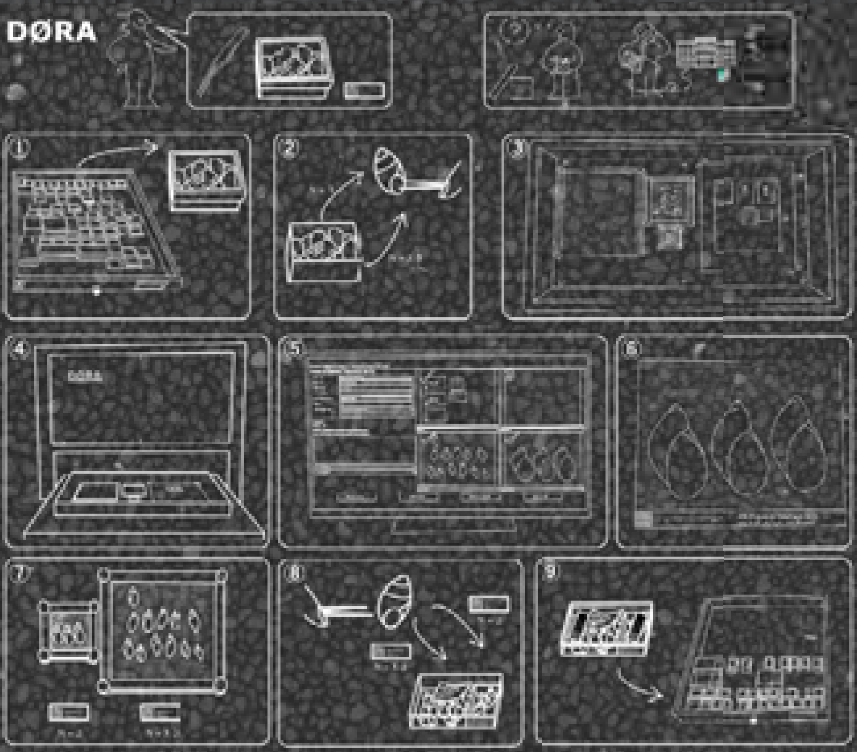
[2] Fraunhofer Institute for Factory Operation and Automation IFF
Sandtorstrasse 22
39106 Magdeburg – Germany

Munich 2022

SETUP



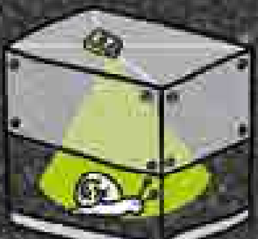
WORKFLOW



RESULTS

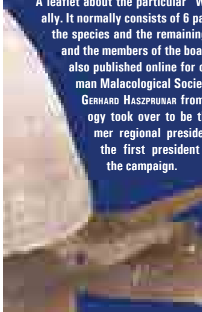
NEXT

5MIN PER LOT



250.000 LOTS IN 4 YEARS

...



In 2002 this campaign was founded by the late Karl-Heinz Beckmann (1948-2007), who was a member of the board of the German Malacological Society ("Deutsche Malakozoologische Gesellschaft" = DMG). The campaign was intended to publish information about the chosen "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 "Weichtier des Jahres" by texts and photographs.

The "Weichtier des Jahres" is elected by a board of trustees ("Kuratorium"), comprising the malacological societies and institutions in Germany. A leaflet about the particular "Weichtier des Jahres" is produced annually. It normally consists of 6 pages of which 4 respectively 5 are about the species and the remaining part is explaining the campaign itself and the members of the board of trustees. The leaflet is printed and also published online for open access on the webpage of the German Malacological Society.

Gerhard Haszprunar from the Bavarian State Collection of Zoology took over to be the public relations officer and the former regional president Wilhelm Weidinger (1939-2018) was the first president and figurehead for the first years of the campaign.

Shortly before his death Karl-Heinz Beckmann handed over the responsibility to Vollrath Wiese and asked him to organize the "Weichtier des Jahres" on behalf of the German Malacological Society.

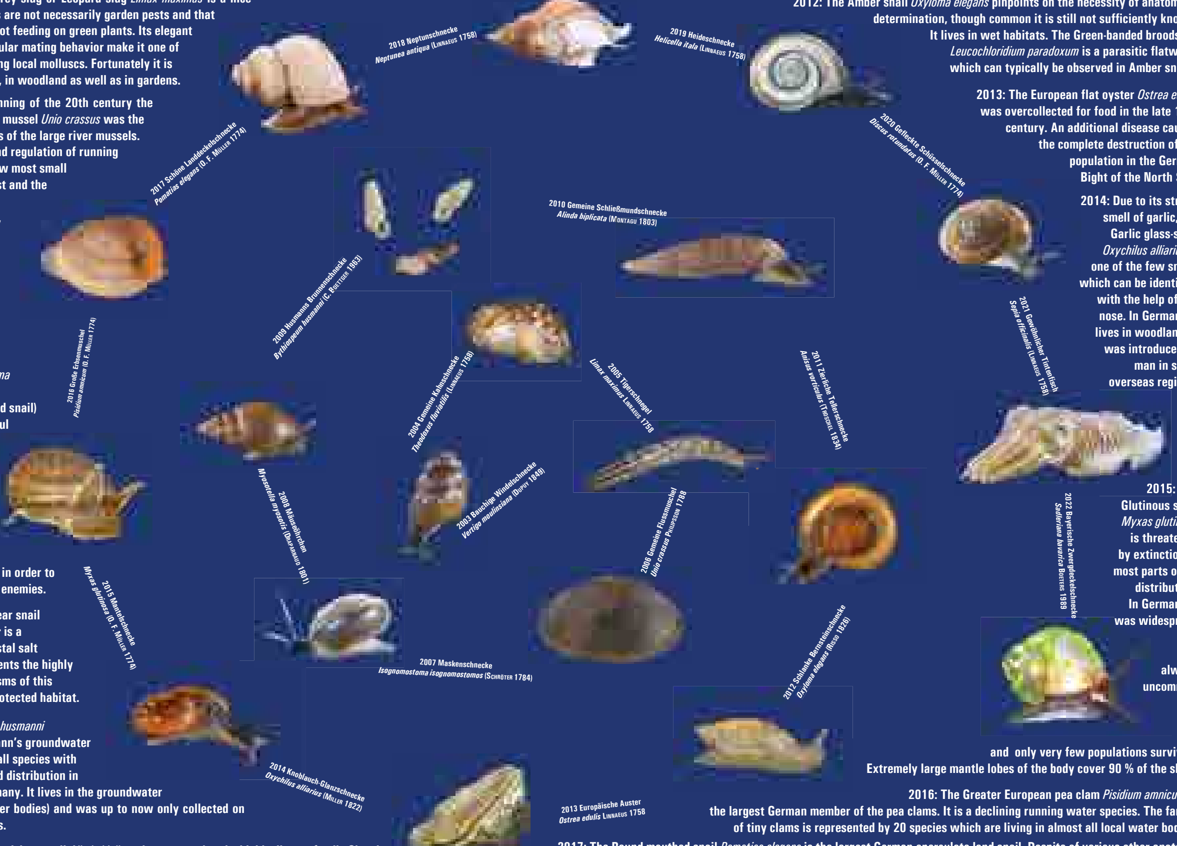
20 years of "Weichtier des Jahres" (German Mollusc of the Year)

by Vollrath Wiese

The „Weichtier des Jahres" (German Mollusc of the Year) project presents interesting molluscs to the public since 2003. In Germany various annual promotions of animals, plants or biotopes are established. When "Weichtier des Jahres" was founded, about a dozen other themes of nature objects already existed.

2003: The first German Mollusc of the Year was *Vertigo moulinsiana*. One of the starting points was, that in 2002 the German Post printed two beautiful stamps with molluscs as representatives of endangered animals. These were the first special mollusc stamps in Germany, including a nice picture of *Vertigo moulinsiana*. With 50 millions of printed stamps this species was broadly introduced to the public. So it was decided to explain a bit more about the biology of this tiny swamp snail, which is only about 2.5 mm in length, specializing to live predominately on *Carex* and protected by the Habitats Directive (FFH) of the European Union.

2004: The River nerite *Theodoxus fluviatilis* is a species of the primeval mollusc group of Neritimorpha. In Germany it is living in larger rivers or on rocky shores of lakes. Its populations are declining and the species is endangered. Two closely related species in Germany are only represented by very small relic populations and are threatened by extinction.



2005: The Great grey slug or Leopard slug *Limax maximus* is a nice example that slugs are not necessarily garden pests and that most species are not feeding on green plants. Its elegant shape and spectacular mating behavior make it one of the most fascinating local molluscs. Fortunately it is still quite common, in woodland as well as in gardens.

2006: At the beginning of the 20th century the Thick shelled river mussel *Unio crassus* was the commonest species of the large river mussels. Due to pollution and regulation of running water systems, now most small populations are lost and the species is highly endangered, in some regions threatened by extinction. Maybe 90 % of their former populations are destroyed.

2007: *Isognomostoma isognomostomos* (translated: Masked snail) is showing beautiful periostracal hairs on its 10 mm shell and has almost closed the opening of the shell with heavy lamellae and teeth in order to protect itself from enemies.

2008: The Mouse ear snail *Myosotella myosotis* is a species of the coastal salt marshes. It represents the highly specialized organisms of this endangered and protected habitat.

2009: *Bythiospeum husmanni* (translated: Husmann's groundwater snail) is a very small species with extremely localized distribution in westernmost Germany. It lives in the groundwater (subterranean water bodies) and was up to now only collected on very few occasions.

2010: The Two lipped door snail *Alinda biplicata* is representing the highly diverse family Clausiliidae which is living in many gardens in central Europe. Its elongated shell has an interesting internal closing system of plicae, lamellae, and the clausilial plate.

2011: The Lesser ramshorn snail *Anisus vorticulus* is one of the few species which are strictly protected by the Habitats Directive of the European Union. Quite often the common *Anisus vortex* is misidentified as the much rarer and not easily determined *Anisus vorticulus*. Originally it seems to be a species of natural floodplains along the larger rivers.

2012: The Amber snail *Oxyloma elegans* pinpoints on the necessity of anatomical determination, though common it is still not sufficiently known. It lives in wet habitats. The Green-banded broodsack *Leucochloridium paradoxum* is a parasitic flatworm which can typically be observed in Amber snails.

2013: The European flat oyster *Ostrea edulis* was overcollected for food in the late 19th century. An additional disease caused the complete destruction of the population in the German Bight of the North Sea.

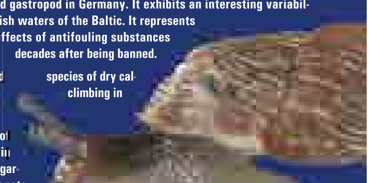
2014: Due to its strong smell of garlic, the Garlic glass-snail *Oxychilus alliarius* is one of the few snails which can be identified with the help of our nose. In Germany it lives in woodland, it was introduced by man in some overseas regions.

2015: The Glutinous snail *Myxas glutinosa* is threatened by extinction in most parts of its distribution. In Germany it was widespread but always uncommon and only very few populations survived. Extremely large mantle lobes of the body cover 90 % of the shell.

2016: The Greater European pea clam *Pisidium amnicum* is the largest German member of the pea clams. It is a declining running water species. The family of tiny clams is represented by 20 species which are living in almost all local water bodies.

2017: The Round-mouthed snail *Pomatias elegans* is the largest German operculate land snail. Despite of various other anatomical specials one of the most unusual facts about it is its way of walking by alternately using the longitudinal parts of its sole.

2018: The Red whelk *Neptunea antiqua* is the largest shelled gastropod in Germany. It exhibits an interesting variability from the offshore regions of the North Sea to the brackish waters of the Baltic. It represents endangered marine molluscs and is an example for toxic effects of antifouling substances decades after being banned.



2019: The Heath snail *Helicella itala* represents the endangered species of dry calcareous grassland. Using pairs of darts before copulation and climbing in the vegetation for aestivation is typical for *Helicella*.

2020: The Rotund disc *Discus rotundatus* is one of the commonest and most widespread molluscs in central Europe. It may be found in most natural gardens, even commonly on the underside of plant pots. It is an example for the many small snails, which are harmless to garden plants.

2021: The Common cuttlefish *Sepia officinalis* as a cephalopod demonstrates the highest evolution among molluscs. Its cuttlebones are commonly collected in beach drift on the shores of the North Sea.

2022: The Bavarian spring snail *Sadleriana bavarica* is an example for very restricted distribution, in Germany only known in one spring of Munich. Therefore it is also representing the 2022 World Congress of Malacology which is hosted by the malacologists of Munich.

References:

Hundreds of publications, exhibits and digital media have presented the molluscs of the year. The leaflets are available for download on the webpage of the German Malacological Society (www.dmg.mollusca.de). Supported by funds of Karl-Heinz Beckmann and later of the DMG the printed leaflets are available for free. Examples for complete printed texts in Journals are the following, for those not available as complete texts in journals the original leaflet citation is given:

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--- (2015): Weichtier des Jahres 2014: Die Knoblauch-Glanzschnecke *Oxychilus alliarius* (Miller 1822). – Conchylia, 45 (1-3): 27-29.
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--- (2018): Weichtier des Jahres 2018. Die Neptunschnecke, *Neptunea antiqua* (Linnaeus 1758). – Conchylia, 49 (1-2): 51-54.
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Address of the author: Dr. Vollrath Wiese, Haus der Natur · Cismar, Bäderstr. 26, 23743 Cismar, Germany, vwiese@hausdernatur.de

In 2022 members of the "Kuratorium Weichtier des Jahres" are:

Deutsche Malakozoologische Gesellschaft [Prof. Dr. Thomas Wilke, Giessen]
Friedrich-Held-Gesellschaft e.V., München [Gerhard Falkner, Wörth-Hörlkofen]
Club Conchylia e.V., Öhringen [Roland Hoffmann, Kronshagen]
Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt a. M. [Prof. Dr. Julia Sigwart]
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Senckenberg Museum für Naturkunde Görlitz [Dr. Heike Reise]
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LIB, Zoologisches Museum Hamburg [Prof. Dr. Bernhard Hausdorf]
Haus der Natur - Cismar (Malakologisches Museum) [Dr. Vollrath Wiese]
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Arbeitskreis Mollusken Rheinland-Pfalz [Klaus Groh, Bad Dürkheim]
Arbeitskreis Mollusken Nordrhein-Westfalen [Hajo Kobialka, Höxter-Corvey]
Arbeitskreis Mollusken Mecklenburg-Vorpommern [Dr. Michael L. Zettler, Rostock]

Special thanks are going to all members of the "Kuratorium Weichtier des Jahres" for their active cooperation to accumulate reliable and interesting information about the molluscs of the year and for their enthusiastic discussions of the facts.

Photos: *Sepia* © Klaus Janke,
Sadleriana © Bastian Brenzinger,
all other photos:
© Haus der Natur - Cismar

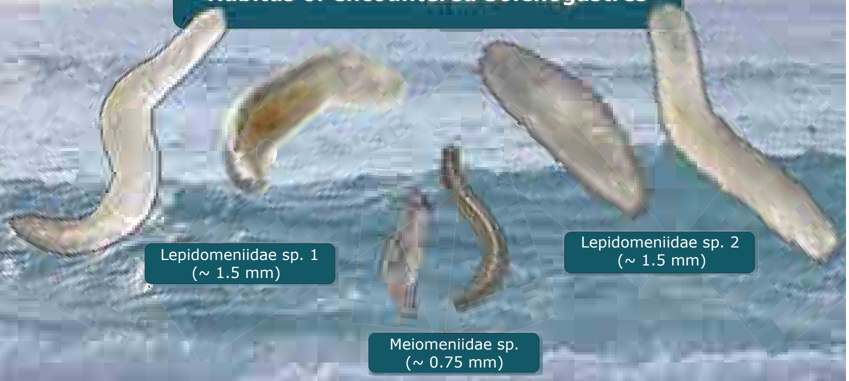
Aloha!
First shallow-water Solenogastres from Hawai'i

Katharina M. Jörger[1], Meghan Yap-Chiongco[2], Kevin M. Kocot[2], Franziska S. Bergmeier[2,3]
[1]Mollusca Department, SNSB-Bavarian State Collection of Zoology, Munich, Germany. Email: joerger@snsb.de
[2]Department of Biological Sciences, The University of Alabama, Tuscaloosa, USA.
[3]Systematic Zoology, LMU Munich, Germany.

Background

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-water. 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 in two different families (Lepidomeniidae and Meiomeniidae), based on scleritome and anatomical data, as well as phylogenetic analyses.

Habitus of encountered Solenogastres



Lepidomeniidae sp. 1 (~ 1.5 mm)

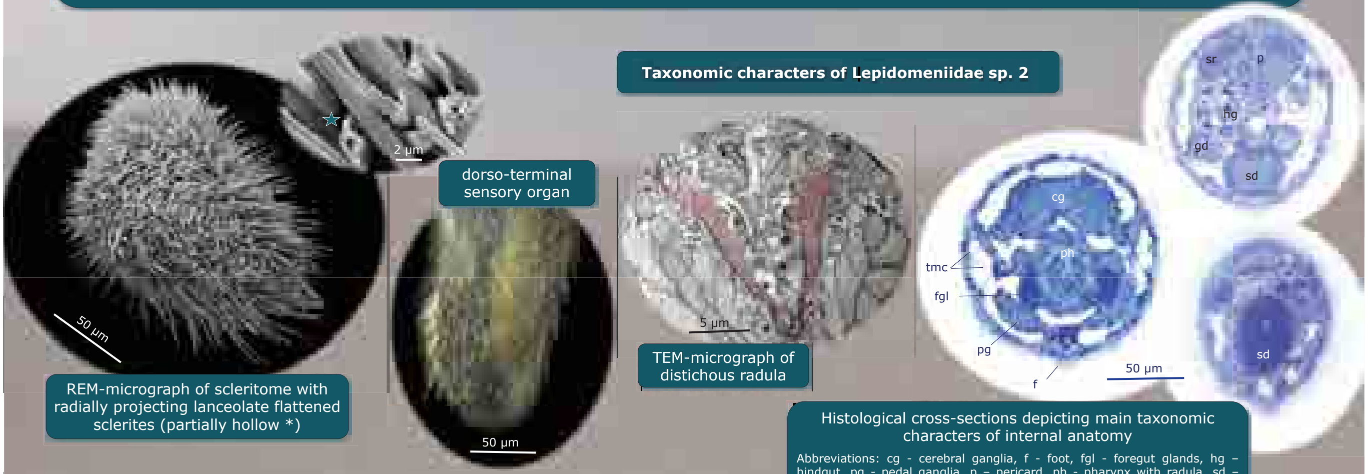
Meiomeniidae sp. (~ 0.75 mm)

Lepidomeniidae sp. 2 (~ 1.5 mm)

Lepidomeniidae from Hawai'i

While Meiomeniidae sp. and Lepidomeniidae sp.1 present characters typical for the respective meiofaunal family, Lepidomeniidae sp. 2 challenges our current classificatory system by a unique mix of characters: In contrast to the otherwise adpressed scleritome in lepidomeniids composed of different types of imbricated scales, which gives them a smooth and shiny appearance, it bears elongate, radially projecting lanceolate sclerites. Other taxonomic characters include a distichous radula and foregut glands of Type A, numerous large, turgescent mesenchymal cells, which fill the entire body cavity, an unusual muscular spawning duct and the presence of a dorso-terminal sensory organ:

Taxonomic characters of Lepidomeniidae sp. 2



REM-micrograph of scleritome with radially projecting lanceolate flattened sclerites (partially hollow *)

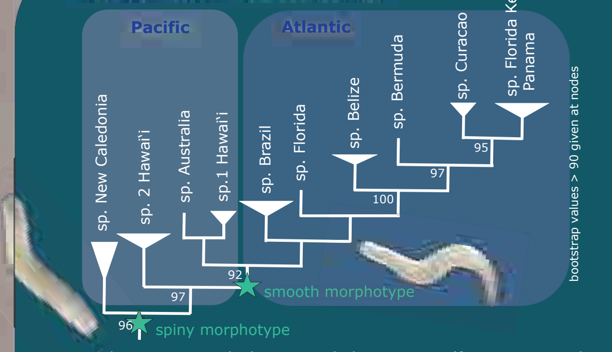
dorso-terminal sensory organ

TEM-micrograph of distichous radula

Histological cross-sections depicting main taxonomic characters of internal anatomy

Abbreviations: cg - cerebral ganglia, f - foot, fgl - foregut glands, hg – hindgut, pg - pedal ganglia, p – pericard, ph - pharynx with radula, sd – spawning duct, sr – seminal receptacle, tmc - turgescent mesenchymal cells

Phylogenetic relationships



Candidate species with this unusual character set (from Hawaii and New Caledonia, both spiny) form a basal grade to all ‚classic' Lepidomeniidae with smooth morphotype (tree modified from ML-analyses based on COI and 16S rRNA).

Conclusions and outlook

- All species encountered on Hawai'i are new to science, and Lepidomeniidae sp. 2 requires either to revise and expand the family diagnosis or to establish a novel family.
- Difficulties to place new findings into the current classificatory system are typical for species inventories in Solenogastres. A community effort is needed to carefully revise the current taxonomy based on integrative datasets. Hypotheses derived from molecular phylogenetic approaches are indispensable to work round to a taxonomy which corresponds to the evolutionary history of the class.

Acknowledgements:
The sampling event was financed and organized by MarineGEO/ Smithsonian Institution. Thanks to Jon Norenburg and the meiofauna gang for support in sorting and to Heidemarie Gensler (LMU) for histological sectioning.

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Computed Microtomography (Micro-CT) in the Anatomical Study and Identification of Solenogastres (Mollusca)

Jesús Martínez Sanjuán[1], Kevin M. Kocot[2], Óscar García-Álvarez[1], María Candás[1] and Guillermo Díaz-Agras[1]
[1]REBUSC, Rede de Estacións Biolóxicas da Universidade de Santiago de Compostela, Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[2]Department of Biological Sciences and Alabama Museum of National History, University of Alabama, Tuscaloosa, AL, United States

Introduction

Despite recent advances, Solenogastres are still an under-studied group of molluscs in all of their aspects. The Microcomputed tomography (Micro-CT) is a technique that allows the visualization of internal anatomy of specimens in a fast, non-destructive way, unlike classical histology. In this research, we wanted to compare the descriptions of *Proneomenia sluiteri*, *Dorymenia menchuescribanae* and *Anamenia gorgonophila* to micro-CT scans of these species to determine if it ispossible to identify them based on the characters visible with micro-CT: genital opening, seminal receptacles, copulatory stylets, respiratory folds, atriobuccal cavity and the general shape of the ventrolateral foregut glands.

Material and methods

Samples were dehydrated in ethanol 80%, 90%, 96%, 24 hours each, and then stained with 1% iodine in ethanol 96% from 3 days to 1 week depending on the size of the specimen. Then, they were dehydrated with hexamethyldisilazane (HMDS) for 2 hours and air dried overnight, and scanned (55 kV, 165 µA and 360º of sample rotation, pixel size of 6.78 µm for *P. sluiteri*, 5.97 µm for *D. menchuescribanae*, and 2.98 and 4.95 µm for both specimens of *A. gorgonophila*, respectively) with a Skyscan 1172 (Bruker, Belgium). Images were reconstructed using NRecon (Bruker, Belgium)and cleaned using CTAn. DataViewer (Bruker, Belgium) was used to obtain 2D transverse sections and CTVox (Bruker, Belgium) was used to visualize 3D images.

Results

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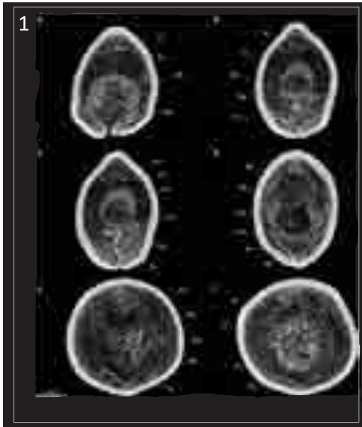

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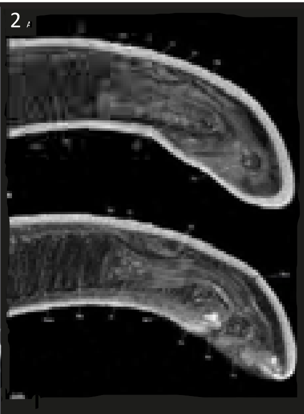

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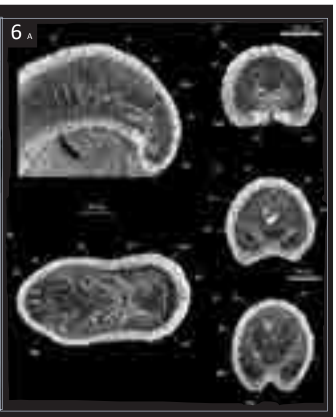

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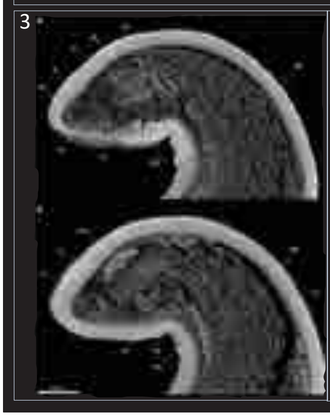

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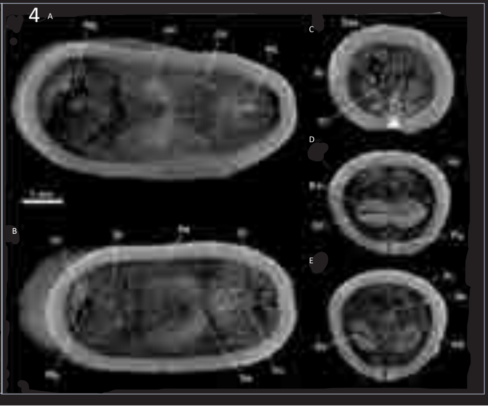

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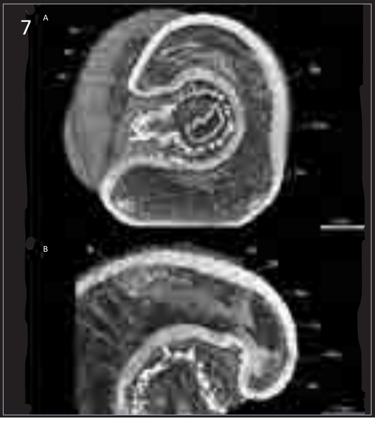

Figure 1. *Proneomenia sluiteri*, cross-section view in different zones of the anterior body. A – Atriobuccal cavity. B, C – Oesophagus. D – Oesophagus entering midgut. E, F – Midgut.
Figure 2. *Proneomenia sluiteri*, sagittal view of the posterior body. A – Spawning duct zone. B – Rectum zone.
Figure 3. *Dorymenia menchuescribanae*, sagittal view of the anterior body. A – Ventrolateral foregut glands zone. B – Pharynx zone.
Figure 4. *Dorymenia menchuescribanae*, dorsal and cross-section view in different zones of the posterior body. A, B – Through spawning duct. C – Through pallial cavity. D – Spawning duct opening and rectum. E – Through pericardium.
Figure 5. *Anamenia gorgonophila* (Alborán), sagittal, dorsal and cross-section view of the anterior body. A – Through pharynx. B – Dorsal view. C – Through atrium. D – Through pharynx. E – Through midgut.
Figure 6. *Anamenia gorgonophila* (Alborán), sagittal, dorsal and cross-section view of the posterior body. A – Through spawning duct. B – Dorsal view. C – Through spawning duct opening. D – Through spawning ducts. E – Through seminal receptacles
Figure 7. *Anamenia gorgonophila* (Iceland), sagittal view of body. A – Anterior body. B – Posterior body.

Abbreviations: Ab, Abdominal spicules; Abc, Atriobuccal cavity; Al, Alcyonacea; At, Atrium; Co, Copulatory stylets; Dc, Dorsal caecum; Di, Diverticles; Dso, Dorsoterminal sense organ; Dvm, Dorsoventral musculature; Gd, Gonopericardioduct; Go, Gonad; Ht, Heart; Mg, Midgut; Mo, Mouth; Oc, Octocorallia; Ov, Oocyte; Pc, Pallial cavity; Pd, Pericardioduct; Pdg, Pedal Gland; Pg, Pedal groove; Ph, Pharynx; Phg, Pharynx glands; Po, Preatrial organ; Pp, Pedal pit; Pr, Pericardium; Pvs, Pallial cavity ventral sac; Re, Rectum; Rs, Radular sac; Sd, Spawning duct; Sdo, Spawning duct opening; Sr, Seminal receptacles; Vfg, Ventral foregut glands; Vs, Ventral sinus.

Conclusions

Microcomputed tomography has proven to be very useful if we want a quick and non-destructive approximation to internal anatomy in solenogasters, and family and even genus level identification can be reached through microCT study. However, it still remains complementary to histological techniques, as the radula and other hard parts were difficult to visualize.

Introduced slug species in Hungary

Ágnes TURÓCI[1,2] & Barna PÁLL-GERGELY[1]

[1]Plant Protection Institute, Centre for Agricultural Research, ELKH, Budapest, Hungary
[2]Doctoral School of Biology, Institute of Biology, ELTE Eötvös Loránd University, Budapest, Hungary

Introduction

The Hungarian molluscan fauna is thoroughly explored (Varga et al., 2005). 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. Secondly, their identification is difficult because of the few reliable identification guides (Rowson et al. 2014, Cameron, 2016) 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 anatomical characters as well. During this work, we discovered 6 new species to the Hungarian fauna, 2 of them are already published (Turóci et al., 2020).

Materials and Methods

From 2019 to 2022 we intensively collected slugs all over Hungary. We payed a special attention to botanical gardens and horticultures of Budapest, assuming that these are potential hotspots of unintentional introductions of slugs. The collected specimens were stored in plastic containers with wet paper towel and disposed in fridge. Photos were taken by Canon EOS 2000d camera with Tamron SP AF90mm F/2.8 Di MACRO 1:1 macro objective. Dissection was implemented with Zeiss Stemi 305 stereomicroscope. After photographing, we killed the specimens in 20%, and preserved in 75% ethanol.

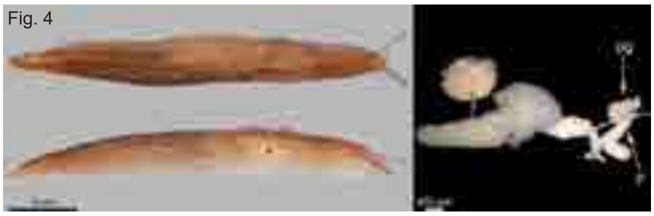

Fig. 4

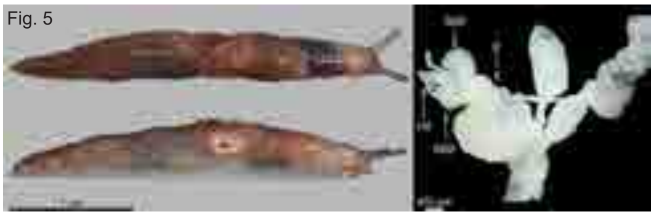

Fig. 5

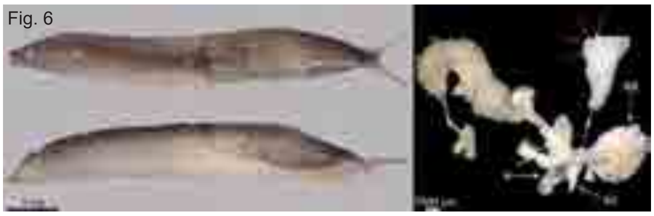

Fig. 6

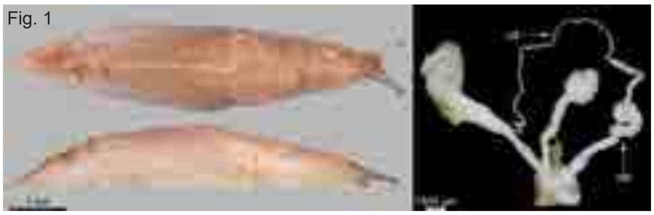

Fig. 1

Fig. 2

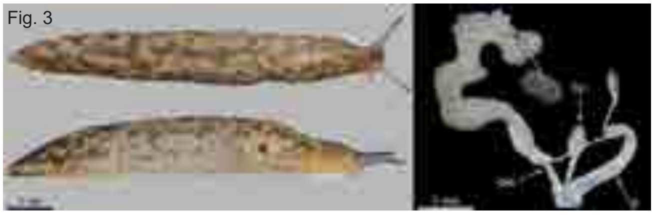

Fig. 3

Results

Tandonia kusceri (H. Wagner, 1931) is a medium sized slug with brownish pink colour and dorsal keel. Epiphallus (epi) long and spirally coiled, vas deferens (vd) long (Fig. 1).

Krynickillus melanocephalus Kaleniczenko, 1851 is a medium sized slug with dirty white colour and deep black head and nape. Penis (p) consists of a thinner basal and a swollen apical portion (Fig. 2).

Limacus maculatus (Kaleniczenko, 1851) is a large bodied slug with greenish-grey colour and darker and lighter spots. Bursa copulatrix (bc) connects to the penis (p), not to the oviductus (ovi) (Fig. 3).

Ambigolimax valentianus (A. Férussac, 1821) is a medium sized slug with light brown or creamy brown colour with two lateral bands. Penis (p) is claviform, broadened to the tip with a thick or lobular, blunt-ended penial gland (pg) (Fig. 4).

Deroceras invadens Reise, Hutchinson, Schunack and Schlitt, 2011 is a small sized slug with brownish coulour but lighter rim around the pneumostome. Proximal penis (p) has two appendages (app) with several penial glands (pg) between them (Fig. 5).

Milax nigricans (Philippi, 1836) is medium sized blackish or black colour and thick dorsal keel. Large accessory gland (ag) connects to the atrium (a). Atrium stimulator (sti) is big, flattened conic shaped with several rows of spine-shaped papillae situated on the base of the stimulator (Fig. 6).

Discussion

The increasing number of introduced molluscs is traditionally explained by the climate change (Mainka & Howard, 2010) and the increased international transportation of goods (Hulme, 2009), mostly agricultural and horticultural products. Since 3 out of the 6 introduced 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, and collect data on the horticultural damage they make.

Acknowledgements

We would like to thank Miklós Rapala for collecting specimens in the horticultures of Budapest.
The authors are grateful to Heike Reise and John M. C. Hutchinson for their contribution.

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Varga, A., Fűköh, L. & Krolopp, E. (2005): Magyar Malakológiai Bibliográfia (1727–2004). *Malakológiai Tájékoztató* [*Malacological Newsletter*], 23: 5–129. [*Hungarian Malacological Bibliography (1727–2004)*] in Hungarian

THE PHYLOGENY OF FRESHWATER MOLLUSC GENUS *BROTIA* H. ADAMS 1866 (GASTROPODA: PACHYCHILIDAE) FROM NORTHEAST INDIA

Anushree Jadhav [1,2], Surya Narayanan [1], Chinta Siddharthan[1] and Aravind Neelavara Ananthram[1,3]
[1]SMS Foundation Center For Biodiversity And Conservation, Ashoka Trust For Research In Ecology And The Environment (ATREE), Bangalore, India
[2]Manipal Academy of Higher Education (MAHE), Manipal, Udupi, India
[3]Yenepoya Research Centre, Yenepoya (Deemed To Be University), University Road, Derlakatte, Mangalore, India
Email: anushree.jadhav@atree.org

INTRODUCTION

- The Pachychilid genus *Brotia* H. Adams 1866 is distributed across south and Southeast Asia, comprising 46 species (Köhler and Glaubrecht 2001, 2006).
- In India, the members of the genus *Brotia* are distributed in the Ganges-Brahmaputra river basin and represented by five valid species (Köhler and Glaubrecht 2001, 2006).
- The major issue with this genus is in regards to the synonymies. Although *Brotia* has recently been revised, the taxonomic issues for the Indian species still need to be addressed (Köhler and Glaubrecht 2001, 2006).
- This study highlights the systematics of Indian *Brotia,* significantly adding to the limited knowledge on Indian freshwater gastropod fauna.

OBJECTIVES

To resolve the phylogenetic relationships of Genus *Brotia* from Northeast India and decipher the divergence time of this genus using molecular data.

METHODS

Fieldwork was carried out in NE India (Fig. 1) and samples were preserved in absolute ethanol. Genomic DNA was extracted using modified CTAB DNA extraction method (Chakraborty et al. 2020) and amplified two mitochondrial markers (COI and 16Sr RNA).

Phylogenetic analysis: Maximum Likelihood, Bayesian Inference, delimitation (ABGD) and divergence time analysis was carried out. SE Asian *Brotia* sequences were downloaded from GenBank. Sequences were manually checked and aligned using ClustalW implemented in MEGA X (Higgins et al. 1994; Tamura et al. 2007). ML analysis was performed using the IQTREE webserver (Trifinopoulos et al. 2016). IQTREE used Modelfinder (Kalyanamoorthy et al. 2017) to find the best-fit models for each partition defined. BI analysis was performed in MrBayes v.3.2 (Ronquist et al. 2012) with default prior settings implementing the best-fit models as per Partitionfinder (Lanfear et al. 2017).

Molecular dating: Analysis was carried out using BEAST 1.8.2 (Drummond et al. 2012) for the concatenated dataset. The tree was calibrated using a substitution rate ranging from 0.0068 to 0.0118 (substitutions per site per million years; uniform prior). Approximate divergence times were estimated using the strict clock (SC) model.

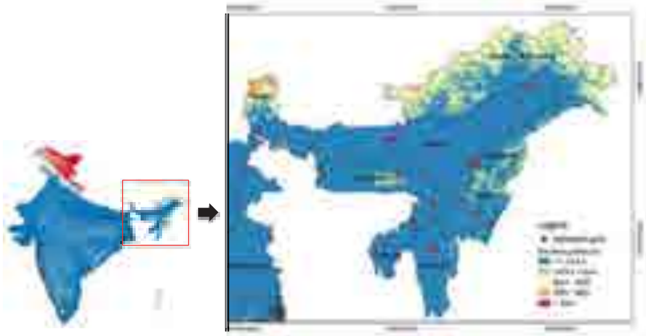


Figure 1: Sampling location for *Brotia* in Northeast India (Inset map showing NE India)

ACKNOWLEDGEMENT

Dept. of Biotechnology, Govt. of India for funding the study.
Unitas Malacologia for travel support
Nipu Kumar Das for the help in fieldwork.

Some examples of *Brotia* spp. from Northeast India used in this study

RESULTS

1) ML and Bayesian analyses: All Indian lineages are nested within the SE Asian clade, with moderate to strong support. The ML tree shows eight well-supported clades (Fig. 2a). The Indian *Brotia* are recovered as paraphyletic. Preliminary phylogenetic analyses retrieved from both ML and BI analyses are largely similar for the Indian lineages, except for some nodes that are unresolved in BI analysis (Fig. 2b). All Indian lineages formed three clades, one with only Indian lineages and is sister to other Indian and SE Asian lineages with low support. Samples from Pobo and Gossaigoan (Assam), both from north of Brahmaputra are recovered as sister with moderate support together sister to samples from Manipur are sister to a Southeast Asian species with moderate support.

2) Species delimitation analysis: ABGD delimitation results suggest eight distinct species.

3) Molecular dating: Indian clades are nested within the Southeast Asian clade, suggesting **"Into-India"** dispersal. The molecular dating results show three major dispersal events between 20 million years ago and 14.49 MYA (Miocene to Mid-Miocene; Fig. 3)

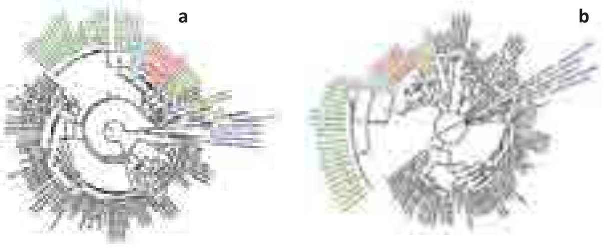
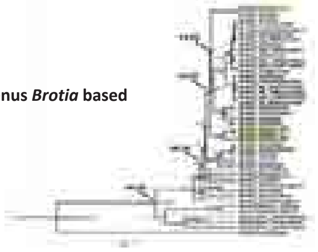


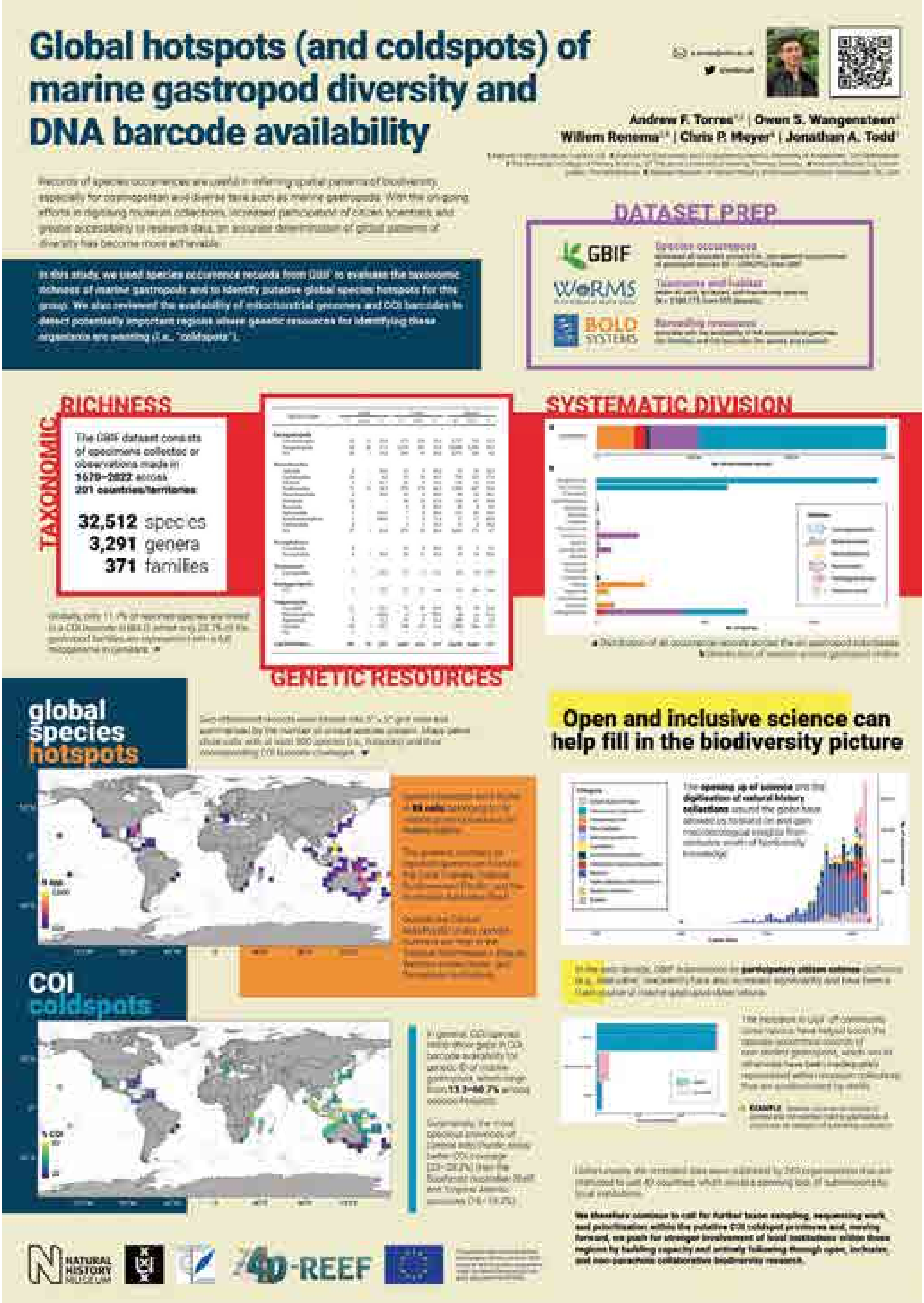
Figure 2: Likelihood (a) and Bayesian phylogenetic tree (b) showing the relationship of *Brotia* spp.

Figure 3: Time calibrated BEAST tree for Genus *Brotia* based on 16S and COI genes.



CONCLUSION

- First ever phylogeny for Genus *Brotia* from India.
- Eight species of *Brotia* in India are recognized by delimitation analysis. Morphology and anatomical analysis remains to be done.
- Members of the genus *Brotia* dispersed Into India during Early and Mid-Miocene.
- Fossil calibrated tree needs to be done to get better idea about the dispersal time.



Global hotspots (and coldspots) of marine gastropod diversity and DNA barcode availability

Andrew F. Torres[1,2] | Owen S. Wangensteen[3]
Willem Renema[2,4] | Chris P. Meyer[5] | Jonathan A. Todd[1]
[illegible]

Records of species occurrences are useful in inferring spatial patterns of biodiversity, especially for cosmopolitan and diverse taxa such as marine gastropods. With the ongoing 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 attainable.

In this study, we used species occurrence records from GBIF to evaluate the taxonomic richness of marine gastropods and to identify putative global species hotspots for this group. We also reviewed the availability of mitochondrial genomes and COI barcodes to detect potentially important regions where genetic resources for identifying these organisms are wanting (i.e., "coldspots").

DATASET PREP

Species occurrences
[illegible]

Taxonomy and habitat
[illegible]

Barcoding resources
[illegible]

TAXONOMIC RICHNESS

The GBIF dataset consists of specimens collected or observations made in **1670–2022** across **201 countries/territories**.

32,512 species
3,291 genera
371 families

Globally, only 11.7% of reported species are linked to a COI barcode in BOLD whilst only 22.7% of the gastropod families are represented with a full mitogenome in GenBank.

GENETIC RESOURCES

[illegible]

SYSTEMATIC DIVISION

a Distribution of all occurrence records across the six gastropod subclasses
b Distribution of species across gastropod orders

global species hotspots

Geo-referenced records were binned into 5° × 5° grid cells and summarised by the number of unique species present. Maps below show cells with at least 300 species (i.e., hotspots) and their corresponding COI barcode coverages.

Species richness is highest in **93 cells** belonging to [illegible]

The greatest numbers of reported gastropods are found in the Coral Triangle [illegible]

Outside the Central Indo-Pacific, [illegible]

COI coldspots

In general, COI coverage [illegible] gaps in COI barcode availability for species ID of marine gastropods, with coverage from **13.3–46.7%** among hotspot provinces.

Surprisingly, the most speciose provinces of Central Indo-Pacific [illegible]

Open and inclusive science can help fill in the biodiversity picture

The **opening up of science** and the **digitisation of natural history collections** around the globe have allowed us to stand on and gain macroecological insights from centuries' worth of biodiversity knowledge.

In the past decade, GBIF submissions for **participatory citizen science** platforms (e.g., [illegible]) have also increased significantly and now form a significant source of marine gastropod observations.

The inclusion in GBIF of community science records have helped boost the reported occurrences of [illegible]

EXAMPLE [illegible]

Unfortunately, the processed data were supplied by 293 organisations that are registered to just 40 countries, which reveals a seeming lack of submissions by local institutions.

We therefore continue to call for further taxon sampling, sequencing work, and prioritisation within the putative COI coldspot provinces and, moving forward, we push for stronger involvement of local institutions within these regions by building capacity and actively following through open, inclusive, and non-parachute collaborative biodiversity research.

NATURAL HISTORY MUSEUM
4D-REEF

[illegible]

How many single-copy orthologous genes from whole genomes reveal deep gastropod relationships?

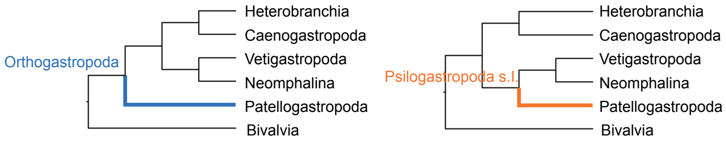
Zeyuan Chen [1,2] and Michael Schrödl [1,2,3]

[1] Mollusca, SNSB-Bavarian State Collection of Zoology, Munich, Bavaria, Germany
[2] Department Biology II, Ludwig-Maximilians-Universität München, Munich, Bavaria, Germany
[3] GeoBio-Center LMU, Munich, Bavaria, Germany

Conflict of gastropods phylogeny using single-copy orthologous genes (SOGs)

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 hypothesis).

With the release of high-quality genomes, orthologous genes can be better identified and serve as powerful candidates for phylogenetic analysis. Several newly published gastropod genome studies have tried to reconstruct gastropod phylogeny using hundreds of SOGs, however, the the conflict still remains when using SOGs in different taxon and gene sets also with different gene coverage.

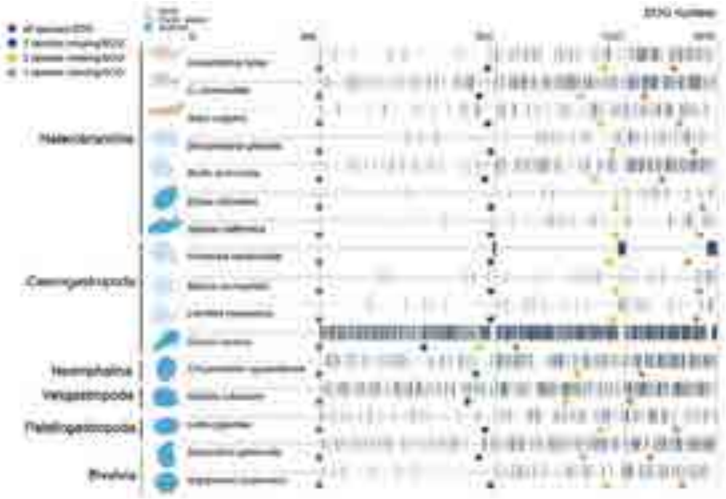


223 SOGs (Chen et al. 2020)

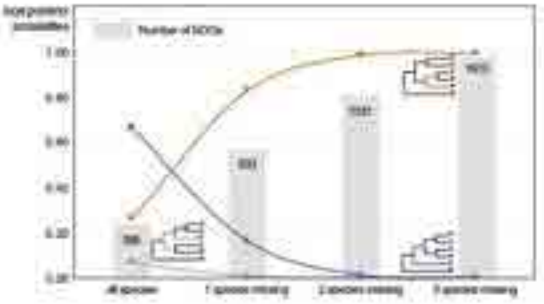
529 SOGs (Lan et al. 2021)
1375 SOGs (Sun et al. 2020)
1357 SOGs (Sun et al. 2019)

Generation of different SOGs datasets

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.

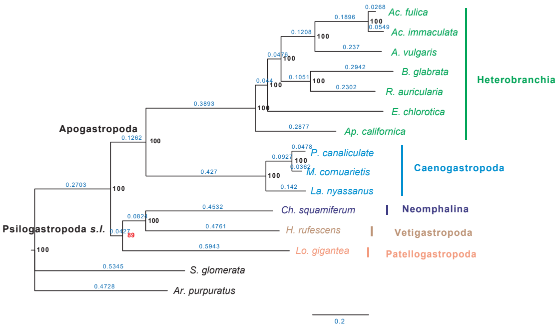


Larger SOGs sets rejected Orthogastropoda and recovered Psilogastropoda



The gastropod relationships constructed with different data sets and the corresponding posterior probabilities of different topology. H: Heterobranchia, C: Caenogastropoda, V: Vetigastropoda, N: Neomphalina, P: Patellogastropoda, B: Bivalve.

Removal of species (*C. consors*) with low SOGs coverage supports Psilogastropoda



Gastropod phylogeny inferred from 847 SOGs identified from 15 species except *C. consors*.

Summary

- As the number of SOGs increased, the inferred topology changed from Orthogastropoda to Psilogastropoda s.l., with considerable support.
- The selection of the **representative species** and use of **sufficient informative sites** greatly influence the analysis of deep gastropod phylogeny.
- Whole genomes of Neritimorpha and Cocculiniformia are entirely missing and other major groups such as Neomphalina and Vetigastropoda rather than being represented by single or few members should be much more densely sampled, including the entire diversity of early branching subclades. Robust reconstruction of deep gastropod relationships will depend on well-assembled whole genomes from **a large** and **balanced taxon** set.

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COMPARISONS AMONG GENOME ASSEMBLIES AND THE IMPORTANCE OF COMPLETE REFERENCE GENOMES. THE PENDING TASK IN MALACOLOGY

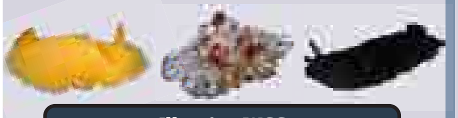
Carles Galià-Camps, Tilman Schell, Alba Enguídanos, Ángel Valdés, Miquel Arnedo, Xavier Turon, Marta Pascual, Carlos Carreras, Manuel Ballesteros, Carola Greve

BACKGROUND

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, only 68 molluscan genomes are publicly available so far, and 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 six low coverage Dendrodorididae genome assemblies, one high-coverage *Dendrodoris* genome assembly and the tunicate *Styela plicata* reference genome.

COMPARING GENOMES

Low-coverage genomes



Illumina WGS (7X)

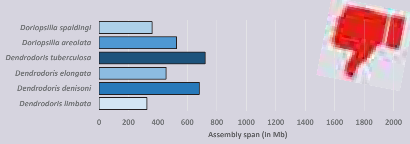
MITOGENOMES

All the mitogenomes could be circularly **recovered**

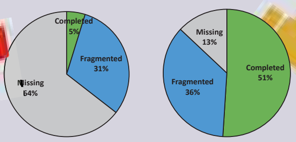
GENOME ESTIMATES

ASSEMBLY SIZE & CONTIGUITY

Compared to other dorid genome sizes (~2000Mb), genome assembly spans are **very small** (<40%) and **variable** (27%-36%)



ASSEMBLY COMPLETENESS



We could recover some single copy ortholog genes, although the number was **variable**

High-coverage genome

Illumina WGS (30X)

MITOGENOME

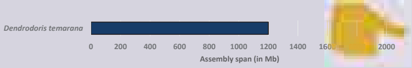
We **successfully circularized** *Dendrodoris temarana* mitogenome

GENOME ESTIMATES

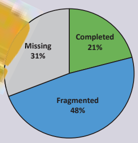
We could test several statistics according to Illumina reads although the results were **unaccurate** and **not reliable** due to coverage

ASSEMBLY SIZE & CONTIGUITY

Compared to other dorid genome size (~2000Mb), genome assembly span **is more than 60%** of the total

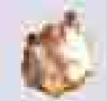


ASSEMBLY COMPLETENESS



We could recover some single copy ortholog genes, although the number was **not that high** and were moslty fragmented

Reference genome



Illumina WGS (30X)

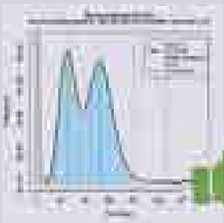
PacBio CLR (100X)

Omni-C Contact Reads (30X)

MITOGENOME

The mitogenome of *Styela plicata* **was successfully circularized**

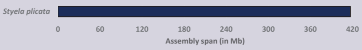
GENOME ESTIMATES



We could **rely on several** statistics according to Illumina reads

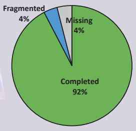
ASSEMBLY SIZE & CONTIGUITY

Compared to the estimated genome size (~420Mb), the genome assembly is **almost complete** (>99%), being **distributed in 16 chromosomes**



ASSEMBLY COMPLETENESS

We recovered **almost all** the single copy ortholog genes



CONCLUSIONS

Low coverage genomes are a nice tool to work with mitogenomes

High coverage genomes recover a more contiguous and complete assembly, allowing some nuclear genomes analyses with few recovered genes

Reference genomes recover a whole representation of the genome, translated to a putative capacity to perform any genomic study on them

PARTNERS

CONTACT

cgaliacamps@gmail.com
@GaliaCampsOmics

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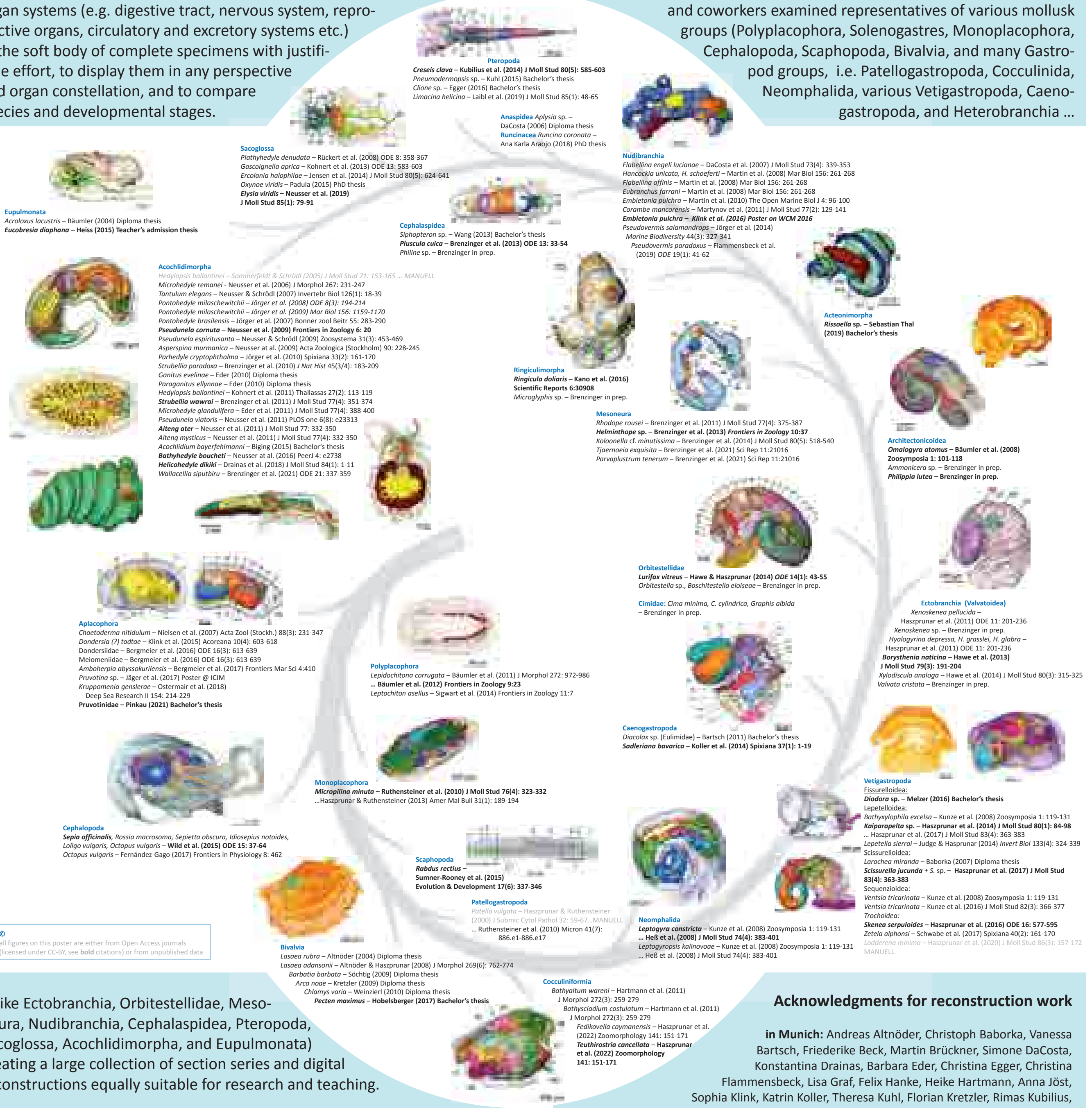
20 years of 3D microanatomical research by the Munich malacologists around Prof. Haszprunar – a synopsis.

Franziska S Bergmeier[1], Bastian Brenzinger[2], Heidemarie Gensler[1], Gerhard Haszprunar[1,2], Andreas Hawe[1], Martin Heß[1], Lisa Hobelsberger[1], Katharina M Jörger[1], Thomas Kunze[1], Peter C Kohnert[2], Timea P Neusser[1], Chiara Pinkau[1], Michael Schrödl[2], Enrico Schwabe[2], Elvira Wild[1]

[1]Biocenter of the Ludwig-Maximilians-University Munich, Germany / [2]SNSB-Bavarian State Collection of Zoology (ZSM), Munich, Germany
Email: hess@bio.lmu.de

The method of computer-assisted 3D reconstruction based on complete series of semi-thin sections has opened up a very fruitful field of research in zoomorphology, also for the study of small mollusk 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. 2003 to 2022 several generations of researchers from LMU, SNSB-ZSM, and coworkers examined representatives of various mollusk groups (Polyplacophora, Solenogastres, Monoplacophora, Cephalopoda, Scaphopoda, Bivalvia, and many Gastropod groups, i.e. Patellogastropoda, Cocculinida, Neomphalida, various Vetigastropoda, Caenogastropoda, and Heterobranchia …



Pteropoda
***Creseis clava* – Kubilius et al. (2014) J Moll Stud 80(5): 585-603**
Pneumodermopsis sp. – Kuhl (2015) Bachelor's thesis
Clione sp. – Egger (2016) Bachelor's thesis
Limacina helicina – Laibl et al. (2019) J Moll Stud 85(1): 48-65

Anaspidea *Aplysia* sp. –
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Runcinacea *Runcina coronata* –
Ana Karla Araojo (2018) PhD thesis

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Plathyhedyle denudata – Rückert et al. (2008) ODE 8: 358-367
Gascoignella aprica – Kohnert et al. (2013) ODE 13: 583-603
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***Elysia viridis* – Neusser et al. (2019)**
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Acroloxus lacustris – Bäumler (2004) Diploma thesis
***Eucobresia diaphana* – Heiss (2015) Teacher's admission thesis**

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Flabellina engeli lucianae – DaCosta et al. (2007) J Moll Stud 73(4): 339-353
Hancockia unicata, H. schoeferti – Martin et al. (2008) Mar Biol 156: 261-268
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***Pluscula cuica* – Brenzinger et al. (2013) ODE 13: 33-54**
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Pontohedyle milaschewitchii – Jörger et al. (2009) Mar Biol 156: 1159-1170
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***Pseudunela cornuta* – Neusser et al. (2009) Frontiers in Zoology 6: 20**
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Scientific Reports 6:30908
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Parvaplustrum tenerum – Brenzinger et al. (2021) Sci Rep 11:21016

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***Omalogyra atomus* – Bäumler et al. (2008)**
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Ammonicera sp. – Brenzinger in prep.
***Philippia lutea* – Brenzinger in prep.**

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***Lurifax vitreus* – Hawe & Haszprunar (2014) *ODE* 14(1): 43-55**
Orbitestella sp., *Boschitestella eloiseae* – Brenzinger in prep.

Cimidae: *Cima minima, C. cylindrica, Graphis albida*
– Brenzinger in prep.

Ectobranchia (Valvatoidea)
Xenoskenea pellucida –
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Xenoskenea sp. – Brenzinger in prep.
Hyalogyrina depressa, H. grasslei, H. glabra –
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… Bäumler et al. (2012) Frontiers in Zoology 9:23
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***Sadleriana bavarica* – Koller et al. (2014) Spixiana 37(1): 1-19**

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Fissurelloidea:
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Lepetelloidea:
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83(4): 363-383
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Ventsia tricarinata – Kunze et al. (2016) J Moll Stud 82(3): 366-377
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***Micropilina minuta* – Ruthensteiner et al. (2010) J Moll Stud 76(4): 323-332**
…Haszprunar & Ruthensteiner (2013) Amer Mal Bull 31(1): 189-194

Cephalopoda
Sepia officinalis, *Rossia macrosoma, Sepietta obscura, Idiosepius notoides,*
Loligo vulgaris, Octopus vulgaris – **Wild et al. (2015) ODE 15: 37-64**
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(2000) J Submic Cytol Pathol 32: 59-67…MANUELL
… Ruthensteiner et al. (2010) Micron 41(7):
886.e1-886.e17

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… Heß et al. (2008) J Moll Stud 74(4): 383-401
Leptogyropsis kalinovoae – Kunze et al. (2008) Zoosymposia 1: 119-131
… Heß et al. (2008) J Moll Stud 74(4): 383-401

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Lasaea rubra – Altnöder (2004) Diploma thesis
Lasaea adansonii – Altnöder & Haszprunar (2008) J Morphol 269(6): 762-774
Barbatia barbata – Söchtig (2009) Diploma thesis
Arca noae – Kretzler (2009) Diploma thesis
Chlamys varia – Weinzierl (2010) Diploma thesis
***Pecten maximus* – Hobelsberger (2017) Bachelor's thesis**

Cocculiniformia
Bathyaltum wareni – Hartmann et al. (2011)
J Morphol 272(3): 259-279
Bathysciadium costulatum – Hartmann et al. (2011)
J Morphol 272(3): 259-279
Fedikovella caymanensis – Haszprunar et al.
(2022) Zoomorphology 141: 151-171
***Teuthirostria cancellata* – Haszprunar**
et al. (2022) Zoomorphology
141: 151-171

… like Ectobranchia, Orbitestellidae, Mesoneura, Nudibranchia, Cephalaspidea, Pteropoda, Sacoglossa, Acochlidimorpha, and Eupulmonata) 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 bivalves from western Portugal

T. Goulding[1], I. Martins[1], M. Gaspar[2], P. Chainho [1,4], M. Rufino[2,3]

[1] MARE - Centro de Ciências do Mar e do Ambiente, Faculdade de Ciências, Universidade de Lisboa, Portugal
[2] IPMA - Instituto Português do Mar e da Atmosfera, Algés, Portugal
[3] CEAUL - Centro de Estatística e Aplicações, Universidade de Lisboa, Portugal
[4] Departamento de Biologia Animal, Faculdade Ciências da Universidade de Lisboa, Portugal
Email: tgoulding@fc.ul.pt

Valuable Species

***R.philippinarum* (*R.phi*), *R.decussatus* (*R.dec*) and *C.edule* (*C.edu*), are valuable species- Legal trade estimated to be worth Eur 10.5 million[1]. However there is substantial national and international illegal trade**

Subject to closures

Regular fluctuations in water quality forces sporadic closure of harvesting grounds. This leads to IUU harvesting and comercialization

Finger-printing tools

We need a quick and cheap method to identify origin of product

Can we use morphometry ?

Morphometrics have been used to differentiate animals from different systems in the past. Does it work for geographically close aquatic systems?

Methods

**Samples (N= 314 *R.phil*; N= 139 *R.dec*; N= 358 *C.edu*) were collected from 5 systems on the west coast of Portugal. Shells were scanned and compared using both traditional morphometric approaches (shell length, width, and height) and geometric morphometric methods using 10 landmarks (LM) .
Landmark positions were rotated, scaled and translated through Generalized Procrustes Analysis (GPA). Centroid size was calculated using the geomorph package and a Principal Component Analysis (PCA) performed in order to try and identify differences in morphometry between species and location.
All analyses was done in R. The tps suite was used to define LMs and for the thin plate spline graphics.**

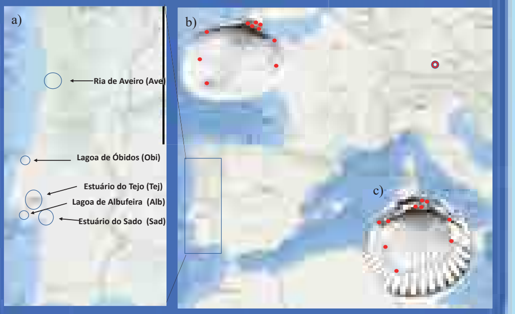

Figure 1- Location of the 5 sampling sites (a), and the selected clam (b) and cockle (c) landmarks

Results

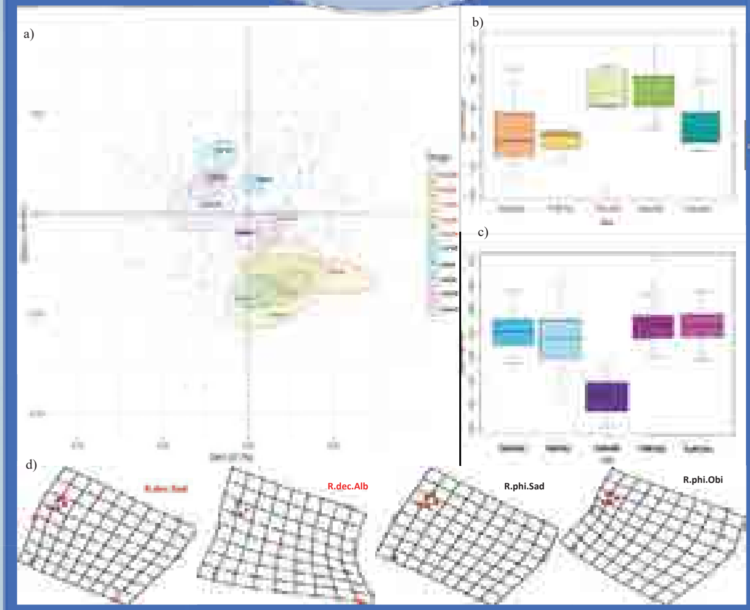

Figure 2- Principal Component Analysis (PCA) of the morphometric landmarks of *Ruditapes decussatus* (*R.dec*), *Ruditapes philippinarum* (*R.phi)* collected at the Sado estuary (Sad), Tejo estuary (Tej), Albufeira coastal lagoon (Alb) and Óbidos coastal lagoon (Obi) and Ria de Aveiro (Ave) (a); *R. decussatus* centroid size and zone boxplot (b), *R. philippinarum* centroid size and zone boxplot (c) and thin plate spline of *R. decussatus in* .Sad and Alb and *R. philippinarum* in Sad and Obi (d) .

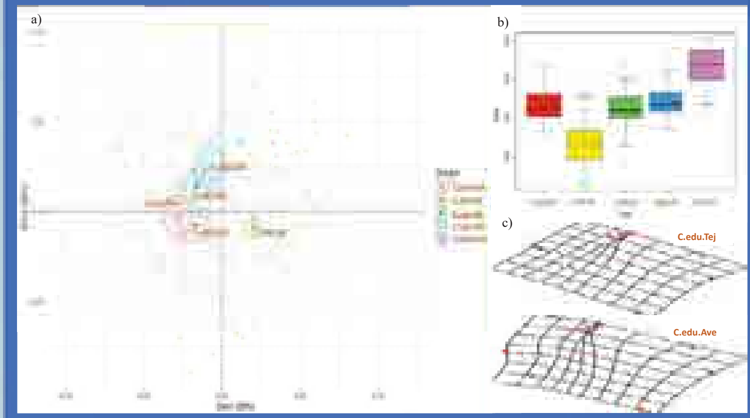

Figure 3- Principal Component Analysis (PCA) of the morphometric landmarks for *Cerastoderma .edule* collected at the Sado estuary (Sad), Tejo estuary (Tej), Albufeira coastal lagoon (Alb) and Óbidos coastal lagoon (Obi) and) and Ria de Aveiro (Ave (a); *C.edule* centroid size and zone boxplot (b) thin plate splinespline of *C.edu.Tej* and *C.edu.Ave* (c)

Discussion

Size differed between collection sites, for both species of clams (fig 2b and 2c). With reference to *R.dec* (fig 2b), the Alb site has on average the largest and Sad the smallest. For *R.phil*, Alb becomes the site with the smallest individuals and Obi the largest.

To visualize shape variation, procrustes coordinates were subjected to a Principal Component Analysis (PCA). PCA was run on the shape variables, produced by Procrustes superimposition (fig 2a). PC1 (37.7%) and PC2 (25.9%) jointly account for 63.6% of the variance with PC3 (12%) and PC4 (8%) accounting for another 20%. Landmark contributions for both PC1 And PC2 include the hinge teeth and the anterior ligament scar, with PC2 also including the point where the pallial sinus and the pallial line meet. Interestingly landmarks representing the posterior ligament scar were of little (≤ 5%) significance to all the PC´s. The PCA appears to differentiate species along both PC1 and PC2. PC1 represents shape changes associated with size (allometric growth), as it is confirmed comparing the pattern shown in the PCA with the centroid size across sites. The exception being *R.phi* from Obi and Tej which appear to group closer to *R.dec* along PC2 when compared to other locations. Csize variation could be one explanation for this however we are also aware that hybridization can occur between these species therefore further genetic testing is needed in order to ascertain whether this may be a reason for this proximity in shape.

Patterns of shape variation were statistically evaluated using the Geomorph package [3,4] and running a nonparametric analysis of variance (ANOVA) with randomized residual permutation procedure[5,6] (RRPP) on the matrix of procrustes coordinates. The model for interspecific comparisons of the shape of the 2 species included the following predictors: species/zone and interactions of these factors (coords ~ Csize + sp + zone + Csize:zone + Csize:sp). The average shape differed significantly between clam species (ANOVA: F =47.161, P=<0.001), zones (ANOVA: F = 7.0178, P=<0.001) and with centroid sizes (ANOVA: F =8.0357, P=<0.001). The interaction of centroid size and zone (ANOVA: F=1.667, p=0.06) and centroid size and species (F=1.1147, p=0.139) was not significant. Overall, these results indicate that the shape changes with size is similar between sites and between species and there is a significant shape difference between sites and between species.

A Thin Plate Spline representation of each species shell from the systems which appear to have the biggest differences in shape from each other are shown in fig 2d) and fig 3c). These were computed by using the consensus configuration (average map) generated from the generalized least-squares orthogonal procrustes analysis (GLS), from all the specimens (of *R.decu*, *R.phi* and *C.edu*) in the study. In essence expressing the shape difference as a deformation on a grid. This allows us to visualize, for example, the difference in proximity between the LMs on the anterior abductor muscle and the lateral hinge tooth between *C.edu.Tej* and *C.edu.Ave*

Regarding *C.edu* the smallest individuals were found in Tej and the largest in Alb (fig 3b). PC1 (29%) and PC2 (24%) for the *C.edu* PCA (Ffig 3a) account for 53% of the variability while PC3 (14%) and PC4 (9%) account for 23%. PC1 seems to group both hinge teeth and a predefined LM located on the pallial scar in the intercostal space between the 9th and 10th ribs (counting from the posterior ligament scar) whereas PC2 groups one point on the hinge teeth with both anterior and posterior abductor scars. The PCA seems to indicate a clear separation of Tej from the other systems along PC2 however this is possibly due to the larger variation of Csize within the sample as indicated by the boxplot (fig 5) .

C.edu average shape varied significantly between zones (ANOVA: F = 14.2557 , P=<0.001) and centroid sizes (ANOVA: F =46.1902 , P=<0.001). However much like the clams , the interaction between centroid size and zone (ANOVA: F=1.457, p=0.09) was not significantly different. This indicates that, the shape changes with size are similar between sites and there is a significant shape difference between sites.

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