SPIXIANA	42	2	193–202	München, Dezember 2019	ISSN 0341–8391

A junior freckled nudibranch: chromatic variability in *Felimida* species from the Eastern Atlantic

(Mollusca, Gastropoda, Chromodorididae)

Juan Moles & Ana Riesgo

Moles, J. & Riesgo, A. 2019. A junior freckled nudibranch: chromatic variability in *Felimida* species from the Eastern Atlantic (Mollusca, Gastropoda, Chromodorididae). Spixiana 42 (2): 193–202.

Nudibranchs are beautiful creatures with marvellous colour patterns that, quoting T. E. Thomson (1976), they are to the molluscs what the butterflies are to arthropods or orchids to angiosperms. For instance, four *Felimida* species belonging to the *"luteorosea"* colour group from the NE Atlantic and the Mediterranean Sea show a yellow spotted pattern above a purple mantle background, i. e. *F. luteopunctata*, *F. luteorosea*, *F. rodomaculata*, *F. rolani*. While the monophyly of *F. luteorosea* and *F. luteopunctata* has been recently recovered using molecular markers, the phylogenetic status of the elusive *F. rodomaculata*, endemic from the Canary Islands, has never been assessed in molecular studies. Although *F. rodomaculata* presents a different chromatic pattern, the lack of sound distinctive morpho-anatomical differences led some authors to suggest this species is a junior synonym of *F. luteopunctata*. Here, we aim to solve the controversial taxonomic status of *F. rodomaculata*. We conducted an integrative approach based on molecular phylogenetics and morphological analysis, including specimens collected at the type locality. Our results indicate that *F. rodomaculata* is, in fact, a chromatic variation of *F. luteopunctata*. Our study reinforces recent evidence of body colour variability within some chromodoridid nudibranchs and the need of caution in the use of this as a diagnostic character in the taxonomy of the group.

Juan Moles (corresponding author), Museum of Comparative Zoology & Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA; and Catalan Opisthobranch Research Group (GROC), Mas Castellar, 17773 Pontós, Spain;
e-mail: jmoles@g.harvard.edu, moles.sanchez@gmail.com

Ana Riesgo, Department of Life Sciences (Invertebrate Division), The Natural History Museum of London, Cromwell Road, SW7 5BD, London, UK

Introduction

Chromodoridid nudibranchs are usually colourful and conspicuous species inhabiting mostly shallow waters of tropical and temperate seas (Padula et al. 2016). Some genera were considered globally widespread, but recent molecular studies have challenged these assumptions. A phylogeny of the Chromodorididae Bergh, 1891 recovered the genus *Chromodoris* Alder & Hancock, 1855 to be polyphyletic (Johnson & Gosliner 2012), in accordance to previous morphological investigations by Ev. Marcus (1971). Subsequently, the species of the genus distributed in the Atlantic and Mediterranean were transferred to the re-erected genus *Felimida* Ev. Marcus, 1971. Among these, the *"luteorosea"* colour group (sensu Ortea & Valdés 1992) includes four species with pinkish to purple mantle colouration and bright yellow punc-

tuation interspersed: *F. luteorosea* (Rapp, 1827), *F. luteopunctata* (Gantès, 1962), *F. rolani* (Ortea, 1988), and *F. rodomaculata* (Ortea & Valdés, 1992). While the first two species are distributed across the North Eastern Atlantic and Mediterranean coasts at shallow depths, *F. rolani* and *F. rodomaculata* are restricted to Cape Verde and the Canary Islands, respectively. *Felimida luteorosea* has been reported to feed upon dendroceratid demosponges such as *Spongionella pulchella* (Sowerby, 1804) and *Aplysilla rosea* (Barrois, 1876) (McDonald & Nybakken 1997). Italian specimens of *F. luteorosea* are suspected to obtain diterpenoid natural products similar to those that occur in the latter sponge (Cimino & Ghiselin 2009). Additional natural products displaying deterrence against fish predators were also found in specimens from both Italy and Spain (Avila 1995). These compounds are typically stored in glands along the mantle border called mantle dermal formations (MDF; Wägele et al. 2006). The species *F. luteopunctata* feeds on *Ircinia* sp. instead, but its chemical ecology has never been assessed (Avila et al. 2018).

Regarding the external morphology, *F. rolani* presents numerous white and yellow spots in the mantle surface. Northern from there, the enigmatic *F. rodomaculata* was described from Fuerteventura and was later observed again in the type locality and the close islands of Lanzarote and Gran Canaria (Ortea et al. 2011). This species presents irregularly arranged big circular or ovate "eroded" dots. Similarly, *F. luteorosea*, known from the entire Mediterranean Sea and the Atlantic coast of the Iberian Peninsula (Perrone 1986, Cervera et al. 2004, Ortea et al. 2011), typically presents large and evenly outlined dots delimited by a white halation. The fourth and last species of the clade, *F. luteopunctata* was described from the Moroccan coast and is found in the western and southern coast of the Iberian Peninsula to Ghana (Cervera et al. 2004, Ortea et al. 2011), this is the only species with fine and profuse punctuation on the mantle and the foot.

Recent phylogenetic studies supported the monophyly of the two species, *F. luteopunctata* and *F. luteorosea*, which together with *F. elegantula* (Philippi, 1844), appeared to be recently diverged species in comparison to other European *Felimida* (Ortigosa et al. 2014). Recently, astounding chromatic variability was reported for some *Felimida* species, such as *F. clenchi* (Russell, 1935) from the Western Atlantic, the amphi-Atlantic *F. binza* (Ev. Marcus & Er. Marcus, 1963) (Padula et al. 2016), and *F. elegantula* from northern and southern Mediterranean Sea (Furfaro et al. 2017). Although sometimes misleading, body colour pattern and radular morphological characteristics have been used to separate *F. rodomaculata* from its congeners (Ortea et al. 2011), but the lack of sound distinctive anatomical differences has led some authors to consider *F. rodomaculata* as a junior synonym of *F. luteopunctata* (Cervera et al. 2004). Here, we aim to shed light on this taxonomical conundrum through an integrative morpho-anatomical, molecular phylogenetic, and species delimitation analyses, allowing a better understanding of the relationship between *F. rodomaculata* and other *Felimida* species.

Material and methods

Sample collection and anatomical analyses

Two specimens of *Felimida rodomaculata* were collected under boulders while snorkelling at 1 m depth in the Canary Islands, NE Atlantic (Fig. 1). These were photographed and measured alive and preserved in 96 % ethanol at −20 °C for both morpho-anatomical and molecular analyses. Additionally, all *Felimida* spp. sequences available from GenBank were gathered (see Table 1).

Specimens were cut open axially with the aid of fine forceps and a stereomicroscope. The reproductive system was depicted with a camera lucida. The labial disc and the radula were obtained from the oral bulb after dissolving the soft tissue in a 10 % NaOH solution for several hours. These were rinsed with distilled water, mounted on metallic stubs covered with carbon stickytabs, and coated with gold-palladium for scanning electron microscopy (SEM). Micrographs were taken with a Zeiss Supra 55VP scanning electron microscope. The material is deposited in the Zoological Museum, University of Bergen (ZMBN).

DNA extraction and amplification

Total genomic DNA was extracted from a small piece of the mantle using the DNeasy Blood and Tissue Kit (Qiagen, CA, USA) and following the manufacturer's protocol. Three molecular markers were amplified following the protocol and primers as described in Moles et al. (2018), namely the mitochondrial genes COI (ca. 658 bp) and 16S (ca. 480 bp) and the nuclear gene H3 (ca. 327 bp). Successful PCR products were purified with EXO-SAP as described in Eilertsen & Malaquias (2013). Sequence reactions were run on an ABI 3730XL DNA Analyser (Applied Biosystems, CA, USA).

Phylogenetic and species delimitation analyses

Chromatograms were visualised, edited, and assembled in Geneious 10.0.2 (Kearse et al. 2012). Consensus sequences were compared against the GenBank nucleotide database using the BLAST algorithm (Altschul et al. 1997). Single gene sequences were aligned with MAFFT v7 (Katoh et al. 2017) and trimmed to a position at which more than 70 % of the sequences had nucleotides. New sequences were deposited in GenBank (Table 1). Saturation was tested for the first, second, and third codon positions of the protein-coding genes COI

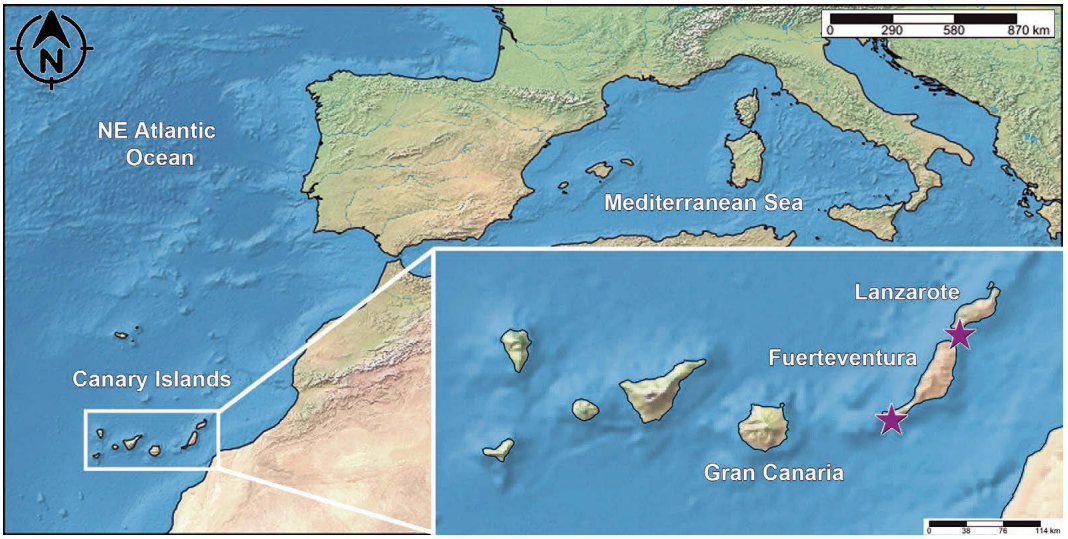

Fig. 1. Map of the NE Atlantic Ocean and close up to the Canary Islands where *Felimida rodomaculata* syn. nov. was collected (purple stars) at Playa Papagayo (Lanzarote) and Puerto de la Cruz (Fuerteventura).

and H3 using MEGA7 (Kumar et al. 2016) by plotting general time-reversible (GTR) pairwise distances against total substitutions (transitions + transversions). GBlocks 0.91b was used on the final trimmed alignment of the non-codifying 16S gene to exclude blocks of hypervariable data, both using stringent settings (Talavera & Castresana 2007). The best-fit model of evolution for each individual gene dataset was run with jModeltest 2.1.7 (Darriba et al. 2012) under the Akaike information criterion (Posada & Buckley 2004). The selected datasets of the three genes were concatenated for analysis. The tree was rooted with *Verconia haliclona* as the sister group of *Felimida* (Ortigosa et al. 2014).

Bayesian inference (BI) was performed on the concatenated alignment of the three genes, using MrBayes ver. 3.2.5 (Ronquist et al. 2011) with a GTR model of sequence evolution (Tavaré 1986), corrections for a discrete gamma distribution, and a proportion of invariant sites (GTR+Γ+I; Yang 1996) specified for each gene partition. Two runs, each with three hot chains and one cold chain, were conducted for 20 million generations, sampling every 2000th generation, using random starting trees. The analysis was performed twice, and 25 % of the runs were discarded as burn-in after checking for stationarity with Tracer v.1.6 (Rambaut et al. 2014). The remaining trees were combined to find the maximum a posteriori probability estimate of phylogeny.

Maximum-likelihood (ML) analyses were conducted using RAxML ver. 8.1.2 (Stamatakis 2014). For the maximum-likelihood searches, a GTR model of sequence evolution with corrections for a discrete gamma distribution (GTR+Γ+I; Yang 1996) was specified for each data partition, and 500 independent searches were conducted. Nodal support was estimated via the rapid bootstrap algorithm (1000 replicates) using the GTR-CAT model (Stamatakis et al. 2008). Bootstrap resampling frequencies were thereafter mapped onto the optimal tree from the independent searches.

To examine the molecular distinctiveness of the different *Felimida* species from the *"luteorosea"* group we used Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012) via the web interface at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html (accessed 6 Dec 2017). ABGD was run for the COI alignment using K80 Kimura measure of distance with transition/transversion ratio equal to 2 and applying default values for P_{min}, P_{max}, and relative gap width.

Results

Phylogeny and species delimitation analysis

No saturation was found for the first, second, and third codon positions of the protein-coding genes COI and H3. Thus, the combined concatenation included three partitions as follows: 658 bp for COI (including the third codon position), 392 bp of 16S after strict settings in GBlocks, and 327 bp for H3 (including the third codon position). The final alignment yielded 1377 bp and contained 44 specimens out of 16 chromodoridid species. The three-genes concatenated BI and ML trees were nearly congruent and both recovered fourteen clades of monophyletic species (with the exception of *Felimida luteopunctata*, and *F. rodomaculata*, see below) with robust support, although the relationships among them are not clear. Both BI and ML recovered a clade with maximum

support containing the NE Atlantic and Mediterranean *"luteorosea"* group: *Felimida elegantula*, *F. luteorosea*, *F. luteopunctata*, and *F. rodomaculata* (Fig. 2). *Felimida luteopunctata* and *F. rodomaculata* were recovered in a single clade with robust support, the genetic proximity of both clades suggest that they might be

Table 1. List of specimens used for the phylogenetic analyses, including sampling localities and GenBank accession numbers.

Species	Locality	COI	16S
Felimida atlantica	Ascension Island, United Kingdom	KX279340	–
Felimida baumanni 1	Guanacaste, Costa Rica	KJ812360	KJ804251
Felimida baumanni 2	Punteras, Costa Rica	KJ812361	KJ804252
Felimida baumanni 3	Guanacaste, Costa Rica	JQ727866	JQ727748
Felimida binza 1	Limón, Costa Rica	KX262399	KX262432
Felimida binza 2	Faial, Azores	KX262402	KX262435
Felimida binza 3	Selvagem Grande, Madeira	KX262408	KX262441
Felimida binza 4	Ilhéu dos Mosteiros, São Miguel Island, Azores	KJ812362	KJ804253
Felimida binza 5	Madeira, Portugal	KJ911273	KJ911253
Felimida clenchi 1	Alagoas, Brazil	KX262397	KX262423
Felimida clenchi 2	Cabo Frio, Brazil	KX262390	KX262429
Felimida clenchi 3	Limón, Costa Rica	KX262388	KX262427
Felimida dalli 1	Santa Lucía Bay, Guerrero, Mexico	KJ911293	KJ911267
Felimida dalli 2	Guanacaste, Punta Carbon, Costa Rica	EU982741	EU982793
Felimida dalli 3	Tres Hermanas Island, Costa Rica	JQ727869	JQ727751
Felimida edmundsi 1	Ilhéu Mosteiros, São Tomé and Príncipe	KJ812351	KJ804240
Felimida edmundsi 2	Azores, Portugal	KJ812350	KJ804239
Felimida elegantula 1	Porto San Paolo, Sardinia, Italy	KJ812356	KJ804245
Felimida elegantula 2	Porto San Paolo, Sardinia, Italy	KJ812357	KJ804247
Felimida elegantula 3	Porto San Paolo, Sardinia, Italy	KJ812358	KJ804248
Felimida krohni 1	Cádiz, Spain	KJ911276	KJ911256
Felimida krohni 2	Guetaria Bay, Basque Country, Spain	KJ911277	KJ911257
Felimida krohni 3	Italy	KJ911278	KJ911258
Felimida luteopunctata 1	Cádiz, Spain	KJ911279	KJ911259
Felimida luteopunctata 2	Cádiz, Spain	KJ911280	KJ911260
Felimida luteopunctata 3	Cádiz, Spain	KJ911281	KJ911261
Felimida luteopunctata 4	Cádiz, Spain	KJ911282	KJ911262
Felimida luteorosea 1	Del Rey Island, Chafarinas, Spain	KJ911283	KJ911263
Felimida luteorosea 2	Guetaria Bay, Basque Country, Spain	KJ911284	KJ911264
Felimida luteorosea 3	Spain	AF249815	–
Felimida luteorosea 4	Greece	KJ812355	KJ804244
Felimida paulomarcioi 1	Brazil	KX279338	–
Felimida paulomarcioi 2	Brazil	KX279339	–
Felimida purpurea 1	Cádiz, Spain	KJ911285	–
Felimida purpurea 2	Cádiz, Spain	KJ911286	KJ911265
Felimida purpurea 3	Ilhéu Mosteiros, São Tomé and Príncipe	KJ812354	KJ804243
Felimida rodomaculata syn. nov. 1	Lanzarote, Canary Islands	MH594463	MH594467
Felimida rodomaculata syn nov. 2	Fuerteventura, Canary Islands	MH594464	MH594468
Felimida sp. 1	Cabo Frio, Brazil	KX279333	KX372565
Felimida sp. 1	Cabo Frio, Brazil	KX279334	KX372566
Felimida sphoni 1	Gulf of Fonseca, El Salvador	–	KJ804249
Felimida sphoni 2	Guerrero, Mexico	KJ911287	KJ911266
Felimida sphoni 3	Punteras, Costa Rica	KJ812359	KJ804250
Verconia haliclona	Port Philip Bay, Australia	EF535117	EF534045

a single species (see Systematics results below). In this sense, ABGD analysis of COI for the *"luteorosea"* group resulted in the identification of only three groups (see Table 2 for intra- and interspecific distances), clustering specimens of. *F. luteopunctata* and *F. rodomaculata* in the same species. The interspecific distances between nominal species ranged from 5.19 to 7.35, while the intraspecific distances among clades ranged from 0 to 2.22. Likewise, the distance between *F. luteopunctata* and *F. rodomaculata* was only 0.65–1.42, therefore suggesting both species may, in fact, be a single one. A second clade containing mainly W Atlantic species and the amphi-Atlantic *F. binza* and the third clade with species from all over the Atlantic was also recovered (Fig. 2).

H3	Reference
–	Padula et al. 2016
KJ812374	Ortigosa et al. 2014
–	Ortigosa et al. 2014
–	Johnson & Gosliner 2012
KX279314	Padula et al. 2016
KX279315	Padula et al. 2016
KX279316	Padula et al. 2016
KJ812375	Ortigosa et al. 2014
KJ911232	Ortigosa et al. 2014
KX279309	Padula et al. 2016
KX279311	Padula et al. 2016
–	Padula et al. 2016
KJ911247	Ortigosa et al. 2014
–	Johnson 2011
–	Johnson & Gosliner 2012
KJ812364	Ortigosa et al. 2014
KJ812363	Ortigosa et al. 2014
KJ812368	Ortigosa et al. 2014
KJ812370	Ortigosa et al. 2014
KJ812371	Ortigosa et al. 2014
KJ911235	Ortigosa et al. 2014
KJ911237	Ortigosa et al. 2014
KJ911236	Ortigosa et al. 2014
KJ911238	Ortigosa et al. 2014
KJ911239	Ortigosa et al. 2014
KJ911240	Ortigosa et al. 2014
KJ911241	Ortigosa et al. 2014
KJ911242	Ortigosa et al. 2014
KJ911243	Ortigosa et al. 2014
–	Wollscheid-Lengeling et al. 2001
–	Ortigosa et al. 2014
–	Padula et al. 2016
–	Padula et al. 2016
KJ911244	Ortigosa et al. 2014
KJ911245	Ortigosa et al. 2014
KJ812367	Ortigosa et al. 2014
MH594465	This study
MH594466	This study
KX279312	Padula et al. 2016
KX279313	Padula et al. 2016
KJ812372	Ortigosa et al. 2014
KJ911246	Ortigosa et al. 2014
KJ812373	Ortigosa et al. 2014
–	Turner & Wilson 2008

Systematics

Class Gastropoda Cuvier, 1795
Subclass Heterobranchia Burmeister, 1837
Order Nudibranchia Blainville, 1814
Family Chromodorididae Bergh, 1891
Genus *Felimida* Ev. Marcus, 1971

Felimida luteopunctata (Gantès, 1962)

Figs 3A–G, 4

Glossodoris luteopunctata Gantès, 1962: 133–135, figs 1–3.
Chromodoris luteopunctata: Cervera et al. 1989: 445–448, figs 1–4; García-Gómez 2002: 215, fig. 50; Sánchez-Tocino 2003: 228–231, figs F11.1–10; Cervera et al. 2004: 109, pl. 2; Trainito 2005: 47; Debelius & Kuiter 2007: 182; Sánchez-Tocino & García-Gómez 2011: 493.
Chromodoris rodomaculata Ortea & Valdés, 1992: 69–85, figs 1, 4, 5; Debelius & Kuiter 2007: 182; Ortea et al. 2011: 162–166, pl. 1C–G, **syn. nov**.
Felimida luteopunctata: Ortea et al. 2011: 166, pl. 1H; Ortigosa et al. 2014: 545, fig. 1B.

Type locality. Temara, NW Morocco, NE Atlantic.

Material examined. One adult, 32 mm, sequenced, preserved in 95 % ethanol (Playa Papagayo, Lanzarote; 28°50'31.88" N 13°47'18.96" W; water depth: 0.5 m) (ZMBN 121360); coll. J. M., 3 August 2016. One adult, 24 mm, dissected and sequenced, preserved in 95 % ethanol (Puerto de la Cruz, Fuerteventura; 28°04'26.56" N 14°29'40.70" W; water depth: 1 m) (ZMBN 121359); coll. J. M., 8 August 2016.

External morphology (Figs 3A, B). Body slightly flattened dorso-ventrally, purple in colour with darker areas interspersed; mantle border ovate, completely covering food except posterior end, broad yellow band present in the periphery, followed by thinner dark-purple line and blue-whitish broader band, white MDFs often seen by transparency (see Fig. 3A). Mantle and foot covered by scattered, usually irregular, yellow and white, small, spots; generally showing larger dots towards mantle edge, these being ovate, eroded in specimens from the Canary Islands. Rhinophores retractile, dark purple, with blue-whitish tip; tiny white punctuation in lamellae, more abundant towards tip. Gill composed

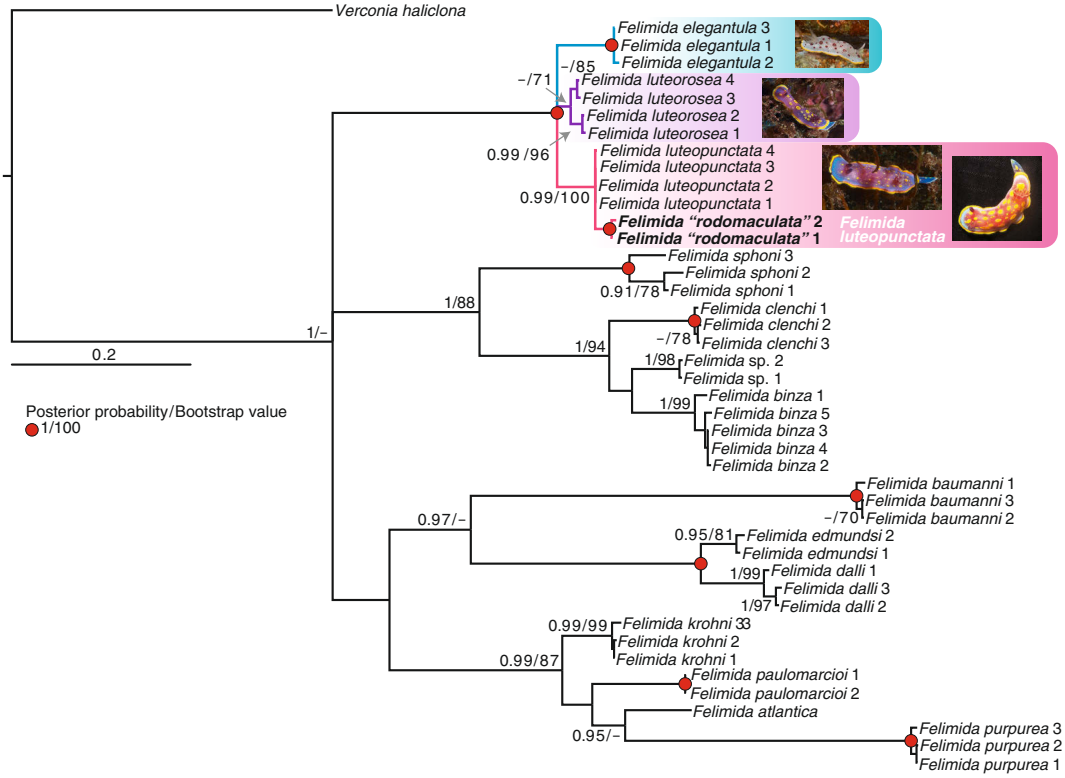

Fig. 2. Phylogenetic tree of Chromodorididae based on the combined COI, 16S, and H3 genes using Bayesian inference (BI) and maximum-likelihood (ML). Numbers on nodes indicate posterior probability values (BI) and bootstrap support values (ML). The tree is rooted with *Verconia haliclona*.

of 12 branchial leaves, unipinnate, retractile; light purple, tiny blue-white punctuations interspersed, more densely found in tip. Oral tentacles short and conical. Anterior part of foot broad, semi-circular, with pointed tips at each side; thin, opaque, white line around foot edge present.

Labial disc and radula (Figs 3C–F). Labial cuticle composed by numerous arched rods with bifid tips (Fig. 3C). Radular formula 49 × 49.1.49. Rachidian teeth triangular in shape, slender, plate-like (Fig. 3D). Lateral teeth unicuspid; first with two denticles in inner side, 3–4 denticles in outer side; progressively becoming more hooked, increasing in size and number of denticles to maximum of 13 per side at middle of half row (Fig. 3E), decreasing in size towards outer side; outer teeth hooked, spatulate, ca. 7 rounded denticles present per side (Fig. 3F).

Reproductive system (Fig. 4)**.** Ovotestis placed in posterior section of viscera (not depicted). Ampulla bean-shaped, slender; spermoviduct thin. Prostate large, convoluted; distal deferent duct long, thin, leading to saccular, elongated penial sheath. Uterine duct thin, connecting proximally to spherical bursa copulatrix (= gametolytic gland); this widely con-

Table 2. Automatic Barcode Gap Discovery (ABGD) intra- and interspecific distances for COI of the closest *Felimida* species from the *"luteorosea"* group.

	F. rodomaculata syn. nov.	*F. luteopunctata*	*F. luteorosea*	*F. elegantula*
F. rodomaculata syn. nov.	0.49			
F. luteopunctata	0.65–1.42	0–0.15		
F. luteorosea	5.24–6.27	5.68–6.07	0.36–2.22	
F. elegantula	6.48–7.24	7–7.35	5.19–6.33	0.15

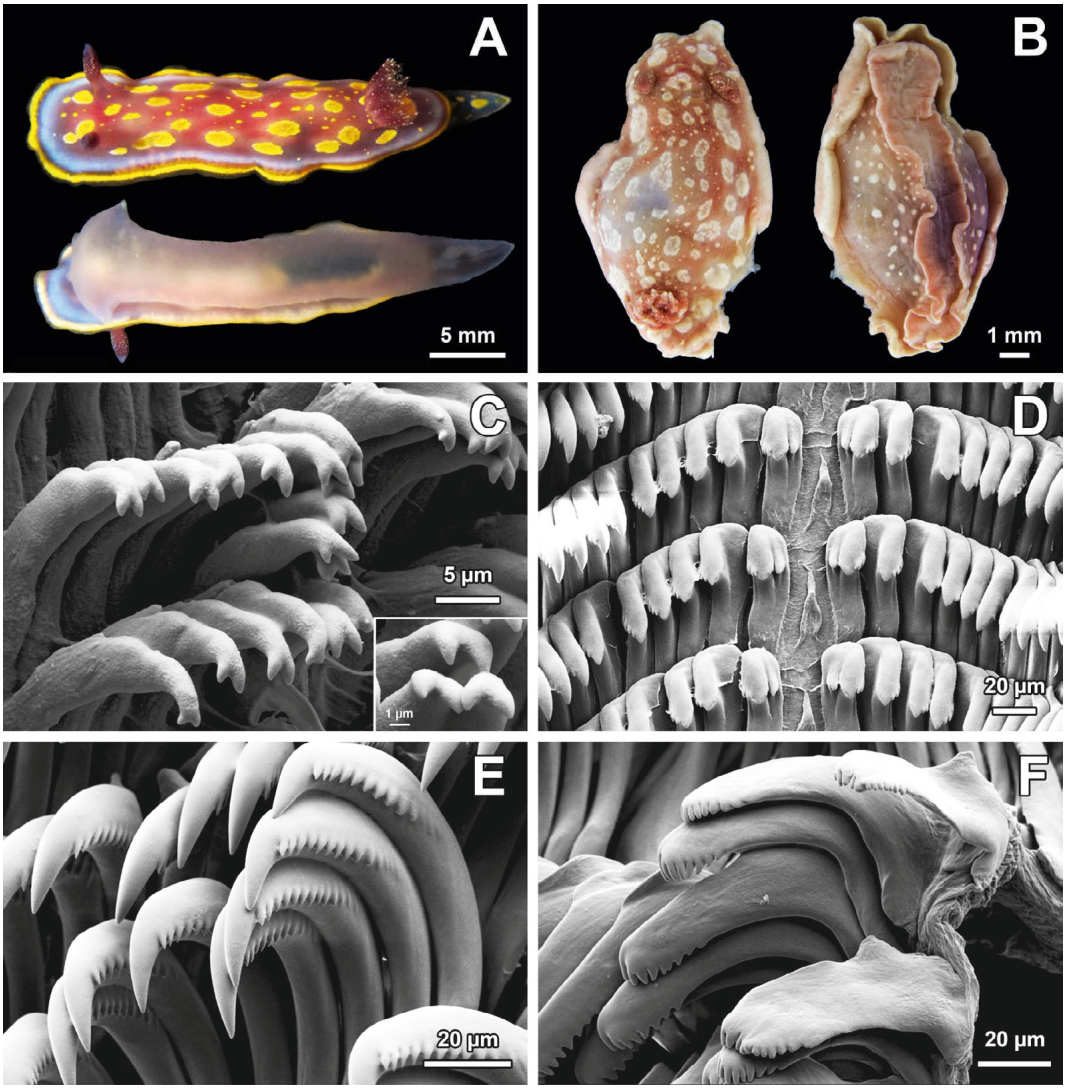

Fig. 3. *Felimida rodomaculata* syn. nov. **A.** Life pictures in dorsal and ventral view of the specimen collected in Lanzarote; **B.** Dorsal and ventral view of the preserved specimen collected in Fuerteventura; **C.** Scanning electron microscopy (SEM) of the elements of the labial armour, close-up of the apical region of the rods; **D.** SEM of the central part of the radula, showing the rachidian and up to 9 of the first lateral teeth; **E.** SEM detail of the 20 to 26 radular lateral teeth; **F.** SEM detail of the last 45 to 49 radular lateral teeth.

nected to a saccular, bean-shaped, seminal receptacle distally; vaginal duct relatively short and thin, connected to saccular, elongated vestibular gland. Nidamental glands wide, macroscopically differentiated in one first wrinkled, granulated, white gland; another larger with smooth surface present behind whole genital system.

Ecology. Found under boulders from the intertidal to 36 m depth (Cervera et al. 1989). Likely feeding on sponges as other *Felimida* species (McDonald & Nybakken 1997).

Geographic distribution. Known from Morocco, Senegal, and Ghana, NW African Coast (Gantès 1962, Ortea et al. 2011); Fuerteventura, Lanzarote, and Gran Canaria, Canary Islands (Ortea et al. 2011, as *F. rodomaculata* syn. nov.); SW Iberian Peninsula from southern Portugal to Granada (Cervera et al. 2004).

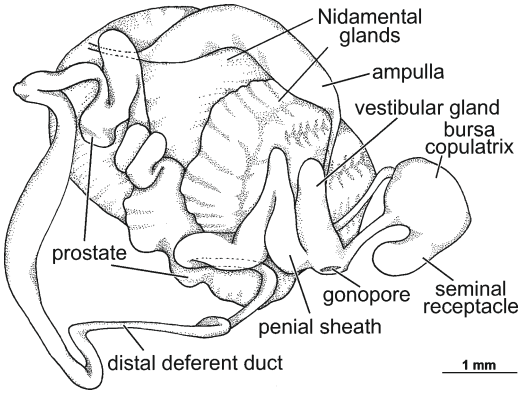

Fig. 4. Schematic representation of the reproductive system of *Felimida rodomaculata* syn. nov. collected in Lanzarote, Canary Islands, NE Atlantic.

Discussion

Our descriptions of the external morphology, radula, and reproductive system agree with Ortea & Valdés (1992) original description of *F. rodomaculata* syn. nov. One of the main anatomical differences stated to tell apart *F. rodomaculata* syn. nov. from *F. luteopunctata* was a wide and flattened seminal receptacle, which is longer in diameter than the bursa copulatrix (Ortea & Valdés 1992; this study), while in *F. luteopunctata* this is shorter and thinner (Ortea et al. 2011). Nonetheless, this character is reported to be variable among specimens of *F. luteopunctata* from different locations (Gantès 1962, Cervera et al. 1989, Sánchez-Tocino 2003), and we believe it is not a valid anatomical difference.

Externally, *F. luteopunctata* is clearly differentiated from *F. luteorosea* by the presence of smaller mantle spots, that can sometimes be larger in size, but they always display an irregular edge, while these have a smooth round edge in *F. luteorosea* (see pl. 1 in Ortea et al. 2011; Fig. 5). Even though *F. rodomaculata* syn. nov. present larger dots similar to *F. luteorosea*, the presence of a dorsal, interspersed, small punctuation is a distinctive characteristic. A lack of a white halation surrounding the dorsal spots and the small white spots on the gills and rhinophores have previously been considered appropriate characters separating *F. luteopunctata* from *F. luteorosea* (Ortea & Valdés 1992), but wide differences have been found among these species too (Debelius & Kuiter 2007,

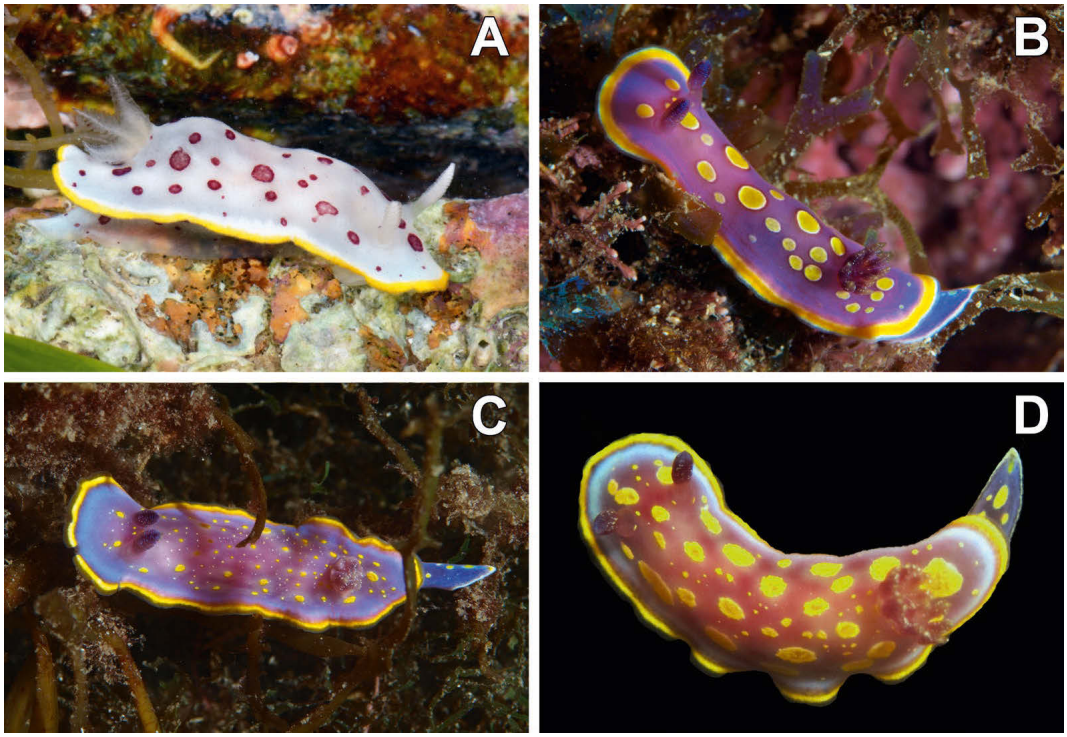

Fig. 5. Underwater pictures of **A.** *Felimida elegantula* from Sardinia, Italy; **B.** *F. luteorosea* from Tarifa, south Spain; **C.** *F. luteopunctata* from Tarifa, south Spain; **D.** *F. rodomaculata* syn. nov. from Lanzarote, Canary Islands.

Ortea et al. 2011) and thus this does not represent a valid diagnostic character.

The specimens of *Felimida rodomaculata* syn. nov. collected from the Canary Islands were characterized by the presence of large, yellow, irregularly-edged spots on the dorsum, with an additional smaller interspersed spotted pattern. The external and most distinctive character of *F. luteopunctata* is the presence of smaller and irregular spots, which sometimes become larger close to the mantle edge (Cervera et al. 1989). But several authors have observed a large variation in the colouration of these species. For instance, small white dots are present in the rhinophores and the gill of both species, as well as in some recorded specimens of *F. luteorosea* (Debelius & Kuiter 2007, Ortea et al. 2011; see Fig. 5). Nonetheless, the latter present smooth and circular spots in the dorsum as a distinctive character. Moreover, both *F. luteopunctata* and *F. rodomaculata* syn. nov. were recovered in a single clade with robust phylogenetic support and ABGD analysis of COI grouped them together, with an interspecific distance comparable to the intraspecific distance of all other chromodoridid species. Given the morphological and molecular evidence provided in this study, we recommend synonymizing *F. rodomaculata* with *F. luteopunctata*. Therefore, chromatic and reproductive anatomical differences claimed to separate both species might be considered as population variability, as already documented for other congener species (Padula et al. 2016, Furfaro et al. 2017). The current distribution of *F. luteopunctata* comprises the southern Iberian Peninsula (Cervera et al. 2004), the oriental Canary Islands (Ortea et al. 2011), and the north-eastern African coast (Gantès 1962, Ortea et al. 2011). Conclusively, this study highlights the need for an integrative study including external colouration, anatomy, and molecular data to discern among *Felimida* species as some species display a wide spectrum of chromatic variability.

Acknowledgements

We are especially grateful to J. Giménez (Estación Biológica de Doñana, Spain) for his help during collecting. We would like to thank M. Chacón who kindly provided the photographs of *F. luteopunctata* and *F. luteorosea* and M. Doneddu for providing the picture of *F. elegantula*. We are indebted to Dr M. A. E. Malaquias and I. Heggstad (University Museum of Bergen, Norway) for their support in the molecular lab and the scanning electron microscopy, respectively. Two anonymous reviewers are recognized by their valuable suggestions. This is the study #5 of the GROC Association. J. Moles was supported by a Ramón Areces postdoctoral fellowship and a short-term grant from the Fundació Montcelimar and the University of Barcelona.

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. 1997. Gapped BLAST and PSI- BLAST: a new generation of protein database search pro- grams. Nucleic Acids Research 25: 3389–3402.

Avila, C. 1995. Natural products of opisthobranch molluscs: a biological review. Oceanography and Marine Biology 33: 487–559.

– – , Núñez-Pons, L. & Moles, J. 2018. From the tropics to the poles: chemical defensive strategies in sea slugs (Mollusca: Heterobranchia). Pp. 71–163 in: Puglisi, M. P. & Becerro, M. A. (eds). Chemical ecology: the ecological impacts of marine natural products. Boca Raton (CRC Press).

Cervera, J. L., García-Gómez, J. C. & Ortea, J. A. 1989. On two rare chromodorid nudibranchs (Opisthobranchia: Chromodorididae) from the eastern Atlantic, with the description of a new species of *Glossodoris*. Journal of Molluscan Studies 55: 445–453.

– – , Calado, G., Gavaia, C., Malaquias, M. A. E., Templado, J., Ballesteros, M., García-Gómez, J. C. & Megina, C. 2004. An annotated and updated checklist of the opisthobranchs (Mollusca: Gastropoda) from Spain and Portugal (including islands and archipelagos). Boletín del Instituto Español de Oceanografía 20: 1–122.

Cimino, G. & Ghiselin, M. T. 2009. Chemical defense and the evolution of opisthobranch gastropods. Proceedings of the California Academy of Sciences 60: 175–422.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

Debelius, H. & Kuiter, R. H. 2007. Nudibranchs of the World. Frankfurt (IKAN-Unterwasserarchiv).

Eilertsen, M. H. & Malaquias, M. A. E. 2013. Systematic revision of the genus *Scaphander* (Gastropoda, Cephalaspidea) in the Atlantic Ocean, with a molecular phylogenetic hypothesis. Zoological Journal of the Linnean Society 167: 389–429.

Furfaro, G., Oliverio, M. & Mariottini, P. 2017. The southernmost record of *Felimida elegantula* (Philippi, 1844) (Gastropoda: Nudibranchia). Marine Biodiversity 47: 579–584.

Gantès, H. 1962. *Glossodoris luteopunctata*, une nouvelle espèce de mollusque nudibranche. Comptes Rendues des Séances Mensuelles de la Société des Sciences Naturelles et Physiques du Maroc 7: 133–135.

García-Gómez, J. C. 2002. Paradigmas de una fauna insólita. Los moluscos opistobranquios del estrecho de Gibraltar (Serie Ciencias) 20. Algeciras (Instituto de Estudios Gibraltareños).

Johnson, R. F. 2011. Breaking family ties: taxon sampling and molecular phylogeny of chromodorid nudibranchs (Mollusca, Gastropoda). Zoologica Scripta 40: 137–157.

– – & Gosliner T. M. 2012. Traditional taxonomic groupings mask evolutionary history: A molecular phylogeny and new classification of the chromodorid nudibranchs. PLoS ONE 7: 29–31.
Katoh, K., Rozewicki, J. & Yamada, K. D. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics: bbx108.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.
Kumar, S., Stecher, G. & Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.
Marcus, Ev. 1971. On some euthyneuran gastropods from the Indian and Pacific Oceans. Journal of Molluscan Studies 39: 355–369.
McDonald, G. R. & Nybakken, J. W. 1997. List of the worldwide food habits of nudibranchs. I. Introduction and the suborder Arminacea. Veliger 40: 157–159.
Moles, J., Avila, C. & Malaquias, M. A. E. 2018. Systematic revision of the Antarctic gastropod family Newnesiidae (Heterobranchia: Cephalaspidea) with the description of a new genus and a new abyssal species. Zoological Journal of the Linnean Society 183: 763–775.
Ortea, J. & Valdés, A. 1992. Descripción de una nueva especie de *Chromodoris* Alder & Hancock, 1855 (Mollusca: Opisthobranchia) de las Islas Canarias, estudio comparado con otras especies atlánticas del grupo cromático "luteorosea". Revista de la Academia Canaria de Ciencias 3: 69–85.
– – , Moro, L., Caballer, M. & Bacallado, J. J. 2011. *Chromodoris luteorosea* (Rapp, 1827) y *Chromodoris luteopunctata* (Gantes, 1962) dos especies de Chromodorididae (Mollusca: Nudibranchia) citadas erróneamente en las islas Canarias. Vieraea 39: 161–166.
Ortigosa, D., Pola, M., Carmona, L., Padula, V., Schrödl, M. & Cervera, J. L. 2014. Redescription of *Felimida elegantula* (Philippi, 1844) and a preliminary phylogeny of the European species of *Felimida* (Chromodorididae). Journal of Molluscan Studies 80: 541–550.
Padula, V., Bahia, J., Stöger, I., Camacho-García, Y., Malaquias, M. A. E., Cervera, J. L. & Schrödl, M. 2016. A test of color-based taxonomy in nudibranchs: molecular phylogeny and species delimitation of the *Felimida clenchi* (Mollusca: Chromodorididae) species complex. Molecular Phylogenetics and Evolution 103: 215–229.
Perrone, A. S. 1986. Opistobranchi (Aplysiomorpha, Pleurobrancomorpha, Sacoglossa, Nudibranchia) del litorale salentino (Mare Jonio) (Elenco-Contrib. Secondo). Thalassia Salentina 16: 19–42.
Posada, D. & Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793–808.
Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21: 1864–1877.
Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. 2014. Tracer v1.6. Available from: http://beast.bio.ed.ac.uk/Tracer.
Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2011. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.
Sánchez-Tocino, L. 2003. Aspectos taxonómicos y biológicos de los Doridoidea (Mollusca: Nudibranchia) del litoral granadino. PhD thesis, Universidad de Granada, Granada, Spain.
– – & García-Gómez, J. C. 2011. Familia Chromodorididae. Pp. 487–496 in: Gofas, S., Moreno, D. & Salas, C. (eds). Moluscos marinos de Andalucía – II. Málaga (Universidad de Málaga, Servicio de Publicaciones e Intercambio Científico).
Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.
– – , Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57: 758–771.
Talavera, G. & Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577.
Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17: 57–86.
Thompson, T. E. 1976. Biology of Opisthobranch Molluscs, Volume 1. 207 pp., London, UK (Ray Society).
Trainito, E. 2005. Nudibranchi del Mediterraneo: guida ai molluschi opistobranchi. Milano (Il Castello).
Turner, L. M. & Wilson, N. G. 2008. Polyphyly across oceans: a molecular phylogeny of the Chromodorididae (Mollusca, Nudibranchia). Zoologica Scripta 37: 23–42.
Wägele, H., Ballesteros, M. & Avila, C. 2006. Defensive glandular structures in opisthobranch molluscs-from histology to ecology. Oceanography and Marine Biology 44: 197.
Wollscheid-Lengeling, E., Boore, J., Brown, W. & Wägele, H. 2001. The phylogeny of Nudibranchia (Opisthobranchia, Gastropoda, Mollusca) reconstructed by three molecular markers. Organisms Diversity & Evolution 1: 241–256.
Yang, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends in Ecology & Evolution 11: 367–372.