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Saved by the Shell: Molecular Analysis Detects the Cryptic Sea Hare, *Aplysia concava* G. B. Sowerby I, 1833 (Mollusca: Heterobranchia: Aplysiidae), from Oceania, with a Redescription

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Abstract: A recent taxonomic revision split the circumglobal sea hare *Aplysia parvula* into 10 constituent taxa, of which only three are likely to be found in the Southern Pacific. This prompted an investigation of animals previously identified as *A. parvula* from Australia. Specimens collected from Eastern Australia and Hunter Island, east of New Caledonia, could not be satisfactorily identified with any of the currently accepted taxa based on morphological diagnostic features listed in the revision; however, the presence of a highly concave shell is diagnostic. Quantification of genetic divergence using Cytochrome Oxidase I (COI) supports the delineation of this species as a distinct taxon, and a phylogenetic reconstruction based on concatenated COI, 16S and H3 markers reveals a sister relationship with the newly described *Aplysia ghanimii* from the Atlantic and Western Indian Oceans and an undescribed species from Japan. As a result, the name *Aplysia concava* G. B. Sowerby, I, 1833 is resurrected for this species. As the original description was based solely on a shell, a redescription is provided here with photographs of living animals and microscope images of internal anatomical structures.

Keywords: *Aplysia parvula*; species complex; integrated taxonomy

Citation: Nimbs, M.J.; Wilson, N.G. Saved by the Shell: Molecular Analysis Detects the Cryptic Sea Hare, *Aplysia concava* G. B. Sowerby I, 1833 (Mollusca: Heterobranchia: Aplysiidae), from Oceania, with a Redescription. *Taxonomy* **2021**, *1*, 48–59. https://doi.org/10.3390/taxonomy1020006

Academic Editor: Barbara Calcinai

Received: 13 March 2021
Accepted: 7 April 2021
Published: 9 April 2021

1. Introduction

Many sea slug species previously thought to have wide, almost circumglobal distributions have been revised based on contemporary species delimitation methods using molecular data (e.g., Valdés et al. [1]; Krug et al. [2]). In most cases, these taxa were found to be complexes of cryptic species with allopatric distributions. A recent revision of the putatively circumtropical *Aplysia parvula sensu lato* Mörch, 1863 by Golestani et al. [3] split this species into ten separate taxa in four distinct genetic lineages. In doing so, the name *Aplysia parvula sensu stricto* was retained for an Atlantic taxon, four names were resurrected for species from the Pacific and Indian Oceans, two species were described for the first time and two remain undescribed [3]. Some of these taxa are short-range endemics, e.g., *Aplysia elongata* (Pease, 1860), which is only known from the Hawaiian Islands, or *Aplysia japonica* G. B. Sowerby II, 1869 from Japan and the Korean Peninsula, whereas *Aplysia ghanimii* Golestani, Crocetta, Padula, Camacho, Langeneck, Poursanidis, Pola, Yokeş, Cervera, Jung, Gosliner, Araya, Hooker, Schrödl and Valdés, 2019 has a broad but disjunct range in the Atlantic and Western Indian Oceans [3].

Golestani et al. [3] provided a list of taxa inquirenda arising from their review of the literature. Many of the available names were based on descriptions of shells that, in most cases, are inadequate for use in contemporary identifications, a problem common among the sea hares [3], [4]. One exception, among the *Aplysia parvula s. l.*, is *Aplysia concava*

G. B. Sowerby I, 1833, redrawn later by von Clessin [5] and Pilsbry [6], from "Australia", which has a distinctive, deeply concave shell (Figure 1A–D). It was synonymised with *Aplysia parvula s. str.* in the 20th century [7]. *Aplysia concava* was not recognised amongst the samples studied by Golestani et al. [3]; however, this is probably the result of opportunistic geographic sampling. Indeed, samples from Australia or New Zealand were not included in their analysis, although material from nearby Papua New Guinea, Vanuatu and Indonesia was.

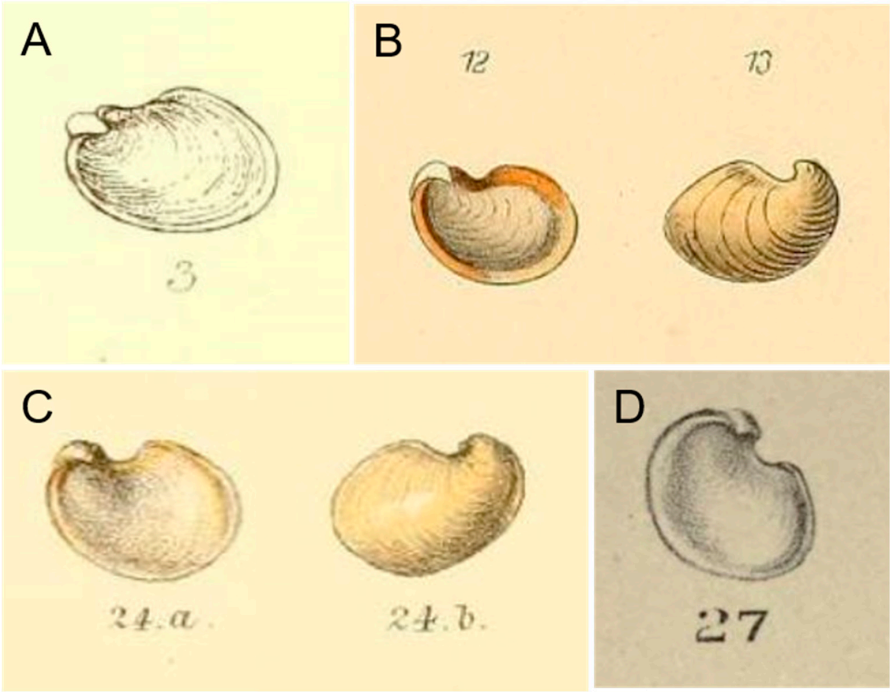

Figure 1. Historic illustrations of shells of Aplysia concava. (**A**) G. B. Sowerby I, 1833, the genera of recent and fossil shells for the use of students in conchology and geology, V. 2, Figure 3, pl. 235; (**B**) von Clessin, 1899, Die familie der Aplysiidae, in Systematisches Conchylien-Cabinet von Martini and Chemnitz, pl. 7, Figures 12 and 13; (**C**) Reeve, 1870, Conchologia Iconica, V. 17, pl. 6, Figure 24a,b; and (**D**) Tryon, 1895, Manual of Conchology, V. 16, pl. 43, Figure 27.

Eastern Australia, particularly the Tasman Sea, is a globally important hotspot for sea hare diversity [4], with 16 species reported from Central New South Wales (NSW) alone, including animals previously identified as *Aplysia parvula s. l.* Of the species described or re-erected by Golestani et al. [3], three are putatively found in the Southern Indo-Pacific: *A. atromarginata* Bergh, 1905, *A. nigrocincta* Von Martens, 1880 and an undescribed species, *Aplysia* sp. 2, in Golestani et al. [3]. Although defining characteristics (molecular, morphological and geographic) were provided for each of these species, some Eastern Australian animals cannot be satisfactorily identified as being any of these three species. Thus, in light of the cryptic diversity now confirmed to exist among *A. parvula s. l.*, it is probable that there remains undiscovered or previously unrecognised diversity among Australian animals. Nevertheless, some specimens of *Aplysia parvula s. l.* from Eastern Australia, while not able to be reconciled to any of the newly defined taxa, have distinctive, highly concave shells, resembling that illustrated for *Aplysia concava* G. B. Sowerby I, 1833. Although *A. concava* was reported from Cloudy Bay Lagoon, Bruni (sic) Island, Tasmania by Tenison-Woods [8], who described the shell as "small, horny, very concave and strongly incurved, sub-auriculate on both sides of the apex, not very common" there have been few references to this species in the literature since.

The aim of this study is to examine and redescribe *Aplysia concava* specimens using modern imaging techniques and to explore its phylogenetic relationship with other species

among *Aplysia parvula s. l.* using data from partial Cytochrome Oxidase I (COI), 16S ribosomal DNA (16S) and Histone 3 (H3) gene sequences. Several specimens exhibiting the species' characteristic shell were sampled from Eastern Australia, along with museum specimens identified using molecular analysis. The present paper provides clarification of the work of G. B. Sowerby I by including details not provided in the original description.

2. Experimental Section

2.1. Source of Material

Eleven specimens from Eastern Australian and one from Hunter Island (east of New Caledonia) were used in this study. These comprised tissue from seven specimens sourced from the collection of the Australian Museum, Sydney (AMS), and four whole specimens collected from various locations in northern NSW by MN (Table 1; Figure 6).

2.2. Molecular Study

DNA was extracted from ~10 mg of foot tissue from each ethanol-preserved specimen using a Qiagen DNeasy 96 Blood and Tissue DNA extraction kit and the supplied protocol. Partial COI sequences were amplified using degenerate primers [9] (jgLCO1490 5′-TITCIACIAAYCAYAARGAYATTGG-3′, jgHCO2198 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′). Universal primers were used to amplify 16S rDNA [10] (16SarL 5′-CGCCTGTTTAACAAAAACAT-3′, 16SbrH 5′-CCGGTCTGAACTCAGATCACGT-3′); and universal primers were used to amplify H3 sequences [11] (H3AF 5′-ATGGCTCGTACCAAGCAGACVGC-3′. H3AR 5′-ATATCCTTRGGCATRATRGTGAC-3′). For each of these, a master mix was prepared using 5.0 µL of MyTaq X 5 PCR buffer, 0.2 µL MyTaq, 0.8 µL primer 1, 0.8 µL primer 2, 16.8 µL molecular-grade H_2O and 1.5 µL of extracted DNA. Polymerase chain reaction (PCR) cycling was carried out using a Bio-Rad T100 thermal cycler. Thermo cycler conditions for PCR amplification of the COI marker were as follows: initial denaturation at 95 °C for 3 min, 8 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 45 s with another 32 cycles of 95 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 45 s. Finally, elongation was carried out at 72 °C for 5 min. For the 16S and H3 markers, PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 35 cycles of further denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 45 s, with a final elongation at 72 °C for 10 min. Visualisation of PCR products was carried out using an Invitrogen E-gel 96, 2% agarose plate to determine the approximate base pair lengths (bp) of COI (658 bp), 16S (422 bp) and H3 (328 bp). PCR products were sequenced at the Australian Genomic Research Facility (AGRF), Perth, WA.

Sequences were de novo assembled using Geneious 11.1.5 [12] and edited by eye. Consensus sequences were then exported and combined with additional sequence data retrieved from GenBank. This includes data from Golestani et al. [3], where COI, 16S and H3 sequences were available for each specimen, and a COI sequence listed as *A. parvula* but identified using BLAST as a match for *Aplysia atromarginata* from French Polynesia (Table 2). Sequences were aligned using the MAFFT plugin [13] using default settings and primers were trimmed from the alignment. Data quality checks were carried out with MEGABLAST [14] and protein translation for COI. An alignment of 16S sequences was uploaded to the online version of GBlocks [15] and processed using relaxed settings to remove ambiguous regions (i.e., containing gaps and/or poorly aligned) [16]. The gene alignments of COI, gblocked 16S and H3 were then concatenated using Geneious.

A phylogenetic tree was produced using W-IQ-Tree [17,18] using default settings and partitioned by gene [19]. W-IQ-Tree incorporates ModelTest [20], which selected HKY + F + G4 as the best-fit model for the first partition, which is scored according to the Bayesian information criterion (BIC) and automatically applied. Trees were visualised using FigTree 1.4.4. *Dolabella auricularia* (Lightfoot, 1786) was used to root trees for analysis as it is sister to *Aplysia* [3,21].

Table 1. Details of specimens used in this study.

Voucher	Locality	Date	Collected by	Length (mm)	GenBank Accession		
					COI	16S	H3
AMS C.481313	Smoky Cape, NSW, Australia	30 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	9	-	-	-
AMS C.481398	Hastings Point, NSW, Australia	27 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	7	MT107263	MT108945	MW854246
AMS C.481399	Hastings Point, NSW, Australia	27 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	19	MT107259	MT108948	MW854247
AMS C.481400	Hastings Point, NSW, Australia	27 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	16	MT107267	MT108950	MW854248
AMS C.481462	Tweed Heads, NSW, Australia	28 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	7	MT107262	MT108947	MW854249
AMS C.481573	Tweed Heads, NSW, Australia	28 February 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	12	MT107265	MT108951	MW854250
AMS C.481686	Kingscliff, NSW, Australia	03 March 2014	J.H. Waterhouse, A.C. Miller, F. Noss, D. Holmes	14	MT107264	MT108944	MW854251
AMS C.546630	Lord Howe Island, NSW, Australia	04 April 2017	K. Layton, A. Reid	9	MT107268	MT108943	MW854252
AMS C.572080	Hunter Island, e. of New Caledonia	04 August 2017	S. Hannam, A. Reid, E.K. Kupriyanova, L. Vogel, I. Middleton	16	MT107261	MT108949	MW854253
AMS C.574818	Sandy Beach, NSW, Australia	04 March 2016	S. D. A. Smith	7	MT107260	MT108946	MW854254
AMS C.574821	Sandy Beach, NSW, Australia	06 April 2016	M. Nimbs	8	MT107266	MT108953	MW854255
AMS C.574820	Newcastle, NSW, Australia	14 December 2016	S. D. A. Smith	14	-	-	-
AMS C.574826	Sandy Beach, NSW, Australia	08 January 2019	M. Nimbs	7	MT107269	MT108942	MW854256
WAM S.29698	Korff's Islet, Coffs Harbour, NSW, Australia	24 May 2019	M. Nimbs	12	-	-	-

Table 2. Details of sequence data retrieved from GenBank used in this study.

Species	Locality	Date	Voucher	GenBank Accession Number		
				COI	16S	H3
Aplysia atromarginata	Moorea, French Polynesia	-	-	KJ522466	-	-
Aplysia atromarginata	Madang, Papua New Guinea	06 December 2012	CPIC 00821	MK422836	MK422738	MK422627
Aplysia elongata	Maliko Bay, Maui, Hawaii, USA	16 January 2011	CPIC 00333	MK422876	MK422773	MK422669
Aplysia elongata	Maliko Bay, Maui, Hawaii, USA	18 June 2011	CPIC 00363	MK422875	MK422772	MK422668
Aplysia ghanimii	Florida, USA	14 April 2013	CPIC 01375	MK422853	MK422752	MK422645
Aplysia ghanimii	Mozambique		CPIC 01384	MK422856	MK422755	MK422648
Aplysia ghanimii	Port d'Ehoala, Madagascar	15 May 2010	CPIC 01370	MK422857	MK422756	MK422649
Aplysia ghanimii	Port d'Ehoala, Madagascar	May 2010	CPIC 01372	MK422858	MK422757	MK422650
Aplysia ghanimii	Rio de Janeiro, Brazil	29 November 2002	ZSM Mol 20040138	MK422863	MK422761	MK422655
Aplysia ghanimii	Stocking Island, Bahamas	19 January 2007	CPIC 00039	MK422848	MK422750	MK422639
Aplysia ghanimii	Stocking Island, Bahamas	19 January 2007	CPIC 00041	MK422847	MK422749	MK422638
Aplysia hooveri	Baja California, Mexico	01 September 2015	CPIC 01845	MK422878	MK422777	MK422673
Aplysia hooveri	Baja California, Mexico	01 September 2015	CPIC 01851	MK422877	MK422776	MK422672
Aplysia japonica,	Gyeongsangbuk-do, Korea	24 August 2011	CPIC 02175	MK422869	MK422764	MK422661
Aplysia japonica	Hokkaido, Japan	12 September 2014	CPIC 01849	MK422867	MK422763	MK422659
Aplysia japonica	Jeju-do, Korea	28 April 2013	CPIC 02176	MK422870	MK422765	MK422662
Aplysia nigrocincta	Latang, Indonesia	11 March 2014	CPIC 01415	MK422888	MK422788	MK422687
Aplysia nigrocincta	Latang, Indonesia	11 March 2014	CPIC 01416	MK422889	MK422789	MK422688
Aplysia nigrocincta	Mozambique		CPIC 01380	MK422885	MK422785	MK422684
Aplysia nigrocincta	Mozambique		CPIC 01382	MK422886	MK422786	MK422685
Aplysia parvula	St. John, U.S. Virgin Is.	15 April 2006	CPIC 00140	MK422834	MK422735	MK422625
Aplysia parvula	St. Peter and St. Paul Is., Brazil	03 May 2011	MZSP 104103	MK422835	MK422737	MK422626
Aplysia sp. 1	Buoys Hole, St. Helena	02 October 2013	ZSM Mol 20190050	MK422838	MK422740	MK422629
Aplysia sp. 1	Lots Wife Ponds, St. Helena	22 January 2014	ZSM Mol 20180052	MK422840	MK422742	MK422631
Aplysia sp. 1	Lots Wife Ponds, St. Helena	22 January 2014	ZSM Mol 20180053	MK422841	MK422743	MK422632
Aplysia sp. 1	Manati Bay, St. Helena	14 October 2013	ZSM Mol 20180051	MK422839	MK422741	MK422630
Aplysia sp. 2	Okinawa, Japan	15 March 1987	HG99	MK422864	MK422762	MK422656
Dolabella auricularia	Napili Bay, Maui, Hawaii, USA	15 June 2011	CPIC 00327	MF669619	MF669573	MF669657

Species hypotheses were also explored using COI sequence data. Within- and between-species mean distances were calculated using MEGA V7.0.26 [22] using the Tamura–Nei substitution model [23]. The Automatic Barcode Gap Discovery (ABGD) algorithm was used to examine the potential for new species [24]. This employs a distance-based analysis of the distribution of pairwise distances in the alignment of barcode COI sequences. As recommended by Puillandre et al. [24], a pairwise distance matrix was developed in MEGA 7, which also selects a best-fit evolutionary model, and this matrix was uploaded to the ABGD webserver (without the outgroup) and processed using the default settings. Haplotype networks were constructed using the TCS network algorithm [25] in PopART [26] software.

2.3. Morphological Study

Animals were photographed alive either *in situ* or in aquaria. Preserved specimens were dissected by a ventral or right lateral incision just above the margin of the foot. Internal anatomy was examined using a Leica Zoom 2000 dissecting microscope. The buccal mass was removed and placed in 10% NaOH for four hours to separate the radula. The radula, penis and shell were isolated from the tissues, rinsed in seawater, critical point dried using a graded ethanol series and mounted for imagery using a Hitachi TM4000plus desktop scanning electron microscope (SEM). The reproductive system was examined and photographed using an Olympus S2 × 7 binocular microscope with an Olympus DP26 overhead-mounted digital camera. Field-collected specimens have been deposited with the Australian Museum, Sydney (AMS) and Western Australian Museum (WAM).

3. Results

3.1. Species Delimitation

PCR amplification and sequencing yielded 658bp of COI, 432 bp of 16S (sequence length reduced to 359 bp after gblock cleaning) and 327 bp of H3. MegaBLAST searches of

the NCBI database identified best matches for all sequences to *Aplysia ghanimii*. ABGD analysis returned nine groups comprising the following taxa: 1. *Aplysia ghanimii*; 2. *Aplysia* sp. from Japan; 3. *Aplysia* sp. from St. Helena; 4. *Aplysia parvula* + *Aplysia atromarginata*; 5. *Aplysia japonica*; 6. *Aplysia nigrocincta;* 7. *Aplysia hooveri*; 8. *Aplysia elongata* and 9. *Aplysia concava*.

Interspecific genetic distances, based on the Tamura–Nei model, delimited *Aplysia concava* as a distinct taxon from all other known species in the *Aplysia parvula* complex with a divergence of 2%, whereas the minimum intraspecific divergence values were >4% for all other *Aplysia* used in this study (Table 3). Delimitation was further supported by a phylogenetic reconstruction based on maximum likelihood using concatenated COI, 16S and H3 markers, which recovered *Aplysia concava* as a maximally supported, monophyletic clade (approximate Bayes/ultrafast bootstrap value = 1/100), sister to *Aplysia ghanimii* + *Aplysia* sp., which comprise Golestani et al. [3] Species Complex 2 (Figure 2).

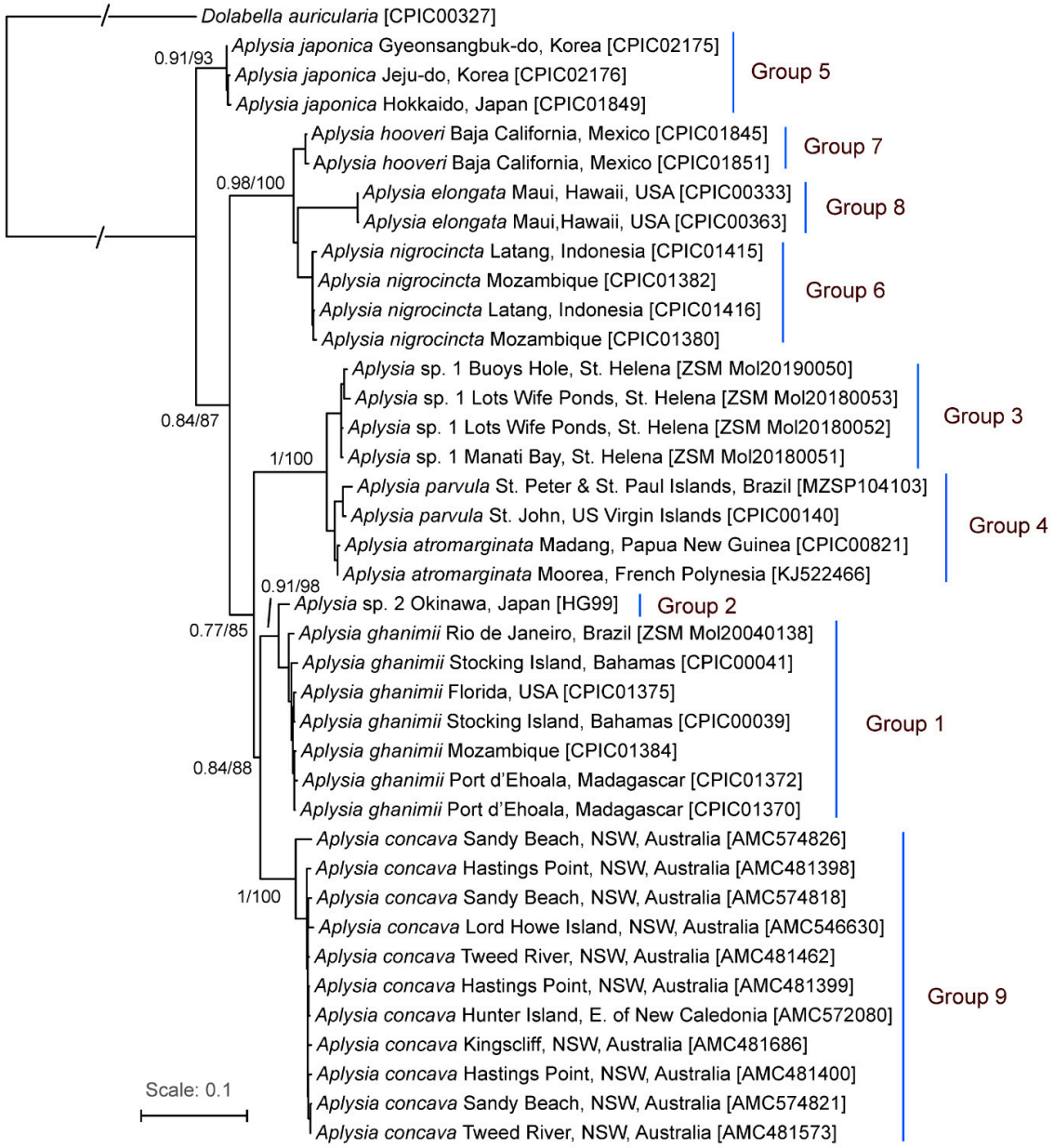

Figure 2. Maximum likelihood tree of *Aplysia parvula s. l.* rooted with *Dolabella auricularia* based on concatenated COI, 16S and H3 markers. Numbers adjacent to branches are approximate Bayes/ultrafast bootstrap support values. Blue bars represent groups identified by ABGD analysis.

Table 3. Maximum intraspecific and minimum interspecific genetic distances calculated using the Tamura–Nei substitution model. Intraspecific distances in bold.

	***Aplysia* sp. 2**	***Aplysia* sp. 1**	***A. parvula***	***A. nigrocincta***	***A. japonica***	***A. hooveri***	***A. ghanimii***	***A. elongata***	***A. concava***	***A. atromarginata***
Aplysia sp. 2	**0.03**									
Aplysia sp. 1	0.11	**0.01**								
A. parvula	0.12	0.06	**0.02**							
A. nigrocincta	0.13	0.14	0.12	**0.01**						
A. japonica	0.12	0.13	0.14	0.12	**0.01**					
A. hooveri	0.12	0.13	0.12	0.05	0.11	**0.01**				
A. ghanimii	0.04	0.09	0.11	0.11	0.11	0.11	**0.01**			
A. elongata	0.13	0.11	0.11	0.12	0.12	0.08	0.12	**0.00**		
A. concava	0.09	0.12	0.12	0.11	0.13	0.10	0.12	0.14	**0.02**	
A. atromarginata	0.11	0.04	0.03	0.12	0.13	0.10	0.10	0.10	0.12	**0.01**

A haplotype network reconstructed using COI sequences for *A. concava* does not show marked geographic structure. Specimens from oceanic islands (Hunter Island, east of New Caledonia and Lord Howe Island, in the Tasman Sea) were separated from other, mainland haplotypes by three and four substitutions, respectively; however, this level of divergence is also present among other mainland Eastern Australian specimens (Figure 3).

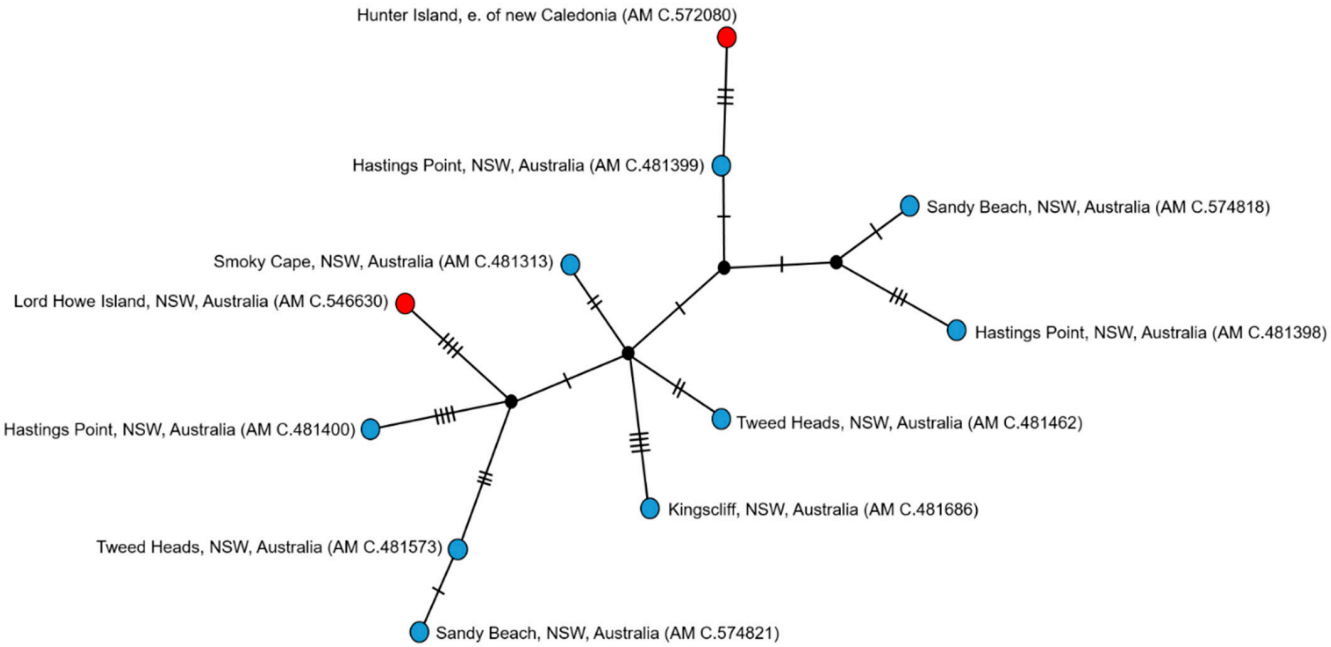

Figure 3. TCS haplotype network based on COI sequence data for the *Aplysia concava* clade. Each circle represents one haplotype. Red circles = Oceanic Island, Blue circles = Mainland Eastern Australia.

SYSTEMATICS

CLASS Gastropoda Cuvier, 1795.

ORDER Aplysiida.

FAMILY Aplysiidae Lamarck, 1809.

GENUS *Aplysia* Linnaeaus, 1767.

SUBGENUS *Pruvotaplysia* Engel, 1936.

Aplysia concava G. B. Sowerby, I, 1833.

(Figures 4 and 5).

Aplysia concava G. B. Sowerby I, 1843: v2, 243, pl. 235, Figure 3. Type locality by subsequent restriction: none given. Type: probably lost, not found at the Natural History Museum, London [3], or in collections at Australian Museums (pers. comm. I. Loch).

—Sowerby II, 1869: v. 17, pl. 6, Figure 24a,b. Locality: 'Australia'.

—Pilsbry, 1895: v.16, pl. 43, Figure 27.

—von Clessin, 1899: 18, pl. 7, Figures 12 and 13.

3.2. Material Examined

Oceania, Hunter Island, east of New Caledonia, 1 spc (sequenced), AMS C,572080, L = 16 mm; Australia, Lord Howe Island, NSW, 1 spc (photographed, sequenced), AMS C.546630, L = 16 mm; Australia, Newcastle, NSW, 1 spc (dissected), AMS C.574820, L = 14 mm; Australia, Sandy Beach, NSW, 3 spcs (3 dissected, 3 sequenced), AMS C.574826 L = 7 mm, AMS C.574821 L = 8 mm AMS C.574818 L = 7 mm; Australia, Korff's Islet, Coffs Harbour, NSW, 1 spc (dissected), WAM S.296298, L = 12 mm; Australia, Smoky Cape, NSW, 1 spc (dissected), AMS C.481313, L = 9 mm; Australia, Hastings Point, NSW, 3 spcs (3 sequenced), AMS C.481398-481400, L = 7–19 mm; Australia, Tweed Heads, NSW, 2 spcs (2 sequenced), AMS C.481462, 481573, L = 7–12 mm; Australia, Kingscliff, NSW, 1 spc (sequenced), AMS C.481686, L = 14 mm.

3.3. Description

3.3.1. External Morphology

Body elongated, with a distinct head and neck. Body widest at visceral region, tapering towards tail and narrowing slightly towards the head and neck. Paired, rolled rhinophores with tapered tips arise on top of the head, posterior to the eye spots. Paired, rolled cephalic tentacles project anterolaterally and may flare along their length, forming a flattened, posteriorly projecting flap. Parapodia do not meet anteriorly, fused high posteriorly, form a fold at their mid-length and do not meet above the mantle. Mantle foramen large, circular, not raised, opening on left side of mantle. Shell visible through foramen. In some, the top of the siphon is level with, in others, it projects above, the parapodia.

3.3.2. Colour Variation

Aplysia concava is highly variable in colour, ranging from dark red to light brown to cream (Figure 4). There is a very fine white parapodial margin and a black submarginal band, which may be discontinuous in light brown to cream animals (Figure 4B–E). The black band may be broad in dark red animals and the marginal band, grey (Figure 4A,F). The foot margins, tips of the cephalic tentacles and rhinophores also exhibit a black margin that may also be discontinuous in some specimens (Figure 4B–E). The mantle foramen has a black margin in dark red animals; however, this is absent in light brown and cream animals. Fine white specks may cluster to form irregular spots all over the body; however, these are absent in dark red animals. The base of the siphon is opaque white in light brown animals.

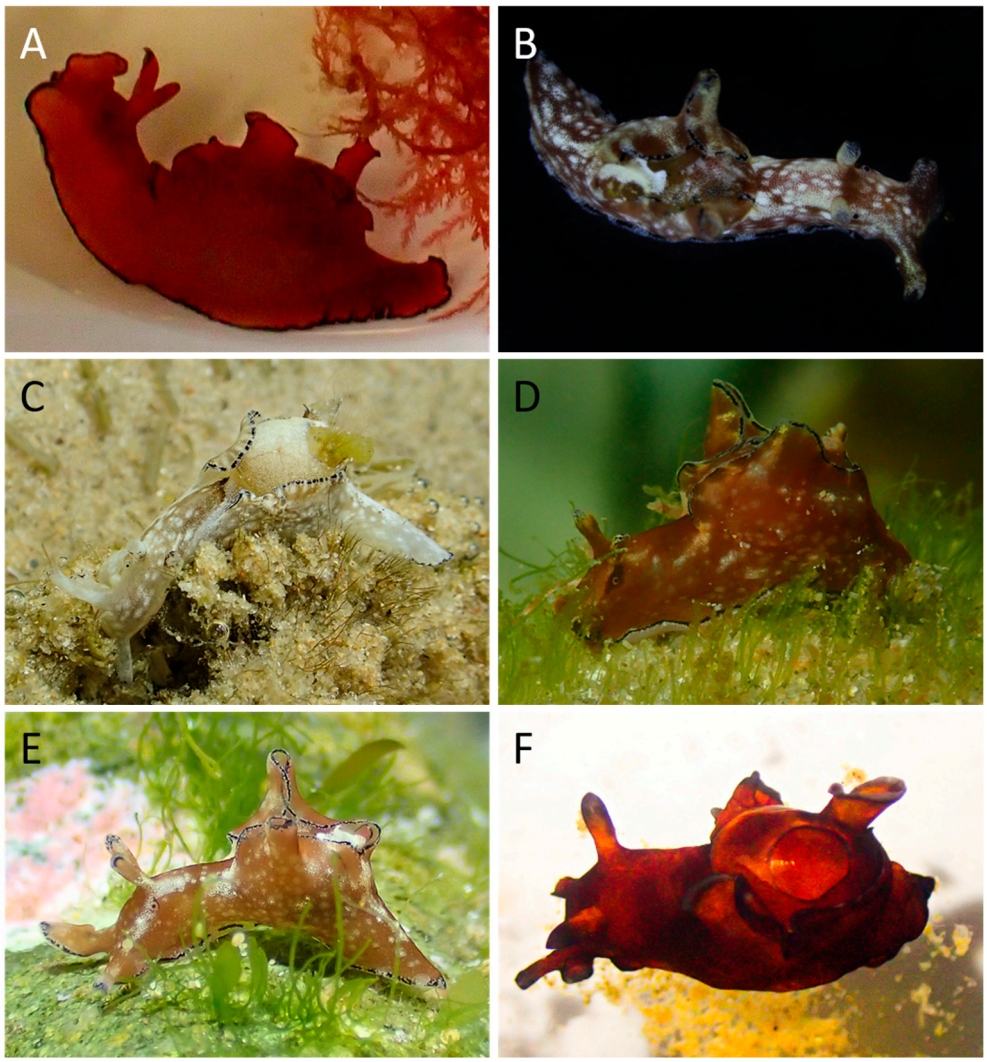

Figure 4. Some of the living animals used in this study: *Aplysia concava*. (**A**) AMS C.572080, Hunter Island, e. of New Caledonia; (**B**) AMS C.546630, Lord Howe Island, NSW; (**C**) AMS C.574820, Newcastle, NSW; (**D**) AMS C.574826, Sandy Beach, NSW; (**E**) AMS C.574821, Sandy Beach, NSW; (**F**) WAM S.29698, Korffs Islet, Coffs Harbour, NSW. Photos: A, B, Australian Museum; C, D, E, F Matt Nimbs.

3.3.3. Internal Morphology

The ovate-auriculate shell is characteristically concave (Figure 5I–K) and the protoconch is highly inflexed so as to be perpendicular to the rest of the shell (Figure 5I) and is consistent with that illustrated for *A. concava* by earlier authors (Figure 1).

Radula ribbon longer than wide (Figure 5E). Radular formula 27 × 2.8.1.8.2 for a 12 mm preserved length specimen from Coffs Harbour, NSW (WAM S.29698) and 19 × 3.6.1.6.3 for an 8 mm preserved specimen from Sandy Beach, NSW (AMS C.574821). Rachidian tooth bilaterally symmetrical with large, serrate central cusp and two smaller, serrate lateral cusps each side (Figure 5A,F). Inner lateral teeth asymmetrical with large, serrate cusp subtended by two smaller cusps distally (Figure 5B). Outer lateral teeth similar, but larger (Figure 5C). Three underdeveloped vestigial teeth lie outside functional outer laterals (Figure 5E). Jaw elements comprise numerous flattened rodlets with stoutly digitate tips (Figure 5G). Penis short, simple, unarmed, with broad, rounded tip. Seminal groove wide, shallow (Figure 5D,H,L).

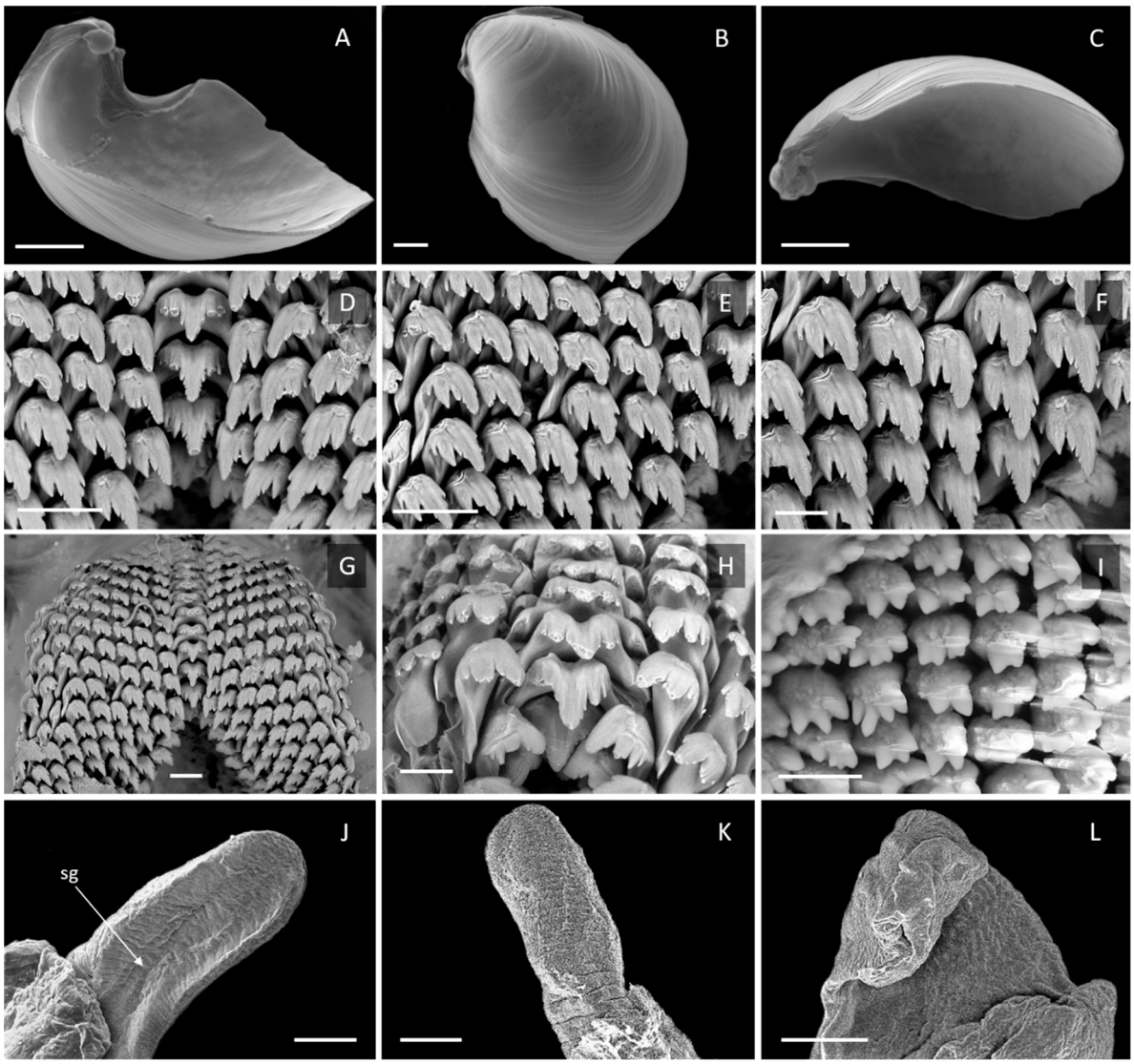

Figure 5. Scanning electron microscope images of *Aplysia concava*: shell **(A)** ventral, (**B**) dorsal, (**C**) lateral view (WAM S.296298); (**D**) rachidian and first three lateral teeth, (**E**) inner laterals, (**F**) outer laterals (WAM S.296298); (**G**) entire anterior radula, (**H**) rachidian and first three lateral teeth, (**I**) tips of jaw rodlets (AMS C.574821); (**J**) penis tip, ventral (sg = sperm groove), (**K**) penis tip, dorsal (WAM S.296298); and (**L**) penis tip (AMS C.574821). Scale bars: a, b, c = 1 mm; d, e, f, g, h, j, k-100 μm; i = 10 μm; l = 150 μm.

4. Discussion

4.1. Diagnosis

Aplysia concava can be distinguished from other *Aplysia* by the presence of a solid or broken black marginal band on the foot, parapodia, rhinophores, cephalic tentacles and by its diminutive adult size. It may be separated from other Southwestern Pacific *Aplysia parvula s. l.* by the presence of a distinctively concave shell, and, depending on the activity of the living animal, the shell may cause the visceral mass to appear swollen (Figure 4C). The parapodia are not able to cover the mantle. In brown specimens, there is an opaque white patch at the base of the siphon (Figure 4B); however, this is absent in dark red specimens. The mantle aperture is large, circular and its margins are not raised.

Although it shares most of the external morphological features of the sister taxa in Golestani et al. [3] Species Complex 2, *A. concava* is allopatric in its distribution. *Aplysia ghanimii* is found in the Atlantic and Western Indian Oceans and *Aplysia* sp. 2 is so far only known in Japan. It can be separated from the potentially sympatric *A. parvula s. l.*

species *A. nigrocincta*, which has characteristic white or light blue parapodial margins without submarginal black banding, and *A. atromarginata*, which exhibits a small, raised, "volcano-like" mantle aperture with dark margins and radiating white lines.

4.2. Range

Aplysia concava has a confirmed range in the Southwestern Pacific, extending south from New Caledonia to Eastern Australia (from the Queensland and NSW border south to the NSW mid-north coast) and east into the Tasman Sea. Its range may include the northern part of New Zealand (MN pers. obs) and, according to Tennyson Woods [8], it extends as far south as Southern Tasmania (Figure 6).

Figure 6. Map of Southern Oceania showing observation and sampling locations of *Aplysia concava* used in this study.

Author Contributions: M.J.N. and N.G.W. conceived and designed the research, contributed material and biological data, conducted molecular work, analysed data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was undertaken as part of a project (MN) titled "Taxonomy and systematics of the Australian Aplysiidae (Mollusca: Heterobranchia)", with funding from the Australian Government's Australian Biological Resources Study National Taxonomy Research Grant Program.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nuccore (accessed on 9 April 2021).

Acknowledgments: Gratitude is extended to Mandy Reid of the Australian Museum, Sydney (AMS) and staff of the Western Australian Museum for assistance, particularly Mia Hillyer for sequencing assistance, Maxine Dawes from Southern Cross University (SCU) for help with SEM, Kirsten Benkendorff, Steve Smith and the SCU Marine Ecology Research Centre and Bob Burn from Museum Victoria. Specimens from NSW were collected under permit issued by NSW DPI (P14/0014-1.1). The authors gratefully acknowledge three reviewers who provided valuable feedback on this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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