Melanella martarum sp. nov. (Gastropoda: Eulimidae): the first parasitic deep-sea snail reported for the Salas y Gomez Ridge. (#91751)

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Melanella martarum sp. nov. (Gastropoda: Eulimidae): the first parasitic deep-sea snail reported for the Salas y Gomez Ridge.

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Eulimidae is a highly diverse family of gastropods that are often parasites of echinoderms. They are cosmopolitan and usually live from the intertidal to great depths. Despite its wide geographic and bathymetric distribution, no species of Eulimidae have been reported for the Salas y Gómez ridge to date. In this study, we describe Melanella martarum sp. nov., which was collected during the EPIC oceanographic cruise onboard RV Mirai (JAMSTEC, Japan) in 2019. Seven specimens were collected with a modified Agassiz on the summit of seamount “Pearl” (Zhemchuznaya) in the Salas y Gómez Ridge (Lat. -25.59, Long. -89.13) at 545 m depth. The morphology of M. martarum sp. nov. was compared with other Melanella species reported for the area, including Chile and Rapa Nui. DNA was extracted and partial sequences of the mitochondrial genes Cytochrome Oxidase 1 (COI) and 16S rDNA and the nuclear gene Histone 3 (H3) were sequenced. Melanella martarum sp. nov. has morphological characteristics that separate it from other species of Melanella, such as the thickness and color of the shell, and the shape of the protoconch. Besides, M. martarum sp. nov. was genetically differentiated from other Melanella spp. sequences (uncorrected p distances from 18,1–8.6% in mitochondrial COI and 16S rDNA to 3% in nuclear H3 sequences). Although there is not much molecular data available for Eulimidae, the phylogenetic analysis confirms the results obtained by morphology, placing the species found on the Salas y Gómez Ridge within the genus Melanella. The current study advances the understanding of the poorly known benthic fauna found on seamounts in the easternmost part of the Sala y Gómez ridge, a location distinguished by a high level of endemism.

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Abstract

Eulimidae is a highly diverse family of gastropods that are often parasites of echinoderms. They are cosmopolitan and usually live from the intertidal to great depths. Despite its wide geographic and bathymetric distribution, no species of Eulimidae have been reported for the Salas & Gómez Ridge to date. In this study, we describe *Melanella martarum* sp. nov., which was collected during the EPIC oceanographic cruise onboard RV Mirai (JAMSTEC, Japan) in 2019. Seven specimens were collected with a modified Agassiz on the summit of seamount "Pearl" (Zhemchuznaya) in the Salas & Gómez Ridge (Lat. -25.59, Long. -89.13) at 545 m depth. The morphology of *M. martarum* sp. nov. was compared with other *Melanella* species reported for the area, including Chile and Rapa Nui. DNA was extracted and partial sequences of the mitochondrial genes Cytochrome Oxidase 1 (COI) and 16S rDNA, and the nuclear gene Histone 3 (H3) were sequenced. *Melanella martarum* sp. nov. has morphological characteristics that separate it from other species of *Melanella*, such as the thickness and color of the shell, and the shape of the protoconch. Besides, *M. martarum* sp. nov. was genetically differentiated from other *Melanella* spp. sequences (uncorrected p distances from 18,1–8.6% in mitochondrial COI and 16S rDNA to 3% in nuclear H3 sequences). Although there is not much molecular data available for Eulimidae, the phylogenetic analysis confirms the results obtained by morphology, placing the species found on the Salas y Gómez Ridge within the genus *Melanella*. The current study advances the understanding of the poorly known benthic fauna found on seamounts in the easternmost part of the Sala y Gómez ridge, a location distinguished by a high level of endemism.

Subjects Biodiversity, Conservation Biology, Marine Biology, Taxonomy, Zoology

Keywords Nazca ridges, Deep-sea, Pacific Ocean, Indo-pacific fauna, Salas & Gómez ridges

Introduction

Eulimidae Philippi, 1853 is a family of marine gastropods that encompass parasites of all extant classes of Echinodermata, presenting a high species richness with diverse body plans and parasitic strategies (Warén, 1983; Takano & Kano, 2014; Takano & Goto, 2021). Currently, there are around 960 valid species (MolluscaBase 2023), but Waren & Gittenberger (1993) provided a gross estimate that there could be more than 4,000 species of Eulimidae. Other authors also highlighted the underestimated diversity of this group. For example, Bouchet et al. (2002) based on a huge sampling effort in New Caledonia estimated that 80% of the eulimids collected may represent undescribed species.

The lack of knowledge about Eulimidae is not only related to the inventory of species but also to the parasitic strategies of this group of snails. Some eulimids are strongly attached to their hosts (i.e., tightly attached ectosymbionts, gall-forming species, and endoparasites) but also free-living ectosymbionts (Dgebuadze et al., 2022). In the former case, these snails can detach from the host more easily when disturbed during sampling with common benthic tools (nets, grabs, dredges), this often makes it difficult to identify the hosts (Takano, Itoh & Kano, 2018; Takano, Kimura & Kano, 2020).

The *Melanella* genus is one of the most species-rich of the family, with more than 200 recognized species (MolluscaBase 2023). *Melanella* is known to live as an ecto- or endoparasite of holothuroids in the orders Holothuriida (formerly Aspidochirotida) and Dendrochirotida (Warén 1983) and it could be host-specific or generalists (Crossland et al. 1993).

In Chile, 15 species of Eulimid gastropods have been reported (10 genera) (Rehder, 1980; Valdovinos, 1999; Osorio, 2023). Most of the species described so far are found on Rapa Nui Island (Easter Is.) (Linse, 1999; Valdovinos, 1999; Osorio, 2023). *Melanella* is the most representative genus with four species: *M. cumingii* (Adams 1854), *M. aciculata* (Pease, 1861) and *M. pisinna* (Rehder 1980) (Rehder, 1980; Osorio, 2023) founded in Rapa Nui Island and *M. subantartica* (Strebel 1908) endemic of the Magellan region (Linse, 1999; Valdovinos, 1999).

Between 1973 and 1987, research expeditions from the former Soviet Union explored 22 seamounts of the Salas & Gómez Ridge (SGR) and the Nazca Ridge (NR) outside the Chilean Exclusive Economic Zone (CEEZ) (mainly west of ~83°W) (Parin, Mironov & Nesis, 1997). This expedition represents only ~3% of the seamounts that make up both dorsal ranges. Within the CEEZ, CIMAR 22 (CONA, 2016) was the first multidisciplinary expedition to study the summit of several seamounts of Salas & Gómez Island and Desventuradas Islands. This new expedition has added new records and/or new species for science, mainly including crustaceans, echinoderms, polychaeta, and mollusks (Mecho et al., 2019; Sellanes et al., 2019; Asorey et al., 2020; Díaz-Díaz et al., 2020; Gallardo et al., 2021). However, no specimens of the Eulimidae family have been collected in this expedition. Furthermore, in the studies carried out outside the CEEZ, no new species have been described or the presence of any specimen of this gastropod family has been reported. But in 2019, during the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) oceanographic cruise, some specimens of this family were collected from the seamount “Pearl” (Zhemchuznaya). So, in the present study, we describe these samples as the first deep-sea parasitic snail species of Eulimidae for the SGR. We also provide genetic data of the new species, assessing its phylogenetic relationships with congeners, as well as insight into its echinoderm host.

Materials & Methods

Material collection and sampling site

Samples were obtained by a modified Agassiz trawl with a mouth of 1.5 m × 0.5 m (width × height) fitted with a net of 12 mm mesh at the cod end and operated in 10 min hauls (bottom contact) at ~3 knots during the oceanographic EPIC cruise onboard the RV Mirai (JAMSTEC, Japan) in 2019. Sampling was performed on the summit of the seamount "Pearl" (Zhemchuznaya, Lat. -25.59, Long. -89.13), at 545 m depth, in SGR. The collected material was preserved in 95% ethanol. Holotype and paratype specimens are housed in Museo Nacional de Historia Natural (MNHNCL) and Sala de Colecciones Biológicas de la Universidad Católica del Norte (SCBUCN), both in Chile (Table 1). Sample collection was performed under the permission of Res. Ext N°3685/2016 from SUBPESCA (Chile) to Universidad Católica del Norte.

Phylogenetic analysis

Genomic DNA was extracted from the whole animal of SCBUCN-5482 using an E.Z.N.A.® Tissue DNA kit (Omega, Bio-Tek, Norcross, GA, USA). To amplify partial sequences of the Histone 3 (H3) nuclear gene and the mitochondrial cytochrome C oxidase I (COI) and 16S rRNA genes, the pairs of primers H3F (ATGGCTCGTACCAAGCAGACVGC) and H3R (ATATCCTTRGGCATRATRGTGAC) (Colgan, Ponder & Eggler, 2000), HCO-1490 (GGTCAACAAATCATAAAGAYATGYG) and LCO-2198 (TAAACTTCAGGGTGACCAAARAAYCA) (Folmer et al., 1994), and 16SAR-16SBR (Palumbi et al., 1991) were used, respectively. The PCR profile for COI started with 5 min at 95°C, followed by 40 cycles of denaturation at 95°C (1 min), annealing at 50°C (1 min), and elongation at 72°C (2 min), with a final elongation phase at 72°C (13 min). A similar PCR profile was set for H3 and 16S rRNA (annealing at 55°C). The resulting amplicons were visualized in agarose 1% gels and the PCR products were sent to Macrogen, Inc. (Korea) for DNA Sanger sequencing.
The COI, 16S rRNA, and H3 sequences (forward and reverse) were visualized and assembled with Geneious Prime 2022. 2.2 (Kearse et al., 2012). Fifteen H3 and 16S rRNA genes and fourteen COI gene sequences of Eulimidae were extracted from NCBI GenBank (Table 2), concatenated, and aligned with the ones of *Melanella martarum* sp. nov., using default MUSCLE (Edgard 2004) parameters. The resulting alignment was used to construct the maximum likelihood phylogenetic tree with the RAxML 8.2.11 software (Guindon et al., 2010) plugin for Geneious Prime 2022.2.2 (Kearse et al., 2012), using the following settings: Nucleotide model = GTR GAMMA, Algorithm=Rapid bootstrapping and search for best-scoring ML tree, bootstrap replicates = 1,000, Partitioning= 16S=1-419, COI = 420-1049, H3 = 1050-1363. Significant bootstrap values (>90)

are reported at the nodes. Sequences of the 3 above-mentioned markers of *Vanikoro helicoidea* (Vanikoridae) were used as an outgroup.

SEM photography

The shell morphology was examined with a Hitachi SU3500 scanning electron microscope (SEM) at the Microscopy Laboratory of the Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile. The shell was dried in a Tousimis, Samdri-780A critical-point dryer using CO_2, mounted on bronze stubs, and coated with gold in a JEOL JFC-100 evaporator. The examined individual was from the same specimen used for the molecular analysis (SCBUCN 5482).

Abbreviations of other repositories: ANSP: Academy of Natural Sciences of Philadelphia at Drexel University, Philadelphia, USA; MCZ: Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; NHMUK: Natural History Museum of the United Kingdom, London, United Kingdom.

New Species Registration

The following information was supplied regarding the registration of a newly described species:
Publication LSID: urn:lsid:zoobank.org:pub:716D01AC-7DA7-4B2D-A4D5-0DD925825BC6
Melanella martarum sp. nov. LSID: urn:lsid:zoobank.org:act:CC4C4BE5-4D7F-4EF3-8926-9B960C01C3CA

Results

Systematic account

Family EULIMIDAE Philippi, 1853
Genus *Melanella* Bowdich, 1822
Type species: *Melanella dufresnii* Bowdich, 1822, by original designation.

Melanella martarum sp. nov.
Figs. 1A-K

Diagnosis: Shell medium in size (up to 8.1 mm long), polished, translucent white, conical, apex minute. Protoconch multispiral, with slightly convex whorls, surface smooth, with no distinction between protoconch I and II; transition to teleoconch marked by sinuous incremental scar. Teleoconch whorls almost flat, incremental scars irregularly spaced, surface smooth. Body whorl about 45% of total shell length in adults, base rounded. Aperture wide, squarish in juveniles, pear-shaped and laterally expanded in adults; outer lip sinuous. Eyes present, anterior body whitish, posterior region orange. Ectoparasitic on holothuroids.

Description (Holotype): Shell conical, apex obtuse, reaching about 6.3 mm long, 2.5 mm wide. Larval shell vitreous, about 4.0 convex whorls, smooth, transition to teleoconch marked by distinct incremental scar. Teleoconch vitreous, not colored, about 6.5 whorls of flat outline; suture shallow, slightly impressed, sloping; subsutural zone about 1/5 of the height of the whorl; surface glossy, smooth, except for incremental scars appearing at irregular intervals. Last whorl about 50% of the shell length; base slightly rounded. Aperture high, 60% of body whorl height, rhomboid in shape, acute posteriorly, slightly rounded anteriorly; outer lip thin, sinuous, opisthocline, retracted near the suture, maximum projection at the middle of the outer lip height; inner lip thin, sinuous, sloping. Not umbilicate.

Description (Paratype SCBUCN-5482): Shell conical, apex obtuse, reaching about 8.1 mm long, 2.6 mm wide. Larval shell vitreous, about 4.0 convex whorls, 480 µm in height, smooth, transition to teleoconch marked by distinct incremental scar. Teleoconch vitreous, not colored, about 9.0 whorls of flat outline; suture shallow, slightly impressed, sloping; subsutural zone about 1/5 of the height of the whorl; surface glossy, smooth, except for incremental scars appearing at irregular intervals. Last whorl about 45% of the shell length; base rounded. Aperture high, 65% of body whorl height, pear-shaped, moderately expanded laterally, acute posteriorly, rounded and spread anteriorly; outer lip thin, very sinuous, opisthocline, retracted near the suture, after strongly projecting, and retracted in the distal region, maximum projection at the middle of the outer lip height; inner lip thin, sinuous, sloping. Not umbilicate.

Holotype: MNHNCL-205421, SL: 6.3 mm, SW: 2.5 mm, AL: 1.7 mm, AW: 1.0 mm, Teleoconch whorls: 6.5. Seamount "Pearl" off the coast of Chile in international waters, Lat. -25.59, Long. -89.13, 545 m depth, February 9th, 2019, RV *Mirai* (JAMSTEC, Japan).

Paratypes (all from type locality): SCBUCN 8611-1, SL: 4.6 mm, SW: 1.9 mm, AL: 1.4 mm, AW: 0.8 mm, Teleoconch whorls: 5; SCBUCN 8611-2, SL: 4.4 mm, SW: 1.8 mm, AL: 1.3 mm, AW: 0.9 mm, Teleoconch whorls: 5; SCBUCN 8611-3, SL: 4.5 mm, SW: 2.0 mm, AL: 1.4 mm, AW: 0.8 mm, Teleoconch whorls: 5; SCBUCN 8612-1, SL: 4.7 mm, SW: 2.1 mm, AL: 1.3 mm, AW: 0.9 mm, Teleoconch whorls: 5; MNHNCL-205422, SL: 3.8 mm, SW: 1.7 mm, AL: 1.4 mm, AW: 0.7 mm, Teleoconch whorls: 4; SCBUCN-5482, SL: 8.1 mm, SW: 2.9 mm, AL: 2.2 mm, AW: 1.4 mm, Teleoconch whorls: 9.

Comparative material: *Melanella aciculata* (Pease, 1861) (Fig 2): Lectotype NHMUK 1962839 (designated by Kay, 1965), Sandwich Islands, Hawaiian Archipelago; Paralectotypes NHMUK 1962840, 4 shells, from type locality; Paralectotype MCZ 31705, 1 shell, Sandwich Islands; Paralectotype MCZ 187747, 1 shell, Hawaiian Islands. *Melanella acicula* (A. Gould, 1849): Syntype ANSP 19773 of *Eulima pisorum* Pilsbry, 1917 [= *M. acicula*], 1 shell, Viti Islands, Fiji (http://clade.ansp.org/malacology/collections/details.php?mode=details&catalognumber=19773#detail).

Distribution, habitat, and parasitic strategy*:* *Melanella martarum* sp. nov. was found tightly attached to *Oneirophanta* cf. *setigera* (Ludwig 1893) (Holothuroidea: Deimathidae) (Fig 3A). The Holothuroid host was found on a hard substrate covered with a finer, sandy-type sediment (Fig 3B) at a depth of 545 meters on the summit of a seamount in international waters off Chile (Lat. -25.59, Long. -89.13). This seamount is known as "Pearl". Ectosymbiont.

Etymology: The species name honors Marta B. Parodi and Marta S. Wainstein, grandmother and mother, respectively, of one of the authors (C.M. Asorey). The Latin genitive case suffix -arum was added as the epithet represents two females.

Species comparison: A few species of Eulimidae are known from this area, including *Melanella*, and most of them are known from shallow waters. *Melanella martarum* sp. nov. resembles the shallow water species *M. aciculata* (Fig. 2A–H), an ectoparasitic species known from the Hawaiian Archipelago, French Polynesia, and Rapa Nui (Osorio 2018, Osorio 2023). However, the newly described species differs from *M. aciculata* by the straighter spire, a consequence of the irregular position of the incremental scars in *M. martarum* sp. nov. The initial whorls of *M. aciculata* have a more regular position of the incremental scars, giving a distorted appearance (Fig. 2G, H). Besides that, *M. martarum* sp. nov has a more acute spire angle, an aperture more expanded laterally, a thinner inner lip and outer lip (Fig. 1D), and the adult of *M. martarum* sp. nov. has a sinuous outer lip (Fig. 1D), while the largest shells of *M. aciculata* have a straighter outer lip (Fig. 2A, C, D, F).

Melanella martarum sp. nov. also resembles *M. acicula*, with a broad distribution in shallow waters of the West Pacific, but the tip of the shell is much more acute in the newly described species. Pilsbry (1917) highlighted the broad apex of *Eulima pisorum* Pilsbry, 1917, a junior synonym of *M. acicula*, while comparing this species with *M. aciculata*. Our comparison with the syntype of *E. pisorum* also reinforces this diagnostic feature. Furthermore, the spire angle of *M. martarum* sp. nov. has a slower increase in diameter, being more acute than in *M. acicula*.

Melanella martarum sp. nov. can be easily distinguished from *Melanella cumingii* (A. Adams, 1854), known from Rapa Nui and other areas of the Indian and Pacific Oceans (Osorio 2018). The straighter, thinner shell with almost flat whorls in the teleoconch of *M. martarum* is much different from the opaque and thicker shell with convex whorls of *M. cumingii*. The latter also reaches greater dimensions (around 20 mm long) (Severns 2011).

Melanella martarum sp. nov. shares the almost flat teleoconch whorls with *Haliella chilensis* Bartsch, 1917, a deep-water species from off Chile, but can be easily distinguished by the acute apex in comparison to the dome-shaped protoconch of the latter species (Bartsch 1917: pl. 43, fig. 6), a common feature of *Haliella* Monterosato, 1878.

Phylogenetic relationship of *Melanella martarum* sp. nov.

We successfully sequenced COI, 16S rRNA, and H3 genes of a single specimen of *M. martarum* sp. nov. The final alignment of the COI resulted in 630 bp, H3 of 314 bp, and the 16S rDNA of 419 bp, and these concatenated sequences produced a final alignment of 1,363 bp. COI presented the greatest intragenus variability, with only an 81.9% similarity with the *M. acicula* sequence. H3 and 16S rDNA presented an identity percentage of 97.0% and 91.4% with *Melanella* sp. CKC-2011, respectively. The identity for H3 and 16s was slightly lower with the *M. acicula* sequences (96.3% and 91.9%), although within the expected percentage identity values between species of the same genus. Consistent identity values between markers are shown in the phylogenetic reconstruction. *Melanella martarum* sp. nov. clustered with other *Melanella* species identified in previous studies and was retrieved as the sister taxon to an unidentified *Melanella* (Fig 4), which was not illustrated in the original publication (Churchill, Strong & Ófoighil, 2011), hampering further comparisons. However, the detailed phylogenetic relationship among congeners is premature, since the genus currently includes more than 200 species, is probably not monophyletic (Souza & Pimenta, 2019), and just a few sequences are available for a species-rich group such as Eulimidae.

Discussion

Based exclusively on the shell morphology, a primary association of the newly described species with the genus *Melanella* could be inferred, despite the plasticity or diverse forms currently included in the genus. *Melanella martarum* sp. nov. has a conical, straight spire, with a sinuous outer lip, more similar to the species historically included in *Polygireulima* Sacco, 1892. The type species of *Polygireulima* is a fossil taxon and no data is available regarding the parasitic association, thus we follow Souza and Pimenta (2019: 429) in the broad concept of *Melanella* with a straight spire, differing from the strongly curved shell of *Melanella dufresnii* Bowdich, 1822 (type species of *Melanella*) (see Souza and Pimenta 2019 for details). *Melanella acicula* (A. Gould, 1849), with a close relationship based on present data, has a similar shell morphology following these features. The lack of illustrations of *Melanella* sp. (CKC-2011) prevents us from checking the shell morphology of this taxon. Assessing with molecular data a broad number of *Melanella* species with these different patterns of straight and curved spires would be interesting for phylogenetic reconstruction and to check about this variation within the genus.

Besides the shell morphology, the type of host also contributed to the generic classification, since *Melanella* comprise species parasitic on holothuroids (Warén 1983; Souza et al., 2018). Despite being collected by an Agassiz trawl, *M. martarum* sp. nov. reached the surface still attached to its host, which is difficult with this type of sampling, especially in the deep-sea. *Melanella martarum* sp. nov. possibly remains strongly attached to the host through the proboscis. Furthermore, the clustering of *M. martarum* sp. nov. with other *Melanella* in the molecular analysis, reinforced our previous assumption, despite the scarcity of molecular data on the genus. Thus, our generic classification could be supported by different types of data.

Most Eulimidae species found in Rapa Nui island are considered endemic. A few extend their distribution to other polynesic islands such as Hawaii, Cook, and Tuamotu (e.g. *M. aciculata*) and only *M. cumingii* has a broad distribution from West Africa to Hawaii (Osorio 2018, Osorio 2023). The new species does not resemble them morphologically, and the available information indicates that it is only found on seamounts in SGR, which is home to a fauna characterized by high levels of endemism (Friedlander et al., 2016). However, due to the possible planktotrophic development of *M. martarum* sp. nov., inferred by the multispiral, conical protoconch, this species probably occurs in surrounding areas. Host specificity and habitat preferences are not well known for a better comprehension of the distribution.

Conclusions

Melanella martarum sp. nov. is the first gastropod of the Eulimidae family reported for seamounts of the Salas & Gómez ridge, an area with a high level of endemism but still poorly explored. The new species has only been observed at the summit of the seamount "Pearl" (Zhemchuznaya) (Lat. -25.59, Long. -89.13), but further sampling is needed in seamounts of the Salas y Gomez Ridge to have a clearer understanding of the geographic distribution of *M. martarum* sp.nov. Despite the scarce molecular data for the family Eulimidae, the phylogenetic reconstruction allowed us to verify the assignment to the genus *Melanella* of this new species. However, the generation of more molecular data is necessary to clarify the taxonomy of the Eulimidae family at the species level.

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Figure 1

Melanella martarum sp. nov.

Melanella martarum sp. nov. A–C. Holotype, SCBUCN 8542. D, E, I–K. Paratype, SCBUCN 5482. F–H. Paratypes, SCBUCN 8611. A, D, F–H. Frontal view; B, E. Dorsal view. C, J. Detail of protoconch, frontal view. I. Detail of teleoconch surfac, close to suturee. K. Detail of protoconch, apical view. Scale bars: A, B, D–H= 1 mm; C, J= 200 µm; I, K= 100 µm.

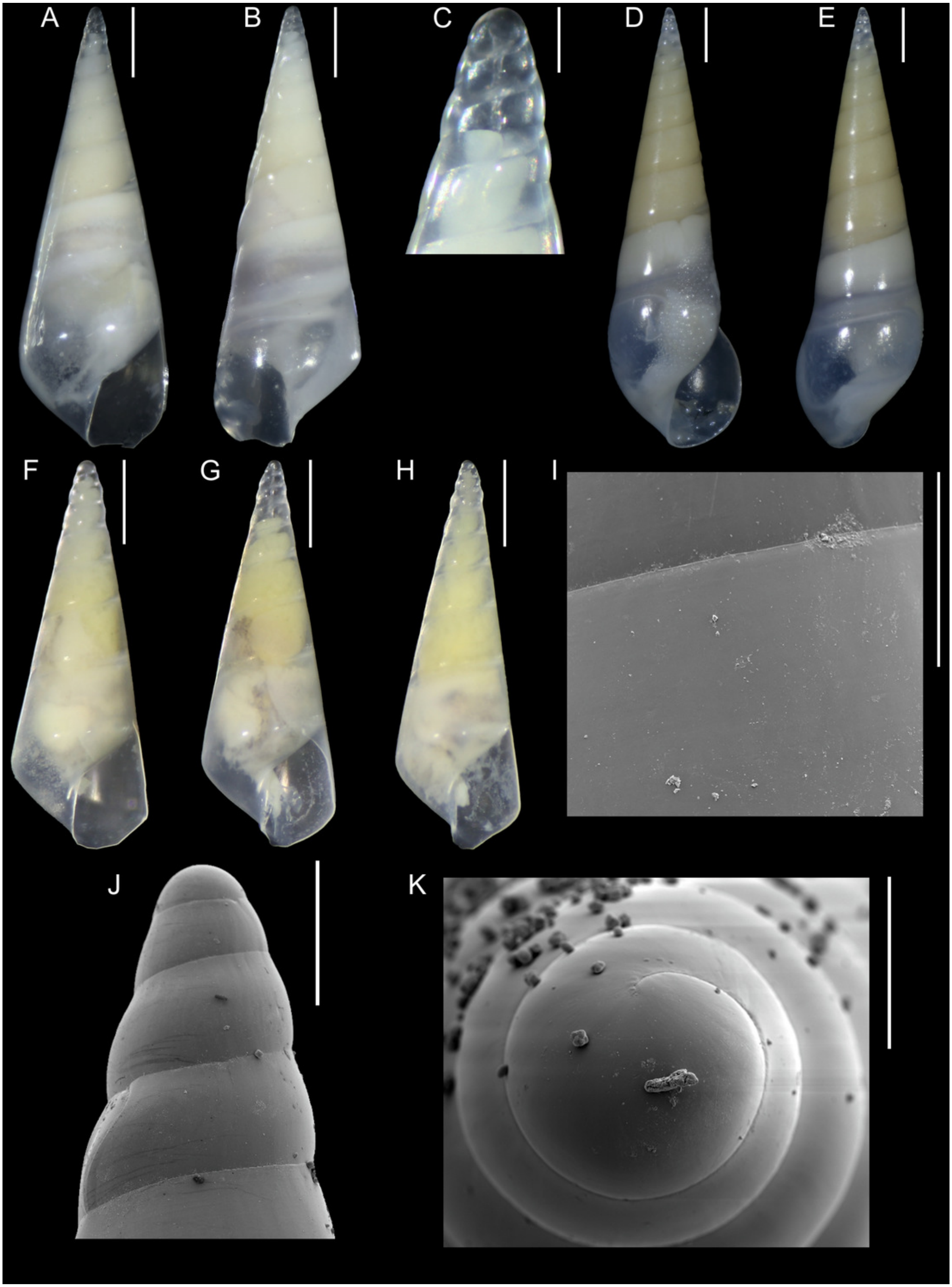
A
B
C
D
E
F
G
H
I
J
K

Figure 2

Melanella aciculata (Pease, 1861).

A, B. Lectotype, NHMUK 1962839. Paralectotypes (A–D, respectively), NHMUK 1962840. G, H. Paralectotype, MCZ 3170. A, C–G. Frontal view. B. Dorsal view. H. Lateral view. Scale bar: 1 mm. Credits: Images A–F, courtesy of Andreia Salvador and photographic unit (NHMUK); G, H: courtesy of Jennifer W. Trimble (MCZ)

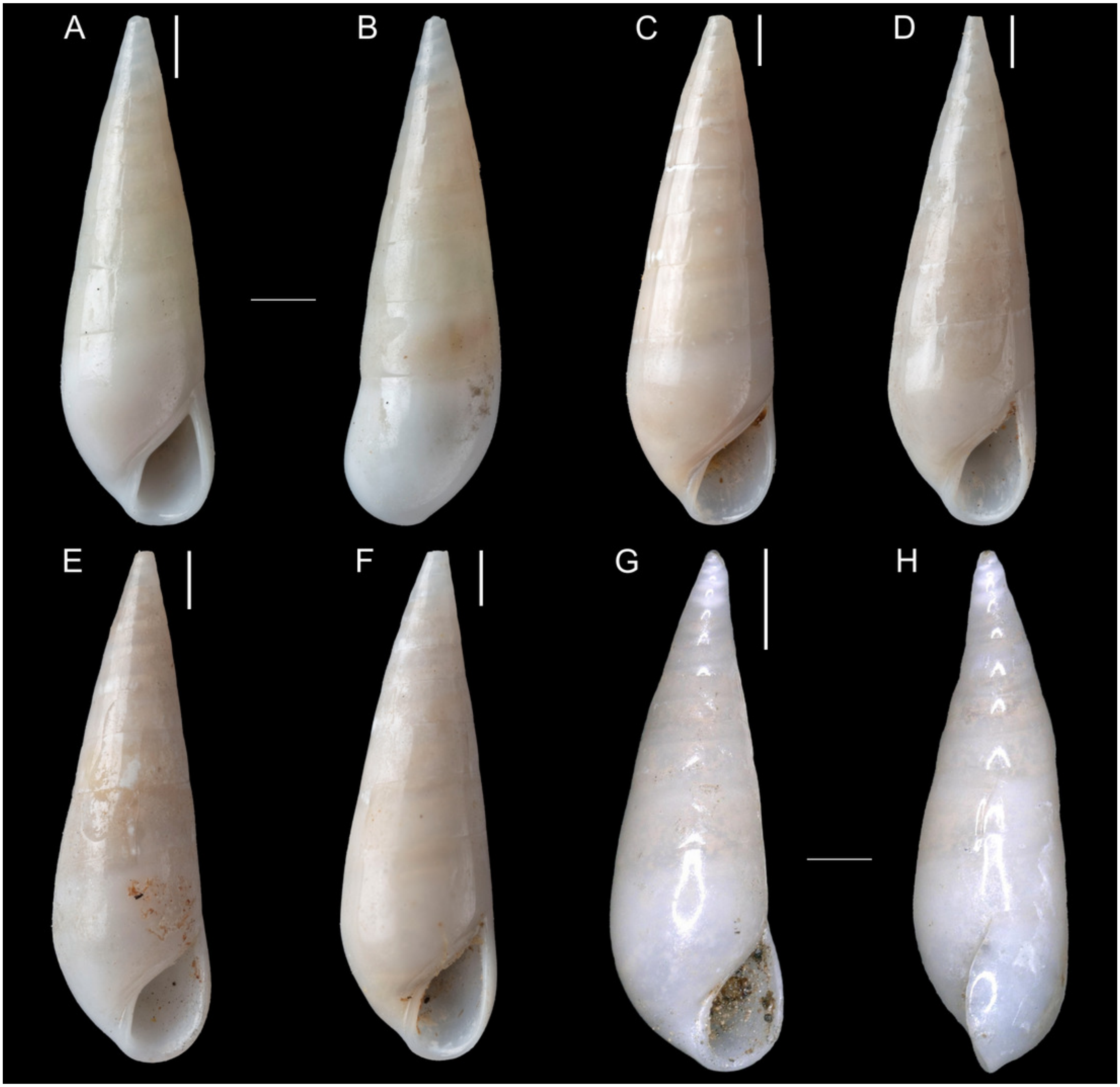
A
B
C
D
E
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Figure 3

Melanella martarum sp. nov. in it holoturian host.

A. *Melanella martarum* sp. nov. SCBUCN-5482 on its holothurian host. Scale bar: 4 mm. The white arrow shows M. martarum sp. nov attached to its holothurian host.B. Screenshot of the camera DeepTowfrom SPG5 seamount. Scale bar: 10 cm. The white arrow shows the host on a sandy substrate.

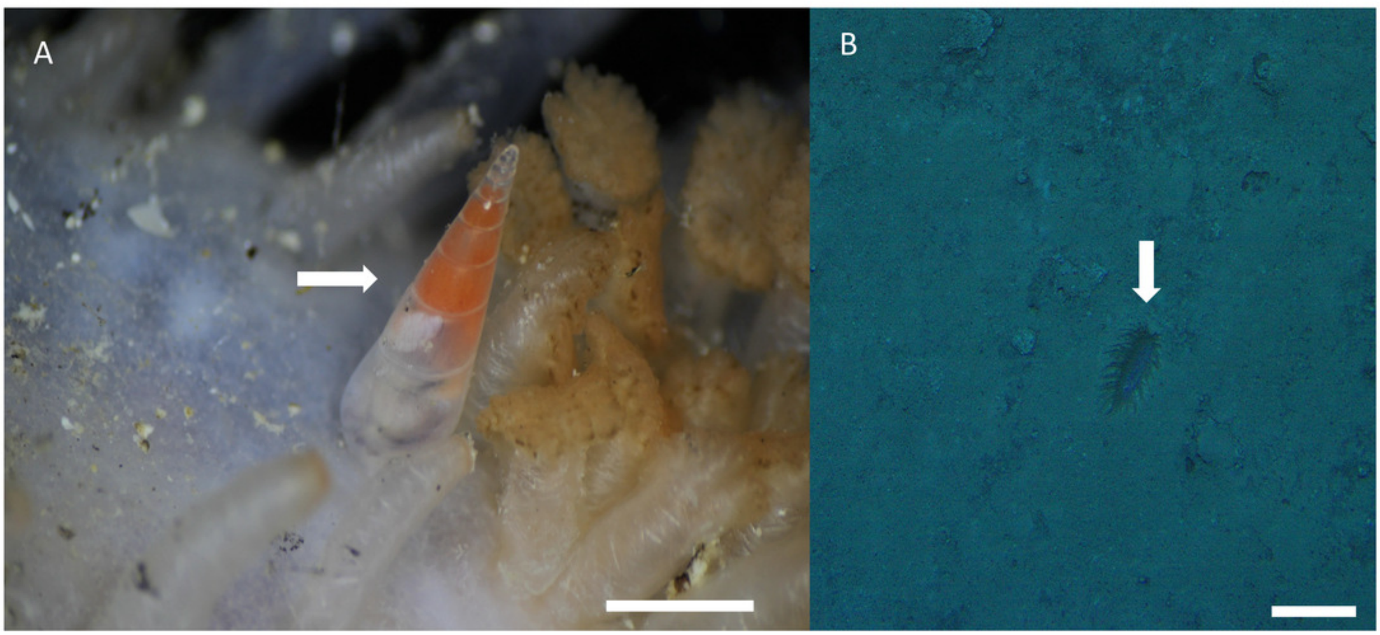

Figure 4

RaxML inferred phylogenetic reconstruction

RaxML inferred phylogenetic reconstruction based on 1363-bp alignment of partial 16S, COI and H3 concatenate sequences of Eulimidae (bootstrap = 1,000). Bootstrap values obtained are shown in each node. *Vanikoro helicoidea* (Vanikoridae) was used as outgroup.

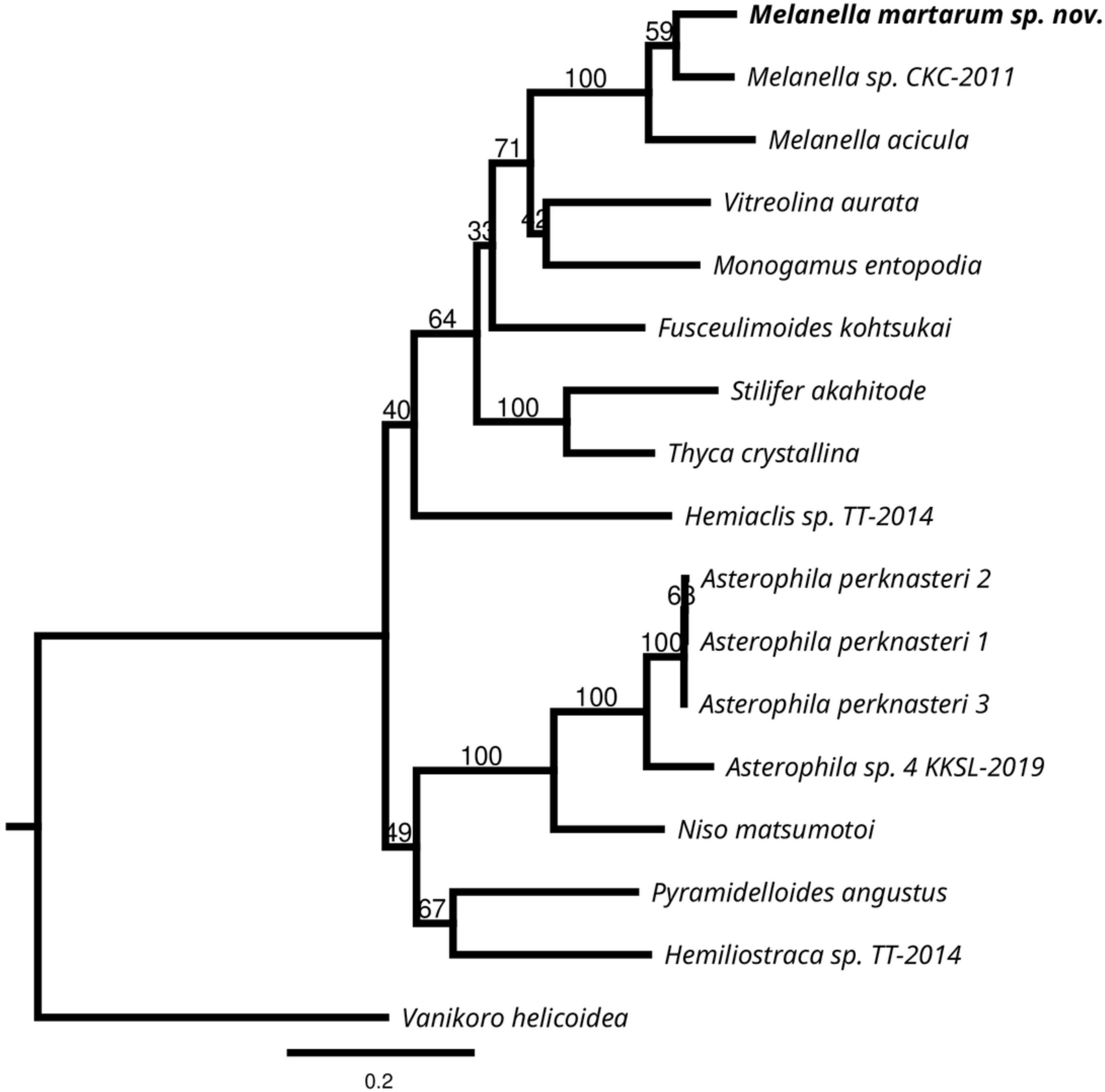
Melanella martarum sp. nov.
Melanella sp. CKC-2011
Melanella acicula
Vitreolina aurata
Monogamus entopodia
Fusceulimoides kohtsukai
Stilifer akahitode
Thyca crystallina
Hemiaclis sp. TT-2014
Asterophila perknasteri 2
Asterophila perknasteri 1
Asterophila perknasteri 3
Asterophila sp. 4 KKSL-2019
Niso matsumotoi
Pyramidelloides angustus
Hemiliostraca sp. TT-2014
Vanikoro helicoidea
59
100
71
33
64
40
100
100
100
100
49
67
0.2

Table 1 (on next page)

Voucher and shell measurements of type specimens of *Mellanella martarum* Sp. nov.

*Sample used by SEM images and molecular analysis.

Table 1: Voucher and shell measurements of type specimens of *Mellanella martarum* Sp. nov. *Sample used by SEM images and molecular analysis.

Specimen	Voucher	Shell high (mm)	Shell width (mm)	Aperture height (mm)	Aperture width (mm)	Number of Teleconcha Whorls
Mellanella martarum sp. nov.	SCBUCN-8611-1	4.61	1.85	1.35	0.75	5
Mellanella martarum sp. nov.	SCBUCN-8611-2	4.42	1.75	1.32	0.88	5
Mellanella martarum sp. nov.	SCBUCN-8611-3	4.50	2.00	1.4	0.77	5
Mellanella martarum sp. nov.	SCBUCN-8612	4.65	2.10	1.26	0.85	5
Mellanella martarum sp. nov.	MNHNCL-205422	3.76	1.65	1.36	0.68	4
Mellanella martarum sp. nov. **(Holotype)**	MNHNCL-205421	6.25	2.45	1.7	1.03	6
* *Mellanella martarum sp. nov.*	SCBUCN-5482	8.14	2.39	NA	NA	9

Table 2 (on next page)

Species used in present analyses with GenBank accession numbers and the authors of each one.

Accession numbers of newly obtained sequences are given in bold.

1 Table 2: Species used in present analyses with GenBank accession numbers and the
2 authors of each one. Accession numbers of newly obtained sequences are given in bold.
3

Family	Species	H3	16S	COI	Published by
Eulimidae	*Hemiaclis sp.*	AB930436	AB930409	AB930465	Takano & Kano 2014
	Hemiliostraca sp.	AB930437	AB930410	AB930466	Takano & Kano 2014
	Melanella acicula	AB930435	AB930408	AB930464	Takano & Kano 2014
	Monogamus entopodia	AB930429	AB930402	AB930458	Takano & Kano 2014
	Niso matsumotoi	AB930440	AB930413	AB930469	Takano & Kano 2014
	Pyramidelloides angustus	AB930441	AB930414	AB930470	Takano & Kano 2014
	Stilifer akahitode	AB930432	AB930405	AB930461	Takano & Kano 2014
	Thyca crystallina	AB930431	AB930404	AB930460	Takano & Kano 2014
	Vitreolina aurata	AB930428	AB930401	AB930457	Takano & Kano 2014
	Asterophila perknasteri 1	MN224387	MN224427	MN224306	Layton etal 2019
	Asterophila perknasteri 2	MN224369	MN224437	MN224310	Layton etal 2019
	Asterophila sp 4 KKSL-2019	MN224372	MN224460	MN224348	Layton etal 2019
	Asterophila perknasteri 3	MN224388	MN224451	MN224362	Layton etal 2019
	Melanella Sp. CKC-2011	JF750989	JF750955	---------	Churchill et al 2011
	Fusceulimoides kohtsukai	LC726229.1	LC726230.1	LC726231.1	Takano et al 2023
	Melanella martarum **sp. nov.**	**OP589975**	**OP575953**	**OP577852**	**This study**
Vanikoridae	*Vanikoro helicoidea*	AB930450	AB930421	AB930487	Takano & Kano 2014

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