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

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Eulimidae is a highly diverse family of gastropods that are often parasites of echinoderms. They are cosmopolitan and 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 trawl on the summit of seamount "Pearl" (Zhemchuznaya) in the Salas & Gómez Ridge (25.59°S, 89.13°W) 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 & 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 & Gómez ridge, a location distinguished by a high level of endemism.

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Melanella martarum sp. nov. (Gastropoda: Eulimidae):

2 the first parasitic deep-sea snail reported for the Salas

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Eulimidae is a highly diverse family of gastropods that are often parasites of echinoderms. They are cosmopolitan and 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 trawl on the summit of seamount "Pearl" (Zhemchuznaya) in the Salas & Gómez Ridge (25.59°S, 89.13°W) 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 & 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 & Gómez ridge, a location distinguished by a high level of endemism.

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





Introduction

- 80 Eulimidae Philippi, 1853 is a family of marine gastropods that encompass parasites of all extant
- 81 classes of Echinodermata, presenting a high species richness with diverse body plans and parasitic
- 82 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
- 84 estimate that there could be more than 4,000 species of Eulimidae. Other authors also highlighted
- 85 the underestimated diversity of this group. For example, Bouchet et al. (2002) based on a huge
- 86 sampling effort in New Caledonia estimated that 80% of the eulimids collected may represent
- 87 undescribed species.
- 88 The lack of knowledge about Eulimidae is not only related to the inventory of species but also to
- 89 the parasitic strategies of this group of snails. Some eulimids are strongly attached to their hosts
- 90 (i.e., tightly attached ectosymbionts, gall-forming species, and endoparasites) but also free-living
- 91 ectosymbionts (Dgebuadze et al., 2022). In the former case, these snails can detach from the host
- 92 more easily when disturbed during sampling with common benthic tools (nets, grabs, dredges),
- 93 this often makes it difficult to identify the hosts (Takano, Itoh & Kano, 2018; Takano, Kimura &
- 94 Kano, 2020).
- 95 The genus *Melanella* is one of the most species-rich of the family, with more than 200 recognized
- 96 species (MolluscaBase 2023). Melanella is known to live as an ecto- or endoparasite of
- 97 holothuroids in the orders Holothuriida (formerly Aspidochirotida) and Dendrochirotida (Warén
- 98 1983) and species can be host-specific or generalist (Crossland et al. 1993).
- 99 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
- 101 Island (Easter Is.) (Linse, 1999; Valdovinos, 1999; Osorio, 2023). Melanella is the most
- representative genus with four species: M. cumingii (A.Adams 1854), M. aciculata (Pease, 1861)
- and M. pisinna (Rehder 1980) (Rehder, 1980; Osorio, 2023) found 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
- 107 Exclusive Economic Zone (CEEZ) (mainly west of ~83°W) (Parin, Mironov & Nesis, 1997).
- 108 These expeditions represent only $\sim 3\%$ of the seamounts that make up both submarine ridges
- ranges. CIMAR 22 was the first multidisciplinary expedition to study the summit of several
- 110 seamounts of Salas & Gómez Island and Desventuradas Islands within the CEEZ (Tapia-Guerra
- et al., 2021). This new expedition has added new records and/or new species for science, mainly
- et al., 2021). 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 family Eulimidae 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
- 117 Science and Technology (JAMSTEC) oceanographic cruise, some specimens of this family were
- 118 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.

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Materials & Methods

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Material collection and sampling site

Samples were obtained by a modified Agassiz trawl with a mouth of 1.5 m \times 0.5 m (width \times height) 126 127 fitted with a net of 12 mm mesh at the cod end and operated in 10 min hauls (bottom contact) at 128 ~3 knots during the oceanographic EPIC cruise onboard the RV Mirai (JAMSTEC, Japan) in 2019. performed the 129 Sampling was on the summit of seamount (Zhemchuznaya, 25.59°S, 89.13°W), at 545 m depth, in SGR. The collected material was preserved 130 in 95% ethanol. Holotype and paratype specimens are housed in Museo Nacional de Historia 131 Natural (MNHNCL) and Sala de Colecciones Biológicas de la Universidad Católica del Norte 132 (SCBUCN), both in Chile (Table 1). Sample collection was performed under the permission of 133

134 Res.

Res. Ext N°3685/2016 from SUBPESCA (Chile) to Universidad Católica del Norte.

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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
- 140 genes, the pairs of primers H3F and H3R (Colgan, Ponder & Eggler, 2000), HCO-1490 and LCO-
- 141 2198 (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
- 144 72°C (13 min). A similar PCR profile was set for H3 and 16S rRNA (annealing at 55°C). The
- 145 resulting amplicons were visualized in agarose 1% gels and the PCR products were sent to
- 146 Macrogen, Inc. (Korea) for DNA Sanger sequencing.
- 147 The COI, 16S rRNA, and H3 sequences (forward and reverse) were visualized and assembled with
- 148 Geneious Prime 2022. 2.2 (Kearse et al., 2012). Fifteen H3 and 16S rRNA genes and fourteen COI
- 149 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
- 153 (Kearse et al., 2012), using the following settings: Nucleotide model = GTR GAMMA,
- 154 Algorithm=Rapid bootstrapping and search for best-scoring ML tree, bootstrap replicates = 1,000,
- 155 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
- 157 (Vanikoridae) were used as an outgroup.



- 158 To check whether the phylogenetic relationships of Melanella martarum sp. nov are consistent
- with the increase in species, 36 COI gene sequences of Eulimidae were extracted from NCBI
- 160 GenBank (Table S1, Supplementary Material) and were aligned with Melanella martarum sp.
- nov., using default MUSCLE (Edgard 2004) parameters. The resulting alignment was used to
- 162 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 I, Algorithm=Rapid bootstrapping and search for best-scoring
- ML tree, bootstrap replicates = 1,000, Partitioning= DNA, gene1codon1 = 1-630\3,
- DNA, gene1codon2 = $2-630\3$, DNA, gene1codon3 = $3-630\3$. The COI sequence of *Vanikoro*
- 167 *helicoidea* (Vanikoridae) AB930487 was used as an outgroup.

SEM images

- 170 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,
- 172 Coquimbo, Chile. The shell was dried in a Tousimis, Samdri-780A critical-point dryer using CO2,
- 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).

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176 Shell measurements

- 177 Shell measurements follow Souza and Pimenta (2019: fig. 1): SL: shell length; SW: shell width;
- BWL: ultimate whorl length; AL: aperture length; AW: aperture width; PCH: protoconch height.
- 179 The spire angle was measured through images of the shell in frontal view, with the vertex
- 180 centralized at the apex and pointing the arcs to the sutures at both sides of the ultimate whorl.

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

186 187

New Species Registration

- 188 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
- 190 Melanella martarum sp. nov. LSID: urn:lsid:zoobank.org:act:CC4C4BE5-4D7F-4EF3-8926-
- 191 9B960C01C3CA

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Results

194

195 Systematic account

- 196 Family EULIMIDAE Philippi, 1853
- 197 Genus Melanella Bowdich, 1822



198 Type species: *Melanella dufresnii* Bowdich, 1822, by monotypy.

- 200 Melanella martarum sp. nov.
- 201 Fig. 1A–H, Fig. 2A–C-K

Diagnosis: Shell medium in size (up to 8.1 mm long), polished, translucent white, conical, apex minute (Fig.1). Protoconch multispiral of about 4 whorls (Fig. 2), 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. Ultimate 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, spire angle 26°. Larval shell vitreous, about 4.0 convex whorls, 500 µm in height, 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 ultimate whorl height, rhomboid in shape, acute above, slightly rounded below; 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, spire angle 20°. 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 ultimate whorl height, pear-shaped, moderately expanded laterally, acute posteriorly, rounded and spread anteriorly; outer lip thickened, 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, 25.59°S,
 89.13°W, 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,
- 238 AW: 0.8 mm, Teleoconch whorls: 5; SCBUCN 8611-2, SL: 4.4 mm, SW: 1.8 mm, AL: 1.3 mm,
- 239 AW: 0.9 mm, Teleoconch whorls: 5; SCBUCN 8611-3, SL: 4.5 mm, SW: 2.0 mm, AL: 1.4 mm,
- 240 AW: 0.8 mm, Teleoconch whorls: 5; SCBUCN 8612-1, SL: 4.7 mm, SW: 2.1 mm, AL: 1.3 mm,
- 241 AW: 0.9 mm, Teleoconch whorls: 5; MNHNCL-205422, SL: 3.8 mm, SW: 1.7 mm, AL: 1.4
- 242 mm, AW: 0.7 mm, Teleoconch whorls: 4; SCBUCN-5482, SL: 8.1 mm, SW: 2.9 mm, AL: 2.2
- 243 mm, AW: 1.4 mm, Teleoconch whorls: 9.

- 245 Comparative material (examined through photographs): Melanella aciculata (Pease, 1861)
- 246 (Fig. 3): Lectotype NHMUK 1962839 (Fig. 3A) (designated by Kay, 1965), Sandwich Islands,
- 247 Hawaiian Archipelago; Paralectotypes NHMUK 1962840 (Fig.3C–F), 4 shells, from type locality;
- 248 Paralectotype MCZ 31705 (Fig. 3G–H), 1 shell, Sandwich Islands; Paralectotype MCZ 187747, 1
- shell, Hawaiian Islands. *Melanella acicula* (A. Gould, 1849): Syntype ANSP 19773 of *Eulima*
- 250 pisorum Pilsbry, 1917 [= M. acicula], 1 shell, Viti Islands, Fiji (see
- 251 http://clade.ansp.org/malacology/collections/details.php?mode=details&catalognumber=19773#d
- 250 Map. France and proof of the first of th
- etail). *Melanella micans* (P.P. Carpenter, 1865): Holotype USNM 14850 of *Eulima micans* P.P. Carpenter, 1865, San Pedro, California, USA; Holotype USNM 267304 of *Melanella mexicana*
- 254 Bartsch, 1917 (junior synonym)], Gulf Coast of Loewer California. *Polygireulima rutila* (P.P.
- 255 Carpenter, 1864): Holotype USNM 14828 of *Eulima rutila* P.P. Carpenter, 1864, Monterey,
- 233 Carpenter, 1804). Holotype Osivivi 14026 of Eutima Futta 1.1. Carpenter, 1804, Wolterey
- 256 California, USA (see http://n2t.net/ark:/65665/38a09710d-1739-4a50-af42-19db3ccb960b).

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Distribution, habitat, and parasitic strategy: Melanella martarum sp. nov. was found tightly

- attached to Oneirophanta cf. setigera (Ludwig, 1893) (Holothuroidea: Deimathidae) in general in
- the area near the base of the introvert (Fig. 4A). The Holothuroid host was found on a hard substrate
- 261 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 (25.59°S,89.13°W). This seamount is known as "Pearl".
- 263 Ectosymbiont.

264265

- Etymology: The species name honors Marta B. Parodi and Marta S. Wainstein, grandmother and
- 266 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
- 271 shallow water species M. aciculata (Fig. 3A-H), an ectoparasitic species known from the
- Hawaiian Archipelago, French Polynesia, and Rapa Nui (Osorio 2018, Osorio 2023). However,
- 273 the newly described species differs from M. aciculata by the straighter spire, a consequence of the
- 274 irregular position of the incremental scars (Bouchet and Warén 1986: 312) in *M. martarum* sp.
- 275 nov. The initial whorls of *M. aciculata* have a more regular position of the incremental scars,
- 276 giving a distorted appearance (Fig. 3G, H). Besides that, M. martarum sp. nov has an aperture



- more expanded laterally, a thinner inner lip and outer lip (Fig. 1C), and the adult of *M. martarum* sp. nov. has a sinuous outer lip (Fig. 1C–D), while the largest shells of *M. aciculata* have a straighter outer lip (Fig. 3A, C–F). The young holotype of *M. martarum* sp. nov. presents a spire angle of 26°, similar to the remaining young specimens (26-27°) and the adult types of *M. aciculata* (25°; n= 5, Fig. 3A–F). However, the adult specimen of *M. martarum* sp. nov. shows a more acute spire angle (20°), which is possibly related to a differentiation of increment of diameter during growth.
- Melanella martarum sp. nov. also resembles M. acicula, with a broad distribution in shallow 284 waters of the West Pacific, but the tip of the shell is much more acute in the newly described 285 species. Pilsbry (1917) highlighted the broad apex of Eulima pisorum Pilsbry, 1917, a junior 286 synonym of M. acicula, while comparing this species with M. aciculata. Our comparison with the 287 syntype of E. pisorum also reinforces this diagnostic feature. The spire angle of the syntype ANSP 288 19773 of E. pisorum, the one with images available (remaining syntypes catalogued as ANSP 289 355044) is about 22°, but this specimen is probably a juvenile, this value is close to the angle of 290 the adult specimen of M. martarum sp. nov. (20°) . 291
- 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 with Melanella persimilis (Kuroda & Habe, 1971), known from the upper slope of Japan (150–250 m) (Hori and Matsuda 2017: pl. 100, fig. 2), the flat outline of teleoconch whorls. However, the former is relatively smaller (13 whorls, 8.1 mm vs. 15 whorls, 20 mm), having a narrower, more pointed apex, more convex base, and laterally expanded aperture.
- Melanella martarum sp. nov. could be distinguished from M. micans, a shallow water species (up to 55 m) known from the Northeast Pacific (Abbott, 1974; McLean & Gosliner, 1996), mainly by the shape of the outer lip and expansion of the aperture. The former has a maximum projection of the outer lip in the middle height, while the latter has its maximum projection close to the distal area. The aperture of M. martarum sp. nov. is strongly expanded laterally in comparison to the almost straight profile of the outer lip of M. micans as seen in the frontal view. Besides that, the protoconch of M. martarum sp. nov. has more convex whorls than M. micans.
- Pacific and is known from depths up to 30 m (Hori and Matsuda 2017: pl. 98, fig. 9), and from *Melanella major* (G.B. Sowerby I, 1834), from Japan and Tropical West Pacific known from depths up to 10 m (Hori and Matsuda 2017: pl. 98, fig. 10), by being smaller (maximum length 8.1 mm *vs.* ~35 mm in *M. martinii* and *M. major*), with straight apical whorls whereas it is curved

Melanella martarum sp. nov. differs from Melanella martinii (A. Adams, 1854), from Indo-West

- 314 in the others, and with sutures not well-impressed which is deeply demarcated in the others.
- Besides that, *M. martarum* sp. nov. can be distinguished by the sinuous outer lip compared to the
- 316 more rectilinear outer lip of *M martini* and *M. major*.



335

- 317 Melanella martarum sp. nov. shares the almost flat teleoconch whorls with Haliella chilensis
- 318 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.
- 320 6), a common feature of *Haliella* Monterosato, 1878.
- 321 Melanella martarum sp. nov. resembles Polygireulima rutila (Carpenter, 1864), known from the
- northeastern Pacific occurring in depths up to 658 m (Bartsch, 1917; Abbott, 1974). These species
- 323 share the flat outline of teleoconch whorls, an elongated spire, and a similar spire angle. However,
- 324 the former has a slightly faster increase in diameter, the base is more convex but could be
- 325 distinguished mainly by the aperture more expanded laterally. The outer lip of *M. martarum* sp.
- 326 nov. advances earlier, just below the suture, reaching its maximum projection in the middle height.
- 327 In P. rutila, the outer lip starts in a straight profile and reaches its maximum projection below the
- middle of its height (see Vanatta 1899: figs. 5–6; images of holotype USNM 14828 of *P. rutila*:
- 329 http://n2t.net/ark:/65665/m3a68bd2aa-cc7a-4973-bdd5-83e4e8674a56). The largest specimen of
- 330 M. martarum (SCBUCN-5482) with a similar number of whorls (~13 whorls) to the holotype of
- P. rutila reaches a considerably larger dimensions (8.1 x 2.4 mm vs. 6.8 x 1.9 mm).
- 332 Holotype USNM 14828, 13 whorls, 6.8 x 1.9 mm

Phylogenetic relationship of *Melanella martarum* sp. nov.

We successfully sequenced COI, 16S rRNA, and H3 genes of a single specimen of M. martarum 336 sp. nov. The final alignment of the COI resulted in 630 bp, H3 of 314 bp, and the 16S rDNA of 337 419 bp, and these concatenated sequences produced a final alignment of 1,363 bp. COI presented 338 the greatest intragenus variability, with only an 81.9% similarity with the M. acicula sequence. 339 H3 and 16S rDNA presented an identity percentage of 97.0% and 91.4% with Melanella sp. CKC-340 2011, respectively. The identity for H3 and 16S was slightly lower with the *M. acicula* sequences 341 (96.3% and 91.9%), although within the expected percentage identity values between species of 342 343 the same genus. Consistent identity values between markers are shown in the phylogenetic reconstructions (Fig 5 and S1). Both the phylogenetic inferences calculated from the COI and with 344 the 3 concatenated markers showed low bootstraps value in the deepest nodes, not being able to 345 separate between different genera (Fig 5 and S1). Melanella martarum sp. nov. clustered with 346 347 other Melanella species identified in previous studies and was retrieved as the sister taxon to an unidentified Melanella (Fig 5), which was not illustrated in the original publication (Churchill, 348 Strong & Ó foighil, 2011), hampering further comparisons. In the phylogenetic reconstruction 349 carried out with COI (Fig S1), the sequences of the Melanella species do not present monophyly 350 (it should be noted that the species reported as *Balcis eburnea*, the accepted name is *Melanella* 351 eburnea Megerle von Mühlfeld 1824). However, the cluster formed between M. martarum and M. 352 acicula is maintained in both phylogenetic reconstructions (Fig 5 and S1). 353

Taking into account these results, the detailed phylogenetic relationship among congeners is premature, since the genus currently includes more than 200 species, and just a few sequences are

available for a species-rich group such as Eulimidae.



Discussion

The type series of *Melanella martarum* sp. nov. is represented by specimens at different growth stages, varying from specimens with four to nine teleoconch whorls and lengths between 3.76–8.14 mm. Figure 1A–K does not represent the specimens at the same scale to avoid discrepancies in size and allow a better observation of shell features in each individual. The young individuals have an angulated ultimate whorl and an aperture with a rhomboid shape (Fig. 1F–H), and the largest specimen (Fig. 1D–E) shows a more rounded outline in the base and aperture. These ontogenetic differences are reasonably common in eulimids (Lyons, 1978: 81; Bouchet and Warén, 1986: 310; Souza et al. 2018: 926). The shape and dimensions of the protoconch are quite similar in all specimens studied, and some of them were collected in the same host. The seven eulimids were collected on four holothuroids, but there is no register of the specific individuals from each host.

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). Another possible generic placement would be *Eulima* Risso, 1826, which is also a "catchall" genus (Bouchet and Warén, 1986; Hoffman and Freiwald, 2020). *Eulima* comprises species mainly with flat teleoconch whorls and elongated shape, as *M. martarum* sp. nov. However, *Eulima* s.s., considering the type species *Eulima glabra* (da Costa, 1778), has a colored shell with brownish spiral bands, a narrower aperture, and a more straight outer lip (Warén, 1989). Hosts known for *Eulima* species are ophiuroids (Bouchet and Warén, 1986).

Melanella acicula (A. Gould, 1849), with a close relationship based on present data, has a similar shell morphology following the features cited previously. 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, although in the phylogenetic reconstruction with the COI *Melanella* was polyphyletic. The lack of monophyly of *Melanella* calls into question the usefulness of the morphological characters used to describe the genus, which has been discussed previously (Bouchet & Waren, 1986, Souza & Pimenta, 2019).

Mollusca endemism is usually high on seamounts (Herrera et al, 2023). In the seamounts of the Nazca and Salas & Gomez ridges, 96% of the gastropod species of the family Turridae and 25% of the Septibranchia bivalves are endemic (Parin et al, 1997). Since *M. martarum* sp. nov is the first record of the Eulimidae; little is known about the rate of endemism of species of this family in Salas & Gomez Ridge. But, on the bathyal slopes of the Azorean seamounts, 38.6% of Eulimidae species (17 of 44) were found to be endemic (Hoffman & Freiwald, 2020).

 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, this species probably occurs in surrounding areas due to the possible planktotrophic development of *M. martarum* sp. nov., inferred by the multispiral, conical protoconch. 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 family Eulimidae 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 reported from 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 family Eulimidae at the species level.

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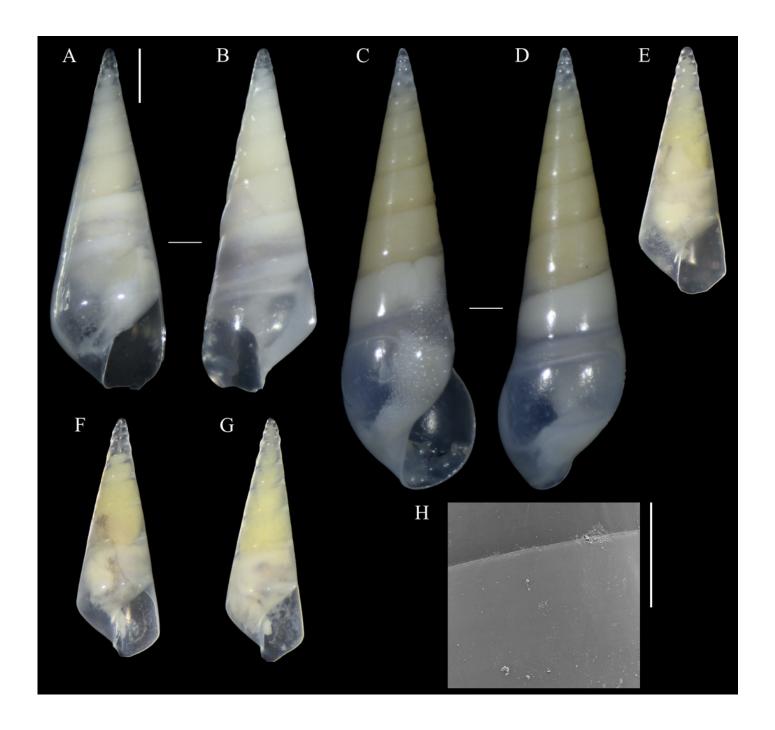
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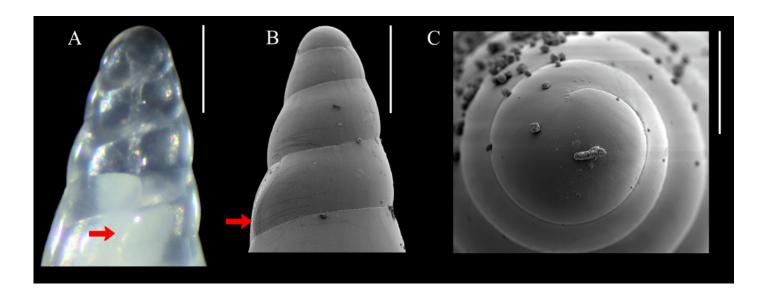
Melanella martarum sp. nov.

A, B. Holotype, SCBUCN 8542. C, D, H. Paratype, SCBUCN 5482. E-G. Paratypes, SCBUCN 8611. A, C, E-G. Frontal view. B, D. Dorsal view. H. Detail of teleoconch surface, close to suture. Scale bars: A-G=1 mm (entire shells at same scale); H=100 μ m.



Melanella martarum sp. nov.

A. Holotype, SCBUCN 8542, detail of protoconch, frontal view. B-C. Paratype, SCBUCN 5482, detail of protoconch, frontal and apical views, respectively. Arrows in A and B indicate transition protoconch-teleoconch. Scale bars: $A-B=200~\mu m$; $C=100~\mu m$.





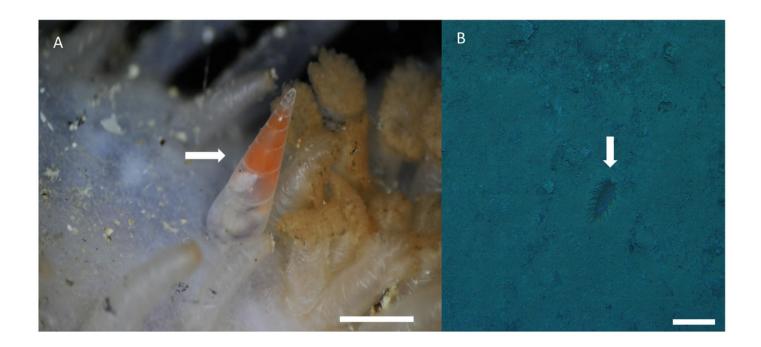
Melanella aciculata (Pease, 1861).

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



Melanella martarum sp. nov. in its holothurian 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 DeepTow from SPG5 seamount. Scale bar: 10 cm. The white arrow shows the host on a sandy substrate.





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 an outgroup.

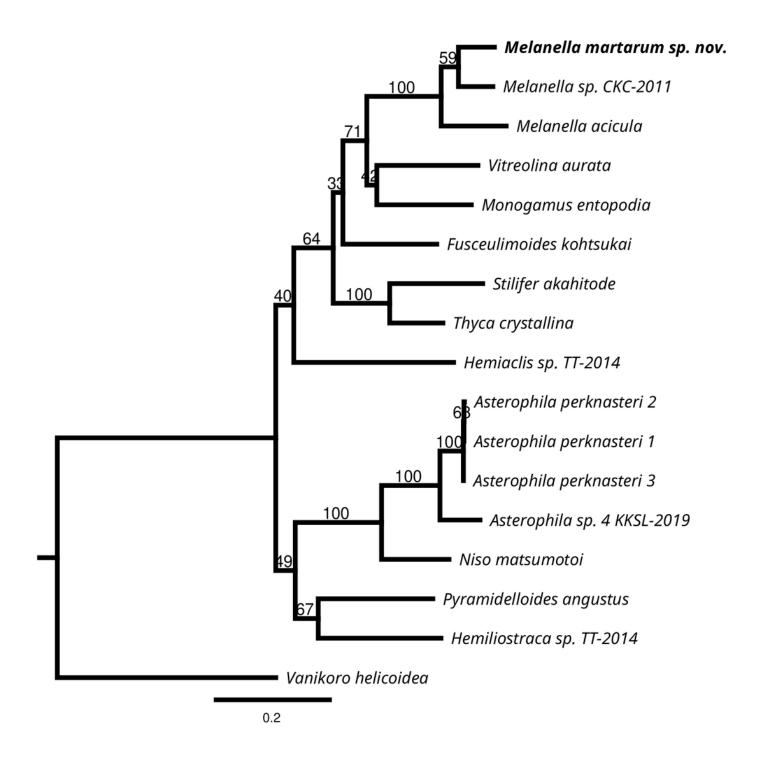




Table 1(on next page)

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



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.

Table 2: 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.

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