



## Phylogeny and systematics of mitriform gastropods (Mollusca: Gastropoda: Neogastropoda)

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Received 26 January 2015; revised 21 March 2015; accepted for publication 25 March 2015

With about 800 Recent species, ‘miters’ are a widely distributed group of tropical and subtropical gastropods that are most diverse in the Indo-West Pacific. They include the two families Mitridae and Costellariidae, similar in shell morphology and traditionally treated as close relatives. Some genera of deep-water Ptychatractidae and Volutomitridae are close to miters in shell morphology, and the term ‘mitriform gastropods’ has been introduced to refer to Mitridae, Costellariidae, and this assortment of convergent forms. The present study aimed at the reconstruction of phylogenetic relationships of mitriform gastropods based on representative taxon sampling. Four genetic markers [cytochrome c oxidase subunit I (*COI*), 16S and 12S rRNA mitochondrial genes, and H3 (Histone 3) nuclear gene] were sequenced for over 90 species in 20 genera, and the molecular data set was supplemented by studies of radula morphology. Our analysis recovered Mitridae as a monophyletic group, whereas the genus *Mitra* was found to be polyphyletic. Of 42 mitrid species included in the analysis, 37 formed a well-supported ‘core Mitridae’ consisting of four major clades, three of them consistent with the subfamilies *Cylindromitrinae*, *Imbricariinae*, and *Mitrinae*, and *Strigatella paupercula* standing out by itself. Basal to the ‘core Mitridae’ are four minor lineages, with the genus *Charitodoron* recognized as sister group to all other Mitridae. The deep-water family *Pyramimitridae* shows a sister relationship to the Mitridae, with high support for a *Pyramimitridae* + Mitridae clade. Our results recover the monophyly of the Costellariidae, which form a well-supported clade that also includes Ptychatractidae, *Columbariinae*, and Volutomitridae, but not Mitridae. Most derived and diverse amongst Costellariidae are species of *Vexillum*, characterized by a bow-shaped, multicuspidate rachidian tooth. Several previously unrecognized deep-water costellariid lineages are revealed. Their members retain some plesiomorphies – in particular a tricuspidate rachidian tooth – that makes them morphologically intermediate between ptychatractids and *Vexillum*. The taxa of Ptychatractidae included in the analysis are not monophyletic, but form three well-supported, unrelated groupings, corresponding respectively to *Ceratoxancus* + *Latiromitra*, *Exilia*, and *Exiliodea*. None of them shows an affinity to Pseudolividae.

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**ADDITIONAL KEYWORDS:** Costellariidae – marine molluscs – *Mitra* – Mitridae – Ptychatractidae – radula – *Vexillum* – Volutomitridae.

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## INTRODUCTION

Almost one-quarter of the diversity of marine molluscs is represented by the order Neogastropoda. With more than 12 000 Recent species classified in 40 families, these predatory and scavenging snails are one of the most evolutionary successful taxa of marine molluscs, playing a key role in marine benthic ecosystems at all latitudes and depths. However, although the superfamilies Conoidea (Puillandre *et al.*, 2008, 2011) and Buccinoidea (Hayashi, 2005; Oliverio & Modica, 2010), and the families Muricidae (Barco *et al.*, 2010) and Cancellaridae (Modica *et al.*, 2011), have been specifically targeted by recent molecular phylogenies, a not inconsiderable fraction of the Neogastropoda – including some ancient and diverse lineages – has remained essentially outside the sphere of molecular systematics. One such untouched group is the so-called ‘miters’, a name that refers to members of the two families Mitridae and Costellariidae that together encompass some 800 species (WoRMS, 2015). Meters are widely distributed in tropical and subtropical waters and reach their highest diversity in the Indo-West Pacific at depths of 0–100 m, where they form a characteristic element of soft-bottom benthic communities. Miter shells have long been popular amongst collectors, and many new species continue to be discovered by amateur taxonomists. Meters usually have high, fusiform shells, with an elongated aperture, strong columellar folds, and an adult size mostly in the 10–50 mm range. Based on this superficial resemblance in shell morphology, a number of costellariid species was originally described under mitrid genera and vice versa. A similar shell is also found in other neogastropod genera, such as *Latiromitra* (Ptychactractidae) or *Microvoluta* (Volutomitridae), and the term ‘mitriform gastropods’ (Ponder, 1972) informally refers to the miters as well as such miter-resembling forms.

The current miters systematics is still largely based on the work carried out by Walter Cernohorsky (1966, 1970, 1976, 1991). However, relationships within both these families remain poorly understood, and the monophyly of neither Costellariidae, nor Mitridae and its subfamilies, has ever been tested. Despite Mitridae and Costellariidae having similar shell morphology, their close relationship has been challenged based on radula morphology and the anatomy of the digestive tract (Iredale, 1929; Cernohorsky, 1966, 1970; Ponder, 1972; Fedosov & Kantor, 2010). What little is available in terms of molecular data likewise suggests that the two families are not close relatives: the single mitrid species sequenced so far clustered with the Muricidae and Olividae, whereas the single costellariid species showed a closer affinity to the deep-water families Ptychactractidae and Volutomitridae (Oliverio & Modica,

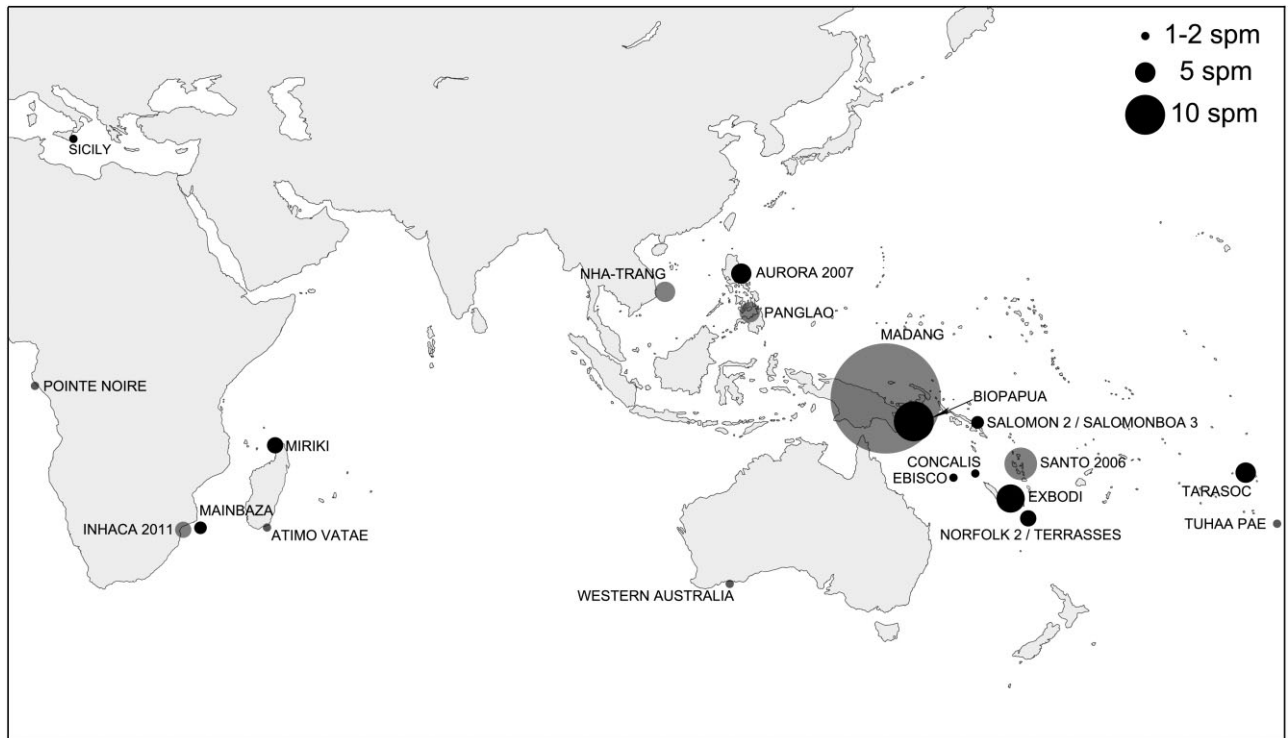
2010; Zou, Li, & Kong, 2011). In fact, the close relationship between Costellariidae and Ptychactractidae was first suggested by Thiele (1929). However, a combination of plesiomorphies in the morphology of Ptychactractidae (the simple radula with tricuspidate rachidian and sickle-shaped laterals; open seminal groove) justified the later placement of this group in the family Turbinellidae (Bouchet & Warén, 1985; Bouchet & Kantor, 2000). A recent morphological study, however, revealed a deep-water member of the family Costellariidae that showed a remarkable similarity to ptychactractids in foregut anatomy and radula morphology (Fedosov & Kantor, 2010); this discovery supports a close relationship between Costellariidae and Ptychactractidae, and renders morphological differences between the two families quite subtle. With this background of contradictory morphological and molecular data, the relationships of Mitridae, Costellariidae, and other lineages of mitriform gastropods remain quite uncertain and speculative, and require a fresh re-evaluation. The purposes of the present study were to map precisely the phylogeny of mitriform gastropods, with an emphasis on the two families of miters, and to describe their relationships within the Neogastropoda.

## MATERIAL AND METHODS

## TAXON SAMPLING

The material used in the present study was collected in a series of shore-based expeditions and deep-water cruises conducted by Museum National d'Histoire Naturelle (MNHN), Institut de recherche pour le Développement (IRD), and Joined Russian Vietnamese Tropical Center in the tropical Indo-Pacific, West Africa, and the Mediterranean (Fig. 1, Table 1). In the field, molluscs were either anaesthetized using magnesium chloride (MgCl<sub>2</sub>), or in some cases shells were drilled, or, in the most recent expeditions, were processed using a microwave oven (Galindo *et al.*, 2014); a tissue clip was then cut and fixed in 95% ethanol. Voucher shells, kept intact for identification, have been deposited in MNHN (Table 1). Taxonomy of the family Mitridae follows Cernohorsky (1976, 1991), taxonomy of the families Costellariidae, Ptychactractidae, and Volutomitridae is based on several sources as indexed in World Register of Marine Species (WoRMS, 2015).

A number of mitriform species in the families Ptychactractidae (genera *Exilia*, *Exilioidea*, *Ceratoxancus*, and *Latiromitra*), Volutomitridae (genera *Volutomitra* and *Microvoluta*), and Turbinellidae (subfamily Vasinae, genus *Vasum*; subfamily Columbariinae, genera *Coluzea* and *Fustifusus*) were included as putative sister groups of the family Costellariidae. A suite of species from 17 other neogastropod families in the superfamilies



**Figure 1.** Map of sampled localities. Black circles mark deep-water cruises, grey circles shallow-water shore-based expeditions. Diameters of circles are proportional to the number of samples used in the present study (see legend in the top right-hand corner; spm, specimens).

Conoidea, Cancellarioidea, Buccinoidea, Muricoidea, Olivoidea, and Pseudolivoidea was included in the analysis to ensure adequate representation of major evolutionary lineages of the Neogastropoda and the likely detection of mitriform gastropod relationships. *Tonna galea* (Caenogastropoda, Tonnoidea, Tonnidae) was used as a non-neogastropod outgroup. Overall classification follows Bouchet & Rocroi (2005).

#### PCR AMPLIFICATION AND DNA SEQUENCING

Total genomic DNA was extracted from muscle tissue using NucleoSpinR 96 Tissues (Macherey–Nagel) and following the manufacturer's instructions. Fragments of the mitochondrial genes cytochrome oxidase I (*COI*), *16S rRNA*, and *12S rRNA*, as well as the nuclear gene *H3* were sequenced (Table 2). PCR reactions were performed in 20  $\mu$ L final volume, containing approximately 3 ng template DNA, 1.5 mM  $MgCl_2$ , 0.26 mM of each nucleotide, 0.3  $\mu$ L of each primer, 5% dimethyl sulphoxide, and 0.75  $\mu$ L of Taq polymerase (Qbiogene).

For amplification of each *16S*, and *H3* only one pair of primers was used (see Table 2). *COI* and *12S* sequences were amplified using two different primer combinations each. Beside standard Folmer's primers a newly designed pair of primers (22COIF - 695COIR)

has been used for the amplification of *COI* fragment in few Mitridae specimens. For most specimens the pair 12SA–12SB was used, producing a fragment of about 370–380 bp. However, owing to poor results with these primers in most Mitridae, the primer pair 12SI–12SIII was used instead for some specimens, resulting in a fragment of about 540 bp (Table 2).

The PCR profile for *COI* started with 5 min at 95  $^{\circ}C$ , followed by 40 cycles of denaturation at 95  $^{\circ}C$  (35 s), annealing at 50  $^{\circ}C$  (35 s), and elongation at 72  $^{\circ}C$  (1 min), with a final elongation phase at 72  $^{\circ}C$  (10 min). Similar PCR profiles were set for *16S* (annealing at 55  $^{\circ}C$ , elongation at 72  $^{\circ}C$  for 50 s), *12S* with the primer pair 12SA–12SB (annealing at 57  $^{\circ}C$ , elongation at 72  $^{\circ}C$  for 50 s), and *H3* (annealing at 57  $^{\circ}C$ , elongation at 72  $^{\circ}C$  for 45 s). For the primer pair 12SI–12SIII a touch-down PCR protocol with gradual decrease of the annealing temperature from 62 to 56  $^{\circ}C$  was used. All genes were sequenced in both directions to confirm the accuracy of each sequence. Sequencing was performed by Eurofins. Chromatograms were edited using CodonCode Aligner v. 3.7.1.1.

#### SEQUENCE ALIGNMENTS

Sequences were aligned for each gene independently using MUSCLE (Edgar, 2004). The accuracy of

Table 1. List of specimens analyzed. Type species of genera shown in bold

Family	Genus	species	MNHN	Expedition	Station	collection data	Clade	BOLD	COI	16S	12S	H3
COSTELLARIIDAE	<i>Thala</i>	sp.	IM-2013-3389	PAPUA NIUGINI	PM25	05°01.1'S 145°47.9'E, intertidal	Thala	MITRI011-15	KR087299	KR088048	KR087384	-
COSTELLARIIDAE	<b><i>Vexillum</i></b>	<b><i>pitcairum</i></b>	IM-2013-13646	PAPUA NIUGINI	PM27	04°59.3'S 145°47.6'E, intertidal	Vexillum s.s.	MITRI007-15	KR087312	KR088059	-	KR088139
COSTELLARIIDAE	<i>costatum</i>		IM-2009-11058	SANTO 2006	DR64	15°27.6'S 167°14.3'E, 6-35 m	Vexillum s.s.	HQ401586.1	HQ401718.1	HQ401655.1	-	-
COSTELLARIIDAE	<i>pagodula</i>		IM-2007-30310	SANTO 2006	DB12	15°36.6'S 167°10.1'E, 10-18 m	Vexillum s.s.	NEOGA293-10	KR087310	-	KR087399	KR088137
COSTELLARIIDAE	<i>deshayesi</i>		IM-2007-30259	SANTO 2006	DS1	15°31.4'S 167°09.7'E, 5 m	Vexillum s.s.	NEOGA274-10	KR087304	-	KR087392	KR088132
COSTELLARIIDAE	<i>micra</i>		IM-2013-4734	PAPUA NIUGINI	PE28	05°11.9'S 145°49.6'E, 10 m	Vexillum s.s.	MITRI001-15	KR087309	KR088057	-	-
COSTELLARIIDAE	<b><i>semifasciatum</i></b>		IM-2013-11594	PAPUA NIUGINI	PM12	05°00.2'S 145°47.6'E, 0-1 m	Vexillum s.s.	MITRI003-15	KR087317	KR088062	KR087404	KR088143
COSTELLARIIDAE	<i>sangaisi</i>		IM-2013-14245	PAPUA NIUGINI	PR68	05°01.6'S 145°48.1'E, 5 m	Vexillum s.s.	MITRI013-15	KR087315	KR088061	KR087402	-
COSTELLARIIDAE	<i>virgo</i>		IM-2013-13079	PAPUA NIUGINI	PD24	05°05.3'S 145°48.6'E, 3-6 m	Vexillum s.s.	MITRI014-15	KR087318	KR088063	KR087405	KR088144
COSTELLARIIDAE	<b><i>exasperatum</i></b>		IM-2013-11680	PAPUA NIUGINI	PR14	05°12'S 145°48.1'E, 2-3 m	Vexillum s.s.	MITRI015-15	KR087305	KR088055	KR087393	KR088133
COSTELLARIIDAE	<i>gloriae</i>		IM-2013-40634	NT2011	ND3	12°10.084'N 109°17.771'E, 6-18 m	Vexillum s.s.	MITRI009-15	KR087306	-	KR087403	KR088134
COSTELLARIIDAE	<i>scitulum</i>		IM-2013-40633	EXBODI	CP3824	21°57'S 166°58'E, 280-296 m	Vexillum s.s.	MITRI005-15	KR087316	-	KR087403	KR088131
COSTELLARIIDAE	<i>dekkersi</i>		IM-2007-30242	PANGLAO 2004	B7	09°35.9'N 123°51.8'E, 4-30 m	Protoelongata	NEOGA265-10	KF671189	KR088054	KR087391	KR088135
COSTELLARIIDAE	<i>herosae</i>		IM-2013-40630	MIRIKY	CP3204	12°37.03'S 48°30.3'E, 59-60 m	Vexillum s.s.	MITRI017-15	KR087307	KR088135	KR087395	KR088133
COSTELLARIIDAE	<i>cancellarioides</i>		IM-2013-40631	TUHAA PAE 2013	AT04	23°25.13'S 149°27.0'W, 12 m	Vexillum s.s.	MITRI012-15	KR087301	KR088051	KR087388	KR088128
COSTELLARIIDAE	<i>rubrum</i>		IM-2013-1747	PAPUA NIUGINI	PB10	05°17.9'S 145°46.7'E, 10 m	Vexillum s.l.	MITRI016-15	KR087314	KR088060	KR087410	KR088141
COSTELLARIIDAE	<i>patriarchalis</i>		IM-2013-10241	PAPUA NIUGINI	PS04	05°10.0'S 145°50.1'E, 12 m	Vexillum s.l.	MITRI008-15	KR087311	KR088058	KR087400	KR088138
COSTELLARIIDAE	<i>acupictum</i>		IM-2013-40635	NT2011	ND3	12°10.084'N 109°17.771'E, 6-18 m	Vexillum s.s.	MITRI004-15	-	-	KR087386	KR088126
COSTELLARIIDAE	<i>nodospiculum</i>		IM-2013-40632	AURORA 2007	CP2794	15°57'N 121°49'E, 453-460 m	Vexillum s.l.	MITRI006-15	-	-	KR087398	KR088136
COSTELLARIIDAE	<i>balutense</i>		IM-2013-40637	BIOPAPUA	CP3747	05°33'S 153°59'E, 458 m	C-I	MITRI020-15	KR087300	KR088050	KR087387	KR088127
COSTELLARIIDAE	<i>cf. choslenae</i>		IM-2007-38378	EXBODI	CP3826	21°52'S 166°51'E, 354-509 m	C-I	MITRI060-15	KR087302	KR088052	KR087389	KR088129
COSTELLARIIDAE	<i>isaoi</i>		IM-2013-40636	BIOPAPUA	CP3748	05°37'S 154°01'E, 398-389 m	C-I	MITRI021-15	KR087308	KR088056	KR087396	-
COSTELLARIIDAE	<i>cf. isaoi</i>		IM-2007-34557	AURORA 2007	CP2709	15°12'N 121°34'E, 244-296 m	C-I	NEOGA771-10	KR087303	KR088053	KR087390	KR088130
COSTELLARIIDAE	<i>pratense</i>		IM-2007-35967	BIOPAPUA	CP3741	09°14'N 152°18'E, 694-766 m	C-I	MITRI069-15	KR087313	-	KR087413	KR088140
COSTELLARIIDAE	<i>Zieritana</i>	<i>zierogelii</i>	IM-2013-15865	PAPUA NIUGINI	PM41	05°08.1'S 145°49.3'E, 0-1 m	Vexillum s.s.	MITRI010-15	KR087326	KR088072	-	-
COSTELLARIIDAE	<i>Zieritana</i>	<i>woldemari</i>	IM-2013-12694	PAPUA NIUGINI	PM8	05°15.3'S 145°46.6'E, 0-1 m	Vexillum s.s.	MITRI002-15	KR087325	KR088071	KR087412	-
COSTELLARIIDAE	Gen.	sp.	IM-2007-30347	SALOMON 2	CP2189	08°20' S 160°02' E, 660-854 m	C-I	NEOGA307-10	KR087243	-	KR087338	-
COSTELLARIIDAE	Gen.	sp.	IM-2007-38296	EBISCO	DW2606	19°37'S 158°42'E, 442-443 m	C-III	MITRI018-15	KR087244	KR087997	KR087339	KR088085
COSTELLARIIDAE	Gen.	sp.	IM-2007-39400	TARASOC	DW3452	16°51'S 151°19'W, 600-705 m	C-III	MITRI022-15	KR087248	KR088001	KR087345	KR088089
COSTELLARIIDAE	Gen.	sp.	IM-2013-40624	BIOPAPUA	CP3719	06°03'S 147°36'E, 410 m	C-I	MITRI074-15	KR087247	KR087999	KR087343	-
COSTELLARIIDAE	Gen.	sp.	IM-2013-40132	PAPUA NIUGINI	CP4055	03°03'S 142°18'E, 370-374 m	C-II	MITRI075-15	KR087246	-	KR087342	-
COSTELLARIIDAE	Gen.	sp.	IM-2013-40627	PANGLAO 2005	CP2381	08°43'N 123°19'E, 259-280 m	C-II	MITRI076-15	KR087245	-	KR087341	KR088087
COSTELLARIIDAE	Gen.	sp.	IM-2013-40628	BIOPAPUA	CP3632	06°56'S 147°08'E, 700-740 m	C-II	MITRI077-15	-	KR087998	KR087340	KR088086
COSTELLARIIDAE	Gen.	sp.	IM-2013-40629	BIOPAPUA	CP3632	06°56'S 147°08'E, 700-740 m	C-II	MITRI078-15	-	KR088000	KR087344	KR088088
COSTELLARIIDAE	Gen.	sp.	IM-2013-40638	PAPUA NIUGINI	CP4079	04°34'S 145°52'E, 960 m	C-III	MITRI023-15	KR087249	-	-	-
COSTELLARIIDAE	Gen.	sp.	IM-2013-4814	PAPUA NIUGINI	CP3949	05°12'S 145°51'E, 380-407 m	C-II	MITRI023-15	KR087249	-	-	-
MITRIDAE	<i>Cancilla</i>	<i>fibula*</i>	IM-2007-38689	TARASOC	DW3441	16°43'S 151°26'W, 350-360 m	M-IV	MITRI058-15	KR087234	-	-	-
MITRIDAE	<i>Cancilla</i>	<i>cf. fibula</i>	IM-2013-40646	EXBODI	CP3822	21°52'S 166°51'E, 341-506 m	M-IV	MITRI030-15	-	KR087991	KR087332	-
MITRIDAE	<i>Charitodoron</i>	sp.	IM-2007-38306	MAINBAZA	CC3157	21°46'S 36°25'E, 1410-1416 m	basal Mitridae	MITRI057-15	KR087240	KR087990	KR087331	KR088076
MITRIDAE	<i>Charitodoron</i>	sp.	IM-2013-40641	INHACA 2011	MR15	26°00.0'S 92°54.4'E, 4 m	basal Mitridae	MITRI040-15	KR087241	-	-	KR088082
MITRIDAE	<i>Domiporta</i>	<i>praesantissima</i>	IM-2013-40642	NT2010	D3	12°10.084'N 109°17.771'E, 6-18 m	M-II	MITRI038-15	KR087253	KR088006	-	KR088092
MITRIDAE	<i>Domiporta</i>	<b><i>flitaris</i></b>	IM-2013-12956	PAPUA NIUGINI	PD23	05°06'S 145°49.2'E, 3-7 m	M-II	MITRI032-15	KR087252	KR088006	-	KR088090
MITRIDAE	<i>Imbricaria</i>	<b><i>conularis</i></b>	IM-2013-18043	PAPUA NIUGINI	PR203	05°10.3'S 145°48.5'E, 1-19 m	M-IV	MITRI044-15	KR087251	KR088004	KR087348	KR088099
MITRIDAE	<i>Imbricaria</i>	<i>olivaeformis</i>	IM-2013-18062	PAPUA NIUGINI	PR214	05°10.2'S 145°50.4'E, 1-8 m	M-IV	MITRI053-15	KR087263	KR088014	KR087356	-
MITRIDAE	<i>Mitra</i>	<i>bernhardina</i>	IM-2013-13618	PAPUA NIUGINI	PR19	05°05.1'S 145°48.6'E, 10 m	basal Mitridae	MITRI041-15	KR087264	KR088015	KR087367	-
MITRIDAE	<i>Mitra</i>	<i>incompta</i>	IM-2007-30175	SANTO 2006	DR78	15°28.4'S 167°15.2'E, 25 m	M-I	NEOGA256-10	KR087273	KR088023	KR087366	KR088104



Table 1. Continued

Family	Genus	species	MNHN	Expedition	Station	collection data	Clade	BOLD	COI	16S	12S	H3
MITRIDAE	<i>Mitra</i>	<i>tuberosa</i>	IM-2007-30311	PANGLAO 2004	R75	09°32.8'N 123°42.1'E, 3–35 m	basal Mitridae	NEOGA294-10	KR087283	KR088033	KR087375	KR088112
MITRIDAE	<i>Mitra</i>	<i>papalis</i>	IM-2007-30114	SANTO 2006	DR87	15°38.5'S 167°15.1'E, 13 m	M-II	NEOGA255-10	KR087276	KR088026	–	–
MITRIDAE	<i>Mitra</i>	<i>sophia</i>	IM-2007-30056	SANTO 2006	DR09	15°34.6'S 167°13.8'E, 12 m	M-II	NEOGA229-10	KR087280	KR088030	KR087372	KR088110
MITRIDAE	<i>Mitra</i>	cf. <i>rosacea</i>	IM-2007-30309	SANTO 2006	AT44	15°36'S 167°03'E, 86–118 m	M-II	NEOGA292-10	KR087267	KR088018	KR087361	KR088103
MITRIDAE	<i>Mitra</i>	<i>christinae</i>	IM-2007-32131	PANGLAO 2005	CP2383	08°45'N 123°18'E, 338–351 m	M-II	NEOGA491-10	KR087269	–	–	–
MITRIDAE	<i>Mitra</i>	<b><i>mitra</i></b>	IM-2013-40639	INHACA 2011	MR15	26°00.0'S 32°54.4'E, 4 m	M-II	MITRI052-15	KR087275	KR088025	KR087368	–
MITRIDAE	<i>Mitra</i>	<i>glabra</i>	IM-2013-40640	Western Australia	WE01	15°16.94'S 124°06.3'E, 11 m	M-I	MITRI042-15	KR087272	KR088022	KR087365	KR088105
MITRIDAE	<i>Mitra</i>	<i>shepmanni</i>	IM-2013-40645	EXBODI	DW3926	18°35'S 164°20'E, 364–473 m	M-IV	MITRI025-15	KR087279	KR088029	KR087371	KR088109
MITRIDAE	<i>Mitra</i>	<i>peculiaris</i>	IM-2013-6168	PAPUA NIUGINI	PR218	05°07.3'S 145°49.4'E	basal Mitridae	MITRI051-15	KR087278	KR088028	KR087370	KR088108
MITRIDAE	<i>Mitra</i>	sp.	IM-2013-40660	BIOPAPUA	CP3728	07°52'S 148°01'E, 498–501 m	M-II	MITRI050-15	KR087281	KR088031	KR087373	–
MITRIDAE	<i>Mitra</i>	<i>corniculata</i>	IM-2013-40661	Sicily, Cyracuse	CP3288	37°0.460'N 15°18.62'E, 6–12 m	M-II	MITRI059-15	KR087270	KR088024	KR087363	KR088107
MITRIDAE	<i>Nebularia</i>	<i>maesta</i>	IM-2013-40648	MIRIKY	PB16	14° 31.9' S 47° 26.54' E, 46–54 m	M-II	MITRI036-15	KR087274	KR088024	KR087367	–
MITRIDAE	<i>Nebularia</i>	<i>connectens</i>	IM-2013-2342	PAPUA NIUGINI	PB15	05°10.7'S 145°47.7'E, intertidal	M-II	MITRI037-15	KR087271	KR088021	KR087364	–
MITRIDAE	<i>Nebularia</i>	<i>pediculus</i>	IM-2013-12705	PAPUA NIUGINI	PB15	05°04.7'S 145°48.9'E, 5 m	M-II	MITRI035-15	KR087282	KR088032	KR087374	KR088111
MITRIDAE	<i>Neocancilla</i>	<b><i>papilio</i></b>	IM-2013-12580	PAPUA NIUGINI	PR24	05°12.3'S 145°48.8'E	M-IV	MITRI034-15	KR087287	KR088037	KR087376	KR088116
MITRIDAE	<i>Neocancilla</i>	<i>clathra</i>	IM-2007-30178	SANTO 2006	DR73	15°22.5'S 167°11.4'E, 10–25 m	M-IV	NEOGA258-10	KR087286	–	–	–
MITRIDAE	<i>Neocancilla</i>	<i>rufescens</i>	IM-2013-40644	INHACA 2011	MD22	25°59.7'S 32°46.8'E, 22 m	M-IV	MITRI033-15	KR087288	KR088038	–	KR088117
MITRIDAE	<i>Pterygia</i>	<b><i>dactylus</i></b>	IM-2013-14989	PAPUA NIUGINI	PM39	05°12.1'S 145°48.4'E, intertidal	M-I	MITRI049-15	KR087291	KR088041	KR087379	KR088120
MITRIDAE	<i>Pterygia</i>	<i>sinensis</i>	IM-2009-15439	ATIMO VATAE	CP3568	25°04.7'S 47°03.4'E, 64–65 m	M-I	MITRI054-15	KR087292	KR088042	KR087380	KR088121
MITRIDAE	<i>Strigatella</i>	<b><i>pauercula</i></b>	IM-2013-15188	PAPUA NIUGINI	PM39	05°12.1'S 145°48.4'E, intertidal	M-III	MITRI031-15	KR087277	KR088027	KR087369	–
MITRIDAE	<i>Subancilla</i>	<i>pugnax</i>	IM-2007-34547	AURORA 2007	CP2716	14°30'N 121°41'E, 335–386 m	M-IV	NEOGA766-10	KR087295	KR088044	–	–
MITRIDAE	<i>Scabricola</i>	<b><i>fusca</i></b>	IM-2013-40643	INHACA 2011	MS8	25°59.5'S 32°52.9'E, 9–17 m	M-IV	MITRI029-15	KR087294	KR088043	KR087381	KR088122
MITRIDAE	<i>Scabricola</i>	<i>casta</i>	IM-2007-31989	PANGLAO 2004	R38	09°29.4'N 123°56.0'E, 6–37 m	M-IV	NEOGA477-10	KR087293	–	–	–
MITRIDAE	<i>Ziba</i>	<b><i>carinata</i></b>	IM-2013-40647	ZANAGA	531DW	04°43.0'S 11°47.0'E, 17 m	M-II	MITRI026-15	–	KR088067	KR087409	KR088148
MITRIDAE	<i>Ziba</i>	cf. <i>abyssicola</i>	IM-2013-40654	EXBODI	CP3829	22°02'S 167°05'E, 350–380 m	M-II	MITRI047-15	KR087233	KR087989	KR087330	KR088075
MITRIDAE	<i>Ziba</i>	<i>fulgetrum</i>	IM-2013-18112	PAPUA NIUGINI	PR240	05°08.2'S 145°48.7'E, 3–20 m	M-IV	MITRI046-15	KR087323	KR088069	KR087411	KR088149
MITRIDAE	<i>Ziba</i>	<i>flammigera</i>	IM-2013-40656	NT2011	ND7	12°10.443'N 109°16.298'E, 15–18 m	M-IV	MITRI028-15	KR087322	KR088068	KR087410	–
MITRIDAE	<i>Ziba</i>	<i>insculpta</i>	IM-2013-40657	NT2011	ND7	12°10.443'N 109°16.298'E, 15–18 m	M-IV	MITRI048-15	KR087324	KR088070	–	KR088150
MITRIDAE	Gen.	sp.	IM-2013-40651	BIOPAPUA	CP3671	04°04'S 151°56'E, 585–601 m	M-II	MITRI027-15	KR087284	KR088034	–	KR088113
MITRIDAE	Gen.	sp.	IM-2013-40655	EXBODI	CP3821	21°53'S 166°50'E, 211–440 m	M-II	MITRI045-15	KR087285	KR088036	–	KR088115
MITRIDAE	Gen.	sp.	IM-2013-40133	PAPUA NIUGINI	CP4035	04°31'S 145°31'E, 380–382 m	M-II	MITRI039-15	–	KR088035	–	KR088114
PYCHATRACTIDAE	<i>Ceratohancus</i>	sp.	IM-2007-39277	TARASOC	DW3394	15°49'S 148°17'W, 500–510 m	Ceratohancus	MITRI055-15	KR087237	–	–	KR088078
PYCHATRACTIDAE	<i>Ceratohancus</i>	<i>teramachii</i>	IM-2007-36797	TERRASSES	DW3077	23°15'S 168°14'E, 420–540 m	Ceratohancus	MITRI061-15	KR087239	–	–	KR088080
PYCHATRACTIDAE	<i>Ceratohancus</i>	<i>leios</i>	IM-2007-38552	NORFOLK 2	DW2060	29°40'S 168°39'E, 582–600 m	Ceratohancus	NEOGA820-10	KR087236	–	–	KR088077
PYCHATRACTIDAE	<i>Ceratohancus</i>	sp.	IM-2007-38552	TARASOC	DW3401	15°51'S 148°18'W, 789–831 m	Ceratohancus	MITRI081-15	KR087238	KR087993	KR087334	KR088079
PYCHATRACTIDAE	<i>Ceratohancus</i>	cf. <i>melichrous</i>	IM-2013-40623	EXBODI	DW3914	19°45'S 165°45'E, 620–725 m	Ceratohancus	MITRI068-15	KR087235	KR087992	KR087333	–
PYCHATRACTIDAE	<i>Exilia</i>	<i>vagrans</i>	IM-2007-34087	SALOMON 2	CP2251	07°28'S 156°14'E, 1000–1050 m	Exilia	NEOGA752-10	KR087258	–	–	KR088097
PYCHATRACTIDAE	<i>Exilia</i>	<i>hilgendorffi</i> s.l.	IM-2007-34612	AURORA 2007	CP2751	15°36'N 121°56'E, 1456–1471 m	Exilia	NEOGA773-10	KR087254	–	–	KR088098
PYCHATRACTIDAE	<i>Exilia</i>	<i>hilgendorffi</i> s.l.	IM-2007-38603	TARASOC	DW3493	17°28'S 149°27'W, 556–565 m	Exilia	MITRI073-15	KR087256	KR088008	KR087351	KR088095
PYCHATRACTIDAE	<i>Exilia</i>	<i>hilgendorffi</i> s.l.	IM-2007-36877	MIRIKY	CP3187	12°30'S 48°08'E, 691–695 m	Exilia	MITRI071-15	KR087255	KR088007	KR087350	KR088094
PYCHATRACTIDAE	<i>Exilia</i>	<i>krigei</i>	IM-2007-38331	MAINBAZA	CC3171	25°59'S 34°42'E, 771–776 m	Exilia	MITRI072-15	KR087257	KR088009	KR087352	KR088096
PYCHATRACTIDAE	<i>Exilioidea</i>	sp.	IM-2007-34633	AURORA 2007	CP2690	14°58'N 123°03'E, 1071–1147 m	Exilioidea	NEOGA777-10	KR087259	KR088010	–	–
PYCHATRACTIDAE	<i>Exilioidea</i>	sp.	IM-2013-40625	PAPUA NIUGINI	CP3963	05°06'S 145°53'E, 960–980 m	Exilioidea	MITRI067-15	KR087260	KR088011	KR087353	–
PYCHATRACTIDAE	<i>Latromitra</i>	<i>barthelowi</i>	IM-2007-35820	SALOMONBOA 3	CP2800	08°41'S 161°04'E, 556–864 m	Latromitra	NEOGA854-10	KR087265	–	KR087358	KR088100

PTYCHACTRIDAE	<i>Latiromitra crosnieri</i>	IM-2007-38178	MIRIKY	no data		Latiromitra	MITRI070-15	KR087266	KR088016	KR087359	KR088101
TURBINELLIDAE	<i>Fustifusus pinicola</i>	IM-2007-43438	TERRASSES	DW3120	22°44'S 167°15'E, 320–380 m	Columbarinae	NEOGA932-10	KR087262	KR088013	KR087355	–
TURBINELLIDAE	cf <i>liriopse</i>	IM-2007-38972	MAINBAZA	CP3139	23°35'S 36°06'E, 1092–1195 m	Columbarinae	MITRI082-15	KR087242	KR087995	KR087336	KR088083
TURBINELLIDAE	<i>groschi</i>	IM-2007-38280	MAINBAZA	CP3143	23°32'S 35°46'E, 264–277 m	Columbarinae	MITRI083-15	–	KR087996	KR087337	KR088084
TURBINELLIDAE	sp.	IM-2009-14647	ATIMO VATAE	TA24	25°23.4'S 44°14.0'E, 15–17 m	Vasinae	MITRI084-15	–	KR088049	KR087385	–
TURBINELLIDAE	<i>turbinellus</i>	LSGB23801				Vasinae	–	HQ834084	HQ833957	HQ833909	HQ834180
VOLUTOMITRIDAE	<i>Microvoluta joloensis</i>	IM-2013-40620	BIOPAPIA	DW3720	06°03'S 147°35'E, 520–523 m	Volutomitridae	MITRI086-15	–	KR088017	KR087360	KR088102
VOLUTOMITRIDAE	<i>Volutomitra glabella</i>	IM-2007-35070	CONCALIS	DW3003	18°34'S 163°08'E, 450–454 m	Volutomitridae	NEOGA824-10	KR087319	KR088064	KR087406	KR088145
VOLUTOMITRIDAE	<i>zizgag</i>	IM-2007-35073	CONCALIS	DW2944	18°59'S 163°24'E, 320–335 m	Volutomitridae	NEOGA826-10	KR087321	KR088066	KR087408	KR088147
VOLUTOMITRIDAE	sp.	IM-2007-38357	TERRASSES	DW3123	22°53'S 167°13'E, 420–450 m	Volutomitridae	MITRI085-15	KR087320	KR088065	KR087407	KR088146
OUTGROUPS											
BELOMITRIDAE	<i>belomitra</i>	IM-2007-38678						JQ950224.1	EU870550.1	EU870525.1	–
BORSONIIDAE	<i>neocaledonia</i>	IM-2007-17857						EU015653	HQ401661	HQ401591	EU015761
BUCCINIDAE	<i>cumingi</i>	NSMK_MS_000480						HM180715.1	JN052941.1	HQ833870.1	HQ834142.1
BUCCINIDAE	<i>amianta</i>	T777						GQ290613.1	GQ290613.1	GQ290634.1	–
BUCCINIDAE	<i>perryi</i>	LSGB232042						JN053003.1	HQ833930.1	HQ833869.1	HQ834141.1
BUCCINIDAE	<i>Phos senticosus</i>	LSGB232091						JN053008.1	JN052944.1	HQ833885.1	HQ834156.1
CANCELLARIDAE	<i>Plesioritron silinoensis</i>	IM-2007-32123						FM999158.1	FM999106.1	FM999075.1	–
CONIDAE	<i>consors</i>	IM-2007-17939						EU015751	HQ401672	HQ401605	EU015858
CONIDAE	<i>pagodus</i>	IM-2007-17914						EU015729	FJ868151	FJ868136	EU015836
FASCIOLARIDAE	<i>Turritatirus turritus</i>	IM-2009-11059						HQ401585.1	HQ401744.1	HQ401651.1	–
MELONGENIDAE	<i>Hemifusus terratanus</i>	LSGB233031						JN053014.1	JN052950.1	HQ833889.1	HQ834160.1
MURICIDAE	<i>rudolphi</i>	LSGB23014						HQ834096	HQ833968	HQ833919	HQ834189
MURICIDAE	<i>Nucella lapillus</i>	MZUR_BAU00187						FM999169.1	FM999119.1	FM999088.1	–
MURICIDAE	<i>haemastoma</i>	MZUR_BAU00696						FM999171.1	FM999121.1	FM999090.1	–
NASSARIIDAE	<i>succinctus</i>	LSGB2340502						HQ834079.1	HQ833949.1	HQ833901.1	HQ834172.1
OLIVELLIDAE	<i>sp</i>	IM-2009-24367	KARUBENTHOS	GD41	16°20.900'S 61°32.217'W, 2 m		MITRI062-15	KR087290	KR088040	KR087378	KR088119
OLIVIDAE	<i>olitua</i>	IM-2007-31988	SANTO 2006	DR09	15°34.600'S 167°13.800'E, 12 m		NEOGA476-10	KR087289	KR088039	KR087377	KR088118
OLIVIDAE	cf. <i>sumatrana</i>	IM-2007-31956	PANGLAO 2005	CP2350	9°31.400'N 124°00.600'E, 602–738 m		MITRI064-15	KR087231	KR087987	KR087328	KR088074
OLIVIDAE	<i>optima</i>	IM-2009-22263	INHACA 2011	MR4	26°6.300'S 32°58.00'E, 17–19 m		MITRI063-15	KR087230	KR087986	KR087327	KR088073
PSEUDOLIVIDAE	sp	IM-2009-31002	AURORA 2007	CP2685	14°59.617'N 123°5.900'E, 1155–1302 m		MITRI065-15	KR087232	KR087988	KR087329	–
PSEUDOLIVIDAE	<i>sepimentum</i>	IM-2013-52003	ZANAGA	D502	4°42.25'S 11°47.0'E, 20 m		MITRI066-15	KR087261	KR088012	KR087354	KR088098
PYRAMIMITRIDAE	<i>efatensis</i>	IM-2013-52073	PAPUA NIUGINI	CP4058	2°54.669'S 142°10.772'E, 535–540 m		MITRI079-15	KR087297	KR088046	–	KR088124
PYRAMIMITRIDAE	<i>efatensis</i>	IM-2013-52074	PAPUA NIUGINI	CP4058	2°54.669'S 142°10.772'E, 535–540 m		MITRI078-15	KR087298	KR088047	KR087383	KR088125
PYRAMIMITRIDAE	<i>superstes</i>	IM-2007-35222					–	KF840066	KF840067	–	–
RAPHITOMIDAE	sp	IM-2013-52046	PAPUA NIUGINI	CP4049	3°19.413'S 143°27.239'E, 422–425 m		MITRI080-15	KR087296	KR088045	KR087382	KR088123
TEREBRIDAE	<i>tinguifera</i>	IM-2007-16735					–	EU015735	EU685670	EU685379	EU015842.1
TONNIDAE	<i>galea</i>	LSGB22802					–	HQ834116	HQ833984	HQ833862	HQ834135
TURRIDAE	<i>babylonia</i>	IM-2007-17754					–	EU015677	HQ401715	HQ401652	EU015786
VOLUTIDAE	<i>melo</i>	LSGB2400102					–	HQ834086	HQ833959	HQ833911	HQ834182

\*Sequences of *Cancilla fibula* from two specimens were assembled in hmyeric concatenated sequence.

**Table 2.** Sequences data and parameters of selected substitution models as defined by hierarchical likelihood ratio tests

Gene	Primers	Reference	annealing temperature	amplification length bp	alignment length bp	N of sequences	substitution model (hLRIs)	I	gamma
<b>COI</b>	HCO-1490 GGTCACAACAAA TCATAAAGAYATGYG	Folmer <i>et al.</i> (1994)	48–50	658	658	120	GTR + I + G	0.44	0.35
	LCO-2198 TAAACTTCAGGG TGACCAARAAYCA	Folmer <i>et al.</i> (1994)							
	22COIF GGAACATTATATAT TCTATTGGAAT	unpublished	48–50	605					
	695COIR TATACYTCMGG RTGACCRAAAATCA	unpublished							
	<b>16S</b> 16SH CCGGTCTGAAC TCAGATCAGG	Palumbi (1996)	55	~550	597	108	TVM + I + G	0.41	0.51
16LC GTTTACCAAAA ACATGGCTTC	Palumbi (1996)								
<b>12S</b> 12SA AAAC TGGGATTAG ATACCCACTAT	Palumbi (1996)	57	~380	620	107	GTR + I + G	0.27	0.62	
12SB GAGGTTGACGGG CGGTGTGT	Palumbi (1996)								
12SI TGCCAGCAGC CGCGGTTA	Oliverio, Mariottini, 2001	57*	~540	620					
12SIII AGACGACGGG CGRTTWTGATC	Oliverio, Mariottini, 2002								
<b>H3</b>	H3F ATGGCTCGTA CCAAGCAGACVGC	Colgan <i>et al.</i> (2000)	55	328	328	84	TyN + I + G	0.59	0.64
	H3R ATATCCTTRG GCATRATRGTGAC	Colgan <i>et al.</i> (2000)							

\*Annealing temperature was gradually decreased from 62 °C to 57 °C.

COI, cytochrome c oxidase subunit I; 16S and 12S, mitochondrial rRNA genes; H3, histone 3 nuclear gene; I, proportion of the invariant sites.

automatic alignments was confirmed by eye using BioEdit v. 7.0.9.0 (Hall, 1999). No indels were detected in the protein-coding genes *COI* and *H3*; fragments of 658 and 328 bp in length were sequenced for these markers, respectively. After the alignment of *12S* in which fragments of different length were amplified, absent positions at the 3'-end were treated as missing data.

#### DATA SETS

Six data sets were analysed. The first three data sets correspond to the three mitochondrial genes, *COI*, *16S*, and *12S*, analysed separately. The fourth data set represents concatenated *COI*, *16S*, and *12S* sequences (1875 positions) and is referred to as MCPV-3mit (MCPV is Mitridae–Costellariidae–Ptychactractidae–Volutomitridae abbreviated). To evaluate the robustness of the mitochondrial phylogeny, a fifth data set corresponding to the nuclear *H3* gene was analysed. Finally, a concatenated data set of 2203 positions (referred to as MCPV-4G) was obtained by adding *H3* sequences to the MCPV-3mit data set.

#### PHYLOGENETIC ANALYSES

Best-fit substitution models were identified for each gene separately, and for each combined data set using MODELGENERATOR v. 85 (Keane *et al.*, 2006) (Table 2). Best-scoring maximum likelihood (ML) trees were estimated using RaxML (Stamatakis, 2006) from 100 independent searches each starting from distinct random trees. Robustness of the nodes was assessed using the thorough bootstrapping algorithm (Felsenstein, 1985) with 1000 iterations. RaxML was performed on the Cipres Science Gateway (<http://www.phylo.org/portal2>), using RAXML-HPC2 on XSEDE.

Bayesian analyses (BAs) were performed by running two parallel analyses in MrBayes (Huelsenbeck, Ronquist, & Hall, 2001). For single gene analyses each run consisted of six Markov chains and 5 000 000 generations with other parameters with default values. For the MCPV-3mit and MCPV-4G data sets each analysis consisted of eight Markov chains of 50 000 000 generations with the number of swaps set to five. The sampling frequency was one tree each 10 000 generations, and the chain temperature was set at 0.02 in all analyses. Convergence of each analysis was evaluated using TRACER 1.4.1 (Rambaut *et al.*, 2014) to check that all effective sample size values exceeded 200. Consensus trees were calculated after omitting the first 25% trees as burn-in. The combined MCPV-3mit and MCPV-4G data sets were separated into five and eight unlinked partitions, respectively: *16S*, *12S*, three codon positions of the *COI* gene (both data sets), plus three codon positions of the *H3* gene (MCPV-4G data set).

Analyses were performed on the Cipres Science Gateway (<http://www.phylo.org/portal2>), using the tool MrBayes 3.2.2 on XSEDE.

#### RADULA MORPHOLOGY

Foregut anatomy of the sequenced specimens was examined or, in some cases, additional specimens conspecific with the sequenced material were dissected. During dissection the buccal mass was isolated and cleaned with diluted bleach (one part of commercially available bleach to three to four parts of water) until tissues were completely dissolved. Cleaned radulae were rinsed in several changes of distilled water and mounted on clear glass cover-slips for scanning electron microscope (SEM) investigation.

### RESULTS

#### DNA SEQUENCES

Of the 106 species studied, the *COI* gene was successfully amplified for 99 species, *16S* and *12S* for 87 species, and *H3* for 69 species. All sequences were deposited in GenBank and Barcode of Life Datasystem (Table 1). For the total count of sequences analysed and details of the substitution models selected, see Table 2.

#### ANALYSES OF SINGLE-GENE DATA SETS

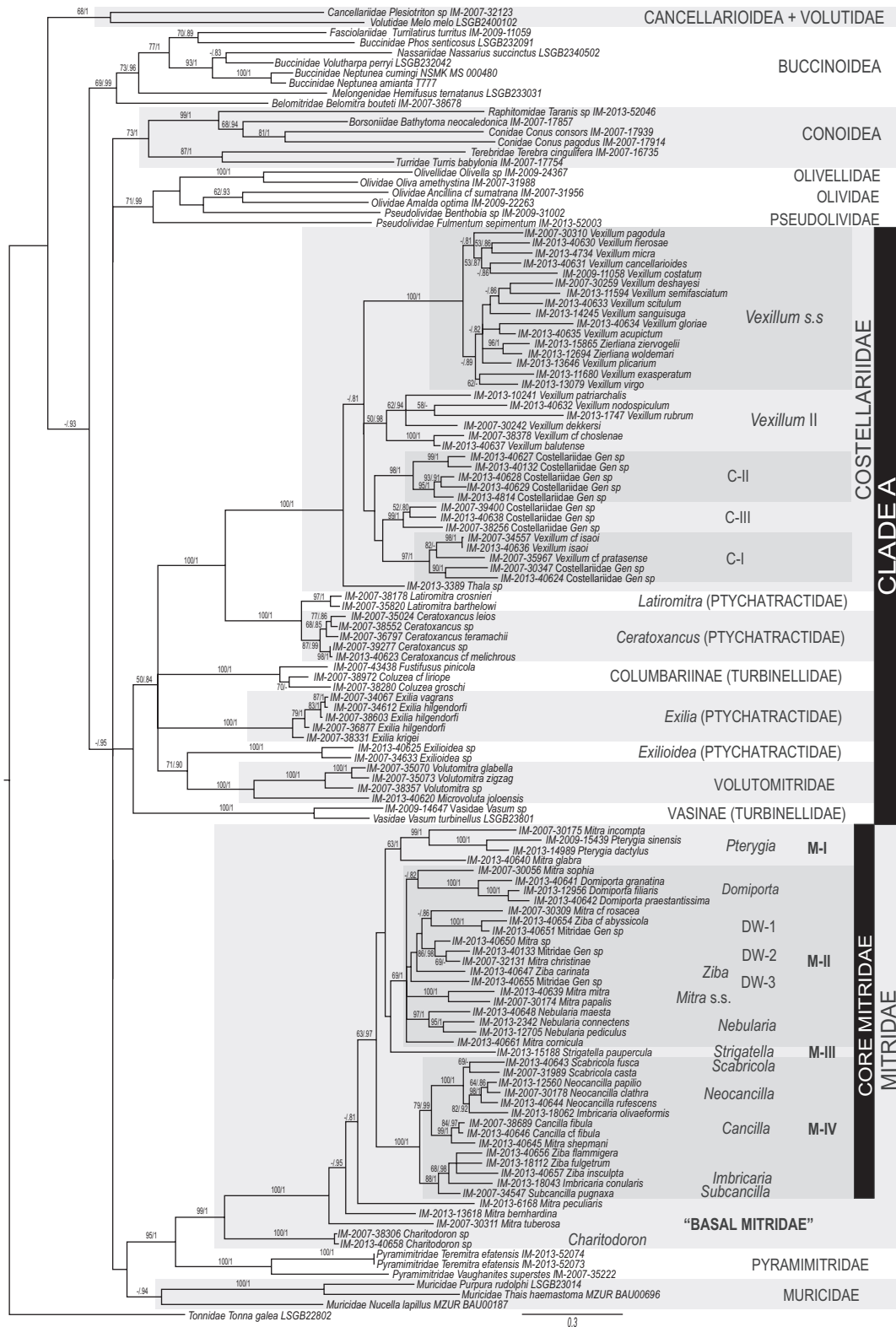
The trees obtained after the single gene analyses are characterized by weak support for most nodes; however, no supported conflicting topologies were found. Because of the weak resolution of the single-gene analyses, their results are not discussed here.

#### ANALYSIS OF MCPV-3MIT DATA SET

The topologies of the consensus trees obtained from BA and ML are largely congruent, with the exception of unresolved relationships at deepest nodes, which show slightly different topologies in the trees obtained with BA and ML, although without support in either case. Here, we mainly discuss the topology of the tree obtained with BA (Fig. 2) and refer to ML bootstrap support values where applicable.

Within Neogastropoda, *Plesiotriton silinoensis* Verhecken, 2011 (Cancellariidae) and *Melo melo* (Lightfoot, 1786) (Volutidae) constitute a well-supported clade [posterior probabilities (PP) = 1, bootstrap support (B) = 68%] that is sister to all other neogastropods. At the next dichotomy, a rather well-supported monophyletic clade corresponding to the superfamily Buccinoidea (PP = 0.99, B = 69%) splits out from the weakly supported main Neogastropoda grouping (PP = 0.95). Six supported major clades can be recognized within the main Neogastropoda grouping, the





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**Figure 2.** Phylogenetic tree of mitriform gastropods and outgroup neogastropod taxa obtained after analysis of the MCPV-3mit data set. The topology corresponds to the tree obtained with Bayesian analysis (BA); support values are indicated for each node as BA posterior probabilities values (when  $\geq 0.8$ )/maximum likelihood bootstrap values (when  $\geq 50$ ). MCPV, Mitridae–Costellariidae–Ptychactractidae–Volutomitridae. Scale bar: 0.3 substitution per site.

relationships to each other being unresolved. Three of these major clades correspond to (1) the superfamily Conoidea (monophyletic, PP = 1, B = 73%), (2) the turbinellid subfamily Vasiniae (monophyletic, PP = 1, B = 100%), and (3) the family Muricidae (monophyletic, PP = 0.94). Members of the families Olividae, Olivellidae, and Pseudolividae together form a fourth major clade (PP = 0.99, B = 71%) that can be referred to Olivoidea. The fifth clade, which is referred to here as 'Clade A', comprises species currently assigned to the four neogastropod families Costellariidae, Turbinellidae (Columbariinae), Volutomitridae, and Ptychactractidae. Finally, the sixth major clade (PP = 1, B = 95%) combines the families Mitridae (PP = 1, B = 95%) and Pyramimitridae (PP = 1, B = 100%), both of them found monophyletic.

#### Clade A

Within Clade A, the species currently assigned to the neogastropod families Costellariidae and Volutomitridae together with the turbinellid subfamily Columbariinae form three well-supported clades (PP = 1, B = 100%), consistent with their accepted taxonomic status. Conversely, the species of Ptychactractidae do not form a single grouping and three distinct clades can be recognized corresponding to (1) the genus *Exilia*, (2) the genus *Exilioidea*, and (3) the genera *Ceratoxancus* and *Latiromitra*. Analysis of the three-genes data set recognizes *Exilioidea* as a sister group to the family Volutomitridae, although with no support, whereas the *Ceratoxancus-Latiromitra* clade clusters with Costellariidae (PP = 1, B = 100%). The affinities of *Exilia* within Clade A remain uncertain.

Within the family Costellariidae, the only species of *Thala* included in the analysis branches out by itself and forms the first offshoot from the costellariid stem, which includes five well-supported clades, the branching order of which is not supported, reflecting the weak resolution of the tree based on the mitochondrial genes. The majority of the species of *Vexillum* cluster in the well-supported grouping *Vexillum s.s.* (PP = 1, B = 100%), forming a long branch on the tree. This clade comprises mostly shallow-water species and includes in particular *Vexillum plicarium* (Linnaeus, 1758), the type species of *Vexillum*, as well as *Zierliana ziervogelii* (Gmelin, 1791), the type species of *Zierliana*. Species of *Vexillum s.s.* tend to form two separate clades, although neither of these is supported. The rather well-supported clade *Vexillum* II (PP = 0.98, B = 50%) includes six species conventionally assigned to *Vexillum*, from both deep-water and from subtidal/intertidal depths, in turn forming two separate clades, a well-supported one with *Vexillum balutense* Herrmann, 2009, and *Vexillum choslenae* Cernohorsky, 1982 (PP = 1, B = 100%), and a weakly supported one combining the other four species. The three remaining costellariid clades are la-

belled C-I (PP = 1, B = 97%), C-II (PP = 1, B = 99%), and C-III (PP = 1, B = 98%) on the molecular tree. These include mostly deep-water forms, representing an essentially uncharacterized and unnamed diversity of the Costellariidae (of 13 sequenced species, only two can be identified with certainty).

#### Mitridae

The monophyly of the family Mitridae (PP = 1, B = 99%) is supported in both BA and ML, as well as close affinity of the Mitridae to the enigmatic deep-water family Pyramimitridae (PP = 1, B = 95%). The first dichotomy of the Mitridae separates the well-supported *Charitodoron* clade (PP = 1, B = 100%) from the rest of the Mitridae (PP = 1, B = 100%). Subsequently, three species, *Mitra tuberosa* Reeve, 1845, *Mitra bernhardina* Röding, 1798, and *Mitra peculiaris* Reeve, 1845, split out from the general mitrid stem, forming three independent lineages, although the lack of support in the corresponding nodes leaves the possibility of alternative branching. From now on, we refer to the combined group including the *Charitodoron* species, *Mitra tuberosa*, *Mitra bernhardina*, and *Mitra peculiaris* as the 'basal Mitridae'. The remaining 37 mitrid species form a rather well-supported core clade (PP = 0.97, B = 63%), which in turn consists of four major groupings (referred to as clades M-I to M-IV), either well supported (M-I, M-II, and M-IV), or consisting of a single species (M-III). Clade M-I (PP = 1, B = 63%) combines two species of *Pterygia* (monophyletic, PP = 1, B = 100%), including its type species, *Pterygia dactylus* (Linnaeus, 1767), and two species currently placed in *Mitra*, *Mitra glabra* Swainson, 1821, and *Mitra incompta* (Lightfoot, 1786). Clade M-II (PP = 1, B = 69%) contains an assortment of *Mitra* species that form several well-supported clades and a set of single-species lineages with unsupported relationships. One of these well-supported clades is formed by *Mitra mitra* (Linnaeus, 1758), the type species of *Mitra*, and the closely related *Mitra papalis* (Linnaeus, 1758), and we consequently refer to it from here onwards as *Mitra s.s.* Another well-supported grouping in the M-II clade corresponds to *Nebularia* (monophyletic, PP = 1, B = 96%). Three clades within M-II are solely represented by deep-water, mostly undescribed, forms (on the tree referred to as Mitridae DW-1 to 3), and we did not find any genus-group name to designate them. Clade M-II also encompasses two lineages that correspond to the genera *Domiporta* (monophyletic, PP = 1, B = 100%) and *Ziba* (only represented by *Ziba carinata* (Swainson, 1824), the type species of the genus). As mentioned before, clade M-III includes a single species, *Strigatella paupercula* (Linnaeus, 1758), the type species of *Strigatella*. Clade M-IV (PP = 1, B = 100%) includes 13 species traditionally classified in the genera *Neocancilla*, *Scabricola*, *Imbricaria*, *Cancilla*, *Ziba*, and *Subcancilla*,

which were all allocated to the subfamily Imbricariinae by Cernohorsky (1991). Three well-supported lineages can be distinguished within this clade, one comprising the monophyletic *Neocancilla* (PP = 1, B = 98%), the monophyletic *Scabricola* (represented by two species of the subgenus *Swainsonia*), and *Imbricaria olivaeformis* (Swainson, 1821). The second lineage combines two closely related species of the genus *Cancilla* (*Cancilla fibula* Poppe, Tagaro & Salisbury, 2009, and *Cancilla cf. fibula*) and *Mitra schepmani* Salisbury & Guillot de Suduiraut, 2003. Finally, the third lineage of clade M-IV combines species traditionally assigned to *Ziba* [*Ziba flammigera* (Reeve, 1844), *Ziba fulgetrum* (Reeve, 1844), and *Ziba insculpta* (A. Adams, 1851)], *Subcancilla* (*Subcancilla pugnax* Poppe, Tagaro & Salisbury, 2009), and *Imbricaria* [represented by *Imbricaria conularis* (Lamarck, 1811), the type species of the genus].

#### ANALYSIS OF MCPV-4G DATA SET

The tree obtained after the BA of the MCPV-4G data set (Fig. 3) is similar to the mitochondrial genes-based tree discussed above and largely congruent with it, but the branching order differs in some clades. In general the four-genes tree has better resolution, and many of the crucial nodes have higher support values. The composition of major neogastropod groupings remains unchanged, although the relationships amongst them are rather weakly supported. Amongst the noteworthy changes are increased supports of Clade A (PP = 1, B = 60%) and of the Volutomitridae + *Exilioidea* grouping (PP = 0.94, B = 68%).

Although the topology of the Mitridae segment of the MCPV-4G tree is nearly identical to the MCPV-3mit tree, the arrangement of Costellariidae clades differs. In the four-genes tree, the most derived position is occupied by *Vexillum s.s.* (PP = 1, B = 100%), which in species composition corresponds to the *Vexillum s.s.* clade of the MCPV-3-mit tree and only slightly differs in internal branching. Conversely, the former clade *Vexillum* II is now split into several separate lineages, which cluster with the *Vexillum s.s.* clade. *Vexillum patriarchalis* (Gmelin, 1791), *Vexillum rubrum* (Broderip, 1836), and *Vexillum nodospiculum* (Cernohorsky, 1870) are closest to *Vexillum s.s.*, and the highly supported clade formed by *Vexillum s.s.* and these three species (PP = 1, B = 85) are thus referred to from here onwards as *Vexillum s.l.* The single-species lineage of *Vexillum dekkersi* forms a sister group to *Vexillum s.l.*; *V. dekkersi* Herrmann, Stossier & Salisbury, 2014, and a set of similar species were recently placed in a separate subgenus *Protoelongata* Herrmann, Stossier & Salisbury, 2014, and this name is therefore used here for that clade. Finally, the well-supported clade (referred to as C-IV) of two deep-

water species, '*Vexillum*' *balutense* and '*Vexillum*' cf. *choslenae*, forms a sister group to the *Vexillum s.l.*-*Protoelongata* clade with high support for the resulting grouping (PP = 1, B = 83%).

Given the increased resolution of the MCPV-4G tree compared with the mitochondrial-genes tree, our results are discussed in the context of the phylogenetic scheme inferred from the four-genes data set. Of the three nominal genera of Costellariidae studied, *Thala* was represented by a single species, so its monophyly was not tested; *Zierliana* comes out monophyletic, but its inclusion in the *Vexillum s.s.* clade implies a revision of its rank; *Vexillum* is undoubtedly polyphyletic, and the extent of its polyphyly depends on how broadly its boundaries are defined (see Discussion).

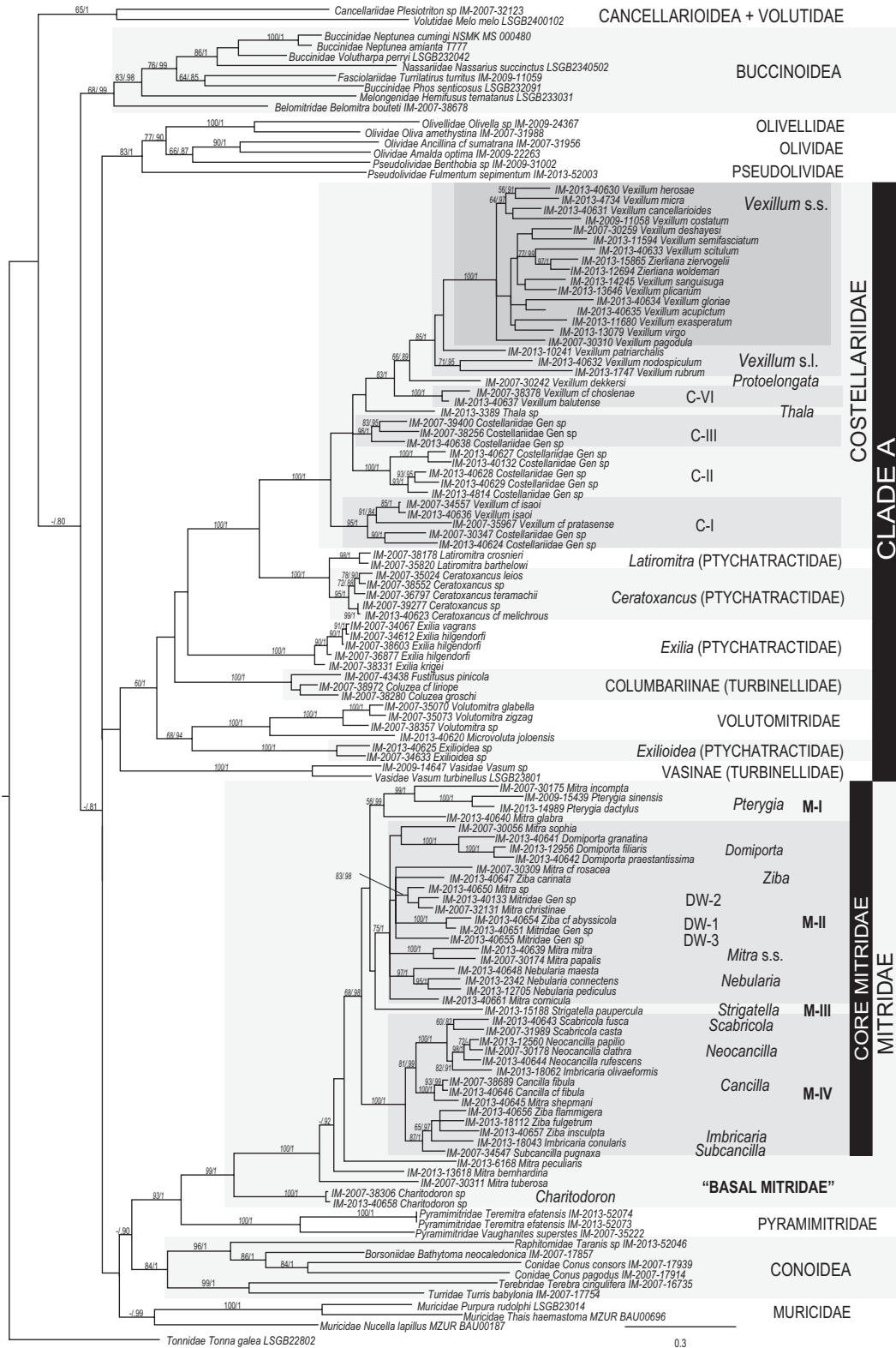
Amongst the mitrid genera, monophyly is supported for *Charitodoron*, *Pterygia*, *Domiporta*, *Neocancilla*, *Cancilla*, and *Scabricola* and for the subgenus *Mitra* (*Nebularia*). Monophyly is not rejected for *Subcancilla*, and *Strigatella*, with single species in each of them studied. The genera *Imbricaria* and *Ziba* are definitely polyphyletic. It is noteworthy that *Ziba carinata*, the type species of *Ziba*, ended up within the clade M-II, separately from three other species traditionally placed in *Ziba*, *Ziba fulgetrum*, *Ziba flammigera*, and *Ziba insculpta*, which cluster in clade M-IV. Finally, the genus *Mitra* appears highly polyphyletic: species conventionally assigned to *Mitra* are found in all four clades of the core Mitridae and also amongst the 'basal Mitridae'.

#### RADULA MORPHOLOGY

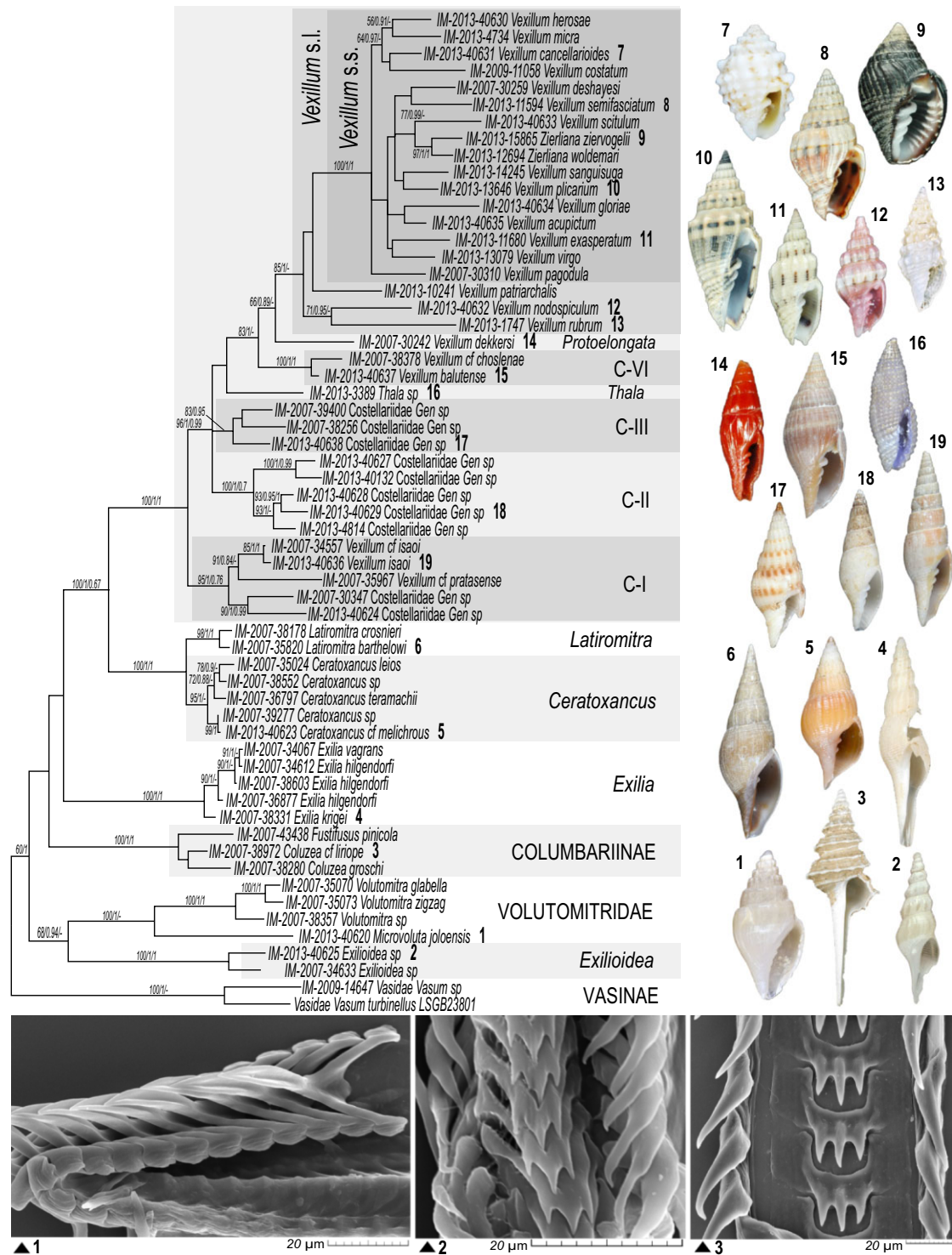
All species in the *Vexillum s.s.* clade are similar in radula morphology: they have triserial radulae with a multicuspidate rachidian and simple, unicuspid laterals (Figs 4, 5). The shape of the rachidian ranges from robust with relatively short cusps, in *Zierliana ziervogeli* and *Vexillum semifasciatum* (Lamarck, 1811) (Fig. 5), to more elaborate, bow-shaped with long pointed cusps in *Vexillum exasperatum* (Gmelin, 1791) or *Vexillum gloriae* Poppe, Tagaro & Salisbury, 2009 (not shown). The number of cusps varies from seven (*Vexillum cancellarioides*, Fig. 5) to more than 20 (*V. exasperatum*). A similar radula is found in the two species of *Vexillum s.l.*, *V. rubrum* and *Vexillum nodulospiculum*.

The other costellariid species (clades C-I-C-IV, *Protoelongata* and *Thala*) have monocuspid laterals similar to those found in *Vexillum s.s.*, but differing in the structure of the rachidian, which bears only three cusps, although it varies in shape, robustness, base width, and orientation, and length of the cusps (Figs 4, 5). '*Vexillum*' *balutense* has a rachidian with two minor intermediate cusps, positioned on both sides of the central cusp (Fig. 5). Lateral teeth vary from slender, sickle-shaped in Costellariidae gen. sp.



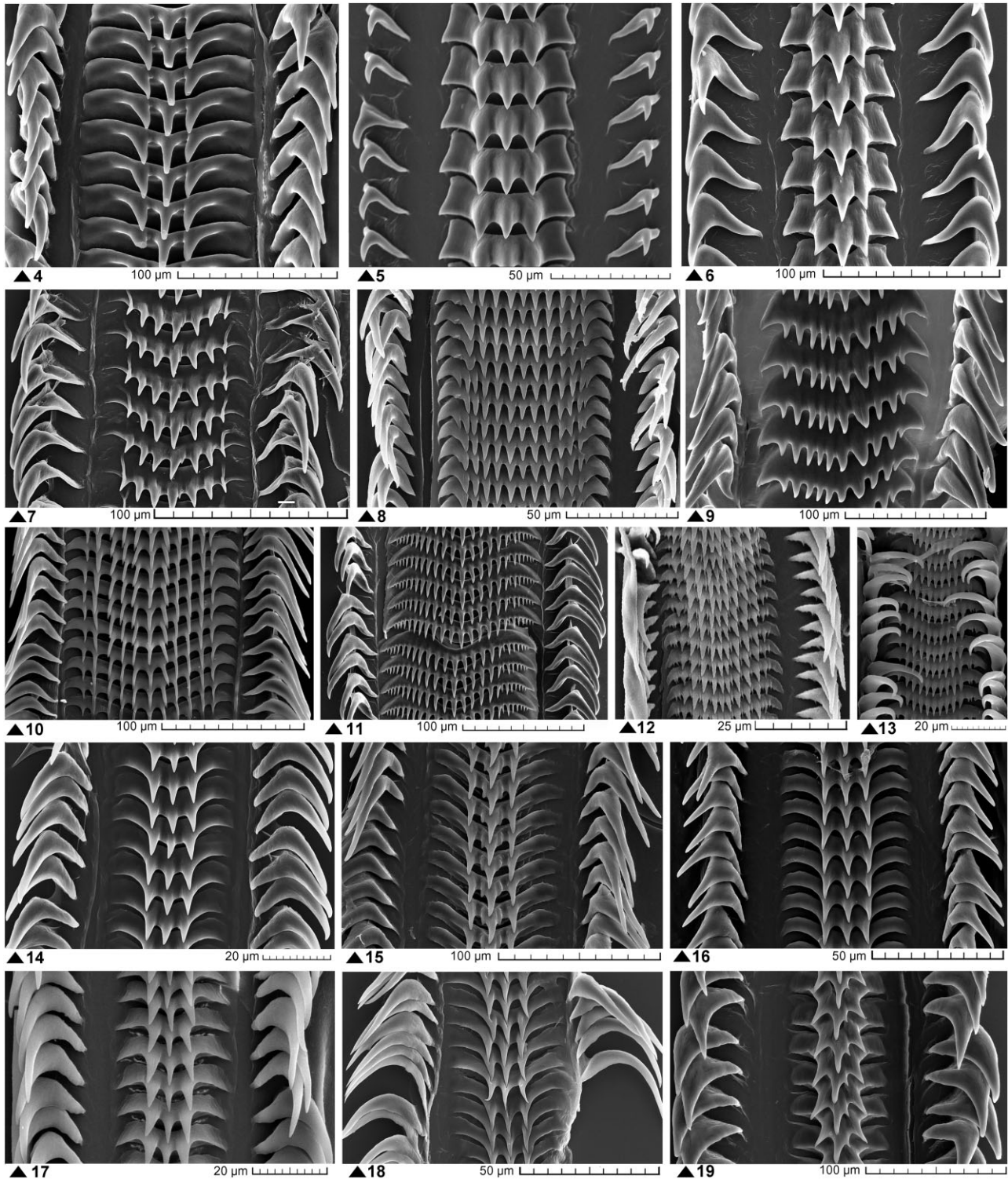


**Figure 3.** Phylogenetic tree of mitriform gastropods and outgroup neogastropod taxa obtained after analysis of the MCPV-4G data set. The topology corresponds to the tree obtained with Bayesian analysis (BA); support values are indicated for each node as BA posterior probabilities values (when  $\geq 0.8$ )/maximum likelihood bootstrap values (when  $\geq 50$ ). MCPV, Mitridae–Costellariidae–Ptychatractidae–Volutomitridae. Scale bar: 0.3 substitution per site.



**Figure 4.** Clade A subtree of the Bayesian tree obtained after analysis of the MCPV-4G data set. Support values are indicated for each node as Bayesian analysis Posterior probabilities values (when  $\geq 0.8$ )/maximum likelihood bootstrap values (when  $\geq 50$ )/BA Posterior probabilities values for the COI single gene analysis (when  $\geq 0.8$ ). Voucher shells for those species studied morphologically are illustrated; numbers in bold correspond to scanning electron microscopy photos of their radulae [shown at the bottom for Volutomitridae (*Microvoluta joloensis* – 1), *Exilioidea* sp. (2), and Columbariinae (*Coluzea cf. liriopae* – 3) and in Figure 5 for *Exilia*, *Ceratoxancus*, *Latiromitra*, and Costellariidae]. MCPV, Mitridae–Costellariidae–Ptychactractidae–Volutomitridae.





**Figure 5.** Radular morphology of Costellariidae. Numbers correspond to the vouchers shown in Figure 4: 4. *Exilia krigei*; 5. *Ceratoxancus cf. melichrous*; 6. *Latiromitra barthelowi*; 7. *Vexillum cancellarioides*; 8. *Vexillum semifasciatum*; 9. *Zierliana ziervogeli*; 10. *Vexillum plicarium*; 11. *Vexillum exasperatum*; 12. *Vexillum nodospiculum*; 13. *Vexillum rubrum*; 14. *Vexillum (Protoelongata) dekkersi*; 15. ‘*Vexillum*’ *balutense*; 16. *Thala* sp.; 17. Costellariidae gen. sp.; 18. Costellariidae Gen. sp.; 19. ‘*Vexillum*’ *isaoi*.

(IM-2013-40629) to robust, almost triangular, in '*Vexillum*' *isaoi* (Kuroda & Sakurai, 1959).

The same general radula morphology characterizes the Ptychactrid genera *Ceratoxancus*, *Latiromitra*, and *Exilia* and, also, the columbariine *Coluzea* cf. *liriope* Harasewych, 1986, but *Exilioidea* is remarkably different (Figs 4, 5). In *Ceratoxancus* cf. *melichrous* Kantor & Bouchet, 1997, and two species of *Latiromitra*, *Latiromitra crosnieri* Bouchet & Kantor, 2000, and *Latiromitra barthelowi* (Bartsch, 1942), there is a robust rachidian bearing three short and blunt cusps on a moderately wide base, and rather slender laterals; in *Exilia krigei* (Kilburn, 1971), the rachidian has a proportionally wider base and closely set, long, and pointed cusps; in *Coluzea* cf. *liriope*, the only distinguishing feature is the very narrow base of the rachidian. The rachidian of *Exilioidea* is characterized by the massive base and three cusps, situated on the tip of a long, blade-like projection. This projection of the rachidian apical segment is directed along the longitudinal axis of the radula and forms a distinct longitudinal furrow, so that the apical part of a tooth interlocks in the furrow of the next one.

Mitridae show considerable variation in shell and radular morphology (Figs 6, 7). A triserial radula with a rather small rachidian and broad, multicuspitate laterals characterize members of clade M-II as well as *Strigatella paupercula* of clade M-III, and *Mitra peculiaris* and *Charitodoron* in the 'basal Mitridae'. Typically, the lateral teeth are nearly rectangular, bear from 14 to more than 20 cusps that are stronger medially and gradually reduce in size towards the margins. The rachidian is about three times narrower than the laterals and bears five to seven subequal, rather short and stout cusps. However, in one unidentified species (IM-2013-40651) of clade M-II, the rachidian and laterals are similar; each tooth has a slightly convex margin that bears 12–13 equal cusps.

The three species in clade M-IV also have triserial radulae, but their morphology differs notably from that seen in clade M-II, and also from each other. The number of cusps on the laterals is reduced to six (*Ziba flammigera*), five [*Scabricola fusca* (Swainson, 1824)], or even one (*Imbricaria olivaeformis*), and one cusp is always much longer and stronger than the others, giving the laterals the appearance of a can opener (Figs 6, 7). Remarkably, the position of the enlarged cusp differs between species: in *Ziba flammigera* the second proximal cusp is the largest, whereas in *Scabricola fusca* it is the most distal one. The morphology of the rachidian also differs between species; *Scabricola fusca* possesses a rachidian resembling a hair comb with long cusps of equal length, but *Imbricaria olivaeformis* and *Ziba flammigera* have rachidians with two central cusps greatly enlarged in comparison with those positioned laterally.

Finally, three species, *Pterygia dactylus* (clade M-I), '*Mitra*' *bernhardina*, and '*Mitra*' *tuberosa* ('basal Mitridae') have monoserial radulae, lacking lateral teeth and retaining rachidians only (Figs 6, 7); these rachidians vary in shape from species to species, and are all greatly reduced in size, not exceeding 20 µm in width.

## DISCUSSION

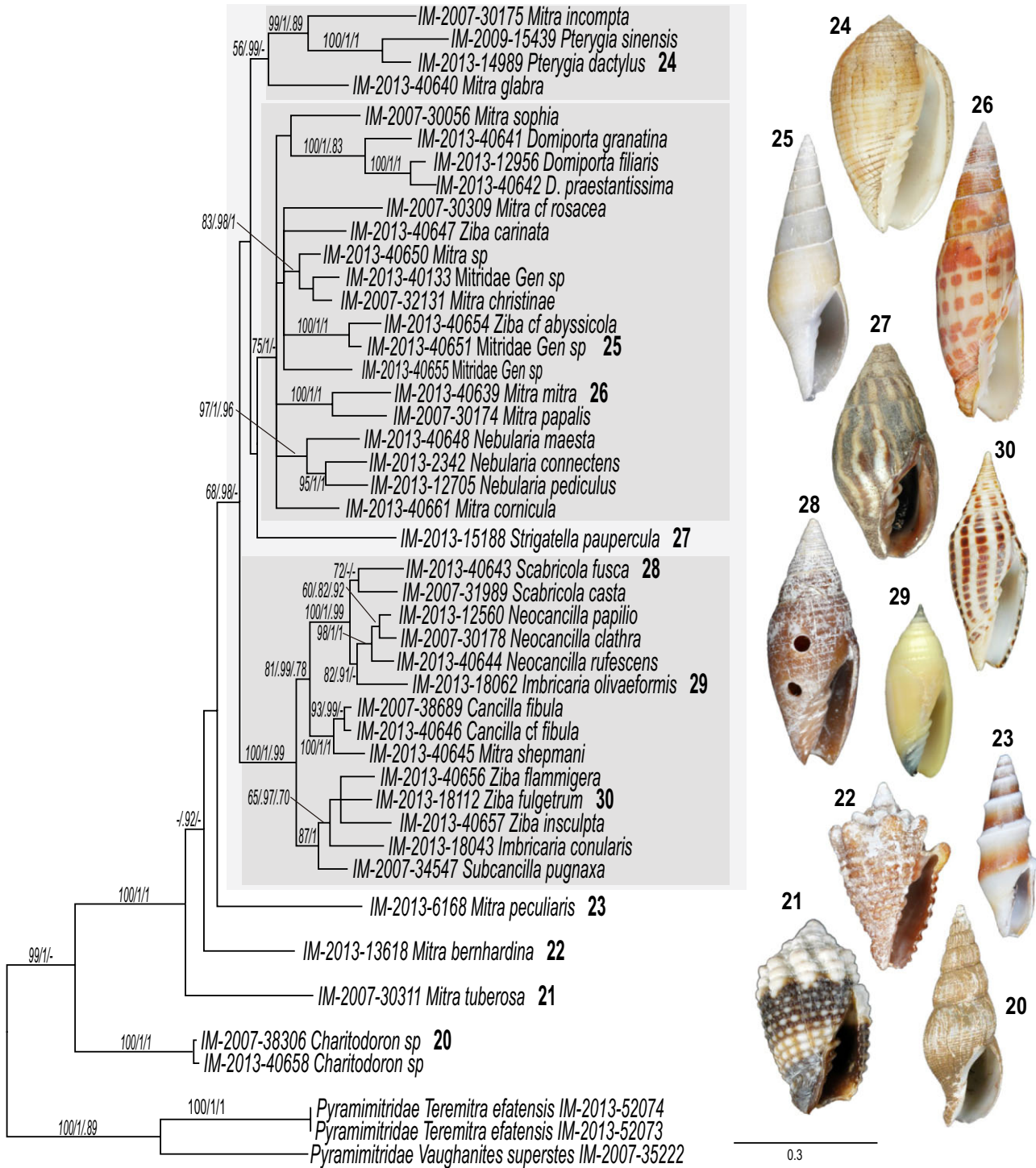
### RADULAR MORPHOLOGY

Our results on radula morphology essentially fall in line with the published data on Costellariidae (Cernohorsky, 1966, 1970; Bandel, 1984; Fedosov & Kantor, 2010), Ptychactridae (Bouchet & Warén, 1988; Kantor & Bouchet, 1997; Bouchet & Kantor, 2000; Kantor, Bouchet & Oleinik, 2001), Mitridae (Cernohorsky, 1966, 1970; Ponder, 1972), Volutomitridae (Bouchet & Kantor, 2004), and Columbariinae (Harasewych, 2004). However, some of our results contradict those published earlier, or deserve special attention as novel and important for future discussion.

The radula of *Exilioidea* agrees with the description and illustrations of Bouchet & Warén (1988). Based on the tricuspidate rachidian and triangular unicuspid laterals, Bouchet & Warén suggested the placement of *Exilioidea* in the family Turbinellidae [which, at the time (Bouchet & Warén, 1985), included Turbinellinae, Ptychactridinae, Columbariinae, and Vasinae]. However, the presence of the strong central projection with its margins half-folded to form a kind of trough recalls the radula of Volutomitridae. The radula of *Exilioidea*, which combines the rather plesiomorphic characters of Ptychactrid radulae and the derived ones of Volutomitridae, supports the relationships of these two groups as suggested by the molecular phylogeny.

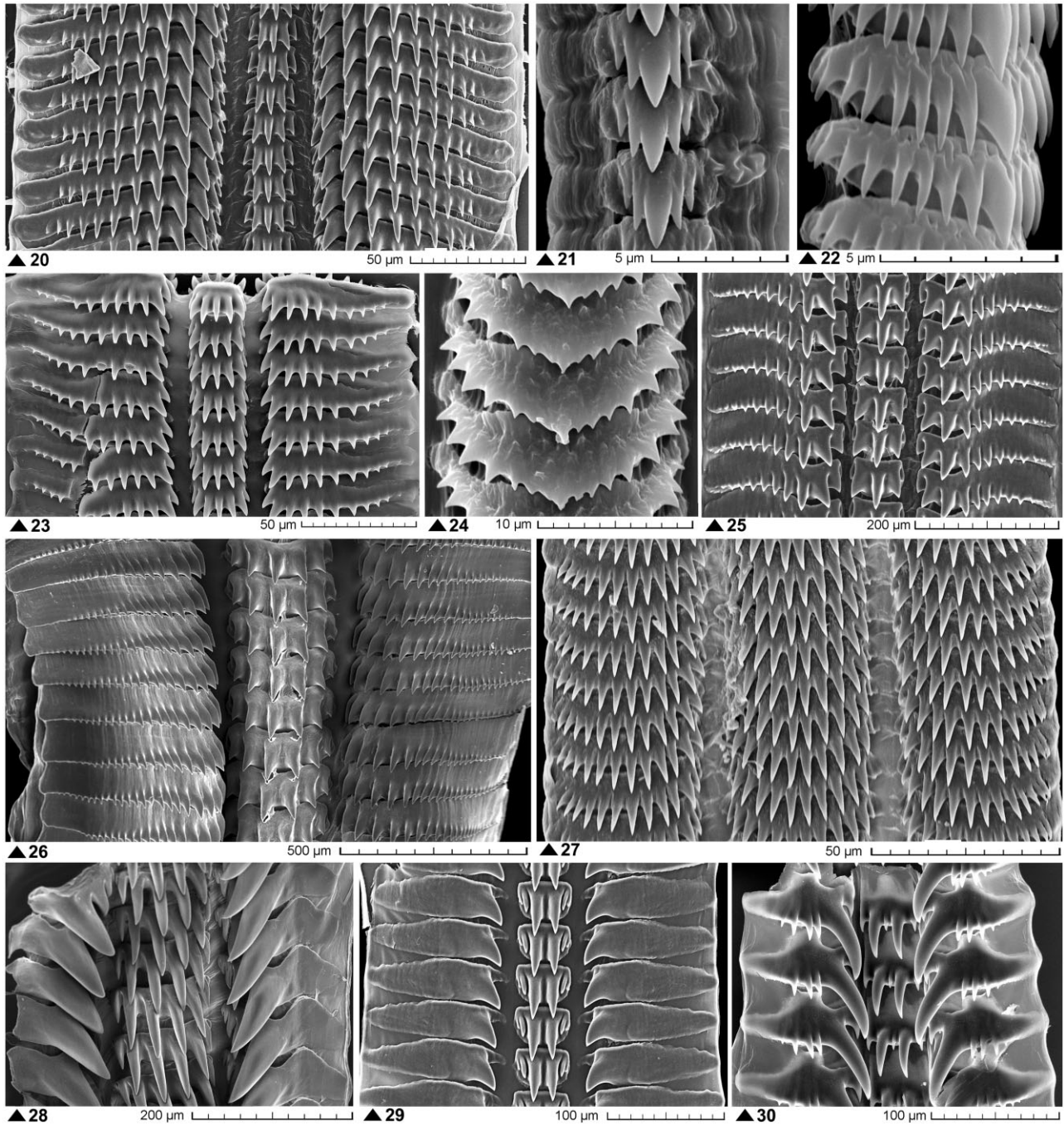
All studied species in the *Vexillum* s.l. clade have radulae with a multicuspitate rachidian, as did those studied by Fedosov & Kantor (2010). Cernohorsky (1970) illustrated the radula of *V. patriarchalis* with a tricuspidate rachidian, matching the radula found by Azuma (1965) in other *Vexillum* species, sometimes classified in the subgenus *Pusia*, such as *Vexillum (Pusia) hizenense* Pilsbry, 1921 [= *Vexillum inerme* (Reeve, 1845)] and *Vexillum (Pusia) australe* (Swainson, 1820) (Ponder, 1998; Robin & Martin, 2004). Corresponding to our results, *V. rubrum* and *V. cancellarioides* (Anton, 1838), which are referable to *Pusia* based on shell morphology, have a multicuspitate rachidian. Furthermore, in our results *Pusia* is not consistent with a single monophyletic grouping, as *V. cancellarioides* is included in the *Vexillum* s.s. clade. Further studies are needed to clarify the position of *V. patriarchalis* in Costellariidae and its relationships to *Vexillum (Pusia) microzonias*, the type of *Vexillum (Pusia)*.





**Figure 6.** Mitridae subtree of the Bayesian tree obtained after analysis of the MCPV-4G data set. Support values are indicated for each node as Bayesian analysis PP values (when  $\geq 0.8$ )/ maximum likelihood bootstrap values (when  $\geq 50$ )/ BA Posterior probabilities values for the COI single gene analysis (when  $\geq 0.8$ ). Voucher shells for those species studied morphologically are illustrated; numbers in bold correspond to electron microscopy photos of their radulae (shown in Fig. 7). MCPV, Mitridae–Costellariidae–Ptychactractidae–Volutomitridae.

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**Figure 7.** Radular morphology of Mitridae. Numbers correspond to the vouchers shown in Figure 6. 20. *Charitodoron* sp.; 21. *Mitra* *tuberosa*; 22. *Mitra* *bernhardina*; 23. *Mitra* *peculiaris*; 24. *Pterygia* *dactylus*; 25. *Strigatella* *paupercula*; 26. *Mitra* *mitra*; 27. Mitridae gen. sp.; 28. *Scabricola* *fusca*; 29. *Imbricaria* *olivaeformis*; 30. *Ziba* *fulgetrum*.

The radula with morphologically identical rachidian and laterals, found in specimen IM-2013-40651, is remarkably close to the one described in *Pleioptygma helenae* (Radwin & Bibbey, 1972), the only Recent species of the family Pleioptygmatidae (Quinn, 1989). This family was erected largely based on the pecu-

liar foregut anatomy and radular morphology of *Pleioptygma helenae*, and a critical reassessment of the status of Pleioptygmatidae is required. Although morphological data can give some hints on its possible relationships, molecular data are critical to resolve this issue. However, it is already clear that a radula with



three morphologically identical teeth in a transversal row cannot be regarded as something outstanding in Neogastropoda; a radula of the same morphology is present also in *Colubraria* (Colubrariidae: Buccinoidea; Oliverio & Modica, 2010).

One of our most interesting results is the finding that several independent mitrid lineages have developed uniserial radulae. To date, uniserial radulae have been reported only in *Pterygia* (Ponder, 1998), but they have most likely been overlooked in other mitrids because of their minute size (7–15 µm broad), which complicates preparation.

#### RELATIONSHIPS AMONGST MAJOR GROUPS OF MITRIFORM GASTROPODS

All our phylogenetic analyses support the monophyly of the families Mitridae, Volutomitridae, and Costellariidae, although the boundaries of the latter are disputable (see below). In the analyses of both the MCPV-4G and MCPV-3-mit data sets, the four genera of Ptychatractidae do not form a monophyletic grouping, but cluster into three unrelated lineages:

1. The *Ceratoxancus*–*Latiromitra* clade shows a close affinity to Costellariidae, a relationship that is highly supported in all analyses (including the analyses of the single-gene data sets), and a logical conclusion is to expand the boundaries of the family Costellariidae to include these two genera as basal offshoots. The possible distant relationship between *Latiromitra*–*Ceratoxancus* and other ptychatractids was suggested by Harasewych (1987) based on the cladistic analysis of a morphological data set. The supposed relationships of *Ceratoxancus* have long remained controversial; Sakurai (1957) suggested an affinity to the Mitridae, and Cernohorsky (1973) to the Volutomitridae. A relationship of *Latiromitra* to *Vexillum* was envisioned by Wenz (1938), who treated *Latiromitra* as a section of *Vexillum*.
2. The genus *Exilia* is more distantly related to this newly defined costellariid–*Ceratoxancus*–*Latiromitra* clade.
3. The close relationship of *Exilioidea* and Volutomitridae is weakly supported by molecular data, but is also suggested by radula morphology.

In conclusion, although the family Ptychatractidae obviously requires revision, it is at present hampered by the lack of material for crucial genera, in particular *Ptychatractus*.

The family Volutomitridae is found to be only distantly related to the Costellariidae, with both Volutomitridae and Costellariidae turning out to be more closely related to genera formerly classified as ptychatractids. In our analyses, the branching within

Clade A thus contradicts the phylogenetic scheme of Oliverio & Modica (2010), who found the volutomitrid genus *Microvoluta* to be most closely related to *Vexillum*; the same scheme was reproduced later by Zou *et al.* (2011). In fact, a close relationship between Costellariidae and Volutomitridae is also contradicted by the morphology of the radula and digestive system (Ponder, 1972; Fedosov & Kantor, 2010). We therefore examined the voucher specimen MNHN IM-2009-4609 *Microvoluta* sp. of Oliverio & Modica's (2010). It is morphologically close to specimens MNHN IM-2013-40628 and MNHN IM-2013-40627 of our deep-water Costellariidae lineage C-II, and its *COI* sequence even suggests that it is conspecific with the former (pairwise distance 0.019). Thus, we assign the specimen studied by Oliverio & Modica's (2010) to Costellariidae, suggesting an initial misidentification that has resulted in a distorted branching in the Ptychatractidae–Volutomitridae–Costellariidae segment of their tree. Admittedly, shell differences between Costellariidae and Volutomitridae are rather subtle, and in day-to-day practice specimens with a bulbous paucispiral protoconch are commonly referred to *Microvoluta*, whereas those with a multispiral protoconch are referred to *Vexillum*.

One of the crucial hypotheses to be confirmed or rejected is whether the families Costellariidae and Mitridae are closely related. Our results unequivocally demonstrate that these two families are unrelated, as they cluster in two different well-supported major groupings of the Neogastropoda. The revealed close affinity between Mitridae and the enigmatic family Pyramimitridae is one of the most unexpected results of our analysis. The family Pyramimitridae was considered extinct until living members of several undoubtedly pyramimitrid genera were discovered in the deep-water fauna of the Indo-Pacific (Kantor *et al.*, 2014). The initial molecular phylogenetic analysis failed to attribute Pyramimitridae to any known neogastropod lineage. Despite Mitridae and Pyramimitridae ending up closely related in the present molecular phylogeny, they differ overly in radula morphology, with wide and flattened multicuspidate laterals and a multicuspidate rachidian in Mitridae, vs. triangular laterals and a pointed unicuspid rachidian in Pyramimitridae. Although limited anatomical data are available at the moment for Pyramimitridae, we can already point out significant differences between the two families. For example the buccal mass occupies a basal position in pyramimitrids, whereas it is apical in mitrids. Members of the family Pyramimitridae have no epiproboscis (see below), but possess a relatively well-developed gland of Leiblein; the latter is lacking in Mitridae. No synapomorphies of the Mitridae–Pyramimitridae clade are known at present.



Published data on the morphology of the monotypic (in the Recent fauna) family Pleioptygmatidae suggest a close relationship to the Mitridae. The anterior alimentary canal of the Mitridae is characterized by the presence of a specific organ – the epiproboscis – that is not found in any other family of Neogastropoda, and is often considered an autapomorphy for the family (West, 1991; Ponder, 1998). We suspect that the ‘proboscis introvert’ described by Quinn (1989) for *Pleioptygma helenae* is, in fact, an epiproboscis, as it does not differ in either morphology or topology from the mitrid epiproboscis (Ponder, 1972). Unfortunately, the narrow geographical range and rarity of *Pleioptygma* make it hard to target for molecular studies, which would be essential to clarify unequivocally its position.

#### DEEP RELATIONSHIPS WITHIN THE NEOGASTROPODA

The current empirical classification of the Neogastropoda (Bouchet & Rocroi, 2005) places the family Ptychactridae in the superfamily Pseudolivoidea. Oliverio & Modica’s (2010) phylogenetic analysis suggested an affinity of the Mitridae to Olivoidea and Pseudolivoidea. However, none of these assignments is supported here, as none of the three evolutionary lineages referable to the Ptychactridae shows a relationship to the two pseudolivids – *Fulmentum sepimentum* (Rang, 1832) and *Benthobia* sp. – included in the present analysis. The latter two taxa fall into a well-supported clade with Olivoidea, separated from Clade A, suggesting only a distant relationship between ptychactrids and Pseudolivoidea.

Ponder & Warén (1988) treated Ptychactridae as a subfamily of Turbinellidae, together with Columbariinae, Vasinae, Turbinellinae, and Tudiculinae. Members of the different turbinellid subfamilies were found to share several anatomical features, e.g. an open seminal groove and a radula with tricuspidate rachidian and simple, unicuspid laterals. These anatomical features were regarded as ancestral for the Neogastropoda, and Turbinellidae *sensu* Ponder & Warén (1988) was often regarded as one of most ancient groups of the Neogastropoda (Ponder, 1998). Our phylogenetic analysis places Columbariinae and various ptychactrids in the well-supported Clade A, with Vasinae as a sister group (although with no significant support). However, ptychactrids, vasines, and columbariines do not form a monophyletic group; instead ptychactrids show closer affinities to Costellariidae and Volutomitridae with their derived anatomy. Therefore, the family Turbinellidae *sensu* Ponder & Warén (1988) and Bouchet & Rocroi (2005) is definitely a nonmonophyletic group based essentially on shared plesiomorphies.

With the most basal relationships amongst Neogastropoda still unresolved, we refrain from a criti-

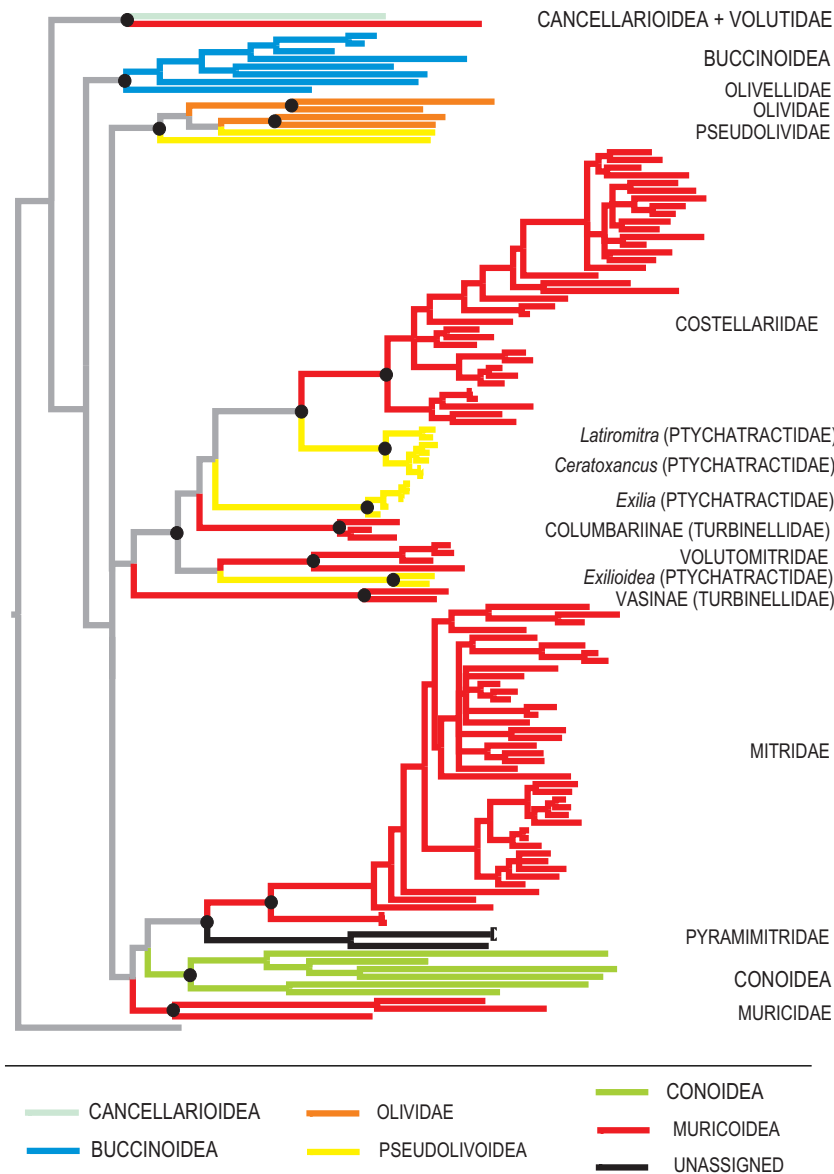
cal revision of the classification at the level of superfamilies. However, some incongruences between the currently accepted systematic arrangement and the present phylogenetic reconstructions (Fig. 8) deserve special mention. Our results confirm the monophyly of the neogastropod superfamilies Buccinoidea and Conoidea, and the monophyly of the Olivoidea and Cancellarioidea is also not rejected. Pseudolivoidea are recovered polyphyletic (see above). The vast majority of the neogastropod evolutionary lineages, including those specifically targeted in the present study, are currently classified in the superfamily Muricoidea (Bouchet & Rocroi, 2005). The families currently classified in Muricoidea (WoRMS, 2015) fall in five inferred major clades: (1) a Muricidae clade; (2) a Vasinae clade; (3) a Mitridae–Pyramimitridae clade; (4) a clade (‘Clade A’) consisting of the families Costellariidae, Turbinellidae (Columbariinae), Volutomitridae, and Ptychactridae; and (5) a Cancellariidae + Volutidae clade (see Fig. 8). Despite the lack of resolution of deep nodes in our trees, at least two points contradict the currently accepted circumscription of the Muricoidea: (1) the inclusion of the ptychactrids in the otherwise muricoid Clade A; and (2) the supported grouping of the volute *Melo melo* with the cancellariid *Plesiotriton silinoensis*. Nevertheless, even the revised position of the Ptychactridae and the exclusion of Volutidae from Muricoidea would not ensure the monophyly of the resulting Muricoidea.

#### RELATIONSHIPS WITHIN THE COSTELLARIIDAE:

##### VEXILLUM VS. ‘BASAL’ LINEAGES

The family Costellariidae is commonly perceived as consisting of the large and extremely diverse genus *Vexillum* and a few, sometimes bizarre, small genera like *Zierliana* or *Visaya*. Our analysis evidences a series of previously unrecognized costellariid lineages, essentially consisting of still-unnamed taxa. The four costellariid lineages C-I–C-IV (Fig. 3) correspond to four to-be-established genera with numerous undescribed new species, which will be named elsewhere.

Although *Vexillum* species with multicuspidate rachidians occupy the most derived position in our phylogenetic trees, costellariids other than *Vexillum*, which are characterized by tricuspidate rachidians, form deep lineages. This radula morphology, which can be regarded as plesiomorphic, is characteristic also for *Ceratoxancus*, *Latiromitra*, and *Exilia*, as well for some other lineages of the superfamily Muricoidea (Fedosov & Kantor, 2010). The difference in radula morphology between *Vexillum* and the ‘basal Costellariidae’ parallels differences in the structure of the foregut glands. Earlier studies on the anatomy of the digestive system of costellariids revealed a large, bulky gland of Leiblein in the only studied species referable to ‘basal Costellariidae’ (Fedosov & Kantor, 2010), and a similar



**Figure 8.** Bayesian analysis tree of the MCPV-4G data set with the current superfamily assignment shown in colour other than grey. MCPV, Mitridae–Costellariidae–Ptychactridae–Volutomitridae.

gland of Leiblein is present in *Latiromitra* (Bouchet & Kantor, 2000), *Ceratoxancus* (Kantor & Bouchet, 1997), and *Exilia* (Harasewych, 1987; Kantor *et al.*, 2001). By contrast, many species of *Vexillum* are characterized by the reduction of the gland of Leiblein and the presence of a long, highly convoluted, glandular tube, originating from the stripped-off folds of the mid-oesophagus, and resembling the venom gland of the Conoidea. An intermediate morphology, with a still noticeable gland of Leiblein and already a rather short tubular duct, was found in *Vexillum cf. salisburyi* Cernohorsky, 1976, and *V. rubrum* (Fedosov & Kantor, 2010), the latter species falling into the *Vexillum s.l.*

group. The origin and evolution of the genus *Vexillum* thus appear to have been linked to the appearance of a multicuspidate rachidian and a rearrangement of the foregut that possibly allowed these snails to develop a new feeding strategy(ies), permitting their explosive radiation. It is however at present unclear whether the multicuspidate rachidian appeared only once in the evolutionary history of the Costellariidae. It could be a synapomorphy of the *Vexillum s.l.* clade (as supported by our MCPV-4G data set), or it could have evolved independently in different Costellariidae lineages. The presence of tricuspidate rachidians in some species of *Vexillum (Pusia)* complicates the picture, and

the affinities of these species need to be further examined. Moreover, the radula of '*V. balutense*' of the 'basal' clade C-IV, with its rachidian bearing two rudimentary cusps flanking the central cusp, may be considered intermediate between the simple tricuspidate and true multicuspidate.

The discovery of multiple costellariid taxa with tricuspidate rachidians and a well-developed, nontubular gland of Leiblein renders the morphological difference between the Costellariidae and traditional Ptychactridae quite subtle. In fact, the foregut anatomy of 'basal Costellariidae' is much closer to the one found in Ptychactridae than it is to the one found in *Vexillum*. Although all costellariids as currently construed lack an operculum, and an operculum is still present in *Ceratoxancus* and *Latiromitra*, we nevertheless suggest the transfer of *Ceratoxancus* and *Latiromitra* from the Ptychactridae to the Costellariidae. Although this rearrangement extends the boundaries of the Costellariidae, it remains a monophyletic group. Altogether, the transfer of *Ceratoxancus* and *Latiromitra* to Costellariidae, the revised status of *Zierliana* and *Protoelongata*, and the future erection of new genera for the newly identified deep-water lineages will significantly impact the generic composition of the family.

#### RELATIONSHIPS WITHIN THE MITRIDAE: SYSTEMATIC CHALLENGES AND MORPHOLOGICAL DIVERSITY

Thiele (1929) recognized three subfamilies in the family Mitridae: Mitrinae, Cyndrininae, and Vexillinae [= Costellariinae]. Cernohorsky (1966, 1970) added the subfamily Imbricariinae. Based on a comprehensive analysis of the anatomy of several species of mitrid gastropods, Ponder (1972) pulled Vexillinae outside Mitridae and synonymized Imbricariinae with Mitrinae. However, the validity of Imbricariinae was restored by Ponder & Warén (1988), and the same three mitrid subfamilies were recognized in Bouchet & Rocroi (2005).

Our phylogenetic analysis supports the monophyly of the family Mitridae and recognizes a 'core Mitridae' group, which is well supported and encompasses almost 90% of the species studied. Clade M-I includes the monophyletic *Pterygia*, the only genus classified in the subfamily Cyndromitridae, and therefore the monophyly of *Pterygia* implies the monophyly of Cyndromitridae. With some reservations we equate clade M-I to a subfamily Cyndromitridae. The well-supported clade M-II includes a diversity of mitrids, including *Mitra* s.s., represented in our data set by *Mitra mitra*, the type species of the genus, and *Mitra papalis*; the name Mitrinae is thus applicable to clade M-II. Clade M-III is represented by the single species *Strigatella paupercula*, the type species of *Strigatella*

that was treated as a separate genus by Cernohorsky (1976) and subsequently placed in *Mitra* as a subgenus (Robin & Martin, 2004; WoRMS, 2015). As it is found to be not immediately related to *Mitra* s.s., and, furthermore, falls outside the Mitridae clade, the status of *Mitra* (*Strigatella*) has to be revised and *Strigatella* ranked as a full genus, if not placed in its own subfamily. Finally, the well-supported clade M-IV includes the genera *Imbricaria*, *Neocancilla*, *Subcancilla*, and *Scabricola* and the name Imbricariinae is available for it. However, two genera that are traditionally classified as imbricariine, *Domiporta* and *Ziba*, turn out to be closer to *Mitra* s.s. than to other imbricariines, and their classification must be revised.

Despite the three mitrid subfamilies (with the newly suggested circumscriptions) being supported, two problems of congruence emerge between the phylogeny of the Mitridae revealed by our analysis and the current classification of the family: (1) the genus *Mitra* turns out to be polyphyletic, comprising multiple unrelated lineages that are spread throughout the mitrid tree, and requires a thorough revision; and (2) some mitrid lineages ('basal Mitridae' and *Strigatella paupercula* of the M-III clade) are not included in any of the three subfamilies. A separate subfamily for each clade of the 'basal Mitridae', as well as for *Strigatella paupercula* of the 'core Mitridae', would need to be established. Moreover, the assignment of the two mitrid species that cluster with *Pterygia* on our molecular tree, but differ in shell morphology, also remains unclear.

The typical mitrid radula with wide multicuspidate laterals characterizes most members of clade M-II (subfamily Mitrinae), but is also found in two 'basal Mitridae', *Charitodoron* sp., and *Mitra peculiaris*. For this reason, we consider this radula morphology ancestral for the family Mitridae. Whereas this morphology seems to have been conserved in most members of the subfamily Mitrinae, in other mitrid clades the radula has undergone transformations, and three different trends can be distinguished:

1. an increase in the number of cusps of the central tooth, resulting in the rachidian and laterals having similar morphology (mitrine species IM-2013-40651 of clade DW2);
2. a reduction in the number of cusps of the laterals, with the notable enlargement of one cusp (species of Imbricariinae);
3. a complete loss of the laterals, concomitant with a considerable size reduction of the radula; a uniserial radula is found in *Mitra tuberosa*, *Mitra bernhardina*, and *Pterygia* spp., in which they are likely to be the result of parallel evolution.

The diversity of radula morphology in the family Mitridae by far exceeds that of Costellariidae, as well as that of many other neogastropod families.

Although this might suggest a variety of feeding strategies, the diet of over 30 Indo-Pacific species of *Mitra*, *Imbricaria*, *Nebularia*, and *Strigatella* is known to consist exclusively of sipunculans (Taylor, 1978, 1984, 1986, 1993; Ponder, 1998; Harasewych, 2009), and Mitridae have been regarded as having the most specialized diet amongst Neogastropoda (Taylor, 1989; Harasewych, 2009). An epiproboscis, which is considered to be an adaptation for feeding on soft-bodied sipunculans (West, 1991), is present in all mitrids studied anatomically (Risbec, 1928; Ponder, 1972, 1998; West, 1991; Harasewych, 2009; Simone & Turner, 2010). Local sipunculan guilds rarely comprise more than ten species; i.e. their diversity is notably lower than that of Mitridae, such that an overlap in prey species amongst syntopic mitrid species is inevitable. A series of studies carried out by Taylor (1978, 1984, 1986, 1989) on Indo-Pacific mitrids showed that most are not species-specific predators, and the same prey species may be consumed by taxonomically closely or distantly related species of snails. For example, the sipunculan *Aspidosiphon tenuis* Sluiter, 1886, was shown to contribute significantly to the diet of at least six mitrids [*Nebularia fraga* (Quoy & Gaimard, 1833), *Nebularia chryzalis* (Reeve, 1844), *Nebularia cucumerina* (Lamarck, 1811), *Mitra acuminata* Swainson, 1824, *Strigatella litterata* (Lamarck, 1811), and *Imbricaria conovula* (Quoy & Gaimard, 1833)]. In addition, any one species of miter may consume three to four different species of sipunculans at any one locality. The broad range of radula types in mitrids, with their feeding specialization on similar prey items, is thus unexpected. It is noteworthy, however, that all the species for which morphological data are available are referable to our 'core Mitridae', although none of the 'basal Mitridae' lineages has been studied to date.

### CONCLUSION

This study revealed a major incongruence between the currently accepted classification of the Mitridae, Costellariidae, and Ptychactridae and the relationships evidenced by molecular phylogenetic trees. Mitriform gastropods do not form a monophyletic group, with Mitridae standing apart from the rest. The families Pleioptygmatidae and Pyramimitridae are most closely related to the Mitridae, the former based on published data on radula and shell morphology, the latter based on the present phylogenetic analysis. Costellariidae display a close relationship to the ptychactrid genera *Ceratoxancus* and *Latiromitra*, and a more distant affinity to *Exilia*, the family Volutomitridae, and the 'turbinellid' subfamilies Columbariinae and possibly Vasiniae. Our results also reject an affinity of Ptychactridae and Pseudolividae, and the superfamily status of Pseudolivoidea is ques-

tioned. Finally, our results also show the need for a revised circumscription of the families Ptychactridae and Turbinellidae, as both are found to be paraphyletic.

The existing taxonomic framework cannot accommodate the revealed diversity of the Costellariidae and Mitridae phylogenetic lineages. A revision of both Costellariidae and Mitridae, with the establishment of multiple new genera for inferred phylogenetic lineages, is needed. In particular, the polyphyly of *Mitra* suggests that a number of species currently assigned to *Mitra* should be reassigned elsewhere, in genera still to be established.

### ACKNOWLEDGEMENTS

The molecular material in this paper originates from numerous shore-based expeditions and deep-sea cruises, conducted respectively by MNHN and Pro-Natura International as part of the *Our Planet Reviewed* programme, and by MNHN and IRD as part of the *Tropical Deep-Sea Benthos* programme. Funders and sponsors include the French Ministry of Foreign Affairs, the Philippines Bureau of Fisheries and Aquatic Research, the Total Foundation, Prince Albert II of Monaco Foundation, Stavros Niarchos Foundation, and Richard Lounsbery Foundation. We thank, amongst others, Virginie Héros, Philippe Maestrati, Pierre Lozouet, Barbara Buge, Ellen Strong, and Laurent Charles for their role in specimen processing during the expeditions and curation. We thank Catherine Rausch (MNHN) and Nadezda Surovenkova for SEM facilities. The present study was largely accomplished during a visiting curatorship of the first author to MNHN in 2013 and 2014, supported also by a Metchnikov post-doctoral fellowship granted by the French Embassy in Russia. The molecular phylogenetic studies were supported by the 'Service de Systématique Moléculaire' (UMS 2700 CNRS-MNHN), the Actions Transversales du Muséum Barcode (principal investigators: Sarah Samadi and Jean-Noël Labat, MNHN), and by grants from the Russian Foundation of Basic Researches: RFBR-14-04-31048-mol-a and RFBR-14-04-00481-a.

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