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Research Article

Molecular and morphological analyses reveal pseudocryptic diversity in *Micromelo undatus* (Bruguière, 1792) (Gastropoda: Heterobranchia: Aplustridae)

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The genus *Micromelo* (family Aplustridae) occurs in almost all tropical and subtropical waters across the globe, with the exception of the Eastern Pacific. Most authors consider *Micromelo undatus* (Bruguière, 1792) as the only valid species in this genus. This study examines populations of specimens identified as *M. undatus* across its geographic range, using morphological and genetic data from two mitochondrial genes (16S and CO1) and one nuclear gene (Histone H3). The results reveal that *M. undatus* is a complex of four species with consistent genetic and anatomical differences. A literature review and evaluation of type material indicates that available names exist for three of the four species. *Micromelo guamensis*, *M. undatus* and *M. scriptus* are resurrected for species found in the Western Pacific, the Atlantic Ocean, and widespread in the Indo-Pacific, respectively. A new name is introduced for another species found in the Western Pacific, namely *Micromelo barbarae* sp. nov. and a neotype is designated for the type species of the genus *M. undatus*.

<http://zoobank.org/urn:lsid:zoobank.org:act:02091D97-0AE1-4AE4-9910-726C4F0ABE2F>

Key words: Atlantic, Gastropoda, indo-west pacific, marine biodiversity, Mollusca, phylogeny, systematics

Introduction

Several lineages of heterobranch sea slugs contain multiple morphological conserved taxa — often pantropical — found across ocean basins. These include sea hares (*e. g.*, the *Dolabrifera dolabrifera* (Rang, 1828), *Bursatella leachii* Blainville, 1817, and *Aplysia parvula* Mörch, 1863 species complexes; Valdés et al., 2018; Golestani et al., 2019; Bazzicalupo et al., 2020) but also sacoglossans (*e. g.*, the *Elysia ornata* (Swainson, 1840) species complex; Krug et al., 2013;), cephalaspideans (*e. g.*, the *Chelidonura hirundinina* (Quoy & Gaimard, 1833) species complex; Zamora-Silva & Malaquias, 2018), and nudibranchs (*e. g.*, *Chromodoris quadricolor* (Rüppell & Leuckart, 1830) group; Tibiriçá et al., 2020). Due to the difficulties taxonomists have

encountered identifying morphological differences between individuals from different ocean basins, most of these taxa have been — and some still are — regarded as widely distributed species with disjunct ranges. However, the breakup of the Tethys Sea, the closure of the Isthmus of Panama and the interruption of east-west communication across tropical and temperate oceans have resulted in a disruption of gene flow and subsequent speciation in different ocean basins, making the existence of pantropical marine invertebrate species unlikely (Hrbek & Meyer, 2003; Malaquias & Reid, 2009; Nikulina et al., 2007).

In this paper we examined another example of a widely distributed species in the tropics *Micromelo undatus* (Bruguière, 1792). This species belongs to the severely understudied shelled sea slug family Aplustridae Gray, 1847 with seven valid genera distributed mostly across tropical and sub-tropical regions in shallow subtidal soft bottoms and apparently specialized

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Fig. 1. Estimated distribution map of *Micromelo* (based on references listed in this paper), with the localities where the specimens examined or sequenced were collected.

on the predation of polychaete worms (Burn & Thompson, 1998; MolluscaBase. (Ed), 2021). The colorful and endearing species *M. undatus* is generally found at depths of 0–5 meters in warm, tropical and subtropical waters across the globe. According to the most recent authoritative accounts, *Micromelo undatus* is regarded as a single, pantropical species with populations in the Caribbean, Atlantic and the entire tropical Indo-Pacific (from Mozambique to the Hawaiian Is.), but is notoriously absent from the Eastern Pacific (Fig. 1; Tibiriçá & Malaquias, 2017; Gosliner et al., 2018). Other authors have used the name *M. guamensis* (Quoy & Gaimard, 1825) to refer to a supposedly distinct Indo-Pacific species (Kay, 1976; Rudman, 1972), but no comprehensive studies have been conducted to estimate the species diversity within this group. Although several species have been described from different localities (Table 1), no dedicated studies have been conducted to estimate the species diversity within *Micromelo*, and all species names have been synonymized with *Micromelo undatus* by Rudman (1972, 1998).

The known variation in colour pattern of *Micromelo undatus* is not necessarily correlated with the geographic origin of the specimens. For example, in Indo-Pacific animals the body margin can be yellow, blue, green or vibrant blue, with opaque white spots being either large and dense or smaller and more evenly distributed (Fig. 2). The shell ranges from having fewer thick, bold red lines to numerous thinner pinkish lines. Evidence of genetic structure and lineage divergence for many groups lacking consistent distinguishing morphological traits as in *Micromelo* has revealed the presence of cryptic and pseudocryptic species (Dawson & Jacobs, 2001; Funk et al., 2012; Herbert, 1998; Matsuda & Gosliner,

2018; McCarthy et al., 2019). Cryptic species refers to two or more genetically distinct lineages that are morphologically indistinguishable (Saez et al., 2003). Pseudocryptis refers to taxa previously thought to be one species but that in fact show molecular differences and when looked upon closely also subtle morpho-anatomical differences (Knowlton et al., 1993; Korshunova et al., 2019; Sørensen et al., 2020). The colour differences between specimens of *M. undatus* may indicate the presence of multiple cryptic or pseudocryptic species, but without genetic analysis, it is unclear whether this variability is interspecific or intraspecific.

The goal of this study is to examine specimens from across the range of *Micromelo* and determine if *M. undatus* is truly a circumtropical species or whether it hides undescribed cryptic diversity using molecular and morphological data. An integrative approach combining DNA sequence data, phylogenetics and morphological characters is used to test the hypothesis that populations from distinct ocean realms have become geographically isolated and allopatric speciation has occurred leading to several species across the world.

Material and methods

Source of specimens

A total of 46 specimens previously identified as *Micromelo undatus* were obtained via SCUBA diving or collected at low tide at multiple localities across the species range (Fig. 1). Newly collected specimens are deposited at the Cal Poly Pomona Invertebrate Collection (CPIC), the California Academy of Sciences, San Francisco (CASIZ), the National Museum

Table 1. List of nominal species names in the genus *Micromelo* with type locality information and taxonomic status.

Name	Type locality	Status	References
<i>M. undatus</i> (Bruguière, 1792)	Discovery Bay, Jamaica	Valid	Rudman (1998), present study
<i>M. nitidulus</i> (Dillwyn [ex. Solander], 1817)	West Indies	Synonym of <i>M. undatus</i>	Rudman (1972), present study
<i>M. guamensis</i> (Quoy and Gaimard, 1825)	Agana, Guam	Valid	Kay (1976), present study
<i>M. elegans</i> (Menke, 1828)	Puerto Rico	Synonym of <i>M. undatus</i>	Rudman (1972), present study
<i>M. ferussaci</i> (Catlow & Reeve, 1845)	Agana, Guam	Synonym of <i>M. guamensis</i>	Present study
<i>M. scriptus</i> (Garrett, 1857)	Hilo, Hawaii	Valid	Present study
<i>M. eximius</i> (Deshayes, 1863)	Réunion Is.	Synonym of <i>M. scriptus</i>	Present study
<i>M. barbarae</i> sp. nov.	Batangas, Luzon, Philippines	Valid	Present study

(Philippines) (NMP), and the University Museum of Bergen (ZMBN). Additional specimens or tissue samples were borrowed from several institutions, including the Natural History Museum of Los Angeles County (LACM), the Bavarian State Collection of Zoology, Munich (ZSM), the California Academy of Sciences, San Francisco (CASIZ), and the Muséum National d'Histoire Naturelle, Paris (MNHN) (Table 2). All specimens were preserved in 70–95% ethanol. Moreover, 11 sequences obtained from GenBank were included in this study.

DNA extraction, amplification and purification

DNA was successfully extracted from thirty-seven ethanol-preserved specimens using an E.Z.N.A. mollusc extraction kit (Omega Bio-Tek, Doraville, U.S.A.) following manufacturer protocols, with the exception of increasing lysis time which was performed overnight. DNA amplification was carried out through polymerase chain reaction (PCR) for each of the specimens for three different gene fragments, including the mitochondrial ribosomal 16S rRNA gene and the cytochrome *c* oxidase subunit I (COI) gene, as well as the nuclear Histone H3 gene. A combination of universal primers and newly designed primers (Table 2) were used to amplify these three gene fragments (Table 3). All PCR reactions contained 37.50 µL of deionized water, 5.00 µL Dream Taq PCR buffer (Thermo Scientific, Waltham, MA), 2.50 µL [10.00 mg/ml] of bovine serum albumin (BSA), 1.00 µL [40.00 mM] of deoxynucleotide triphosphates, 1.00 µL [10.00 µM] of each primer, 0.25 µL [5 mg/ml] of DreamTaq (Thermo Scientific, Waltham, MA), and 2.00 µL of DNA. PCR conditions with the universal primers for 16S and Histone H3 were as follows: initial denaturation at 94° C for 2 minutes, followed by 30 cycles of denaturation at 94° C for 30 seconds, annealing at 57.2° C for 30 seconds, elongation at 68° C for 1 minute, followed by a final elongation step at 68° C for 7 minutes. For the COI primers, an initial denaturation at 95° C for 3 minutes, followed by 30 cycles of

denaturation at 94° C for 45 seconds, annealing at 60° C for 45 seconds, elongation at 72° C for 2 minutes, followed by a final elongation step at 72° C for 10 minutes was used. Gel electrophoresis (30 minutes at 100 volts) using 1% agarose TBE buffer (tris-borate-EDTA) and ethidium bromide was used to visualize amplification of chosen markers, and included a positive control and a negative control. Samples with positive PCR results were then purified using a Genejet purification kit (Thermo Scientific, Waltham, MA) following manufacturer protocols, with the exception of elution into 35.00 µL of elution buffer instead of 45.00 µL. The DNA concentration of the purified PCR product was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). Sanger sequencing was outsourced to SourceBioscience (Santa Fe Springs, CA) using 10.00 µL of the purified PCR product and primer dilutions with a concentration of 4.00 µM.

Sequence alignment and phylogenetic analyses

Forward and reverse sequence reads were assembled, edited, and the consensus sequences for each gene were extracted using Geneious v11.1.5 (Kearse *et al.*, 2012). All consensus sequences as well as the sequences from GenBank were aligned for each gene fragment using MUSCLE v 3.8.4 (Edgar, 2004) implemented in Geneious. Individual gene alignments were concatenated in Geneious. Bayesian phylogenetic analyses (BI) were conducted using MrBayes v3.2.6 (Ronquist *et al.*, 2012) with five specimens of *Bullina* used to root the tree. Models of evolution were determined with PartitionFinder v2 (Lanfear *et al.*, 2017). Analyses were conducted with the models of evolution listed in Table S1 and two runs of six chains for 10 million generations with a sampling interval of 1000 generations and burn-in set to 25%. Maximum likelihood analyses (ML) were conducted 10 times for the concatenated and individual gene alignments using RAxMLGUI v1.0 (Silvestro & Michalak, 2012), the GTR model, 10,000 repetitions



Fig. 2. Photographs of living animals in the *Micromelo undatus* species complex. **A–C.** *Micromelo undatus*, (A) specimen from Brazil (ZMBN 103322), (B) specimen from Brazil (ZMBN 88216, isolate KF117), (C) specimen from Cape Verde (ZMBN 94146, isolate KF121). **D.** *Micromelo guamensis*, specimen from Panglao, the Philippines (MNHN 42238). **E–G.** *Micromelo scriptus*, (E) specimen from Taiwan (ZMBN 116943, isolate KF130), (F) specimen from Mozambique (ZMBN 94236, isolate KF124), (G) Specimen from Maui, Hawaiian Is. (CPIC 329, isolate KF2). **H–J.** *Micromelo barbarae* sp. nov., (H) specimen from Japan (ZMBN 106876, isolate KF128, paratype), (I) specimen from the Philippines (CASIZ 208475 A, isolate KF79, paratype), (J) specimen from the Philippines (holotype, NMP – 041297).

and the bootstrap + consensus option. Posterior probabilities (PP) ≥ 0.9 and Bootstrap (BS) values $\geq 70\%$ were considered significant (Hillis & Bull, 1993; Huelsenbeck & Rannala, 2004). FigTree v1.4.3 (Rambaut, 2014) was used to visualize the trees.

Species delimitation analysis

The COI and 16S sequence datasets including 37 and 39 specimens, respectively, were used to conduct the Automatic Barcode Gap Discovery (ABGD) species delimitation analyses (Puillandre *et al.*, 2012). The web-based version of ABGD was used to compare interspecific distances to intraspecific distances to provide statistical support for the recovery of candidate species from the datasets. Pairwise *p*-distances were calculated in MEGA X (Kumar *et al.*, 2018) using uncorrected distances as well as the Kimura-2 model (Kimura, 1980).

Population genetics

A haplotype network was created using the COI mitochondrial dataset of 37 sequences in order to visualize relationships between haplotypes and corresponding geographic locations. The alignment was uploaded into the program PopArt v1.7 (Leigh & Bryant, 2015) and the TCS (Clement *et al.*, 2000) option was implemented to construct the network.

Morphological analyses

Photographs of live animals were observed and compared. Anatomical dissections were performed on at least two individuals for each of the recovered candidate species, except for *Micromelo guamensis* for which we had only GenBank sequences and no material was available to dissect. All photographs were taken using a Leica EZ4D dissecting microscope. The body was removed from the shell and the empty shells were photographed from two angles: apex and apertural views. The digestive system was removed and photographed. The buccal bulb was placed into 10% sodium hydroxide (NaOH) for 2–5 days to dissolve the tissues surrounding the radula. The radula was rinsed in ultrapure water, cleaned using an ultrasonicator, mounted on a stub, and sputter coated with gold for examination with a Jeol JSM-601 scanning electron microscope (SEM). The penis was dissected and photographed. The penis was dried by soaking in hexamethyldisilazane overnight and also mounted for SEM examination.

Results

Phylogenetic analyses

Bayesian and maximum likelihood analyses of all-three genes combined (Fig. 3A) and the single gene alignments (Supplementary Fig. S1) produced trees with similar topologies, but node support was generally lower in the individual gene trees. The concatenated analyses reveal four clades within specimens of *Micromelo*, although one of the clades was not supported in the ML analysis. Two clades, including individuals from the western Pacific had maximum support (PP = 1, BS = 100) in both BI and ML analyses; one is composed of specimens from the Philippines, Guam, and Papua New Guinea and the other by specimens from the Philippines and southern Japan. Two additional sister clades were recovered, one with specimens from the eastern and western tropical Atlantic (PP = 1, BS = 98) and the other with specimens from the entire Indo-West Pacific from Mozambique to Hawaii. The latter clade was marginally supported in the BI analysis, but not supported in the ML analysis (PP = 0.90, BS < 50). The same clades were recovered in the COI and 16S analyses, but the latter clade was not supported. The H3 trees lack specimens from one of the clades and only recovered one clade with all four candidate species.

Species delimitation using ABGD analysis

The ABGD analysis of the COI sequence data using the Kimura-2 model suggested that the specimens identified as *M. undatus* represented three distinct species while using uncorrected *p*-distances four species were rendered. The ABGD analyses of the 16S dataset suggested both using uncorrected *p*-distances and the Kimura 2 model four distinct species (Fig. 3B, C, Tables 4, 5). These species corresponded to the clades recovered in the phylogenetic analyses (Fig. 3A).

Haplotype network

The haplotype network of the COI sequence data revealed geographic structure among four different regions (Fig. 4). A distinct haplogroup occurs in the Atlantic, represented by specimens from Brazil, Canary Islands, Ascension Island, Cape Verde and Curaçao separated from the rest by 7–34 nucleotide substitutions. Another haplogroup is widespread in the Indo-Pacific region, represented by specimens from Hawaiian Is., Mozambique, Samoa, French Polynesia, and Taiwan separated from the rest by 7–30 nucleotide substitutions. Two distinct Western Pacific haplogroups were also recovered, one containing representatives from the Philippines and Guam, and the other composed of

Table 2. List of specimens sequenced for this study including final species name, locality, collection date, museum voucher number, isolate number and GenBank accession number. Accession numbers in bold represent novel sequences generated by this study. Species names based on the conclusions of this paper.

Species name	Isolate	Voucher	Locality	Collection date	H3	16S	COI
<i>M. scriptus</i>	KF2	CPIC 329A	Maliko Bay, Maui, Hawaiian Is.	16 Jun 2011	MZ099799	MZ048386	MZ048445
<i>M. scriptus</i>	KF3	CPIC 329B	Maliko Bay, Maui, Hawaiian Is.	16 Jun 2011	MZ099788	MZ048375	MZ048434
<i>M. scriptus</i>	KF4	CPIC 329C	Maliko Bay, Maui, Hawaiian Is.	16 Jun 2011	MZ099783	MZ048370	MZ048429
<i>M. scriptus</i>	KF5	CPIC 329D	Maliko Bay, Maui, Hawaiian Is.	16 Jun 2011	MZ099789	MZ048376	MZ048435
<i>M. scriptus</i>	KF7	CPIC 347A	Honokeana Bay, Maui, Hawaiian Is.	17 Jun 2011	MZ099790	MZ048377	MZ048436
<i>M. scriptus</i>	KF8	CPIC 347B	Honokeana Bay, Maui, Hawaiian Is.	17 Jun 2011	MZ099782	MZ048369	MZ048428
<i>M. scriptus</i>	KF9	CPIC 347C	Honokeana Bay, Maui, Hawaiian Is.	17 Jun 2011	MZ099791	MZ048378	MZ048437
<i>M. scriptus</i>	KF11	CPIC 1656A	Maliko Bay, Maui, Hawaiian Is.	22 Jun 2016	MZ099784	MZ048371	MZ048430
<i>M. scriptus</i>	KF12	CPIC 1656B	Maliko Bay, Maui, Hawaiian Is.	22 Jun 2016	MZ099786	MZ048373	MZ048432
<i>M. scriptus</i>	KF14	CPIC 1676A	Mama's Fish House, Maui, Hawaiian Is.	23 Jun 2016	MZ099792	MZ048379	MZ048438
<i>M. scriptus</i>	KF15	CPIC 1676B	Mama's Fish House, Maui, Hawaiian Is.	23 Jun 2016	MZ099779	MZ048366	MZ048425
<i>M. scriptus</i>	KF16	CPIC 1697A	Mama's Fish House, Maui, Hawaiian Is.	25 Jun 2016	–	MZ048365	MZ048424
<i>M. scriptus</i>	KF17	CPIC 1697B	Mama's Fish House, Maui, Hawaiian Is.	25 Jun 2016	MZ099793	MZ048380	MZ048439
<i>M. scriptus</i>	KF18	CPIC 1697C	Mama's Fish House, Maui, Hawaiian Is.	26 Jun 2016	MZ099794	MZ048381	MZ048440
<i>M. scriptus</i>	KF124	ZMBN 94236	Inharrime, Mozambique	3 Feb 2014	MZ099785	MZ048372	MZ048431
<i>M. scriptus</i>	KF125	ZMBN 94272	Inharrime, Mozambique	9 Aug 2013	MZ099795	MZ048382	MZ048441
<i>M. scriptus</i>	KF126	ZMBN 94265	Inharrime, Mozambique	2 Jan 2012	MZ099796	MZ048383	MZ048442
<i>M. scriptus</i>	KF127	ZMBN 94269	Inharrime, Mozambique	2 Nov 2012	MZ099797	MZ048384	MZ048443
<i>M. scriptus</i>	KF36	ZSM- Mol-20060031	Savaii Is., Samoa	21 Aug 2005	MZ099780	MZ048367	MZ048426
<i>M. scriptus</i>	KF130	ZMBN 116943	Magang, Taiwan	16 May 2017	MZ099798	MZ048385	MZ048444
<i>M. scriptus</i>	FP01	MNHN-IM- 2013-52843	Marquesas Is., French Polynesia	17 Jan 2012	MZ099781	MZ048368	MZ048427
<i>M. scriptus</i>	FP02	MNHN-IM- 2013-86142	Marquesas Is., French Polynesia	18 Jan 2012	MZ099787	MZ048374	MZ048433
<i>M. scriptus</i>	–	–	Samoa	–	–	GQ845194	GQ845175
<i>M. undatus</i>	KF25	CPIC 02572	Playa Santa Cruz, Curaçao	23 Oct 2018	MZ099807	MZ048394	MZ048452
<i>M. undatus</i>	KF41	ZSM- Mol-20130108	Ascension Is.	10 Sep 2012	MZ099802	MZ048389	MZ048447
<i>M. undatus</i>	KF117	ZMBN 88216A	Alagoas, Brazil	26 Jan 2012	MZ099803	MZ048390	MZ048448
<i>M. undatus</i>	KF118	ZMBN 88216B	Alagoas, Brazil	26 Jan 2012	MZ099804	MZ048391	MZ048449

(continued)

Table 2. Continued.

Species name	Isolate	Voucher	Locality	Collection date	H3	16S	COI
<i>M. undatus</i>	KF131	ZMBN 88216C	Alagoas, Brazil	26 Jan 2012	MZ099810	–	MZ048454
<i>M. undatus</i>	KF134	ZMBN 88216E	Alagoas, Brazil	26 Jan 2012	MZ099808	MZ048395	–
<i>M. undatus</i>	KF135	ZMBN 88216F	Alagoas, Brazil	26 Jan 2012	MZ099800	MZ048387	–
<i>M. undatus</i>	KF120	ZMBN 81650	São Tiago Is., Cape Verde	1 Aug 2008	MZ099805	MZ048392	MZ048450
<i>M. undatus</i>	KF121	ZMBN 94146	São Vicente Is., Cape Verde	17 Jul 2013	MZ099806	MZ048393	MZ048451
<i>M. undatus</i>	KF122	ZMBN 94147	Boavista Is., Cape Verde	18 Jul 2013	MZ099809	–	MZ048453
<i>M. undatus</i>	KF123	ZMBN 106851	Santa Luzia Is., Cape Verde	14 Apr 2016	MZ099801	MZ048388	MZ048446
<i>M. undatus</i>	–	BMNH 20030800	Tenerife, Canary Is.	–	KJ022944	KJ022778	DQ974653
<i>M. barbarae</i> sp. nov.	KF78	CASIZ 217409	Calumpang Peninsula, Luzon, the Philippines	14 Sep 2016	MZ099774	MZ048361	MZ048422
<i>M. barbarae</i> sp. nov.	KF79	CASIZ 208475A	Puerto Galera, the Philippines	31 Mar 2015	MZ099776	MZ048363	MZ048423
<i>M. barbarae</i> sp. nov.	KF80	CASIZ 208475B	Puerto Galera, the Philippines	31 Mar 2015	MZ099777	MZ048364	–
<i>M. barbarae</i> sp. nov.	KF128	ZMBN 106876	Hachijō-jima, Japan	24 May 2016	MZ099775	MZ048362	–
<i>M. guamensis</i>	–	–	Guam	–	–	GQ845195	GQ845176
<i>M. guamensis</i>	–	MNHN-IM- 2007- 42238	Panglao, the Philippines	–	–	–	DQ974652
<i>M. guamensis</i>	–	–	Papua New Guinea	–	–	DQ991933	–
<i>Bullina</i> sp.	KF58	MNHN IM- 2013-86148	Koumac, New Caledonia	Sep 2018	MZ099770	MZ048357	–
<i>Bullina</i> sp.	KF115	MNHN IM- 2013-86150	Koumac, New Caledonia	Sep 2018	MZ099771	MZ048358	–
<i>B. lineata</i>	KF59	MNHN IM- 2013-86151	Koumac, New Caledonia	Sep 2018	MZ099772	MZ048359	MZ048421
<i>B. lineata</i>	KF132	MNHN IM- 2013-86149	Koumac, New Caledonia	Oct 2019	MZ099773	MZ048360	–
<i>B. lineata</i>	–	–	NSW, Australia	–	–	GQ845196	AY296847

representatives from the Philippines. These were separated by 24–50 and 22–50 nucleotide substitutions, respectively.

Morphological analyses

There are consistent interspecific morphological differences among representative specimens of the clades recovered in the phylogenetic analyses, which also correspond to the haplogroups in the haplotype network and the candidate species from the species delimitation analyses. These differences included aspects of internal morphology such as radular formula, digestive system and penial differences and are discussed in the Systematics section below.

Systematics

Class Gastropoda Cuvier, 1975

Subclass Heterobranchia Gray, 1840
Superfamily Acteonoidea d'Orbigny, 1842
Family Aplustridae Gray, 1847
Genus *Micromelo* Pilsbry, 1895

Micromelo Pilsbry, 1893–1895 [1895]: 391–392. Type species: *Bulla undata* Bruguière, 1792, by original designation.

Diagnosis. Shell up to 30 mm, thin, fragile, ovate, globose, without operculum; body whorl large; spire sunken, with two whorls; columella thickened; sculpture of faint punctuated spiral grooves. Aperture wide, narrowing posteriorly. Shell color white to cream with dark red to light pink wavy longitudinal lines crossed by three spiral lines. Live animal body approximately twice the length of the shell. Body color translucent white with opaque white spots; margin color ranges from vibrant blue to green and yellow. Mantle consists of

Table 3. Primers used to amplify Histone H3, 16S and COI genes.

Gene	Primer	5'–3' Sequence	Source
Histone H3	HexAF (F)	ATGGCTCGTACCAAGCAGACGGC	(Colgan et al., 1998)
	HexAR (R)	ATATCCTTGGGCATGATGGTGAC	(Colgan et al., 1998)
16S rRNA	16Sar-L (F)	CGCCTGTTTATCAAAAACAT	(Palumbi, 1996)
	16Sbr-H (R)	CCGGTCTGAACTCAGATCACGT	(Palumbi, 1996)
Cytochrome c oxidase I	LCO1490 (F)	GGTCAACAAATCATAAAGATATTGG	(Folmer et al., 1994)
	HCO2198 (R)	TAAACTTCAGGGTGACCAAAAAATCA	(Folmer et al., 1994)
	MmeloCOI-1 (F)	GTATATGATGTGGTCTGGTAGG	Present study
	MmeloCOI-1 (R)	GCTGATACAAAATTGGATCTCC	Present study

anterior and posterior lobe; anterior lobe divided into two extensible appendages on each side of the headshield. Foot broad, oval extending beyond the mantle on all sides.

Internal morphology. Radular formula 19–29 × 10–13.1.0–1.1.10–13. Rachidian tooth reduced or absent. Lateral teeth hook-shaped; increasing in size outward. Innermost tooth narrower with 2–6 denticles, rest of inner lateral teeth larger, hook-shaped, with 4–8 denticles; mid-lateral teeth with 1–5 denticles; outer teeth with no denticles. Penis located posterior to right headshield appendage, striated, with two retractor muscles at base and a stylet.

Remarks. The genus name *Micromelo* was introduced by Pilsbry (1893–1895) as distinct from the closely related genera *Hydatina* Schumacher, 1817 and *Aplustrum* Schumacher, 1817, by having a “spirally sculptured shell” and the live animal with “two, instead of four tentacular processes on the head disk”. Pilsbry (1893–1895) originally designated *Bulla undata* Bruguière, 1792 as the type species, but also included *Bulla guamensis* Quoy and Gaimard, 1825 and *Bulla eximia* Deshayes, 1863 as members of this new genus.

Micromelo undatus (Bruguière, 1792)
(Figs 2A–C, 5, 6)

Bulla undata Bruguière, 1789–1792 (1792): 380.
Type locality unknown.

Bulla nitidula Dillwyn [ex. Solander], 1817: 483.
Type locality: West Indies.

Bulla elegans Menke, 1828: 13, 125 [non *Bulla elegans* J. E. Gray, 1825: family Haminoeidae]. Type locality: “Porto-rico” [=Puerto Rico]

Type material. No original type material is known to exist for either *Bulla undata*, *Bulla nitidula* or *Bulla elegans* (see remarks). Neotype (here designated): Discovery Bay, Jamaica, Mar 2006, 18 mm preserved length (voucher number LACM 3768; ex. CPIC 2573).

Material examined. Alagoas, Brazil, 26 Jan 2012, 6 specimens 12–14 mm preserved length (ZMBN 88216 A–F), specimens A–C and E–F sequenced (isolates KF117 [Fig. 2B], KF118, KF131, KF133, KF134, KF135). Taliarte wreck, Boavista Is., Cape Verde, 18 Jul 2013, 1 specimen 9 mm preserved length (ZMBN 94147), sequenced (isolate KF122). Tarrafal, São Tiago Is., Cape Verde, 1 Aug 2008, 1 specimen 12 mm preserved length (ZMBN 81650), sequenced (isolate KF120). Baía das Gatas, São Vicente Is., Cape Verde, 17 Jul 2013, 1 specimen 8 mm preserved length (ZMBN 94146), sequenced (isolate KF121 [Fig. 2C]). Praia do Palmo a Tostão, Santa Luzia Is., Cape Verde, 14 Apr 2016, 1 specimen 5 mm preserved length (ZMBN 106851), sequenced (isolate KF123).

External morphology (Figs 2A–C, 5A, B). Body oval to elongate, translucent grey, covered with large, oval opaque white spots, densely arranged – distance between spots about same size as the spots themselves. Mantle, rhinophores, and foot with a marginal yellow-orange band followed by a pale blue or green diffuse area. Headshield as long as wide, prominently narrower mid-length. Shell oval, nearly round, almost as long as wide. Posterior part of the aperture forming a smooth rounded edge with apex. Outer edge of the aperture curved.

Internal morphology (Figs 5C–F, 6). Digestive system with nearly spherical buccal mass, with similar length and width. Oesophagus and salivary glands elongated, the latter having numerous folds, covering a large portion of digestive gland; oesophagus twice as long as buccal bulb. Radular formula 21–29 × 10–13.1.1.1.10–13. Rachidian tooth present, narrow, elongate. Innermost tooth smaller, narrower than rest of inner lateral teeth, with 2–4 denticles and prominent upper extension; other inner lateral teeth with 5–8 denticles, hook-shaped. Mid-lateral teeth with 1–7 denticles, hook-shaped, with elongate cusps. Outermost teeth with sharp, long cusps and no denticles. Penis elongate, with two strong muscles at the base and a

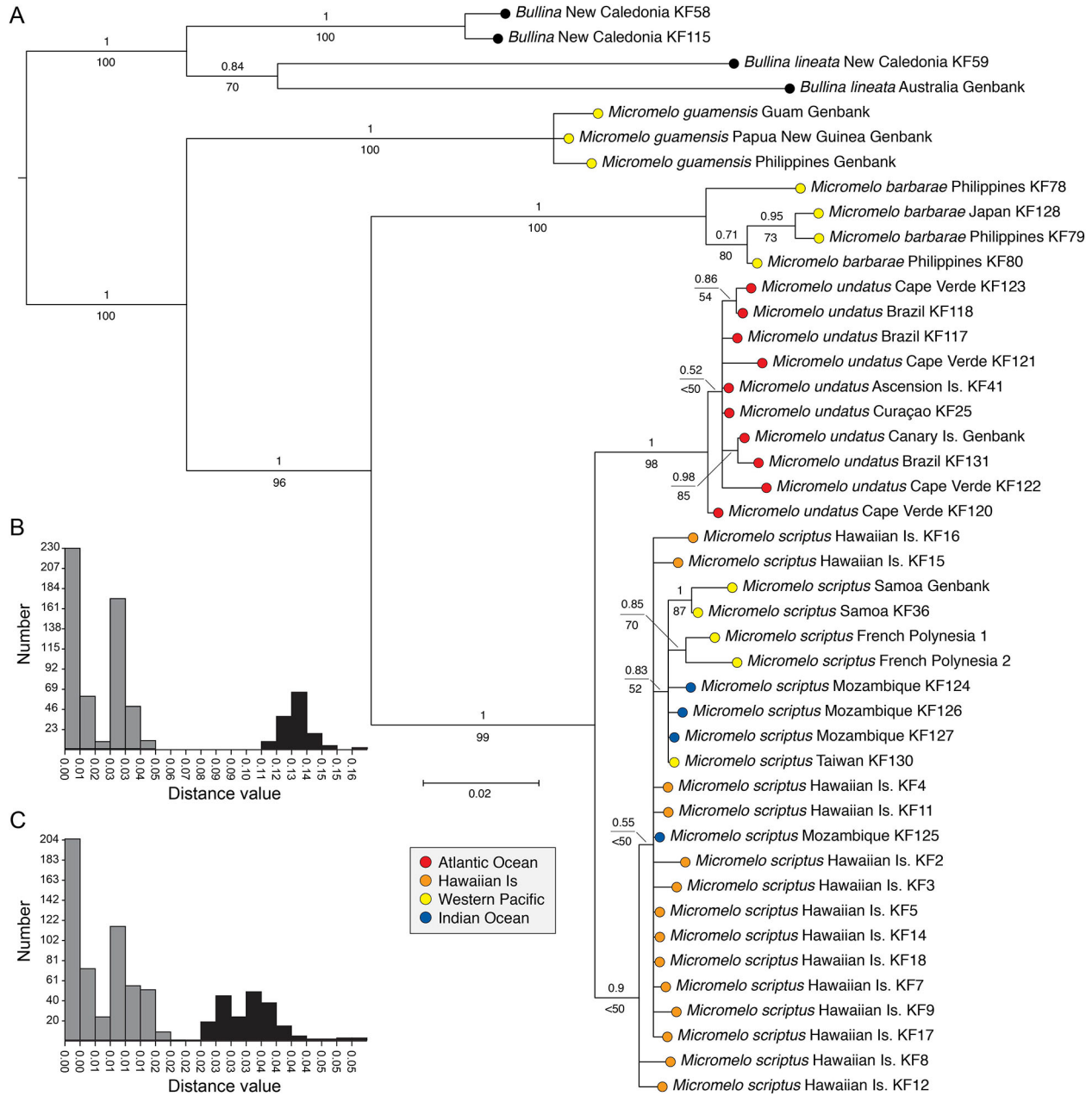


Fig. 3. **A.** Bayesian consensus tree of the concatenated 16S, COI and H3 genes. Posterior probabilities from the Bayesian analysis are listed above each branch; bootstrap values from Maximum Likelihood analysis are listed below each branch. **B.** Results of ABGD analysis using the COI gene showing pairwise *p*-distances (Kimura 2 model) among candidate species. **C.** Results of ABGD analysis using the 16S gene showing pairwise *p*-distances (Kimura 2 model) among candidate species.

subapical penial stylet. Penial stylet straight with a longitudinal groove.

Geographic range. Ascension I. (Padula *et al.*, 2017), Cape Verde Is (Valdés, 2005), Canary Is. (Moro *et al.*, 2003), Azores (Nordsieck, 1972), the Caribbean coast of Costa Rica, St. Lucia (Valdés *et al.*, 2006), Jamaica (present study), Curaçao (present study), and Brazil (Padula *et al.*, 2012),

Remarks. Specimens of *Micromelo* from the Atlantic Ocean examined in this paper were recovered as a well-supported clade in all phylogenetic analyses, a distinct haplogroup in the haplotype network, and as a candidate species in the ABGD analyses, with the exception of the Kimura-2 analysis of COI sequences. In addition, several morphological differences with other specimens here examined support considering these animals as a valid, distinct species. *Micromelo undatus* has the most

Table 4. Results of the ABGD species delimitation analyses of COI sequence data using uncorrected and Kimura 2 model *p*-distances.

Model	Species	Isolate Numbers
Uncorrected <i>p</i> -distances	<i>Micromelo guamensis</i> <i>Micromelo barbarae</i> sp. nov. <i>Micromelo undatus</i>	DQ974652, GQ845176 KF78, KF79 KF123, KF41, KF117, KF118, KF120, KF121, KF25, KF122, KF131, DQ974653
	<i>Micromelo scriptus</i>	KF2, KF12, KF8, KF16, KF9, KF3, KF126, KF124, KF130, KF127, KF36, KF15, KF17, KF7, KF125, KF18, KF14, KF11, KF5, KF4, FrenchPolynesia-1, FrenchPolynesia- 2, GQ845175
Kimura-2 <i>p</i> -distances	<i>Micromelo guamensis</i> <i>Micromelo barbarae</i> sp. nov. <i>Micromelo undatus</i> + <i>scriptus</i>	DQ974652, GQ845176 KF78, KF79 KF121, KF122, KF120, KF131, KF117, KF25, KF123, KF118, KF41, KF2, KF12, KF8, KF16, KF9, KF3, KF126, KF124, KF130, KF127, KF36, KF15, KF17, KF7, KF125, KF18, KF14, KF11, KF5, KF4, FrenchPolynesia-1, FrenchPolynesia-2, GQ845175, DQ974653

Table 5. Results of the ABGD species delimitation analyses of 16S sequence data using both uncorrected and Kimura-2 model *p*-distances.

Species	Isolate Numbers
<i>Micromelo guamensis</i> <i>Micromelo barbarae</i> sp. nov. <i>Micromelo undatus</i>	GQ845195, DQ991933 KF78, KF79, KF80, KF128 KF135, KF123, KF41, KF117, KF118, KF134, KF120, KF121, KF25, KJ022778
<i>Micromelo scriptus</i>	KF16, KF15, GQ845194, GQ845175, KF36, FrenchPolynesia-1, KF8, KF4, KF11, KF124, KF12, FrenchPolynesia-2, KF3, KF5, KF7, KF9, KF14, KF17, KF18, KF125, KF126, KF127, KF130, KF2

conspicuous internal differences of all the species recognized herein, including a round instead of oval shell, a substantially longer penis with a longitudinal groove in the stylet, a round buccal mass with elongated oesophagus and very elongated salivary glands, and a radula with the largest amount of teeth rows and largest number of denticles. Interestingly, we did not recover any phylogeographic structure in this species with a separation between eastern and western Atlantic (Fig. 3A). Instead, there seems to be relative genetic homogeneity across the Atlantic realm suggesting either a recent process of dispersal or possible gene flow between both sides of the Atlantic.

The name *Bulla undata* Bruguière, 1792 was introduced by Bruguière (1789–1792) based on the pre-Linnaean illustration of “*Concha veneris umbilicata, tenuis, striis undatis vel ramificatis depicta*” by Lister et al. (1688: pl. 715, fig. 74) and subsequent consistently non-binominal references (Favanne, 1780: pl. 27, fig. 3; Klein, 1753: 82; Martini, 1769: 283, text figs.

4–5 [p. 274]). Lister et al. (1688: pl. 715, fig. 74) illustration clearly represents a shell of *Micromelo* as described by Pilsbry (1893–1895), but no type locality is provided. Bruguière (1789–1792) also included a description of the shell and indicated that the main shell characteristics that distinguish this species from other *Bulla* are the transversal striations and undulating longitudinal lines. No type material is known to exist and consequently it is difficult to determine the identity of the species with certainty. D’Orbigny (1853) reported *B. undata* from Cuba, which constitutes the first geographic record of this species. Pilsbry (1893–1895) designated *B. undata* as the type of the new genus *Micromelo*, and regarded it as an Atlantic species, while assigned the names *Bullaea guamensis* Quoy and Gaimard, 1825 and *Bulla eximia* Deshayes, 1863 for Pacific and Indian Ocean species, respectively. Because the genus *Micromelo* contains a species complex, rather than a single circumtropical species, including an Atlantic species and several Indo-Pacific species,

Table 6. Summary of most useful characters for diagnosis of species of *Micromelo*.

Species /character	<i>M. undatus</i>	<i>M. guamensis</i>	<i>M. scriptus</i>	<i>M. barbarae</i> sp. nov.
Shell	Nearly round; posterior part of aperture forming smooth rounded edge with apex	?	Elongate, with convex sides; apex extending beyond posterior end of aperture	Elongate, with convex sides; apex extending slightly beyond posterior end of aperture; protoconch with black pigment
Colour pattern	Large, oval opaque white patches, densely arranged; distance between patches about same size as patches themselves	Large, compound opaque white patches, densely arranged, almost touching; distance between patches smaller than patches themselves	Small, oval or compound opaque white patches, widely spaced; distance between patches equal or larger than patches themselves	Large, oval or compound opaque white patches, widely spaced; distance between patches equal or larger than patches themselves
Colour of body margins	Yellow-orange band followed inwards by pale blue or green diffuse bands	Light vibrant blue band followed inwards by dark blue or purple diffuse band	Yellow-orange band followed inwards by pale blue or green diffuse band	Yellow-orange band followed inwards by pale green diffuse band
Cephalic lobes	Posterior margins wavy	Posterior margins slightly wavy	Posterior margins smooth	Posterior margins slightly wavy
Oesophagus and salivary glands	Oesophagus about twice as long as buccal bulb, salivary glands many times longer	?	Oesophagus twice as short as buccal bulb, salivary glands about same length as oesophagus	Oesophagus as long as buccal bulb; salivary glands shorter
Radula	10–13.1.1.1.10–13; rachidian tooth present; innermost tooth with 2–4 denticles; inner lateral teeth with 5–8 denticles; mid-lateral teeth with 1–7 denticles; outermost teeth with sharp, long cusps, smooth	?	9.1.0–1.1.9; rachidian tooth present or absent; innermost tooth with 4–5 denticles; inner lateral teeth with 5–6 denticles and shorter upper extension; mid-lateral teeth with 0–2 denticles; outermost teeth hook-shaped, smooth	9.1.1.1.9; rachidian tooth present; innermost tooth with 4–6 denticles; inner lateral teeth with 4–8 denticles, with prominent upper extension; mid-lateral teeth with 0–3 denticles; outermost teeth hook-shaped, smooth
Male reproductive system	Penis elongate; penial stylet straight with longitudinal groove	?	Penis oval, almost as wide as long; penial stylet curved; with swollen tip, not grooved	Penis short, round; penial stylet slightly curved, not grooved
Distribution	Eastern and Western Atlantic Ocean	Western Pacific	Indo-West Pacific	NW tropical Pacific

without type locality information the name *B. undata* cannot be assigned with certainty to any of the species recognized in this paper. Whereas Lister *et al.* (1688) did not provide a type locality for the specimen illustrated, indirect evidence may help narrow down the origin of this specimen. The bulk of the specimens illustrated by Lister *et al.* (1688) came from the Courten and Sloane collections (Roos, 2013). In fact, Lister *et al.* (1688) dedicated the first part of their work to the “illustrious and excellent man William Courten, of the Middle Temple, London, as a mark of appreciation for the help received by the loan of specimens for illustration”. Courten had a public museum of curiosities in

a suite of ten rooms in the Temple, London, including artwork, specimens of flora and fauna, and archaeological objects (Roos, 2013). Courten lived much abroad and had family interests in Barbados through his paternal grandfather, Sir William Courten (Wilkins, 1953). This probably accounts for the common appearance of Barbados on the legends of Lister *et al.* (1688) plates (Wilkins, 1953). Thus, if the shell of *B. undata* illustrated by Lister *et al.* (1688) came from the Courten museum, it is probable that its origin could be traced to Barbados. However, Courten amassed a large collection of specimens purchased from various sources and with diverse origins (Kusukawa, 2017), thus the origin of any

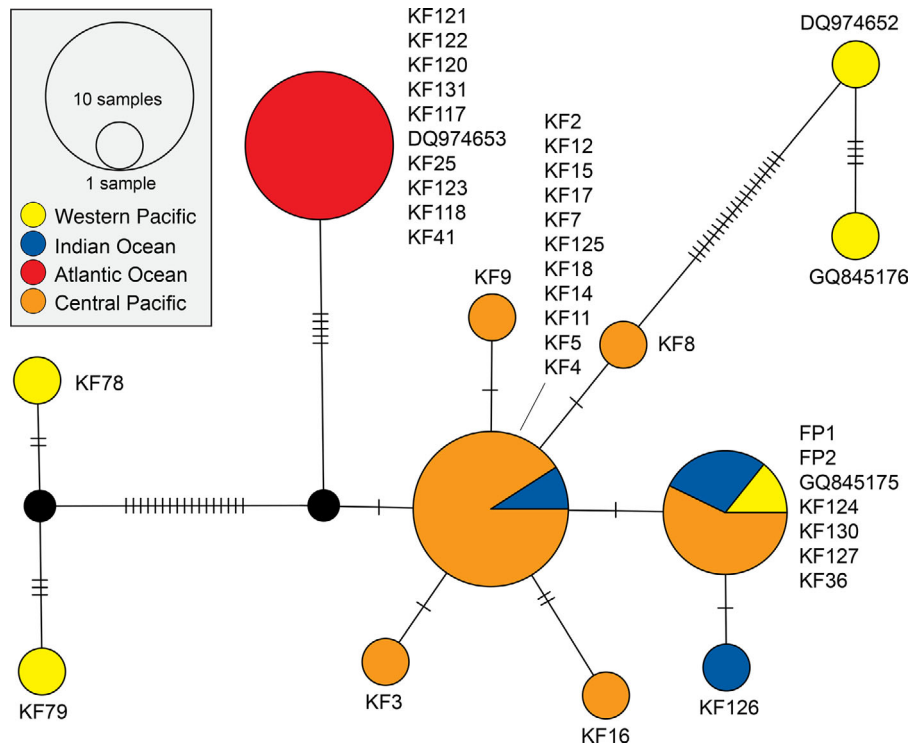


Fig. 4. Haplotype network based on COI sequence data. Colors represent the geographic region of origin of specimen as in Fig. 2. Circle sizes indicate the number of individuals represented. Spaces between dashes represent nucleotide differences. Widespread network lower right = *M. scriptus*. Red = *M. undatus*. Yellow top right = *M. guamensis*. Lower left ones = *M. barbaraee*. See Table 2 for specific geographical locations.

particular specimen cannot be determined with certainty, unless it was indicated in the legend. In 1702 Courten's collection was bequeathed to Sir Hans Sloane (Wilkins, 1953), the founder of the British Museum, who also contributed specimens to Lister et al. (1688)'s work. When Sloane went to Jamaica in 1687, Martin Lister asked him to bring back specimens, not only of shells, but of what he termed 'naked snails' or slugs (Roos, 2013). Sloane's early collection was mainly from the West Indies, primarily Jamaica, but also Carolina and Bahamas (Wilkins, 1953). Although later Sloane also acquired substantial collections from Japan, China and India (Wilkins, 1953), those specimens could not had been included in Lister et al. (1688)'s work. If the shell of *B. undata* came from the Sloane collection, it is likely that its origin could be traced to Jamaica or the West Indies, but as in the case of the Courten collection, this cannot be determined with certainty.

Attempts to locate the original shell of *B. undata* illustrated in Lister et al. (1688) were unsuccessful. The Lister collection was donated to the Ashmolean Museum, whose natural history collections became the Oxford University Museum of Natural History in 1860. According to Collections Manager Mark Carnall (personal communication, December 12, 2020), the Oxford

University Museum of Natural History currently has no malacological specimens from the Lister collection. The remains of the Sloane collections are at the Mollusca Section of the Natural History Museum of the UK, London. According to Senior Curator of Marine Gastropoda and Historical Mollusca Collections, Andreia Salvador (personal communication, December 11, 2020), the original specimen of *B. undata* is not present in this institution either. Despite the fact that the type locality of *B. undata* is unknown, it seems probable that the specimen illustrated by Lister et al. (1688: pl. 715, fig. 74) was collected either in Barbados or Jamaica, although this cannot be verified with certainty. Moreover, the name *B. undata* has been consistently applied to Atlantic specimens (d'Orbigny, 1853, Pilsbry, 1893–1895, Rudman, 1972). Therefore, in order to preserve nomenclatural stability, retain the widely used name *B. undata*, which has been consistently applied to the Atlantic species, and permanently resolve taxonomic issues, the same specimen (voucher number LACM 3768; ex. CPIC 2573) is here designated as the neotype for *Bulla undata*, *B. nitidula* and *B. elegans*. According to the Code (ICZN, 1999), a neotype is validly designated when there is an exceptional need and only when that need is stated expressly. In this case, the

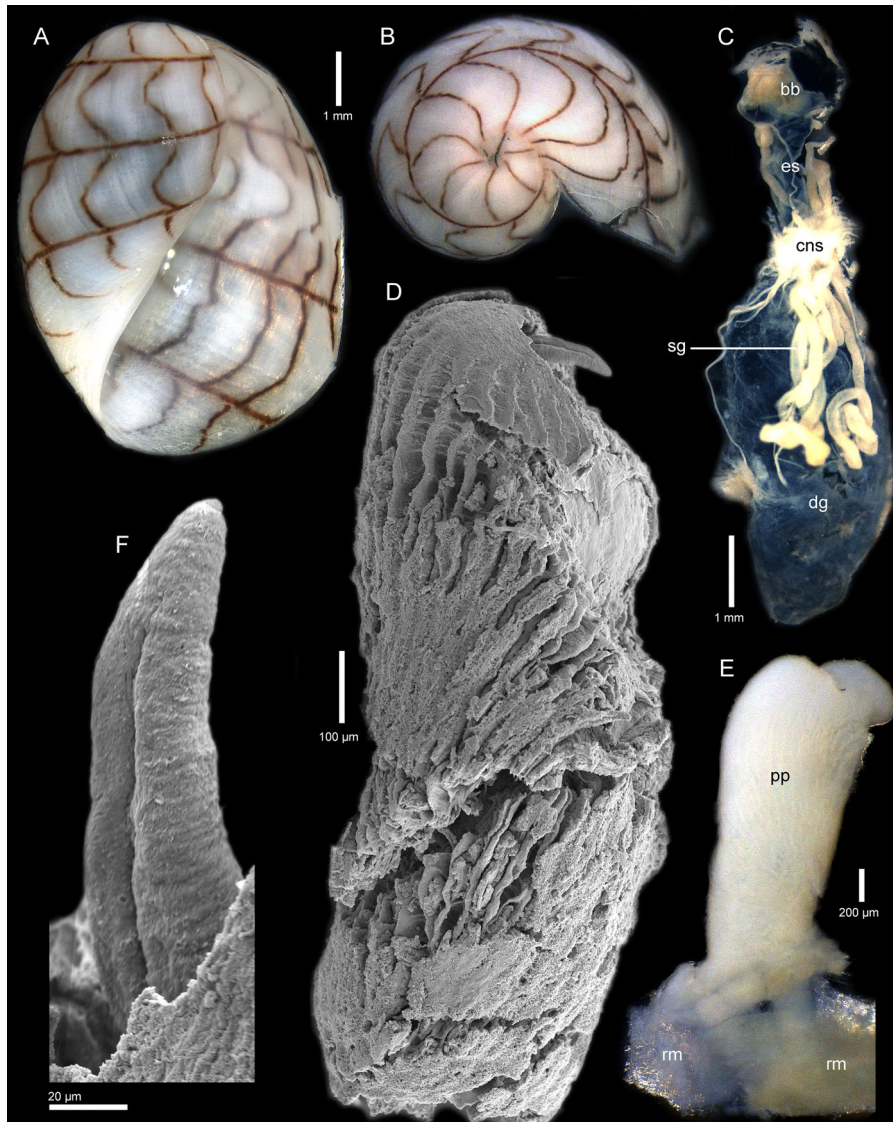


Fig. 5. Anatomy of *Micromelo undatus*. **A–B.** Photographs of the shell and digestive system of a specimen from Brazil (ZMBN 88216C), aperture view (A), apical view (B). **C.** Digestive system of a specimen from Brazil (ZMBN 88216C). **D–F.** Photograph and scanning electron micrographs of the penis and penial stylet of a specimen from Brazil (ZMBN 88216F). **E.** Photograph of the penis of a specimen from Brazil (ZMBN 88216E). Abbreviations: bb, buccal bulb; cns, central nervous system; dg, digestive gland; es, esophagus; pp, penis; rm, retractor muscle; sg, salivary gland; st, stylet.

designation of a neotype is needed to maintain nomenclatural stability by preserving the usage of the widely accepted name *Micromelo undatus* as well as to clarify its type locality and taxonomic status of this nominal species.

Dillwyn (1817) introduced the name *Bulla nitidula* Dillwyn, 1817 with a short description and a reference to a manuscript name in the Portland Catalogue and authorship attributed to Solander. However, we have been unable to find any references to “*Bulla nitidula*” in Lightfoot (1786). Dillwyn (1817) also included *Bulla undata* in the synonymy of *B. nitidula*, with references

to descriptions and illustrations by Bruguière (1789–1792), Lister et al. (1688), and Martini (1769). Dillwyn (1817) provided a text description of the shell and indicated that Humphreys established the range as the West Indies. Years later, A. Adams (1855) illustrated a specimen of *B. nitidula* from St. Vincent (pl. 120, figs. 15–16) but assigned the authorship of this species to Lister instead of Dillwyn (A. Adams, 1855: 565–566), based on the same reference provided by Bruguière (1789–1792). All these descriptions and illustrations are consistent with the characteristics of *Micromelo*. The type material of *B. nitidula* is

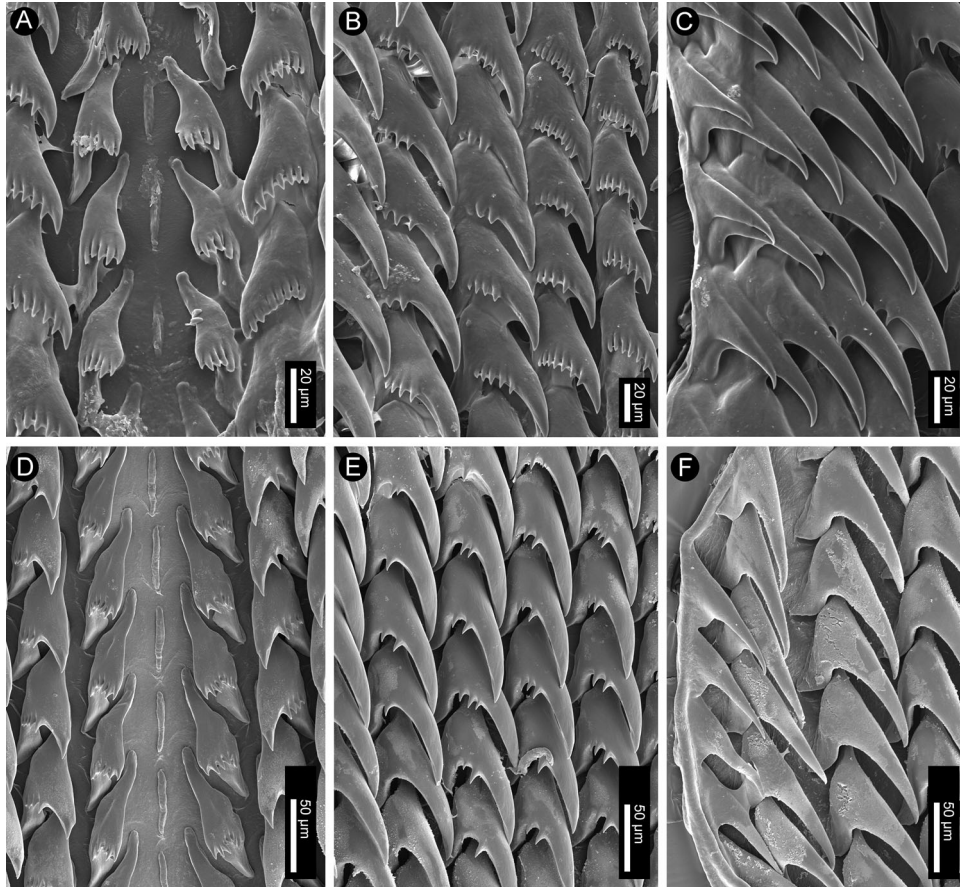


Fig. 6. *Micromelo undatus*, scanning electron micrographs of radulae. A–C. Specimen from Cape Verde (ZMBN 94147), rachidian and innermost teeth (A), mid-lateral teeth (B), outermost teeth (C). D–F. Specimen from Brazil (ZMBN 88216D), rachidian and innermost teeth (D), mid-lateral teeth (E), outermost teeth (F).

untraceable, a few shells from the Duchess of Portland collection are at Natural History Museum of London, UK (Dance, 1966), however, according to A. Salvador (pers. comm.) the type material of *B. nitidula* is not present in this institution. However, based on the type locality, there is no doubt *Micromelo nitidulus* belongs to the Atlantic species of this genus. Because the same specimen is here designated as the neotype for *B. undata* and *B. nitidula* these two names become objective synonyms.

Menke (1830: 125) described *Bulla elegans* [non *Bulla elegans* J. E. Gray, 1825: family Haminoeidae] with a short Latin text and also a reference to Lister et al. (1688: pl. 715, fig. 74) indicated the habitat as “Porto-rico” [= Puerto Rico]. The characteristics of this species are also consistent with those of *Micromelo*. Since this study recovered a single Atlantic species, the name *Bulla elegans* is regarded as a synonym of *Micromelo undatus* and this synonymy is fixed by the designation of a neotype for both *B. undata* and *B. elegans*. Menke’s collection was sold after his death to a natural history dealer, M.J. Landauer of Frankfurt, who

dispersed it by selling specimens to various private collectors (Zilch, 1961). Therefore, the location of the original type material of *B. elegans* is unknown.

Micromelo guamensis (Quoy and Gaimard, 1825)
(Fig. 2D)

Bullaea guamensis Quoy and Gaimard, 1824–1826 (1825): 423–425, pl. 66, figs. 10–12. Type locality: “Agagna” [=Agana], Guam.

Bulla ferussaci Catlow and Reeve [ex. Quoy and Gaimard], 1845: 113. Type locality: “Agagna” [=Agana], Guam.

Type material. Probably lost, the syntype of *Bullaea guamensis* Quoy and Gaimard, 1825 at MNHN (IM-2000-27697) is likely mislabelled (see Remarks).

External morphology (Fig. 2D). Body elongate, translucent grey, covered with large, compound opaque white

spots, densely arranged, almost touching – distance between spots much smaller than the spots themselves. Mantle, rhinophores, and foot with a marginal light vibrant blue band followed by a dark blue or purple diffuse area. Headshield elongate, prominently narrower mid-length. Shell not observed.

Internal morphology. No material examined.

Geographic range. Guam (Burdich, 2021; Göbbeler & Klussmann-Kolb, 2010; Quoy & Gaimard, 1824–1826), Papua New Guinea (Medina *et al.*, 2011), the Philippines (Malaquias *et al.*, 2009), Kwajalein reef quarry, Marshall Islands (Johnson & Johnson, 2021).

Remarks. The results of this study confirm that some specimens of *Micromelo* from the Western Pacific Ocean constitute a distinct species, recovered both in the phylogenetic and species delimitation analyses. Although no specimens of *M. guamensis* were available for dissection, it was possible to examine the external morphology based on photos of the animal collected in the Philippines and from which a COI sequence was available (GenBank Accession Number DQ974652). This species is externally morphologically distinct and characterized by having opaque white spots on the body that are larger and more densely arranged than in all other species, and margins lacking a yellow band.

Quoy & Gaimard (1824–1826) introduced the new name *Bullaea guamensis* Quoy & Gaimard, 1825 based on an undetermined number of specimens collected in “Agagna” [=Agana], Guam. Quoy & Gaimard (1824–1826) also used the vernacular name “Bullée Férussac” to refer to this species in both the text and the figure legend. One of the animals was illustrated alive, as well as one shell Quoy & Gaimard (1825: pl. 66, figs. 10–11). Both illustrations, as well as the written description are consistent with the current use of the name *Micromelo*. However, the syntype (MNHN-IM-2000-27697) illustrated in the Muséum National d’Histoire Naturelle collection website (<http://coldb.mnhn.fr/catalog-number/mnhn/im/2000-27697>) is not consistent with the original description, and clearly represents a species of the cephalaspid heterobranch *Smaragdinella* A. Adams, 1848. Because the species here recognized is the only *Micromelo* species in this study occurring in Guam, which is the type locality of *M. guamensis*, and more importantly, the characteristics of the live animals closely match the original drawing by Quoy & Gaimard (1825: pl. 66, fig. 10), we propose using the name *M. guamensis* for this species.

Catlow and Reeve (1845) listed the new name *Bulla ferussaci* with a reference to the description of *B.*

guamensis by Quoy & Gaimard (1825: pl. 66, figs. 10–12) but with no description. Because *Bulla ferussaci* was introduced before 1931 and is accompanied by an indication, it is an available name (ICZN, 1999: Article 12). Since *B. ferussaci* is based on the same description and has the same type[s] as *B. guamensis*, it is a junior objective synonym of the latter.

Micromelo scriptus (Garrett, 1857)
(Figs 2E–G, 7, 8)

Bulla scripta Garrett, 1857: 103. Type locality: Hilo, Hawaiian Is.

Bulla eximia Deshayes, 1863: 55–56, pl. 7, figs. 23–24. Type locality: Réunion Island.

Type material. Syntypes of *Bulla scripta* Garrett, 1857, probably lost. Syntype of *Bulla eximia* Deshayes, 1863 at MNHN (IM-2000-27690).

Material examined. Maliko Bay, Maui, Hawaiian Is., 16 Jun 2011, 0–1 m depth, 4 specimens 2–6 mm preserved length (CPIC 0329), specimens A–D sequenced (isolates KF2–5 [Fig. 2G]); 22 Jun 2016, 2 specimens 4–7 mm preserved length (CPIC 1656), specimens A–B sequenced (isolates KF11–12). Honokeana Bay, Maui, Hawaiian Is., 17 Jun 2011, 3 specimens 2–5 mm preserved length (CPIC 0347). Mama’s Fish House, Maui, Hawaiian Is., 23 Jun 2016, 0–1 m depth, 2 specimens 7–10 mm preserved length (CPIC 1676), specimens A–B sequenced (isolates KF14–15); 3 specimens, 7–11 mm preserved length (CPIC 1697), specimens A–C sequenced (isolates KF16–18). Magang, New Taipei City, Taiwan, 1 specimen 11 mm preserved length (ZMBN 116943), sequenced (isolate KF130 [Fig. 2E]). Zavora, Inharrime, Mozambique, 2 Nov 2012, 1 specimen 4 mm preserved length (ZMBN 94269), sequenced (isolate KF127); 2 Jan 2012, 1 specimen 10 mm preserved length (ZMBN 94265), sequenced (isolate KF126); 9 Aug 2013, 1 specimen 6 mm preserved length (ZMBN 94272), sequenced (isolate KK125); 3 Feb 2014, 1 specimen 11 mm preserved length (ZMBN 94236), sequenced (isolate KF124 [Fig. 2F]).

External morphology (Figs 2E–G, 7A, B). Body elongate, translucent grey, covered with small, oval or compound opaque white spots, generally widely spaced – distance between spots equal or larger than the spots themselves. Mantle, rhinophores, and foot with a marginal yellow-orange band followed by a pale blue or green diffuse area. Headshield as long as wide, prominently narrower mid-length. Shell elongate, longer than

wide, with convex sides. Apex extending beyond the posterior end of the aperture.

Internal morphology (Figs 7C–F, 8). Digestive system with an oval buccal bulb, slightly longer than wide, with two large retractor muscles attached proximally. Oesophagus twice as short as buccal bulb, wide. Salivary glands relatively short, covering a small portion of the digestive gland. Radular formula 21–23 × 9.1.0–1.1.9. Rachidian tooth present or absent, very narrow and elongate. Innermost tooth elongate, smaller, narrower than rest of inner lateral teeth, with 4–5 denticles and a very elongate and prominent upper extension. Other inner lateral teeth hook-shaped, with 5–6 denticles and a shorter upper extension. Mid-lateral teeth hook-shaped with 0–2 denticles. Outermost teeth with the same shape but no denticles. Penis oval, almost as wide as long, with a subapical curved stylet having a swollen tip, not grooved.

Geographic range. Hawaiian Is. (Bertsch & Johnson, 1981; Kay, 1976), Mozambique (Gosliner et al., 2008; King & Fraser, 2014; MacNae & Kalk, 1958; Tibirić & Malaquias, 2017), and South Africa (Gosliner, 1987; Gosliner et al., 2008), Samoa (present study), Taiwan (present study), French Polynesia (present study).

Remarks. The Bayesian phylogenetic analysis and most of the species delimitation analyses, recovered a species of *Micromelo* widespread in the Indo-Pacific region, ranging from the Hawaiian Is. to the Indian Ocean. This species is closely related to the Atlantic species *M. undatus* and the maximum likelihood analysis as well as the Kimura-2 ABGD analyses failed to recover them as either well supported or distinct. However, because these two species occur in different ocean basins and display several morphological differences are here regarded as distinct.

Garrett (1857) introduced *Bulla scripta* Garrett, 1857 based on shell[s] collected in shallow pools in the West coast of Hilo, Hawaiian Is. The species description includes no illustrations but a short text in which the characteristics of *Micromelo* are recognizable, including a whitish shell with three distant transverse black lines crossed by several longitudinal waived lineations. While the description is incomplete, because there are no other species names introduced from the Hawaiian Islands and there is only one species of *Micromelo* present in this region, we assign the name *Micromelo scriptus* to this species, which ranges from the Indian Ocean to the Central Pacific Ocean.

Deshayes (1863) described and illustrated *Bulla eximia* Deshayes, 1863 based on shell specimens from

Réunion Island. Deshayes recognized the similarities between *Bulla eximia* and *Bulla undata* and suggested they could be variations of the same species. As in the case of *B. guamensis*, Deshayes' description and illustrations of *Bulla eximia* are consistent with a species of *Micromelo*. The only remaining syntype of *Bulla eximia* (MNHN-IM-2000-27690) was illustrated by Valdés and Héros (1998) and is also consistent with the original description.

According to Černohorsky (1987) the type specimens of the taxa described by Andrew Garrett are primarily at the Bernice P. Bishop Museum, Honolulu, although several types are also in the Academy of Natural Sciences of Drexel University, Philadelphia. According to Norine Yeung, Malacology Curator at the Bernice P. Bishop Museum, there are no type specimens for *Bulla scripta* among the collections. Also, a search of the Academy of Natural Sciences of Drexel University online database revealed no type specimens of *B. scripta*. Therefore, the type material of *B. scripta* is probably lost.

Micromelo scriptus is distinguishable from other species of *Micromelo* by having widely spaced white spots on the body, with the distance between spots equal or larger than the spots themselves, a headshield as long as wide, prominently narrower mid-length, with posterior smooth margins compared with *M. undatus*, an elongate shell with the apex extending beyond the posterior end of the aperture, an oesophagus and salivary glands relatively short, and an oval penis, almost as wide as long, with a subapical curved stylet having a swollen tip.

Micromelo barbarae sp. nov.

(Figs 2H–J, 9, 10)

Zoobank registration. urn:lsid:zoobank.org:act:02091D97-0AE1-4AE4-9910-726C4F0ABE2F

Holotype. NMP – 041297, (formerly CASIZ 217409), Balanoy dive site (13.6864°N, 120.8912°E), Calumpan Peninsula, Mabini, Batangas, Luzon, Philippines, 4 Apr 2016, 6–33 m depth, collected by T. Gosliner, 4 mm preserved length, sequenced (isolate KF78).

Paratypes. CASIZ 208475 A–B, 2 specimens, one dissected, Boulders dive site (13.51104°N, 120.98422°E), Puerto Galera, Mindoro Oriental, Philippines, 31 Mar 2015, 6–33 m depth, 10 mm preserved length (CASIZ 208475 A–B), specimens A–B sequenced (isolates KF79–80). Hachijō-jima, Hachijo, Japan, 24 May 2016, 1 specimen, 8 mm preserved length (ZMBN 106876), sequenced (isolate KF128).

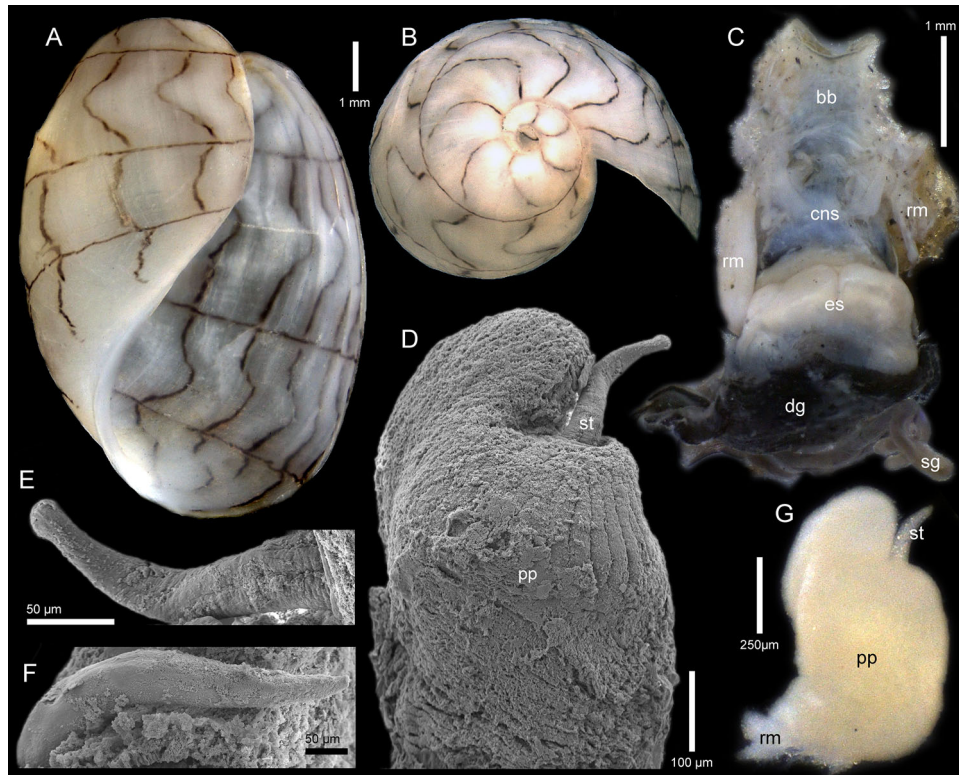


Fig. 7. Anatomy of *Micromelo scriptus*. **A–B.** Shell of specimen from the Hawaiian Is. (CPIC 1697 A), aperture view (A), apical view (B). **C.** Digestive system of a specimen from Mozambique (ZMBN 94269 D). **D–F.** Scanning electron micrograph of penis and penial stylets of specimens from the Hawaiian Is., penis (CPIC 0329 A) (D), penial stylet (CPIC 0329 A) (E), penial stylet (CPIC 1697 A) (F). **G.** Photograph of the penis of a specimen from the Hawaiian Is., (CPIC 0329 A). Abbreviations: bb, buccal bulb; cns, central nervous system; dg, digestive gland; es, esophagus; pp, penis; rm, retractor muscle; sg, salivary gland; st, stylet.

Type locality. Balanoy dive site (13.6864°N, 120.8912°E), Calumpán Peninsula, Mabini, Batangas, Luzon, Philippines.

Etymology. This species is named in honor of Barbara Weller, grandmother of the first author, for inspiring her love of marine science and genetics from a young age and in appreciation for funding her first year of graduate school tuition.

External morphology (Figs 2H–J, 9A, B): Body elongate, translucent grey, covered with large, oval or compound opaque white spots, widely spaced – distance between spots equal or larger than the spots themselves. Mantle, rhinophores, and foot with a marginal yellow-orange band followed by a pale green diffuse area. Headshield longer than wide, with large posterior extensions, narrower close to the anterior end of the body. Shell elongate, longer than wide, with convex sides. Apex extending slightly beyond the posterior end of the aperture. Protoconch with black pigment.

Internal morphology (Figs 9C–F, 10). Digestive system with an oval, elongate buccal bulb, with two short salivary glands, not reaching the digestive gland. Oesophagus as long as the buccal bulb. Radular formula $23 \times 9.1.1.1.9$. Rachidian tooth present, narrow and elongate. Innermost tooth elongate, smaller, narrower than rest of inner lateral teeth, with 4–6 denticles and a prominent upper extension. Other inner lateral teeth with 4–8 denticles, hook-shaped with a prominent upper extension. Mid-lateral teeth with 0–3 denticles, hook-shaped. Outermost teeth with same shape but no denticles. Penis is short and round with two large proximal retractor muscles and a subapical slightly curved stylet.

Geographic range. Philippines and Japan (present study).

Remarks. *Micromelo barbara* sp. nov. is genetically distinct from other species recovered in this study. The specimens of *M. barbara* sp. nov. examined showed a distinct black marking on the protoconch as well as a short, rounded penis with a slightly curved stylet, an

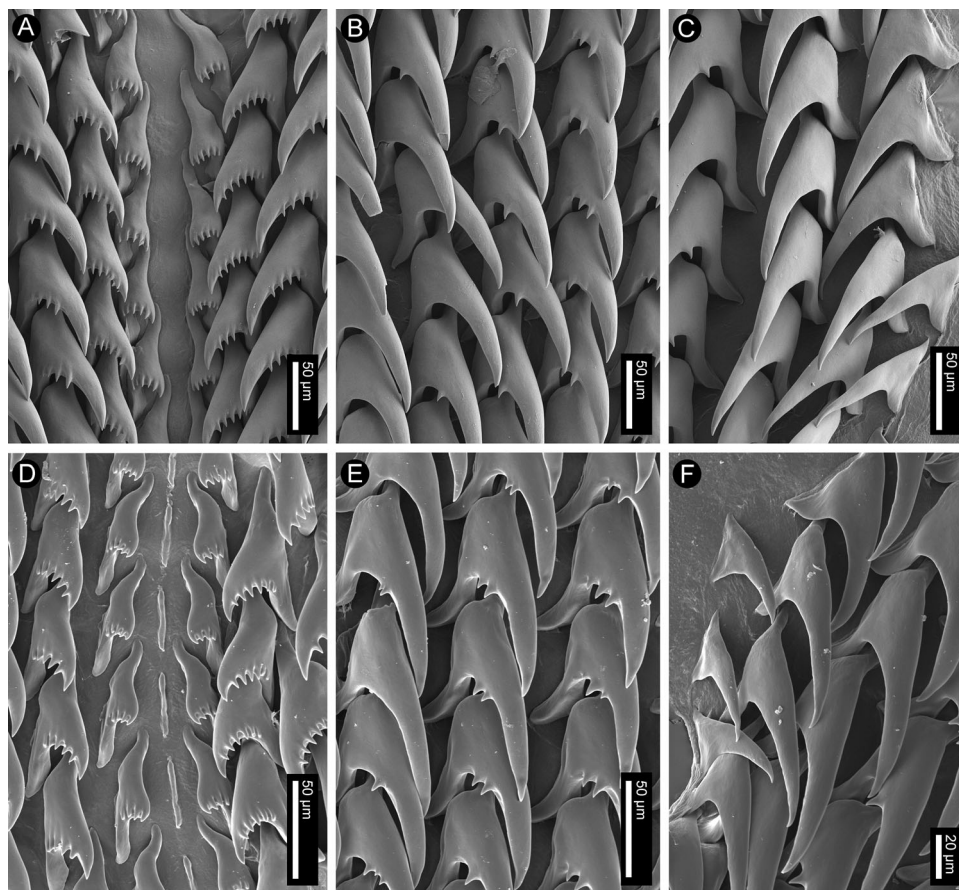


Fig. 8. *Micromelo scriptus*, scanning electron micrographs of radulae of. **A–C.** Specimen from the Hawaiian Is. (CPIC 1697B), innermost teeth (A), mid-lateral teeth (B), outermost teeth (C). **D–F.** Specimen from Mozambique (ZMBN 94269), rachidian and innermost teeth (D), mid-lateral teeth (E), outermost teeth (F).

elongated buccal mass, short salivary glands and a radula with fewer rows of teeth and fewer denticles than the other species examined. External distinguishing characteristics of this species include the presence of widely spaced white patches on the body, a longer than wide headshield, with large posterior extensions, and a shell with the apex extending slightly beyond the posterior end of the aperture.

Discussion

The results of this study confirm that *Micromelo undatus*, previously thought to be a single species with a pantropical range (Gosliner et al., 2018; Rudman, 1972), is actually a species complex (Table 6). Species delimitation analysis of COI mtDNA and 16S mtDNA sequence data recovered four distinct candidate species (with the exception of the COI Kimura-2 result that recovered only three candidate species – the widespread Indo-Pacific and the Atlantic species were not recovered

as distinct). The four candidate species also represent different haplogroups in the haplotype network analysis, further supporting their distinctiveness. Moreover, phylogenetic analysis revealed four clades within *Micromelo*, which correspond to the candidate species recovered in the ABGD analysis and the haplogroups. However, only three of the four clades were well supported in both maximum likelihood and Bayesian analyses, as the widespread Indo-Pacific clade (*M. scriptus*) was not supported in the maximum likelihood analysis. This clade/candidate species is the only one distributed across the entire Indo-West Pacific, and the samples here examined include mainly the two extremes of the range (Mozambique and the Hawaiian Is.), while the coverage for intermediate localities is limited. This could explain why the analyses here presented only achieved partially supported phylogenetic recovery for this clade. However, because *M. scriptus* is sister to the Atlantic clade and these two clades are clearly distinguishable morphologically and geographically, there is no question they constitute different species.

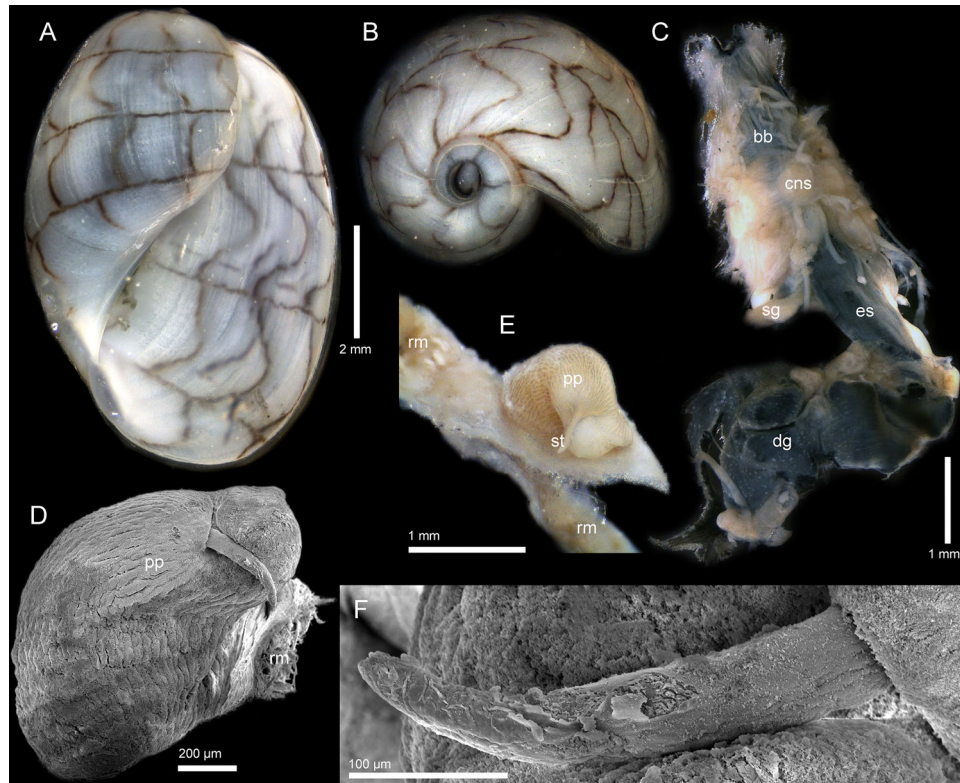


Fig. 9. Anatomy of *Micromelo barbarae* sp. nov. **A–B.** Shell of a specimen from Japan (ZMBN 106876, paratype), aperture view (A), apical view (B). **C.** Digestive system of a specimen from the Philippines (CASIZ 208475 A, paratype). **D–F.** Scanning electron micrographs of penis and penial stylet of a specimen from the Philippines (CASIZ 208475 A, paratype). **E.** Photograph of the penis of a specimen from the Philippines (CASIZ 208475 A, paratype). Abbreviations: bb, buccal bulb; cns, central nervous system; dg, digestive gland; es, esophagus; penis; rm, retractor muscle; sg, salivary gland; pp, st, stylet.

A review of the literature, geographic information, and available type material indicates that three of the four species have valid available names that can be applied to them. The name *M. undatus* is here proposed for the species distributed across the Atlantic Ocean. The name *M. guamensis* is resurrected for a species found in Guam, Papua New Guinea, the Philippines, and Marshall Islands with features matching those of the original description of this nominal species. The species widespread across the Indo-Pacific matches well the original description of *M. scriptus* and therefore this name is applied to it. There is no name available for another species found in the Western Pacific with representatives from the Philippines and Japan, thus, it is here described as *M. barbarae* sp. nov.

The external color pattern can be used to distinguish species to a certain degree. For example, the size and shape of the white spots on the body and the distance between them varies between species (see systematics section). However, the colour pattern shows natural intraspecific variation within all four species, although some colours are more common than others. Specifically, yellow body margin color is more likely to

be found in the Atlantic species *M. undatus*, and a vibrant blue body margin is often seen in the southwestern Pacific species *M. guamensis*. This could be explained by the hypothesis that body colour may depend to a certain degree on prey composition, if for instance pigment is sequestered as in many sacoglossan species (Cruz *et al.*, 2015; Middlebrooks *et al.*, 2012). *Micromelo* are known to sequester chemicals, such as toxins, from their cirratulid polychaete prey for protection (Gosliner *et al.*, 2018).

The results of this study are consistent with findings of other studies on pantropical sea slug studies originally thought to be one species but were then genetically revealed to be complexes of different species existing in disjunct ocean basins (Alexander & Valdés, 2013; Carmona *et al.*, 2014; Krug *et al.*, 2013). In *Micromelo*, the ranges of three of the species, namely *Micromelo barbarae* sp. nov., *M. guamensis* and *M. scriptus* partially overlap, mainly in the Coral Triangle, an area of high biodiversity and high diversification rates located between Malaysia, the Philippines, Indonesia, and Papua New Guinea (Hoeksema, 2007). There are several hypotheses have been proposed to explain the drivers of

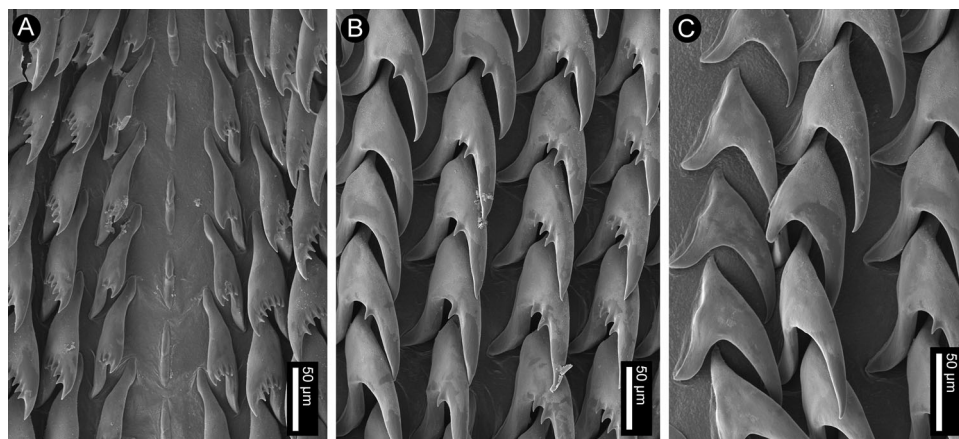


Fig. 10. *Micromelo barbarae* sp. nov., scanning electron micrographs of the radula of a specimen from the Philippines (CASIZ 208475 A, paratype). **A.** Rachidian and innermost teeth. **B.** Mid-lateral teeth. **C.** Outermost teeth.

diversity in the Coral Triangle. One proposal is that gene flow from non-overlapping species distributions in adjacent regions end up in the Western Pacific by which it becomes a Center of Accumulation for diversity (Ladd, 1960). Another hypothesis is that the Coral Triangle is a Center of Overlap where species distributions from the Indian and Pacific Oceans overlap and combine to form increased biodiversity (Woodland, 1983). A third idea is the Center of Origin hypothesis (Ekman, 1953), which suggests that speciation occurs at higher rates within the Coral Triangle with subsequent dispersal to other regions. In the case of *Micromelo*, the fact that *M. barbarae* sp. nov. and *M. guamensis* exist solely in the Western Pacific Ocean and were the first of the four species to diverge (Fig. 3A) appears to support the Center of Origin hypothesis.

Another intriguing aspect of the biogeography of *Micromelo* is the absence of species from the Eastern Pacific. For several pantropical species complexes, the closure of the Isthmus of Panama approximately three million years ago, is hypothesized to have provided the barrier to gene flow necessary for speciation to occur between Atlantic and Eastern Pacific geminate taxa (Knowlton et al., 1993; Leigh et al., 2014). If this is the case in *Micromelo*, the absence of Eastern Pacific species could be explained by a local extinction event caused by the changes in current flow, water temperature and salinity after the Isthmus formed as had occurred in several other cases (Lessios, 2008). An alternate hypothesis, based on the fact that *Micromelo* is present in the Western Indian Ocean and that *M. scriptus* is sister to the Atlantic species *M. undatus*, is that *Micromelo* has never occurred in the Eastern Pacific and perhaps the Atlantic species is the result of dispersal across South Africa into the Atlantic during warmer periods of the Plio-Pleistocene as suggested for other

sea slug groups (Churchill et al., 2014; Golestani et al., 2019).

The advent of molecular systematics has led to the discovery of previously unrecognized pseudocryptic diversity (Carmona et al., 2014; Golestani et al., 2019; Lindsay & Valdés, 2016; Pola et al., 2012; Sørensen et al., 2020). Early identifications of *Micromelo* have been based solely upon morphological observations. This study provides the first integrative approach to the taxonomy of this understudied bubble snail genus. Incorporation of molecular and morphological data in this study revealed a species complex of four genetically and morphologically distinct species in what was formerly believed to be a pantropical species. Even with a limited sample size, consistent differences were found among individuals collected in different ocean basins. Further understanding of the ecology of this genus such as larval developmental modes, feeding preferences, and reproductive strategies are required in order to further examine important factors that may play a role in speciation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplemental material

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