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1 Molecular phylogeny and biogeography of the living Pleurotomariidae (Mollusca: Gastropoda),
2 with the description of a new genus.

3

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5

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12

13 Running head: MOLECULAR PHYLOGENY OF LIVING PLEUROTOMARIIDAE

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15

ABSTRACT

16
17 The once diverse family Pleurotomariidae had a widespread global distribution spanning shallow
18 water faunas throughout the Mesozoic but is presently known only from bathyal habitats along
19 the western margins of the Atlantic and Indo-Pacific Oceans at temperate and tropical latitudes.
20 We evaluate the relationships among surviving lineages of Pleurotomariidae using partial
21 sequences of two mitochondrial genes and three nuclear genes for 22 of the 45 named Recent
22 species level taxa for which sequence data were available for two or more genes. Phylogenetic
23 analyses partition these species among five lineages, including the new genus *Bouchetitrochus*
24 that is described herein. Of the five lineages, two are present in both the western Atlantic and the
25 western Indo-Pacific Oceans, one is endemic to the western Atlantic and two are endemic to the
26 western Indo-Pacific.

27

INTRODUCTION

28
29 The family Pleurotomariidae, among the oldest surviving lineages within Gastropoda, was a
30 common component of the global shallow water fauna throughout the Mesozoic but was thought
31 to be extinct until the first living specimens were discovered at bathyal depths in the second half
32 of the 19th century (Fischer & Bernardi, 1856). The transition from continental shelf to bathyal
33 environments occurred during the late Paleogene (Jablonski *et al.*, 1983), possibly a consequence
34 of increased predation pressure in shallower waters (Vermeij, 1977). The increased sampling of
35 deep-sea habitats by commercial fisheries as well as by biological surveys has resulted in the
36 discoveries of additional pleurotomariid taxa. Forty-five extant species and subspecies,
37 partitioned among four genera, have thus far been named within the family Pleurotomariidae,
38 although not all are currently accepted (Molluscabase, 2023). Continued exploration of deep-sea
39 habitats will undoubtedly result in the discovery of additional species as well as range
40 expansions of presently known species.

41 To date, the majority of pleurotomariid species have been described primarily on the
42 basis of shell morphology, with descriptions published in the last decade including details of
43 anatomical features such as radular morphology as well as molecular data (usually partial
44 sequences of the mitochondrial cytochrome *c* oxidase I gene used by the Barcode of Life) (e.g.
45 Anseeuw *et al.*, 2015; Zhang, Zhang & Wei, 2016; Anseeuw, Bell & Harasewych, 2017). Over
46 the past several decades there have been a number of studies investigating the evolutionary
47 relationships among the basal taxa within Gastropoda using anatomical as well as molecular
48 data. Despite multiple efforts, the relationships of Pleurotomariidae have not yet been robustly
49 resolved. Most analyses based on morphological data (e.g. Haszprunar, 1988; Ponder &
50 Lindberg, 1996; Sasaki, 1998) and some based on molecular data (e.g., Cunha & Giribet, 2019;

51 Lee *et al.* 2019; Uribe, Sei & Harasewych, 2022) include Pleurotomariidae within the order
52 Vetigastropoda, while other molecular studies (e.g., McArthur & Harasewych, 2003, Aktipis &
53 Giribet, 2012; Uribe *et al.* 2022) place Pleurotomariidae outside of Vetigastropoda, or as an
54 outgroup to a paraphyletic Vetigastropoda.

55

56 In the present study, we evaluate the relationships among living Pleurotomariidae based
57 on selected individual exemplars of 22 of the 45 named species level taxa for which sequence
58 data are available for two or more of the five genes (two mitochondrial and three nuclear) that
59 have been regularly used alone or in combination in previous studies of gastropod phylogeny.
60 We evaluate the results in terms of the geographic distributions of the resulting clades and
61 describe one of the main clades as a new genus.

62

63 MATERIALS AND METHODS

64 *Sampling*

65 Most of the newly sequenced samples were collected during several expeditions organized
66 by the Muséum National d'Histoire Naturelle, Paris – MNHN (EBISCO, CONCALIS,
67 NORFOLK 2, TERRASSES, KANADEEP and KANACONO in New Caledonia, PAPUA
68 NIGINI, MADEEP and BIOPAPUA in Papua-New-Guinea, SALOMON 1 in the
69 Solomons, ZhongSha 2015 and NanHai 2014 in Taiwan, MAINBAZA and BIOMAGLO
70 in the Mozambique Channel, MIRIKY in Madagascar, GUYANE 2014 in French Guyana
71 and KARUBENTHOS 2 in Guadeloupe). Also sequenced were samples of
72 pleurotomariids in the collections of the National Museum of Natural History,
73 Smithsonian Institution (USNM) that were collected by the senior author during dives

74 aboard the research submersibles *R/S Johnson-Sea-Link I & II*, *R/S Clelia*, *R/S Nekton*
75 *Delta*, *R/S Idabel* and *R/S Curasub* throughout the tropical western Atlantic over a period
76 of three decades. Additional samples were provided by amateur malacologists. All
77 specimens included in the analysis together with outgroups (*Cyathernia naticoides* Warén
78 & Bouchet, 1989, Neomphalidae, Neomphaliones, and *Turbo castanea* Gmelin, 1791,
79 Turbinidae, Vetigastropoda) are summarized in Table 1.

80

81 *DNA sequencing*

82 Newly produced sequences were generated by two labs, using slightly different protocols: the
83 Service de Systématique Moléculaire (UMS2700), at the MNHN and the Laboratories of
84 Analytical Biology at the National Museum of Natural History in Washington DC (USNM).

85

86 In the MNHN, DNA was extracted from ethanol-preserved tissue samples using the
87 NucleoSpin 96 Tissue Core Kit (Macherey-Nagel), following the manufacturer's
88 recommendations, and in combination with the epMotion 5075 robot (Eppendorf). Fragments of
89 the mitochondrial cytochrome oxidase subunit I (COI) and 16S rRNA, and the nuclear 28S
90 rRNA and histone 3 (H3) genes were amplified using universal primers LCO1490/HCO2198
91 (Folmer *et al.*, 1994), 16SH/16SLC (Palumbi, 1996), C1/D2 (Jovelin & Justine, 2001; Dayrat *et*
92 *al.*, 2001) and H3F/H3R (Colgan, Ponder & Egglar, 2000), respectively. Amplification consisted
93 of an initial denaturation step at 94° C for 4 min, followed by 35/40 cycles of denaturation at 94°
94 C for 30 s, annealing at 48° C for COI, 55° C for 16S and 28S, and 53° C for H3 for 30 s,
95 followed by extension at 72° C for 1 min. The final extension was at 72° C for 5 min. PCR
96 products were purified and sequenced by the Eurofins sequencing facility.

97
98 At USNM, DNA was extracted from frozen or ethanol-preserved tissue samples (~25 mg
99 buccal muscle) using the DNeasy Tissue Kit (Qiagen) following the manufacturer's animal tissue
100 protocol. Fragments of the mitochondrial cytochrome oxidase subunit I (COI) and 16S rRNA,
101 and nuclear 18S rRNA were amplified using universal primers LCO1490/HCO2198 (Folmer *et*
102 *al.*, 1994), 16SH/16SLC (Palumbi, 1996), and 18S rRNA primers (Holland, Hacker & Williams,
103 1991; Harasewych *et al.*, 1997), respectively. PCR amplifications used the Promega GoTaq hot
104 start master mix (Promega M7132) according to manufacturer's instructions but modified to
105 reduce the reaction volume to 20 μ L. Cycling parameters: initial denaturation at 95° C for 3
106 minutes, followed by 45 cycles of denaturation at 94° C for 30 seconds, annealing at 48° C for
107 45 seconds and extension at 72° C for 2 minutes, with final extension at 72° C for 5 minutes.
108 Resulting PCR products were visualized by agarose gel electrophoresis (1.5% agarose), purified
109 with ExoSAP-IT (Affymetrix), and sequenced using the BigDye Terminator v3.1 Cycle
110 Sequencing Kit (Applied Biosystem). Reactions were purified using Millipore Sephadex plates
111 and sequenced on an ABI 3730XL automated DNA analyzer. Sequencher v. 4.7 (Gene Codes)
112 was used to visualize, trim, and assemble contigs from forward and reverse electropherograms.

113

114 All new sequences have been deposited in GenBank and/or BOLD (Barcode of
115 Life Datasystem) (Table 1). Additional sequences were downloaded from GenBank, as
116 noted in Table 1.

117

118 *Phylogenetic reconstruction*

119 Individual exemplars were selected to represent 22 species for which sequence data were
120 available for the highest number of genes to reduce the proportion of missing data (Table 1). In
121 some cases, chimeric sequences were produced by concatenating sequences for different genes
122 from different specimens of the same species. In these cases, the conspecificity of the samples
123 was confirmed morphologically, or by comparing COI sequences of the different specimens.
124 Sequences were automatically aligned using the Clustal algorithm in Bioedit v.7.0.5.3 (Hall,
125 1999). The five genes were combined into a single dataset of 3,026 bp (COI: 658 bp, 16S: 557
126 bp, 18S: 549 bp, 28S: 936 bp, H3: 326 bp).

127
128 Phylogenetic reconstructions were performed using RAxML (Maximum Likelihood – ML)
129 (Stamatakis, 2006) and MrBayes (Bayesian Inference – BI) (Huelsenbeck *et al.*, 2001). For the
130 ML analyses, robustness of the nodes was assessed using bootstrapping, with 1,000 iterations.
131 The BI consisted of 2 runs of eight Markov chains, each 30,000,000 generations long, with a
132 sampling frequency of one tree each 3,000 generations.
133 Each gene, and each codon position for the COI and H3 genes, was treated as an unlinked
134 partition, for a total of nine partitions. For the BI, each partition followed a GTR model, with a
135 gamma-distributed rate variation across sites approximated in four discrete categories and a
136 proportion of invariable sites. For the ML analysis, each partition followed a GTR model. For the
137 BI, convergence of each run was evaluated using Tracer 1.7 (Rambaut *et al.*, 2018) to check that
138 ESS values were all greater than 200. A consensus tree was then calculated after omitting the
139 first 25% of trees as burn-in.
140 All the analyses were performed on the Cipres Science Gateway (<http://www.phylo.org/portal2>),
141 using the tools MrBayes 3.2.3 on XSEDE and RAXML-HPC2 on XSEDE (8.1.11).

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RESULTS

Phylogenetic analyses of the concatenated dataset using BI and ML each produced a tree with identical topology and with most branches well supported (Fig. 1). The two *Entemnotrochus* P. Fischer, 1885 species, *E. adansonianus* (Crosse & Fischer, 1861) and *E. rumphii* (Schepman, 1879) form a highly supported clade [Bayesian posterior probability / ML bootstrap value / (PP/BS) = 1/100] that diverges from all remaining Pleurotomariidae (the “*Perotrochus* lineage”) that comprise a similarly well-supported clade (1/98). This “*Perotrochus* lineage” is composed of three highly supported clades. Seven Western Atlantic species in the genus *Perotrochus* P. Fischer, 1885 form a clade (1/88) that includes *Perotrochus quoyanus* (P. Fischer & Bernardi, 1856), the type species of *Perotrochus*. Five western Pacific species that had been originally described in the genus *Perotrochus* form another highly supported clade (1/100) for which the new genus *Bouchetitrochus* (type species *Bouchetitrochus pseudogranulosus* (Anseeuw, Puillandre, Utge & Bouchet, 2015)) is here proposed. The remaining pleurotomariids are included in a third well-supported clade (0.99/98) that includes *Bayerotrochus midas* (Bayer, 1965), the only western Atlantic species, *Bayerotrochus africanus* (Tomlin, 1848), from the western Indian Ocean, a well-supported clade (1/91) containing four western Pacific species of *Bayerotrochus* Harasewych, 2002, and a clade comprising three species of *Mikadotrochus* Lindholm, 1927 (1/98). Although the monophyly of each of these three clades is strongly supported, relationships among them in the five gene tree are not resolved with significant support.

DISCUSSION

165 Relationships among the genus level groups in the five gene tree are less well resolved than those
166 reported in several previously published single gene trees (e.g., Harasewych *et al.*, 1997;
167 Harasewych, 2002; Anseeuw *et al.* 2015; 2017; Zhang *et al.*, 2016) although the number of
168 species-level taxa and relationships among them may vary in some instances. *Perotrochus*
169 *pyramus* Bayer, 1967, originally described in the genus *Perotrochus*, had been transferred to
170 *Bayerotrochus* (Harasewych, 2002) but appears as member of *Perotrochus* in our tree. The
171 species *Perotrochus gotoi* Anseeuw, 1990, had been transferred to *Mikadotrochus*
172 (Molluscabase, 2023, based on Anseeuw (2010)), but emerges as a member of the new genus
173 *Bouchetitrochus* in this study, as do the species originally proposed as *Perotrochus caledonicus*
174 Bouchet & Métivier, 1982, *Perotrochus deforgesi* Métivier, 1990, *Perotrochus wareni* Anseeuw
175 *et al.*, 2015 and *Perotrochus pseudogranulosus* Anseeuw *et al.*, 2015. *Perotrochus vicdani*
176 Kosuge, 1980, is here shown to be referable to the genus *Mikadotrochus* with strong support.
177 Generic assignments of the remaining species in our tree are concordant with those reported in
178 Molluscabase (2023). The genus *Bayerotrochus* is not recovered as monophyletic in our five-
179 gene tree because it includes *Mikadotrochus*. However, *Bayerotrochus* and *Mikadotrochus* were
180 previously recovered as sister taxa with strong support in published trees based on partial COI
181 gene sequences (e.g., Anseeuw *et al.*, 2015, 2017). Additional data to complete the five gene data
182 matrix may improve resolution of these nodes in the five gene tree.

183

184 The deepest divergence among the living Pleurotomariidae is between the genus
185 *Entemnotrochus* (Fig. 2A), easily recognized by a large, conical shell with a long, narrow slit
186 spanning approximately half a whorl, and a wide, deep umbilicus, and all remaining living
187 species (here referred to as the “*Perotrochus* lineage”, Figs 2B-E), which have smaller shells

188 with more rounded whorls, a broader, shorter slit that spans less than a quarter of a whorl and a
189 nacreous callus along the columella rather than an wide, open umbilicus.

190

191 The lineages leading to *Entemnotrochus* and *Perotrochus* are reported to have diverged
192 during the Middle Triassic (Szabó, 1980) or Lower Jurassic (Benfrika, 1984) (see Harasewych,
193 2002: fig. 9). Bose, Das & Mondal (2021: Appendix 1) compiled a list of 149 species of
194 Cenozoic Pleurotomariidae and apportioned them among seven genera, noting that a number of
195 species that had originally been described as *Pleurotomaria* DeFrance, 1826 could not be
196 accurately assigned to genera due to poor preservation. Of the seven Cenozoic genera of
197 Pleurotomariidae, *Conotomaria* Cox, 1959 became extinct at the end of the Paleocene, while
198 *Chelotia* Fischer, 1885 and *Leptomaria* Eudes-Deslongchamps, 1864 became extinct at the end
199 of the Eocene. Their study shows that the genera *Entemnotrochus* and *Perotrochus* both
200 appeared in the early Eocene, *Mikadotrochus* dates to the Middle Miocene, and *Bayerotrochus* is
201 recorded only from the Recent fauna. Both *Entemnotrochus* and *Perotrochus* had wide
202 distributions in the early Cenozoic, but the closure of the Tethys Sea during the mid-Miocene
203 blocked global equatorial currents resulting in the isolation of the Atlantic from the Indo-West
204 Pacific faunas (Hou & Li, 2017). This resulted in a vicariant division of pleurotomariid faunas
205 into those inhabiting the western margins of the Atlantic Ocean at temperate and tropical
206 latitudes from those along the western margins of the Indian and Pacific Oceans at similar
207 latitudes.

208

209

210 The genus *Entemnotrochus* was widespread and diverse during the Paleogene throughout
211 and beyond the Neo-Tethys Sea, with a dozen species ranging from the west coast of the United
212 States, throughout Europe and India to Japan and Taiwan (Bose *et al.*, 2021), yet only a single
213 Recent species *E. rumphii* is known to occur in the western Pacific Ocean, ranging from central
214 Japan southward through the Philippines, to Indonesia (Fig. 3A). Another species, *E.*
215 *adansonianus* (with two subspecies), occurs in the western Atlantic, ranging from Bermuda
216 southward throughout the Florida Keys and Bahamas, into the Gulf of Mexico and throughout
217 the Caribbean to Maranhao, Brazil (Rosenberg, 2009).

218
219 Over forty species attributed to the genus *Perotrochus* based on shell morphology were also
220 widespread during the Cenozoic. However, molecular data discern two distinct clades among the
221 Recent members of the *Perotrochus* phenotype. The clade that is endemic to the western Atlantic
222 (Fig. 3B) retains the name *Perotrochus* as it includes the type species of the genus (Fig. 2B). The
223 clade inhabiting the eastern part of the range (Fig. 3B), which is named *Bouchetitrochus* herein
224 (Fig. 2C), is presently known only from the vicinity of New Caledonia to the Central Philippines
225 and southern Japan. The genus *Bayerotrochus* (Fig. 2D, Fig. 3C) the most diverse and wide
226 ranging pleurotomariid genus in the Indo-West Pacific region, includes a dozen species ranging
227 from Japan southward to New Zealand and westward to South Africa. Although this genus has
228 not been reported from fossil records (Bose *et al.*, 2021), the presence of a single species of
229 *Bayerotrochus* in the western Atlantic fauna indicates that this genus originated prior to the
230 closing of the Neo-Tethys Seaway. Earliest records of the genus *Mikadotrochus* (Fig. 2E), a
231 clade that is the sister group to the Indo-West Pacific species of *Bayerotrochus* and ranges from

232 Japan to the Philippines and the South China Sea (Fig. 3D), are from the Lower Miocene,
233 suggesting that it likely diverged after the closing of the Neo-Tethys Sea.

234
235 In the western Atlantic, pleurotomariid genera stratify bathymetrically (Fig. 4), while
236 species segregate geographically, so that species occur in allopatry (Harasewych, 2002: fig.
237 12B). The single species of *Entemnotrochus* has the broadest geographic range and occurs at the
238 shallowest depths (100–350 m). The genus *Perotrochus*, with multiple species, occurs at greater
239 depths (200–550 m) than *Entemnotrochus*, and spans a similar range, from Bermuda to
240 southernmost Brazil. Greatest species diversity is on the Caribbean tectonic plate, believed to be
241 a section of Pacific Ocean floor that has drifted into the Atlantic since the Cretaceous (Malfait &
242 Dinelman, 1972), although some alternative hypotheses suggest that the Caribbean evolved in
243 place (James, Lorente & Pindel, 2009). Two species inhabiting the coast along the United States
244 form a separate clade that is sister group to the clade inhabiting the Caribbean. When it becomes
245 available, sequence data for *Perotrochus atlanticus* Rios & Matthews, 1968, which occurs along
246 the coast of Brazil, will reveal if it is more closely related to its congeners from the southern
247 Caribbean, or to the species from the coast of North America from which it may have been
248 isolated by the intrusion of the Caribbean plate. Only a single species of *Bayerotrochus* is
249 currently recognized in the western Atlantic fauna. Much of its geographic range overlaps with
250 those of *Entemnotrochus* and *Perotrochus*, although it occurs at greater depths (500–800 m) than
251 *Perotrochus*. However, it has not been reported from Bermuda, the Gulf of Mexico, the southern
252 coast of the Caribbean Sea or off the coast of Brazil. This may be due to the absence of
253 submersible observations or collections at appropriate depths in these areas.

254

255 Information on bathymetric distributions of Indo-West Pacific pleurotomariids is more
256 generalized (Fig. 4), as most records were collected by dredging or trawling and report depths
257 within a certain range. The depths reported for *Entemnotrochus rumphii* and various species of
258 *Mikadotrochus* largely overlap, as do depth ranges for many species *Bayerotrochus* and
259 *Bouchetitrochus* (Harasewych, 2002: fig. 12A). However, *Bayerotrochus* and *Bouchetitrochus*
260 tend to occur at somewhat greater depths than *Entemnotrochus* and *Mikadotrochus*, especially in
261 regions where their geographic distributions overlap.

262
263 Additional genetic data for species level taxa not represented in our tree will no doubt
264 enhance our understanding of patterns of evolution of pleurotomariids both geographically and
265 throughout the Cenozoic, as will more detailed population level studies of relationships of
266 congeneric Recent species. Development and refinement of methodologies to analyze characters
267 of shell morphology in order to allocate fossil species into lineages that are concordant with
268 patterns supported by molecular data is another avenue of promising research.

269

270 SYSTEMATICS

271 Class Gastropoda Cuvier, 1795

272 Order Vetigastropoda Salvini-Plawen, 1980

273 Family Pleurotomariidae Swainson, 1840

274 Genus *Bouchetitrochus* New Genus

275 Type Species: *Perotrochus pseudogranulosus* Anseeuw, Puillandre, Utge & Bouchet, 2015.

276

277 *Description:* Shell of moderate size, with basal diameter 35–40 mm, rarely reaching over 55 mm.
278 Shell shape trochoidal, depressed to conical, with solid shell construction for the small size.
279 Slit narrow, forming a selenizone situated at or just below mid-whorl. Shell thin but solid
280 (*Bouchetirochus caledonicus*, *B. pseudogranulosus*), with thicker prismatic layer in some
281 species (*B. wareni*, *B. gotoi*). Surface sculpture with pronounced spiral cords that may appear
282 beaded at intersections with axial ribs. A microsculptural pattern of fine radiating threads is
283 visible between the dominant ribs in some species (*B. pseudogranulosus*). No significant
284 differences in dominant sculpture on surface areas above and below the selenizone. Color
285 mottled brownish-red with a tendency to crimson patches, producing a checkerboard pattern that
286 is more intense below the selenizone. Basal disc slightly convex to flattened, sculptured by well
287 impressed, straight to heavily beaded spiral cords. Aperture horizontally oblong. Nacreous layer
288 visible along the inner slit lips within the aperture, which may be either completely or only
289 partially covered (*B. gotoi*, *B. wareni*), depending on the thickness of the prismatic layer.
290 Operculum small, very small to absent.

291

292 *Etymology:* Named in honor of Prof. Philippe Bouchet, who organized many expeditions
293 throughout the Indo-Pacific, resulting in the discovery of many new species of Pleurotomariidae,
294 including several of the species included in *Bouchetirochus*.

295

296 *Remarks:* Members of the genera *Perotrochus* and *Bouchetirochus* are readily distinguished
297 with significant support on the basis of their DNA sequences in single gene (e.g., Anseeuw *et al.*
298 2015; 2017; Zhang *et al.*, 2016) as well as in multiple gene phylogenetic analyses. However,
299 both genera appear to share a plesiomorphic shell morphology that results in a lack of readily

300 recognizable shell characteristics capable of differentiating between them. When compared to
301 *Perotrochus quoyanus*, the type species of *Perotrochus*, the species of *Bouchetitrochus* share
302 many similarities in gross morphology that have lead authors to initially assign these Indo-
303 Pacific species to the genus *Perotrochus*. Morphological similarities shared by both genera
304 include: small shell diameters, small, thin opercula, lack of an open umbilicus, presence of a
305 callus pad, a broadly conical shell shape, weakly concave, relatively short slits (1/6th of basal
306 diameter) and a dominant spiral sculpture and weak axial cords, producing only weakly
307 cancellated spiral cords.

308

309 A recent analysis of the relationships among Cenozoic species of pleurotomariids (Bose
310 *et al.*, 2021: fig. 4) using multivariate analyses of 15 shell characters included multiple Recent
311 species of *Perotrochus* and *Bouchetitrochus* but was not able to differentiate these genera.
312 Positions of species of both genera overlapped in plots of principal component morphospace
313 (both in PC1 vs. PC2 and PC2 vs. PC3 plots) (Das, personal communication).

314

315 *Bouchetitrochus pseudogranulosus*, the type species, differs from the other four species
316 included in this new genus, by its more regular conical shell, more flattened basal disc profile,
317 more lustrous shell surface, a slightly longer slit length, a more intense color pattern and
318 microsculpture of fine radiating threads on the surface of the whorls.

319

320 Included species: *Bouchetitrochus pseudogranulosus*, *B. caledonicus*, *B. deforgesi*, *B. gotoi*, *B.*
321 *wareni*.

322

323 *Geographic distribution:* The distributions of the five species of *Bouchetitrochus* are limited to
324 the western Pacific Ocean. Four species (*B. caledonicus*, *B. wareni*, *B. deforgesii* and *B.*
325 *pseudogranulosus*) are restricted to areas of the New Caledonian plateau and adjacent Coral Sea,
326 where they each occur at depths ranging from 180–600 m. One species (*B. gotoi*) has a broader
327 distribution, extending from the southern Philippines towards Indonesia and further eastwards
328 extending in NW Pacific waters towards Japan, occurring at depths of 200–360 m.

329

330

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332

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337 ([dx.doi.org/10.17600/8100010](https://doi.org/10.17600/8100010)), NORFOLK 2 ([dx.doi.org/10.17600/3100030](https://doi.org/10.17600/3100030)), TERRASSES
338 ([dx.doi.org/10.17600/8100100](https://doi.org/10.17600/8100100)), KANADEEP ([dx.doi.org/10.17600/17003800](https://doi.org/10.17600/17003800)) and
339 KANACONO ([dx.doi.org/10.17600/16003900](https://doi.org/10.17600/16003900)) in New Caledonia, PAPUA NIGINI
340 ([dx.doi.org/10.17600/18000841](https://doi.org/10.17600/18000841)), MADEEP ([dx.doi.org/10.17600/14004000](https://doi.org/10.17600/14004000)) and BIOPAPUA
341 ([dx.doi.org/10.17600/10100040](https://doi.org/10.17600/10100040)) in Papua-New-Guinea, SALOMON 1
342 ([dx.doi.org/10.17600/1100090](https://doi.org/10.17600/1100090)) in the Solomons, ZhongSha 2015 and NanHai 2014 in Taiwan,
343 MAINBAZA and BIOMAGLO ([dx.doi.org/10.17600/17004000](https://doi.org/10.17600/17004000)) in the Mozambique Channel,
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484 FIGURE CAPTIONS

485 **Figure 1.** Phylogenetic tree obtained by Bayesian Inference based on the five-gene dataset. The
486 posterior probabilities (when > 0.95) and the bootstrap support (when > 80) are provided for
487 each node.

488

489 **Figure 2.** Type species of the genera of living Pleurotomariidae. A. *Entemnotrochus*
490 *adansonianus*, Andros Island, Bahamas. B. *Perotrochus quoyanus* Roatan, Honduras. C.
491 *Bouchetitrochus pseudogranulosus*, Coral Sea, New Caledonia. D. *Bayerotrochus midas* Roatan,
492 Honduras. E. *Mikadotrochus beyrichii*, Japan. Scale bars = 5 cm for adjacent species. All
493 specimens from the Patrick Anseeuw collection.

494

495 **Figure 3.** Maps showing the geographic distribution of living species within each of the five
496 genera of living Pleurotomariidae. A. *Entemnotrochus*. B. *Perotrochus* and *Bouchetitrochus*
497 new genus. C. *Bayerotrochus*. D. *Mikadotrochus*.

498

499 **Figure 4.** Distribution of living species of Pleurotomariidae by depth based on museum and
500 literature records.

501

Table 1. List of taxa and genes used in phylogenetic analyses. GenBank sequences in **BOLDFACE** are first reported in this study.

TAXON	VOUCHER	LOCATION	DEPTH (M)	NO. OF GENES	GENBANK ACCESSION NUMBERS					BOLD ID NO..
					COI	16S	18S	28S	HISTONE	
<i>Cyathernia naticoides</i> Warén & Bouchet, 1989	MCZ:Mala:378348	East Pacific Rise		5†	DQ093518	DQ093472	AY923888	FJ977685	DQ093498	
<i>Turbo castanea</i> Gmelin, 1791	MCZ:Mala:378777	Abaco, Bahamas		5†	FJ977763	FJ977713	FJ977650	AM404008	FJ977742	
<i>Entemnotrochus adansonianus</i> (Crosse & Fischer, 1861)	MNHN-IM-2013-61163	Guadeloupe	210-240	5†	OQ075929	OQ075937	ON563470	OQ075949	OQ075959	PLERO065-22
<i>Entemnotrochus rumphii</i> (Schepman, 1879)	USNM 888698	Amami-O-Shima, Japan	120	2	L78911		L78889			
<i>Perotrochus pyramus</i> Bayer, 1967	MNHN-IM-2013-60460	Guadeloupe	432-482	4	OQ075928	OQ075936		OQ075947	OQ075958	PLERO064-22
<i>Perotrochus charlestonensis</i> Askew, 1988	USNM 1080831	Marathon, FL, USA	180-299	3	MK559363*	MK559363*	ON563483			
<i>Perotrochus amabilis</i> (Bayer, 1963)	USNM 1080832	Marathon, FL, USA	178-181	3†	KY432519		ON563481		AY923965	
<i>Perotrochus maureri</i> Harasewych & Askew, 1993	USNM 869919	Charleston, SC, USA	200-237	2	L78914		L78892			
<i>Perotrochus lucaya</i> Bayer, 1965	USNM 888619	San Salvador, Bahamas	520	3†	L78916	KY671216	L78891			
<i>Perotrochus quoyanus</i> (P. Fischer & Bernardi, 1856)	MNHN-IM-2013-60292	Guadeloupe	323-347	5†	OQ075933	OQ075943	L78890	OQ075955	OQ075966	PLERO066-22
<i>Perotrochus sunderlandorum</i> (Petuch & Berschauer, 2017)	USNM 1153967	Curaçao	168	2	ON586603	KY671222				
<i>Bouchetitrochus pseudogranulosus</i> (Anseeuw, Puillandre, Utge & Bouchet, 2015)	MNHN-IM-2009-7495	Chesterfield Islands	348-354	4	KR087196	OQ075942		OQ075954	OQ075965	PLERO033-15
<i>Bouchetitrochus gotoi</i>	USNM 894500	Amami-O-Shima, Japan	180-200	2	KY432520	KY671205				
<i>Bouchetitrochus caledonicus</i> (Bouchet & Métivier, 1982)	MNHN-IM-2007-36302	New Caledonia	270	4	KR087189	OQ075941		OQ075953	OQ075963	PLERO041-15
<i>Bouchetitrochus deforgesii</i> (Métivier, 1990)	MNHN-IM-2007-32086	Chesterfield Islands	300-323	4†	OQ075934	OQ075945		OQ075948	OQ075968	PLERO062-22
<i>Bouchetitrochus wareni</i> (Anseeuw, Puillandre, Utge & Bouchet, 2015)	MNHN-IM-2007-34685	New Caledonia	380-430	4	KR087222	OQ075938		OQ075950	OQ075960	PLERO020-15
<i>Bayerotrochus midas</i> (Bayer, 1965)	SBMNH uncat. tissue			5†	AY923930	KY671212	AY923892	FJ977668	AY923966	
<i>Bayerotrochus africanus</i> (Tomlin, 1848)	MNHN-IM-2007-38002	Madagascar	244-300	4	OQ075930	OQ075939		OQ075951	OQ075951	PLERO063-22
<i>Bayerotrochus belauensis</i> Anseeuw, Bell & Harasewych, 2017	USNM 905393	Palau	212	2	KY432527	KY671232				
<i>Bayerotrochus. boucheti boucheti</i> (Anseeuw & Poppe, 2001)	MNHN-IM-2007-34676	New Caledonia	450-495	5	KU693174 = KY432525	OQ075944	ON563471	OQ075956	OQ075967	PLERO060-15
<i>Bayerotrochus. boucheti mirificus</i> Anseeuw, 2016	MNHN-IM-2009-7484	Chesterfield Islands	543-544	4	KU693173	OQ075935		OQ075946	OQ075957	PLERO061-15
<i>Mikadotrochus vicdani</i> (Kosuge, 1980)	Toba Aquarium	Bohol, Philippines		4	OQ075931	OQ075940		OQ075952	OQ075962	PLERO068-22

<i>Mikadotrochus beyrichi</i> (Hilgendorf, 1877)	Not available	Boso Peninsula, Japan		3	AM049331		AM048636	AM048695		
<i>Mikadotrochus hirasei</i> (Pilsbry, 1903)	MNHN-IM-2013-43545	Japan	150	3†	OQ075932		AF417117		OQ075964	PLERO067-22

*Appropriate gene portion taken from complete mitochondrial genome sequence.

† *Cyathermia naticoides* is a composite using an 18S sequence from a different specimen.

† *Turbo castanea* is a composite using a 28S sequence from a different specimen.

† *Entemnotrochus adansonianus* is a composite using an 18S sequence from a specimen from Marathon, FL, USA (voucher = USNM 1080839)

† *Perotrochus amabilis* is a composite using a Histone sequence from a specimen from Louisiana (voucher = LACM152930)

† *Perotrochus lucaya* is a composite using a 16S sequence from a specimen from Grand Bahama Island, Bahamas (voucher = USNM 880236)

† *Perotrochus quoyanus* is a composite using an 18S sequence from a specimen from Guadeloupe (voucher = USNM 888646)

† *Bouchetitrochus deforgesi* is a composite using a 28S sequence from a different specimen (MNHN-IM-2009-7486).

† *Bayerotrochus midas* is a composite using a 28S sequence is from MCZ:Mala:378259 and a 16S sequence is from a specimen from Roatan , Honduras DNR.

† *Mikadotrochus hirasei* is a composite using an 18S sequence from a different specimen, also from Japan.







