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Systematics and biogeography of *Pleurobranchus* Cuvier, 1804, sea slugs (Heterobranchia: Nudipleura: Pleurobranchidae)

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Species of *Pleurobranchus* (Mollusca: Gastropoda: Heterobranchia: Nudipleura: Pleurobranchidae) are commonly found worldwide, but there is a substantial amount of confusion regarding the ranges and identification of individual species. Difficulties in phylogenetic reconstruction and identification of pleurobranchids using morphological traits has resulted in complex classification schemes, with several species having disjunct ranges across physical and biogeographical barriers (including the tropical Indo-Pacific, the eastern Pacific, and the Atlantic). A sizeable number of species of *Pleurobranchus* has been described; however, many of these species are morphologically and biogeographically similar to others, and probably constitute synonyms. This paper provides a phylogenetic framework of classification for *Pleurobranchus* based on the mitochondrial genes *cytochrome c oxidase I* (*COI*) and *16S* rDNA and the nuclear gene *histone 3* (*H3*) using Bayesian and maximum likelihood approaches. Molecular phylogenies obtained recovered most of the well-established species of *Pleurobranchus* and some morphological characters were found to have taxonomic value for delimiting species in this group. Automatic barcode gap discovery (ABGD) analyses substantiated the distinctiveness of units/species recovered in the phylogenetic analyses, with some exceptions. Morphological descriptions for the 14 species recovered in the molecular phylogeny and discussions on the biogeography and colour variation are included.

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INTRODUCTION

Pleurobranchus (Nudipleura, Pleurobranchidae) is a group of side-gilled heterobranch sea slugs, typically found intertidally and in the shallow subtidal of tropical and subtropical regions worldwide. According to Willan (1987), the main synapomorphies of *Pleurobranchus* are the presence of a tuberculate mantle and gill rachis, a cleft anterior border of the mantle, and flaps surrounding the genital aperture in mature adults. The monophyly of *Pleurobranchus* has been confirmed by morphoanatomical cladistics approaches (Willan, 1987; Cervera *et al*., 2000; Martynov & Schrödl, 2009). Martynov & Schrödl (2009) provided evidence that *Pleurobranchus* is a derived rather than a basal pleurobranchoidean clade, which was subsequently confirmed by Göbbeler & Klussmann-Kolb (2010). Species of *Pleurobranchus* are exclusively carnivorous and have a reduced internal shell. Some species of *Pleurobranchus* secrete chemicals for defence that are potentially beneficial to human health, including *Pleurobranchus albiguttatus* and *Pleurobranchus forskalii*, which contain compounds (cytotoxic lissoclimide-type diterpenes) that have shown promising toxicity against melanomas and solid tumours (Fu *et al*., 2004).

Over 70 species of *Pleurobranchus* (including synonyms) have been identified and described, many based solely on characteristics of the internal shell and some on external coloration (Martynov & Schrödl, 2009). As the shell of *Pleurobranchus* lacks distinctive traits (Willan, 1987), the recognition and identification of many of these species is problematic. In recent years, experts have disagreed on the value of certain morphological traits for classification and phylogenetic reconstruction of pleurobranchids (Willan, 1987; Gosliner & Bertsch, 1988), which also has implications for our ability to recognize and identify species.

Difficulties in phylogenetic reconstruction and identification of species in *Pleurobranchus* using morphological traits has resulted in complex classification schemes, with several species having disjunct ranges across physical and biogeographical barriers. A good example of a problematic taxon is the species *Pleurobranchus areolatus*, which has been reported in both the Caribbean Sea and eastern Pacific, despite the fact that these two regions have been separated for more than 3.1 million years by the formation of the Isthmus of Panama (Coates & Obando, 1996). By contrast, some species seem to have more restricted ranges and are found only in specific localities, such as *Pleurobranchus reticulatus*, which is reportedly found only in the Gulf of Guinea, off the coast of western Africa (Rang, 1832; Neves, Cervera & Calado, 2007), and *Pleurobranchus membranaceus*, which is found exclusively in the Mediterranean and north-eastern Atlantic (Bergh, 1897; Cervera *et al*., 2006).

Of the over 70 published species names of *Pleurobranchus*, only 14 have been used routinely in field guides and recent publications. These include *P. membranaceus* (Montagu, 1815) and *Pleurobranchus testudinarius* Cantraine, 1835, from the Mediterranean, *Pleurobranchus garciagomezi* Cervera, Cattaneo-Vinetti & Edmunds, 1996, and *P. reticulatus* Rang, 1832, from the eastern Atlantic, *Pleurobranchus evelinae* Thompson, 1977, from the Caribbean, *Pleurobranchus areolatus* Mörch, 1863, from the eastern Pacific, Caribbean, and eastern Atlantic, *Pleurobranchus hilli* (Hedley, 1894) from Southern Australia, and *P. albiguttatus* (Bergh, 1905), *P. forskalii* Rüppell & Leuckart, 1828, *Pleurobranchus grandis* Pease, 1868, *Pleurobranchus mamillatus* Quoy & Gaimard, 1832, *Pleurobranchus nigropunctatus* (Bergh, 1907), *Pleurobranchus peronii* Cuvier, 1804, and *Pleurobranchus weberi* (Bergh, 1905) from the Indo-Pacific. Of other species assigned to *Pleurobranchus*, some have been transferred to either *Berthella* or *Berthellina* by previous publications (Burn, 1962; Macnae, 1962; MacFarland, 1966; Edmunds & Thompson, 1972; Thompson, 1977; Ev. Marcus, 1984; Willan, 1984; Willan & Bertsch, 1987; Gosliner & Bertsch, 1988).

The main focus of this paper is to reconstruct the phylogeny of *Pleurobranchus* based on molecular data. We have attempted to clarify the taxonomic status of the existing species within *Pleurobranchus*, including the *P. areolatus* species complex, using two mitochondrial genes [*16S* rDNA and *cytochrome c oxidase I* (*COI*)] and one nuclear gene (*histone 3*, *H3*). Additionally, some morphological traits were re-examined for some species and combined with the molecular data. This paper also includes a review of the relevant literature to determine valid names for each species in *Pleurobranchus* in order to achieve nomenclatural stability for this group.

MATERIAL AND METHODS

SPECIMENS

DNA from 167 specimens of *Pleurobranchus* was extracted, but sequences were obtained for only 72 (Table 1). Forty-five specimens were obtained from the collections of the Natural History Museum of Los Angeles County (LACM), 68 specimens from the California Academy of Sciences Invertebrate Zoology collection (CASIZ) in San Francisco, one specimen from the Natural History Museum of Crete (NHMC), seven specimens from Zoologische Staatssammlung, Munich (ZSM), five specimens from the Museo de Zoología, University of Costa Rica (MZUCR), two specimens from the Zoological Museum, University of Bergen (ZMBN), and two specimens from the Museu de Zoologia, Universidade de São Paulo (MZSP). Twenty-two

Table 1. List of specimens examined in this paper for which sequences were obtained, including locality, museum collection numbers, and GenBank accession numbers for the three genes sequenced. The first column indicates the preliminary identification of the specimen based on available literature and the external colour of the animals. The last column indicates the final identification based on molecular and anatomical data. Sequences shorter than 200 bp were not deposited in GenBank but are marked with an asterisk and provided in Appendix S2

Preliminary identification	Locality	Voucher no.	GenBank accession no.			Final identification
			COI	*H3*	*16S*	
Pleurobranchaea meckelii	–	GenBank	FJ917499	EF133470	FJ917439	–
Pleurehdera haraldi	Sand Island, Palmyra Atoll	CASIZ 174202	KM521726	KM521620	–	–
Berthella stellata	St James, Jamaica	CPIC 00655	KM521691	KM521621	KM521594	–
Berthella stellata	Islan Canal de Afuera, Panama	LACM 153343	KM521692	KM521622	–	–
Berthella sp.	Muros de Nalón, Asturias, Spain	CPIC 00445	KM521693	KM521623	–	–
Berthellina delicata	Maui, Hawaii, USA	CPIC 00349	KM521689	KM521624	KM521593	–
Pleurobranchus albiguttatus	Honokeana Bay, Maui, Hawaii, USA	CPIC 00352	–	KM521688	–	*Pleurobranchus varians*
P. albiguttatus	Maliko Bay, Maui, Hawaii, USA	CPIC 00357	KM521701	KM521626	KM521598	*P. varians*
P. albiguttatus	Honokeana Bay, Maui, Hawaii, USA	CPIC 00409	KM521702	KM521627	KM521599	*P. varians*
P. albiguttatus	Madang, Papua New Guinea	CASIZ 191409	KM521736	KM521657	–	*P. albiguttatus*
P. albiguttatus	Honokeana Bay, Maui, Hawaii, USA	CPIC 00351	KM521700	KM521625	KM521597	*P. varians*
P. albiguttatus	Plettenberg Bay, South Africa	CASIZ 075983	–	KM521682	KM521612	*Pleurobranchus nigropunctatus*
P. albiguttatus	Madang, Papua New Guinea	CASIZ 191076	KM521746	KM521662	–	*P. albiguttatus*
Pleurobranchus areolatus	Yucatán Peninsula, México	CPIC 00196	KM521711	KM521629	–	*P. areolatus*
P. areolatus	Yucatán Peninsula, México	CPIC 00208	KM521712	KM521630	KM521604	*P. areolatus*
P. areolatus	Bahamas	LACM 173235	KM521706	KM521628	–	*P. areolatus*
P. areolatus	La Paz, Baja California, México	LACM A.9555	KM521735	KM521644	–	*Pleurobranchus digueti*
P. areolatus	Jalisco, México	LACM A.8477	–	KM521683	*	*P. digueti*
P. areolatus	Costa Rica, Pacific, Cocos Island	CASIZ 073374	KM521718	KM521645	–	*P. digueti*
P. areolatus	Costa Rica	MZUCR 6178	KM521720	KM521643	–	*P. digueti*
P. areolatus	Costa Rica	MZUCR 6990	KM521721	KM521642	–	*P. digueti*
P. areolatus	Panama	MZUCR 6201	KM521724	KM521647	–	*P. digueti*
P. areolatus	Jalisco, México	CASIZ 175782	KM521725	KM521656	–	*P. digueti*
P. areolatus	Guanacaste, Costa Rica	CASIZ 175736	KM521719	KM521640	–	*P. digueti*
P. areolatus	Costa Rica	MZUCR 6994	KM521722	KM521641	–	*P. digueti*
P. areolatus	Panama	MZUCR 6986	KM521723	KM521646	–	*P. digueti*
P. areolatus	Espiritu Santo Island, Vanuatu	CASIZ 175771	KM521705	KM521650	–	*P. varians*
P. areolatus	Costa Rica	–	KM521715	KM521639	–	*P. areolatus*
P. areolatus	Costa Rica	–	KM521714	KM521638	–	*P. areolatus*
P. areolatus	Panama	CPIC 00929	KM521709	KM521649	–	*P. areolatus*
P. areolatus	Panama	–	KM521710	KM521648	–	*P. areolatus*
P. areolatus	Brazil	MZSP103373	KM521737	KM521659	–	*Pleurobranchus reticulatus*
P. areolatus	Brazil	MZSP103380	KM521738	KM521660	–	*P. reticulatus*
Pleurobranchus crossei	Bahamas	LACM 173236	KM521708	KM521631	KM521605	*P. areolatus*
P. crossei	Florida, USA	CPIC 00829	KM521707	KM521681	–	*P. areolatus*
P. crossei	Palm Beach, Florida, USA	CPIC 00847	KM521753	–	*	*P. areolatus*
Pleurobranchus evelinae	Conch Point, Isla de Colon, Panama	LACM 2004–12.1	KM521713	KM521632	KM521606	*P. areolatus*
Pleurobranchus forskalii	Great Barrier Reef, Australia	CASIZ 078704	KM521727	KM521663	KM521603	*P. forskalii*

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P. forskalii	Madang, Papua New Guinea	CASIZ 191034	KM521739	KM521668	–	*P. forskalii*
P. forskalii	Madang, Papua New Guinea	CASIZ 191164	KM521740	KM521669	–	*P. forskalii*
P. forskalii	Philippines	–	KM521729	KM521666	–	*P. forskalii*
P. forskalii	Philippines	CASIZ 176077	KM521728	KM521667	–	*P. forskalii*
Pleurobranchus garciagomezi	Boca das Caldirinhes, Faial Islands, Azores	ZMBN 81687	–	KM521686	–	*P. reticulatus*
P. garciagomezi	Faial Island, Azores	ZMBN 81685	–	KM521685	–	*P. reticulatus*
P. garciagomezi	São Vicente, Cape Verde	–	KM521754	KM521613	–	*P. reticulatus*
P. garciagomezi	Boavista, Cape Verde	–	–	KM521614	–	*P. reticulatus*
P. garciagomezi	Canary Islands, Spain	–	KM521744	–	–	*P. reticulatus*
Pleurobranchus grandis	Luzon Islands, Philippines	CASIZ 177651	KM521698	KM521633	KM521596	*P. weberi*
P. grandis	New Caledonia	–	KM521697	KM521652	–	*P. grandis*
P. grandis	Sulawesi, Indonesia	ZSM 20034031	KM521696	KM521678	KM521609	*P. grandis*
Pleurobranchus hilli	Australia	GenBank	FJ917497	–	FJ917438	*P. hilli*
Pleurobranchus mamillatus	Maalaea Bay, Maui, Hawaii, USA	CPIC 00818	KM521747	KM521661	–	*P. mamillatus*
Pleurobranchus membranaceus	Ardrichaig Pinnacles, Loche Fyne, Scotland	–	KM521748	KM521675	–	*P. membranaceus*
P. membranaceus	Fraoch Eilean, Minard Island, Loche Fyne, Scotland	–	KM521749	KM521676	–	*P. membranaceus*
P. membranaceus	Fraoch Eilean, Minard Island, Loche Fyne, Scotland	–	KM521750	KM521677	–	*P. membranaceus*
P. membranaceus	France	GenBank	FJ917496	–	FJ917437	*P. membranaceus*
P. membranaceus	France	ZSM 20070301	KM521752	KM521680	KM521611	*P. membranaceus*
P. membranaceus	Croatia	ZSM 20100634	KM521751	KM521679	KM521610	*P. membranaceus*
Pleurobranchus peronii	Maliko Bay, Maui, Hawaii, USA	CPIC 00358	KM521703	KM521664	KM521600	*P. varians*
P. peronii	Kapalua Bay, Maui, Hawaii, USA	CPIC 00405	KM521704	KM521665	KM521601	*P. varians*
P. peronii	Luzon Islands, Philippines	CASIZ 177491	KM521716	KM521672	KM521607	*P. peronii*
P. peronii	Luzon Islands, Philippines	CASIZ 177440	KM521730	KM521673	KM521608	*P. peronii*
P. peronii	Madang, Papua New Guinea	CASIZ 191141	KM521741	KM521658	–	*P. albiguttatus*
P. peronii	Madang, Papua New Guinea	CASIZ 191300	KM521742	KM521670	–	*P. forskalii*
P. peronii	Madang, Papua New Guinea	CASIZ 191359	KM521743	KM521671	–	*P. forskalii*
P. peronii	Madang, Papua New Guinea	CASIZ 191434	KM521745	KM521674	–	*P. peronii*
P. reticulatus	Guinea	GenBank	FJ917498	–	–	*P. reticulatus*
P. weberi	Luzon Islands, Philippines	CASIZ 181282	KM521699	KM521651	–	*P. weberi*
Pleurobranchus sp.	Honokeana Bay, Maui, Hawaii, USA	CPIC 00342	KM521717	KM521636	KM521602	*P. albiguttatus*
Pleurobranchus sp.	Kapalua Bay, Maui, Hawaii, USA	CPIC 00404	KM521731	KM521637	KM521595	*P. peronii*
Pleurobranchus sp.	Indonesia	CASIZ 139587	KM521732	KM521654	–	*P. peronii*
Pleurobranchus sp.	Hawaii, USA	CASIZ 166753	KM521733	KM521655	–	*P. peronii*
Pleurobranchus sp.	Australia	–	KM521734	KM521653	–	*P. peronii*
Pleurobranchus sp.	Greece	CPIC 00444	KM521694	KM521634	–	*Berthellina* sp.
Pleurobranchus sp.	Costa Rica	–	KM521690	KM521635	–	*Berthellina* sp.
Pleurobranchus sp.	Crete, Greece	NHMC 52.37	–	KM521687	–	*P. testudinarius*
Pleurobranchus sp.	Cagarras, Brazil	–	–	KM521619	–	*P. testudinarius*
Pleurobranchus sp.	Gales, Brazil	–	–	KM521618	–	*P. testudinarius*

COI, cytochrome c oxidase I; H3, histone 3.

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specimens were obtained by direct collection via snorkelling and SCUBA diving and were deposited in the Cal Poly Pomona Invertebrate Collection (CPIC). All specimens deposited at these museums were collected and exported with appropriate permits from each country in which collections were made. Fifteen specimens do not have voucher numbers from any institution, as only small tissue samples were taken from these animals before they were returned to the ocean. All specimens were preserved in 70 or 95% ethanol for long-term storage. Sequences for four individuals were downloaded from GenBank, *Pleurobranchaea meckelii* (FJ917499, EF133470, FJ917439), *P. hilli* (FJ917497, FJ917438), *P. membranaceus* (FJ917496, FJ917437), and *P. reticulatus* (FJ917498).

DNA extraction

DNA extraction was performed using either a hot Chelex protocol or DNeasy Blood and Tissue Kits (Qiagen). Approximately 1–3 mg tissue was taken from the foot of the animals and cut into fine pieces for extraction for each protocol. For the Chelex extraction, the tissue was rinsed and rehydrated using 1.0 mL Tris-EDTA (TE) buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, pH 8.0) for 20 min. A 10% (w/v) Chelex 100 (U.S. Standard 100–200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the mixture was then centrifuged, 975.00 μL of the supernatant was removed, and 175.00 μL of the Chelex solution was added. Samples were then incubated at 56 °C in a water bath for 20 min, heated to 100 °C in a heating block for 8 min, and the supernatant was used for PCR.

The DNeasy protocol supplied by the manufacturer was followed, with some modifications. The elution step was modified such that the first elution was collected using 100.00 μL Buffer AE and was allowed to incubate at room temperature for 5 min. In a new tube, a second elution step was conducted using 200.00 μL Buffer AE and was also allowed to incubate at room temperature for 5 min. The first elution, and in some cases the second elution, was used for PCR. Multiple extraction protocols were implemented owing to the fact that some tissue samples could not be amplified when using a particular extraction method. The use of the DNeasy extraction protocol was only used as an alternative to the Chelex extraction when it became apparent that the DNA could not be successfully extracted using the latter technique.

Primers

Colgan's universal *H3* primers (Colgan *et al*., 1998), Folmer's universal *COI* primers (Folmer *et al*., 1994), and Palumbi's universal *16S* primers (Palumbi, 1996) were used to amplify the regions of interest for some specimens (Table 2). However, internal primers for *16S* and external primers (as compared with the universal primers) for *COI* designed for another group of sea slugs (Ornelas-Gatdula, Dupont & Valdés, 2011) were also used, resulting in additional partial sequences for some specimens.

PCR amplification and sequencing

The master mix (for each sample) was prepared using 34.75 μL H_2O, 5.00 μL PCR buffer (ExACTGene, Fisher Scientific), 5.00 μL 25 mM $MgCl_2$, 1.00 μL 40 mM deoxynucleotide triphosphates, 1.00 μL 10 μM primer 1, 1.00 μL primer 2, 0.25 μL 5 mg mL^{-1} Taq, and 2.00 μL extracted DNA. Reaction conditions for *H3* and *16S*

Table 2. Forward (F) and reverse (R) PCR primers used to amplify regions of the nuclear *histone 3* (*H3*) gene and mitochondrial *cytochrome c oxidase* I (*COI*) and *16S* genes

Name	Sequence 5′–3′	Source
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)
16Sar-FAP (F)	AAA GAC GAG AAG ACC CTT AGA GTT TT	Ornelas-Gatdula *et al*. (2011)
16Sbr-FAP (R)	AAA ACT CTA AGG GTC TTC TCG TCT TT	Ornelas-Gatdula *et al*. (2011)
COI		
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)
LCO-NAF (F)	GCC TTT TCA ACA AAC CAT AAA GA	
HCO-NAR (R)	CCA TCC TGG TAA AAT TAA AAT ATA	

rRNA were as follows: an initial denaturation for 2 min at 94 °C, 35 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 72 °C, and a final elongation for 7 min at 72 °C. Reaction conditions for *COI* were an initial denaturation for 3 min at 95°C, 39 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. PCR products yielding bands of appropriate size (approximately 375 bp for *H3*, 195 bp for the *16S* fragments, and 695 bp for *COI*) were purified using the Montage PCR Cleanup Kit (Millipore).

Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 4.0 μM to send out for sequencing with the PCR products. PCR products were diluted to between 5 and 30 ng μL^{-1} for sequencing. Sequencing was outsourced to Eton Biosciences (San Diego, CA).

Sequence analyses

Sequences for each gene were assembled and edited using GENEIOUS PRO 4.8.5 (Drummond *et al*., 2009). GENEIOUS was also used to extract the consensus sequence and to construct the alignment for each gene using the default parameters. The sequences were not trimmed after alignment. The total length of *H3* was 328 bp, approximately 450 bp for *16S*, and 658 bp for *COI*. A combined analysis was conducted using all genes concatenated (Fig. 1).

The levels of saturation for each gene were investigated using the substitution saturation test developed by Xia *et al*. (2003) and Xia & Lemey (2009) implemented in the program DAMBE (Xia & Xie, 2001).

To assess whether *COI*, *H3*, and *16S* have significantly conflicting signals, the incongruence length difference (ILD) test (Mickevich & Farris, 1981; Farris *et al*., 1994), implemented in PAUP*4.0 as the partition homogeneity test (Swofford, 2002), was conducted for all three genes combined.

Bayesian and maximum likelihood analyses were conducted for all genes concatenated and for each gene separately. To determine the best-fit model of evolution for the Bayesian analysis, the Akaike information criterion (Akaike, 1974) was executed in MrModeltest v. 2.3 (Nylander, 2004), using PAUP*4.0 (Swofford, 2002; Table 3). The Bayesian analyses were implemented in MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001), with partitioning by gene (unlinked). The Metropolis-coupled Markov chain Monte Carlo analysis was run with two runs of six chains for 10 000 000 generations, with sampling every 100 generations, and a default burn-in of 25%. Clades with posterior probabilities ≥ 0.95 ($\alpha = 0.05$) were considered significant (Alfaro, Zoller & Lutzoni, 2003). The maximum likelihood analyses were implemented in GARLI 2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006) and grid computing (Cummings & Huskamp, 2005) through The Lattice Project (Bazinet & Cummings, 2008), which created a web service for GARLI (Bazinet & Cummings, 2011) that uses a special programming library and associated tools (Bazinet *et al*., 2007). Following the general computational model of a previous phylogenetics study (Cummings *et al*., 2003), which used an earlier grid computing system (Myers & Cummings, 2003), the required files were distributed amongst hundreds of computers where the analyses were conducted asynchronously in parallel. Partitioned maximum likelihood analyses were conducted on the total data matrix, with each partition set to its optimal model (as described above), with these models unlinked and employing their own rates (i.e. using the settings linkmodels = 0 and subsetspecificrates = 1). A bootstrap analysis was run with 2000 replicates under the default parameters of the web service. Postprocessing of the phylogenetic inference results was carried out using DendroPy (Sukumaran & Holder 2010) and the R system for statistical computing (R Development Core Team, 2011). The estimation of the number of replicates required to recover the 'best' topology follows Regier *et al*. (2009). *Pleurobranchaea meckelii* was used as the outgroup for both analyses because of the hypothetical basal position of *Pleurobranchaea* with respect to *Pleurobranchus* (Willan, 1987).

Date estimates for uncalibrated nodes in the Bayesian consensus tree were derived by using r8s 1.7 (Sanderson, 2003) using the finalization of the closure of the Isthmus of Panama as the calibration point, estimated as 3.1–3.5 Mya (Coates & Obando, 1996). Several analyses using the Langley–Fitch, nonparametric rate, and penalized likelihood methods were run. For the Langley–Fitch method, analyses were run using the Powell, truncated Newton, and quasi-Newton algorithms as well as the local molecular clock procedure. The nonparametric rate method analysis was run with the Powell algorithm and the penalized likelihood analyses with the Powell and truncated Newton algorithms. The results of the analyses are reported in Table 4.

In order to compute the theoretical maximal limit of the intraspecific diversity (using a coalescent model) in *Pleurobranchus*, the automatic barcode gap discovery (ABGD) method (Puillandre *et al*., 2012) was implemented for *COI* sequence data. ABGD identifies in the entire distribution of pairwise distances which gap (superior to the maximal limit of the intraspecific diversity) potentially corresponds to the so-called 'Barcoding gap', a hypothetical limit between intra- and interspecific diversity (Puillandre *et al*., 2012). Inference of the limit and gap detection is then

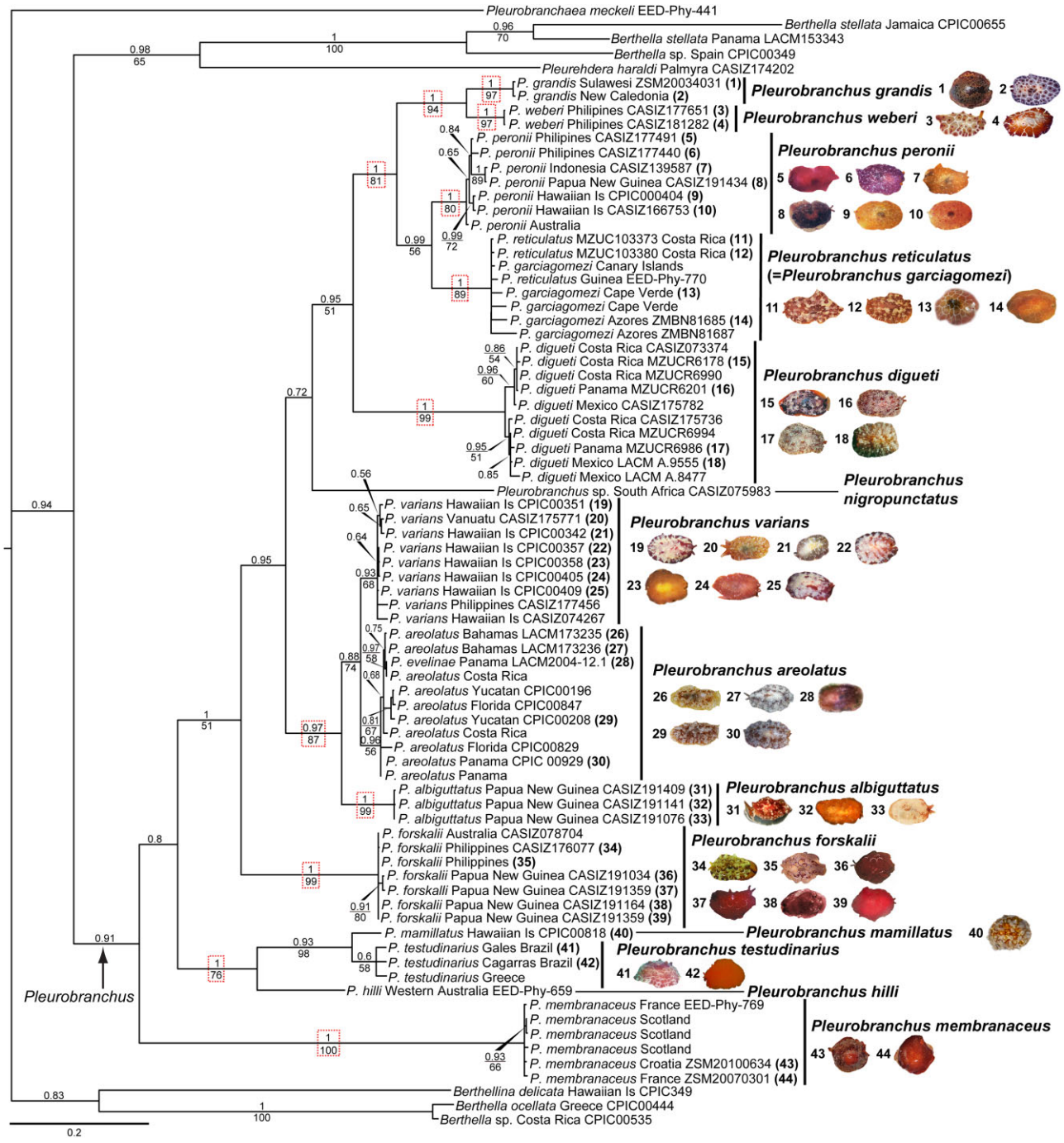

Figure 1. Bayesian consensus phylogenetic tree of *cytochrome c oxidase I*, *histone 3*, and *16S* rDNA genes concatenated for *Pleurobranchus*. Posterior probabilities and bootstrap values from the maximum likelihood analysis are presented for each clade above and below each branch, respectively. Well-supported clades are indicated with a red square around the support values. Bold numbers following the species names indicate the specimen illustrated on the right side of each branch.

recursively applied to previously obtained groups to get finer partitions until there is no further partitioning. The online version of the software (http://wwwabi.snv.jussieu.fr/public/abgd/) was used to analyse the *COI* and *16S* data sets. MEGA 4.0 (Tamura *et al.*, 2007) was used to build the distance matrices for *COI* and *16S* using a Tamura Nei model. The data were analysed using the two available models: Jukes–Cantor

Table 3. Summary of each data set used for analysis with the best-fit evolutionary models and estimated parameters

Parameters	*COI*	*H3*	*16S*
No. of specimens used in the study	67	64	22
No. of included characters	663	328	466
Best-fit model	GTR + I + G	GTR + I + G	HKY + G
Frequency A	0.2773	0.2298	0.3388
Frequency C	0.1042	0.3061	0.1302
Frequency G	0.1623	0.2605	0.1961
Frequency T	0.4561	0.2036	0.3349
R-matrix [A-C]	0.0000	2149277.5	–
R-matrix [A-G]	34.7806	10929979.0	–
R-matrix [A-T]	1.6842	4467196.5	–
R-matrix [C-G]	7.8775	1261227.25	–
R-matrix [C-T]	66.3054	18705996.0	–
R-matrix [G-T]	1.0000	1.0000	–
Γ shape (G)	0.5933	2.4256	–
Proportion of invariable sites (I)	0.5450	0.7014	0

COI, cytochrome c oxidase I; H3, histone 3; HKY, Hasegawa Kishino Yano; GTR, general time reversible.

Table 4. Results from r8s molecular clock analyses, including mean and SD for divergences (in millions of years) for *Pleurobranchus*, using different methods and algorithms. Node codes (letters) are labelled in Figure 34

Node	Langley–Fitch/local molecular clock	Langley–Fitch/Powell	Langley–Fitch/quasi-Newton	Langley–Fitch/truncated Newton	Nonparametric rate smoothing/Powell	Penalized likelihood/Powell	Penalized likelihood/truncated Newton
A	9.95	9.95	9.97	9.97	15.53	12.98	15.58
B	7.97	7.97	7.99	7.99	13.17	10.8	13.15
C	6.26	6.26	6.27	6.27	11.14	8.98	11.13
D	4.69	4.69	4.7	4.7	7.82	5.98	7.82
E	3.91	3.91	3.91	3.91	5.88	4.32	5.89
F	3.55	3.55	3.56	3.56	5.03	3.65	5.04
G	2.96	2.96	2.96	2.96	3.91	2.8	3.92
H	2.18	2.18	2.18	2.18	2.73	1.92	2.73
I	0.89	0.89	0.89	0.89	1.02	0.71	1.02
J	1.42	1.42	1.42	1.42	1.72	1.2	1.72
K	1.39	1.39	1.39	1.39	2.75	1.97	2.76
L	0.69	0.69	0.69	0.69	1.51	1.09	1.54
M	2.87	2.87	2.87	2.87	6.19	4.93	6.18
SD	2.12E-08	2.12E-08	1.71E-09	1.14E-09	5.80E-02	5.29E-02	3.63E-02

(JC69) and Kimura (K80). The program requires two user-specified values: P (prior limit to intraspecific diversity) and X (proxy for minimum gap width). To evaluate the effect on the data sets, X values from 0.1 to 5 were tested and the maximum Pmax value was extended from 0.1 to 0.2 (as in Jörger *et al*., 2012).

Morphological examination

In order to obtain the maximum support for our species determinations, photographs of each specimen were compared to determine the extent of variation in external morphology and colour patterns. We then dissected one to three specimens of all the species examined. The shell of each specimen was dissected and the surrounding tissue removed manually. The buccal mass of each specimen was then removed and the tissue surrounding the jaws and radula was dissolved using 10% sodium hydroxide (NaOH). Shells, jaws, and radulae were rinsed in water, dried, mounted, and sputter-coated for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the Natural History

Museum of Los Angeles County. The features of the reproductive system of all species available were examined and drawn using a dissecting microscope Nikon SMZ-100 with the aid of a *camera lucida* attachment. The reproductive morphology was illustrated as observed; however, several of the specimens dissected were small or poorly preserved and some reproductive organs and connections were difficult to observe.

Type material

An effort was made to determine the location of the type specimens of all the species of *Pleurobranchus* deposited in museums and other research institutions from all over the world. The institutional abbreviations are as follows: BMNH, The Natural History Museum, London; USNM, Natural History Museum, Smithsonian Institution; MNHN, Muséum National d'Histoire Naturelle, Paris; ZMUC, Zoologisk Museum, Københavns Universitet, Copenhagen; ANSP, Academy of Natural Sciences of Drexel University, Philadelphia; MCZ, Museum of Comparative Zoology at Harvard University; BPBM, Bernice Pauahi Bishop Museum, Honolulu; NL, Naturalis, Leyden; MNCN, Museo Nacional de Ciencias Naturales, Madrid; MCHN, Museo Civico di Storia Naturale di Genova; AM, Australian Museum, Sydney; TAU, Tel Aviv University; IOUSP, Instituto Oceanográfico da Universidade de São Paulo. Some type specimens were examined morphologically when it was necessary to solve outstanding taxonomic problems.

RESULTS

Molecular analyses

The saturation analyses showed insignificant levels of saturation for *H3* and *COI* [Index of substitution saturation (Iss) < Index of substitution saturation critical (Iss.c), $P = 0.000$] even when the third codon positions of these two genes were analysed independently. *16S* has some saturation (Iss < Iss.c, $P = 0.6629$), but it was within the limits of usefulness for phylogenetic analyses. The ILD test showed no significant conflicting signals amongst *H3*, *COI*, and *16S* ($P = 0.94$), *COI* and *H3* ($P = 0.58$), *COI* and *16S* ($P = 0.99$), or *H3* and *16S* ($P = 0.97$).

The Bayesian and maximum likelihood analyses of the concatenated data set (*H3* + *COI* + *16S*) (Fig. 1), as well as the discrete mitochondrial (*COI*, *16S*) and nuclear (*H3*) gene analyses (Appendix S1), resulted in largely congruent trees. The values given below were obtained from the concatenated data set analyses; values from the single gene analyses are available in Appendix S1. None of the analyses recovered *Pleurobranchus* as a monophyletic group as support values are low: posterior probability (PP) = 0.91, bootstrap support value (BS) < 50. Within *Pleurobranchus*, *P. membranaceus* is monophyletic (PP = 1, BS = 100) and appears to be one of the most basal species of *Pleurobranchus*, although the basal topology of the tree is unresolved owing to poor support. Amongst all other species of *Pleurobranchus*, a clade containing *P. hilli*, *P. mamillatus*, and *P. testudinarius* (PP = 1, BS = 76) is sister to the rest of the species, which form a monophyletic group in the Bayesian tree (PP = 1, BS = 51). *Pleurobranchus forskalii* (PP = 1, BS = 99) comes out as sister to the remaining species of *Pleurobranchus*, which are also monophyletic in the Bayesian tree (PP = 0.95). This clade is further divided into two reciprocally monophyletic clades, the first (PP = 0.97, BS = 87) containing *P. albiguttatus* from the Indo-Pacific (PP = 1, BS = 99), which is sister to a poorly supported clade (PP = 0.88, BS = 74) containing specimens identified as *Pleurobranchus varians* from the Central Pacific (PP = 0.93, BS = 68) and *P. areolatus* from the Caribbean (PP = 0.96, BS = 56). In the second clade, one unidentified *Pleurobranchus* sp. (CASIZ075983) from South Africa comes out as sister to the rest, including specimens identified as *P. areolatus* from the eastern Pacific, here called *Pleurobranchus digueti* (PP = 1, BS = 99). *Pleurobranchus digueti* is sister to a clade containing two more reciprocally monophyletic clades (PP = 1, BS = 81). The first of these (PP = 0.99, BS = 56) contains *P. peronii* from the Indo-Pacific (PP = 1, BS = 80) and a well-supported subclade containing two Brazilian specimens originally identified as *P. areolatus* together with *P. reticulatus* from Guinea and *P. garciagomezi* from the eastern Atlantic (PP = 1, BS = 89). There is no structure within this subclade. The second clade, sister to *P. peronii* + *P. reticulatus* + *P. garciagomezi*, is also well supported (PP = 1, BS = 94), and contains specimens identified as *P. weberi* (PP = 1, BS = 97) and *P. grandis* from the Indo-Pacific (PP = 1, BS = 97).

In all of the ABGD analyses specimens identified as *P. digueti*, *P. forskalii*, *P. mamillatus*, *P. hilli*, and *P. membranaceus* were all recovered as individual groups, whereas those identified as *P. varians*, *P. areolatus*, and *P. albiguttatus* were recovered as a single group. In all analyses conducted using Jukes–Cantor distances, specimens of *P. peronii* were recovered as a single group, and specimens identified as *P. garciagomezi* and *P. reticulatus* were grouped together. In the analyses conducted using Kimura distances, individuals identified as *P. peronii*, *P. garciagomezi*, and *P. reticulatus* were recovered in a single group. In the majority of analyses, individuals identified as *P. weberi* and *P. grandis* were separated into separate groupings, with the exception of two analyses (using Kimura distances, the first using all default parameters except X = 0.1 and the second using all default parameters except $P = 0.2$), where *P. weberi* and *P. grandis* were recovered as a single group.

The r8s analysis (Table 4) indicated that three clades of *Pleurobranchus* diverged around 9.5 Mya, one

splitting further into *P. mamillatus* and *P. hilli* (6.31 Mya). *Pleurobranchus forskalii* diverged from the largest *Pleurobranchus* clade around 7.88 Mya. Following that, this clade split 6.81 Mya into two clades, one containing *P. varians* and the other *P. grandis*. The next split was the divergence of *P. digueti* from the *P. grandis* clade 5.28 Mya, followed by the split of the *P. grandis*/*P. weberi* clade from the *P. reticulatus*/*P. peronii* clade 4.16 Mya. *Pleurobranchus reticulatus* and *P. peronii* then diverged 3.02 Mya. Finally, *P. albiguttatus* split from the *P. varians*/*P. areolatus* clade 4.35 Mya, followed by the *P. varians*/*P. areolatus* split 3.10 Mya.

SYSTEMATICS

Based on the molecular data and analyses, 14 species of *Pleurobranchus* are recognized as valid. In this section, brief descriptions of these species as well as a diagnosis of *Pleurobranchus* are provided in order to facilitate species identification and future work. The species descriptions are organized chronologically by original description date to simplify reading.

PLEUROBRANCHUS CUVIER, 1804

Diagnosis: Nudipleura side-gilled slugs with a tuberculate mantle and gill rachis, cleft anterior border of the mantle, and flaps surrounding the genital aperture of mature adults. Rhinophores involute and basally joined together, which pulsate regularly in living animals. Penial gland absent, radular teeth typically simple and hamate, jaws composed of denticulate elements. All species with an oval body, mantle notched anteriorly, and a slightly protruding oral veil. Bipinnate gill not visible from dorsal side and covered by mantle, placed on the right-hand side of the body and united with the body for the majority of its length.

Remarks: The first comprehensive taxonomic review of *Pleurobranchus* and closely related taxa was conducted by Bergh (1897). This was followed by two monographical revisions by Vayssière (1898, 1901) who divided *Pleurobranchus* into several genus-level taxa (*Pleurobranchus sensu stricto*, *Oscanius* Leach in Gray, 1847, *Susania* Gray, 1957, *Oscaniella* Bergh, 1897) based on one or a few traits. Willan (1987) synonymized these taxa with *Pleurobranchus*, and this is currently accepted by most authors. The diagnosis above is based on Willan's (1987) revision, which provided an updated diagnosis of *Pleurobranchus* based on a phylogenetic analysis.

PLEUROBRANCHUS PERONII CUVIER, 1804 (FIGS 2A–H, 3B, F, 4, 5, 6)

Pleurobranchus peronii Cuvier, 1804: 266–276, figs 1–6 (type locality: 'Mer des Indes' [Indian Ocean])

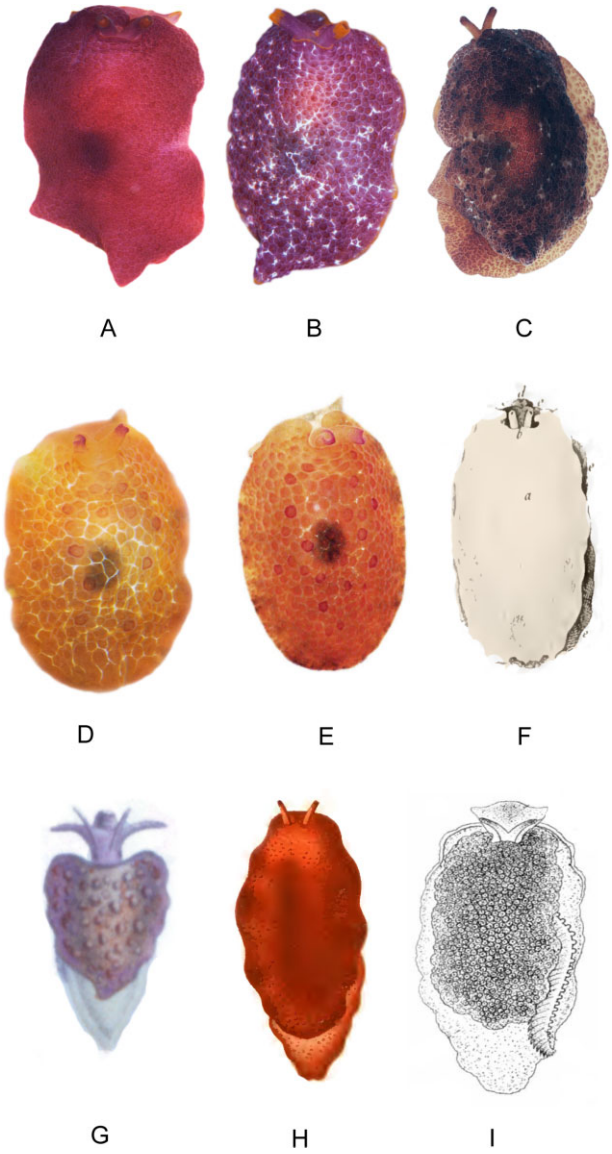

Figure 2. Colour variation in *Pleurobranchus peronii*. A, *P. peronii* from Luzon, Philippines (CASIZ177491). B, *P. peronii* from Luzon, Philippines (CASIZ177440). C, *P. peronii* from Madang, Papua New Guinea (CASIZ191434). D, *P. peronii* from the Hawaiian Islands (CPIC00404). E, *P. peronii* from the Hawaiian Islands (CASIZ166753). F, *P. peronii*, original description illustration from the Indian Ocean (Cuvier, 1804). G, *Pleurobranchus cornutus*, original description illustration from Indonesia (Quoy & Gaimard, 1832–1833). H, *Oscaniella purpurea*, illustration by Bergh (1905) from East Timor. I, *Pleurobranchus hirasei*, original description illustration from Japan (Baba, 1971).

Pleurobranchus cornutus Quoy & Gaimard, 1832–1833: 298–299, pl. 22, figs 20–24 (type locality: Bay of Ambon, Ambon Island, Indonesia) **syn. nov.**

Pleurobranchus ovalis Pease, 1868: 79, pl. 9, fig. 3 (type locality: Tahiti)

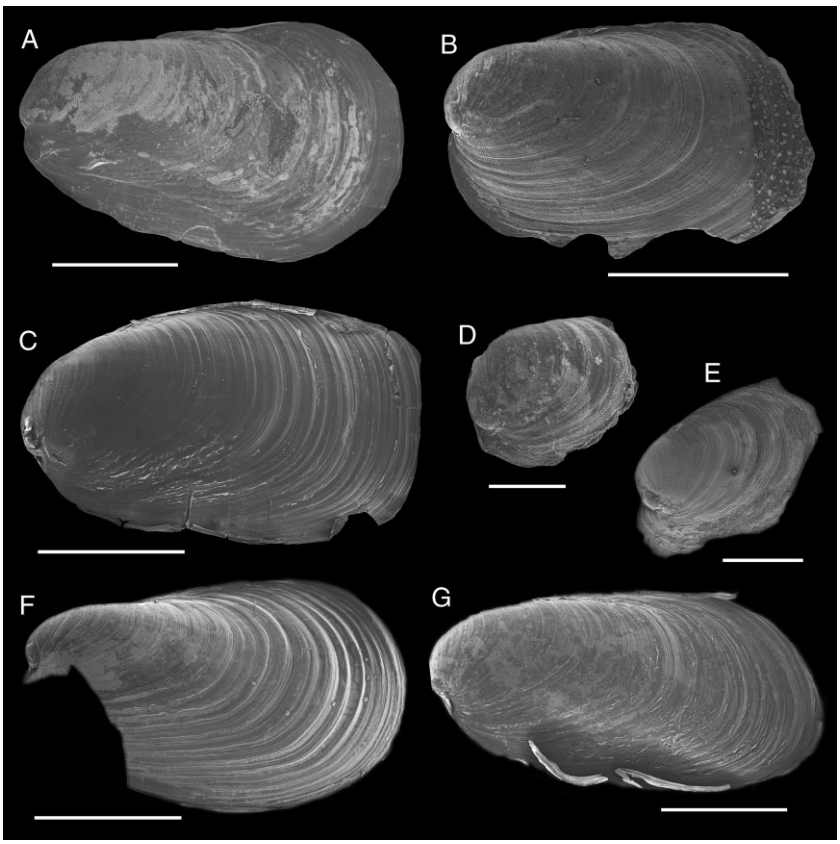

Figure 3. Scanning electron micrographs of shells. A, *Pleurobranchus varians* from the Hawaiian Islands (CPIC00351), scale bar = 1 mm. B, *Pleurobranchus peronii* from the Hawaiian Islands (CPIC00404), scale bar = 2 mm. C, *Pleurobranchus mamillatus* from the Hawaiian Islands (CPIC00818), scale bar = 2 mm. D, *P. varians* from the Hawaiian Islands (CPIC00358), broken, only apex illustrated, scale bar = 500 μm. E, *Pleurobranchus weberi* from Luzon, Philippines (CASIZ177651), scale bar = 1 mm. F, *P. peronii* from Luzon, Philippines (CASIZ177491), scale bar = 2 mm. G, *Pleurobranchus forskalii* from the Great Barrier Reef, Australia (CASIZ078704), scale bar = 2 mm.

Pleurobranchus giardi Vayssière, 1896b: 354–356 (type locality: Camiguin, Luzon, Philippines) **syn. nov.**

Oscaniella purpurea Bergh, 1897: 95–99, pl. 8, figs 28–39 (type locality: Philippines).

Pleurobranchus winckworthi White, 1946: 52–54, figs 1–3 (type locality: Karachi, Pakistan)

Pleurobranchus papillatus Risbec, 1951: 156–157, fig. 15 (type locality: Artillerie, Nouméa, New Caledonia) **syn. nov.**

Pleurobranchus inhacae Macnae, 1962: 174–176, fig. 5 (type locality: Inhaca Island, Mozambique) **syn. nov.**

Pleurobranchus xhosa Macnae, 1962: 176–177, fig. 6 (type locality: Inhaca Island, Mozambique) **syn. nov.**

Pleurobranchus hirasei Baba, 1971: 24–28, pl. 3, figs 1–9 (type locality: Sagami Bay, Japan) **syn. nov.**

Type material: Syntype of *P. peronii* (MNHN), syntype of *P. giardi* (MNHN). Type material of *P. cornutus* and *P. papillatus* is not known to exist, not found at MNHN (Valdés & Héros, 1998). Type material of *P. purpurea* is not known to exist, not found at ZMUC. Type material of *P. winckworthi, P. inhacae*, and *P. xhosa* is not known to exist, not found at BMNH. Holotype and four paratypes of *P. hirasei* are known to exist, location unknown. Type material not examined.

Material examined: Luzon, Philippines (CASIZ177491); Luzon, Philippines (CASIZ177440); Madang, Papua New Guinea (CASIZ191434); Hawaiian Islands, USA (CASIZ166753); Kapalua Bay, Maui, Hawaiian Islands, USA (CPIC00404).

Distribution: Known from South Africa, Madagascar, and Tanzania to the western Pacific of Australia, Papua New Guinea, Indonesia, Malaysia, Korea, and Japan to the Hawaiian Islands (Gosliner, Behrens & Valdés, 2008).

External morphology and coloration: Body size up to 60 mm long (Gosliner *et al*., 2008). Rhinophores with multiple horizontal striations along the length. The foot sometimes projects posteriorly from the mantle. Gill rachis tuberculate at the base of the pinnae. Adult

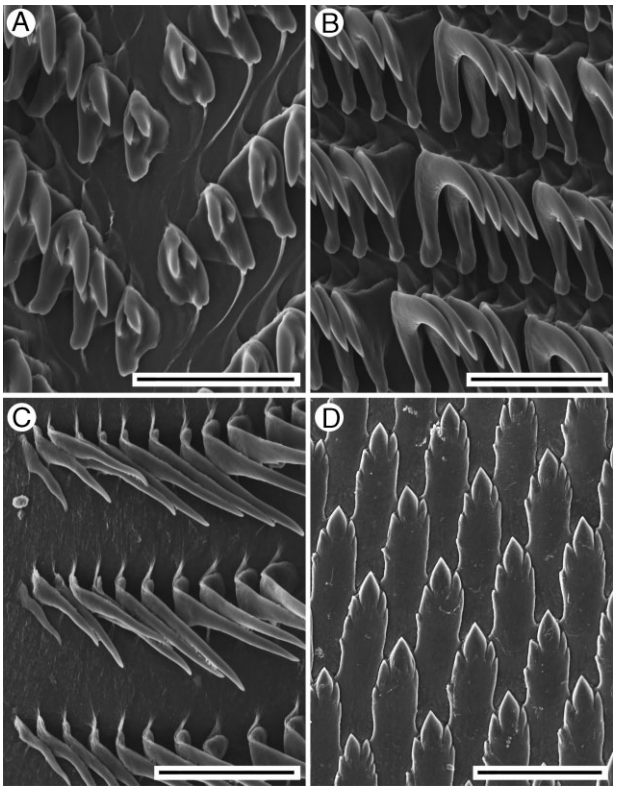

Figure 4. Scanning electron micrographs of the radula and jaws of *Pleurobranchus peronii* from the Hawaiian Islands (CPIC00404). A, innermost lateral teeth, scale bar = 30 μm. B, mid-lateral teeth, scale bar = 30 μm. C, outermost lateral teeth, scale bar = 30 μm. D, jaw elements, scale bar = 50 μm.

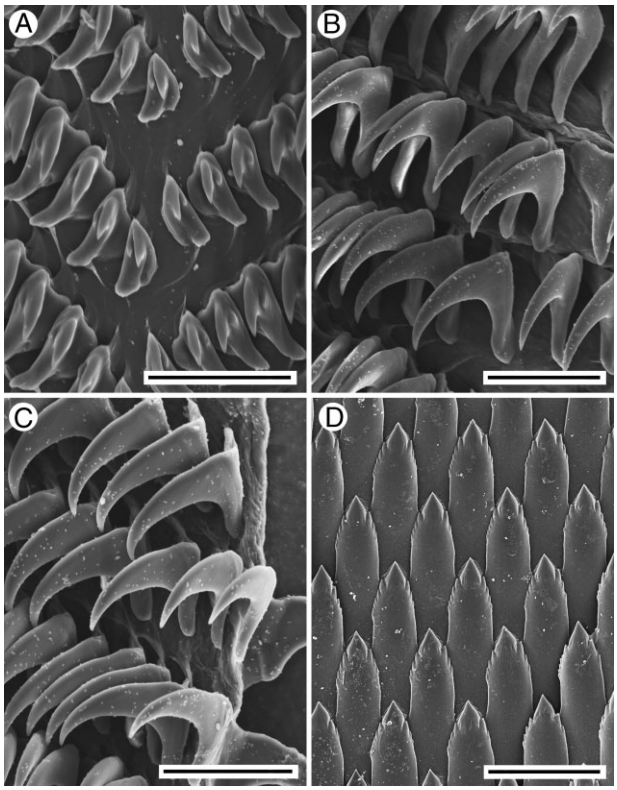

Figure 5. Scanning electron micrographs of the radula and jaws of *Pleurobranchus peronii* from Luzon, Philippines (CASIZ177491). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 100 μm.

tubercles polygonal in shape, larger and more flattened than those in *P. albiguttatus*. Juvenile tubercles conical or hemispherical, getting bigger as the individuals mature. In some specimens, tubercles similar in size, in others smaller in size towards the edge of the mantle.

Background colour orange to dark red in adults and juveniles often a somewhat transparent white. Some tubercles darker; in adults this pigment concentrated at the edges of the tubercle. White, reticulated lines usually found on much of the mantle. Oral veil and rhinophores are the same colour as the mantle and the tips of the rhinophores are often a different colour than that along their length (Fig. 2A–E).

Internal anatomy: Shell oval in shape and the same width along its length. The protoconch is roughly 400 μm, has about one whorl, and lacks any ornamentation (Fig. 3B, F).

Elongated cruciform elements in jaws have a prominent cusp flanked by two to three short denticles on each side (Figs 4D, 5D). Radular formula 43 × 59.0.59 (CPIC 00404) and 54 × 78.0.78 (CASIZ 177491). Radular teeth smooth and hook-shaped and the inner lateral teeth are the shortest. Mid-lateral teeth 2–3× the length of the inner lateral teeth, and outermost lateral teeth 2–3× the length of the mid lateral teeth. (Figs 4A–C, 5A–C).

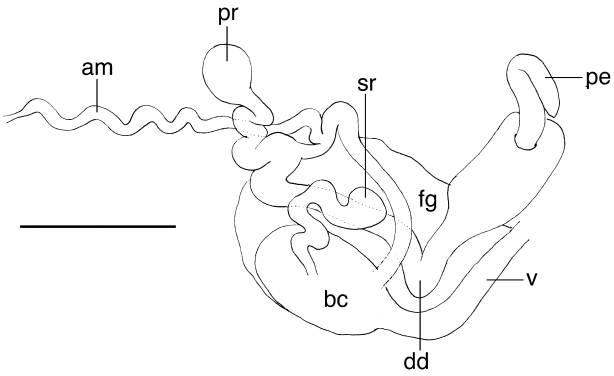

Figure 6. Reproductive system of *Pleurobranchus peronii* from Luzon, Philippines (CASIZ177491), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

Reproductive system (Fig. 6) triaulic *sensu* Cervera *et al*. (2000), with the sperm storage organs connected to the female gland complex only at the common genetial atrium. Ampulla simple, muscular, splitting into two ducts, one connecting to the bursa copulatrix and the other to the prostate. No connection with the female gland complex was observed as in Cervera *et al*. (2000) for *P. areolatus*, but this is probably because of the small size of the reproductive system examined. Bursa copulatrix rounded connecting to two ducts; one opens into the vagina and the other to the short, rounded, irregular seminal receptacle. Vaginal duct straight and simple. Prostate long, simple, and connected by the deferent duct to the large and simple penis.

Remarks: Pleurobranchus peronii was originally described and illustrated by Cuvier (1804) and is the type species of *Pleurobranchus*. *Pleurobranchus peronii* differs from similar species of *Pleurobranchus* (such as *P. forskalii* and *P. varians*) by the dark ring of pigment along the edges of some of the polygonal tubercles. The body colour of *P. peronii* ranges from dark purple to red or orange, but the distinctive darker pigment present along some tubercles remains. Additionally, *P. peronii* is distinguishable from other species by having a different colour on the tips of the rhinophores than at the base.

A review of the literature revealed that several species of *Pleurobranchus* are synonymous with *P. peronii*. These include *Pleurobranchus cornutus* Quoy & Gaimard, 1832, *Pleurobranchus ovalis* Pease, 1860, *Pleurobranchus giardi* Vayssière, 1896, *Pleurobranchus purpurea* (Bergh, 1897), *Pleurobranchus winckworthi* White, 1946, *Pleurobranchus papillatus* Risbec, 1951, *Pleurobranchus inhacae* Macnae, 1962, *Pleurobranchus xhosa* Macnae, 1962, and *Pleurobranchus hirasei* Baba, 1971 (Fig. 2F–I). *Pleurobranchus purpurea* was synonymized with *P. peronii* by Edmunds & Thompson (1972) and Er. Marcus (1965) owing to similarities in external morphology. Edmunds & Thompson (1972) also synonymized *P. winckworthi* with *P. peronii*. Rudman (1999b) suggested that *P. ovalis* was a colour form of *P. peronii*. Additionally, *P. cornutus*, *P. giardi*, *P. papillatus*, *P. inhacae*, *P. xhosa*, and *P. hirasei* match the description of *P. peronii*, having small, simple tubercles, some of which have darker rings of pigment around the edges.

Pleurobranchus membranaceus (Montagu, 1815) (Fig. 7F, G)

Pleurobranchus tuberculatus Meckel, 1808: 26, 33, pl. 38, figs 33–37, 40 (type locality: Sicily, Italy)

Lamellaria membranacea Montagu, 1815: 184–186, pl. 12, fig. 3 (type locality: Kingsbridge, Devon, UK)

?*Pleurobranchus lesuerii* Blainville, 1825: 470, pl. 43, fig. 2 (type locality: not specified)

?*Pleurobranchus contarinii* Vérany, 1846 (type locality: Gulf of Genoa, Mediterranean Sea)

?*Pleurobranchus denotarisii* Vérany, 1846: 16, 19 (type locality: Gulf of Genoa: Mediterranean Sea)

Gymnotoplax barashi Ev. Marcus, 1977: 418–420, figs 1–5, 8–11 (type locality: Haifa Bay, Mediterranean Sea)

Type material: The type material of *P. tuberculatus* is not known to exist. Types of *P. membranaceus* and *P. lesuerii* are not known to exist, not found at MNHN (Valdés & Héros, 1998). Types of *P. contarinii* and *P. denotarisii* are not known to exist, not found at MCHN. Syntypes of *G. barashi* are not known to exist, not found at TAU (Mienis, 2014).

Distribution: Known from the UK and France in the North Atlantic and Spain, Greece, and Croatia in the Mediterranean (Rudman, 2001).

External morphology and coloration: Description provided in Thompson & Slinn (1959).

Internal anatomy: Description provided in Thompson & Slinn (1959) and Martynov & Schrödl (2009).

Remarks: Pleurobranchus membranaceus was first described and illustrated by Montagu (1815), but Pilsbry (1895) considered this species to be a synonym of *P. tuberculatus* Meckel, 1808. However, a number of more recently published papers have maintained *P. membranaceus* as a valid species and *P. tuberculatus* as a synonym despite the fact that *P. tuberculatus* is an older name (Edmunds, 1968; Thompson, 1969, 1970, 1976, 1983; Cervera, Cattaneo-Vietti & Edmunds, 1996; Cervera *et al*., 2000, 2006; Martynov & Schrödl, 2009; Göbbeler & Klussmann-Kolb, 2010). The similarities in morphology between the two species include the presence of reddish-brown, small tubercles that irregularly cover the mantle, and a foot that extends well beyond the posterior edge of the mantle (Fig. 7F–G). Given these similarities, we agree that these two species are synonyms. However, despite the fact that *P. membranaceus* is currently used as the valid name for this species, we are unable to propose a Reversal of Precedence, as stated in the International Code of Zoological Nomenclature (ICZN, 1999) because Pruvot-Fol (1954) used the name *P. tuberculatus* as valid. Therefore, this case will have to be referred to the International Commission on Zoological Nomenclature at a later date.

Other species names also considered to be synonyms of *P. membranaceus* are *P. lesuerii* Blainville, 1825, *P. contarinii* Vérany, 1846, and *P. denotarisii* Vérany, 1846 by Pilsbry (1895), and *Gymnotoplax barashi* Ev. Marcus, 1977 by Ev. Marcus (1984). We could not confirm these synonymies owing to incomplete original descriptions with the exception of *G. barashi*, which was synonymized with *P. membranaceus* by Willan (1978).

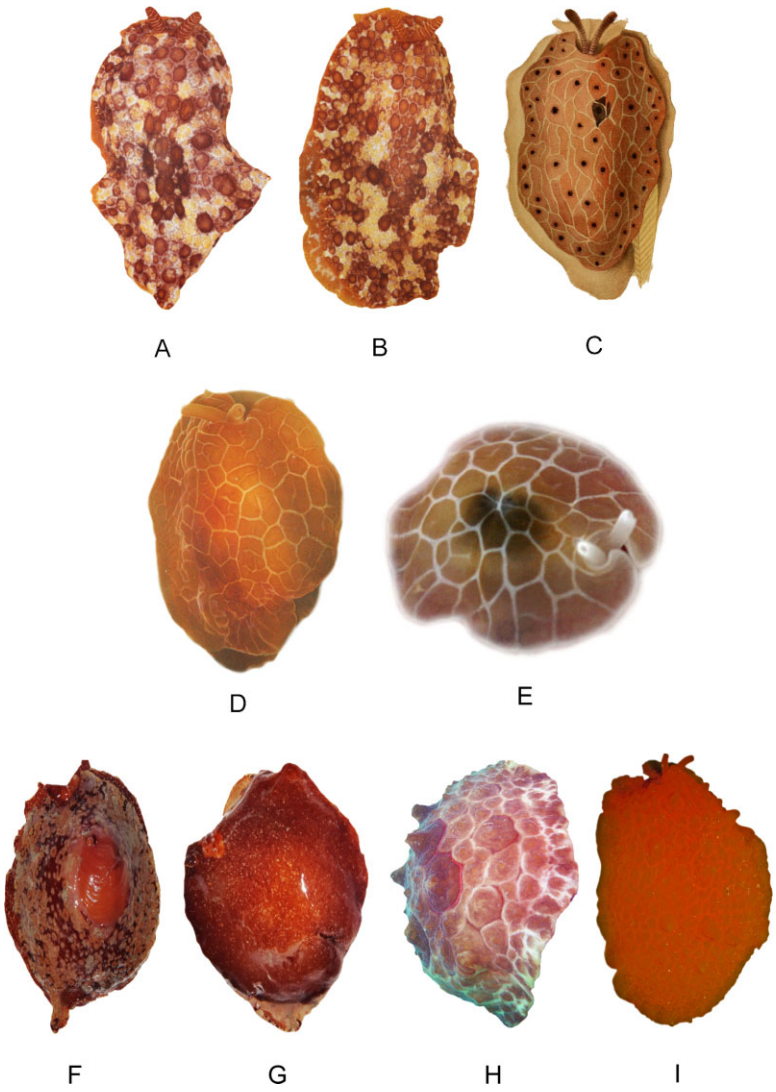

Figure 7. Colour variation in *Pleurobranchus reticulatus*, *Pleurobranchus membranaceus*, and *Pleurobranchus testudinarius*. A, *P. reticulatus* from Brazil (MZSP103373). B, *P. reticulatus* from Brazil (MZSP103380). C, *P. reticulatus*, original description illustration from Principe Island (Rang, 1832). D, *Pleurobranchus garciagomezi* from Azores (ZMBN81685). E, *P. garciagomezi* from Cape Verde (no voucher no.). F, *P. membranaceus* (ZSM20100634). G, *P. membranaceus* from France (ZSM20070301). H, *P. testudinarius* from Santa Catarina, Brazil (photo by Edson Júnior). I, *P. testudinarius* from Rio de Janeiro, Brazil (photo by Fernando Moraes).

PLEUROBRANCHUS FORSKALII RÜPPELL & LEUCKART, 1828 (FIGS 8–11)

'Lepus marinus' Forsskål, 1776 (type locality: not specified)

Pleurobranchus forskalii Rüppell & Leuckart, 1828: 18–20, pl. 5, fig. 2A, B (type locality: Red Sea)

Pleurobranchus perrieri Vayssière, 1896a: 126–128, pl. 4, figs 2–4 (type locality: not specified; cited from Philippines; Ambon, Moluccas, Indonesia; Tahiti) **syn. nov.**

Oscanius semperi Vayssière, 1896a: 134, 135, pl. 4, fig. 1 (type locality: Philippines) **syn. nov.**

?*Susania karachiensis* White, 1946: 55, 56, figs 8–10, pl. 5, fig. 7 (type locality: Karachi, Pakistan)

?*Susania ceylonica* White, 1948: fig. 2 (type locality: 'Cheval Parr, Ceylon' [Sri Lanka])

Type material: Sixteen syntypes of *P. perrieri* (MNHN). The type material of *P. semperi* is not known to exist, not found at MNHN (Valdés & Héros, 1998). Types of *P. karachiensis* and *P. ceylonicus* are not known to exist, not found at BMNH. No type material examined.

Material examined: Philippines (voucher number unknown); Madang, Papua New Guinea (CASIZ191034);

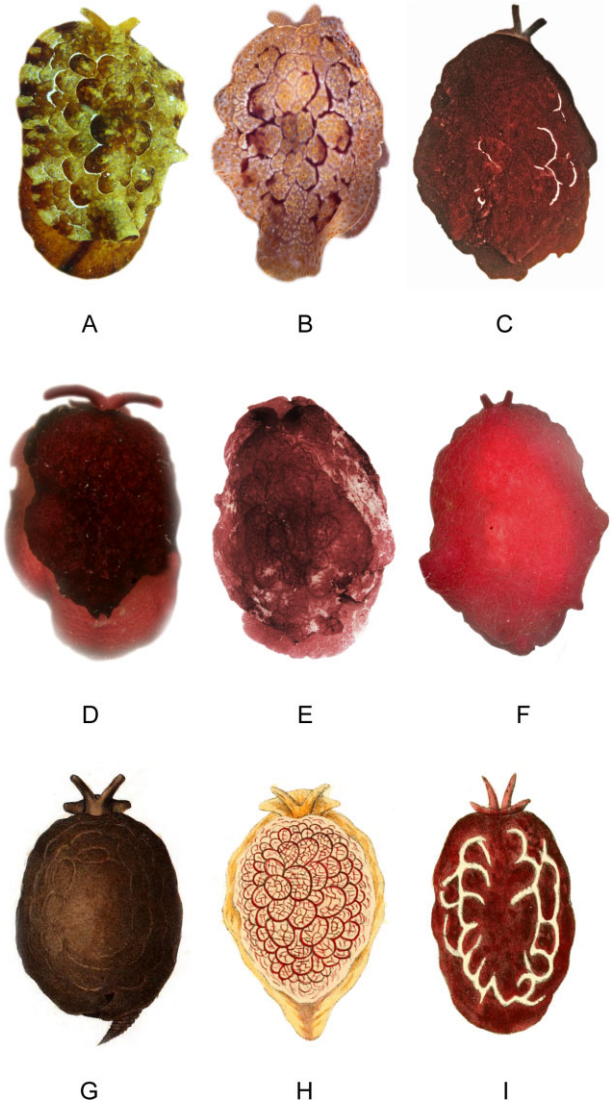

Figure 8. Colour variation in *Pleurobranchus forskalii*. A, *P. forskalii* from the Philippines (CASIZ176077). B, *P. forskalii* from the Philippines (no voucher no.). C, *P. forskalii* from Madang, Papua New Guinea (CASIZ191034). D, *P. forskalii* from Madang, Papua New Guinea (CASIZ191359). E, *P. forskalii* from Madang, Papua New Guinea (CASIZ191164). F, *P. forskalii* from Madang, Papua New Guinea (CASIZ191300). G, *P. forskalii*, original description illustration from the Red Sea (Rüppell & Leuckart, 1828). H, *Pleurobranchus semperi*, original description illustration from the Philippines (Vayssière, 1896a). I, *Pleurobranchus perrieri*, original description illustration from the tropical Indo-Pacific (Vayssière, 1896a).

Madang, Papua New Guinea (CASIZ191359); Philippines (CASIZ176077); Madang, Papua New Guinea (CASIZ191164); Madang, Papua New Guinea (CASIZ191300); Great Barrier Reef, Australia (CASIZ078704).

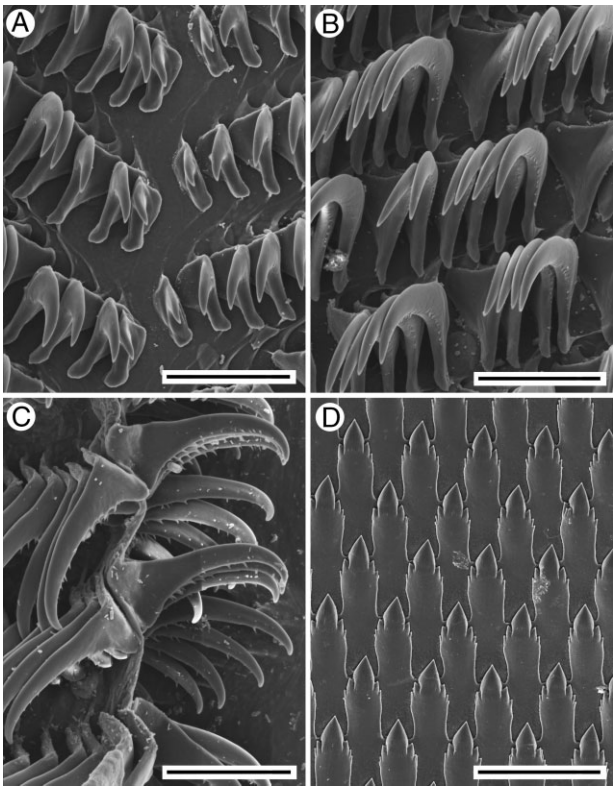

Figure 9. Scanning electron micrographs of the radula and jaws of *Pleurobranchus forskalii* from the Great Barrier Reef, Australia (CASIZ078704). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 100 μm.

Distribution: Known from Tanzania, the Red Sea, Australia, Fiji, New Guinea, Indonesia, Philippines, Guam, Japan, and Australia (Gosliner *et al*., 2008).

External morphology: Body size up to 300 mm long (Gosliner *et al*., 2008). Rhinophores with multiple horizontal striations along the length. Posterior end of the foot projecting from the mantle in some specimens. Gill rachis tuberculate at the base of the pinnae and axes of the pinnae tuberculate. Broad, compound tubercles in adults usually outlined in coloured arcs.

Background colour in mature animals light, mottled orange-brown or dark red. Tubercles outlined with white arcs in dark animals and dark arcs in light animals. Tubercles not outlined in some specimens. Oral veil and rhinophores same colour as the mantle and white-tipped in juveniles (Fig. 8A–F).

Internal anatomy: Shell oval in shape and narrower at the anterior end than the posterior. Protoconch roughly 600 μm, has about one whorl and lacks any ornamentation.

Elongated cruciform elements in jaws have a prominent cusp flanked by two to three short denticles on

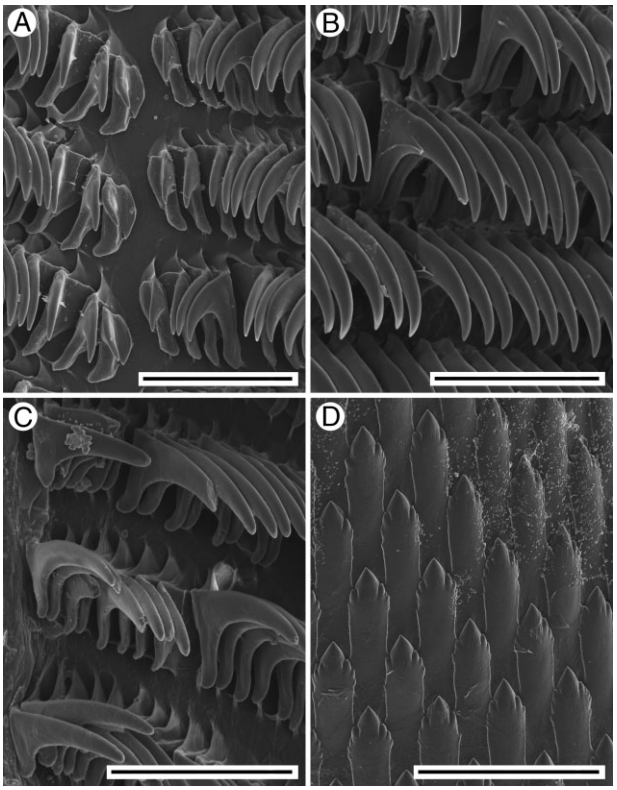

Figure 10. Scanning electron micrographs of the radula and jaws of *Pleurobranchus forskalii* from the Philippines (CASIZ176077). A, innermost lateral teeth, scale bar = 100 μm. B, mid-lateral teeth, scale bar = 100 μm. C, outermost lateral teeth, scale bar = 100 μm. D, jaw elements, scale bar = 200 μm.

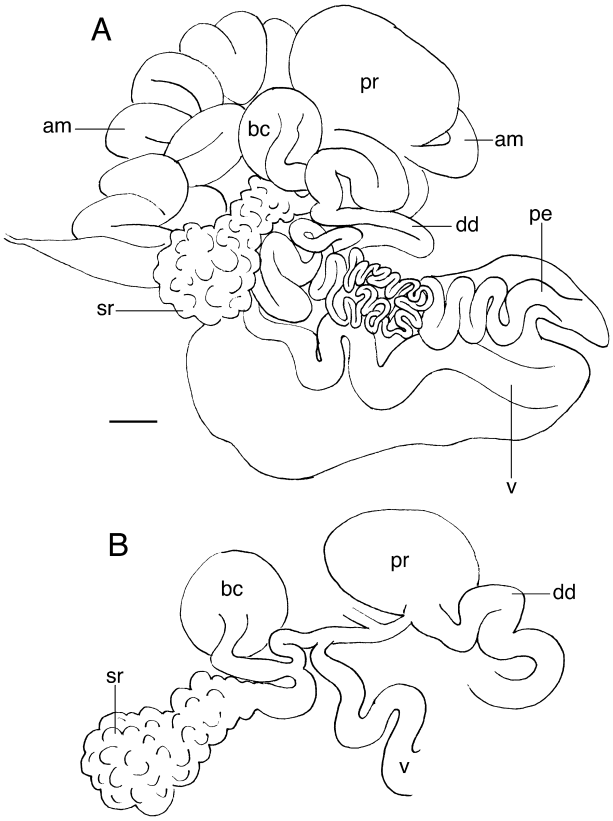

Figure 11. Reproductive system of *Pleurobranchus forskalii* from the Philippines (CASIZ176077). A, reproductive system; B, detail of some reproductive organs. Scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

each side (Figs 9D, 10D). Radular formula 37 × 87.0.87 (CASIZ 078704) and 67 × 123.0.123 (CASIZ 176077). Radular teeth smooth and hook-shaped, innermost lateral teeth the shortest. Fine denticulations can be observed in some teeth (Fig. 9B) but this appears to be a preservation artefact. Mid-lateral teeth roughly 2–3× the length of the inner teeth and outermost lateral teeth 1–2× larger than the mid-lateral teeth (Figs 9A–C, 10A–C).

Reproductive system (Fig. 11) triaulic. Ampulla long, thick, convoluted muscular duct connecting directly into the prostate. Prostate large and rounded, narrowing into a long deferent duct, progressively becoming thin and convoluted. The prostate also connects to the female gonoduct by a duct that connects first to the female gland complex, then to the vagina, and finally to seminal receptacle and bursa copulatrix. Deferent duct connecting directly to the large penis. Seminal receptacle large, elongate, and irregularly shaped, bursa copulatrix large and oval.

Remarks: Rüppell & Leuckart (1828) described *P. forskalii* from a figure, originally published by Forsskål (1776), labelled 'Lepus marinus', from Massawa, Eritrea (Red Sea). Yaron, Schiøtte & Wium-Andersen (1986) reproduced the original figure in Forsskål (1776), which represents a pleurobranchid, closely resembling the specimens here assigned to *P. forskalii*. Yaron *et al*. (1986) considered that the original description of 'Lepus marinus' is valid as it is accompanied by an indication to a work by Avicenna. However, Rudman (1993) considered 'Lepus marinus' a vernacular name and therefore invalid. Rudman (1993) submitted a petition to the International Commission on Zoological Nomenclature requesting that *P. forskalii* be considered the valid name for this species, as there was currently another species of *Pleurobranchus* described from the Mediterranean with a similar name (*Pleurobranchus forskahli* Delle Chiaje, 1822). In Opinion #1767, the Commission accepted the suppression of *P. forskahli*, placing it as a synonym of *P. testudinarius*, and considered *P. forskalii* a valid species name.

In 1896, Vayssière published two new species of *Pleurobranchus* (*P. perrieri* and *P. semperi*) that we con-

sider to be synonyms of *P. forskalii*. The original description illustrations of *P. perrieri* and *P. semperi* have the distinguishing colour pattern of *P. forskalii*, the opaque white or black circles or semicircles that are often present on the dorsum (Fig. 8G–I). Additionally, *P. karachiensis* and *P. ceylonicus*, described by White (1946, 1948) are probably synonyms of *P. forskalii* as well. *Pleurobranchus karachiensis* was described as having 'large and small tubercles arranged in circles and forming a regular pattern. The colour of the preserved specimen is grey with brown rings that outline the surface of the tubercles.' This matches the description of some specimens of *P. forskalii*, which have opaque black markings that form a semicircle around small tubercles arranged in clusters. *Pleurobranchus ceylonicus* was also described as having a golden-brown body with darker brown tubercles that were each encircled by a bright blue line. In some specimens of *P. forskalii*, the compound tubercles almost seem to be one entity that is often surrounded by an opaque line. In addition, the small size and colour of the specimen is consistent with a juvenile *P. forskalii*. Colour illustrations, however, were not provided for either *P. karachiensis* or *P. ceylonicus*, so we cannot be completely sure of their identity.

PLEUROBRANCHUS MAMILLATUS QUOY & GAIMARD, 1832 (FIGS 3C, 12I, J, 13)

Pleurobranchus mamillatus Quoy & Gaimard, 1832–1833: 294–296, pl. 22, figs 1–6 (type locality: Port Louis, 'Ile de France' [Mauritius])

?*Pleurobranchus moebii* Vayssière, 1896a: 128–130, pl. 4, figs 5, 6 (type locality: Querimba Islands, Mozambique)

Type material: Two syntypes of *P. mamillatus* (MNHN). Type material of *P. moebii* is not known to exist, not found at MNHN (Valdés & Héros, 1998). No type material was examined.

Material examined: Maalaea Bay, Maui, Hawaiian Islands, USA (CPIC00818).

Distribution: Known from Tanzania, South Korea, Japan, and the Hawaiian Islands (Gosliner *et al*., 2008).

External morphology and coloration: Body size up to 500 mm long (Gosliner *et al*., 2008). Rhinophores smooth. Foot does not project from the mantle. Gill rachis tuberculate at the base of the pinnae. Tubercles round and weakly compound and become more elongate from the edge of the mantle to the centre of the dorsum. Secondary tubercles flat around the edge of the mantle.

Background colour in juveniles cream to yellow or light brown, and in adults light brown to dark violet. Tubercles partially outlined in bright violet arcs and sometimes spots. Oral veil and rhinophores same colour as the mantle, sometimes with a different pigment at the tips of the rhinophores (Fig. 12I).

Internal anatomy: Shell oval in shape and the same width along its length. Protoconch roughly 250 μm, has about one whorl and lacks any ornamentation (Fig. 3C).

Elongated cruciform elements in jaws have a prominent cusp flanked by two to three short denticles on each side (Fig. 13D). Radular formula 49 × 109.0.109 (CPIC 00818). Radular teeth smooth and hook-shaped and the inner lateral teeth are the shortest. Mid-lateral teeth 4–5× the length of the inner lateral teeth, and outermost lateral teeth roughly the same as the mid-lateral teeth. (Fig. 13A–C).

Remarks: Pleurobranchus mamillatus was originally described as having a series of strikingly elevated tubercles on the mantle, with some smaller tubercles arranged in clusters (Quoy & Gaimard, 1832–1833). Additionally, this species most often has a tan, orange to reddish, or black body colour (Fig. 12J). Both Pilsbry (1895) and Thompson (1970) considered *P. mamillatus* to be a valid species.

One species that may be a potential synonym of *P. mamillatus* is *Pleurobranchus moebii* Vayssière, 1896. In the original description, *P. moebii* is described as having large, protruding papillae (Vayssière, 1896a), which is consistent with the description of *P. mamillatus*. However, as the only illustration provided by the author is that of the shell, this synonymy is difficult to confirm. Additionally, juveniles of *P. forskalii* are commonly confused with juveniles of *P. mamillatus*, although juveniles of *P. forskalii* lack the violet arcs and spots prevalent in *P. mamillatus*.

PLEUROBRANCHUS RETICULATUS RANG, 1832 (FIG. 7A–E)

Pleurobranchus reticulatus Rang, 1832: pl. 1 (type locality: Probably San Antonio Bay, Principe Island, São Tomé, and Principe)

?*Pleurobranchus garciagomezi* Cervera, Cattaneo-Vietti & Edmunds, 1996: 150–156, figs 1–4, pl. 1 (type locality: Albacora Bay, Ilha do Sal, Cape Verde Islands)

Type material: Type material of *P. reticulatus* is not known to exist, not found at MNHN (Valdés & Héros, 1998). Holotype of *P. garciagomezi* (MNCN 15.05/15840) and paratype (MCHN).

Material examined: Saco da Pedra Reef, Alagoas, north-eastern Brazil (MZSP 103373) and Saco da Pedra Reef, Alagoas, north-eastern Brazil (MZSP 103380)

Distribution: Originally described from the Gulf of Guinea, West Africa (Neves *et al*., 2007), and recorded as *P. areolatus* from Brazil (Marcus, 1976; García, Troncoso & Domínguez, 2002; Padula *et al*., 2012), Madeira (see Cervera *et al*. 2004), and the Ascension islands (Padula *et al*. 2014b). If the synonymy of

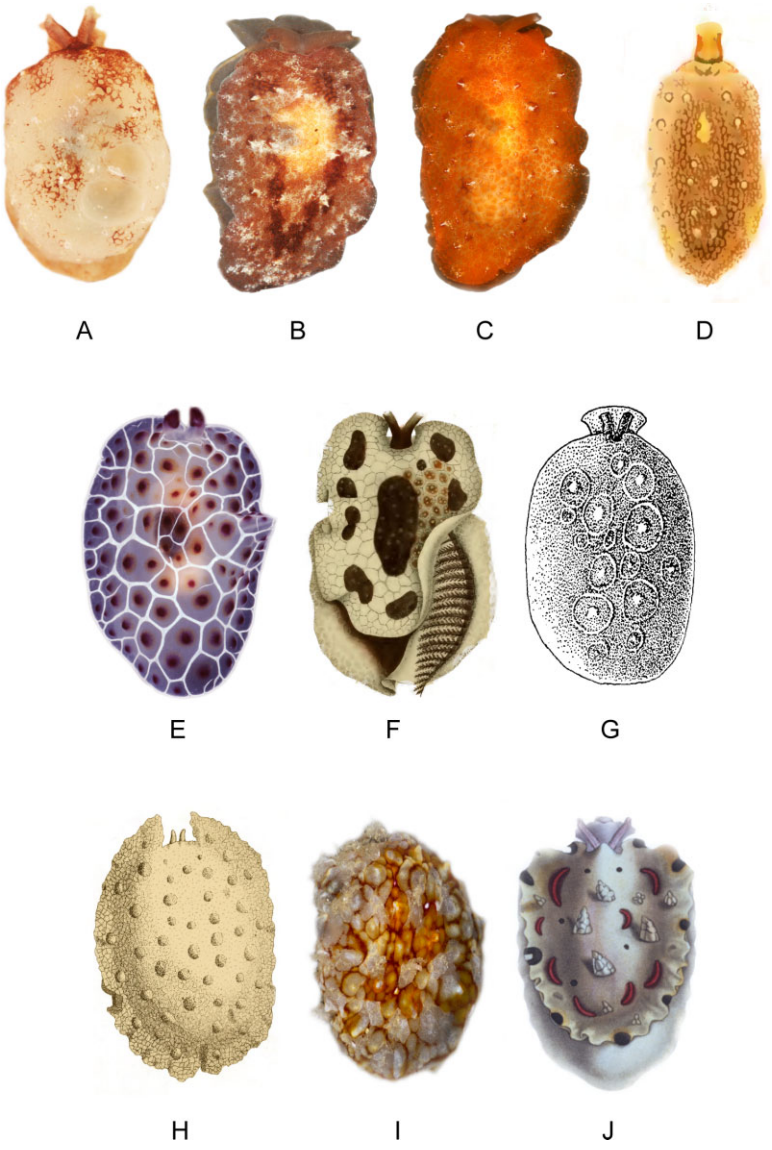

Figure 12. Colour variation in *Pleurobranchus albiguttatus*, *Pleurobranchus grandis*, *Pleurobranchus hilli*, and *Pleurobranchus mamillatus*. A, *P. albiguttatus* from Madang, Papua New Guinea (CASIZ191076). B, *P. albiguttatus* from Madang, Papua New Guinea (CASIZ191409). C, *P. albiguttatus* from Madang, Papua New Guinea (CASIZ191141). D, *P. albiguttatus*, original description illustration from Indonesia (Bergh, 1905). E, *P. grandis*, juvenile from New Caledonia (no voucher no.). F, *P. grandis*, original description illustration from Huahine, French Polynesia (Pease, 1868). G, *Pleurobranchus iouspi*, original description illustration from Brazil (Ev. Marcus, 1984). H, *P. hilli*, original description illustration from New South Wales, Australia (Hedley, 1894). I, *P. mamillatus* from the Hawaiian Islands (CPIC00818). J, *P. mamillatus*, original description illustration from Mauritius (Quoy & Gaimard, 1832–1833).

P. garciagomezi is confirmed, the species is also recorded from the Canary Islands, Cape Verde, and the Azores (Cervera *et al*., 2006; Malaquias *et al*., 2009).

External morphology and coloration: Descriptions provided in Neves *et al*. (2007) and Cervera *et al*. (1996). Photographs of Brazilian specimens provided by García *et al*. (2002), Padula *et al*. (2012), and in the present study (Fig. 7A, B).

Internal anatomy: Descriptions provided in Neves *et al*. (2007) and Cervera *et al*. (1996).

Remarks: Pleurobranchus reticulatus was originally described and illustrated by Rang (1832). The species was then redescribed by Neves *et al*. (2007), where they included data on the anatomy of the reproductive system and the development of colour pattern in this species. This species can be recognized by the thinner white

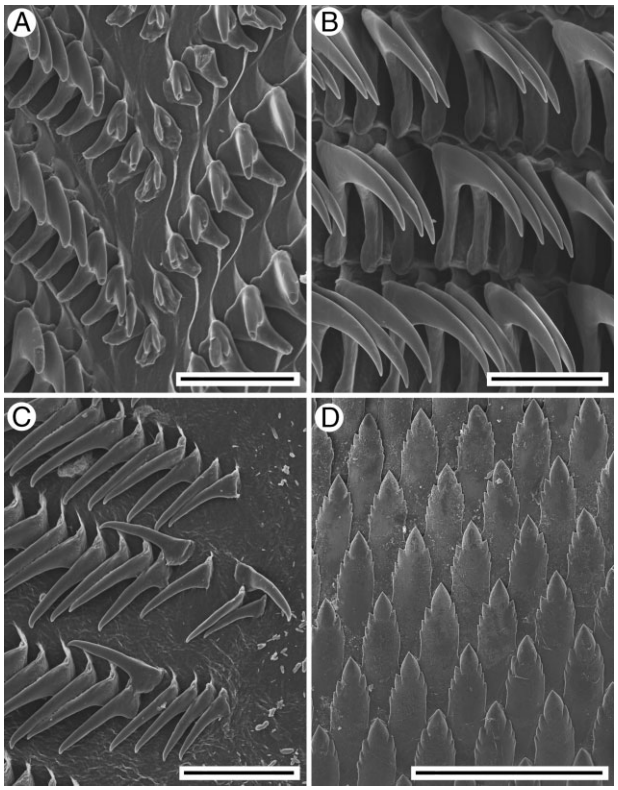

Figure 13. Scanning electron micrographs of the radula and jaws of *Pleurobranchus mamillatus* from the Hawaiian Islands (CPIC00818). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 200 μm.

reticulations on the mantle and the presence of dark tubercles inside the polygonal areas in the adults (Fig. 7C).

Cervera *et al*. (1996) described *P. garciagomezi* and separated this species morphologically from *P. reticulatus* by the thicker white reticulations on the mantle and the lack of dark tubercles in the adult specimens. The type locality of *P. garciagomezi* is Cape Verde, but the species has been subsequently reported from the Azores, the Canary Islands, and Madeira (Cervera *et al*., 1996; Rudman, 2000). Molecular data obtained in the present study from specimens collected in Cape Verde, the Azores, and the Canary Islands (Fig. 7D, E) are genetically similar to a Guinean specimen identified as *P. reticulatus* (FJ917498) by Göbbeler & Klussmann-Kolb (2010) and to specimens collected in Brazil, originally identified as *P. areolatus*. As there are no photographs available of the *P. reticulatus* specimen from the Canary Islands, it is not possible to confirm it belongs to this species; however, the specimens from Brazil are externally clearly distinct from other specimens of *P. garciagomezi* collected to date, with a reddish mantle (Fig. 7A–B) lacking the characteristic thicker white reticulations. This suggests that this species, as do many other species of *Pleurobranchus*, may display substantial chromatic variation. Both the phylogenetic and ABGD analyses indicated that *P. garciagomezi* is not distinct from the specimens assigned to *P. reticulatus*, but we are not definitely synonymizing these two species because we are not confident about the correct identification of the specimen of *P. reticulatus* from the Canary Islands. Further evidence is necessary to fully determine the relationship between *P. garciagomezi* and *P. reticulatus*.

PLEUROBRANCHUS TESTUDINARIUS CANTRAINE, 1835 (FIG. 7H, I)

Pleurobranchus testudinarius Cantraine, 1835: 385 (type locality: Naples, Italy)

?Pleurobranchus iouspi Ev. Marcus, 1984: 68–70, figs 57–61 (type locality: São Paulo, Brazil)

Type material: Type material of *P. testudinarius* is not known to exist, not found at MNHN (Valdés & Héros, 1998). According to Ev. Marcus (1984), type of *P. iouspi* was deposited at IOUSP but it is no longer there or at MZUSP (M. Petti & D. Cavallari, pers. comm.).

Distribution: Known from the Mediterranean Sea and Brazil (Marcus, 1971; Rudman, 1999a; Cunha *et al*. 2014).

External morphology and coloration: Description provided in Cattaneo-Vietti (1986). In addition, Brazilian specimens can be completely orange as well (Fig. 7I).

Internal anatomy: Description provided in Cattaneo-Vietti (1986) and Martynov & Schrödl (2009).

Remarks: Cantraine (1835) originally described *P. testudinarius* from the Mediterranean, and it has been deemed valid by Pilsbry (1895), Bergh (1897), and Cervera *et al*. (1996). The species was later recorded from Brazil based on a single specimen (Marcus, 1971). *Pleurobranchus testudinarius* can be distinguished by large, polygonal tubercles present on the mantle that 'occupy a mesh of a rose-carmine network' (Pilsbry, 1895).

Although *P. iouspi* was described and illustrated from São Paulo, Brazil, by Ev. Marcus (1984) and was determined to be valid by Cervera *et al*. (1996), very few accounts of this species exist in the literature. Morphologically, *P. iouspi* is very similar to *P. testudinarius* described by Cantraine (1835) from the Mediterranean. Both have large, complex tubercles that cover the mantle, although the colour patterns could not be compared because *P. iouspi* was described from a preserved specimen. Our molecular data indicate that these two species are genetically identical. However, we have only nuclear data from all three specimens, two from Brazil and one from the Mediterranean. As such, and

because of the morphological similarity between these two species, it is very probable that they are synonyms, but this needs to be confirmed by additional evidence.

PLEUROBRANCHUS VARIANS PEASE, 1860 (FIGS 3A, D, 14A–G, 15–17)

Pleurobranchus varians Pease, 1860: 25 (type locality: 'Sandwich Islands' [Hawaiian Islands, USA])

Type material: Type material of *P. varians* is not known to exist, not found at BMNH.

Material examined: Honokeana Bay, Maui, Hawaiian Islands, USA (CPIC00351); Maliko Bay, Maui, Hawaiian Islands, USA (CPIC00358); Honokeana Bay, Maui, Hawaiian Islands, USA (CPIC00342); Maliko Bay, Maui, Hawaiian Islands, USA (CPIC00357); Kapalua Bay, Maui, Hawaiian Islands, USA (CPIC00405); Honokeana Bay, Maui, Hawaiian Islands, USA (CPIC00409); Espiritu Santo Island, Vanuatu (CASIZ175771).

Distribution: Known from the Hawaiian Islands and Vanuatu.

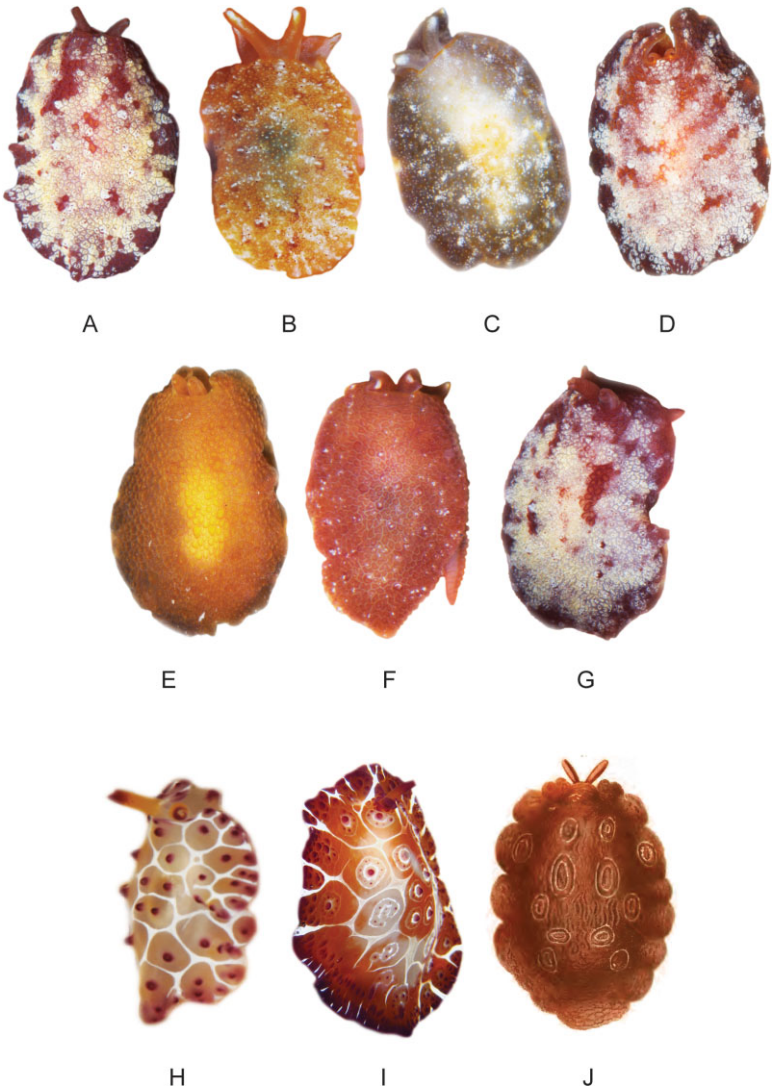

Figure 14. Colour variation in *Pleurobranchus varians* and *Pleurobranchus weberi*. A, *P. varians* from the Hawaiian Islands (CPIC00351). B, *P. varians* from Vanuatu (CASIZ175771). C, *P. varians* juvenile from the Hawaiian Islands (CPIC00342). D, *P. varians* from the Hawaiian Islands (CPIC00357). E, *P. varians* from the Hawaiian Islands (CPIC00358). F, *P. varians* from the Hawaiian Islands (CPIC00405). G, *P. varians* from the Hawaiian Islands (CPIC00409). H, *P. weberi* juvenile from Luzon, Philippines (CASIZ177651). I, *P. weberi* from Luzon, Philippines (CASIZ181282). J, *P. weberi*, original description illustration from Indonesia (Bergh, 1905).

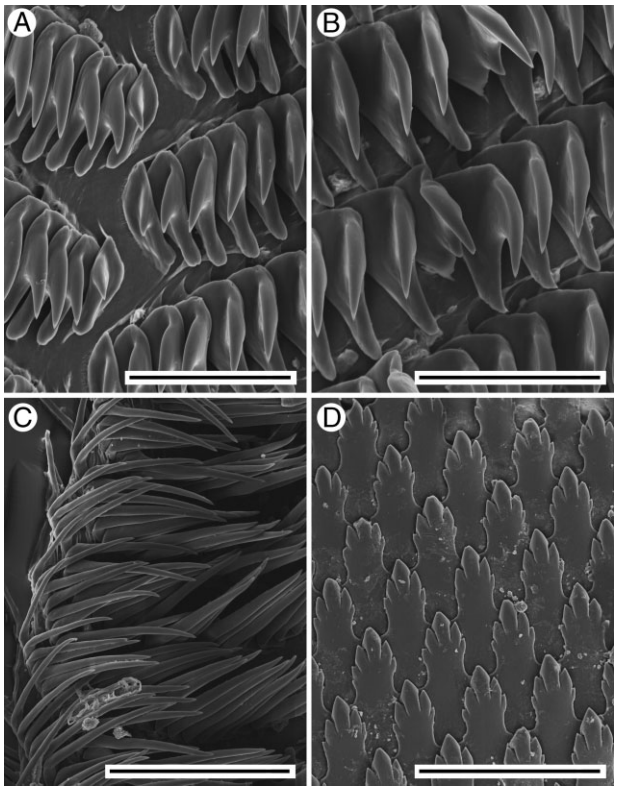

Figure 15. Scanning electron micrographs of the radula and jaws of *Pleurobranchus varians* from the Hawaiian Islands (CPIC00351). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 100 μm. D, jaw elements, scale bar = 100 μm.

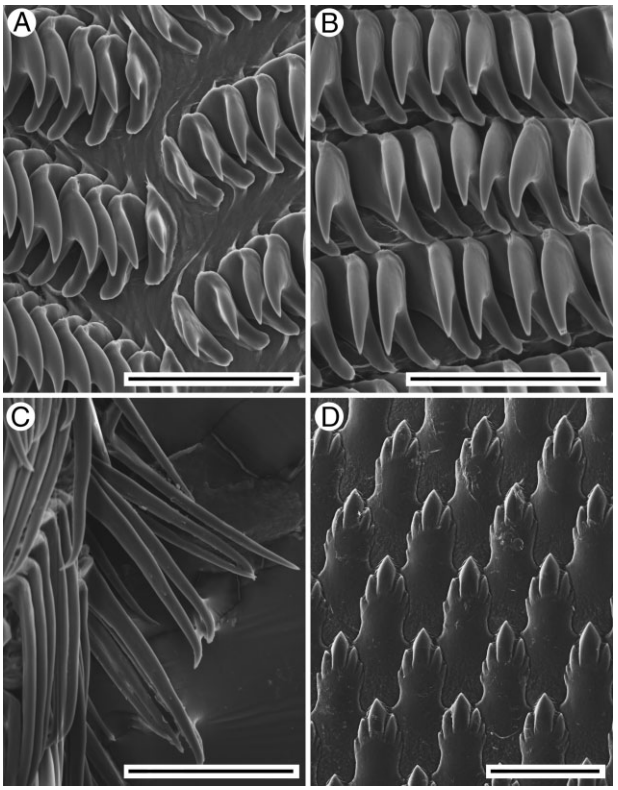

Figure 16. Scanning electron micrographs of the radula and jaws of *Pleurobranchus varians* from the Hawaiian Islands (CPIC00358). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 50 μm.

External morphology and coloration: Body size up to 70 mm long. Rhinophores with multiple horizontal striations along the length. Foot does not project from the mantle. Gill rachis tuberculate at the base of the pinnae. Tubercles polygonal in shape and larger, more flattened than those in *P. albiguttatus*. Tubercles similar in size in some specimens, whereas in others tubercles become smaller towards the edge of the mantle.

Background colour orange to dark red. Opaque white pigment on some of the tubercles in some specimens, similar to *P. albiguttatus*. Oral veil and rhinophores same colour as the mantle (Fig. 14A–G).

Internal anatomy: Shell oval in shape and the posterior end is narrower than the anterior end. Protoconch roughly 300 μm, has about one whorl and lacks any ornamentation (Fig. 3A, D).

Elongated cruciform elements on jaws have a prominent cusp flanked by two to three short denticles on each side (Figs 15D, 16D). Radular formula 38 × 65.0.65 (CPIC 00351), 51 × 72.0.72 (CPIC 00357), and 51 × 58.0.58 (CPIC 00358). Radular teeth smooth and hook-shaped and the inner lateral teeth are the shortest. Mid-lateral teeth 1–2× the length of the inner lateral teeth, and outermost lateral teeth 5–6× the length of the mid lateral teeth. (Figs 15A–C, 16A–C).

Reproductive system (Fig. 17) diaulic. Ampulla simple, thick, muscular duct splitting into two ducts, one connecting to the bursa copulatrix and the other to the prostate. No connection with the female gland complex was observed, but this could be because of the small size of the reproductive system. Bursa copulatrix large and rounded connecting to two other ducts, one opening into the vagina and the other to the short, somewhat rounded and irregular seminal receptacle. Vaginal duct straight and simple. Prostate large, oval and connected by a short duct to the large, irregular penis.

Remarks: The name *P. varians* was originally introduced by Pease (1860) for an animal collected in the Hawaiian Islands. Kay (1979) considered *P. varians* to be unidentifiable, and since then, this name has not been used as valid in the literature. Our phylogenetic analyses show that a subset of animals from the Central Pacific (Fig. 1), but mainly from the Hawaiian Islands,

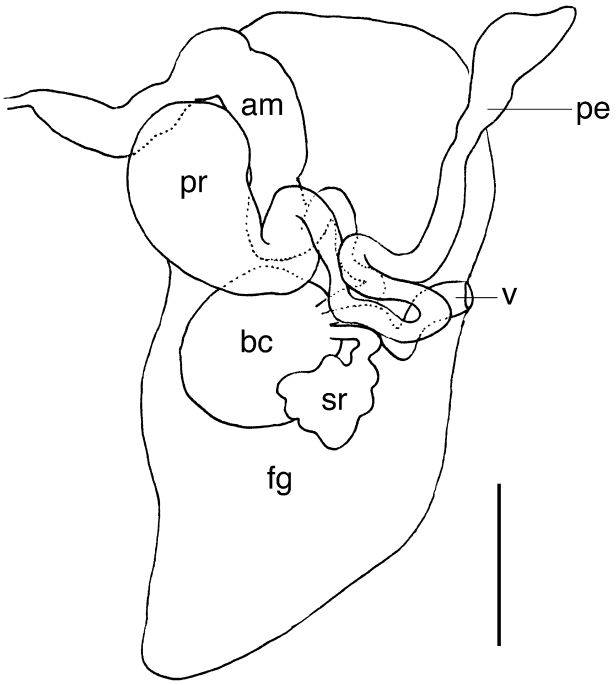

Figure 17. Reproductive system of *Pleurobranchus varians* from the Hawaiian Islands (CPIC00357), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

are genetically distinct from the other species of *Pleurobranchus* included in the analysis. These specimens externally match the original description of *P. varians*; therefore, we reinstate the species name *P. varians* for this subset of specimens as they are genetically distinct from other species of *Pleurobranchus*.

Members of this species have recently been incorrectly identified as *P. albiguttatus* (Gosliner *et al*., 2008), a similar-looking species from the Indo-Pacific, leading to the decline in the use of the name *P. varians*. *Pleurobranchus varians* differs from *P. albiguttatus* by its larger, more polygonal-shaped tubercles. Additionally, the white pigment found on some specimens of *P. varians* is far more prevalent than on specimens of *P. albiguttatus. Pleurobranchus albiguttatus* seems to have a broad range of colour forms, whereas *P. varians* has a sharp distinction between only two colour forms, one with a large coverage of white pigment, one with none whatsoever.

PLEUROBRANCHUS AREOLATUS MÖRCH, 1863 (FIGS 18A–G, 19C–F, 20–22)

Pleurobranchus areolatus Mörch, 1863: 28, 29 (type locality: St Thomas, US Virgin Islands)

Pleurobranchus atlanticus Abbott, 1949: 73–78, pl. 5, figs 1–10 (type locality: Biscayne Bay, Florida, USA)

Pleurobranchus crossei Vayssière, 1896b: 353–354, fig. 1 (type locality: Caribbean Sea) **syn. nov.**

Pleurobranchus evelinae Thompson, 1977: 108–110, figs 12E, F, 13C–E (type locality: St. Ann's Bay, Jamaica) **syn. nov.**

Susania gardineri White, 1952: 106, 107: pl. 6, fig. 1 (type locality: Dry Tortugas, Florida, USA)

Pleurobranchus reesi White, 1952: 107–109: pl. 6, fig. 2 (type locality: Dry Tortugas, Florida, USA) **syn. nov.**

Pleurobranchus emys Ev. Marcus, 1984: 70, figs 62–66 (type locality: Santa Marta, Colombia) **syn. nov.**

Type material: Type material of *P. areolatus* is not known to exist, not found at ZMUC. Holotype of *P. atlanticus* (USNM 574352), four paratypes (USNM 574342), one paratype shell (ANSP 184350), and another paratype shell (MCZ 165951). Holotype of *P. crossei* in MNHN. Holotype of *P. evelinae* (BMNH 19773W). Types of *P. gardineri* and *P. reesi* are not known to exist, not found at BMNH. No type material examined.

Material examined: Bahamas (LACM173235); Bahamas (LACM173236); Conch Point, Colón Island, Panama (LACM2004-12.1); Bocas del Toro, Panama (voucher number unknown); Panama, Caribbean Sea (Box4.No.60PanCar); Costa Rica (MZUCR-INB0001495902); Limón, Costa Rica (MZUCR-INB0003758925).

Distribution: Known from Venezuela, Jamaica, St Thomas, Aruba, St Maarten/St Martin, Brazil, Panama, México, Costa Rica, Bahamas, Puerto Rico, and Bermuda (Valdés *et al*., 2006).

External morphology and coloration: Body size up to 150 mm long (Valdés *et al*., 2006). Rhinophores with horizontal striations from the base to the tip. Posterior end of the foot projects from the mantle in some specimens. Gill rachis located at the base of the pinnae and axes of the pinnae tuberculate. Tubercles polygonal in shape. Tubercles similar in size in some specimens, in others they are smaller in size towards the edge of the mantle. Pedal gland visible in the posterior region of the pedal sole in some specimens.

Background colour light brown to deep violet, some animals with dark outlines. Opaque white pigment on some tubercles in some specimens, often arranged in a symmetrical pattern anterior to posterior, in other specimens no white pigment visible. Oral veil and rhinophores same colour as the mantle, sometimes with opaque white spots. Foot semitransparent and yellowish-brown with greyish-brown irregular spots (Fig. 18A–G).

Internal anatomy: Shell oval in shape and the posterior end is narrower than the anterior end. Protoconch roughly 400 μm, has about one whorl, and lacks any ornamentation (Fig. 19C–F).

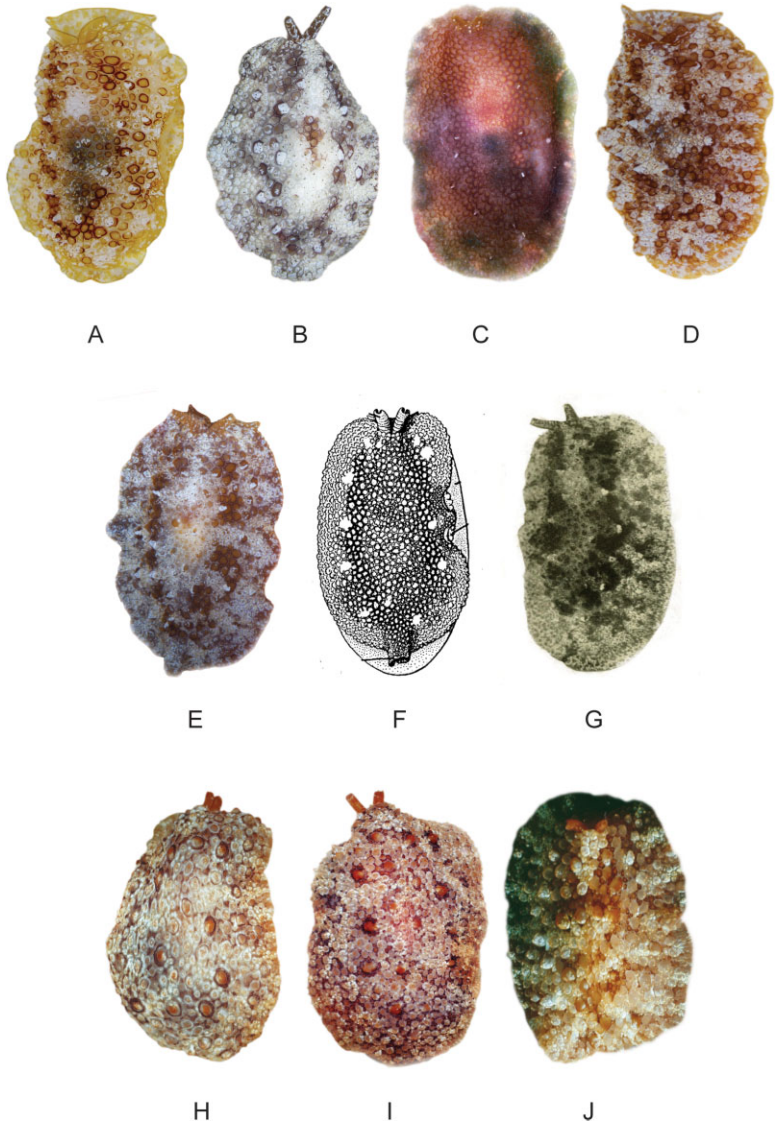

Figure 18. Colour variation in *Pleurobranchus areolatus* and *Pleurobranchus digueti*. A, *P. areolatus* from Bahamas (LACM173235). B, *P. areolatus* from Bahamas (LACM173236). C, *P. areolatus* juvenile from Colón Island, Panama (LACM2004-12.1). D, *P. areolatus* from Yucatán, México (CPIC00208). E, *P. areolatus* from Panama (CPIC00929). F, *Pleurobranchus evelinae*, original description illustration from Jamaica (Thompson, 1977). G, *Pleurobranchus atlanticus*, original description illustration from Florida (Abbott, 1949). H, *P. digueti* from Panama (MZUCR6986). I, *P. digueti* from Panama (MZUCR6201). J, *P. digueti* from Baja California, México (LACMA.9555).

Elongated cruciform elements on jaws have a prominent cusp flanked by one to two short denticles on each side (Figs 20D, 21D). Radular formula 73 × 115.0.115 (CPIC 00929). Radular teeth smooth and hook-shaped and the inner lateral teeth are the shortest. Mid-lateral teeth are 2× the length of the innermost and outermost lateral teeth roughly 5× the length of the mid-lateral teeth. (Figs 20A–C, 21A–C).

Reproductive system (Fig. 22A–B) triaulic. Ampulla convoluted, muscular duct splitting into two ducts, one connecting to the bursa copulatrix and the other to the prostate and the female gland complex. Bursa copulatrix large and rounded with two other ducts connecting to it, one opens to the vagina and the other to the short, somewhat rounded and irregular seminal receptacle. Vaginal duct straight and simple. Prostate long, simple, and connected by a short duct to the large and variable penis.

Remarks: Mörch (1863) described *P. areolatus* from the island of St Thomas in the Caribbean Sea. It has subsequently been considered a valid species by

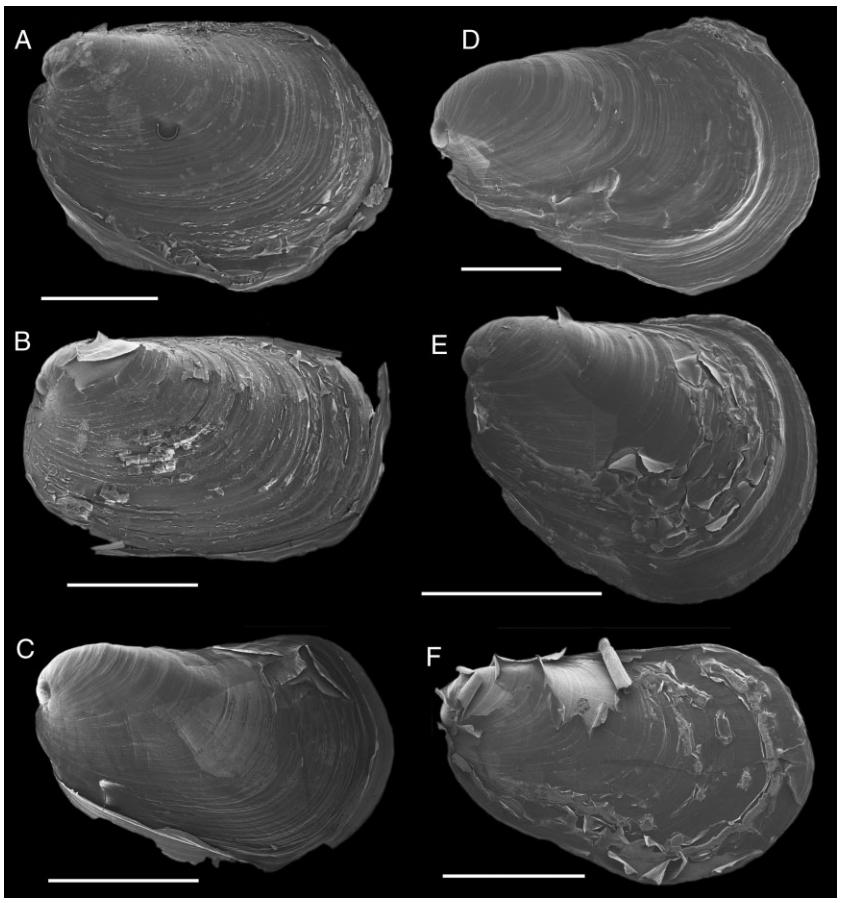

Figure 19. Scanning electron micrographs of shells. A, *Pleurobranchus digueti* from Baja California, México (LACMA.9555), scale bar = 2 mm. B, *P. digueti* from Baja California, México (LACM66-25), scale bar = 2 mm. C, *Pleurobranchus areolatus* from Colón Island, Panama (LACM2004-12.1), scale bar = 2 mm. D, *P. areolatus* from Bahamas (LACM173236), scale bar = 1 mm. E, *P. areolatus* from Costa Rica (MZUCR-INB0001495902), scale bar = 2 mm, F, *P. areolatus* from Limón, Costa Rica (MZUCR-INB0003758925), scale bar = 2 mm.

Pilsbry (1895), Bergh (1905), Marcus & Marcus (1962), and Thompson (1977). Several other species of *Pleurobranchus* have been introduced for Caribbean specimens (see list of synonyms), based on external colour differences. In the present study we sequenced several specimens covering the colour range of Caribbean *Pleurobranchus* (Fig. 18A–E) and concluded they were all the same species.

Although the type material of *P. areolatus* is lost, Bergh (1897: 111–113, pl. 9, figs 31–41) dissected and illustrated two of Mörch's type specimens. The illustrations of the penis, radula, jaw, and shell are very similar to those of the specimens here assigned to *P. areolatus*, confirming that our specimens are correctly identified.

Marcus & Marcus (1967) extended the range of *P. areolatus* into Baja California in the eastern Pacific and Edmunds (1968) reported *P. areolatus* from the western coast of Africa. The specimen identified by Edmunds (1968) as *P. areolatus* was later re-examined and identified as *P. reticulatus* by Cervera *et al*. (1996), a species described from that region. Bertsch & Smith (1973) argued that the range extension proposed by Marcus & Marcus (1967) implied that *P. digueti*, a species described from the eastern Pacific, should be considered a synonym of *P. areolatus*. They found anatomical similarities between these two species that they provided as evidence of this synonymy.

The molecular data do not support the synonymy between *P. digueti* and *P. areolatus* (Fig. 1). Additionally, further investigation into morphological characteristics has provided evidence that supports the molecular data in separating these two species. Externally, these two species have a similar coloration that ranges from light brown to dark red. Both *P. areolatus* and *P. digueti* have opaque white pigment present on the mantle, although in *P. areolatus* the abundance and concentration of this white pigment seem to be much more variable. The morphology of the tubercles is also different. In *P. areolatus* the

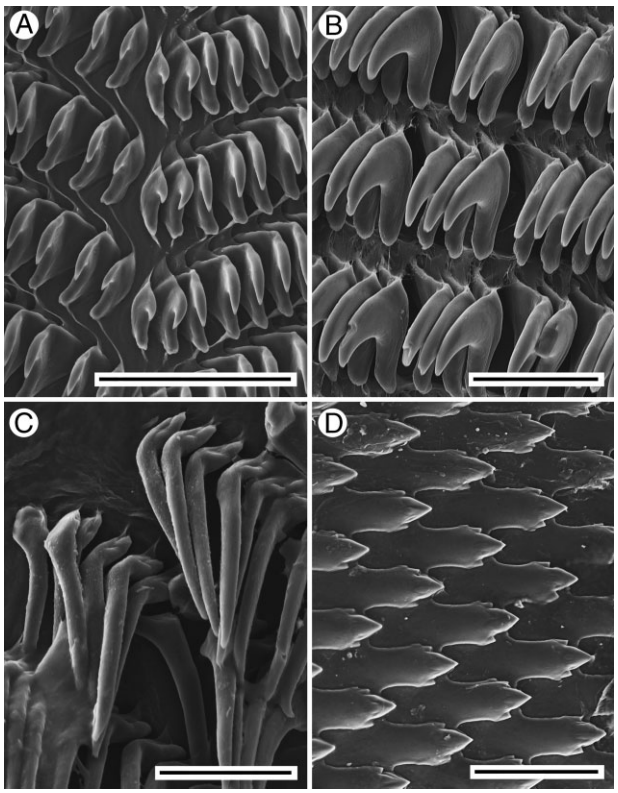

Figure 20. Scanning electron micrographs of the radula and jaws of *Pleurobranchus areolatus* from Colón Island, Panama (LACM2004-12.1). A, innermost lateral teeth, scale bar = 100 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 100 μm.

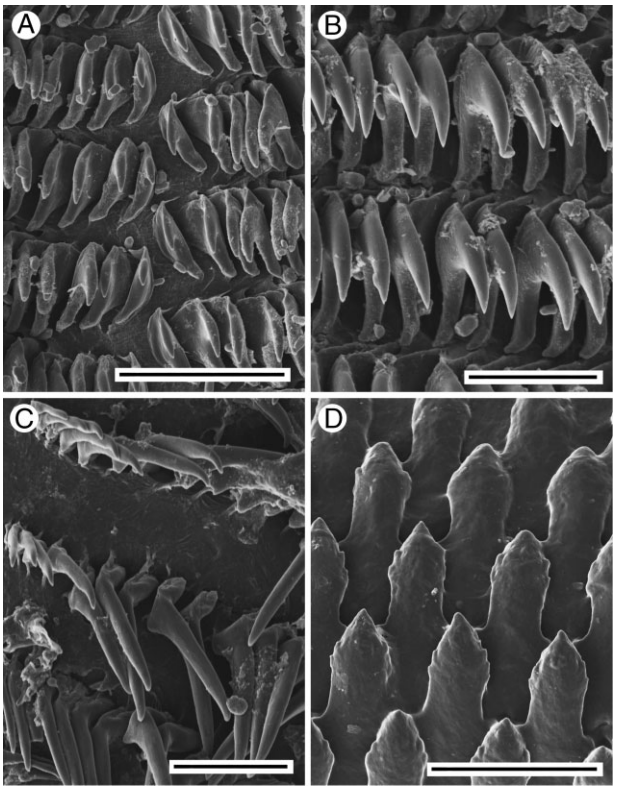

Figure 21. Scanning electron micrographs of the radula and jaws of *Pleurobranchus areolatus* from the Bahamas (LACM173236). A, innermost lateral teeth, scale bar = 100 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 100 μm.

tubercles are polygonal, relatively flat, and fairly regular, and in some individuals the tubercles decrease in size towards the edge of the mantle. Conversely, individuals of *P. digueti* have very large, spike-shaped tubercles that cover part of the mantle and are surrounded by small, polygonal tubercles such as we see in *P. areolatus*. The rhinophores are similar in these two species, both having bands of superficial opaque white pigment present, but these are more conspicuous in *P. areolatus*.

There are also numerous differences in internal morphology. Whereas both *P. areolatus* and *P. digueti* have similar radular formulas and smooth, hook-shaped radular teeth (Figs 20, 21, 23, 24), the radulae of *P. areolatus* are much shorter than those of *P. digueti*. The jaws of *P. digueti* generally have more denticles on the elongated cruciform elements than individuals of *P. areolatus* (Figs 20D, 21D, 23D, 24D). The shells of these two species also differ. Although both species have oval-shaped shells, in *P. areolatus* the posterior region of the shell is much narrower than the anterior portion (Fig. 19C–F), whereas in *P. digueti* the width is uniform throughout the length of the shell (Fig. 19A, B). Finally, the reproductive system of these two species is substantially different. Both the ampulla and the prostate are much longer and thinner in *P. digueti*, as is the deferent duct (Fig. 25). The seminal receptacle is also much larger in *P. digueti* (Fig. 25) and far more irregular than in *P. areolatus* (Fig. 22). Lastly, the bursa copulatrix seems to be smaller in *P. digueti*, and the duct connecting it to the vagina much wider (Fig. 25).

As mentioned above, the Brazilian morphotype generally identified as *P. areolatus* (Fig. 7A–B) represents, in fact, *P. reticulatus*. However, the presence of *P. areolatus* in Brazil is confirmed based on the specimen illustrated by Padula *et al*. (2012) as *P. atlanticus*.

Pleurobranchus grandis Pease, 1868 (Fig. 12E, F)

Pleurobranchus grandis Pease, 1868: 78, 79, pl. 10, fig. 2 (type locality: Huahine, French Polynesia)

?*Pleurobranchus blainvillii* Lesson, 1830: 143, pl. 51, fig. 1 (type locality: Point Venus, Tahiti)

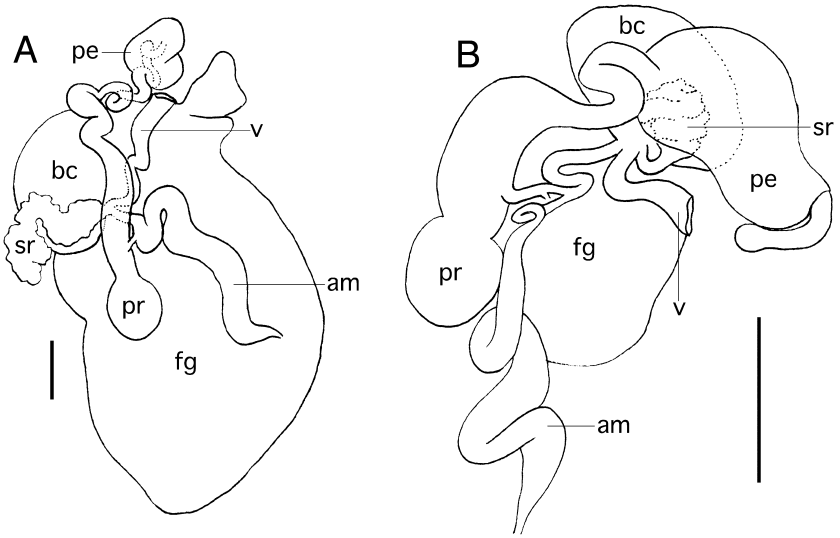

Figure 22. Reproductive system of *Pleurobranchus areolatus*. A, specimen from Limón, Costa Rica (MZUCR-INB0003758925); B, specimen from Colón Island, Panama (LACM2004-12.1), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

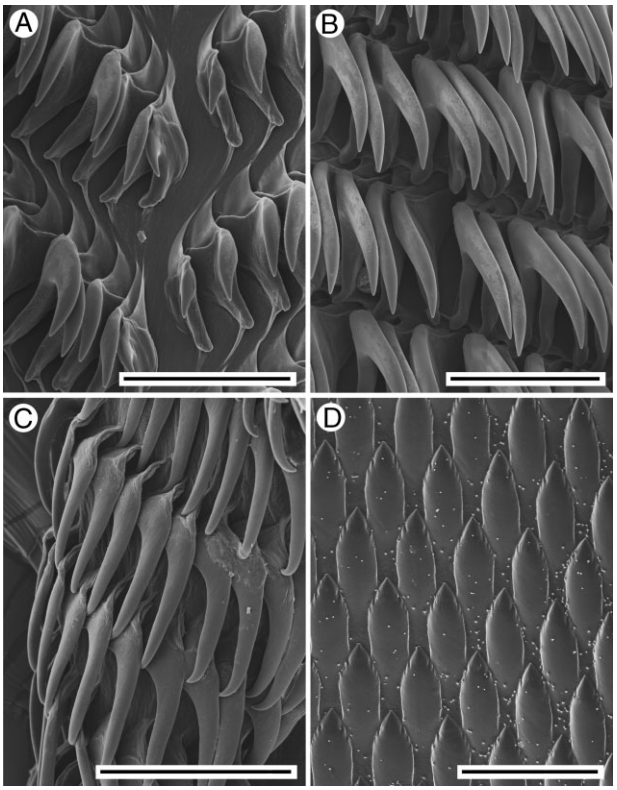

Figure 23. Scanning electron micrographs of the radula and jaws of *Pleurobranchus digueti* from Baja California, México (LACMA.9555). A, innermost lateral teeth, scale bar = 100 μm. B, mid-lateral teeth, scale bar = 100 μm. C, outermost lateral teeth, scale bar = 100 μm. D, jaw elements, scale bar = 200 μm.

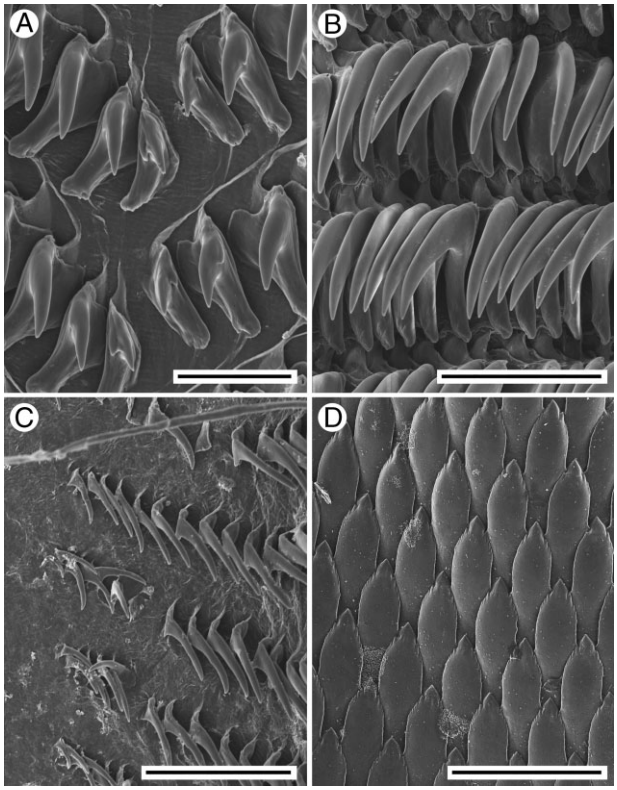

Figure 24. Scanning electron micrographs of the radula and jaws of *Pleurobranchus digueti* from Baja California, México (LACM66-25). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 100 μm. C, outermost lateral teeth, scale bar = 100 μm. D, jaw elements, scale bar = 200 μm.

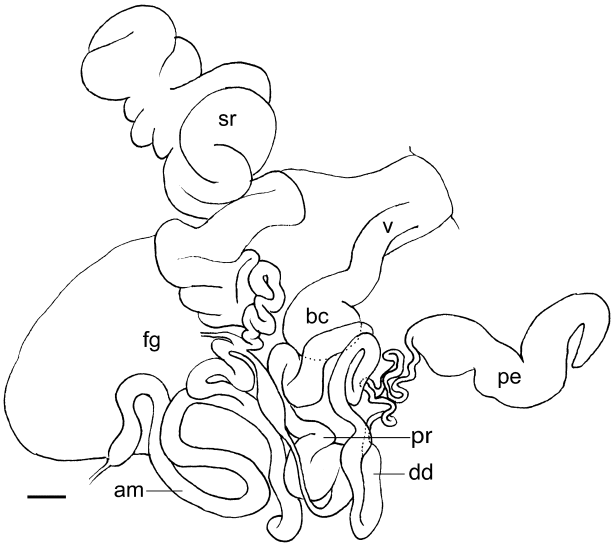

Figure 25. Reproductive system of *Pleurobranchus digueti* from Baja California, México (LACMA.9555), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

?*Pleurobranchus violaceus* Pease, 1863: 510 (type locality: 'Sandwich Islands' [Hawaiian Islands])

Type material: Type material of *P. grandis* is not known to exist, not found at BMNH or BPBM. Types of *P. blainvillii* are not known to exist, not found at MNHN (Valdés & Héros, 1998). Types of *P. violaceus* are not known to exist, not found at BMNH or BPBM.

Material examined: Juvenile from New Caledonia (no voucher no.).

Distribution: Known from New Guinea, the Philippines, Fiji, Society Islands, and New Caledonia (Gosliner *et al.*, 2008).

External morphology and coloration: Description provided in Er. Marcus & Ev. Marcus (1970).

Internal anatomy: Description provided in Er. Marcus & Ev. Marcus (1970) and Martynov & Schrödl (2009).

Remarks: Pleurobranchus grandis was originally described and illustrated by Pease (1868) and has been recognized as a valid species by Pilsbry (1895) and Bergh (1897).

Based on the original descriptions, two other species names, *P. blainvillii* Lesson, 1830 and *P. violaceus* Pease, 1863, may be synonymous with *P. grandis*. The colour patterns of these two species were both described as having a violet-coloured mantle with bluish-white striae (Lesson, 1830) or darker purple granules that give the mantle a reticulated appearance (Pease, 1860). Both could potentially be descriptions of the juvenile form of *P. grandis*, which has a translucent, light purple body with white reticulated lines and dark purple tubercles on the mantle. Given that the descriptions are not exact matches and no dimensions of the animals were given, we are unable to determine their identity with certainty. Additionally, adults of *P. grandis* tend to have tubercles arranged in clusters and have varying proportions of red, yellow, white, and black pigment on the mantle (Fig. 12E, F). Neither of the two descriptions is similar to a mature *P. grandis*. If these three species were found definitively to be synonymous, *P. grandis* and *P. violaceus* would have to be synonymized with *P. blainvillii*, as it is the senior name. However, the descriptions of these two species are not complete enough for us to confidently recognize this synonymy; therefore, *P. grandis* is considered a valid species for the purposes of this paper.

PLEUROBRANCHUS HILLI (HEDLEY, 1894) (FIG. 12H)

Oscanius hilli Hedley, 1894: 126–128, pl. 7 (type locality: Broken Bay, New South Wales, Australia)

Type material: Holotype (AM C.645.001) and three paratypes (AM C.240097.001).

Distribution: Known from south-eastern Australia from central New South Wales to Tasmania, Victoria, and South Australia (Rudman, 2003).

External morphology and coloration: Description provided in Thompson (1970).

Internal anatomy: Description provided in Thompson (1970).

Remarks: The original description and illustration of this species was published by Hedley (1894) as *Oscanius hilli* from New South Wales, Australia, and this species has been recognized as valid by Pilsbry (1895), Bergh (1897), Thompson (1970), and Burn (2006).

In addition to the unique range of this species compared with other members of *Pleurobranchus*, *P. hilli* is distinguishable externally from other species in this group by the large, warty, irregular tubercles on the mantle (Fig. 12H). No other described *Pleurobranchus* occurring in this region match the description of *P. hilli*.

PLEUROBRANCHUS DIGUETI ROCHEBRUNE, 1895 (FIGS 18H–J, 19A, B, 23–25)

Pleurobranchus digueti Rochebrune, 1895: 240 (type locality: Mogote, Bahía de La Paz, México)

Type material: Five syntypes known to exist (MNHN; BPBM 258974). No type material examined.

Material examined: Panama, Pacific Ocean (MZUCR 6986, MZUCR 6201); La Paz, Baja California Sur,

México (LACM A.9555); Ceralvo Island, Baja California, México (LACM 66-25).

Distribution: Known from Santa Barbara, California, USA, Gulf of California, México, to Colombia and Islas Galápagos, Ecuador (Behrens & Hermosillo, 2005).

External morphology: Body size up to 106 mm long. Rhinophores with horizontal striations from the base to the tips. Posterior end of the foot projecting from the mantle in some specimens. Gill rachis tuberculate at the base of the pinnae. Tubercles large, tentacular-shaped with pointed apices, surrounded by smaller, polygonal tubercles. Tubercles smaller towards the edge of the mantle in some specimens. Pedal gland visible in the posterior region of the pedal sole.

Background colour light brown, with opaque white pigment on some tubercles. Oral veil and rhinophores same colour as the mantle, sometimes with opaque white spots. Foot semitransparent and yellowish brown with greyish-brown, irregular spots (Fig. 18H–J).

Internal anatomy: Shell oval in shape and the same width along its length. Protoconch roughly 250 μm, has about one whorl and lacks any ornamentation (Fig. 19A–B).

Elongated cruciform elements on jaws have a prominent cusp flanked by one to two short denticles on each side (Figs 23D, 24D). Radular formula 70 × 123.0.123 (LACM A.9555), 36 × 67.0.67 (LACM 66-25), and 64 × 119.0.119 (LACM 70-1). Radular teeth smooth and hook-shaped and the innermost lateral teeth are the shortest. Mid-lateral teeth are 5× the length of the inner teeth and the outermost lateral teeth are only slightly longer than the mid-lateral teeth (Figs 23A–C, 24A–C).

Reproductive system (Fig. 25) triaulic. Ampulla long, thin, convoluted muscular duct splitting into three ducts, one connecting to the female gland complex, one to the seminal receptacle and the other to the prostate. Seminal receptacle large, elongate, and irregular. Prostate long, thin, and convoluted narrowing into a long and convoluted deferent duct. Deferent duct connects to a large and elongated penis. Bursa copulatrix large and oval and connects directly to the vagina.

Remarks: Pleurobranchus digueti was described by Rochebrune (1895) from a specimen collected by Diguet in Mogote, Bahía de la Paz, Baja California del Sur, México, also in 1895. Pilsbry (1895) redescribed *P. digueti* based on an additional specimen, collected by W. K. Fisher from La Paz. MacFarland (1966) then included extensive anatomical studies under the name *P. digueti*. The molecular and morphological information here presented clearly indicates that *P. digueti* is a valid and distinct species (see *P. areolatus*).

PLEUROBRANCHUS ALBIGUTTATUS (BERGH, 1905) (FIGS 12A–D, 26B, 27, 28)

Oscaniella albiguttata Bergh, 1905: 58–60, pl. 2, fig. 3, pl. 11, figs 10–17 (type locality: Pasi Tanette, South Sulawesi, Indonesia)

Type material: Three syntypes of *P. albiguttatus* from Siboga station 213 mentioned in the original description, two of these syntypes in NL (ZMA.MOLL.139284).

Material examined: Madang, Papua New Guinea (CASIZ191076, CASIZ191141, CASIZ191409).

Distribution: Known from Tanzania, the Red Sea, Reunion, the Comores, Australia, New Caledonia, Papua New Guinea, Indonesia, the Philippines, Japan, Palau, and Guam (Gosliner *et al*., 2008).

External morphology and coloration: Body size up to 65 mm long (Gosliner *et al*., 2008). Rhinophores with multiple horizontal striations along the length. Foot sometimes projecting posteriorly from the mantle. Tubercles conical or hemispherical in profile and polygonal from above. Tubercles often smaller than those of *P. varians* or *P. peronii* and in some specimens decrease in size towards the edge of the mantle.

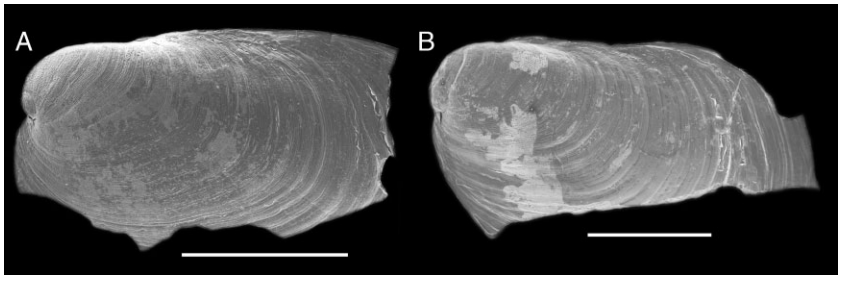

Figure 26. Scanning electron micrographs of the radula and jaws of shells. A, *Pleurobranchus weberi* from Luzon, Philippines (CASIZ181282), scale bar = 2 mm. B, *Pleurobranchus albiguttatus* from Madang, Papua New Guinea (CASIZ191076), scale bar = 1 mm.

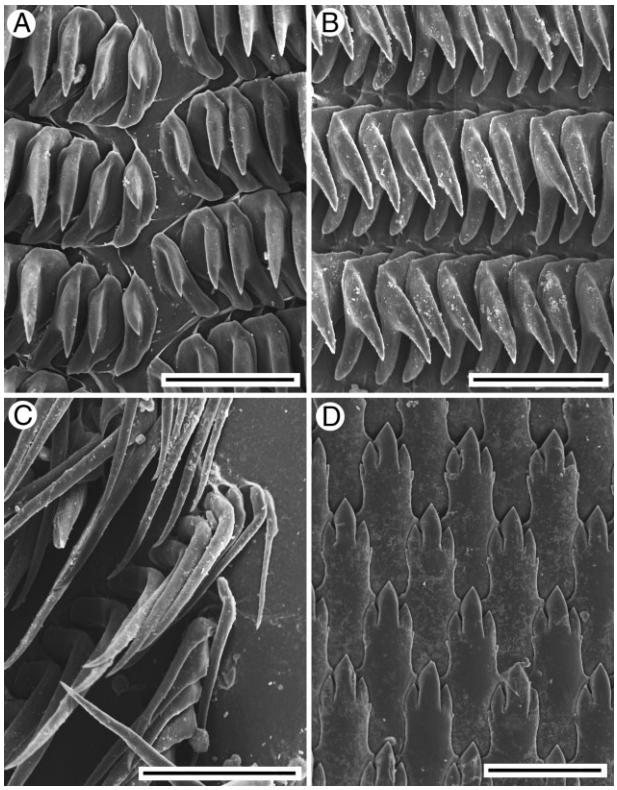

Figure 27. Scanning electron micrographs of the radula and jaws of *Pleurobranchus albiguttatus* from Madang, Papua New Guinea (CASIZ191076). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 50 μm. D, jaw elements, scale bar = 50 μm.

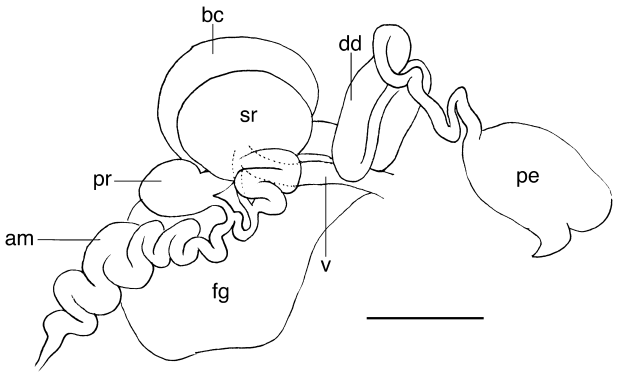

Figure 28. Reproductive system of *Pleurobranchus albiguttatus* from Madang, Papua New Guinea (CASIZ191076), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

Background colour orange to dark red. White pigment sometimes present on the tubercles, and the amount of white pigment highly variable. Oral veil and rhinophores same colour as the mantle (Fig. 12A–D).

Internal anatomy: Shell is oval in shape and seems to narrow anteriorly. Protoconch is roughly 275 μm, has about one whorl, and lacks any ornamentation (Fig. 26B).

Elongated cruciform elements on jaws have a prominent cusp flanked by one large denticle on each side (Fig. 27D). Radular formula 53 × 72.0.72 (CASIZ 191076). Radular teeth smooth and hook-shaped and the innermost lateral teeth are the shortest. Mid-lateral teeth 1–2× the length of the inner lateral teeth, and outermost lateral teeth roughly 4–6× the length of the mid lateral teeth. (Fig. 27A–C).

Reproductive system (Fig. 28) diaulic. Ampulla tightly packed, convoluted, muscular duct splitting into two ducts, one connecting to the bursa copulatrix and seminal receptacle and the other to the prostate. No connection with the female gland complex was observed, but this could be because of the small size of the reproductive system. Bursa copulatrix large and rounded with two other ducts connecting to it, one opening into the vagina and the other to the large and rounded seminal receptacle. Vaginal duct straight and simple. Prostate short and rounded, connected by a long, convoluted deferent duct to the large penis.

Remarks: Bergh (1905) originally illustrated and described this species, placing it in the genus *Oscaniella* Bergh, 1897, which Thiele (1931) synonymized with *Pleurobranchus*. Later, Cervera *et al*. (1996) recognized the validity of *P. albiguttatus*.

Pleurobranchus albiguttatus is distinguishable from *P. peronii*, the most similar species morphologically to it, by its uniform conical tubercles, which often have opaque white markings covering part or the entire surface of some tubercles. In addition, the rhinophores have a uniform colour from the base to the tips.

Gosliner *et al*. (2008) listed *P. nigropunctatus* (Bergh, 1907) as a synonym of *P. albiguttatus*, although no evidence was provided. The original description of *P. nigropunctatus* states that the specimens were covered by polygon-shaped areas up to 3–5 mm in diameter, which may or may not contain a very pronounced black centre. This colour pattern is not consistent with *P. albiguttatus*. Based on this disparity in external morphology, we propose that *P. nigropunctatus* may be a valid species. However, we have no specimens from the type locality of *P. nigropunctatus*, and as such we are unable to provide definitive evidence for this claim.

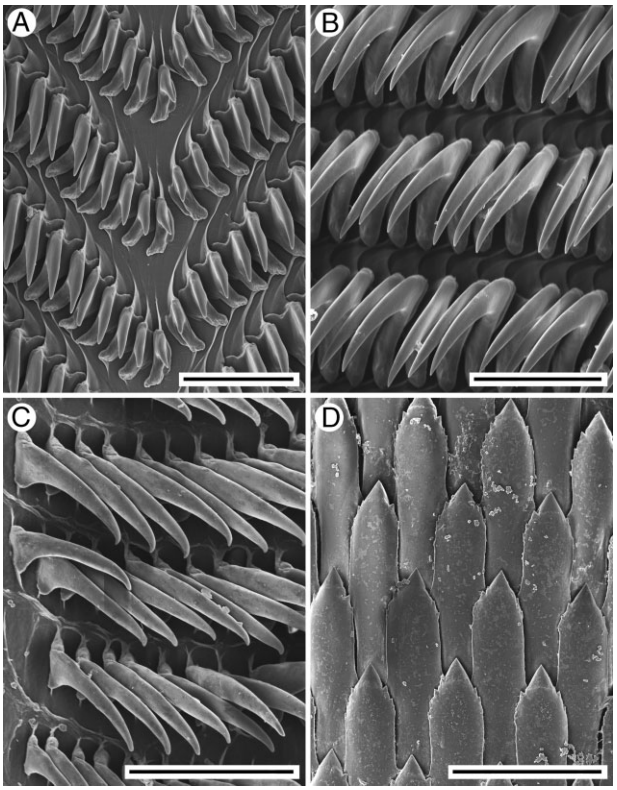

Figure 29. Scanning electron micrographs of the radula and jaws of *Pleurobranchus weberi* from the Hawaiian Islands (CASIZ160491). A, innermost lateral teeth, scale bar = 100 μm. B, mid-lateral teeth, scale bar = 100 μm. C, outermost lateral teeth, scale bar = 100 μm. D, jaw elements, scale bar = 200 μm.

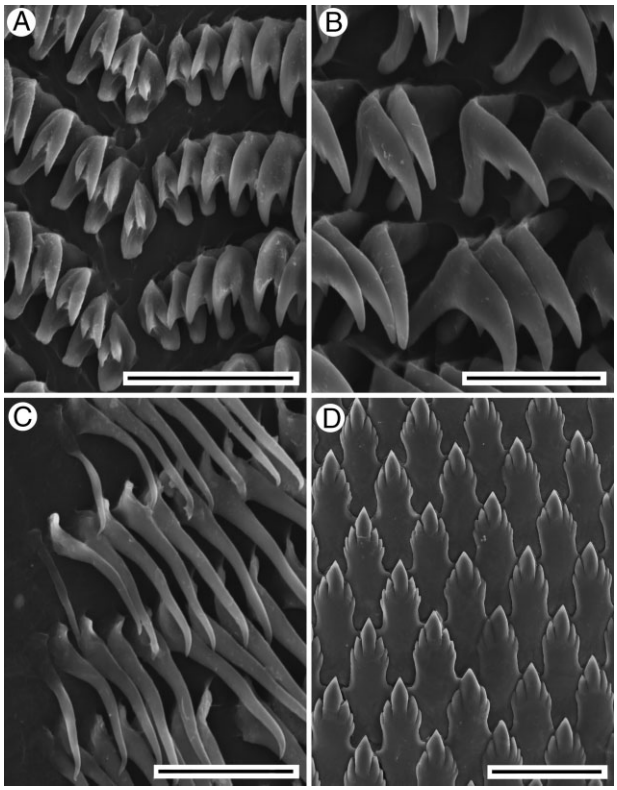

Figure 30. Scanning electron micrographs of the radula and jaws of *Pleurobranchus weberi* from Luzon, Philippines (CASIZ177651). A, innermost lateral teeth, scale bar = 30 μm. B, mid-lateral teeth, scale bar = 20 μm. C, outermost lateral teeth, scale bar = 20 μm. D, jaw elements, scale bar = 50 μm.

PLEUROBRANCHUS WEBERI (BERGH, 1905) (FIGS 14H–J, 26A, 29–31)

Oscanius weberi Bergh, 1905: 53–55, pl. 2, fig. 1, pl. 6, figs 1–6 (type locality: Selayar Island, Indonesia)

?*Pleurobranchus tessellatus* Pease, 1861: 245 (type locality: Pacific Ocean)

Type material: Four syntypes of *Oscanius weberi* mentioned in the original description, one from station 213 (not in NL), one from station 303 in NL (ZMA.MOLL.139379), two from station 201 (not in NL).

Material examined: Makena Bay, Maui, Hawaiian Islands, USA (CASIZ160491); Luzon, Philippines (CASIZ177651, CASIZ181282).

Distribution: Known from Indonesia and the Philippines (Gosliner *et al*., 2008) and the Hawaiian Islands (present study).

External morphology: Body size up to 200 mm long (Gosliner *et al*., 2008). Rhinophores smooth. Mantle completely covers the foot. Gill rachis heavily tuberculate at both the base of the pinnae and along its length. Tubercles widely spaced and conical in adults, surrounded by rings of flattened secondary tubercles. In some specimens, these rings collect sand. No secondary tubercles present in juveniles.

Background colour in mature animals cream to orange or red; multiple colours present in some individuals. Tubercles usually with darker pigment than the rest of the mantle, surrounded by one or two rings of white pigment or sand. Juveniles translucent white with orange pigment and white reticulations on the mantle. Tubercles dark purple. Oral veil and rhinophores same colour as the mantle in adults, orange in juveniles, sometimes with purple tips (Fig. 14H–J).

Internal anatomy: Shell oval in shape and the same width along its length. Protoconch roughly 300 μm, has about one whorl, and lacks any ornamentation (Fig. 26A).

Elongated cruciform elements on jaws have a prominent cusp flanked by one to four short denticles on each side (Figs 29D, 30D, 31D). Radular formula 42 × 60.0.60 (CASIZ 177651) and 55 × 109.0.109 (CASIZ 181282). Radular teeth smooth and hook-shaped and

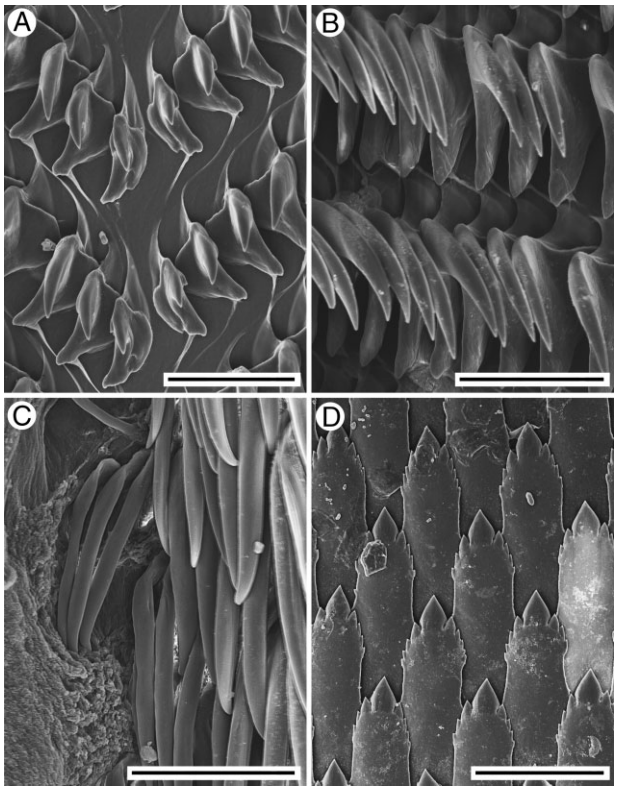

Figure 31. Scanning electron micrographs of the radula and jaws of *Pleurobranchus weberi* from Luzon, Philippines (CASIZ181282). A, innermost lateral teeth, scale bar = 50 μm. B, mid-lateral teeth, scale bar = 50 μm. C, outermost lateral teeth, scale bar = 30 μm. D, jaw elements, scale bar = 100 μm.

the innermost lateral teeth are the shortest. The mid-lateral teeth are roughly 5× the length of the inner teeth and the outermost lateral teeth are only slightly longer than the mid-lateral teeth (Figs 29A–C, 30A–C, 31A–C).

Reproductive system (Fig. 32) triaulic. Ampulla long, thin, convoluted muscular duct that splits into three ducts, one connecting to the female gland complex, one to the seminal receptacle, and the other to the deferent duct, which connects to the prostate. Seminal receptacle large and rounded. Prostate large and rounded, narrowing into a long and convoluted deferent duct that narrows further into the penis. Penis large and irregularly shaped. Bursa copulatrix large and oval, connecting directly to the vagina.

Remarks: Bergh (1905) described and illustrated *P. weberi*, originally *Oscanius weberi*, which can be distinguished from other Indo-Pacific species of *Pleurobranchus* by its very distinct dark red pigment and two opaque white concentric circles surrounding the tubercles on its mantle. Juveniles of this species are very similar to juveniles of *P. grandis*, with a translucent, light purple mantle with reticulated white lines and small, dark purple tubercles. These seem to be distinguishable from *P. grandis* by the orange pigment surrounding the tubercles and on the rhinophores.

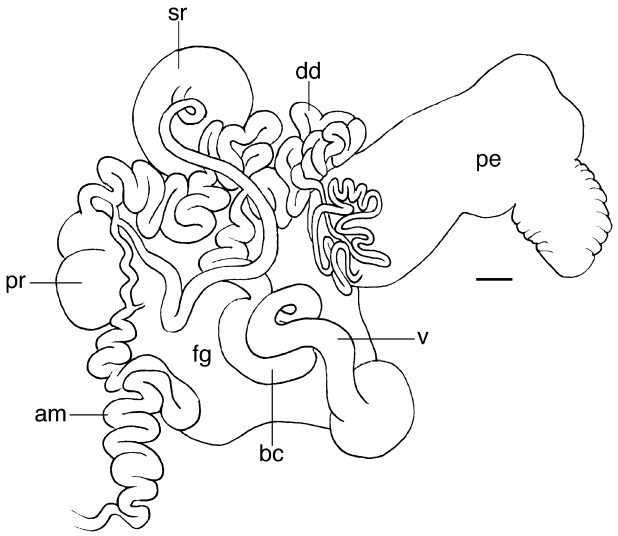

Figure 32. Reproductive system of *Pleurobranchus weberi* from the Hawaiian Islands (CASIZ160491), scale bar = 1 mm. Abbreviations: am, ampulla; bc, bursa copulatrix; dd, deferent duct; fg, female gland complex; pe, penis; pr, prostate; sr, seminal receptacle; v, vagina.

Pleurobranchus tessellatus Pease, 1861, was considered a synonym of *P. grandis* by Rudman (2008). However, the original description indicates that this species is cream in colour with an opaque white reticulation, and irregularly maculated with reddish brown. This is consistent with the specimens here assigned to *P. weberi*. As the type material of *P. tessellatus* cannot be examined, and is not known to exist, this synonymy is regarded as tentative.

PLEUROBRANCHUS NIGROPUNCTATUS (BERGH, 1907)

Oscaniella nigropunctata Bergh, 1907: 37–39, pl. 4, figs 22–26, pl. 11, figs 9–18 (type locality: Infanta, Western Cape, South Africa)

Type material: Type material not known to exist, not found at ZMUC.

Distribution: Known only from South Africa (Gosliner, 1987).

Remarks: Originally described by Bergh (1907) as *Oscaniella nigropunctata*, *P. nigropunctatus* (Bergh, 1907), as explained above, is a potential synonym of *P. albiguttatus* or, as suggested by Rudman (1999b), a synonym of *P. peronii*. On the contrary, Gosliner (1987) considered it a valid species. The description of *P. nigropunctatus* indicates that there are darker

polygonal areas towards the edges of the mantle that supposedly have a very pronounced black centre. In addition to the absence of distinct black spots on specimens of *P. albiguttatus*, the description of *P. nigropunctatus* does not include the presence of white colour on the mantle, which is often a trait to distinguish individuals of *P. albiguttatus*. One specimen included in our molecular data is from South Africa and seems to be genetically distinct from other *Pleurobranchus*. We believe that this may be *P. nigropunctatus*, but more specimens are needed for confirmation.

DISCUSSION

Taxonomy

The analysis of the molecular data produced well-resolved and generally well-supported phylogenetic consensus trees at the species level (Fig. 1). However, none of the analyses recovered *Pleurobranchus* as monophyletic. The most likely explanation is that the genes used in this study are not the most appropriate to resolve deep nodes within Nudipleura, but they provide valuable data to resolve the relationships amongst species. In this study, we used recently developed standards in the delimitation of problematic sea slug groups, using morphological differences, reciprocal monophyly (based on mitochondrial and nuclear gene phylogenies), and different species delimitation analyses to validate the species taxonomy (Ornelas-Gatdula *et al*., 2012; Padula, Wirtz & Schrödl, 2014b). Although most species are well supported in the phylogenetic analysis and their molecular distinctiveness is substantiated in the ABGD analyses, there are some exceptions. For example, the species *P. varians*, *P. areolatus*, and *P. albiguttatus* were grouped together in all ABGD analyses, and the monophyly of *P. varians* and *P. areolatus* is poorly supported in both Bayesian and maximum likelihood analyses of the concatenated sequences. However, *P. albiguttatus* is clearly monophyletic. The lack of support for *P. varians* and *P. areolatus* is particularly surprising, as the ranges of these two species are far apart (Central Pacific and Caribbean, respectively) and they are morphologically distinct. Because of this, they are here considered distinct valid species. Another example of problematic species includes *P. peroni*, *P. reticulatus*, and *P. garciagomezi*, which are grouped together in some ABGD analyses. In this case, the lack of enough sequence data for *P. garciagomezi* and some taxonomic uncertainties discussed above may be the cause for these results. However, *P. peroni* and *P. reticulatus* are clearly monophyletic and morphologically distinct and therefore considered as two different species.

Based on trees here obtained and a re-evaluation of the original descriptions of all species of *Pleurobranchus*, we propose a new taxonomic classification for this group, as described above and summarized in Table 5. Furthermore, after reviewing the original descriptions of all nominal species, some species previously considered members of *Pleurobranchus* were transferred to other genera and some descriptions were too incomplete for us to determine the identity of these species (Table 6).

Reproductive anatomy

Willan (1987) considered the reproductive system of *Pleurobranchus* to be diaulic, whereas Cervera *et al*. (2000) regarded *Pleurobranchus* as having a modified triaulic system. Valdés, Gosliner & Ghiselin (2010) discussed the differences between the arrangement of the reproductive systems of *Pleurobranchus* and other pleurobranchid groups, which display considerable variation. In the present study we observed considerable variation within *Pleurobranchus*, with some species having a direct connection between the male and female parts of the gonoduct and lacking a uterine duct, as indicated by Cervera *et al*. (2000), and others having a true uterine duct, connecting the prostate and the bursa copulatrix to the female gland complex, as in other triaulic systems such as dorid nudibranchs (Valdés *et al*., 2010). As mentioned above, some of the specimens examined were small and the reproductive organs difficult to examine; therefore, the observations shown in this study will need further verification using histology and/or examination of large, well-preserved specimens. However, it appears that *Pleurobranchus* displays a considerable diversity of genital arrangements and could include a combination of diaulic and triaulic species as well as different types of triaulic systems, with some species having the uterine duct located on the ampula–prostate connection and others having this duct located on the male–female gonoduct connection. This diversity of arrangements may prove useful in the species-level taxonomy for this group (Martynov & Schrödl, 2009).

Biogeography

The most common biogeographical structure in temperate and tropical sea slugs, as well as in many other groups of marine invertebrates, is exemplified by different groups of dorid nudibranchs, described by Gosliner & Johnson (1999), Valdés (2001), Garavoy, Valdés & Gosliner (2001), Dorgan, Valdés & Gosliner (2002), and Padula & Valdés (2012). In these groups there is a primary dichotomy in the phylogeny, with one clade containing species from the Indo-Pacific and the other containing species from the eastern Pacific and Atlantic (Fig. 33). This is consistent with the biogeographical patterns found in other marine invertebrates

Table 5. Proposed synonymies of species of *Pleurobranchus*

Species	Synonyms	Possible synonyms
Pleurobranchus peronii Cuvier, 1804	*Pleurobranchus cornutus* Quoy & Gaimard, 1832, *Pleurobranchus giardi* Vayssiere, 1896, *Pleurobranchus ovalis* Pease, 1868, *Oscaniella purpurea* Bergh, 1897, *Pleurobranchus winckworthi* White, 1946, *Pleurobranchus papillatus* Risbec, 1951, *Pleurobranchus inhacae* Macnae, 1962, *Pleurobranchus xhosa* Macnae, 1962, *Pleurobranchus hirasei* Baba, 1971	None
Pleurobranchus membranaceus Montagu, 1815	*Pleurobranchus tuberculatus* Meckel, 1808, *Lamellaria membranacea* Montagu, 1815, *Gymnotoplax barashi* Ev. Marcus, 1977	*Pleurobranchus lesuerii* Blainville, 1825, *Pleurobranchus contarinii* Vérany, 1846, *Pleurobranchus denotarisii* Vérany, 1846
Pleurobranchus forskalii Rüppell and Leuckart, 1828	'Lepus marinus' Forsskål, 1776, *Pleurobranchus perrieri* Vayssiere, 1896, *Oscanius semperi* Vayssiere, 1896	*Susania ceylonica* White, 1948
Pleurobranchus mamillatus Quoy & Gaimard, 1832	None	*Pleurobranchus moebii* Vayssiere, 1896
Pleurobranchus reticulatus Rang, 1832	None	*Pleurobranchus garciagomezi* Cervera, Cattaneo-Vietti & Edmunds, 1996
Pleurobranchus testudinarius Cantraine, 1835	None	*Pleurobranchus iouspi* Ev. Marcus, 1984
Pleurobranchus varians Pease, 1860	None	None
Pleurobranchus areolatus Mörch, 1863	*Pleurobranchus crossei* Vayssiere, 1896, *Pleurobranchus atlanticus* Abbott, 1949, *Pleurobranchus evelinae* Thompson, 1977, *Susania gardineri* White, 1952, *Pleurobranchus reesi* White, 1952, *Pleurobranchus emys* Ev. Marcus, 1984	None
Pleurobranchus grandis Pease, 1868	None	*Pleurobranchus blainvillii* Lesson, 1830 *Pleurobranchus violaceus* Pease, 1863
Pleurobranchus hilli (Hedley, 1894)	None	None
Pleurobranchus digueti Rochebrune, 1895	None	None
Pleurobranchus albiguttatus (Bergh, 1905)	None	None
Pleurobranchus weberi (Bergh, 1905)	None	*Pleurobranchus tessellatus* Pease, 1863
Pleurobranchus nigropunctatus (Bergh, 1907)	None	None

such as *Nerita*, a group of marine gastropods (Frey & Vermeij, 2008), the fiddler crab genus *Uca* (Levinton, Sturmbauer & Christy, 1996), the shrimp genus *Penaeus* (Lavery *et al*., 2004), the sea urchin genus *Eucidaris* (Lessios, Kessing & Robertson, 1999), and multiple clades within Littorinidae (Reid, Dyal & Williams, 2010). Valdés (2004) hypothesized that the pattern observed in dorid nudibranchs was determined by vicariant events related to the break-up of the Tethys Sea, including the formation of the Arabian Land Bridge 23 Mya (Harzhauser *et al*., 2007), the East Pacific Barrier about 10 Mya (Lessios, Kessing & Pearse, 2001), and the

Table 6. Proposed classification changes to species assigned to *Pleurobranchus* and a list of the species that could not be identified

Species	New classification
Berthella or *Berthellina*	
Pleurobranchus elongatus Cantraine, 1835	*Berthella* sp.
Pleurobranchus dehaanii Cantraine, 1841	*Berthellina* sp.
Pleurobranchus brevifons Philippi, 1844	*Berthella* sp.
Pleurobranchus perforatus Philippi, 1844	*Berthella* sp.
Pleurobranchus marginatus Pease, 1860	*Berthella* sp.
Pleurobranchus rufus Pease, 1860	*Berthellina delicata* (Pease, 1861)
Pleurobranchus ornatus Cheeseman, 1878	*Berthella martensi* (Pilsbry, 1896)
Pleurobranchus monterosatoi Vayssiere, 1880	*Berthella* sp.
Pleurobranchus strubelli Bergh, 1897	*Berthella* sp.
Pleurobranchus lacteus Dall & Simpson, 1901	*Berthella* sp.
Placobranchiopsis niveus Verrill, 1901	*Berthella* sp.
Pleurobranchus caledonicus Risbec, 1928	*Berthella* sp.
Unidentified *Pleurobranchus*	*Berthella* sp.
Pleurobranchus oblongus Andouin, 1827	NA
Pleurobranchus calyptraeoides Forbes, 1843	NA
Pleurobranchus limacoides Forbes, 1843	NA
Pleurobranchus scutatus Forbes, 1843	NA
Pleurobranchus sordidus Forbes, 1844	NA
Pleurobranchus savi Vérany, 1846	NA
Oscaniella affinis Bergh, 1897	NA
Oscaniella diversicolor Bergh, 1897	NA
Oscanius petersi Bergh, 1897	NA
Oscanius semonis Bergh, 1897	NA
Oscaniella styphla Bergh, 1897	NA
Pleurobranchus lowei Watson, 1897	NA
Pleurobranchus hornelli Farran, 1905	NA
Oscaniella dubia Bergh, 1905	NA
Pleurobranchus griseus Bergh, 1905	NA
Oscaniella inermis Bergh, 1905	NA
Pleurobranchus latipes Bergh, 1905	NA
Oscaniella lugubris Bergh, 1905	NA
Oscaniella modesta Bergh, 1905	NA
Oscanius papuligerus Bergh, 1905	NA
Oscaniella purpurescens Bergh, 1905	NA
Oscanius sibogae Bergh, 1905	NA
Pleurobranchus obses White, 1946	NA

Isthmus of Panama 3.1–3.4 Mya (Coates & Obando, 1996). The implication of this hypothesis is that all the groups that follow this pattern were poorly diversified before the closure of the east–west communication in tropical and temperate oceans.

However, the phylogeny of *Pleurobranchus* differs considerably from this pattern (Fig. 34). In *Pleurobranchus* there is only one species found in the Caribbean, *P. areolatus*. Yet, contrary to the pattern mentioned above, *P. areolatus* is not sister to *P. digueti*, the only eastern Pacific species of *Pleurobranchus*. Additionally, Indo-Pacific species of *Pleurobranchus* are paraphyletic and are more closely related to the Atlantic species of *Pleurobranchus* than the extant

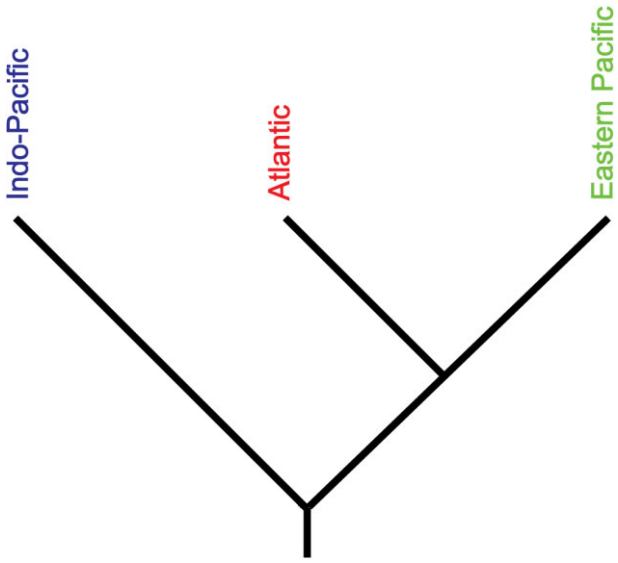

Figure 33. Geographical area cladogram for groups of dorid nudibranchs as presented by Valdés (2004).

eastern Pacific species. One hypothesis for this is that *Pleurobranchus* diversified before the break-up of the Tethys, but our molecular clock analysis estimated divergence times that are inconsistent with that premise. Some other marine invertebrate groups also break away from the common pattern, including the sea urchin group *Diadema* (Lessios *et al*., 2001), the hooded shrimps of *Betaeus* and *Betaeopsis* (Anker & Baeza, 2012), and the sea slug *Pontohedyle* (Jörger *et al*., 2012).

Our molecular clock analysis (Fig. 34, Table 4) indicates that the majority of the diversification in *Pleurobranchus* occurred in the Indo-Pacific between 1 and 10 Mya. The divergence between *Pleurobranchus* and *Berthella* is estimated to have occurred in the 10–15 Mya range and the deepest branch in *Pleurobranchus* is dated within the 8–13 Mya range. The dates for the divergence between *Pleurobranchus* and *Berthella* are significantly more modern than the estimates by Göbbeler & Klussmann-Kolb (2010), who placed the origin of *Pleurobranchus* in the Oligocene–Miocene transition. However, Göbbeler & Klussmann-Kolb's (2010) molecular clock estimates for the early diversification of *Pleurobranchus* in the middle Miocene coincide with the data presented here. The limited sampling of other Pleurobranchomorpha in the present study may account for this difference.

Considering the unique biogeographical structure of *Pleurobranchus* and the estimated divergence times for the various clades, we speculate that *Pleurobranchus* cladogenesis has been produced by a complexity of factors. For instance, a series of small vicariant events in the periphery of the Indo-Pacific region has been suggested to produce complex patterns of diversifica-

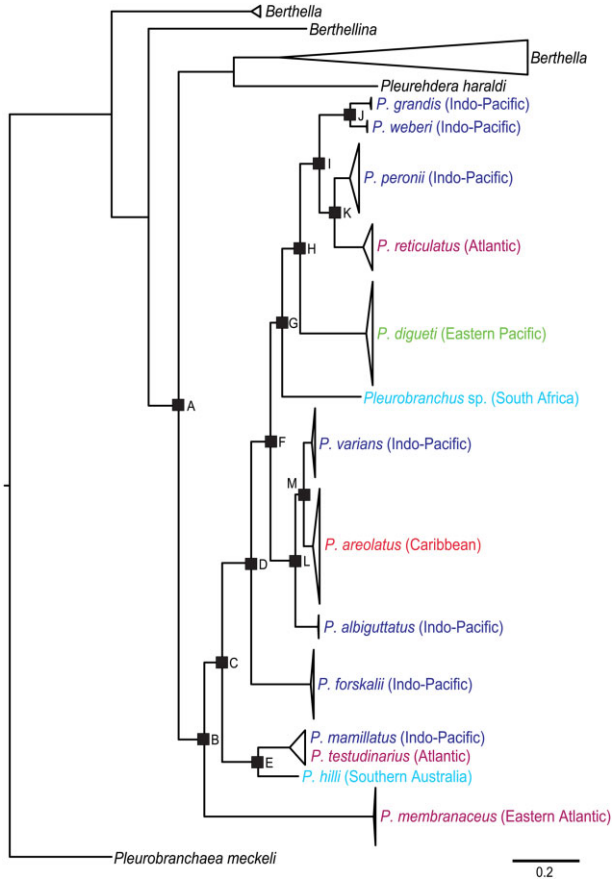

Figure 34. Simplified version of the Bayesian tree from Figure 1. Dark blue-coloured names indicate Indo-Pacific species, pink indicates eastern Atlantic species, red indicates Caribbean species, light blue indicates temperate Indo-Pacific species, and green indicates eastern Pacific species. Additionally, nodes with a black square are labelled with the proposed time of divergence from the molecular clock analysis run by r8s (Table 4) in millions of years ago (Mya).

tion in other invertebrates and sea slugs (Gosliner & Johnson, 1999; Garavoy, Valdés & Gosliner, 2001; Lessios *et al.*, 2001). A similar hypothesis could be used to explain some of divergences within *Pleurobranchus*, including the split of *P. weberi* and *P. grandis*, the split of the ancestor of the *P. weberi* and *P. grandis* clade and the ancestor of *P. peronii* and *P. reticulatus*, and the split of *P. albiguttatus* and the ancestor of *P. varians* and *P. areolatus*. These species appear to have diverged from other populations around the periphery of their original range, including those from the eastern Atlantic, eastern Pacific, and Caribbean, creating the unusual biogeographical patterns found in *Pleurobranchus*. These peripheral divergences may have been initiated by vicariant events or dispersal, but there is no definitive evidence supporting either hypothesis.

The divergence of *P. hilli* and *P. mamillatus* + *P. testudinarius* is especially interesting. The common ancestor of these species may have been isolated in the southern Indo-Pacific, then diverged into *P. hilli* and *P. mamillatus* + *P. testudinarius* as a result of a vicariant process caused by the expansion of the Subantarctic Front and adjacent temperate waters (Verducci *et al.*, 2009). The individuals of this hypothetical ancestor that were better adapted to colder waters evolved into *P. hilli*, a temperate species, and those that were better adapted to warmer temperatures evolved into *P. mamillatus* + *P. testudinarius*. This has also been suggested in *Nerita*, a group of primarily tropical marine gastropods (Frey & Vermeij, 2008), and the sea urchin clade *Diadema* (Lessios *et al.*, 2001).

The lack of sister species relationships between the eastern Pacific and the Caribbean species is also surprising. One possibility is that the ancestors of both *P. digueti* and *P. areolatus* had sister species that went extinct after the closure of the Isthmus of Panama, leaving the observed pattern. The dramatic temperature shifts of these two areas after the closure of the Isthmus of Panama has been suggested as a major factor in the large number of extinctions that occurred during this period (Vermeij & Petuch, 1986; Allmon *et al.*, 1993; Jackson *et al.*, 1993; Vermeij & Rosenberg, 1993; O'Dea *et al.*, 2007). Examples in the recent literature have revealed that interpretation of biogeographical signals in phylogenetic trees can be misleading because of lack of fossil data (Edwards *et al.*, 2011), such as is found in *Pleurobranchus*.

The other species in the Atlantic, *P. reticulatus* and *P. garciagomezi*, seem to have secondarily dispersed from the Indo-Pacific around 3 Mya, at the end of the Pliocene. This divergence is consistent with the strengthening of the Benguela current, which could have caused an allopatric split. This pattern is concordant with that found in species of *Diadema* (Lessios *et al.*, 2001).

Significance of colour

Gosliner & Behrens (1990) estimated that 50 per cent of 'opisthobranch' molluscs are aposematic, displaying bright external colour patterns that warn predators that the slug is toxic or unpalatable. Experimental data have shown that these bright external colorations are more effective in preventing predation than distasteful cryptic species (Tullrot, 1994). However, most species of *Pleurobranchus* are nocturnal, potentially limiting the need to warn predators, and appear to have cryptic coloration and blend in while on rocks and coral rubble (Thompson & Slinn, 1959), indicating that aposematism does not seem to be a method of protection for species of *Pleurobranchus*. One species of *Pleurobranchus*, *P. garciagomezi*, is known to be active

during the day (Fontes, Tempera & Wirtz, 2001), but unlike other species of *Pleurobranchus* does not have the bright external coloration associated with aposematism.

In 'opisthobranch' molluscs, colour pattern has been used to make taxonomic decisions on species boundaries and definitions. The established paradigm in 'opisthobranch' systematics is that members of the same species share the same, or similar, colour pattern whereas members of other species have differing colour patterns. Recent molecular studies, however, have shown that in some sea slugs, colour patterns are extremely variable within the same species (Pola *et al*., 2006; Ornelas-Gatdula *et al*., 2012; Valdés, Ornelas-Gatdula & Dupont, 2013). Other studies have revealed the existence of cryptic species that form part of a species complex long thought to be one or few widespread species (Carmona *et al*., 2011, 2013, 2014; Jörger *et al*., 2012; Krug *et al*., 2013; Padula *et al*., 2014a).

In *Pleurobranchus* there seems to be both phenotypic plasticity and cryptic diversity. For example, in the Hawaiian Islands, specimens of *P. varians* were previously identified as *P. albiguttatus* (Fig. 14A, C–D, G) because the colour patterns found on individuals of these two species seem to be remarkably similar. Based on the molecular data we now know that although these two species do not seem to differ in external morphology, they do differ genetically and geographically. *Pleurobranchus varians* is found in the Central Pacific, whereas *P. albiguttatus* is found in the rest of the Indo-Pacific. Another example of difficult-to-identify *Pleurobranchus* is members of *P. forskalii* and *P. peronii*, as both have a purple colour form with small, simple tubercles that are extremely difficult to distinguish in the field (Figs 2C, 8D–F).

One example of within-species variation occurs in *P. forskalii*. Members of this species differ in colour depending on whether they are adults, which are a dark reddish-purple colour and often have a series of white semicircles on the mantle, or juveniles, which are light brown in colour and often have dark brown or black semicircles surrounding complex tubercles on the mantle. Another interesting example of within-species variation is the two colour morphs of *P. varians* and *P. areolatus*. Both species contain individuals that are either uniform in colour, normally reddish or yellow, or specimens that have tubercles variable in colour and may be partially covered in opaque, mottled white. These two species can be difficult to identify based on photographs without knowing the locality, because specimens found in the same localities have different colorations, and specimens found in the Hawaiian Islands and the Caribbean are very similar to each other. However, we found some external patterns that are consistent within some species, and may therefore be reliable in field identifications. These are discussed in the individual species descriptions in the Systematics section, and include patterns such as the distinctive white semicircular markings on dark reddish-purple adult individuals of *P. forskalii*. At this point the evolutionary significance and biological role of the colour variation amongst individuals and species within this group are unknown.

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REFERENCES

Abbott RT. 1949. A new Florida species of the tectibranch genus *Pleurobranchus*. *The Nautilus* **62:** 73–78.

Akaike H. 1974. A new look at the statistical model identifications. *IEEE Transactions on Automatic Control* **19:** 716–723. doi: 10.1109/TAC.1974.1100705.

Alfaro ME, Zoller S, Lutzoni F. 2003. Bayes or Bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biological Evolution* **20:** 255–266. doi: 10.1093/molbev/msg028.

Allmon WD, Rosenberg G, Portell R, Schindler K. 1993. Diversity of Atlantic coastal plain mollusks since the Pliocene. *Science* **260:** 1626–1629.

Anker A, Baeza JA. 2012. Molecular and morphological phylogeny of hooded shrimps, genera *Betaeus* and *Betaeopsis* (Decapoda, Alpheidae): testing the center of origin biogeographic model and evolution of life history traits. *Molecular Phylogenetics and Evolution* **64:** 401–415.

Baba K. 1971. *Pleurobranchus hirasei* n. sp., proposed for a mollusk formerly known as *Oscanius testudinarius*: Hirase 1927, from Japan (Opisthobranchia: Notaspidea). *Venus* **30:** 23–28.

Bazinet AL, Cummings MP. 2008. The Lattice Project: a grid research and production environment combining multiple grid computing models. In: Weber MHW, ed. *Distributed & Grid Computing – Science Made Transparent for Everyone. Principles, Applications and Supporting Communities*. Marburg: Rechenkraft.net, 2–13.

Bazinet AL, Cummings MP. 2011. Computing the tree of life – leveraging the power of desktop and service grids. In: Institute of Electrical and Electronics Engineers International Symposium on Parallel and Distributed Processing Workshops and PhD Forum, Anchorage: IEEE Computer Society, 1896–1902.

Bazinet AL, Myers DS, Fuetsch J, Cummings MP. 2007. Grid services base library: a high-level, procedural application program interface for writing Globus-based Grid services. *Future Generation Computer Systems* **23:** 517–522.

Behrens DW, Hermosillo A. 2005. *Eastern Pacific Nudibranchs*. Monterey: Sea Challengers.

Bergh LSR. 1897. Malacologische Untersuchungen. Band 7, Heft 1-2 (Die Pleurobranchiden). In: Semper C, ed. *Reisen im Archipel der Philippinen*, 1–115. pls. 1-8.

Bergh LSR. 1905. *Die Opisthobranchiata der Siboga-Expedition, Monographie 50*. Leiden: Netherlands: EJ Brill.

Bergh LSR. 1907. The Opisthobranchiata of South Africa. *Transactions of the South African Philosophical Society* **17:** 1–144.

Bertsch H, Smith AA. 1973. Observations on three opisthobranchs (Mollusca: Gastropoda) of the La Paz Area, Baja California, México. *The Southwestern Naturalist* **18:** 165–176.

Blainville HMD. 1825. *Manuel de Malacologie et de Conchyliologie*. Paris: Leurault.

Burn R. 1962. On the new pleurobranch subfamily Berthellinae (Mollusca: Gastropoda); A revision and new classification of the species of New South Wales and Victoria. *Memoirs of the National Museum of Victoria* **25:** 129–148.

Burn R. 2006. A checklist and bibliography of the Opisthobranchia (Mollusca: Gastropoda) of Victoria and the Bass Strait area, south-eastern Australia. *Museum of Victoria Science Reports* **10:** 1–42.

Cantraine FJ. 1835. Mollusques – diagnoses ou descriptions succintes de quelques espèces nouvelles de mollusques, qui feront partie de l'ouvrage: malacologie méditerranéenne et littorale, et comparaison des coquilles qu'on trouve dans les collines subapenines avec celles qui vivent encore dans nos mers. *Bulletin de Academie Royale de Sciences, Bruxelles* **2:** 380–407.

Carmona L, Lei BR, Pola M, Gosliner TM, Valdés A, Cervera JL. 2014. Untangling the *Spurilla neapolitana* (Delle Chiaje, 1841) species complex: a review of the genus *Spurilla* Bergh, 1864 (Mollusca: Nudibranchia: Aeolidiidae). *Zoological Journal of the Linnean Society* **170:** 132–154.

Carmona L, Malaquias MAE, Gosliner TM, Pola M, Cervera JL. 2011. Amphi-Atlantic distributions and cryptic species in Sacoglossan sea slugs. *Journal of Molluscan Studies* **77:** 401–412.

Carmona L, Pola M, Gosliner TM, Cervera JL. 2013. A tale that morphology fails to tell: a molecular phylogeny of Aeolidiidae (Aeolidida, Nudibranchia, Gastropoda). *PLoS ONE* **8:** e63000.

Cattaneo-Vietti R. 1986. Pleurobranchomorpha from Italian seas (Mollusca: Opisthobranchia). *The Veliger* **28:** 302–309.

Cervera JL, Calado G, Gavaia C, Malaquias MAE, Castaño JT, Ballesteros M, Megina Martínez C, García-Gómez JC. 2004. An annotated and updated checklist of the opisthobranchs (Mollusca: Gastropoda) from Spain and Portugal (including islands and archipelagos). *Boletín del Instituto Español de Oceanografía* **20:** 5–115.

Cervera JL, Calado G, Gavaia C, Malaquias MAE, Templado J, Ballesteros M, García-Gómez JC, Megina C. 2006. An annotated and updated checklist of the opisthobranchs (Mollusca: Gastropoda) from Spain and Portugal (including islands and archipelagos). *Boletín del Instituto Español de Oceanografía* **20:** 1–122.

Cervera JL, Cattaneo-Vietti R, Edmunds M. 1996. A new species of Notaspidean of the genus *Pleurobranchus* Cuvier, 1804 (Gastropoda, Opisthobranchia) from the Cape Verde archipelago. *Bulletin of Marine Science* **59:** 150–157.

Cervera JL, Gosliner TM, Garcia Gomez JC, Ortea JA. 2000. A new species of *Berthella* Blainville, 1824 (Opisthobranchia: Notaspidea) from the Canary Islands (eastern Atlantic Ocean), with a re-examination of the phylogenetic relationships of the Notaspidea. *Journal of Molluscan Studies* **66:** 301–311.

Coates AG, Obando JA. 1996. The geologic evolution of the Central American Isthmus. In: Jackson JBC, Budd AF, Coates AG, eds. *Evolution and environment in tropical America*. Chicago: University of Chicago Press, 21–56.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46:** 419–437.

Cummings MP, Handley SA, Myers DS, Reed DL, Rokas A, Winka K. 2003. Comparing bootstrap and posterior

probability values in the four-taxon case. *Systematic Biology* **52:** 477–487.

Cummings MP, Huskamp JC. 2005. Grid computing. *Educause Review* **40:** 116–117.

Cunha CM, Padula V, Magalhães ARM, Ferreira FM, Ferreira JF. 2014. Moluscos. In: Lindner A, ed. *Vida Marinha de Santa Catarina*. Florianópolis: Universidade Federal de Santa Catarina, 39–51.

Cuvier GL. 1804. Mémoire sur la Phyllidie et sur le Pleurobranche, deux nouveaux genres de mollusques de l'ordre des gastéropodes, et voisins des patelles et des oscabrions, dont l'un est nu et dont l'autre porte une coquille cachée. *Annales du Muséum National d'Histoire Naturelle* **5:** 266–276.

Dorgan KM, Valdés A, Gosliner TM. 2002. Phylogenetic systematics of the genus *Platydoris* (Mollusca, Nudibranchia, Doridoidea) with descriptions of six new species. *Zoologica Scripta* **30:** 199–213.

Drummond AJ, Kearse M, Heled J, Moir R, Thierer T, Ashton B, Wilson A, Stones-Havas S. 2009. GENEIOUS v. 4.8. Available at: http://www.geneious.com/. Accessed 03 March 2010.

Edmunds M. 1968. Opistobranchiate Mollusca from Ghana. *Proceedings of the Malacological Society of London* **38:** 83–100.

Edmunds M, Thompson TE. 1972. Opisthobranchiate Mollusca from Tanzania Part IV Pleurobranchomorpha, Dendronotoidea and Arminoidea. *Proceedings of the Malacological Society of London* **40:** 219–234.

Edwards CJ, Suchard MA, Lemey P, Welch JJ, Barnes I, Fulton TL, Barnett R, O'Connell TC, Coxon P, Monaghan N, Valdiosera CE, Lorenzen ED, Willerslev E, Baryshnikov GF, Rambaut A, Thomas MG, Bradley DG, Shapiro B. 2011. Ancient hybridization and an Irish origin for the modern polar bear matriline. *Current Biology* **21:** 1251–1258. doi: 10.1016/j.cub.2011.05.058.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. *Cladistics* **10:** 315–319.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Fontes J, Tempera F, Wirtz P. 2001. On some interesting opisthobranchs (Mollusca, Gastropoda) from the Azores. *Arquipélago* **18A:** 85–87.

Forsskål P. 1776. *Icones rerum naturalium, quas in itinere orientali depingi curavit*. Hauniae: Carsten Niebuhr.

Frey MA, Vermeij GJ. 2008. Molecular phylogenies and historical biogeography of a circumtropical group of gastropods (Genus: *Nerita*): implications for regional diversity patterns in the marine tropics. *Molecular Phylogenetics and Evolution* **48:** 1067–1086.

Fu X, Palomar AJ, Hong EP, Schmitz FJ, Valeriote FA. 2004. Cytotoxic lissoclimide-type diterpenes from the molluscs *Pleurobranchus albiguttatus* and *Pleurobranchus forskalii*. *Journal of Natural Products* **67:** 1415–1418.

Garavoy JM, Valdés A, Gosliner TM. 2001. Phylogeny of the genus *Rostanga* (Mollusca, Nudibranchia). *Proceedings of the California Academy of Sciences* **56:** 213–272.

García FJ, Troncoso JS, Domínguez M. 2002. New data on the benthic opisthobranch molluscs from the Archipelago of Fernando de Noronha (Brazil), with description of a new species of *Aegires* Lovén, 1844. *Iberus* **20:** 45–56.

Göbbeler K, Klussmann-Kolb A. 2010. Out of Antarctica? New insights into the phylogeny and biogeography of the Pleurobranchomorpha (Mollusca, Gastropoda). *Molecular Phylogenetics and Evolution* **55:** 996–1007.

Gosliner TM. 1987. *Nudibranchs of Southern Africa*. Monterey: Sea Challengers.

Gosliner TM, Behrens DW. 1990. Special resemblance, aposematic coloration and mimicry in opisthobranch gastropods. In: Wicksten M, ed. *Symposium on the adaptive significance of color in invertebrates*. College Station: Texas A&M University Press, 127–138.

Gosliner TM, Behrens DW, Valdés A. 2008. *Indo-Pacific nudibranchs and sea slugs, a field guide to the world's most diverse fauna*. Gig Harbor: Sea Challengers; San Francisco: California Academy of Sciences.

Gosliner TM, Bertsch H. 1988. A review of the genus *Berthella* (Opisthobranchia: Notaspidea) from the Pacific coast of North America. *The Veliger* **31:** 46–67.

Gosliner TM, Johnson RF. 1999. Phylogeny of *Hypselodoris* (Nudibranchia: Chromodorididae) with a review of the monophyletic clade of Indo-Pacific species, including descriptions of twelve new species. *Zoological Journal of the Linnean Society* **125:** 1–114.

Harzhauser M, Kroh A, Mandic O, Piller WE, Göhlich U, Reuter M, Berning B. 2007. Biogeographic responses to geodynamics: a key study all around Oligo-Miocene Tethyan Seaway. *Zoologischer Anzeiger* **246:** 241–256.

Hedley C. 1894. On some naked Australian marine Mollusca, Part I. *Proceeding of the Linnean Society of New South Wales* **9:** 126–128.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755. doi: 10.1093/bioinformatics/17.8.754.

ICZN. 1999. *International code of zoological nomenclature*. London: International Trust for Zoological Nomenclature.

Jackson JBC, Jung P, Coates AG, Collins LS. 1993. Diversity and extinction of tropical America molluscs and emergence of the Isthmus of Panama. *Science* **260:** 1624–1626.

Jörger KM, Norenburg JL, Wilson NG, Schrödl M. 2012. Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. *BMC Evolutionary Biology* **12:** 245.

Kay AE. 1979. *Hawaiian marine shells: reef and shore fauna of Hawaii. Section 4, Mollusca*. Honolulu: Bishop Museum Press.

Krug PJ, Vendetti JE, Rodriguez AK, Retana JN, Hirano YM, Trowbridge CD. 2013. Integrative species delimitation in photosynthetic sea slugs reveals twenty candidate species in three nominal taxa studied for drug discovery, plastid symbiosis or biological control. *Molecular Phylogenetics and Evolution* **69:** 1101–1119.

Lavery S, Chan TY, Tam YK, Chu KH. 2004. Phylogenetic relationships and evolutionary history of the shrimp genus *Penaeus s.l.* derived from mitochondrial DNA. *Molecular Phylogenetics and Evolution* **31:** 39–49.

Lessios HA, Kessing BD, Pearse JS. 2001. Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution* **55:** 955–975.

Lessios HA, Kessing BD, Robertson RD. 1999. Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. *Evolution* **53:** 806–817.

Lesson RP. 1830. Mollusques, annelids et vers. In: Duperry LJ, ed. *Voyage autour du monde execute par ordre du Roi, sur la Corvette de S. M. La Coquille pendant les années 1822, 1823, 1824 et 1825, sous le ministrére et conformément aux instructions de S. E. M. Le Marquis de Clarmont-Tonnerre, Ministre de la Marine, Zoologie 2*. Paris: Bertrand.

Levinton J, Sturmbauer C, Christy J. 1996. Molecular data and biogeography: resolution of a controversy over evolutionary history of a pan-tropical group of invertebrates. *Journal of Experimental Marine Biology and Ecology* **203:** 117–131.

MacFarland FM. 1966. Studies of opisthobranchiate mollusks of the Pacific coast of North America. *Memoirs of the California Academy of Sciences* **6:** 1–546.

Macnae W. 1962. Notaspidean opisthobranchiate molluscs from Southern Africa. *Annals of the Natal Museum* **15:** 167–181.

Malaquias MAE, Calado GP, Padula V, Villani G, Cervera JL. 2009. Molluscan diversity in the North Atlantic Ocean: new records of opisthobranch gastropods from the Archipelago of the Azores. *Marine Biodiversity Records* **2:** e38.

Marcus E. 1965. Some Opisthobranchia from Micronesia. *Malacologia* **3:** 263–286.

Marcus E. 1971. Opisthobranchs from northern Brazil. *Bulletin of Marine Science* **20:** 922–951.

Marcus E. 1976. Marine euthyneura gastropods from Brazil (3). *Studies on Neotropical Fauna and the Environment* **11:** 5–23.

Marcus E. 1977. On a pleurobranchid from the Mediterranean. *The Veliger* **19:** 418–420. 1 pl.

Marcus E. 1984. The western Atlantic warm water Notaspidea (Gastropoda, Opisthobranchia), Part 2. *Boletim de Zoologia, Universidade São Paulo* **8:** 43–76.

Marcus E, Marcus E. 1962. Opisthobranchs from Florida and the Virgin Islands. *Bulletin of Marine Science of the Gulf and Caribbean* **12:** 450–488.

Marcus E, Marcus E. 1967. Tropical American opisthobranchs. *Studies in Tropical Oceanography* **6:** 1–137.

Marcus E, Marcus E. 1970. Opisthobranch mollusks from the southern tropical Pacific. *Pacific Science* **24:** 155–179.

Martynov AV, Schrödl M. 2009. The new Arctic side-gilled sea slug genus *Boreoberthella* (Gastropoda, Opisthobranchia): Pleurobranchoidean systematics and evolution revisited. *Polar Biology* **32:** 53–70.

Meckel JF. 1808. *Beyträge zur vergleichenden Anatomie. Volume 1*. Leipzig: Reclam.

Mickevich MF, Farris JS. 1981. The implications of congruence in Menidia. *Systematic Zoology* **30:** 351–370.

Mienis HK. 2014. Fourth addition to the catalogue of type specimens in the mollusc collection of the Tel Aviv University. (08/20/2014). Available at: http://www.mnh.tau.ac.il/en/?cmd=collections.1496

Montagu G. 1815. Descriptions of several new or rare animals, principally marine, discovered on the South Coast of Devonshire. *Transactions of the Linnean Society of London, Zoology* **11:** 1–26.

Mörch OAL. 1863. Contributions à la faune malacologique des Antilles Danoises. *Journal de Conchyliologie* **11:** 21–43.

Myers DS, Cummings MP. 2003. Necessity is the mother of invention: a simple grid computing system using commodity tools. *Journal of Parallel and Distributed Computing* **63:** 578–589.

Neves R, Cervera JL, Calado G. 2007. Redescription of the tropical West African Pleurobranchid *Pleurobranchus reticulatus* Rang, 1832 (Gastropoda: Opisthobranchia). *Journal of Conchology* **39:** 265–270.

Nylander JAA. 2004. *MrModeltest v. 2*. Evolutionary Biology Centre, Uppsala University. Available at: http://www.abc.se/~nylander/. Accessed 22 May 2008.

O'Dea A, Jackson JBC, Fortunato H, Smith JC, Croz LD, Johnson KG, Todd JA. 2007. Environmental change preceded Caribbean extinction by 2 million years. *Proceedings of the National Academy of Sciences, USA* **104:** 5501–5506.

Ornelas-Gatdula E, Camacho-García Y, Schrödl M, Padula V, Hooker Y, Gosliner TM, Valdés A. 2012. Molecular systematics of the '*Navanax aenigmaticus*' species complex (Mollusca, Cephalaspidea): coming full circle. *Zoologica Scripta* **41:** 374–385.

Ornelas-Gatdula E, Dupont A, Valdés A. 2011. The tail tells the tale: taxonomy and biogeography of some Atlantic *Chelidonura* (Gastropoda: Cephalaspidea: Aglajidae) inferred from nuclear and mitochondrial gene data. *Zoological Journal of the Linnean Society* **163:** 1077–1095. doi: 10.1111/j.1096-3642.2011.00749.x.

Padula V, Araújo AK, Matthews-Cascon H, Schrödl M. 2014a. Is the Mediterranean nudibranch *Cratena paregrina* (Gmelin, 1791) present on the Brazilian coast? Integrative species delimination and description of *Cratena minor* n. sp. *Journal of Molluscan Studies* **80:** 575–584. doi:10.1093/mollus/eyu052.

Padula V, Bahia J, Correira MD, Sovierzoski HH. 2012. New records of opisthobranchs (Mollusca: Gastropoda) from Alagoas, Northeastern Brazil. *Marine Biodiversity Records* **5:** e57. Available at: http://dx.doi.org/10.1017/S1755267212000346

Padula V, Valdés A. 2012. Phylogeny and biogeography of *Paradoris* (Nudibranchia, Discodorididae), with the description of a new species from the Caribbean Sea. *The Veliger* **51:** 165–176.

Padula V, Wirtz P, Schrödl M. 2014b. Heterobranch sea slugs (Mollusca: Gastropoda) from Ascension Island, South Atlantic Ocean. *Journal of the Marine Biological Association of the United Kingdom*. http://dx.doi.org/10.1017/S0025315414000575

Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular*

systematics. Sunderland: Sinauer & Associates Inc., 205–247.

Pease WH. 1860. Descriptions of new species of Mollusca from the Sandwich Islands. *Proceedings of the Zoological Society of London* **28:** 18–36.

Pease WH. 1861. Descriptions of new species of Mollusca from the Pacific Islands. *Proceedings of the Zoological Society of London* **29:** 242–247.

Pease WH. 1863. [Letter. On errors and omissions in former communications]. *Proceedings of the Zoological Society of London* **31:** 510.

Pease WH. 1868. Descriptions of marine Gasteropodae, inhabiting Polynesia. *American Journal of Conchology* **4:** 71–80.

Pilsbry HA. 1895. 1896. Philinidae, Gastropteridae, Aglajidae, Aplysiidae, Oxynoeidae, Runcinidae, Umbraculidae, Pleurobranchidae. In: Tyron GW. *Manual of Conchology* **16:** 1–252.

Pola M, Vallés Y, Cervera JL, Medina M, Gosliner TM. 2006. Taxonomic status of *Tambja abdere* and *Tambja fusca* based on morphological and molecular evidence, with comments on the phylogeny of the subfamily Nembrothinae (Nudibranchia, Polyceridae). *Annales Zoologici Fennici* **43:** 52–64.

Pruvot-Fol A. 1954. Mollusques Opisthobranches. *Faune de France* **58:** 1–460.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21:** 1864–1877.

Quoy JRC, Gaimard JP. 1832–1833. *Voyage de découvertes de l'Astrolabe exécuté par ordre du Roi, pendant les annees 1826-1827-1828-1829, sous le commandement de Dumont d'Urville MJ*. Zoologie, Mollusca 2: 1–686, Atlas pls. 1–26. Paris: J. Tastu.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/

Rang S. 1832. *Pleurobranchus reticulatus*. *Magasin de Zoologie* **2.**

Regier JC, Zwick A, Cummings MP, Kawahara AY, Cho S, Weller S, Roe A, Baixeras J, Brown JW, Parr C, Davis DR, Epstein M, Hallwachs W, Hausmann A, Janzen DH, Kitching IJ, Solis MA, Yen S-H, Bazinet AL, Mitter C. 2009. Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BMC Evolutionary Biology* **9:** 280.

Reid DG, Dyal P, Williams ST. 2010. Global diversification of mangrove fauna: a molecular phylogeny of *Littoraria* (Gastropoda: Littorinidae). *Molecular Phylogenetics and Evolution* **55:** 185–201.

Risbec J. 1951. Notes sur les Tectibranches de Nouvelle-Calédonie. *Journal de la Société des océanistes* **7:** 123–158.

Rochebrune ATde. 1895. Diagnose de mollusques nouveaux, provenant du voyage de M. Diguet en Basse-Californie. *Bulletin du Muséum National d'Histoire Naturelle, Paris* **1:** 239–243.

Rudman WB. 1993. *Pleurobranchus forskalii* Rüppell and Leuckart, [1828] and *P. testudinarius* Cantraine, 1835 (Mollusca, Gastropoda): proposed conservation of the specific names. *Bulletin of Zoological Nomenclature* **50:** 16–19.

Rudman WB. 1999a. *Pleurobranchus testudinarius* Cantraine, 1835. Available at: http://www.seaslugforum.net/factsheet/pleutest. Accessed 01 July 2014.

Rudman WB. 1999b. *Pleurobranchus peroni* Cuvier, 1804. Available at: http://www.seaslugforum.net/factsheet/pleupero. Accessed 01 July 2014.

Rudman WB. 2000. *Pleurobranchus garciagomezi* Cervera, Cattaneo-Vietti & Edmunds, 1996. Available at: http://www.seaslugforum.net/factsheet/pleugarc. Accessed 01 July 2014.

Rudman WB. 2001. *Pleurobranchus membranaceus* (Montagu, 1815). Available at: http://www.seaslugforum.net/factsheet/pleumemb. Accessed 01 July 2014.

Rudman WB. 2003. *Pleurobranchus hilli* (Hedley, 1894). Available at: http://www.seaslugforum.net/factsheet/pleuhill. Accessed 01 July 2014.

Rudman WB. 2008. Comment on Re: *Pleurobranchus* from Hurghada, Red Sea by Binyamin Koretz. Available at: http://www.seaslugforum.net/find/21359. Accessed 01 July 2014.

Rüppell E, Leuckart FS. 1828. *Atlas zu der Reise im nordlichen Afrika von Eduard Rüppell. Neue wirbellose thiere des Rothen Meeres*. Frankfurt: Brönner.

Sanderson MJ. 2003. R8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* **19:** 301–302.

Sukumaran J, Holder MT. 2010. DendroPy: a Python library for phylogenetic computing. *Bioinformatics* **26:** 1569–1571.

Swofford DL. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4*. Sunderland: Sinauer & Associates Inc.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24:** 1596–1599.

Thiele J. 1931. *Handbuch der systematischen Weichtierkunde,* 2nd edn. Stuttgart: G. Fischer, 377–788.

Thompson TE. 1969. Acid secretion in Pacific Ocean gastropods. *Australian Journal of Zoology* **17:** 755–764.

Thompson TE. 1970. Eastern Australian Pleurobranchomorpha (Gastropoda, Opisthobranchia. *Journal of Zoology, London* **160:** 173–198.

Thompson TE. 1976. *Biology of opisthobranch molluscs, 1*. London: The Ray Society.

Thompson TE. 1977. Jamaican opisthobranch molluscs I. *Journal of Molluscan Studies* **43:** 93–140.

Thompson TE. 1983. Detection of epithelial acid secretions in marine mollusks: review of techniques, and new analytical methods. *Comparative Biochemistry and Physiology* **74A:** 615–621.

Thompson TE, Slinn DJ. 1959. On the biology of the opisthobranch *Pleurobranchus membranaceus*. *Journal of the Marine Biological Association of the United Kingdom* **38:** 507–524.

Tullrot A. 1994. The evolution of unpalatability and warning coloration in soft-bodied marine invertebrates. *Evolution* **48:** 925–928.

Valdés A. 2001. Depth-related adaptations, speciation processes and evolution of color in the genus *Phyllidiopsis* (Mollusca: Nudibranchia). *Marine Biology* **139:** 485–496.

Valdés A. 2004. Phylogeography and phyloecology of dorid nudibranchs (Mollusca, Gastropoda). *Biological Journal of the Linnean Society* **83:** 551–559.

Valdés A, Gosliner TM, Ghiselin MT. 2010. Opisthobranchs. In: Leonard JL, Córdoba-Aguilar A, eds. *The evolution of primary sexual characters in animals*. New York: Oxford University Press, 148–172.

Valdés A, Hamann J, Behrens DW, DuPont A. 2006. *Caribbean sea slugs*. Gig Harbor: Sea Challengers.

Valdés A, Héros V. 1998. The types of Recent and certain fossil opisthobranch molluscs in the Muséum national d'Histoire naturelle, Paris. *Zoosystema* **20:** 695–742.

Valdés A, Ornelas-Gatdula E, Dupont A. 2013. Color pattern variation in a shallow-water species of opisthobranch mollusc. *Biological Bulletin* **224:** 35–46.

Vayssière A. 1896a. Description des coquilles de quelques espèces nouvelles ou peu connues de Pleurobranchidés. *Journal de Conchyliologie* **44:** 113–137. pls 4–5.

Vayssière A. 1896b. Description de deux especes nouvelles de Pleurobranchides. *Journal de Conchyliologie* **44:** 353–356.

Vayssière A. 1898. Monographie de la famille des Pleurobranchidés. *Annales des Sciences Naturelles, Zoologie et Paléontologie* **8:** 209–402. pls 13–28.

Vayssière A. 1901. Monographie de la famille des Pleurobranchidés (Deuxième et dernière partie). *Annales des Sciences Naturelles, Zoologie et Paléontologie* **12:** 1–85. pls. 1-6.

Vérany GB. 1846. *Catalogo degli animali invertebrati marini del Golfo di Genova e Nizza*. Genova: Tipografia Ferrando.

Verducci M, Foresi LM, Scott GH, Sprovieri M, Lirer F, Pelosi N. 2009. The Middle Miocene climatic transition in the Southern Ocean: evidence of paleoclimatic and hydrographic changes at Kerguelen plateau from planktonic foraminifers and stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology* **280:** 371–386.

Vermeij GJ, Petuch EJ. 1986. Differential extinction in tropical America molluscs: endemism, architecture and the Panama land bridge. *Malacologica* **27:** 29–41.

Vermeij GJ, Rosenberg G. 1993. Giving and receiving: the tropical Atlantic as a donor and recipient region for invading species. *American Malacological Bulletin* **10:** 181–194.

White KM. 1946. On three new species of Pleurobranchidae from Karachi. *Proceedings of the Malacological Society of London* **27:** 52–56.

White KM. 1948. On a collection of marine mollusks from Ceylon. *Proceedings of the Malacological Society of London* **27:** 199–205.

White KM. 1952. On a collection of molluscs from Dry Tortugas, Florida. *Proceedings of the Malacological Society of London* **29:** 106–120. pl. 6.

Willan RC. 1978. An evaluation of the notaspidean genera *Pleurobranchopsis* Verrill and *Gymnotoplax* Pilsbry (Opisthobranchia: Pleurobranchinae). *Journal of Conchology* **29:** 337–344.

Willan RC. 1984. The Pleurobranchidae (Opisthobranchia: Notaspidea) of the Marshall Islands, central-west Pacific ocean. *The Veliger* **27:** 37–53.

Willan RC. 1987. Phylogenetic systematics of the Notaspidea (Opisthobranchia) with reappraisal of families and genera. *American Malacological Bulletin* **5:** 215–241.

Willan RC, Bertsch H. 1987. Description of a new pleurobranch (Opisthobranchia: Notaspidea) from Antarctic waters, with a review of notaspideans from southern Polar Seas. *The Veliger* **29:** 292–301.

Xia X, Lemey P. 2009. Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme A-M, eds. *The phylogenetic handbook: a practical approach to DNA and protein phylogeny,* 2nd edn. Cambridge, MA: Cambridge University Press, 615–630.

Xia X, Xie Z. 2001. DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92:** 371–373. doi: 10.1093/jhered/92.4.371.

Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26:** 1–7. doi: 10.1016/S1055-7903(02)00326-3.

Yaron I, Schiøtte T, Wium-Andersen G. 1986. A review of molluscan taxa described by P. Forsskål and C. Niebuhr with citation of original descriptions, discussion of type-material available and selection of some lectotypes. *Steenstrupia* **12:** 157–203.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD dissertation, The University of Texas at Austin.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Bayesian consensus phylogenetic trees for *cytochrome c oxidase I* (*COI*), *histone 3* (*H3*), and *16S* for *Pleurobranchus*. Posterior probabilities and bootstrap values from the maximum likelihood analysis are presented for each clade above and below each branch, respectively. Robustly supported monophyletic groups are marked with an asterisk (*).

Appendix S2. 16S rDNA sequences shorter than 200 bp, not accepted by GenBank.