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Stargazing under the sea: molecular and morphological data reveal a constellation of species in the *Berthella stellata* (Risso, 1826) species complex (Mollusca, Heterobranchia, Pleurobranchidae)

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Abstract

Molecular and morphological evidence support the view that the widely distributed species *Berthella stellata* (Risso, 1826) is a species complex of at least eight different species. The closely related species *Berthella plumula* (Montagu, 1803), examined for comparison, is also a complex of two species; the name *B. plumula* is retained for the Atlantic species and the name *Berthella perforata* (Philippi, 1844) is proposed for the Mediterranean species. The *B. stellata* species complex forms a monophyletic group when the Eastern Pacific species *Berthella strongi* (MacFarland, 1966) is included. Based on a critical review of the literature, the name *Berthella stellata* is retained for the Eastern Atlantic and Mediterranean species, and the name *Berthella pellucida* (Pease, 1860) is resurrected for a species found in the Hawaiian Islands. Two new species from the Caribbean region (*Berthella nebula* sp. nov., *Berthella vialactea* sp. nov.) and one from the Eastern Pacific (*Berthella andromeda* sp. nov.) are described herein, but the status of the Brazilian species *B. tupala* Er. Marcus, 1957 remains uncertain. Two possible new species from the Eastern Pacific, represented by one specimen each, were recovered in the phylogenetic analyses but not formally described. It is hypothesized that additional species of this complex may occur in other parts of the Indo-Pacific tropics, particularly in the Indian Ocean.

Keywords Systematics · Taxonomy · Cryptic species · Biogeography · New species

Introduction

Morphology-based approaches to heterobranch sea slug systematics have sometimes produced conservative classification schemes, resulting in nominal species having large, disjunct geographic ranges across different ocean basins(e.g., Thompson and McFarlane 1967; Bebbington 1977). This is due in part to the lack of distinguishing morphological characters among recently diverged taxa, known as cryptic speciation (Churchill et al. 2014; McCarthy et al. 2019), but also to

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a tendency by authors to regard small morphological differences as intraspecific variation (discussed by Jörger et al. 2012 and Hoover et al. 2015). Molecular approaches have shown that most of such species with broad geographic distributions are in fact cryptic or pseudocryptic species complexes, with much narrower ranges (e.g., Ornelas-Gatdula et al. 2012; Carmona et al. 2014; Uribe et al. 2018; Valdés et al. 2017). The identification of undescribed cryptic diversity has important implications for reliable estimates of biodiversity and species richness (Jörger and Schrödl 2013). Moreover, identifying new sea slug species and unraveling their phylogenetic relationships can shed light on evolutionary processes (Churchill et al. 2013) or have implications for other fields of science (Lindsay and Valdés 2016).

Berthella stellata (Risso, 1826) is an example of a nominal species of sea slug with a large and disjunct geographic range across several ocean basins (Gosliner and Bertsch 1988). It was originally described from the Mediterranean Sea, as having a yellowish white, oval body with scattered opaque white "star-like" markings on the mantle (Risso 1826). Subsequently, several other species or subspecies of *Berthella* with dorsal scattered opaque white markings and/or some variation of an opaque white color patch near the center of the mantle have been described from various regions, including *Berthella pellucida* (Pease, 1860) from the Hawaiian Islands, *Berthella tupala* Er. Marcus, 1957 from Brazil, *Berthella postrema* Burn, 1962 from eastern Australia, and *Berthella stellata albocrossata* Heller & Thompson, 1983 from the Red Sea. Gosliner and Bertsch (1988) conducted a detailed anatomical review of all those taxa, based on specimens collected from the Mediterranean, the Caribbean Sea, and the Pacific and Indian Oceans. Their results revealed a great deal of variation in external and internal morphology within nominal species, and no consistent morphological variation among taxa. This conclusion supported Thompson's (1985) documentation of substantial variation in color pattern, but no correlation with geographic origin in *B. stellata* from the Mediterranean Sea. Gosliner and Bertsch (1988) suggested that the best explanation for the observed morphological variation in this group is to consider the variation to be intraspecific. Based on this approach, these authors considered *B. pellucida*, *B. tupala*, *B. postrema*, and *B. stellata alboscrossata* to be junior synonyms of *B. stellata*, which gave *B. stellata* a widespread distribution across several ocean basins, as Gosliner and Bertsch (1988) also reported this species from the Eastern Pacific for the first time.

In this paper, we examine the molecular and morphological variation among *B. stellata* populations across the known range of this species. The main goal is to determine whether *B. stellata* could constitute a complex of geographically isolated species. In the molecular analyses, we also included specimens of the closely related species *Berthella strongi* (MacFarland, 1966) and *Berthella plumula* (Montagu, 1803) for comparison. This resulted in additional taxonomic changes addressed in this paper.

Materials and methods

Source of specimens

A total of 30 specimens and tissue samples of specimens identified as *B. stellata* were obtained from various geographic locations covering a substantial portion of the known range of this putative species (Table 1). Sequences for one additional specimen of *B. stellata* (from Italy) were downloaded from GenBank. Additionally, seven specimens of *Berthella strongi* (MacFarland, 1966) from the Eastern Pacific were obtained for this study, based on the fact that our preliminary phylogenies indicated a close relationship with *B. stellata*; DNA of two of the specimens of *B. strongi* did not amplify; thus, they were only used for morphological examination. For phylogenetic analysis comparison, sequences of four specimens of *Berthella plumula* (Montagu, 1803) and one of *Berthella californica* (Dall, 1900) (outgroup) were included in the analyses. Specimens were deposited at the California State Polytechnic University Invertebrate collection (CPIC), the California Academy of Sciences Invertebrate Zoology collection (CASIZ), the Natural History Museum of Los Angeles County (LACM), the Muséum National d'Histoire Naturelle, Paris (MNHN), and the Zoologische Staatssammlung, Munich (ZSM). All the specimens collected for this study were preserved in 95% ethanol.

DNA extraction, amplification, and sequencing

DNA was extracted from all the specimens using a hot Chelex® (Bio-Rad, Hercules, CA) protocol. First, a small piece (1–3 mg) of tissue was removed from the foot of the animal and cut into smaller pieces using a sterile razor blade. The pieces of tissue were then placed in a 1.75-mL tube along with 1.0 mL of Tris-EDTA (TE) buffer (10 mM Tris, 1.0 mM ethylenediaminetetraacetic acid, pH 8.0) and placed on a rotator for at least 20 min to rehydrate the tissue. The solution was then centrifuged for 3 min at 21,130×*g* and 975.0 μL of the supernatant was removed afterwards without disturbing the pellet. Next, 175.0 μL of a 10% (w/v) Chelex® 100 (US Standard 100–200 mesh, sodium form), previously prepared using TE buffer, was added to the tube containing the tissue. The samples were then placed in a water bath at 56 °C for at least 20 min and then in a heating block at 100 °C for exactly 8 min. After the heating stages, the samples were centrifuged for 3 min at 21,130×*g*. The supernatant was used for PCR.

Universal Histone H3 (Colgan et al. 1998) and COI primers (Folmer et al. 1994) were used to successfully amplify the gene fragments of interest for all the specimens (Table 2).

Table 1 List of specimens sequenced for this study including final species names, locality, museum voucher numbers, isolate numbers, collection date, and GenBank accession numbers

Species	Locality	Voucher	Isolate	Date	GenBank accession numbers		
					16S	COI	H3
B. andromeda sp. nov.	Mazatlán, Mexico	LACM 3654	SG15	23 Oct 13	MK542708	MK542746	MK542774
B. andromeda sp. nov.	Mazatlán, Mexico	CPIC 01418	SG16	24 Oct 13	MK542709	–	MK542775
B. andromeda sp. nov.	El Tomatal, Baja California, Mexico	CASIZ 182217A	SG41	31 Dec 09	MK542710	–	MK542776
B. nebula sp. nov.	Bocas del Toro, Panama	CASIZ 172853	SG44	15 Jun 05	MK542711	–	MK542777
B. nebula sp. nov.	Anse Noir, Martinique	CPIC 02097	SG84A	31 Mar 15	MK542712	MK542747	MK542778
B. nebula sp. nov.	Anse Noir, Martinique	CPIC 02098	SG84B	1 Apr 15	MK542713	MK542748	MK542779
B. nebula sp. nov.	Anse Marette, Martinique	MNHN IM-2000-34532	SG85B	Mar 15	MK542714	MK542749	MK542780
B. nebula sp. nov.	Anse Marette, Martinique	CPIC 02102	SG85D	Mar 15	MK542715	MK542750	MK542781
B. nebula sp. nov.	St. James, Jamaica	CPIC 00655	JG57	18 Jul 11	KM521594	KM521591	KM521621
B. nebula sp. nov.	Anse Marette, Martinique	CPIC 02437	SG103	17 Mar 18	–	MK542751	MK542782
B. pellucida	Maui, Hawaiian Is.	CPIC 01714	SG70A	27 Jun 16	MK542716	MK542752	MK542783
B. pellucida	Maui, Hawaiian Is.	CPIC 01714	SG70B	27 Jun 16	MK542717	MK542753	MK542784
B. pellucida	Maui, Hawaiian Is.	CPIC 01689	SG98	25 Jun 16	MK542718	MK542754	MK542785
B. stellata	Porto, Portugal	CPIC 01391	SG17	13 Jul 15	MK542719	MK542755	MK542786
B. stellata	Elba Is., Italy	ZSM 20013043	SG29	1 Jul 01	MK542720	MK542756	MK542787
B. stellata	Elba Is., Italy	ZSM 20013041	SG32	1 Jul 01	MK542721	MK542757	MK542788
B. stellata	Trapani, Sicily, Italy	ZSM 20110680	SG66A	21 Oct 11	MK542722	MK542758	–
B. stellata	Trapani, Sicily, Italy	ZSM 20110680	SG66B	21 Oct 11	MK542723	MK542759	–
B. stellata	Es Caials, Catalonia, Spain	CPIC 02105	SG74A	24 May 16	MK542724	MK542760	MK542789
B. stellata	Es Caials, Catalonia, Spain	CPIC 02106	SG74B	24 May 16	MK542725	MK542761	MK542790
B. stellata	Muros de Nalón, Asturias, Spain	CPIC 00445	JG74	28 Aug 10	MK542726	KM521593	KM521623
*B. stellata**	Italy	–	–	–	FJ917430	FJ917488	–
B. strongi	Pt. Sur Lighthouse, California	CPIC 01411	SG10	18 May 17	MK542727	–	MK542791
B. strongi	Naples, Santa Barbara, California	CPIC 01410	SG11	3 Dec 09	MK542728	–	MK542792
B. strongi	La Jolla, California	CPIC 01408	SG14	4 Jun 00	MK542729	MK542762	MK542793
B. strongi	Punta Rosarito, Baja California, Mexico	CASIZ 122181A	SG54	29 May 00	MK542730	–	MK542794
B. strongi	El Tomatal, Baja California, Mexico	CASIZ 182722A	SG55	31 Dec 10	MK542731	–	MK542795
B. vialactea sp. nov.	Anse Marette, Martinique	CPIC 02096	SG83	28 Jan 17	MK542732	–	MK542796
B. vialactea sp. nov.	Anse Marette, Martinique	CPIC 02099	SG85A	Mar 14	MK542733	MK542763	MK542797
B. vialactea sp. nov.	Anse Marette, Martinique	MNHN IM-2000-34531	SG85C	Mar 14	MK542734	MK542764	MK542798
B. vialactea sp. nov.	Le Diamant, Martinique	CPIC 02434	SG100	26 Mar 17	MK542735	MK542765	MK542799
B. vialactea sp. nov.	Le Diamant, Martinique	CPIC 02435	SG101	26 Mar 17	–	MK542766	–
B. vialactea sp. nov.	Le Diamant, Martinique	CPIC 02436	SG102	26 Mar 17	MK542736	–	MK542800
B. sp. 1	Canal de Afuera, Veraguas, Panama	LACM 153343	JG78	21 May 03	MK542737	KM521592	KM521622

Table 1 (continued)

Species	Locality	Voucher	Isolate	Date	GenBank accession numbers		
					16S	COI	H3
B. sp. 2	Isla Darwin, Galapagos Is., Ecuador	CASIZ 097563	SG39	13 May 94	MK542738	–	MK542801
B. perforata	Galenzana, Elba Is., Italy	ZSM 20013109	SG18	1 Jul 01	MK542739	MK542767	–
B. perforata	Galenzana, Elba Is., Italy	ZSM 20012312	SG56	12 Apr 01	MK542740	MK542768	–
B. cf. *postrema*	Koumac, New Caledonia	MNHN KM322-AV0584	SG104	27 Sep 18	MK542741	MK542769	MK542802
B. plumula	Ballyhenry Is., Northern Ireland, UK	CASIZ 193034	SG53	4 Aug 13	MK542742	MK542770	MK542803
B. plumula	Finistère, Brittany, France	ZSM 20050612	SG58	23 May 05	MK542743	MK542771	–
B. plumula	Finistère, Brittany, France	ZSM 20034195	SG64	28 Jun 03	MK542744	MK542772	MK542804
*B. plumula**	France (Atlantic)	–	–	–	FJ917435	FJ917493	–
B. californica	Ventura, California	CPIC 01328	SG1	8 Nov 14	MK542745	MK542773	MK542805

CASIZ California Academy of Sciences Invertebrate Zoology, *CPIC* Cal Poly Pomona Invertebrate Collection, *LACM* Natural History Museum of Los Angeles County, *MNHN* Museum National d'Histoire Naturelle, Paris, *ZSM* Zoologische Staatssammlung, Munich

*Sequences obtained from GenBank

Universal 16S rRNA primers (Palumbi 1996) were initially used to amplify the 16S rRNA partial region but produced suboptimal results. Thus, new *Berthella*-specific primers were designed to amplify a portion of the region of interest (Table 2).

The PCR master mix was prepared using 37.25 μL of deionized water, 5.00 μL of Dream Taq PCR buffer (Fischer Scientific, Hampton, NH), 2.5 μL of 10 mg mL^{-1} bovine serum albumin (BSA), 1.00 μL of 40 mM deoxynucleotide triphosphates, 1.00 μL of 10 μM primer 1, 1.00 μL of 10 μM primer 2, 0.25 μL of 5 mg mL^{-1} of Dream Taq (Fischer Scientific, Hampton, NH), and 2.00 μL of extracted DNA. The reaction conditions for the 16S rRNA and Histone H3 genes were the same and as follows: an initial denaturation for 2 min at 94 °C, followed by 30 cycles of (1) denaturation for 30 s at 94 °C, (2) annealing for 30 s at 50 °C, and (3) elongation for 1 min at 68 °C, and a final elongation for 7 min at 68 °C. Reaction conditions for COI were an initial denaturation for 3 min at 95 °C, 35 cycles of (1) denaturation for 45 s at 94 °C, (2) annealing for 45 s at 45 °C, and (3) elongation for 2 min at 72 °C, and a final elongation for 10 min at 72 °C. An agarose gel electrophoresis with ethidium bromide was run with PCR products and viewed under UV light to identify samples producing bands of appropriate size (~ 375 bp for Histone H3, ~ 495 bp for 16S rRNA, and ~ 695 bp for COI). PCR products were purified using a GeneJET PCR purification kit (Thermo Scientific, Waltham, MA) by following the manufacturer's protocol. DNA concentration in purified PCR products was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). The primers were diluted to 4.0 μM and the PCR products were diluted to between 5 and 30 ng μL^{-1} before being submitted to Source BioScience (Santa Fe Springs, CA) for Sanger sequencing.

Phylogenetic analyses

The sequences obtained for each gene were edited, assembled, and extracted using Geneious Pro R8 (Kearse et al. 2012). Sequences were aligned using the MUSCLE (Edgar 2004) plugin in Geneious and the alignments were also concatenated in Geneious. The program jModelTest (Posada 2008) was used to execute the Akaike information criterion (Akaike 1974) to determine the best-fit model of evolution for each gene sequence: GTR + I + G (16S), GTR + I (COI), GTR + I (H3), and GTR + I + G (concatenated data set). Bayesian and maximum likelihood phylogenetic analyses were conducted on the concatenated sequences and each gene individually. The Bayesian analyses were implemented in MrBayes 3.2.1 (Ronquist et al. 2012), partitioned by gene with two runs of six chains for 10 million repetitions, with a sampling interval of 1000 repetitions and burn-in of 25%. The maximum likelihood analyses were conducted in raxmlGUI 1.0 (Silvestro

Table 2 List of forward (F) and reverse (R) primers used to amplify the nuclear Histone H3 gene and the mitochondrial 16S rRNA and COI genes

Gene name	Primer name	Sequence 5′–3′	Source
Histone H3	HexAF (F)	ATG GCT CGT ACC AAG CAG ACG GC	Colgan et al. (1998)
	HexAR (R)	ATA TCC TTG GGC ATG ATG GTG AC	Colgan et al. (1998)
16S rRNA	16Sar-L (F)	CGC CTG TTT ATC AAA AAC AT	Palumbi et al. (1996)
	16Sbr-H (R)	CCG GTC TGA ACT CAG ATC ACG T	Palumbi et al. (1996)
16S rRNA	16Sar-L (F) modified	CGA CTA GTT TAC CAA AAA CAT	Present study
	16Sbr-H (R) modified	CCG GTC TGA ACT CAG ATC ATG T	Present study
Cytochrome *c* oxidase I	LCO1490 (F)	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. (1994)
	HCO2198 (R)	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. (1994)

and Michalak 2012) for the concatenated alignment of all three genes concatenated and each gene individually, using the bootstrap + consensus option and the GAMMAGI model with 10,000 bootstrap repetitions.

Species delimitation analysis

The Automatic Barcode Gap Discovery (ABGD) analysis was implemented using the COI and 16S rRNA mitochondrial sequences to determine the number of species present in the dataset, based on the gap in the distribution of pairwise distances among sequences. MEGA 7.0.16 (Kumar et al. 2016) was used to calculate the pairwise distances for the data set using the Kimura 2-parameter (K2) and Tamura-Nei (TN) models. The distance data matrix was then analyzed using the ABGD webtool (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) (Puillandre et al. 2012) at default settings ($X = 1.5$, $P = 0.001$–0.1).

Morphological analyses

Based on availability of material, 1 to 5 specimens from each species recovered in the species delimitation analysis were examined morphologically to substantiate the molecular findings. Photographs of the animals were obtained after collection of the specimens in the field. Photographs of 1 to 5 specimens from each clade recovered in the molecular analyses were compared to one another for similarities and differences in external morphology. The shell and the buccal mass containing the jaw and radula were dissected using a Leica EZ4D stereo dissecting microscope. The buccal mass and the shell were placed in a 10% NaOH solution for about 45 min to dissolve excess tissue. The jaw was then removed and placed in deionized water for about 5 min to remove any residual NaOH before being mounted on an SEM stub. The radula and the shell were left in the NaOH solution for up to 3 days to dissolve any remaining tissue and then placed in deionized water for 5 min and mounted on a SEM stub. These structures were then sputter coated. Images were obtained using a Jeol JSM-6010 scanning electron microscope (SEM) at the California State Polytechnic University, Pomona. The reproductive system of each specimen was also dissected and drawn using a Nikon SMZ-100 dissecting microscope with a *camera lucida* attachment. Specimens in which all reproductive organs were present were considered sexually mature.

Results

Phylogenetic analyses

Posterior probabilities (PP) ≥ 0.9 were treated as significant (Huelsenbeck and Rannala 2004), and bootstrap (BS) values ≥ 70% were treated as significant (Hillis and Bull 1993). Both Bayesian and maximum likelihood analyses of the concatenated gene sequences from specimens identified as *B. stellata*, *B. strongi*, and *B. plumula* produced trees with the same topologies but with differences in support values (Fig. 1a). In both trees, specimens identified as *B. stellata* did not form a monophyletic group as *B. strongi* was nested within *B. stellata* (Fig. 1a). Both analyses returned two main clades, one containing the specimens identified as *B. stellata* and *B. strongi*, and the other including the specimens identified as *B. plumula* (Fig. 1a). Specimens identified as *B. stellata* formed six well-supported to fully supported clades. These clades are as follows: one clade including the Hawaiian and New Caledonia specimens (the Hawaiian specimens are also monophyletic within this clade); two sister clades including the Caribbean specimens; one clade including the specimens from the Pacific coast of Panama and the Galapagos Islands; one clade including the specimens from the Pacific coast of Mexico; and one clade containing the specimens from the eastern Atlantic and the Mediterranean (Fig. 1a). Specimens identified as *B. plumula* formed a fully supported clade. Specimens identified as *B. plumula* from the Mediterranean grouped together with complete support, and *B. plumula* specimens from the eastern Atlantic formed another fully supported clade (Fig. 1a). The single-gene trees (Figs.

S1–S3) were similar to the consensus tree and recovered similar relationships. The 16S rRNA tree (Fig. S1) was nearly identical to the consensus tree (Fig. 1a). However, the COI trees were poorly resolved basally but did resolve critical nodes at the base of the *B. stellata* species complex (Fig. S2). On the contrary, the Histone H3 tree was not well-resolved at the species level and was unable to recover the two Caribbean species and the Eastern Pacific species as reciprocally monophyletic (Fig. S3).

Molecular species delimitation

The ABGD analysis of the mitochondrial COI and 16S rRNA sequences produced the same results (Table 3) with the exception that there was no COI sequence data for isolate SG39. Both analyses recovered 11 distinct candidate species (10 in COI) using both the K2 and TN distance matrices (Table 3). The 11 candidate species correspond to the clades recovered in the concatenated Bayesian and ML analyses (Fig. 1a). The distribution of pairwise distances are presented in Fig. 1b for COI and Fig. S4 for 16S rRNA.

Morphological analyses

There were consistent differences in one or more aspects of external and/or internal morphology of the candidate species recovered as distinct in the ABGD analysis. In some cases, species were indistinguishable based on external coloration and patterns, but characters of internal morphology exhibited consistent and clear differences (Table 4).

Systematics

Family Pleurobranchidae Gray, 1827

Genus *Berthella* de Blainville, 1824

Berthella stellata (Risso, 1826) (Figs. 2a–c, 3, 4, and 5a)

Pleurobranchus stellatus Risso, 1826: 41. Type locality: Nice, France.

Type material: Probably lost (Arnaud 1978).

Material examined: Muros de Nalón, Asturias, Spain, 28 Aug 2010, 1 specimen 9 mm preserved length, dissected, leg. A. Valdés (CPIC 00445). Praia de Gondarém, Porto, Portugal, 13 Jul 2015, 1 specimen 5 mm preserved length, dissected, leg. A. Valdés et al. (CPIC 01391). Galenzana, Elba, Italy, Jul 2001, 1 specimen 11 mm preserved length, dissected, leg. P. Durán et al. (ZSM 20013041); 1 specimen 11 mm preserved length, leg. P. Durán et al. (ZSM 20013043). Bastione, Sicily, Italy, 21 Oct 2011, 2 specimens 4.5–7 mm preserved length, dissected, leg. V. Padula (ZSM 20110680). Es Caials, Catalonia, Spain, 24 May 2016, 1 specimen 16 mm preserved length, dissected, leg. M. Ballesteros (CPIC 02161).

Diagnosis: Animal translucent cream to reddish brown, with numerous small opaque white markings and/or a single Y-shaped, irregular, or transverse patch; gill with 11 pinnae, 7 free of body wall; shell oval, convex; protoconch 213–233 μm in diameter with 1.5 whorls; radular inner and mid-lateral teeth hook-shaped lacking denticles; outer teeth with a single cusp; jaw elements with 3–6 pointed denticles; bursa copulatrix about 4 times as large as seminal receptacle, connected semiserially; penial gland elongate; penis conical, retractable.

Description: Body broad, convex to flat in lateral view, oval to rounded in dorsal view, with mantle covering foot on all sides (Fig. 2a–c). Mantle translucent cream, or yellowish brown to reddish brown, covered with irregular ridges. Few to numerous opaque white markings scattered throughout, forming stellate or irregular patterns throughout mantle in most specimens. Dorsal white markings forming “Y” shaped to irregular pattern or transverse bar near central notum in some specimens. Foot translucent white to light brown. Oral veil broad, trapezoidal, translucent white to light brown with few to numerous scattered small opaque white spots. Rhinophores enrolled, fused together at base, emerging between mantle and oral veil, translucent white to opaque yellowish or reddish brown, sometimes with few scattered white spots along, and one white spot at tip of each rhinophore. Gill bipinnate, long, occupying three fourth of body length, with 11 pinnae (7 in portion of gill not attached to body wall) on each side of smooth rachis in a 5-mm specimen from Portugal (CPIC 01391) and with 18 pinnae (13 in portion of gill not attached to body wall) in 16 mm specimen from Spain (CPIC 02161).

Shell oval, convex (Fig. 3a, c, e) covering entire body. Sculpture of punctate radial grooves crossing distinct concentric growth lines (Fig. 3f). Shell dimensions: 8.75 × 5.5 mm (11 mm preserved length specimen from Elba, Italy—ZSM 20013041); 3.25 × 2 mm (7 mm preserved length specimen from Sicily, Italy—ZSM 20110680); 2.75 × 1.7 mm (4.5 mm preserved length specimen from Sicily, Italy—ZSM 20110680). Protoconch oval, 213–233 μm in diameter (Fig. 3b, d).

Radular formula 57 × 69.0.69 in 11 mm specimen from Elba Is., Italy (ZSM 20013041) and 54 × 65.0.65 in 11 mm specimen from Elba, Italy (ZSM 20013043). Inner teeth simple (Fig. 4a), hook-shaped with single short cusp. Mid-lateral teeth (Fig. 4b) simple, hook-shaped with cusp size increasing toward outer margin. Outer teeth (Fig. 4c) elongate, slender sometimes with or without a secondary denticle in same specimen. Jaw elements in distal view with central cusp bearing 3–6 pointed denticles on each side in both specimens examined (Fig. 4d); denticulation not always symmetrical. Jaw elements in proximal view with indentation near center of cusp (Fig. S5a).

Reproductive system (Fig. 5a) androdiaulic. Ampulla long, curved, not convoluted, branching into short oviduct and long, slender prostate; ampulla nearly twice as long as the prostate. Penial gland elongate, convoluted, narrowing proximally for

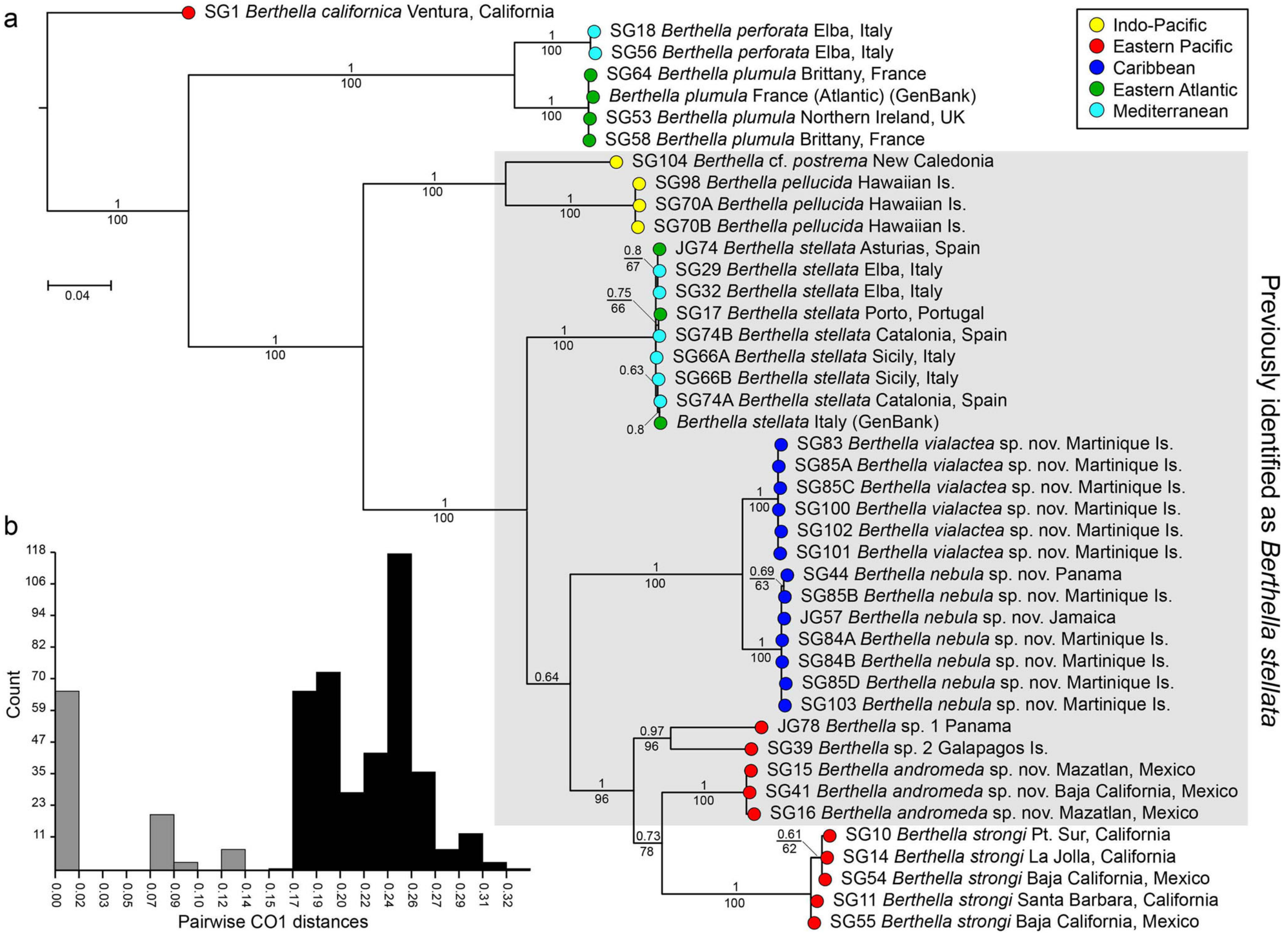


Fig. 1 **a** Bayesian consensus phylogenetic tree of *Berthella strongi* (MacFarland, 1966) and members of *Berthella stellata* (Risso, 1824) and *B. plumula* (Montagu, 1803) species complexes, with *B. californica* (Dall, 1900) as the outgroup. Posterior probabilities indicated above branches, and maximum likelihood bootstrap support values indicated below branches. Colored dots on the branch tips indicate the geographic region of origin for the specimen. Specimen labels include isolate number, species name, and locality. Species names are the final names proposed in this study; specimens previously identified as *B. stellata* shaded in gray. **b** Distributions of pairwise distances between sequences and Automatic Barcode Gap Discovery (ABGD) results for COI. Intraspecific distances indicated by white bars and interspecific distances by black bars

almost one third its length before joining short deferent duct. Seminal receptacle elongate, narrowing proximally for almost one half its length before joining stalked, rounded bursa copulatrix and vaginal canal; bursa copulatrix about 4 times as large as seminal receptacle, connected semiserially. Vaginal duct twice as long as deferent duct, opening ventral to penis. Penis conical, retractable.

Geographic range: Eastern and western Mediterranean and northeastern Atlantic Ocean, from northern Spain to Cape Verde, including the Azores, Madeira, and the Canary Islands (Thompson 1981; Cattaneo-Vietti 1986; Gosliner and Bertsch 1988; Cervera et al. 2004; Valdés 2005; Öztürk et al. 2014).

Remarks: Risso (1826) introduced the name *Pleurobranchus stellatus* Risso, 1826 based on an undetermined number of specimens collected from “coralligenous depths” near Nice, France. Risso (1826) described this species as having a round, oval body, whitish yellow in color, with small white star-shaped dots on the mantle and the internal organs visible as a dark area. Other authors assigned Mediterranean pleurobranchids with numerous star-like white spots to *P. stellatus* (e.g., Delle Chiaje 1828; Philippi 1844). In the following years, a great deal of confusion surrounded the identity of this species. Vayssière (1880) regarded *P. stellatus* as a synonym of *Bulla plumula* Montagu, 1803 (type locality Milton Sands, south coast of Devon, England), but Mazzarelli (1891) suggested that *P. stellatus* was distinct from *B. plumula* (under *Pleurobranchus*) because of the absence of star-like markings in previous records of *B. plumula* from the Mediterranean Sea (e.g., de Monterosato 1874; Vayssière 1880) which are characteristic of *P. stellatus* (Risso 1826; Delle Chiaje 1828). Mazzarelli (1891) described his material of *P. stellatus* as being yellow, with eight small four-pointed white stars, arranged regularly near to the margin. Pilsbry (1895–1896) agreed that

Table 3 Results of the ABGD species delimitation analyses of COI and 16S rRNA sequence data, using the K2 and TN models. Species recovered are organized in columns with the final names presented in the text. Isolate numbers are arranged by the species to which they belong, and missing data is indicated by dashes

Species	COI	16S
B. andromeda	SG15, —, —	SG15, SG16, SG41
B. nebula	—, SG84A, SG84B, SG85B, SG85D, JG57, SG103	SG44, SG84A, SG84B, SG85B, SG85D, JG57, SG103
B. pellucida	SG70A, SG70B, SG98	SG70A, SG70B, SG98
B. plumula	SG53, SG58, SG64, GB	SG53, SG58, SG64, GB
B. stellata	SG17, SG29, SG32, SG74A, SG74B, SG66A, SG66B, JG74	SG17, SG29, SG32, SG74A, SG74B, SG66A, SG66B, JG74
B. strongi	—, —, SG14, —, —	SG10, SG11, SG14, SG54, SG55
B. vialactea	—, SG85A, SG85C, SG100, SG101, —	SG83, SG85A, SG85C, SG100, SG101, SG102
B. perforata	SG18, SG56	SG18, SG56
B. sp. 1	—	SG39
B. sp. 2	JG78	JG78
B. cf. *postrema*	SG104	SG104

P. stellatus and *B. plumula* (under *Pleurobranchus*) were distinct species and retained the name *P. stellatus* for Mediterranean and temperate Eastern Atlantic records (from the Azores to Cape Verde), while using the name *B. plumula* for records from Northern Europe. This distinction was based on internal anatomical differences, including the presence of denticulate jaw elements and smooth lateral teeth as well as more gill pinnae and a more quadrate shell in *P. plumula* (see Pilsbry 1895–1896: 194). Bergh (1897) disagreed and maintained both *B. plumula* and *P. stellatus* as synonyms, under the genus *Pleurobranchus*, without explicit justification. Subsequently, Vayssiére (1898) examined a specimen sent to him by Mazzarelli and admitted that *P. stellatus* (which he transferred to the genus *Bouvieria* Vayssière, 1897) was different from *B. plumula* (which he moved to the genus *Berthella*), based on a detailed study of the characteristics of these species. Vayssiére (1898) described his specimen of *B. stellata* as having a single, large dorsal white patch resembling a star, which he considered a modification of the eight stars described by Mazzarelli (1891). Contrary to Pilsbry (1895–1896), Vayssière (1898) considered that *B. plumula* ranges from Northern Europe to the Western Mediterranean and includes semitransparent pale yellow to reddish yellow animals. Bergh (1897) following Vayssière (1898) regarded *B. plumula* as a member of *Berthella* but did not comment on the synonymy with *B. stellata*. More recently, Pruvot-Fol (1954) considered both *B. plumula* and *B. stellata* as distinct and valid species of *Berthella*, both present in the Mediterranean, and this opinion is currently widely accepted (e.g., Cattaneo-Vietti 1986; Cervera et al. 2004; Thompson 1981).

The distinction between *B. plumula* and *B. stellata* can be problematic, due to the fact that *B. stellata* is highly variable in color and Mediterranean and Atlantic specimens of *B. plumula* are morphologically different (Thompson 1981). Contrary to Thompson's (1981) assertion, *B. plumula* from the Mediterranean Sea [renamed *B. perforata* (Philippi, 1844) below] can also possess dorsal stellate markings (Fig. 21, m), making it very similar to some specimens of *B. stellata* in external coloration. Montagu (1803) described the mantle of *B. plumula* as having reticulate markings. This character was also observed in specimens of *B. plumula* included in this study, as well as other records of this species (e.g., Pruvot-Fol 1954; Thompson 1976). These reticulate markings are also present in Mediterranean or Atlantic representatives of *B. stellata*, but often not as clearly visible (Rudman 2003). Therefore, the presence of a conspicuous reticulate pattern along with the presence of a central large opaque white patch or concentration of white pigment can be reliably used to distinguish *B. stellata* from *B. plumula* in the region. As discussed below, other species of the *B. stellata* species complex from the tropical Indo-Pacific have conspicuous reticulate dorsal patterns.

For this paper, we examined several specimens from the Mediterranean and Eastern Atlantic identified as *B. stellata*. These specimens varied in color from translucent cream to reddish brown and were covered with either numerous small opaque white markings over the entire dorsum (star-like or irregularly shaped), and/or a single large, opaque white patch near central notum (shaped as a "Y," a transverse bar, or irregular). All these specimens formed a monophyletic group, distinct from specimens identified as *B. plumula* (animals with a conspicuous reticulate pattern and lacking concentrations of opaque white pigment on the center of the dorsum) also collected in the Mediterranean and Eastern Atlantic. Additionally, specimens of *B. stellata* grouped together in the ABGD analysis of the COI mitochondrial sequences of the available specimens including other members of the *B. stellata* species complex (Table 3), which further corroborates that Northeastern Atlantic and Mediterranean *B. stellata* constitutes a distinct species.

Table 4 Comparative table of the main diagnostic characteristics for species in the *Berthella stellata* complex examined morphologically in this paper

Species	Ground color	Dorsal pattern	Gill	Shell	Radula	Jaw elements	Reproductive system
B. stellata (Risso, 1826)	Translucent cream to reddish brown	Numerous small opaque white markings and or single Y-shaped, irregular, or transverse bar	Occupying 3/4 of body length, 11 pinnae (7 free of body wall)	Oval, convex; protoconch 213–233 μm in diameter, 1.5 whorls	Inner and mid-lateral teeth hook-shaped with no denticles; outer teeth with single cusp; formulae 54–57 × 65–69.0.65–69	3–6 pointed denticles	Bursa copulatrix about 4 times as large as seminal receptacle, connected semiserially; penial gland elongate; penis conical, retractable
B. pellucida (Pease, 1860)	Colorless translucent with reticulate pattern	Scattered opaque white and brown spots	Occupying 1/2 of body length, 8–11 pinnae (all free of body wall)	Oval, convex near protoconch, flattened near anterior margin; protoconch 275–300 μm in diameter, 1.5 whorls	Inner and mid-lateral teeth hook-shaped with no denticles; outer teeth bifid; formula 60 × 63.0.63	3–4 denticles	Bursa copulatrix and seminal receptacle connected serially, similar in size; penial gland wide, curved; penis oval, retractable
B. cf. *postrema* Burn, 1962	Translucent, with numerous opaque white specs; reticulate pattern	T-shaped	Occupying less than 1/2 of body length, 8 pinnae (all free of body wall)	–	Inner and mid-lateral teeth hook-shaped with no denticles; outer with singe cusp and secondary denticle; formula 51 × 39.0.46	2–4 pointed to rounded denticles	Bursa copulatrix and seminal receptacle similar in size, connected serially; short, oval penial gland; penis stalked, oval, retractable
B. andromeda sp. nov.	Translucent white to yellowish white, with numerous opaque white spots	2 transverse bars at same level near central mantle	Occupying 1/2 of body length, 6 pinnae (3 free of body wall)	Oval, convex near protoconch, somewhat flattened near anterior margin; protoconch 200–210 μm in diameter, 1.5 whorls	Inner and mid-lateral teeth hook-shaped with no denticles; outer teeth with single cusp; formula 49 × 54.0.50	3–6 rounded to pointed denticles	Bursa copulatrix about 4 times as wide as seminal receptacle, connected serially; penial gland elongate, curved; penis oval, retractable
B. nebula sp. nov.	Off-white to grayish white with numerous opaque white spots	T-shaped or Y-shaped	Occupying less than 1/2 of body length, bipinnate, 8–10 pinnae (5–6 free of body wall)	Oval, convex, translucent honey brown, covering almost entire length of preserved animal; protoconch 210–250 μm in diameter, 1 whorl	Inner and mid-lateral teeth hook-shaped with strong secondary denticle; outer teeth bifid; formula 56 × 48.0.48	4–6 pointed to rounded denticles	Bursa copulatrix about 8 times as large as seminal receptacle connected semiserially; penial gland elongate; penis oval, retractable
B. vialactea sp. nov.	Translucent white to milky white with numerous opaque white spots	T-shaped, Y-shaped, or irregularly shaped	Occupying 1/2 of body length, 6–10 pinnae (2–4 free of body wall)	Oval, convex, translucent white; protoconch 450 μm in diameter, 1.5 whorls	Inner and mid-lateral teeth hook-shaped some with weak secondary denticle; outer teeth with single cusp; formula 58 × 57.0.57	3–6 denticles	Seminal receptacle about 1/2 as large as bursa copulatrix, connected semiserially; penial gland convoluted; penis oval, retractable

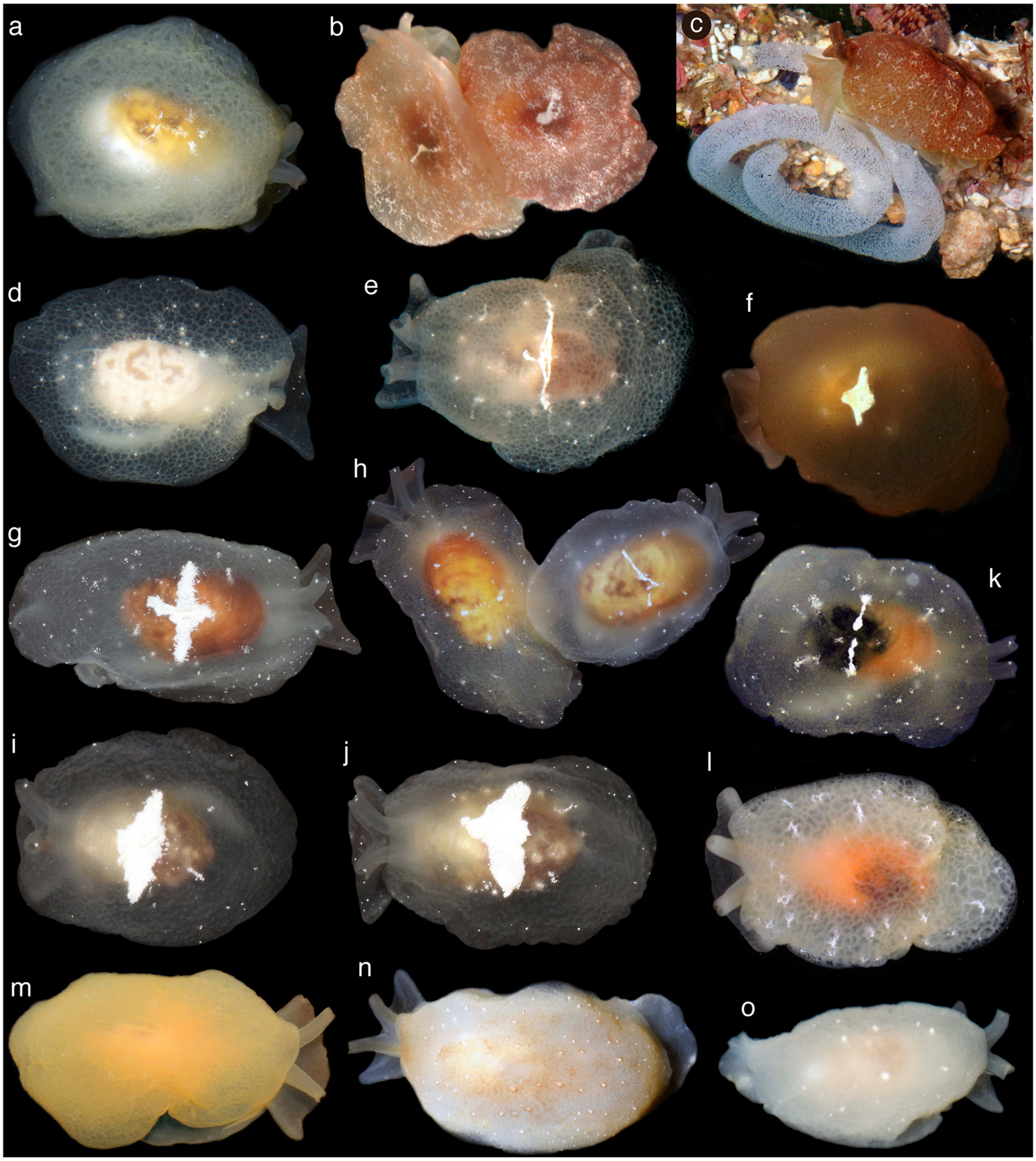


Fig. 2 Live animals of the species here examined. **a** *Berthella stellata* (Risso, 1826), Asturias, Spain (CPIC 00445); **b** *Berthella stellata*, Elba, Italy, two specimens (ZSM20013041 and ZSM20013043); **c** *Berthella stellata*, Catalonia, Spain, with egg mass (CPIC 02105); **d** *Berthella pellucida* (Pease, 1860), Maui, Hawaiian Islands (CPIC 01714); **e** *Berthella* cf. *postrema* Burn, 1962, Koumac, New Caledonia (MNHN KM322-AV0584); **f** *Berthella* sp. 2, Galapagos Islands (CASIZ 97563). **g** *Berthella nebula* sp. nov., Martinique (CPIC 02437); **h** *Berthella nebula* sp. nov., two specimens, Martinique (CPIC 02097). **i** *Berthella vialactea* sp. nov., Martinique (CPIC 02434); **j** *Berthella vialactea* sp. nov., Martinique (CPIC 02436); **k** *Berthella andromeda* sp. nov., Mazatlán, Mexico (CPIC 01418); **l** *Berthella perforata* (Philippi, 1844), Elba, Italy (ZSM 20012312); **m** *Berthella plumula* (Montagu, 1803), Northern Ireland (CASIZ 193034); **n** *Berthella strongi* (MacFarland, 1966), Cayucos, California (CPIC 02076); **o** *Berthella strongi* (MacFarland, 1966), Cayucos, California (CPIC 02075)

Berthella pellucida (Pease, 1860) (Figs. 2d, 5e, 6, and 7)

Pleurobranchus pellucidus Pease, 1860: 24. Type locality: Sandwich Islands [= Hawaiian Islands].

Type material: Untraceable, not at USNM.

Material examined: Maui, Hawaiian Islands, 25 June 2016, 1 specimen 7 mm preserved length, dissected, leg. A. Valdés et al. (CPIC 01689); 27 June 2016, 2 specimens 3–6 mm preserved length, dissected, leg. A. Valdés et al. (CPIC 01714).

Diagnosis: Animal colorless, translucent, with a reticulate pattern and scattered opaque white and brown spots; gill occupying one half of body length, with 8–11 pinnae, all free of body wall; shell oval, convex near protoconch, flattened near

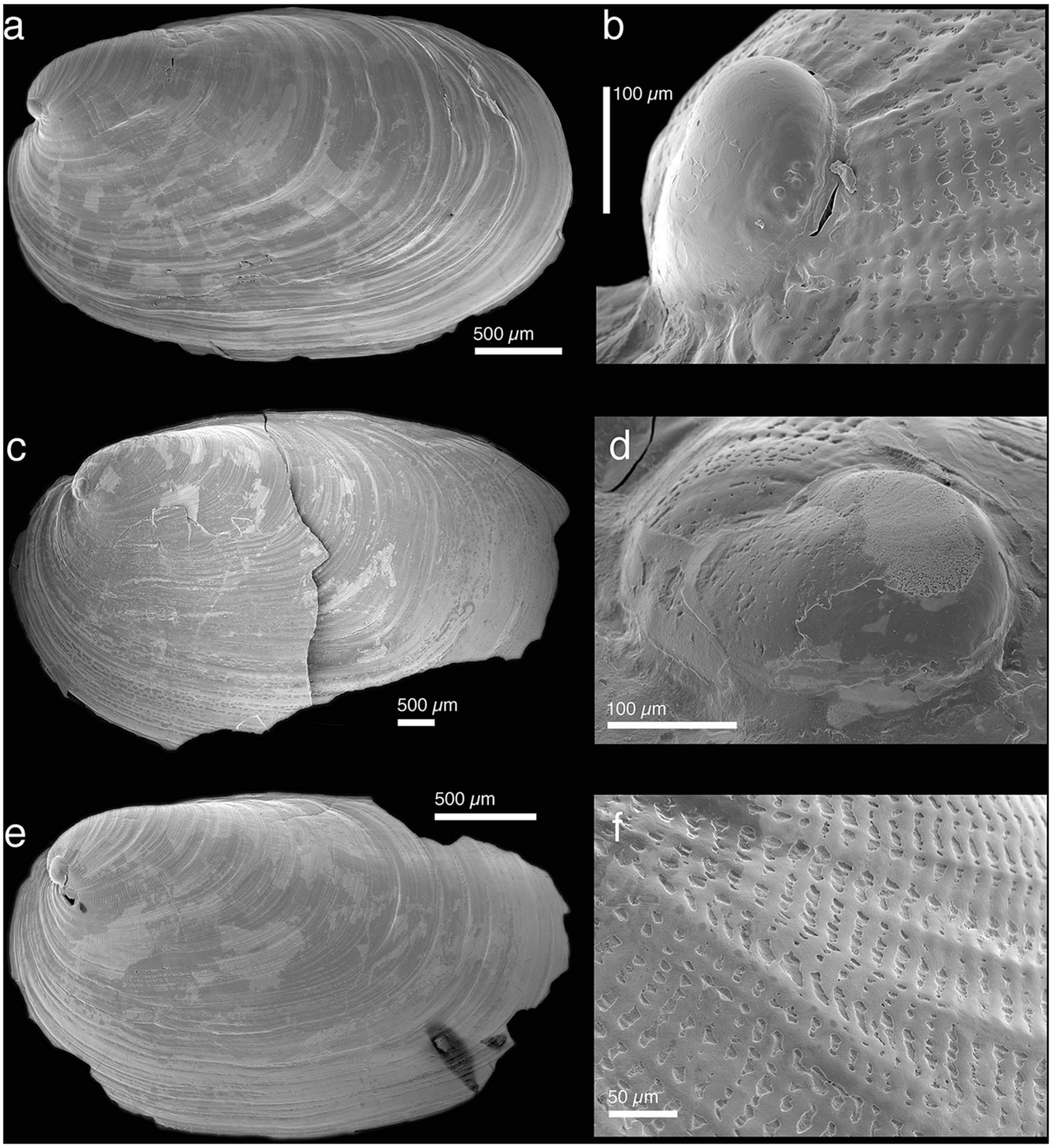


Fig. 3 *Berthella stellata* (Risso, 1826), scanning electron micrographs of the shells. **a**, **b** Specimen from Sicily, Italy (ZSM 20110680), dorsal view of the teleoconch (**a**), detail of the protoconch (**b**). **c**, **d** Specimen from Elba, Italy (ZSM 20013041), dorsal view of the teleoconch (**c**), detail of the protoconch (**d**). **e**, **f** Specimen from Sicily, Italy (ZSM 20110680), dorsal view of the teleoconch (**e**), detail of the sculpture (**f**)

anterior margin; protoconch 275–300 μm in diameter with 1.5 whorls; radular inner and mid-lateral teeth hook-shaped lacking denticles; outer teeth bifid; jaw elements with 3–4 denticles; bursa copulatrix and seminal receptacle connected serially, similar in size; penial gland wide, curved; penis oval, retractable.

Description: Body broad, oval (Fig. 2d). Mantle oval, covering foot on all sides. Mantle smooth, reticulated, colorless translucent with numerous scattered opaque white and brown spots. Oral veil broad, trapezoidal, translucent white with 4 to 5 scattered white spots near center. Rhinophores grayish white, rolled, joined at base, emerging between mantle and oral veil. Gill bipinnate, occupying one half of body length, with 8 pinnae on each side of smooth rachis in a 3 mm specimen (CPIC 01714), 10 pinnae in 6 mm specimen (CPIC 01714), and 11 pinnae in 7 mm specimen (CPIC 01689), none in portion of gill not attached to body wall, all from the Hawaiian Islands.

Shell oval, covering entire body, convex near protoconch, flattened near anterior margin (Fig. 6a, c). Sculpture of transverse grooves crossing distinct growth lines. Protoconch (Fig. 6b, d) oval, about 275–300 μm in diameter, 1.5 whorls. Shell dimensions: 2.73 × 1.68 mm (3 mm specimen—CPIC 01714), 5.50 × 3.13 mm (6 mm specimen—CPIC 01714) all from the Hawaiian Islands.

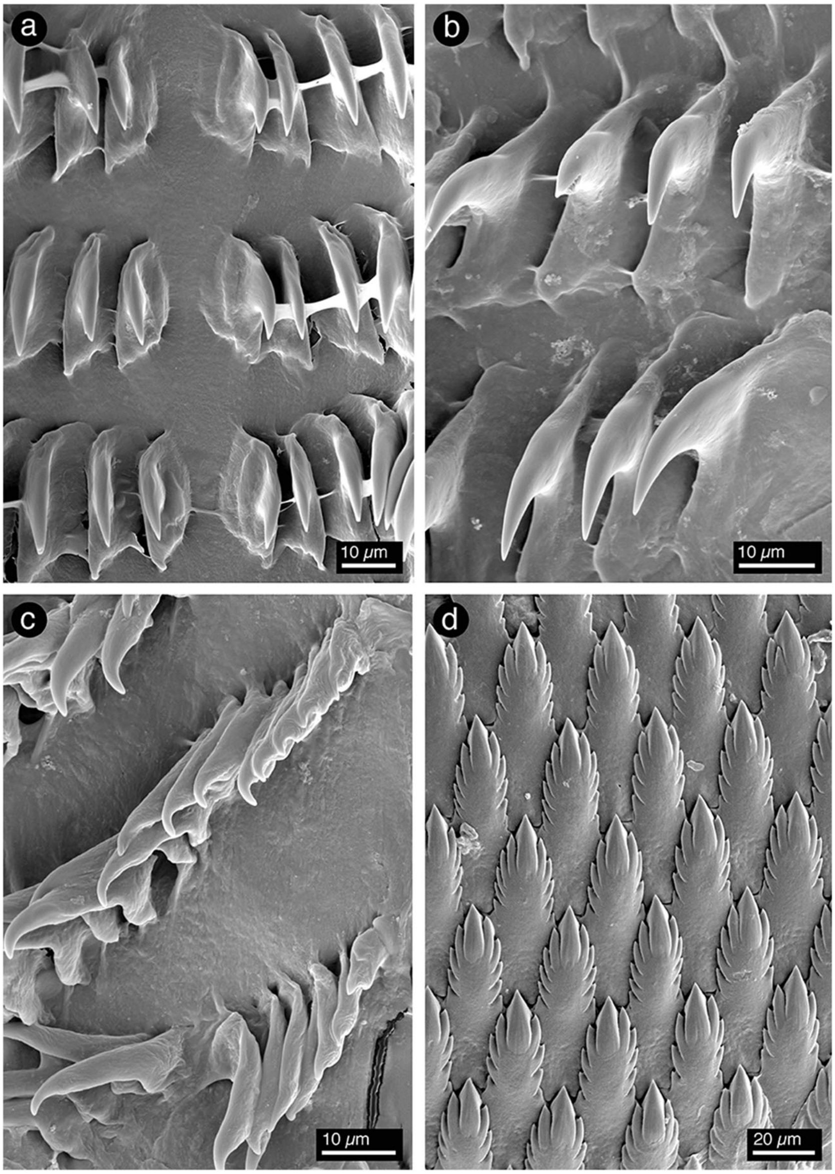


Fig. 4 *Berthella stellata* (Risso, 1826), scanning electron micrographs of the radular teeth and jaw elements of specimens from Elba, Italy. **a** Innermost lateral radular teeth (ZSM 20013041); **b** Mid-lateral radular teeth (ZSM 20013041); **c** Outer lateral radular teeth (ZSM 20013041); **d** Jaw elements (ZSM 20013043)

Radular formula 56 × 45.0.45 in 6 mm specimen. Inner (Fig. 7a) and mid-lateral teeth (Fig. 7b) simple, hook-shaped, cusp size increasing toward outer margins. Outer teeth (Fig. 7c) erect, slender hooks, some with second denticle. Jaw elements in distal view with central cusp bearing 3–4 denticles on each side (Fig. 7d). Denticulation generally symmetrical. Jaw elements in proximal view with triangular indentation near center of cusp, small circular indentation near base of some elements (Fig. S5e).

Reproductive system (Fig. 5e) androdiaulic. Ampulla elongate, slender, curved, but not convoluted, branching near base into short oviduct and prostate; ampulla as long as the prostate. Prostate elongate, convoluted in middle. Penial gland twice as wide as prostate, curved, but not convoluted, joining deferent duct near penis. Seminal receptacle similar in shape to penial gland, but thinner, about 20% shorter, joining vaginal duct near rounded bursa copulatrix; bursa copulatrix and seminal receptacle connected serially, similar in size. Vaginal duct twice as short as deferent duct, opening ventral to penis. Penis oval, retractable.

Geographic range: The range of this species is confirmed to include the Hawaiian Islands (Pease 1860; Kay 1979; Gosliner and Bertsch 1988; present study). Other Indo-Pacific records assigned to *B. pellucida* could not be verified with molecular data.

Remarks: Pease (1860) introduced the name *P. pellucidus* based on specimens from the Hawaiian Islands, described as translucent whitish with the dorsum minutely reticulated. Subsequently, this species was reported from New Caledonia (Risbec 1928) and Japan (Baba 1969). Thompson (1970) transferred *Pleurobranchus pellucidus* to *Berthella* and reported it from Queensland, Australia. Burn (1962) described

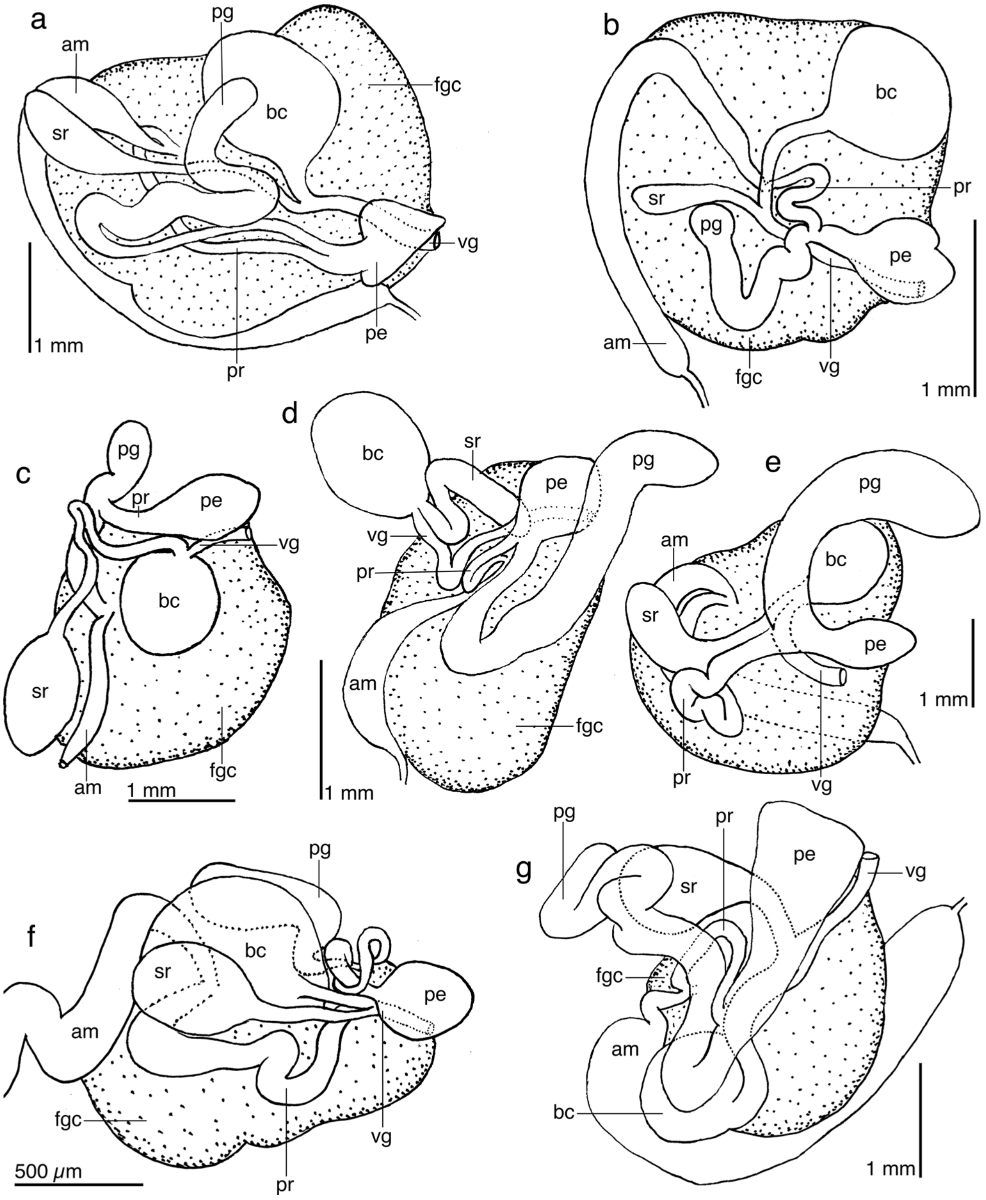


Fig. 5 Drawings of the reproductive systems of species examined in this study; **a** *Berthella stellata* (Risso, 1824), specimen from Elba, Italy (ZSM 20013043); **b** *Berthella nebula* sp. nov., holotype, Martinique Is., Caribbean Sea (MNHN IM-2000-34532); **c** *Berthella* cf. *postrema* Burn, 1962, specimen from Koumac, New Caledonia (MNHN KM322-AV0584); **d** *Berthella andromeda* sp. nov., specimen from Mazatlán, Mexico (CPIC 01418); **e** *Berthella pellucida* (Pease, 1860), specimen from Maui, Hawaiian Islands, Pacific Ocean (CPIC 01714); **f** *Berthella vialactea* sp. nov., holotype, Martinique Is., Caribbean Sea (MNHN IM-2000-34531); **g** *Berthella strongi* (MacFarland, 1966), specimen from Cayucos, California (CPIC 02075). am ampulla, bc bursa copulatrix, fgc female gland complex, pe penis, pg penial gland, pr prostate, sr seminal receptacle, vg vagina

the new species *Berthella postrema* Burn, 1962 (type locality Collaroy, New South Wales, Australia) but did not compare it with *B. pellucida*. Willan (1984) synonymized *B. postrema* Burn, 1962 with *B. pellucida* and reported specimens from the Marshall Islands and Guam for the first time. More recently, Gosliner and Bertsch (1988) synonymized both *B. pellucida* and *B. postrema* with *B. stellata*. This opinion is now accepted and subsequent records of similarly colored animals from the Western Pacific region have been assigned to *B. stellata* (e.g., Carlson and Hoff 2003; Wägele et al. 2006; Cobb 2008; Nakano 2018; Gosliner et al. 2018).

The three specimens from the Hawaiian Islands here examined, originally identified as *B. stellata*, clustered together in a monophyletic group, and the ABGD analysis of the COI mitochondrial sequences confirmed they constitute a distinct species in the *B. stellata* species complex (Table 3). These animals are characterized by having a reticulate pattern on the dorsum that was mentioned in the original description of *B. pellucida* (Pease 1860) and subsequent references from the Hawaiian Islands (Kay 1979). Gosliner and Bertsch (1988) examined the radular teeth, jaw elements, and reproductive system of a specimen of *B. pellucida* from the Hawaiian Islands, which generally agree well those of the present material, including the presence of elongate outermost teeth with a secondary denticle (Gosliner and Bertsch 1988: fig. 11F; Fig. 7C) and the reproductive system with the bursa copulatrix and seminal receptacle of about the same size, connected serially, and a large and curved penial gland (Gosliner and Bertsch 1988: fig. 12B; Fig. 5E). Based on the genetic differences between the animals from the Hawaiian Islands and other

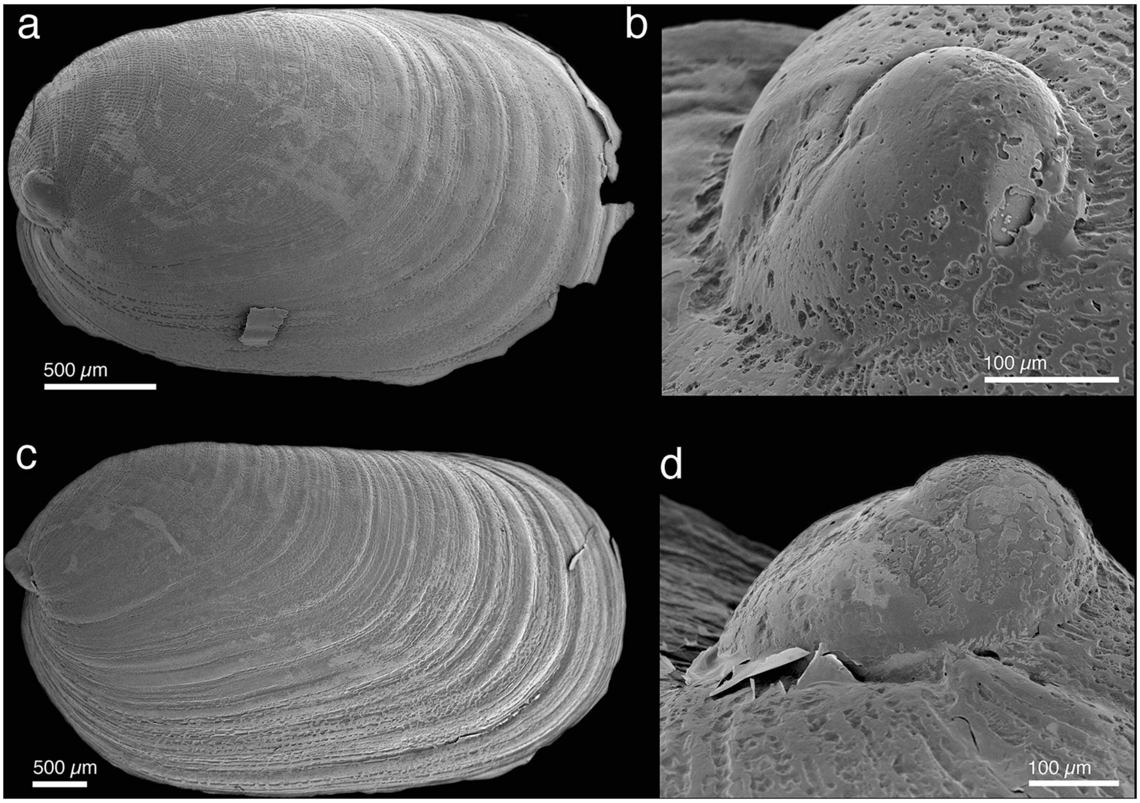


Fig. 6 *Berthella pellucida* (Pease, 1860), scanning electron micrographs of the shells of specimens from the Hawaiian Islands. **a**, **b** Specimen from Maui, Hawaiian Islands (CPIC 01714), dorsal view of the teleoconch (**a**), detail of the protoconch (**b**). **c**, **d** Specimen from Maui, Hawaiian Islands (CPIC 01714), dorsal view of the teleoconch (**c**), detail of the protoconch (**d**)

members of the *B. stellata* species complex, we resurrect the name *B. pellucida* for the Hawaiian species. We can only confirm the presence of *B. pellucida* with certainty in the Hawaiian Islands, and the other specimen here examined from the Indo-Pacific region is genetically and morphologically distinct (see description of *B.* cf. *postrema*).

Berthella strongi (MacFarland, 1966) (Figs. 2n, o, 5g, 8, and 9)

Pleurobranchus strongi MacFarland, 1966: 89–93, pl. 6, figs. 3–7, pl. 15, figs. 1–15, pl. 16, figs. 13–14. Type locality: Various localities along the California coast: Point Pinos, Monterey Bay, Point Lobos, Carmel Bay, Cabrillo Point, Pescadero Point (Monterey County); Santa Cruz Island; White Point (Los Angeles County).

Type material: Syntype: White Point, San Pedro, California (USNM 575224).

Material examined: La Jolla, California, USA, 6 Apr 2000, 1 specimen 5 mm preserved length, leg. J. Goddard (CPIC 01408). Southside of Pt. Sur, California, 18 May 2007, 1 specimen 7 mm preserved length, leg. J. Goddard (CPIC 01411). Naples, Santa Barbara County, California, 3 Dec 2009, specimen 10 mm preserved length, leg. J. Goddard (CPIC 01410). Cayucos, California, USA, 29 Apr 2017, 1 specimen 7 mm preserved length, leg. J. Goddard (CPIC 02075); 1 specimen 9 mm preserved length, dissected, leg. J. Goddard (CPIC 02076).

Diagnosis: Animal beige with small worm-like ridges, some with reddish brown edges; numerous variably sized tubercles, each with white apex; gill with 12 pinnae, 6 free of body wall; shell elongate, convex near protoconch, flattened near anterior margin; protoconch 216 μm in diameter; radular inner and mid-lateral teeth hook-shaped with no denticles; outer teeth simple or bifid; jaw elements with 3–6 denticles; bursa copulatrix as large as seminal receptacle, connected semiserially; penis oval, retractable.

Description: Body elongate-ovate (Fig. 2n, o). Mantle oval, covering foot on sides but not posterior portion. Mantle beige with small worm-like ridges, some ridges near central notum with reddish brown edges; numerous variably sized tubercles scattered throughout mantle, each tubercle with white spot at apex. Some large tubercles near central mantle with reddish-brown base, less prominent apical white spot. Oral veil broad, trapezoidal, translucent white. Rhinophores rolled, emerging between mantle and oral veil, fused together about one third of exposed length. Rhinophoral lateral half same color as mantle, medial half translucent white. Foot translucent white, left, right margins solid white. Few small scattered white spots on exposed posterior portion of foot. Gill of both specimens examined relatively short, occupying one third of body length,

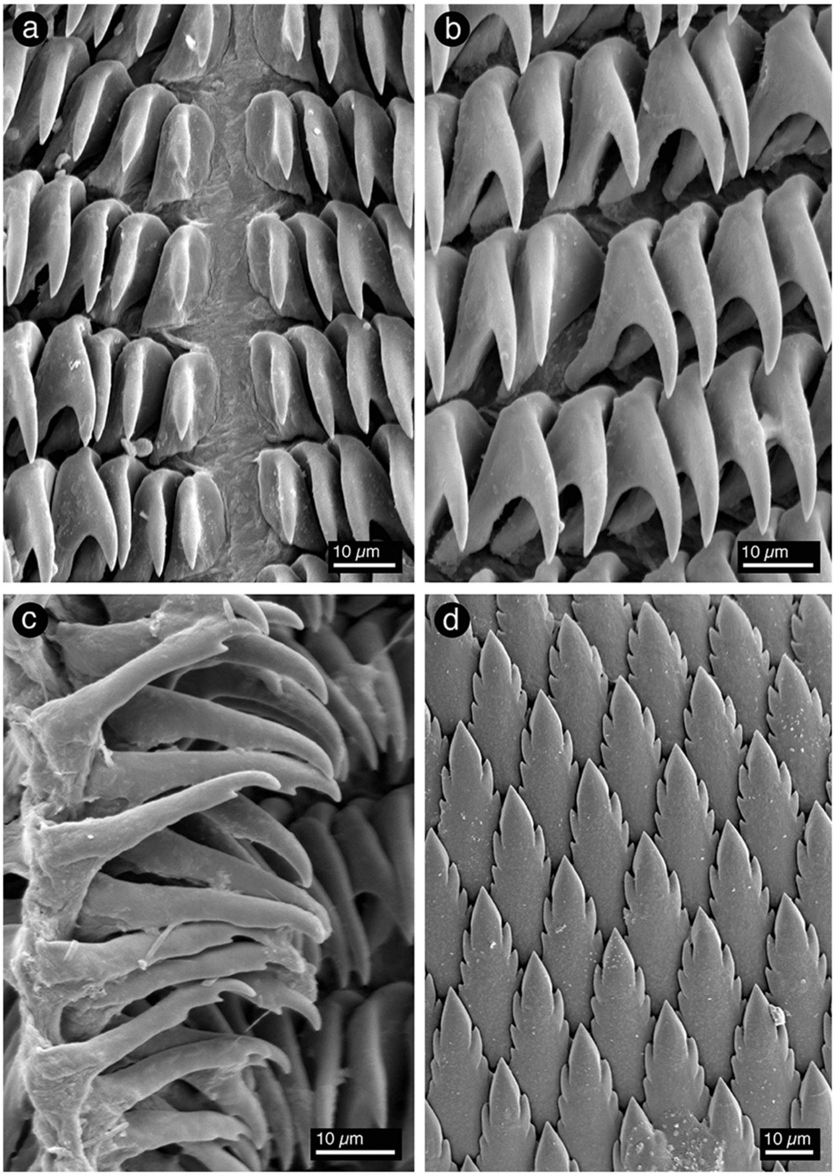


Fig. 7 *Berthella pellucida* (Pease, 1860), scanning electron micrographs of the radular teeth and jaw elements of a specimen from Maui, Hawaiian Islands (CPIC 01714); **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** Jaw elements

bipinnate with 12 primary pinnae (6 in portion of gill not attached to body wall) on each side of smooth rachis.

Shell elongate, oval with left, right margins almost parallel (Fig. 8b). Convex near protoconch, flattened near anterior margin. Sculpture of transverse grooves crossing distinct growth lines. Protoconch (Fig. 8a) diameter 216 μm, shell length 10.5 mm, shell width 2.95 mm in 9 mm specimen (CPIC 02076).

Radular formula 60 × 63.0.63 in 9 mm specimen (CPIC 02076). Innermost teeth (Fig. 9a) simple, hook-shaped. Inner lateral teeth (Fig. 9b) simple with cusps generally longer than innermost teeth. Last 3 to 4 outer teeth (Fig. 9c) with shorter cusp than inner lateral teeth. Some outer teeth with secondary denticle in the same specimen (Fig. 9d). Jaw elements in distal with central cusp bearing 3–6 denticles on each side (Fig. 9e); denticulation not always symmetrical. Denticle size decreasing toward base.

Reproductive system (Fig. 5g) androdiaulic. Ampulla wide, elongated, curved, but not convoluted, narrowing proximally before branching into short oviduct, connecting to prostate and well-developed female gland complex; ampulla nearly twice as long as the prostate. Prostate not convoluted, joining wide deferent duct leading to penis. Penial gland elongated, convoluted, joining deferent duct. Bursa copulatrix spherical and stalked. Seminal receptacle bulbous and elongated, as large as bursa copulatrix. Bursa copulatrix and seminal receptacle connected semiserially to narrow, non-convoluted vaginal duct. Vaginal duct twice as long as deferent duct, opening ventral to penis. Penis oval, retractable.

Geographic range: Eastern Pacific Ocean, from northern California to Punta Rosarito and El Tomatal, Baja California, and possibly as far north as Vancouver Island, British Columbia, during strong El Niño events (Goddard

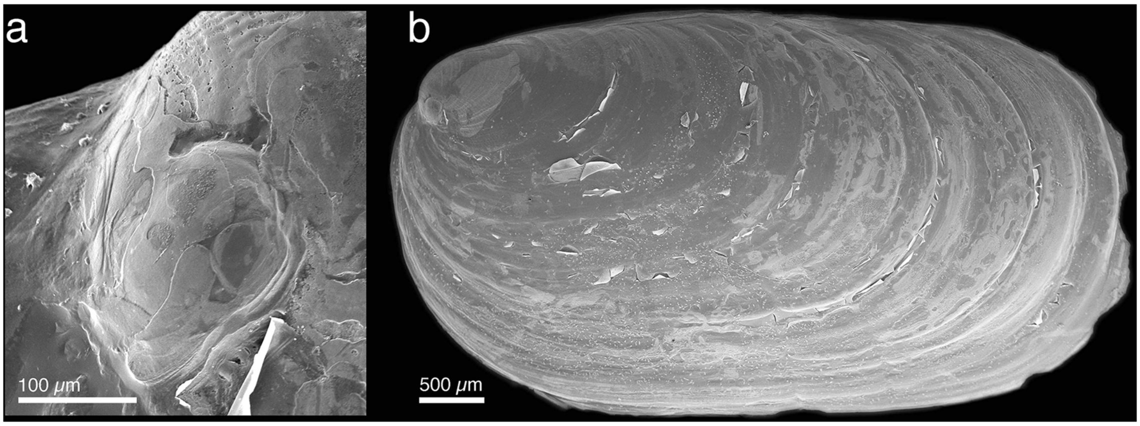
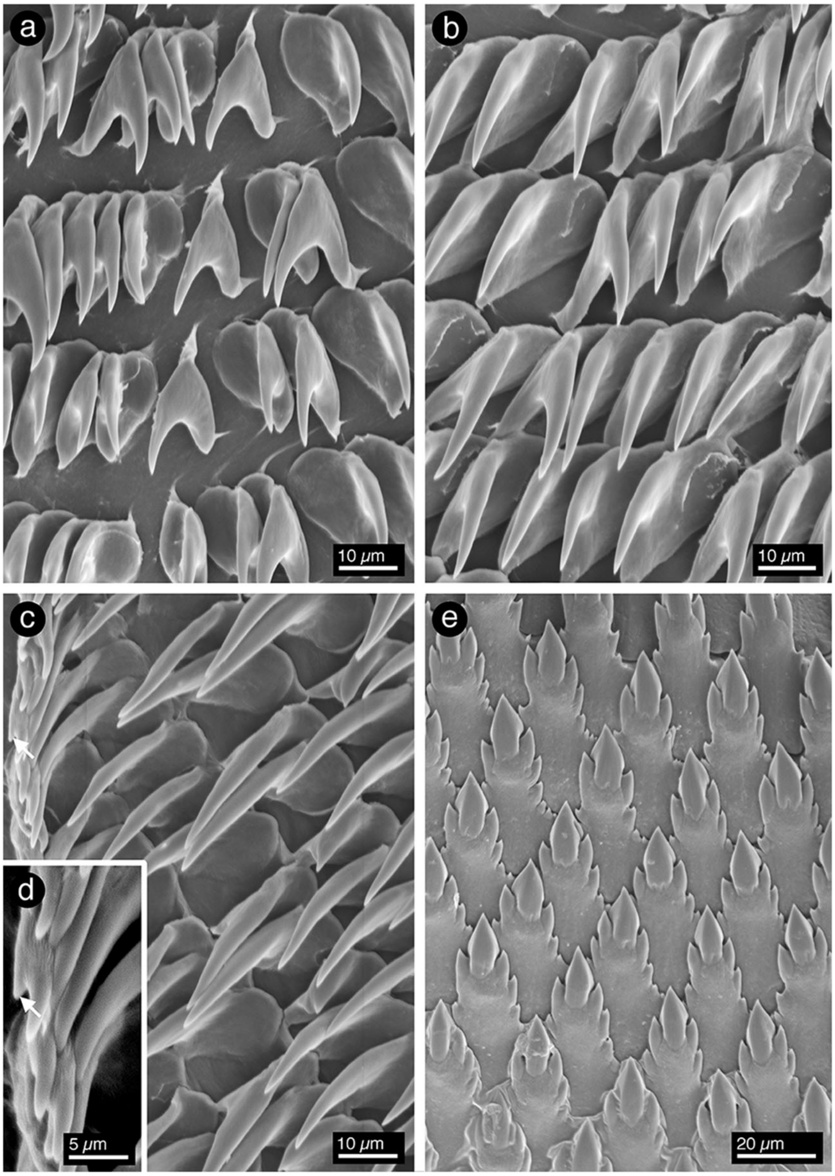


Fig. 8 *Berthella strongi* (MacFarland, 1966), scanning electron micrographs of the shell of specimen from Cayucos, California, USA (CPIC 02076). **a** Detail of protoconch; **b** Dorsal view of teleoconch

Fig. 9 *Berthella strongi* (MacFarland, 1966), scanning electron micrographs of the radular teeth and jaw elements of a specimen from Cayucos, California, USA (CPIC 02076). **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** magnification of outer teeth; **e** Jaw elements. White arrows indicate the position of secondary denticles



and Schickel 2000; Goddard and Green 2013; Goddard et al. 2018).

Biology: The diet of *B. strongi* has been determined by field observations and laboratory feeding trials to be plakinid sponges including the species *Oscarella carmela* Muricy and Pearse, 2004 (Goddard 2007). *Berthella strongi* lays coiled cylindrical egg masses with eggs averaging 89 μm in diameter deposited one per capsule; planktotrophic veligers with eyespots and type 1 shells averaging 137 μm long hatched after 16 days at 12–16 °C (Goddard 2002; Goddard and Green 2013).

Remarks: *Berthella strongi* was originally described as *Pleurobranchus strongi* by MacFarland (1966) and later transferred to *Berthella* by Gosliner and Bertsch (1988) based on the fact that the gill rachis lacks tuberculation.

Although *B. strongi* has never been synonymized with *B. stellata*, it was included in this study because in preliminary molecular analysis, *B. strongi* was consistently nested within specimens identified as *B. stellata*. These results were here confirmed, and *B. strongi* was found to form a monophyletic group with Eastern Pacific species of the *B. stellata* species complex. The ABGD analysis of the COI mitochondrial sequences of the available specimens in the *B. stellata* species complex recovered *B. strongi* as distinct from all the other species (Table 3), confirming its validity. Gosliner and Bertsch (1988) found consistent differences between this species and specimens identified as *B. stellata*. Gosliner and Bertsch (1988) indicated the radular teeth of *B. strongi* have a narrower, more elongate cusps than any of those found in *B. stellata*, the outer teeth of *B. strongi* are never elongate or bifid, and more significantly, the penial gland of *B. strongi* is always highly convoluted, whereas that of *B. stellata* has a maximum of 1–2 convolutions. The characteristics of the animals examined herein are consistent with those of the specimens examined by Gosliner and Bertsch (1988), except that some outer radular teeth of *B. strongi* are bifid (Fig. 9(d)).

Berthella cf. *postrema* Burn, 1962 (Figs. 2e, 5c, and 10)

Berthella postrema Burn, 1962: 140–143, text figs. 1B, 2B, 4, pl. 1, fig. 2, pl. 2, figs. 3–4. Type locality: Long Reef, Collaroy, New South Wales, Australia.

Type material: Holotype: Long Reef, Collaroy, New South Wales, Australia, 16 Nov 1958 (Museums Victoria F20145), not examined.

Material examined: Koumac, New Caledonia, 27 Sep 2018, 1 specimen 4 mm preserved length, dissected, leg. A. Valdés (MNHN KM322-AV0584).

Diagnosis: Animal translucent, with numerous opaque white specs, a reticulate pattern, and a single T-shaped dorsal patch; gill with 8 pinnae all free of body wall; radular inner and mid-lateral teeth hook-shaped, lacking denticles; outer teeth with a singe cusp and a secondary denticle; jaw elements with 2–4 pointed to rounded denticles; bursa copulatrix and seminal receptacle similar in size, connected serially; penial gland short, oval; penis stalked, oval, retractable.

Description: Body oval (Fig. 2e). Mantle broad, covering foot on all sides except posterior portion. Mantle translucent, reticulated, with numerous opaque white specs scattered throughout. Internal organs brownish to pale cream. Some dorsal white spots forming "T"-shaped pattern near central mantle. Translucent white shell, brown visceral mass visible through translucent mantle. Oral veil broad, trapezoidal, translucent white, not reticulated. Rhinophores rolled, fused for about one fourth their length, emerging between oral veil and mantle, same color as oral veil. Gill bipinnate, occupying less than one half of body length, with 8 pinnae (none in portion of gill not attached to body wall) on each side of smooth rachis in a 4-mm specimen (MNHN KM322-AV0584).

The translucent white shell was damaged and could not be examined complete.

Radular formula 51 × 39.0.46. Inner teeth (Fig. 10a) and mid-lateral teeth (Fig. 10b) hook-shaped. Outer teeth (Fig. 10c) with one accessory denticle; outermost 5–8 teeth decreasing in size. Jaw elements in distal view (Fig. 10d) with pointed central cusp bearing 2–4 pointed to rounded denticles on each side; denticulation not always symmetrical. Jaw elements in proximal view (Fig. S5f) with elongate indentation near center of cusp.

Reproductive system (Fig. 5c) androdiaulic. Ampulla elongate, slender, not convoluted. Short, oval penial gland emerging from female gland mass at base of ampulla; ampulla as long as the prostate, nearly twice as wide. Seminal receptacle almond-shaped, with long, curved stalk leading to spherical bursa copulatrix. Bursa copulatrix and seminal receptacle similar in size, connected serially to narrow, non-convoluted vaginal duct. Vaginal duct emerging from bursa copulatrix near base of seminal receptacle. Stalked, oval penis emerging from about middle of penial gland.

Geographic range: *Berthella postrema* was originally described from New South Wales, Australia. It has been reported from several localities in the Western Pacific but the identity of those records in uncertain. The species here described as *B.* cf. *postrema* has been reported from New Caledonia as *B. pellucida* (Risbec 1928) and *B. stellata* (Hervé 2010).

Remarks: Burn (1962) described *Berthella postrema* based on several specimens collected from New South Wales, Australia. When comparing this species with *B. postrema* [sic.], probably an error for *Berthella medietas* Burn, 1962, the diagnostic characteristics for *B. postrema* were the median position of the anus in relation to the gill membrane, the stronger sculpture of the shell, the shape of the mandibular elements, and the form of the radular teeth (Burn 1962). Willan (1984) synonymized *B. postrema* with *B. pellucida* as he did not observe significant differences between specimens from the Marshall Islands, assigned to *B. pellucida*, and additional material from temperate eastern Australia. However, Willan (1984) hinted at some differences in the reproductive system between these two populations

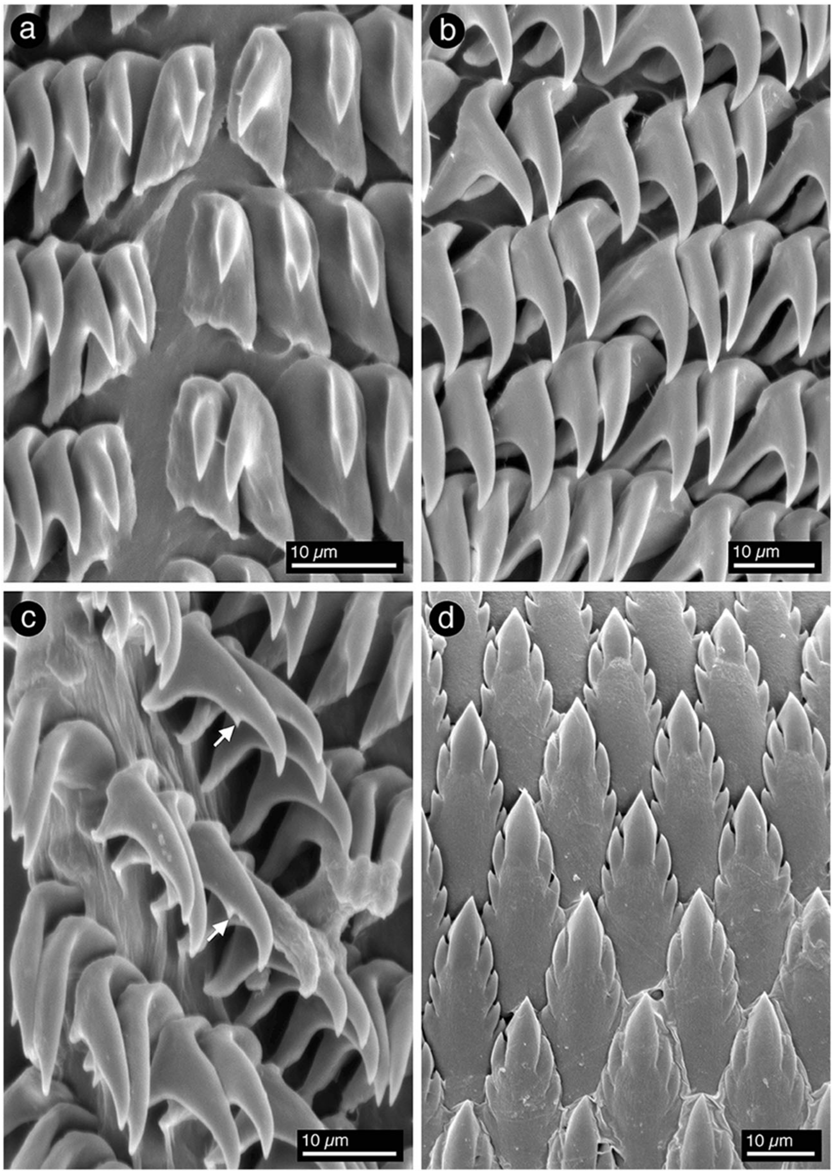


Fig. 10 *Berthella* cf. *postrema* Burn, 1962, scanning electron micrographs of the radular teeth and jaw elements of a specimen from Koumac, New Caledonia (MNHN KM322-AV0584); **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** Jaw elements. White arrows indicate the position of secondary denticles

and mentioned the lack of denticles in the jaw elements of this specimens from the Marshal Islands. Subsequently, Gosliner and Bertsch (1988) synonymized *B. pellucida* with *B. stellata*.

For this study, we had no access to specimens from eastern Australia. However, the internal anatomy of a single specimen from New Caledonia is consistent with the original description of *B. postrema* and to some extent to a specimen from Hastings Point, Australia, examined by Gosliner and Bertsch (1988), and is here tentatively assigned to this species. The characters of the radular teeth and jaw elements of the present material agree with those in the original description of *B. postrema*. The jaw elements of the New Caledonia animals have 4 denticles on each side (Fig. 10d) as in the 14-mm-long specimen described in original description of *B. postrema* (Burn 1962: fig. 2B) and in specimen from Hastings Point examined by Gosliner and Bertch (Gosliner and Bertsch 1988: fig. 9G). Also, the outermost radular teeth have simple tips and are elongate in both the New Caledonia material (Fig. 10c) and Burn's (Burn 1962: fig. 2B) illustration, but they are bifid and elongate in the specimen from Hastings Point (Gosliner and Bertsch 1988: fig. 11G). Finally, the reproductive system of *B. postrema* (Burn 1962: fig. 4) has a very short vagina and a serially connected bursa copulatrix, as in the material from New Caledonia here examined. This is different from *B. pellucida*, which has a much longer vaginal duct. However, due to the small size of the specimen examined, these observations may be unreliable; hence, the New Caledonia material is assigned to *B. postrema* tentatively.

Berthella cf. *postrema* was recovered as a distinct species in the ABGD analysis of the specimens in the *B. stellata* species complex. As mentioned by Willan (1984), *Berthella* cf. *postrema* greatly resembles *B. pellucida* in its external morphology and coloration, i.e., both species have a translucent reticulated mantle. The specimen of *B.* cf. *postrema* here examined possessed a "T"-shaped opaque white mark near the center of the mantle, but this pattern was absent in the specimens of *B. pellucida* examined in this study. This could be because the *B.* cf. *postrema* specimen was a juvenile and the *B. pellucida* specimens examined were adults. Willan (1984) reported juveniles of *B. pellucida* having a central opaque white cross-like pattern on the mantle. The main morphological difference between *B.* cf. *postrema* and *B. pellucida* can be found in the reproductive systems of these species. One notable difference is the presence of a convoluted prostatic portion in the deferent duct of *B. pellucida* (Fig. 5e) not found in *B.* cf. *postrema* (Fig. 5c).

Other records of members of the *B. stellata* species complex from the Western Pacific could not be assigned to any species (Willan 1984; Carlson and Hoff 2003; Wägele et al. 2006; Cobb 2008; Nakano 2018; Gosliner et al. 2018) due to the lack of specimens for molecular analysis. Additional material from the tropical Indo-Pacific is necessary to determine how many species occur in this region.

Berthella stellata albocrossata Heller & Thompson, 1983

Berthella stellata albocrossata Heller and Thompson 1983: 328–329, figs. 5A, C. Type locality: Harvey Reef, Sudan.

Remarks: This subspecific name was introduced by Heller and Thompson (1983) based on a specimen from the Sudanese Red Sea, which possessed a more precisely delineated dorsal cross-like pattern compared to specimens from the Mediterranean Sea. Without providing any clear justification, Heller and Thompson (1983) stated that *B. stellata* is a Mediterranean species that invaded the Red Sea. This idea was further developed by Thompson (1985), who argued that the presence of *B. stellata albocrossata* in the Red Sea was the result of anti-Lessepsian migration (from the Mediterranean Sea to the Red Sea through the Suez Canal) of *B. stellata* and the subsequent evolution of a more symmetrical dorsal cross, due to the clearer waters in the Red Sea. The rationale behind this hypothesis was the fact that no records of *B. stellata* from the Red Sea had existed prior to the opening of the Suez Canal (Thompson 1985). Perrone (1984) reported a record of *B. stellata albocrossata* from Pazzi Island, Salentino Peninsula, Italy, which seemingly contradicts Thompson's (1985) argument, but subsequently Perrone (1986) assigned this material to *B. stellata*. Gosliner and Bertsch (1988) synonymized *B. stellata albocrossata* with *B. stellata* because its characteristics fit within the variability of this species.

We did not have access to any specimens from the Indian Ocean for this study, but other records from this region (assigned to *B. stellata*) include animals with a high degree of variability in the shape of the dorsal central white patch (Gosliner 1987—as *B. tupala*; Wells and Bryce 1993; Kazmi et al. 1996—under *B. tupala*; Coleman 2008; Flodrops 2008; Bhave 2009; Apte et al. 2010; Cadet 2011). Some of them have a very well-defined dorsal cross-shaped patch as in Heller and Thompson's (1983) original description (Cadet 2011), but others have broken and loosely formed stars (Wells and Bryce 1993; Coleman 2008; Flodrops 2008; Bhave 2009; Cadet 2011), or no distinct central patch at all (Gosliner 1987; Apte et al. 2010). Therefore, there are no obvious consistent external traits that characterize Indian Ocean animals. However, because of the geographic distance between the type locality of *B. stellata albocrossata* and other Indo-Pacific nominal species, *B. pellucida* (Hawaiian Islands) and *B. postrema* (Eastern Australia), it is possible the Indian Ocean animals identified as *B. stellata albocrossata* or *B. stellata* could constitute a distinct species. Due to the lack of material for molecular work, we are unable to confirm this point, and therefore, *B. stellata albocrossata* is here regarded as a taxon inquirendum.

Berthella andromeda sp. nov. (Figs. 2k, 5d, 11, and 12)
http://zoobank.org/150E8F54-72B8-4747-A3AF-0B7542717159

Holotype: Mazatlán, Mexico, 23 Oct 2013, 5 mm preserved length, dissected, leg. A. Valdés (LACM 3654).

Other material examined: Mazatlán, Mexico, 23 Oct 2013, 1 specimen 6 mm preserved length, dissected, leg. A. Valdés (CPIC 01418).

Diagnosis: Animal translucent white to yellowish white, with numerous opaque white spots and two transverse bars near center; gill with 6 pinnae, 3 free of body wall; shell oval, convex near protoconch, somewhat flattened near anterior margin; protoconch 200–210 μm in diameter with 1.5 whorls; radular inner and mid-lateral teeth hook-shaped lacking denticles; outer teeth with a single cusp; jaw elements with 3–6 rounded to pointed denticles; bursa copulatrix about 4 times as wide as seminal receptacle, connected serially; penial gland elongate, curved; penis oval, retractable.

Description: Body elongate-ovate (Fig. 2k). Mantle oval, covering foot on all sides. Mantle smooth, translucent white to yellowish white, with numerous opaque white spots scattered throughout. Dorsal white spots forming two transverse bars at same level near central mantle. Rhinophores rolled, emerging below mantle, fused about one half their length, translucent white, each one bearing one opaque white spot on dorsal tip. Gill bipinnate, occupying one half of body length, with 6 primary pinnae (3 in portion of gill not attached to body wall) on each side of smooth rachis in the 5 mm holotype (LACM 3654).

Shell covering entire body, oval, convex near protoconch, somewhat flattened near anterior margin (Fig. 11a, c). Sculpture of transverse grooves crossing distinct growth lines. Protoconch (Fig. 11b, d) oval 200–210 μm in diameter, with 1.5 whorls. Shell dimensions: 4.17 × 2.83 mm (5 mm holotype—LACM 3654) and 5.75 × 3.40 mm (6 mm specimen—CPIC 01418).

Radular formula 49 × 54.0.50 in 6 mm specimen (CPIC 01418). Inner teeth (Fig. 12a) simple hooks. Mid-lateral teeth

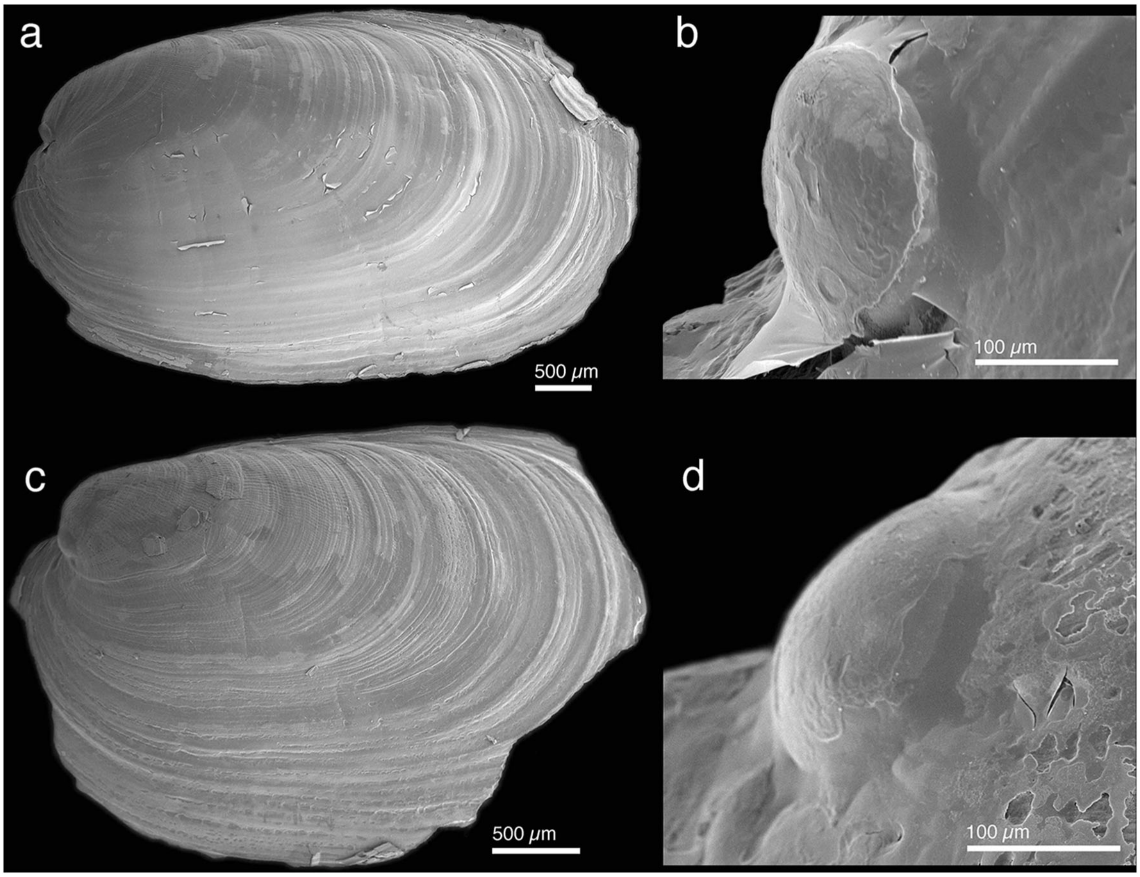


Fig. 11 *Berthella andromeda* sp. nov., scanning electron micrographs of the shells of specimens from the Eastern Pacific. **a**, **b** Specimen from Mazatlán, Mexico (CPIC 01418), dorsal view of the teleoconch (**a**), detail of the protoconch (**b**); **c**, **d** Holotype, Mazatlán, Mexico (LACM 3654), dorsal view of the teleoconch (**c**), detail of the protoconch (**d**)

(Fig. 12b) simple hooks, increasing in size laterally. Last 6–8 outer teeth decreasing in size laterally (Fig. 12c), some with small, rounded secondary denticle. Jaw elements distal view with pointed, sometimes bifid central cusp in same specimen bearing 3–6 rounded to pointed denticles on each side (Fig. 12d). Denticulation not symmetrical. Jaw elements in proximal view with round indentation near center of cusp (Fig. S5d).

Reproductive system (Fig. 5d) androdiaulic. Ampulla short, curved, but not convoluted, narrowing proximally leading to oviduct. Prostate short, curved, emerging at base of oviduct; ampulla nearly five times as long as the prostate. Penial gland elongate, curved, with two folds, narrowing proximally before connecting to penis. Seminal receptacle elongate, curved narrowing before joining base of oval bursa copulatrix; bursa copulatrix about 4 times as wide as seminal receptacle, connected serially. Vaginal duct nearly twice as long as deferent duct, curved in middle emerging at base of bursa copulatrix and seminal receptacle connection. Penis oval, retractable.

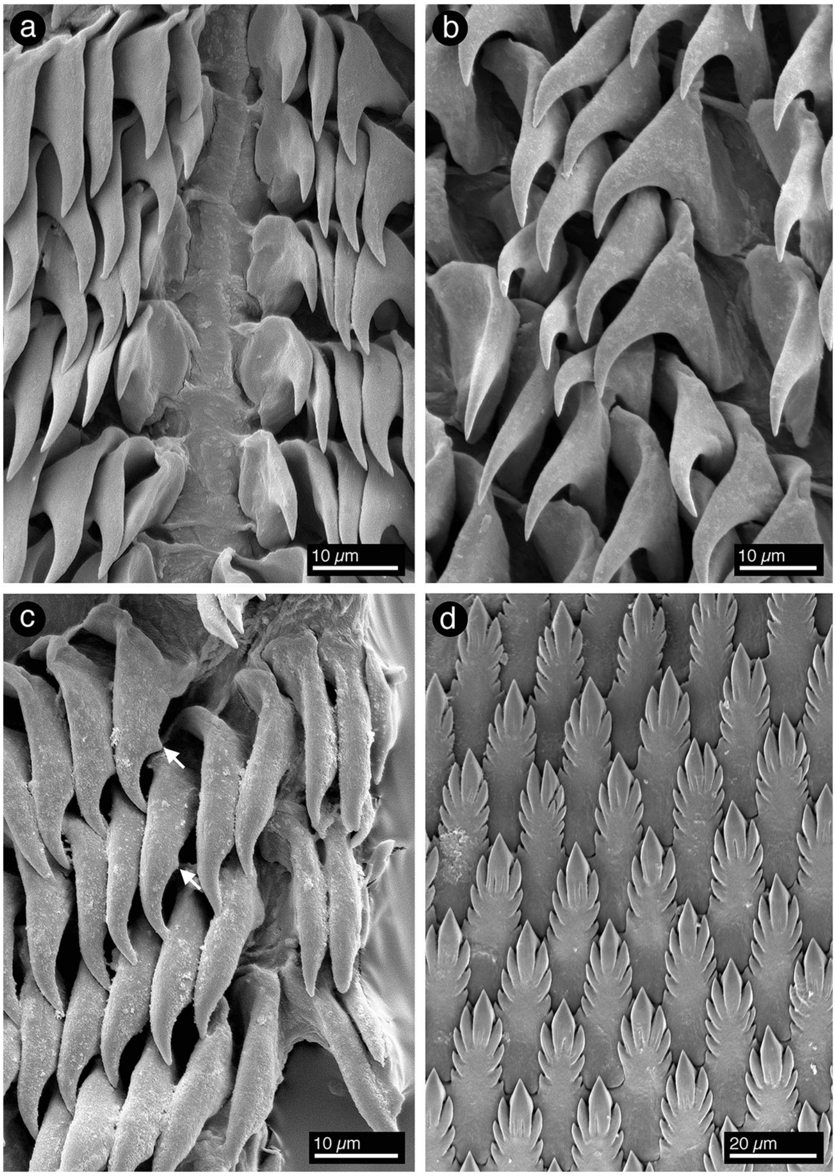
Geographic range: Eastern Pacific, from Baja California, Mexico (Gosliner and Bertsch 1988), to possibly Costa Rica (Camacho-García et al. 2005).

Biology: *Berthella andromeda* sp. nov. has been observed feeding on the plakinid sponge *Oscarella* sp. (Goddard 2010).

Derivatio nominis: Named after the galaxy Andromeda, the nearest major galaxy to our Milky Way.

Remarks: Molecular data here presented show that *B. andromeda* sp. nov. is distinct from the other species in the *B. stellata* species complex. The three sequenced specimens of *B. andromeda* sp. nov. grouped together in the ABGD analysis of the COI mitochondrial sequences of the specimens identified as *B. stellata* (Table 3). *Berthella andromeda* sp. nov. was originally reported as *B. stellata* from the Pacific coast of Mexico by Gosliner and Bertsch (1988). The external morphology and the morphology of the shell, jaw elements, and radular teeth of the specimens collected by Gosliner and Bertsch (1988) agree very well with the present material. The only other species known from the Pacific coast of Mexico similar to *B. andromeda* sp. nov. in external morphology is *B. strongi*. However, molecular data presented here show that they are distinct. *Berthella andromeda* sp. nov. can also be distinguished from *B. strongi* by its external morphology. *Berthella strongi* has a beige mantle (Gosliner and Bertsch 1988; present study) with numerous small dorsal white tubercles some of which have a brown base (present study: Fig. 2n, o). In contrast, the mantle of *B. andromeda* sp. nov. is smooth, translucent white to yellowish white (present study)

Fig. 12 *Berthella andromeda* sp. nov., scanning electron micrographs of the radular teeth and jaw elements of a specimen from Mazatlán, Mexico (CPIC 01418). **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** Jaw elements. White arrows indicate the position of secondary denticles



(Fig. 2k). Moreover, *B. andromeda* sp. nov. usually has an opaque white transverse bar near the center of the mantle (Gosliner and Bertsch 1988; Goddard 2010; present study: Fig. 2k), a character not observed in *B. strongi*. Gosliner and Bertsch (1988) reported the presence of a yellow tubercle at each lateral tip of the dorsal opaque white transverse bar in a specimen identified as *B. stellata* from the Pacific coast of Mexico. Such tubercles, however, were not observed in the present material and could be a morphological variation in *B. andromeda* sp. nov. Another difference between *B. andromeda* sp. nov. and *B. strongi* is in the morphology of their shells. The lateral margins of the shell of *B. strongi* (Fig. 8b) are almost parallel, whereas the lateral margins of the shell of *B. andromeda* sp. nov. (Fig. 11a, c) are not. Finally, the reproductive anatomy of these two species is distinct. Whereas in *B. andromeda* sp. nov. the seminal receptacle and bursa copulatrix are connected serially, they are connected semiserially in *B. strongi*. Additionally, the bursa copulatrix of *B. andromeda* sp. nov. is much larger than the seminal receptacle, whereas they are about the same size in *B. strongi*. The ampulla of *B. strongi* is proportionally much larger than that of *B. andromeda* sp. nov.

Records from Costa Rica (e.g., Camacho-García et al. 2005) are provisionally assigned to this species; however, they could belong to *Berthella* sp. 1, an undescribed species from Panama discussed below.

Berthella nebula sp. nov. (Figs. 2g, h, 5b, 13, and 14)
http://zoobank.org/B81A077B-7F39-4A16-BF2E-BA7D1BEC6D29

Holotype: Martinique, Mar 2014, 6 mm preserved length, dissected, leg. Yan Buske (MNHN IM-2000-34532).

Other material examined: St. James, Jamaica, 18 Jul 2011, 1 specimen 4 mm preserved length, leg. Jessica Goodheart (CPIC 00655). Anse Noir, Martinique, 31 Mar 2015, 1 specimen 6 mm preserved length, dissected, leg. Yan Buske (CPIC 02097); 1 Apr 2015, 1 specimen 5.5 mm preserved length, dissected, leg. Yan Buske (CPIC 02098). Anse Marette, Martinique, Mar 2014, 1 specimen 6 mm preserved length, leg. Yan Buske (CPIC 02102); 17 Mar 2018, 1 specimen 5 mm preserved length, leg. Yan Buske (CPIC 02437). Punta Caracol, Bocas del Toro, Panama, 16 Jun 2005, 1 specimen 10 mm preserved length, leg. S. Fahey (CASIZ 172853).

Diagnosis: Animal off-white to grayish white, with numerous opaque white spots and T-shaped or Y-shaped central patch; gill occupying with 8–10 pinnae, 5–6 free of body wall; shell oval, convex, translucent honey-brown, covering almost entire length of preserved animal; protoconch 210–250 μm in diameter with1 whorl; radular inner and mid lateral teeth hook-shaped with strong secondary denticle; outer teeth bifid; jaw elements with 4–6 pointed to rounded denticles; bursa copulatrix about 8 times as large as seminal receptacle connected semiserially; penial gland elongate; penis oval, retractable.

Description: Body elongate oval (Fig. 2g, h). Mantle broad, covering foot on all sides, smooth, translucent white to off-white to grayish white with numerous opaque white spots scattered throughout. Some dorsal white spots forming variably shaped pattern sometimes resembling a “T” or “Y” near central mantle. Oral veil broad, trapezoidal, translucent white with few to numerous opaque white spots near anterior and side margins. Rhinophores rolled, fused for about two thirds their length, emerging between oral veil and mantle, translucent white, each with one opaque white spot on dorsal tip. Gill small, occupying less than one half of body length, bipinnate, with 10 pinnae (6 in portion of gill not attached to body wall) on each side of smooth rachis in 6 mm specimen from Anse Noir, Martinique (CPIC 02097), and 8 pinnae (5 in portion of gill not attached to body wall) in the 6 mm holotype, Martinique (MNHN IM-2000-34532).

Shell covering entire body, oval, convex, translucent light honey brown, covering almost entire length of preserved animal (Fig. 13a, c). Sculpture of transverse grooves crossing distinct growth lines. Protoconch oval, 210–250 μm in diameter (Fig. 13b, d), with 1 whorl. Shell dimensions: 5.15 × 3.25 mm (5.5 mm specimen—CPIC 02098) and 5.95 × 3.45 mm (6 mm holotype—MNHN IM-2000-34532), all from Martinique.

Radular formula 56 × 48.0.48 in the 6 mm holotype, Martinique (MNHN IM-2000-34532). Inner teeth (Fig. 14a) hook-shaped with one basal denticle. First 3–6 inner lateral teeth with one basal denticle; basal denticles decreasing in size laterally. Mid-lateral teeth (Fig. 14b) hook-shaped, increasing in size toward outer margin. Three to six outermost teeth (Fig. 14c) smooth, decreasing in size laterally; ~ 9 preceding teeth erect, slender, with secondary denticle. Jaw elements in distal view with pointed central cusp bearing 4–6 pointed to rounded denticles on each side (Fig. 14d); denticulation not symmetrical. Jaw elements in proximal view with indentation near center of cusp (Fig. S5b).

Reproductive system (Fig. 5b) androdiaulic. Ampulla elongate, slender, curved but not convoluted, branching at base into oviduct and prostate; ampulla nearly four times as long and wide as the prostate. Prostate with two folds. Penial gland elongate, curved in middle, mildly convoluted near base. Prostate and penial gland joining at base of deferent duct leading to penis. Bursa copulatrix rounded, stalked, joining elongate, slender seminal receptacle semiserially at base of vaginal canal; bursa copulatrix about 8 times as large as seminal receptacle. Penis oval, retractable.

Geographic range: Known from Martinique Is. (present study), Jamaica (present study), the Caribbean coast of Mexico (Gosliner and Bertsch 1988), Puerto Rico (Marcus and Marcus 1970), and the Caribbean coast of Panama (present study).

Derivatio nominis: The name *nebula* refers to interstellar clouds of dust or ionized gases.

Remarks: The phylogenetic and ABGD analyses using the COI mitochondrial sequences of the specimens identified as *B. stellata* recovered *B. nebula* sp. nov. as distinct from the other specimens included in the analysis (Fig. 2, Table 3), including a second species from the Caribbean region described below.

The only available species name for the *B. stellata* complex in the western Atlantic is *B. tupala* Er. Marcus, 1957, originally described from Brazil. Er. Marcus (1957) introduced the name *B. tupala* based on a single specimen collected from São Paulo and described it as having a “light ochre” notum with a dorsal trapezoidal pattern and a few dorsal opaque white spots. Since the original description of *B. tupala* several specimens of *Berthella* from the western Atlantic including the Caribbean Sea that possessed a translucent white to yellowish white mantle with dorsal opaque white spots, sometimes forming some variation of a pattern near the center of the mantle, was reported by several authors as members of this species (Marcus and Marcus 1967; Marcus and Marcus 1970; Bertsch 1975). Even though all these records assigned to *B. tupala* generally agree in external morphology, some intraspecific variation was reported regarding aspects of their internal morphology, including denticulation of jaw elements and radular teeth. For example, in the original description of *B. tupala*, Er. Marcus (1957) described the jaw elements of this species as having two rounded denticles on each side of a rounded central cusp; however, Bertsch (1975) reported a specimen from the Caribbean coast of Panama with jaw elements that completely lacked any denticulation. Marcus and Marcus (1970) recorded a specimen from Puerto Rico with some jaw elements having a triangular indentation and some

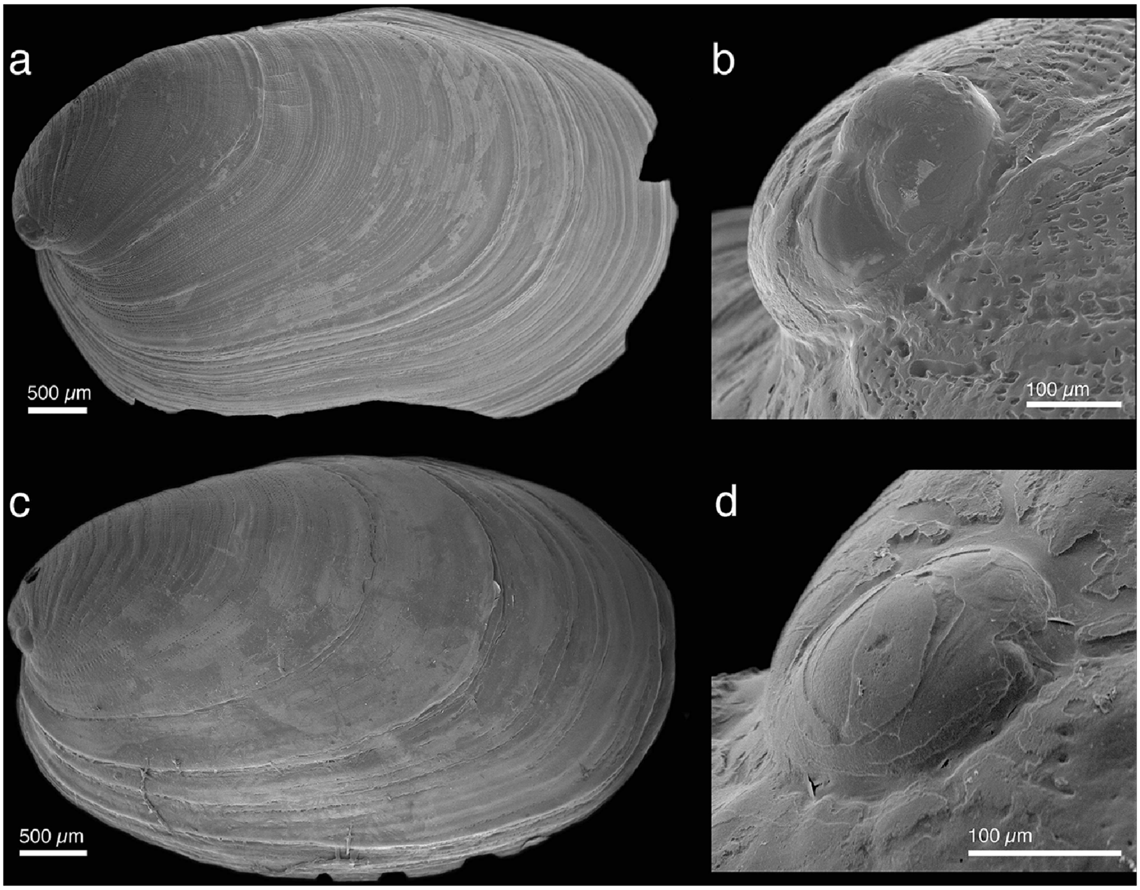


Fig. 13 *Berthella nebula* sp. nov., scanning electron micrographs of the shells of specimens from the Caribbean. **a**, **b** Holotype, Martinique Is. (MNHN IM-2000-34532), dorsal view of the teleoconch (**a**), detail of the protoconch (**b**); **c**, **d** Specimen from Martinique Is. (CPIC 02098), dorsal view of the teleoconch (**c**), detail of the protoconch (**d**)

jaw elements having no indentation at all. Variation was also observed in the denticulation of the outer radular teeth. Er. Marcus (1957) described the six to eight outermost teeth of *B. tupala* as having a secondary cusp; however, Marcus and Marcus (1967) reported three specimens of *B. tupala* from Florida with the five outer teeth having smooth cusps, and only the preceding ten rows having a secondary cusp. Gosliner and Bertsch (1988) examined a specimen collected from the Caribbean coast of Mexico, which they found to be morphologically similar to the original description of *B. tupala* by Er. Marcus (1957), except for some differences in the reproductive anatomy, attributed to error. Gosliner and Bertsch (1988) were unable to find consistent differences between the Caribbean material and other records of *B. stellata* from other ocean basins, and regarded *B. tupala* as a synonym of *B. stellata*. More recently, Alvim and Pimenta (2015) recorded several specimens identified as *B. stellata* from various localities in Brazil. These specimens possessed two different shell morphologies, some specimens were small and had delicate, white shells, whereas others were larger and had strongly calcified, brown shells. Alvim and Pimenta (2015) also described some variability in the radular morphology; for example, the outer lateral teeth of a specimen with a white shell were described as bifid, whereas the outer radular teeth of a specimen with a brown shell were described as "smooth," or having one cusp. Again, Alvim and Pimenta (2015) considered this variability to be intraspecific and maintained the taxonomic scheme proposed by Gosliner and Bertsch (1988).

The detailed anatomical studies by Gosliner and Bertsch (1988) and Alvim and Pimenta (2015) concluded that *B. stellata* is a single species with a widespread distribution in the western Atlantic and beyond. On the contrary, our molecular data revealed the presence of two distinct, sympatric species in the Caribbean region as well as additional species in other ocean basins. Because there is no molecular data available for specimen from southern Brazil, we are unable to determine with certainty which—if any—of the two Caribbean species could correspond to *B. tupala*. Moreover, based on the variability described by Alvim and Pimenta (2015), it is possible that two cryptic species could co-occur in Brazil. The two Caribbean species recovered in our analyses also possess white and brown shells respectively, as well as similar differences in the radular morphology.

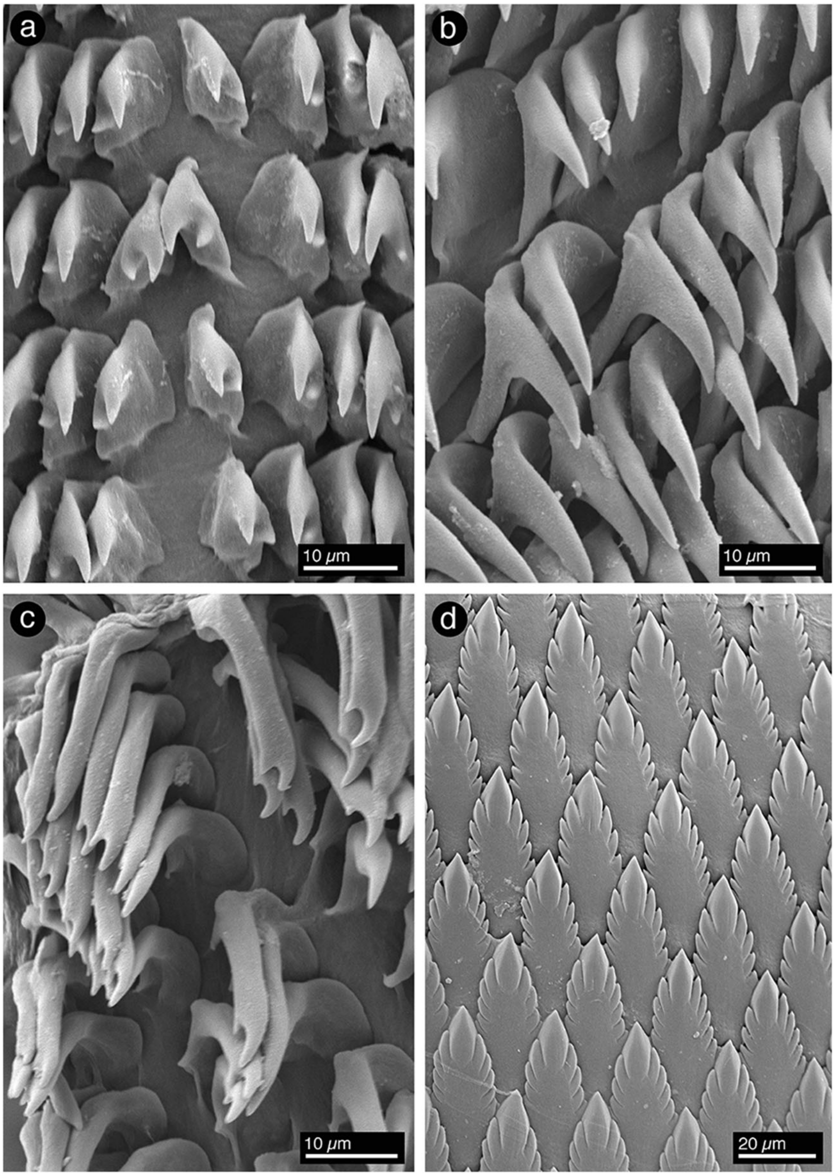


Fig. 14 *Berthella nebula* sp. nov., scanning electron micrographs of the radular teeth and jaw elements of the holotype, Martinique Is. (MNHN IM-2000-34532); **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** Jaw elements

We compared the morphology of *B. nebula* sp. nov. to the original description of *B. tupala* as we did not have access to the type material. The external morphology of *B. nebula* sp. nov. generally agrees with the original description of *B. tupala* and both species have brown shells; however, characters of the radular teeth and the jaw elements differ from those originally described for *B. tupala* or there are too variable to reach a definitive conclusion. The innermost radular teeth and the succeeding five to six inner lateral teeth of *B. nebula* sp. nov. have a basal denticle. Er. Marcus (1957) did not illustrate the innermost radular teeth of *B. tupala*; however, Ev. Marcus and Er. Marcus (1967) recorded three specimens identified as *B. tupala* from Florida and stated that the innermost radular teeth had a small denticle near the base. Ev. Marcus and Er. Marcus (1967) indicated that this basal denticle was also present in the type material of *B. tupala* from São Paulo, Brazil, but did not provide any illustrations of the innermost radular teeth of the type material. Therefore, there is no reference for comparison between the innermost radular teeth of *B. nebula* sp. nov. and those of the type specimen of *B. tupala*. Alvim and Pimenta (2015) described and illustrated inner radular teeth from the same Brazilian material with and without a denticle, suggesting this trait is not taxonomically useful. The jaw elements of *B. nebula* sp. nov. have 3–6 rounded to pointed denticles on each side of the pointed central cusp. Er. Marcus (1957) in the original description of *B. tupala* described and illustrated the jaw elements as having two rounded denticles on each side of a rounded central cusp. Marcus and Marcus (1970) reported a specimen of *B. tupala* from Puerto Rico with jaw elements bearing up to five pointed denticles on each side of the pointed central cusp. Marcus and Marcus (1970) stated that after seeing this specimen, they re-examined the type material of *B. tupala* from Brazil and found that the shape of the cusps and the number of denticles on the jaw elements varied from one area to the other. Marcus and Marcus (1970) did not, however, provide any illustrations of the jaw elements of the original material of *B. tupala*. They only provided illustrations of the jaw elements of the specimen from Puerto Rico. In the absence of illustrations or a clear account of the nature of variation in the jaw elements of the type specimen of *B. tupala*, it cannot be determined whether the jaw elements of *B. nebula* sp. nov. agree with those of the holotype of *B. tupala*. Er. Marcus (1957) illustrated the reproductive system of *B. tupala*, which lacks a seminal receptacle. Gosliner and Bertsch (1988) concluded that this was due to error. The overall morphology of the reproductive system of *B. tupala* and *B. nebula* sp. nov. is similar, but the penial gland of *B. nebula* sp. nov. is proportionally shorter, wider, and more convoluted than that of *B. tupala.*

In summary, because of the lack of molecular data from Brazilian specimens, we cannot determine with certainty whether any of the two Caribbean species recovered in our analyses correspond to *B. tupala*. *Berthella nebula* sp. nov. is the most similar morphologically (both have brown shells), but differences in the reproductive anatomy and inconsistencies in the radular and jaw morphology prevent us from reaching a conclusion. The more comprehensive study of Brazilian material by Alvim and Pimenta (2015) was based on specimens with different shell colorations and morphologies and could represent two cryptic species. For all these reasons, we are describing *Berthella nebula* sp. nov. as a new species. Further molecular work is required to determine the identity of the specimens from Brazil.

Based on the results of this study, Indian Ocean records of *B. tupala* (Gosliner 1987; Kazmi et al. 1996) most likely belong to a different species, but this cannot be confirmed until specimens from this region are examined.

Berthella vialactea sp. nov. (Figs. 2i, j, 5f, 15, and 16)
http://zoobank.org/0EE95656-5A1A-4E6B-9A76-95A5D2507057

Holotype: Martinique, Mar 2014, 6 mm preserved length, dissected, leg. Yan Buske (MNHN IM-2000-34531).

Other material examined: Anse Marette, Martinique, 28 Jan 2017, 1 specimen 4 mm long preserved length, leg. Y. Buske (CPIC 02096); Mar 2014, 1 specimen 6 mm preserved length, leg. Y. Buske (CPIC 02099). Le Diamant, Martinique, 26 Mar 2017, 1 specimen 3 mm long preserved length, leg. Y. Buske (CPIC 02434); 26 Mar 2017, 1 specimen 4 mm long preserved length, leg. Y. Buske (CPIC 02435); 26 Mar 2017, 1 specimen 4 mm long preserved length, leg. Y. Buske (CPIC 02436).

Diagnosis: Animal translucent white to milky white, with numerous opaque white spots, central patch T-shaped, Y-shaped, or irregularly shaped; gill with 6–10 pinnae, 2–4 free of body wall; shelloval, convex, translucent white; protoconch 450 μm in diameter with 1.5 whorls; radular inner and mid-lateral teeth hook-shaped some with weak secondary denticle; outer teeth with single cusp; jaw elements with 3–6 denticles; seminal receptacle about one half as large as bursa copulatrix, connected semiserially; penial gland convoluted; penis oval, retractable.

Description: Body elongate, oval (Fig. 2i, j). Mantle broad, covering foot on all sides, smooth, translucent white to milky white with numerous opaque white spots scattered throughout. Dorsal white spots forming "T"-shaped to "Y"-shaped or irregularly shaped patch near central mantle. Oral veil broad, trapezoidal, translucent white with some opaque white spots. Rhinophores emerging between mantle, oral veil, rolled, fused together about one half their length. Gill bipinnate, occupying one half of body length, with 6 primary pinnae (2 in portion of gill not attached to body wall) on each side of smooth rachis in a 4-mm specimen (CPIC 02096), 10 pinnae (4 in portion of gill not attached to body wall) in a 6-mm specimen (CPIC 02099), and 10 pinnae (4 in portion of gill not attached to body wall) in the 6-mm holotype (MNHN IM-2000-34531), all from Martinique.

Shell (Fig. 15a) oval, convex, covering entire body. Sculpture of transverse grooves crossing distinct growth lines. Protoconch (Fig. 15b) oval, about 450 μm in diameter, with 1.5 whorls. Shell dimensions 5.17 × 3.5 mm in the 6 mm holotype from Martinique (MNHN IM-2000-34531).

Radular formula 58 × 57.0.57 in the 6-mm holotype from Martinique (MNHN IM-2000-34531). Innermost teeth (Fig. 16a) hook-shaped, some with very small basal denticle. Inner lateral teeth (Fig. 16b) simple, hook-shaped, increasing in size laterally. Outer teeth erect, typically smooth, some with a secondary denticle (Fig. 16c). Last 8–10 outer teeth decreasing in size (Fig. 16c). Jaw elements in distal view with central cusp bearing 3–6 denticles on each side (Fig. 16d); denticulation not symmetrical. Some denticles pointed; some denticles rounded. Jaw elements in proximal view with elongate indentation near center of cusp, widening toward the base (Fig. S5c).

Reproductive system (Fig. 5f) androdiaulic. Ampulla elongate, convoluted, narrowing proximally into oviduct. Prostate emerging at base of oviduct, with onefold about halfway to penis. Penial gland convoluted near base before entering penis. Bursa copulatrix spherical. Seminal receptacle oval, about one half bursa copulatrix. Bursa copulatrix and seminal receptacle connected semiserially at base leading to short vaginal duct. Deferent duct twice as long as vaginal duct, opening into penis dorsal to vagina. Penis oval, retractable.

Geographic range: Known from the Martinique Island, Caribbean Sea (present study).

Derivatio nominis: The species named *vialactea* refers to the Via Lactea, or the Milky Way, the galaxy that contains our Solar System.

Remarks: The ABGD analysis of the COI mitochondrial sequences recovered *B. vialactea* sp. nov. as a distinct species in the *B. stellata* species complex (Table 3). This species is very similar to the sympatric *B. nebula* sp. nov. in external morphology but is clearly different from that species based on molecular data, as well as some internal morphological traits here presented.

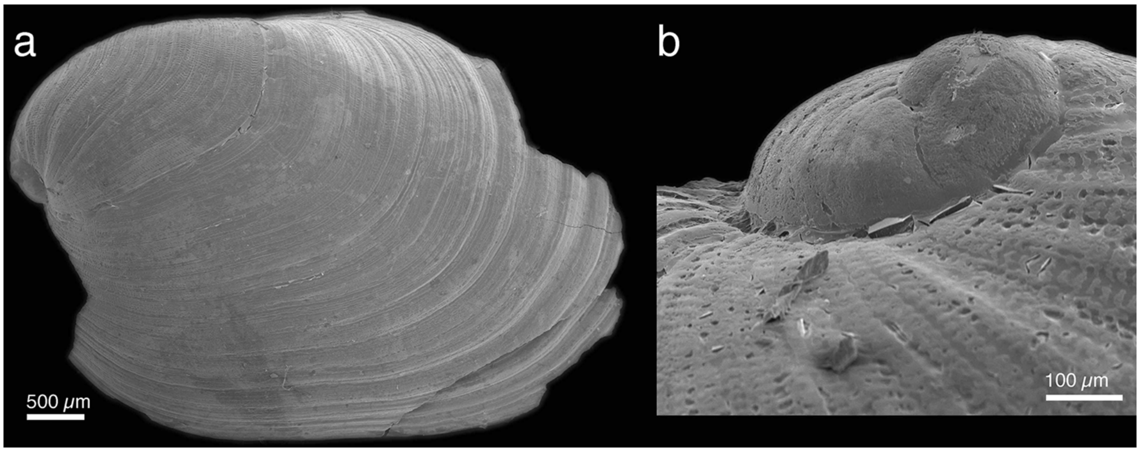
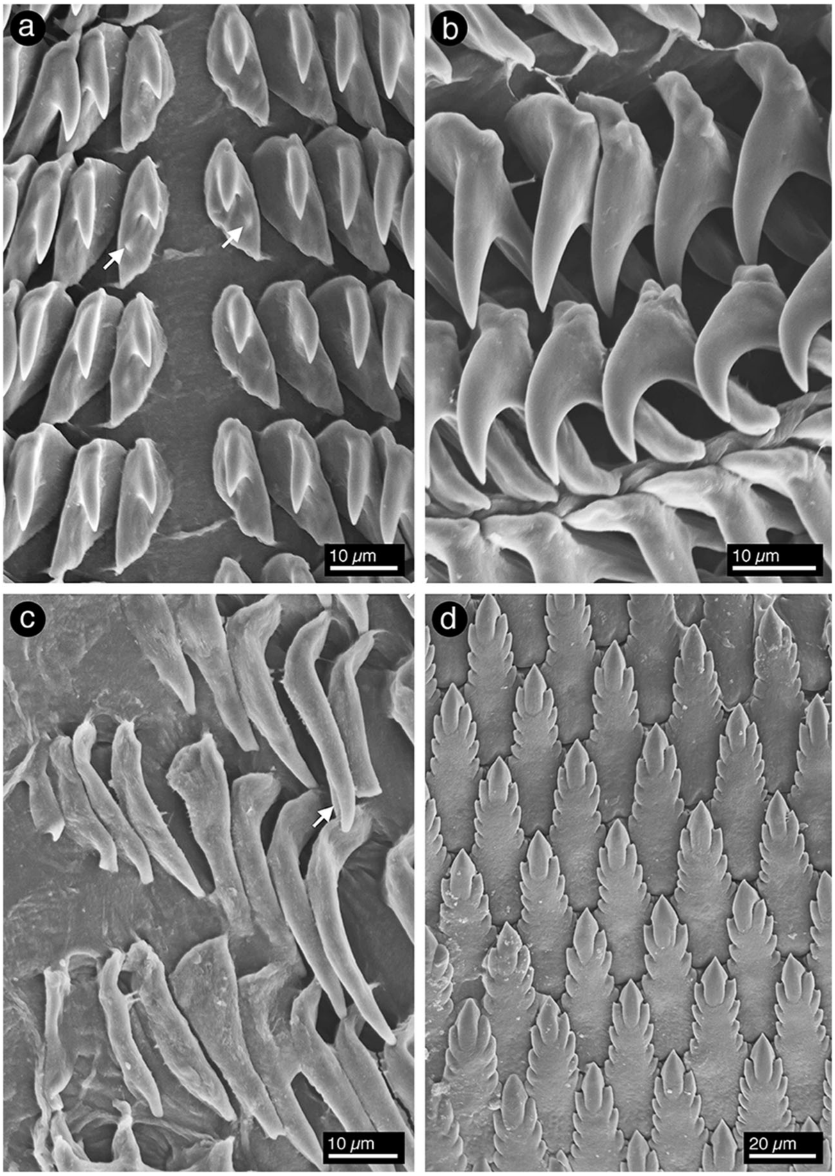


Fig. 15 *Berthella vialactea* sp. nov., scanning electron micrographs of the shell of the holotype, Martinique Is., Caribbean Sea (MNHN IM-2000-34531). **a** Dorsal view of teleoconch; **b** Detail of protoconch

Fig. 16 *Berthella vialactea* sp. nov., scanning electron micrographs of the radular teeth and jaw elements of the holotype, Martinique Is., Caribbean Sea (MNHN IM-2000-34531). **a** Innermost lateral radular teeth; **b** Mid-lateral radular teeth; **c** Outer lateral radular teeth; **d** Jaw elements. White arrows indicate the position of secondary denticles



The innermost radular teeth of *B. nebula* sp. nov. all have a basal denticle (Fig. 14a), but not all the innermost radular teeth of *B. vialactea* sp. nov. have a basal denticle (Fig. 16a). Moreover, the first 5 to 6 rows of the inner lateral teeth have a basal denticle in *B. nebula* sp. nov. but not in *B. vialactea* sp. nov. The outermost radular teeth have a second denticle in *B. nebula* sp. nov. (Fig. 14c) whereas in *B. vialactea* sp. nov., the outermost teeth generally have a smooth cusp, although in some a second denticle is present (Fig. 16c). The reproductive systems of the two species are also different, and *B. nebula* sp. nov. has a shorter prostate and a more elongate penial gland than *B. vialactea* sp. nov. (Fig. 5b, f). *Berthella vialactea* sp. nov. should also be compared to *B. tupala* from the western Atlantic Ocean due its similarity in external morphology to that species. *Berthella vialactea* sp. nov. is readily differentiated from *B. tupala* based on the shell color, which was described as brown in *B. tupala* (Marcus 1957) and it is white in *B. vialactea* sp. nov. Some of the specimens described by Alvim and Pimenta (2015) from Brazil with a white shell could belong to this species, but this needs to be tested with molecular data.

Berthella sp. 1

Material examined: Isla Canal de Afuera, Panama, 21 May 2003, 1 specimen 10 mm preserved length, leg. A. Hermosillo (LACM 153343), morphology not examined.

Geographic range: Known from the Pacific coast of Panama (present study).

Remarks: This specimen formed a well-supported clade with a specimen from the Galapagos Islands (*Berthella* sp. 2) in both the Bayesian and ML analyses, which is sister to the clade including the Eastern Pacific species *B. andromeda* sp. nov. and *B. strongi*. The length of the branches for *Berthella* sp. 1 and *Berthella* sp. 2 are longer than other branches for distinct species (Fig. 1) suggesting that they might be a separate species. However, the COI mitochondrial DNA of this specimen did not amplify after multiple attempts and there are no photos of the live animal available. Thus, we cannot confirm it constitutes a distinct species. More specimens from this locality must be obtained and sequenced to determine their taxonomic status.

Berthella sp. 2 (Fig. 2f)

Material examined: NE side of Isla Darwin, Galapagos Islands, 13 May 1994, 1 specimen 11 mm preserved length, leg. T.M. Gosliner (CASIZ 97563), morphology not examined.

Geographic range: Known from the Galapagos Islands (present study).

Remarks: This species formed a well-supported clade with the *Berthella* sp. 1 from Panama which was sister to the clade including the Eastern Pacific species *B. andromeda* sp. nov. and *B. strongi*.

This specimen was recovered as a distinct species in the ABGD analysis of the COI mitochondrial sequences of the specimens included in the study (Table 3). This species is similar in external morphology to *B. andromeda* sp. nov. from the Eastern Pacific in that it has a central opaque white pattern on its dorsum. However, the central pattern in the present material is in the shape of a cross whereas the central pattern in *B. andromeda* sp. nov. is in the shape of a transverse bar. Another difference is ground color which is translucent white to grayish white in *B. andromeda* sp. nov. but brown in the present material. It appears that *Berthella* sp. 2 is a distinct species but it is not described herein due to the lack of sufficient material.

Discussion

Berthella stellata is a species complex

Molecular species delimitation analyses using COI mtDNA sequence data, as well as morphological comparisons among candidate species (Table 4), indicate that the nominal circumtropical species *B. stellata* is a complex of at least six distinct species. A review of the literature revealed that the valid names for these species are *B. stellata* for the northeastern Atlantic Ocean and the Mediterranean Sea; *B. nebula* sp. nov. and *B. vialactea* sp. nov. for the Caribbean Sea; *B. andromeda* sp. nov. for the Eastern Pacific Ocean; *B. pellucida* from the Hawaiian Islands; and *B.* cf. *postrema* from the Western Pacific Ocean. Two additional species were recovered in the Eastern Pacific but are not described due to the limited material available. Phylogenetic analysis also revealed a strong biogeographic signal among the species studied and the fact that another Eastern Pacific species, *B. strongi*, is part of the *B. stellata* species complex. Moreover, *B. plumula* is also a complex of two different species with allopatric ranges in the Eastern Atlantic and Mediterranean.

These results are not surprising as several other nominal species of heterobranch sea slugs with unusually large disjunct geographic ranges have been shown to be species complexes (Ornelas-Gatdula et al. 2012; Carmona et al. 2014; Uribe et al. 2018; Valdés et al. 2017). One important contributing factor resulting in species with very large, disjunct geographic ranges across different ocean basins is a historical tendency to consider small morphological differences in similar individuals as intraspecific variation (see Jörger et al. 2012; Hoover et al. 2015). This approach can be problematic as it disregards convergence as well as cryptic or pseudocryptic speciation, ultimately leading to an underestimation of biodiversity and species richness (Jörger et al. 2012; Hoover et al. 2015). The results of the present study add to the mounting body of evidence challenging conservative morphological approaches to systematics and taxonomy. In the case of *B. stellata*, a single character, namely the presence of an opaque white patch, cross, or bar near the center of the mantle, was used to identify any species of *Berthella* from anywhere in the world as *B. stellata*

(Gosliner and Bertsch 1988). Differences in external coloration and the morphology of the shell, radular teeth, jaw elements, and reproductive systems were considered intraspecific variation (Gosliner and Bertsch 1988). The justification proposed by Gosliner and Bertsch (1988) for considering the observed variation as intraspecific was that much of this variation was seen in single populations in the same geographic area. The main assumption by Gosliner and Bertsch (1988) was that all the individuals with similar external morphologies (e.g., a dorsal opaque white patch) in a given population belonged to the same species. Our results show that this assumption is not always correct. We found two sympatric genetically distinct species, namely *B. nebula* sp. nov. and *B. vialactea* sp. nov. from Martinique Island in the Caribbean Sea, both with an opaque white pattern near the center of the mantle. These species are very similar externally but are different with respect to some internal morphological characters including shell color, denticulation of radular teeth, and arrangement of reproductive systems. However, we did find intraspecific variation in members of the *B. stellata* complex. For example, according to the results of our molecular species delimitation analysis, *B. stellata* populations in the Mediterranean Sea and the Northeastern Atlantic Ocean belong to the same species. However, specimens from these populations exhibit differences in external coloration (see *B. stellata* description and remarks above). These findings underscore the importance of using an integrative molecular and morphological approach in delineating species of heterobranch sea slugs.

Berthella plumula is also a species complex

Specimens of *Berthella plumula* (Montagu, 1803) were included in this study for comparison, primarily because this species has been considered a synonym of *B. stellata* in the past (Vayssière 1880; Bergh 1897), and the external differentiation between the two species can be problematic. *Berthella plumula* is currently considered to have an extensive geographic range in the Northeastern Atlantic from Norway to the Canary Islands and in the Mediterranean Sea (Thompson 1976; Cervera et al. 2004); however, there are some morphological differences between Mediterranean and Atlantic specimens. As mentioned above, the phylogenetic and species delimitation analysis confirmed the distinctiveness of *B. plumula* and *B. stellata*. Additionally, specimens identified as *B. plumula* from the Mediterranean and the Northeastern Atlantic (Northern Ireland and France) formed two distinct monophyletic groups, sister to one another. The ABGD analysis of the COI mitochondrial sequences confirmed that they constitute distinct species. Resolving outstanding taxonomic issues regarding *B. plumula* was not a goal of this study but in light of the new evidence, we feel it necessary to provide names for the species recovered in the analyses. Montagu (1803) originally introduced the name *B. plumula* based on specimens collected in England, described as having a pale yellow reticulated mantle. This color pattern agrees with that of the specimens here examined from Northern Ireland (Fig. 2m). Additional descriptions and online photographic records of specimens identified as *B. plumula* from the northeastern Atlantic Ocean (Thompson 1976; Picton 2002) also show these specimens possessing a pale yellow, strongly reticulated dorsum. Based on the type locality and the consistency in color pattern, the name *B. plumula* is retained for the Northeastern Atlantic species. Therefore, a new name is needed for the Mediterranean animals.

A review of the literature revealed that the oldest available name for the Mediterranean species is most likely *Pleurobranchus perforatus* Philippi, 1844 (type locality Catania, Sicily, Italy). *Pleurobranchus perforatus* is currently regarded as a synonym of *B. plumula* (Thompson 1976). Philippi (1844) described *P. perforatus* based on a preserved specimen with no traces of its original color, but having the dorsum perforated all over with deeply impressed points. This resembles the dorsal morphology of Atlantic and Mediterranean specimens assigned to *B. plumula*. Therefore, *P. perforatus* in the binomen *Berthella perforata* is here regarded as the valid name for the Mediterranean population. *Berthella porosa* de Blainville, 1824, also considered to be a synonym of *B. plumula* (Thompson 1976), was originally described from England (de Blainville 1824) and therefore should not be used for Mediterranean animals. Another possible candidate, *Sigaretus stomatellus* Risso, 1826, originally described from Nice, has been regarded as a synonym of *B. plumula* (Locard 1886; Clessin 1899), but this is probably a misinterpretation of Cantraine (1835), who mentioned that *S. stomatellus* was a synonym of "*Helix pellucida* L. Gmelin." Risso (1826) described the shell of *S. stomatellus* as yellow with a pearly interior, which is not consistent with *Berthella*. Bergh (1887) considered *S. stomatellus* a member of *Marsenia*. Finally, *Pleurobranchus brevifrons* Philippi, 1844 (type locality not stated, Sicily) was described in the same paper as *B. perforata* and could also be a synonym. However, Philippi's (1844) description is vague and it is difficult to determine the identity of *P. brevifrons* with enough certainty. Bergh (1898) reported *B. plumula* from the Red Sea, but this is probably a misidentification.

The identification of specimens of *B. perforata* is problematic due to the color variation in this species (Fig. 2l). Some of the specimens examined in this study display a pattern of stellate markings similar to that of specimens of *B. stellata* while others are yellowish or whitish, with no stellate markings, resembling *B. plumula*.

Biogeography and diversity

This study included samples representing most of the range of the *B. stellata* species complex, including the Western, Central, and Eastern Pacific Ocean, the Eastern and Western

Atlantic, and the Mediterranean Sea. However, members of the species complex have also been recorded from the Indian Ocean (Heller and Thompson 1983; Gosliner 1987; Wells and Bryce 1993; Kazmi et al. 1996; Flodrops 2008; Coleman 2008; Bhave 2009; Apte et al. 2010; Cadet 2011). We did not have access to any specimens from the Indian Ocean, for which the name *Berthella stellata albocrossata* Heller & Thompson, 1983 is available; thus, we cannot verify whether they constitute a distinct species. Photographic records of Indian Ocean specimens (Wells and Bryce 1993; Coleman 2008; Cadet 2011) show a strongly reticulated mantle, similar to that of specimens of *B. pellucida* and *B.* cf. *postrema* here examined. We hypothesize that the Indian Ocean animals are probably closely related to other Indo-Pacific taxa, but this will need to be confirmed with molecular data. Considering the species diversity of the *B. stellata* species complex in the Atlantic Ocean (at least 3 species) and the Eastern Pacific (2 described species + 2 additional undescribed species), it is likely that the tropical Indo-Pacific contains additional unnamed taxa. Additional evidence for additional Indo-Pacific diversity comes from the fact that the specimens from the Hawaiian Islands are genetically distinct from the single animal studied from New Caledonia and they constitute different species. The single specimen from New Caledonia was tentatively assigned to *B. postrema* due to the strong morphological similarities between this animal and the original description of this species from temperate Australia (Burn 1962). However, we had no access to specimens from Australia for comparison. Further research may reveal that the animals from temperate waters of Australia are genetically distinct and a new name (or more names) may be needed for specimens recorded from tropical regions of the Western Pacific (Risbec 1928; Baba 1969; Thompson 1970; Willan 1984; Carlson and Hoff 2003; Wägele et al. 2006; Cobb 2008; Nakano 2018; Gosliner et al. 2018). Unfortunately, specimens of the *B. stellata* species complex are typically rare in this region, and adequate collections for molecular work from the Indo-Pacific region are lacking.

Previous studies of the phylogenetic systematics and biogeography of several heterobranch sea slug lineages have shown a recurrent (but not universal) biogeographical pattern, in which species from the Indo-Pacific form a monophyletic group, sister to a clade containing species from the Eastern Pacific and Atlantic Ocean (Gosliner and Johnson 1999; Ornelas-Gatdula et al. 2012; Valdés 2004). The phylogenetic relationships here recovered for the *B. stellata* complex also exhibit this pattern: the Eastern Pacific clade is sister to the Atlantic clade (Fig. 1), and the Indo-Pacific species formed a clade sister to the clade containing Eastern Pacific and the Atlantic species. The hypothesis proposed to explain this biogeographical pattern involves three main consecutive vicariant events: (1) the initial fragmentation of the Tethys Sea after the collision between Africa and Eurasia which separated the Indo-Pacific from the Atlantic (Hrbek and Meyer 2003; Williams and Reid 2004), (2) the formation of the East Pacific Barrier, a 5000-km stretch of water that separated the Eastern Pacific from the Central Pacific (Briggs 1961; Lessios and Robertson 2006), and (3) the closure of the Isthmus of Panama, which separated the Eastern Pacific and the Western Atlantic (Bacon et al. 2015; Montes et al. 2015; O'Dea et al. 2016). For the formation of the observed pattern, it is necessary that these three events are consecutive in time, because marine organisms should evolve in response to vicariant events sequentially (Valdés 2004). Recent estimates of the closure of the Isthmus of Panama place this event as early as the Middle Miocene (Montes et al. 2015) or even during the Oligocene-Miocene transition (Bacon et al. 2015). If those estimates are correct, much earlier estimates for the formation of the East Pacific Barrier are needed to explain the phylogenetic pattern observed in the *B. stellata* species complex. This would also imply that the *B. stellata* species complex is much older than divergence estimates for larger clades in the closely related genus *Pleurobranchus* (Goodheart et al. 2015). In light of these data, the more recent estimate for the formation of the Isthmus of Panama (O'Dea et al. 2016) appears to be more realistic.

None of the vicariant events discussed above provides an explanation for the existence of two sympatric, sister species of the *B. stellata* complex in the Caribbean. While an unknown vicariant event may have been responsible for the formation of these two species, they could have also resulted from ecological speciation within their current range. Several other examples of recently evolved sympatric taxa of heterobranch sea slugs in the Western Atlantic (e.g., Ornelas-Gatdula and Valdés 2012; Espinoza et al. 2014; Valdés et al. 2017) suggest that further research in this complex region could provide important insights into the underlying mechanisms resulting in divergence and speciation of marine taxa (Ellingson and Krug 2016).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.

Data availability The datasets generated during and/or analyzed during the current study are available in the the GenBank repository, https://www.ncbi.nlm.nih.gov/genbank/

Author contribution HG and AV conceived and designed research. MS, JHRG, MB, TMG and YB contributed material and biological data. HG conducted molecular work. HG and AV analyzed data and wrote the manuscript. All authors read and approved the manuscript.

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