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Here be dragons – phylogeography of *Pteraeolidia ianthina* (Angas, 1864) reveals multiple species of photosynthetic nudibranchs (Aeolidina: Nudibranchia)

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The aeolid *Pteraeolidia ianthina* (Angas, 1864) is a strikingly-coloured aeolid nudibranch, informally known as the 'Blue Dragon'. It is recognised as an unusually widespread Indo-Pacific species, with variation in colouration and morphology, and biogeographic differences in zooxanthellae (dinoflagellate symbionts of the genus *Symbiodinium*). This variation hints at possible cryptic species, which was tested here using phylogenetic analyses of mitochondrial DNA data (COI, 16S). Our results showed multiple well-supported clades with slight but consistent differences in radular morphology and colouration, and thus we clarify one of the three available names. A temperate NSW clade showed a more elongate and pointed central radular tooth and lacked white body colouration, in comparison to a more variable tropical clade, which had a shorter and more blunt central tooth. The type locality of *Pteraeolidia ianthina* is Sydney Harbour, New South Wales (NSW), Australia, and according to our study, does not occur outside NSW. *Pteraeolidia semperi* (Bergh, 1870) and *P. scolopendrella* (Risbec, 1928) are removed from synonymy with *P. ianthina*. Wider phylogeographic sampling is required before resolving the availability of the two remaining names, and subclades within the tropical clade, but there is evidence to suggest multiple cryptic species exist. The biogeographic differences in symbionts, and the importance of their role in life history, suggests that changes in symbiosis may have helped drive divergence via local adaptation in the host nudibranchs.

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ADDITIONAL KEYWORDS: aeolid – cryptic species – delimitation – symbiosis – zooxanthellae.

INTRODUCTION

The identification of cryptic species has increased dramatically with the use of DNA sequence data (Knowlton, 1993; Hebert, Cywinska & Ball, 2003; Ratnasingham & Hebert, 2007). It is widely accepted that genetic data continue to reveal diversity not predicted by traditional morphological characters (Bickford *et al*., 2007). Molecular systematics provides a framework with which to test this diversity, independent of phenotypic, ontogenetic, or ecological differences. Knowlton's (1993) review of marine cryptic species demonstrated that most cryptic species show some differentiation of habitat use or life history characteristics. Thus, any widespread species with variable morphology or ecological differences should be treated with a degree of caution until it has been examined in a molecular systematic framework.

The aeolid nudibranch *Pteraeolidia ianthina* (Angas, 1864), informally known as the 'Blue Dragon', is a widespread and variable species, occurring throughout the Indo-Pacific, from tropical through to temperate waters. The distribution of *P. ianthina* occurs from the south-western Indian Ocean off South Africa, through the Red Sea, across the Coral Triangle of the Indo-West Pacific, to the Hawaiian Islands in the East, Japan in the North, and southerly to eastern Australia (type locality Sydney Harbour). *Pteraeolidia ianthina* is one

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of the few species with a highly efficient symbiosis with zooxanthellae (dinoflagellates in *Symbiodinium*). This discovery was first detailed in Rudman (1982) and further investigations have demonstrated that its symbionts photosynthesize, respire and multiply *in situ* (Hoegh-Guldberg & Hinde, 1986; Hoegh-Guldberg, Hinde & Muscatine, 1986). *Pteraeolidia ianthina* is thought to obtain its symbionts from cnidarian prey (Hadfield, 1976; Gosliner, 1980; Kempf, 1984; Willan, 1989), and can survive for extended periods of time by relying on the photosynthetic products of its symbionts (Kempf, 1984; Burghardt, Stemmer & Wägele, 2008). Importantly, *Pteraeolidia* appears to show distinct symbiont assemblages concordant with biogeographical boundaries (Loh, Cowlishaw & Wilson, 2006).

Morphologically, *Pteraeolidia ianthina* shows high variability of characters such as body colour pattern, as well as arrangement and length of dorsal cerata. Burn (1989) noted that individuals that shared colouration in tropical waters in eastern Australia and South Australia (the latter receives westward tropical currents for part of the year), looked different to animals from temperate New South Wales. The most recent revision (Rudman, 1982) recognizes *P. ianthina* (Angas, 1864) as valid, with *P. semperi* (Bergh, 1870) and *P. scolopendrella* (Risbec, 1928) as synonyms. Yorifuji *et al*. (2012) employed a molecular approach to show there were at least two groups that might represent different species. All of their samples were from the northwest Pacific, so they were unable to resolve the status of any group with *Pteraeolidia ianthina*, the only name in common use at present.

The genetic diversity of *Pteraeolidia* symbionts has already been investigated in some areas (Ishikura *et al*., 2004; Loh *et al*., 2006; Yorifuji *et al*., 2012). *Pteraeolidia* appears to host a very high diversity of clades of *Symbiodinium* compared to most single species of reef-building corals, forams, anemones or zoanthids (Baker, 2003). One study showed latitudinal changes in symbiont identity, with temperate, subtropical and tropical animals hosting different symbiont assemblages (Loh *et al*., 2006). The clades of *Symbiodinium* known from *Pteraeolidia* possibly reflect different ecotypes that are adapted to different environmental conditions, and thus *Pteraeolidia* may provide an important model system to contrast with coral-zooxanthellae symbioses.

The combination of high variability of morphological characters, the wide distribution and known ecological differences in *Pteraeolidia ianthina* are suggestive of a cryptic species complex, rather than a single species. Alternatively, the polymorphic traits known throughout its range may be due to phenotypic plasticity, perhaps influenced by the retention of diverse symbionts. Previous cryptic species complexes in Nudibranchia have been successfully revealed with mitochondrial markers (e.g. Wilson, Schrödl & Halanych, 2009; Pola, Camacho-Garcia & Gosliner, 2012), and we apply a similar approach here, integrated with morphology, to elucidate the diversity and phylogeography of the taxon *Pteraeolidia*. The acquisition of a photosynthetic system for nutrition is thought to be a key characteristic driving evolutionary radiations in heterobranch slugs; 'solar-powered' sea slugs appear to have diversified much more than their non-symbiotic relatives (Wägele, 2004; Wägele *et al*., 2010). Thus, *Pteraeolidia* might be expected to host cryptic species; its current low diversity status, based on morphological characters, provides significant opposition to that hypothesis. This is of particular importance if *Pteraeolidia* is to be utilized as an alternative host model system to Cnidaria, to investigate the interaction of host and symbiont evolution in metazoan-zooxanthellae symbioses.

METHODS

Forty-two animals were collected from 12 localities throughout the Indo-Pacific during 2002–2011 (Table 1, Fig. 1). Most were fixed in 96% ethanol, or fixed in 10% neutral-buffered formalin with a tissue subsample taken in ethanol. Samples were deposited in the Australian Museum, Sydney (AMS); some were accessed from the Florida Museum of Natural History, USA (FMNH) Invertebrate collection or Genetic Repository. Outgroup data was accessed through GenBank (Table 1). The phylogeny of Aeolidina is not yet well-understood, so we selected outgroups based on available studies. The Facelinidae was not recovered as monophyletic in Carmona *et al*., (2013), so outgroups here were selected from both clades that contained facelinid species, as well as a representative from Piseinotecidae, which represented another independent clade close in that topology. Because existing data on *Pteraeolidia* (Yorifuji *et al*., 2012) derive from a different genome (nuclear), and from a different set of individuals, it was not possible to do any combined analyses.

Tissue was extracted with the Qiagen DNeasy kit (Qiagen, Maryland, USA) according to manufacturer's instructions. Polymerase chain reactions (PCR) were carried out with 1–5 μl of genomic extract using Illustra PuRe *Taq* RTG PCR beads (GE Healthcare). We amplified two partial mitochondrial genes, cytochrome oxidase I (COI) and 16S rDNA with universal primers (Folmer *et al*., 1994; Palumbi *et al*., 1991, respectively). COI amplifications used a standard barcoding protocol that denatures at 95 °C for 3 min; followed by five cycles of 95 °C for 40 s, 45 °C for 40 s, and 50 s of extension at 72 °C; followed by 40 cycles at 51 °C annealing, and a final 5 min extension at 72 °C. Partial 16S was amplified as above, but with 35 cycles of annealing at 50 °C. PCR amplicons were purified with ExoSap-IT and outsourced for sequencing on an ABI capillary 3700 or 3730 at Macrogen (Korea).

Table 1. GenBank and voucher accession information for *Pteraeolidia* and outgroups analysed in this study

Specimen code	COI	16S	Voucher
Sydney A	NA	JN687514	AMS C.474030
Sydney B	NA	JN687521	lost
Sydney D	JN687487	JN687520	AMS C.474031
Sydney E	KJ200956	KJ201006	AMS C.474032
Sydney F	KJ200957	KJ201007	AMS C.474033
Sydney G	KJ200958	KJ201008	AMS C.474034
Sydney H	JN687485	JN687517	AMS C.474035
Sydney I	KJ200959	KJ200982	AMS C474137
Port Stephens A	JN687488	JN687519	AMS C.474036
Port Stephens B	KJ200960	KJ200996	AMS C.474037
Port Stephens C	KJ200961	KJ200997	AMS C.474038
Port Stephens D	KJ200962	KJ200998	AMS C.474039
Port Stephens E	JN687484	JN687515	AMS C.474040
Port Stephens F	NA	JN687513	AMS C.474041
Port Stephens G	NA	JN687516	AMS C.474042
Port Stephens H	KJ200963	KJ200999	AMS C.474043
Eden	JN687486	JN687518	AMS C.474044
Tuamotus	KJ200964	KJ201009	UF 400267
Moorea	KJ200965	KJ200992	FL MBIO 41683
Heron B	JN687481	JN687524	AMS C.474026
Lizard 171	JN687483	JN687523	unknown
Lizard 191	KJ200966	KJ200991	unknown
Papua New Guinea	KJ200967	KJ200995	AMS C.474022
Sulawesi A	JN687478	KJ201000	AMS C.474021
Sulawesi B	JN687482	JN687522	AMS C.474020
Sulawesi C	KJ200968	KJ201001	AMS C.474013
Sulawesi E	KJ200969	KJ200002	AMS C.474014
Sulawesi G	KJ200970	KJ201003	AMS C.474016
Sulawesi I	KJ200971	KJ201004	AMS C.474017
Sulawesi J	KJ200972	KJ201005	AMS C.474018
Maui A	NA	KJ200983	AMS C.474045
Maui B	JN687480	JN687526	AMS C.474046
Maui C	KJ200973	KJ200984	AMS C.474047
Maui D	KJ200974	KJ200985	AMS C. 474171
Maui E	KJ200975	KJ200986	AMS C.474049
Maui F	KJ200976	KJ200987	AMS C.474050
Maui G	KJ200977	KJ200989	AMS C.474051
Maui H	KJ200978	KJ200990	AMS C.474052
French Frigate Shoals	KJ200979	KJ200988	UF 415599
Oahu 1	JN687479	JN687525	AMS C.474060
Oahu 2	KJ200980	KJ200993	AMS C.474061
Oahu 3	KJ200981	KJ200994	AMS C.474062
Godiva quadricolor	HM162756	HM162680	CASIZ176385
Cratena peregrina	HQ616752	HQ616715	MNCN15.05/53691
Sakuraeolis enosimensis	HM162758	HM162682	CASIZ178876
Favorinus elenalexiarum	HM162755	HM162679	CASIZ178875
Favorinus branchialis	HQ616761	HQ616724	MNCN15.05/53695
Pisenotecus sp.	HM162694	HM162604	CASIZ177740

Bi-directional sequences were assembled and edited with Sequencher v5 (Gene Codes Corporation, Ann Arbor, USA).

Sequences were assembled in Se-Al (Rambaut, 1996). Because there were no indels or deletions, COI sequences were aligned by eye, and checked for stop codons by translation. 16S sequences were aligned using the autostrategy in MAFFT (Katoh *et al*., 2002). The concatenated dataset was analysed using RAxML (Stamatakis, 2006) implemented in the raxMLGUI v

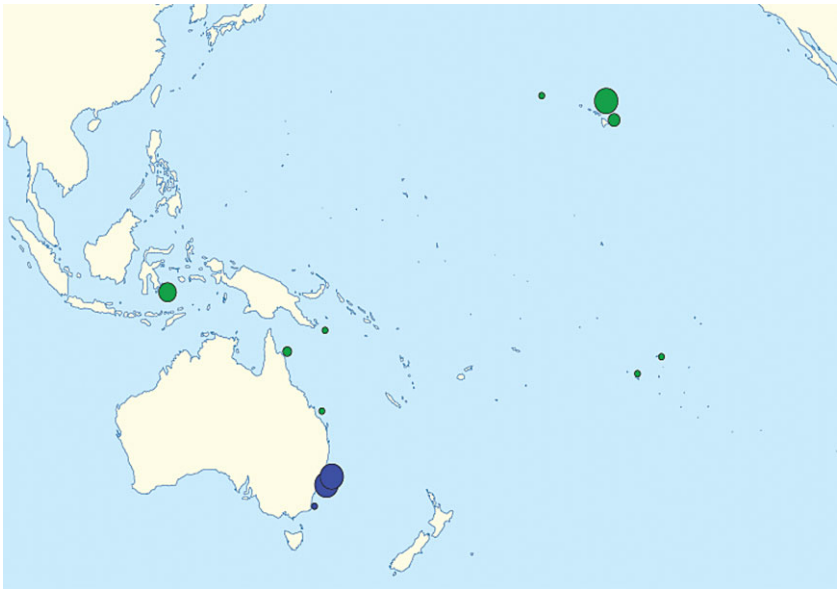

Figure 1. Distribution of samples sequenced in this study. Blue indicates samples determined to be *Pteraeolidia ianthina*, green represents *P. 'semperi'*. Size of circle indicates numbers of specimens, see Table 1 for exact numbers.

Table 2. Uncorrected average COI pairwise differences among *Pteraeolidia* by geographic region

	NSW	Tuamotus	Moorea	GBR	PNG	Sulawesi	Hawaii
NSW	**0.0045**						
Tuamotus	0.1688	**NA**					
Moorea	0.1643	0.1484	**NA**				
GBR	0.1601	0.1400	0.1234	**0.0183**			
PNG	0.1739	0.1424	0.1274	0.0866	**NA**		
Sulawesi	0.1697	0.1364	0.1214	0.0890	0.0525	**0.0213**	
Hawaii	0.1752	0.1514	0.1104	0.0993	0.0710	0.0759	**0.0029**

Bold styling indicates intra-regional differences.

1.3 (Silvestro & Michalak, 2011), partitioning the two genes but implementing joint branch lengths, and the GTR+G model for both (see RAxML manual 2.2.3, to avoid the correlation of the proportion of invariant sites with the alpha parameter of the gamma distribution). Clade support was assessed with 1000 'thorough' bootstrap (BS) replicates. Single gene sets were analysed the same way. We also explored removing variable parts of the alignment using Gblocks (Castresana, 2000) (Fig. S3); under the least stringent conditions, the recovered dataset consisted of 93% of the original positions. Maximum parsimony was also carried out in PAUP v4.0a126 on a reduced concatenated dataset (retaining only two individuals in from the Sydney and Hawaii regions respectively to reduce tree space; Maui E, French Frigate Shoals, Port Stephens A, Sydney D), using a stepwise addition starting tree with ten random sequence addition replicates, and TBR branch swapping. The topology was assessed with 10 000 full heuristic bootstrap replicates.

For species delimitation analyses on the COI data set (excluding the three shorter COI sequences Sulawesi B, Lizard 171, Port Stephens E), the Automated Barcoding Gap Discovery tool was implemented (Puillandre *et al*., 2012). This algorithm infers from the data a one-sided confidence limit for intraspecific divergence. Then it finds the first significant gap beyond this limit, and repartitions the data iteratively until there is no further partitioning. Further, Statistical Parsimony (haplotype network) analyses were carried out in TCSv1.21 at 95% connection limits, with gaps treated as missing data (Clement, Posada & Crandall, 2000). We also calculated uncorrected COI p-distances among subclades in PAUP v4.0a134 (Table 2).

For radular data, buccal bulbs were excised from slugs, and soaked in 10% KOH solution for about 6 h. The released radulae were subsequently cleaned by rinsing in distilled, filtered water and stored in 100% ethanol. Radulae were mounted on a carbon tab/aluminium stub, air dried, sputter coated in gold

(EMITECH K550) and imaged under the electron scanning microscope at the Australian Museum (Zeiss EVO LS15 SEM, using a Robinson Backscatter Detector).

RESULTS

Phylogeny and species delimitation analyses

Single gene analyses were concordant but less-supported than the concatenated maximum-likelihood (ML) topology shown in Fig. 2 (see Figs S1–S3). Removing variable parts of the 16S alignment did not change the ingroup topology, and lowered support for only a single node in the '*semperi*' clade, reducing support for a sister group relationship between the Hawaiian clade and the Papua New Guinea (PNG)/Indonesian clade (ML bootstrap went from 65 to below 50). All analyses reported here refer to the full data set. The genus *Pteraeolidia* was strongly supported as monophyletic in all analyses (Fig. 2, ML = 100, MP = 100), and the clade referring to *P.* '*semperi*' is comprised of a series of putative cryptic species. The phylogeny reflected two distinct sister clades. The clade containing animals from temperate NSW showed little genetic variation, which we refer to as *Pteraeolidia ianthina* (Fig. 2, ML = 100, MP = 100). Its sister clade contained the tropical/subtropical animals, and showed strong geographic structure and varying amounts of genetic variation within and among clades. For the present, we refer to that clade as the tropical clade or the *P.* '*semperi*' clade *sensu lato* (Fig. 2, ML = 93, MP = 97). A subclade within the '*semperi*' clade containing Indonesian (Sulawesi) animals with varying branch lengths (Fig. 2, ML = 96, MP = 99), was sister to an individual from PNG (Fig. 2, ML = 91, MP = 97). Together, these were sister (Fig. 2, ML = 90, MP = 96) to a Hawaiian subclade (Fig. 2, ML = 100, MP = 100), which showed very low genetic variation. Sister to the Indonesia-PNG-Hawaii subclade (Fig. 2, BS ML 98, MP 96) was a clade of animals from the Great Barrier Reef (Fig. 2, ML = 100, MP = 100), which showed more variable branch lengths. The Tuamotus and Society Island (Moorea) animals formed a basal grade respectively to the rest of the tropical '*semperi*' clade. Uncorrected p-distances are shown in Table 2, and show a maximum of 2.1% intraclade variation, and a maximum of 17.52% between *P. ianthina* and the Hawaiian subclade in *P.* '*semperi*'. The lowest between subclade distance within *P.* '*semperi*' occurred between PNG and Indonesia, with 5.25%.

The Automated Barcoding Gap Discovery (ABGD) method resulted in six groups using default starting priors. This result was robust irrespective of whether Kimura or Jukes-Cantor corrected distances or simple distances were used. The six groups comprised of the NSW animals in one group (*Pteraeolidia ianthina*), the Great Barrier Reef animals in another, PNG+Sulawesi together, all Hawaiian animals, and singletons from Moorea and Tuamotus (Fig. 2).

The haplotype network analysis on the same data resulted in nine unconnected networks (membership overlaid onto Fig. 2). These were identical to the ABGD results, except that the Great Barrier Reef animals were separated into two groups; the PNG animal was separated from the Indonesian ones; and one Indonesian animal was separated from the rest (Fig. 2).

Systematics (following World Register of Marine Species, Jan 2014)

GASTROPODA Cuvier, 1817

HETEROBRANCHIA sensu Haszprunar, 1985

NUDIBRANCHIA Cuvier, in de Blainville, 1817

DEXIARCHIA, Schrödl, Wägele & Willan, 2001

AEOLIDIOIDEA, Gray, 1827

FACELINIDAE Bergh, 1899

Pteraeolidia Bergh, 1875

Flabellina semperi Bergh 1870 (type by monotypy)

***Pteraeolidia semperi* (Bergh, 1870)**

Type locality: Philippines, Pacific Ocean (Figs 3A-C, 4A,C,E, 5A,C,E)

Flabellina semperi Bergh, 1870: 18–30

Pteraeolidia semperi (Bergh, 1870) Bergh, 1875: 652; Eliot, 1903: 255; Bergh, 1905; Eliot in Hornell 1909: 144; Eliot, 1913: 44; Baba, 1949: 182–183; Risbec, 1953: 161–163; Risbec, 1956: 31; Marcus & Marcus, 1960: 921–922; E. Marcus, 1965: 280

F. scolopendrella Risbec 1928: 259–260. Type locality: New Caledonia, Pacific Ocean.

misidentified as *Pteraeolidia ianthina* (Angas, 1864) Ev. Marcus & E. Marcus 1970:211; Gosliner, 1980:60; Johnson & Boucher 1983:34.

Material examined: (also Table 1): One specimen UF 400267, French Polynesia, Tuamotu Islands, Aratika Atoll (15°29′36.07″S, 145°26′22.74″W), 5 m, coll. Machel Malay, 5 June 2006; one specimen FL MBIO.41683, French Polynesia, Society Islands, Moorea, W side Opunohu Bay (17°49′41 S, 149°86′20 W), 10–20 m, coll. Greg Rouse & Fred Pleijel, 10 Dec 2010; one specimen AMS C.474026, Australia, Great Barrier Reef, Heron Island, NE Bernies Bay (23°25′58.74″S, 151°57′18.34″E), 0–1 m, coll. Daniel Jackson, 16 Jan 2003; one specimen, lost, Australia, Great Barrier Reef, Lizard Island, Casuarina Beach (14°41′22.74″S, 145°27′58.07″E), coll. Sabrina Bleidissel, 2008; one specimen, lost, Australia, Great Barrier Reef, Lizard Island, Loomis Reef (14°41′00.43″S, 145°26′57.92″E), coll. Sabrina Bleidissel, 2008; one specimen AMS C.474022,

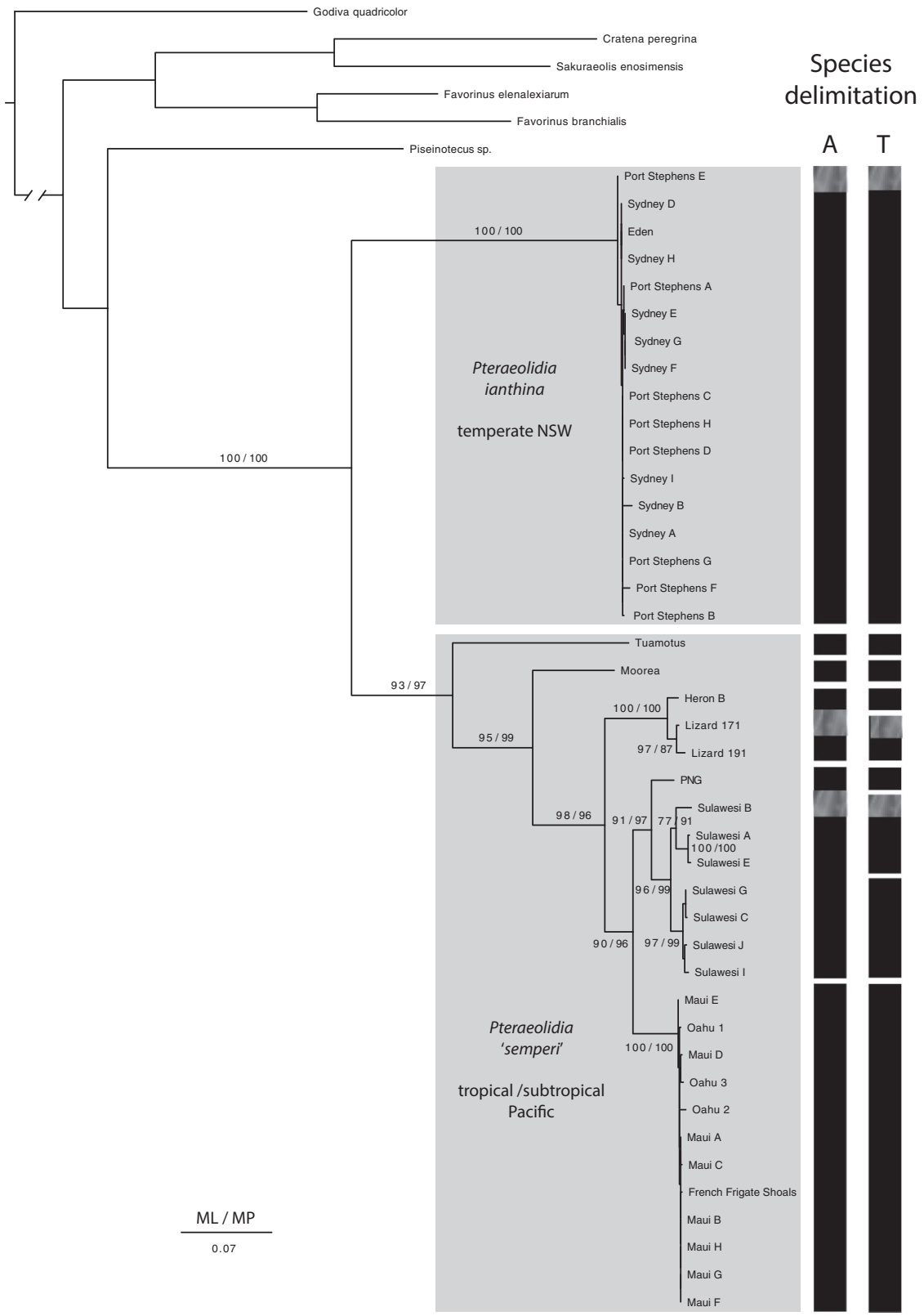

Figure 2. Maximum-likelihood topology of *Pteraeolidia* phylogeny based on combined COI and 16S data sets. Maximum-parsimony support also shown. Black bars indicate results of species delimitation analyses on COI data set; ABGD (A) and statistical parsimony (T). Within those bars, missing data is shown with textured grey.

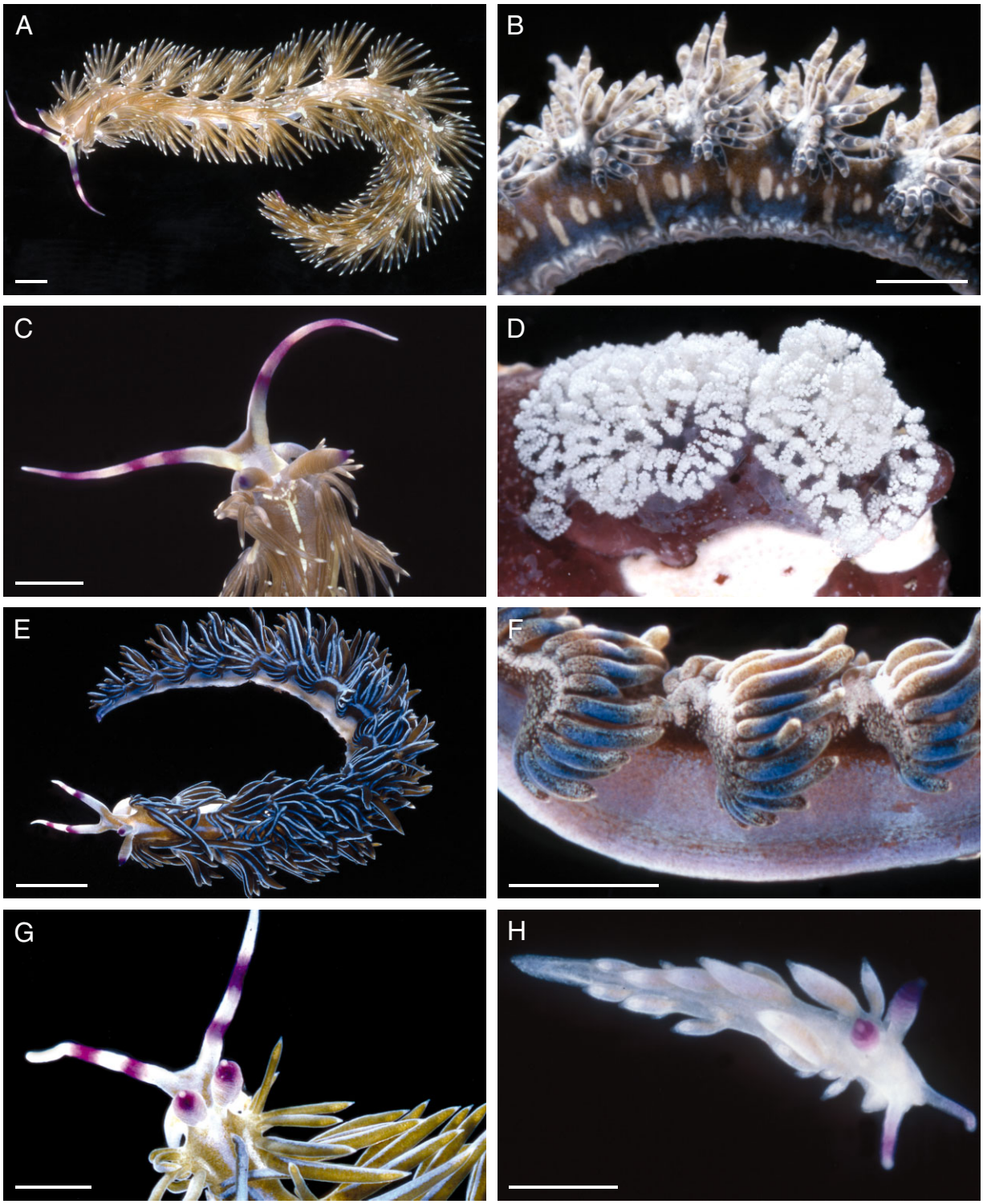

Figure 3. Comparison of live images of tropical clade *Pteraeolidia 'semperi'* (A–C) and *P. ianthina* (D–H). A, Adult tropical *Pteraeolidia*, AMS C.153588, Darwin. B, Markings of lateral sides, tropical *Pteraeolidia*, AMS C.126088, Lizard Is. C, Three bands on oral tentacles of tropical *Pteraeolidia*, AMS C.153588, Darwin. D, Egg masses of *P. ianthina*, AMS C.155772, Solitary Is., Coffs Harbour. E, Adult *P. ianthina*, AMS C.124698, Port Stephens. F, No markings present on lateral sides of *P. ianthina*, AM C.133292, North Bondi, Sydney. G, Two bands on oral tentacles of *P. ianthina*, AMS C.149567, Eden. H, Juvenile *P. ianthina*, AM C.149567, Eden. Scale bar is 10 mm in all cases except G (5 mm), and B and H (2.5 mm). All photographs are by Bill Rudman, except B by John Fields, and E by Heather McLennan.

Papua New Guinea, Louisiade Archipelago, NW Misima Island, nr Gulewa (10°37′49.34″S, 152°44′41.88″E), 2.5 m, coll. Nerida Wilson & Greg Rouse, 14 Aug 2006; one specimen AMS C.474021, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Kaledupa, Sampela Buoy 2 (5°28′46.14″S, 123°44′24.54″E), 6 m, coll. David Thompson & Nerida Wilson, 19 Aug 2002; one specimen AMS C.474020, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Kaledupa, Sampela Buoy 2 (5°28′46.14″S, 123°44′24.54″E), 6 m, coll. Nerida Wilson, 15 Jul 2002; one specimen AMS C.474013, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Hoga, Home Reef Buoy 4 (5°28′13.54″S, 123°45′24.85″E), 12 m, coll. Nerida Wilson, 4 Aug 2002; one specimen AMS C.474014, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Hoga, North Wall 2 (5°27′1.09″S, 123°46′6.06″E), 19 m, coll. Nerida Wilson, 13 Sep 2002; one specimen AMS C.474016, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Hoga, Coral Gardens (5°26′44.30″S, 123°45′19.33″E), 10 m, coll. David Thompson & Nerida Wilson, 15 Sep 2002; one specimen AMS C.474017, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Kaledupa, Double Spur (5°27′53.03″S, 123°42′9.51″E), 8 m, coll. Coral Horn, 18 Aug 2002; one specimen AMS C.474018, Indonesia, SE Sulawesi, Tukangbesi Archipelago, Hoga, Coral Gardens (5°26′44.30″S, 123°45′19.33″E), 11 m, coll. Coral Horn, 10 Sep 2002; eight specimens AMS C474045-C.474047, C.474171, C.474049-C.474052, Hawaiian Islands, Maui, Makena (20°39′21.24″N, 156°26′37.26″W), 18 m, coll. Pauline Fiene, Feb 2003; one specimen UF 415599, Hawaiian Islands, French Frigate Shoals (23°45′21.47″N, 166°07′35.87″W), 32 m, coll. Corey Pittman, 11 Oct 2006; three specimens AMS C.474060-C.474062, Hawaiian Islands, Oahu, wreck of the Yo-256 (21°15′38.76″N, 157°50′15.00″W), 32 m, coll. Greg Rouse, Feb 2006.

Diagnostic clade characters (which will require further delineation with broader geographic sampling): Oral tentacles with three or more purple bands, the two bands closest to the head may be very close together and partially fused (see Fig. 3). White markings on head, particularly anterior to rhinophores. Dorsal and lateral body typically show white vertical bars or spots. Cerata and body often show white, green or yellow and mottled markings. Cerata relatively short in comparison to body length. Size up to 150 mm.

Radula: Radular formula ranges from 15–36 × 0.1.0 (Bergh, 1870; Baba, 1949; Marcus & Marcus, 1960; Gosliner, 1980; Rudman, unpubl. obs.; AMS.C.131625 18(+2)x0.1.0; AMS C.96581 22(+2)x0.1.0; AMS C.129222 22(+2)x0.1.0; AMS C.152525 26(+1)x0.1.0; AMS C.152524 23(+1)x0.1.0; AMS C.99008 18x0.1.0; AMS C.129221 16(+1)x0.1.0;, Figs 4, 5). Each rachidian tooth with large median cusp. Margin of rachidian tooth with 5–12 relatively long denticles (Bergh, 1870; Eliot, 1903; Baba, 1949; Gosliner, 1980). Central rachidian tooth relatively blunt and broad with uppermost pair of denticles closely attached to central cusp (without a deep gap). Radular denticles generally arcuated towards central cusp.

Distribution: Widespread tropical Indo-Pacific. From NSW, Australia, northwards through the Great Barrier Reef, northeast to Hawaii, northwest to Japan, through the Coral Triangle, to the Red Sea, to the southerly limits of Indian Ocean reaching South Africa and Western Australia, through parts of South Australia.

Depth range: intertidal to 32 m (AMS C.128126 and C.474060 respectively).

Symbiosis: Highly efficient with long-term retention of zooxanthellae (Wägele & Johnsen, 2001; Burghardt *et al*., 2008). Symbionts from most areas remain untested and unknown. Specimens from Singapore and Indonesia host *Symbiodinium* clades C and D, although specimens from southern QLD and the Great Barrier Reef are so far only known to host clade C (Loh *et al*., 2006).

Remarks

Burn (1965) made *P. semperi* (Bergh, 1870) a subjective junior synonym of *P. ianthina*, notionally on the basis of priority (*F. ianthina* was described 6 years before *F. semperi*). This was followed by Marcus & Marcus (1970, Madagascar) and reluctantly, by Gosliner (1980, Hawaii). However, Bergh (1875) clearly designated *P. semperi* as the type by monotypy, and this cannot be ignored. Therefore *Pteraeolidia semperi* is recognised as the type species of *Pteraeolidia*. *Pteraeolidia scolopendrella* (Risbec, 1928) from New Caledonia is an available name that may be connected to a cryptic lineage in future studies. However, Risbec (1953) was persuaded to list *P. scolopendrella* as a synonym of *P. semperi*, and was presumably unaware of Bergh's species at the time of describing *P. scolopendrella*. We adopt the conservative position of leaving *P. scolopendrella* in synonymy until a revision can be done examining material from type locality.

***Pteraeolidia ianthina* (Angas, 1864)**

Type locality: Port Jackson (enclosing Sydney Harbour)

(Figs 3D-H, 4B,D,E, 5B,D,E, 6, 7)

Flabellina ianthina Angas, 1864: 66–67, pl. 6, fig. 6.

Pteraeolidia ianthina (Angas, 1864) Burn, 1965: 89–90; Rudman, 1982: 178–183

Material examined: (also Table 1): Eight specimens AMS C.474030 – AMS C.474135, Australia, Sydney, Bare Island (33°59′30.40″S, 151°13′56.67″E), 5 m, coll. William Loh & Melissa Cowlishaw, 2002; one specimen, AMS C.474137,

Australia, Sydney, Clovelly, Gordons Bay (33°54′59.31″S, 151°15′49.15″E), 8 m, coll. Nerida Wilson & Lauren Hughes, 31 Jul 2011; eight specimens AMS C.474036-C.474043, Australia, Port Stephens, Nelson Bay, The Pipeline (32°43′3.64″S, 152°8′28.44″E), 5 m, coll. David and Leanne Atkinson, 20 May 2003; one specimen AMS C.474044, Australia, Eden, Chipmill Wharf (37°6′24.66″S, 149°55′37.85″E), 4 m, coll. Nerida Wilson, 3 April 2007.

Diagnostic species characters: Oral tentacles with two purple bands (see Fig. 3). No white markings on head. Lateral body with purple and brown markings, no white vertical bars or spots. Cerata do not show white markings away from tip, although entire animal may be pale in animals lacking an active zooxanthellae symbiosis. Cerata relatively long in comparison to body length. Size up to 100 mm.

Radula: Radular formula ranges from 12–27 × 0.1.0 (Angas, 1864; Rudman, 1982; Rudman, unpub. obs.; AMS C.133292 27x0.1.0; AMS C.114580 25x0.1.0; AMS C.63054 22(+2)x0.1.0; AMS C.114580 12(+2)x0.1.0; AMS C.1436 17(+4)x0.1.0, Figs 4, 5). Each rachidian tooth with large

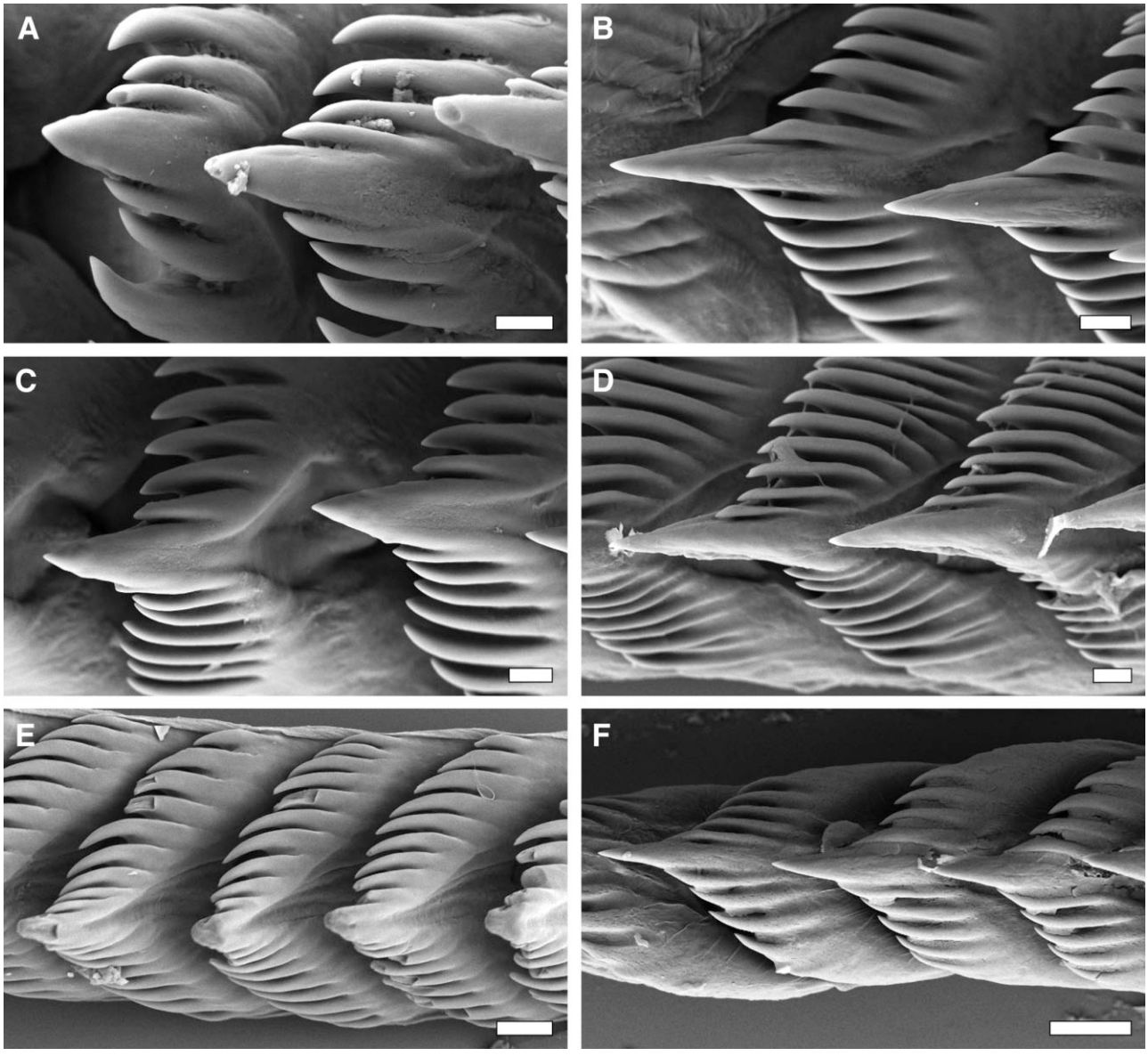

Figure 4. Scanning electron microscopy of *Pteraeolidia* radulae. A,C, E. Dorsal view *P. 'semperi'* (respectively Sulawesi I, AMS C.474017; Maui E, AMS C.474049; Heron B, AMS C.474026). B,D,F. Dorsal view *P. ianthina* (respectively Port Stephens H, AMS C.474043; Sydney E, AMS C.474032; Eden, AMS C.474044). Scale bar represents 10 μm for all, except D and E, where it is 20 μm.

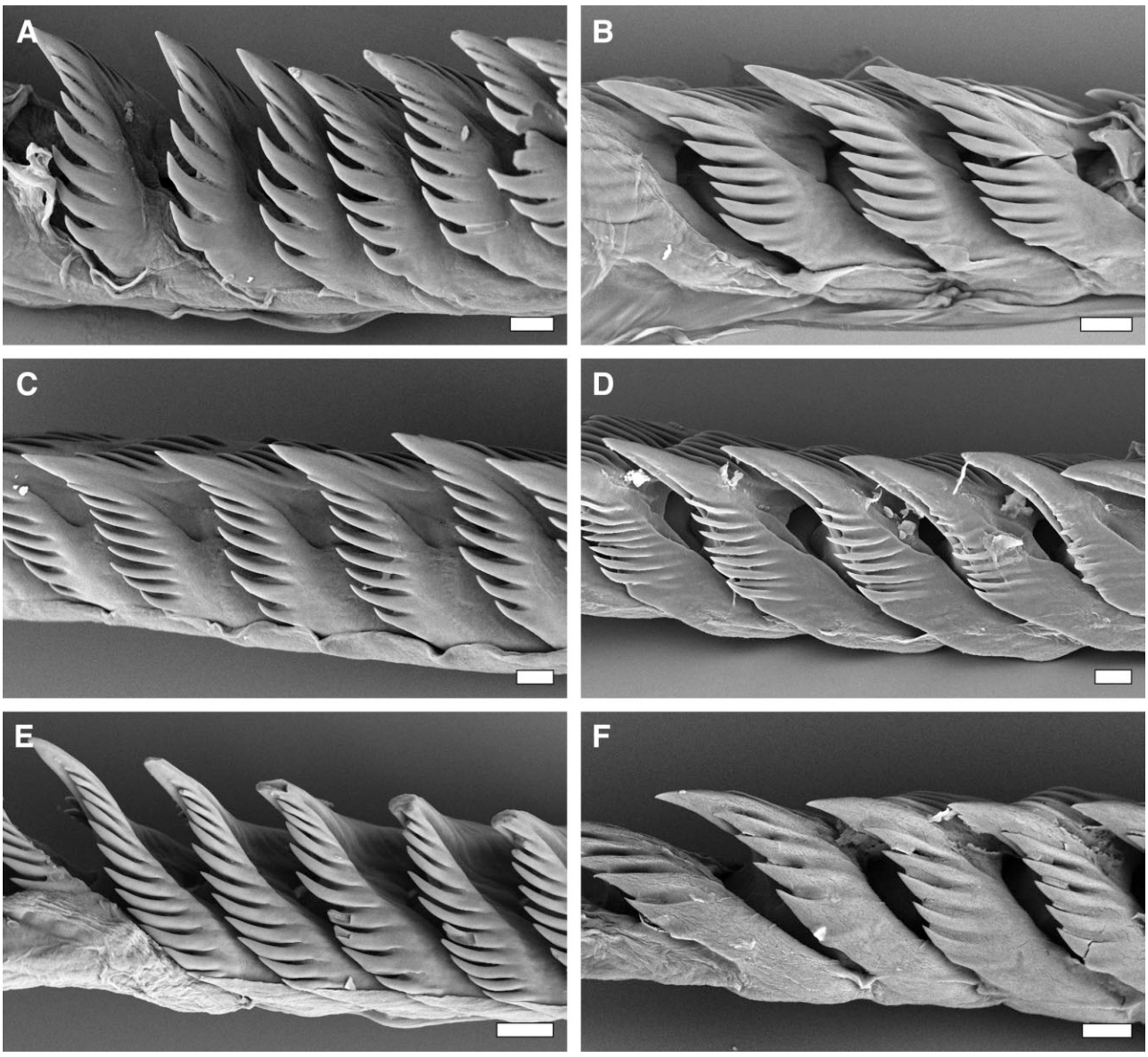

Figure 5. Scanning electron microscopy of *Pteraeolidia* radulae. A,C,E. Lateral view *P. 'semperi'* (respectively Sulawesi I, AMS C.474017; Maui E, AMS C.474049; Heron B, AMS C.474026). B,D,F. *P. ianthina* (respectively Port Stephens H, AMS C.474043; Sydney E, AMS C.474032; Eden, AM S.474044). Scale bar represents 20 μm for all except B, where it is 10 μm.

median pointed cusp. Margin of rachidian tooth with relatively long denticles. Central rachidian tooth elongated, pointed, with deep gap between central tooth and innermost pair of denticles. Radular denticles generally only slightly arcuated towards central cusp.

Distribution: Temperate eastern Australia, New South Wales, from Eden (southern NSW), northwards to the Solitary Islands, Coffs Harbour, NSW (Fig. 6).

Depth range: 4–30 m (AMS C.474044 & C,146981 respectively).

Symbiosis: Highly efficient, with long-term retention of zooxanthellae (Hoegh-Guldberg & Hinde, 1986). Specimens from NSW host *Symbiodinium* clades A and B (Loh *et al*., 2006).

Remarks

This species is easily recognisable from Angas' drawings. The figure published with the species description (Angas, 1864) shows some minor colouration differences from the original notebook drawing, which we reproduce here (Fig. 7). The main differences are

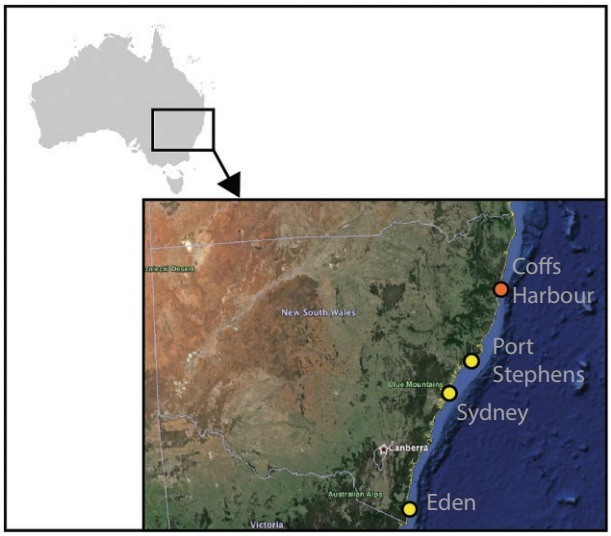

Figure 6. Extent of known distribution of *Pteraeolidia ianthina*. Yellow circles indicate sequenced specimens, orange circle indicates photographic record. Museum records inside these northern and southern boundaries not shown.

Figure 7. Angas' original unpublished notebook drawing of *Flabellina ianthina*, approximately 1852. This particular drawing was later edited and reproduced in the Journal de Conchyliologie (Angas, 1864) lacking some colour veracity.

that the original publication (Angas, 1864) whitened the body colouration that is normally brown with symbionts, dorsal markings have been erased, and the colour of the cerata are violet/purple instead of blue. The notebook drawing is a much more realistic interpretation, and is worthy of circulation. It is believed that the hand-coloured drawings in his sketchbook 'Nudibranches of Port Jackson' were produced in approximately 1852 (http://australianmuseum.net.au/Nudibranches-of-Port-Jackson).

Although the name has been applied widely through the Indo-Pacific, we show here that it should be restricted to animals from temperate New South Wales, Australia. The exact distributional boundaries are yet to be determined, but so far this species appears to be found only in New South Wales, Australia.

DISCUSSION

Our study shows strong genetic structuring of slugs with geography (Fig. 2). Where there are multiple samples from a single area, genetic diversity can be extremely low (e.g. NSW, Hawaii) or slightly more diverse (e.g. Sulawesi). The type locality of *Pteraeolidia ianthina* (Angas, 1864) is Sydney Harbour, so the name can be fixed to the subclade found only in temperate NSW. This name is applied in this manner for the rest of the publication. Tropical fish vagrants often occur in waters around Sydney (Booth *et al*., 2007) courtesy of the southward East Australia Current, and there is one record of a tropical vagrant *P. semperi* occurring in waters just north of Sydney Harbour (Hansen, 1999). However, Angas' drawing easily clarifies his species as the more typical temperate dweller, and not a tropical vagrant.

The taxonomic status of the sister clade to *P. ianthina* is more complicated. The other available names, *P. semperi* (Bergh, 1870), and *P. scolopendrella* Risbec, 1928, must be removed from synonymy, but their validity remains uncertain until sampling from each of the type localities can be incorporated into phylogeographic analyses. It is possible that *P. scolopendrella* will remain a synonym of *P. semperi*. The genetic groups of Yorifuji *et al*. (2012) remain uncertain; they report animals from the Philippines (the generalised type locality of *P. semperi*) in both of their genetic groups (termed A and B), so it may not be possible to resolve the identity of that species. It is still possible that the temperate group described by Yorifuji *et al*. (2012) is part of the true *P. ianthina* clade, but two factors negate this. Firstly, the geographic

distance between the NSW and Japan is overly large compared to the strong genetic structuring we see between other, geographically closer locations in our study. Secondly, although some zooxanthellae-hosting fauna have distributions that range from Japan to eastern Australia (Rodriguez-Lanetty & Hoegh-Guldberg, 2002), the clades of *Symbiodinium* hosted by the slugs also differ; temperate NSW slugs contain *Symbiodinium* clades A and B, while temperate Japanese specimens are known to host *Symbiodinium* clades A and D.

Our study establishes the known distribution of *P. ianthina* as New South Wales, Australia (see Fig. 6). The range of sequenced specimens (Port Stephens, south to Eden) exceeds 500 km, and if combined with AM photographic records from Coffs Harbour, extends to over 800 km. In comparison, the range of the Hawaiian *semperi* clade (from French Frigate shoals to Maui) exceeds 1000 km. Both of these groups show remarkably little variation in their within-clade sequences. Considering geographic distances, the 10 km separating the Sulawesi samples hardly seems reasonable to sustain the more diverse sequences seen in that clade, and it is likely other vicariant factors such as historical sea level change may be responsible.

Relatively deep genetic divergence is demonstrated between the two lineages present in the geographically close Tuamotus and Society Island (Moorea) samples in the Central Pacific. The geographic distance between these two samples is approximately 500 km, equivalent to the distance covered by all true NSW *P. ianthina* specimens (and half that of the Hawaiian subclade range). Only 0.25% uncorrected pairwise COI divergence was found across all specimens in NSW *P. ianthina*, while the uncorrected pairwise COI difference between the Tuamotus and Society Island (Moorea) samples was 14.8% (Table 2). Similarly large (but polyphyletic) divergence was found among three lineages of *Pontohedyle* slugs found on Moorea (Jörger *et al*., 2012), and may be indicative of more widespread complex evolutionary organismal histories in the Central Pacific region. Certainly some faunal connectivity is known between the Central Pacific and the Hawaiian Archipelago (see references in Toonen *et al*., 2011), but that pattern is not represented by our phylogeny, where the Central Pacific individuals are a basal grade to the rest of the clade, and the Hawaiian samples are one of the most derived clades (Fig. 2).

Reproduction may vary among cryptic lineages, which may help to delineate them. Rose & Hoegh-Guldberg (1982) reported that *P. ianthina* from Sydney laid white eggs masses that took 11 days to hatch as lecithotrophs at 25 °C. To date, reproduction for *P. semperi* is unknown. Differences in reproduction have previously been used to identify cryptic species in nudibranchs (Rose, 1985; Brodie & Calado, 2006), and it is unclear if any differences occur among lineages of *Pteraeolidia*. Interestingly, *P. ianthina* is the only nudibranch to date known to exhibit some form of parental care. Immediately after oviposition, adults are seen to encircle and cover egg masses until close to hatching, and this was observed both in the laboratory and in the field (Rose & Hoegh-Guldberg, 1982). This behaviour is probably facilitated by the ability to retain zooxanthellae so the parent may receive nutrition while still attending the egg mass, and by the ability to retain nematocysts from their prey, to act as a form of defence against potential attackers. Despite much diving activity in the tropical Indo-Pacific, reports of this brood-protecting habit from *Pteraeolidia* anywhere outside New South Wales are conspicuously absent.

Given the extended range of *Pteraeolidia* throughout the Indo-Pacific (and with our sampling limited only to the Pacific), it is almost certain that other lineages will be discovered. So far, our limited sampling has not recovered multiple lineages/groups at one location. However, such overlap was reported by Yorifuji *et al*. (2012) where specimens from their slug groups A and B were co-located at Kagoshima, Japan, and Batangas, Philippines. Although Yorifuji *et al*. (2012) did not report the identity of any *Symbiodinium* symbionts, previous work reported Japanese *Pteraeolidia* from Hayama Bay to host clades A and D (Ishikura *et al*., 2004). This area is close to one of the strictly temperate sample sites of Yorifuji *et al*. (2012). There is much to learn about the *Pteraeolidia-Symbiodinium* relationship. Most studies identifying symbionts have used only PCR amplicon-based techniques, and not qPCR, or other more sensitive methods for detecting less abundant symbionts (Apprill & Gates, 2007; Mieog *et al*., 2007; Fitzpatrick *et al*., 2012). However, the less abundant symbionts may not affect the ecology of the host-symbiont as significantly as the dominant strain of *Symbiodinium*, and may not matter over evolutionary timescales.

The co-occurrence of multiple lineages of *Pteraeolidia* at one site provides a good test of the specificity of the symbiont-host relationship. If the symbiont assemblage is strictly associated with the slug lineage, it is concordant with species-specific symbioses. But if the symbionts are selected by the host for their local adaptation, and possibly temperature-related distribution, we would expect different lineages of slug to house the same symbionts at a single location since the slugs would be exposed to the same environmental conditions. These are questions that should be pursued in these localities (Kagoshima, Japan; Batangas, Philippines) and will help shed light on whether climate-related distributional shifts may disrupt the symbiosis. Responses to climate-induced changes typically result in distributional shifts of organisms (Moritz & Agudo, 2013). In the case of *Pteraeolidia*, it is not clear if host slugs would take their potentially less-adapted symbionts

into new ranges, or take up more locally-adapted partners. This possible flexibility in symbiont assemblages has been discussed for corals as the 'Adaptive Bleaching Hypothesis' (Buddemeier & Fautin, 1993) and is still heavily debated in the light of climate change and global warming. Given that many investigated specimens of *Pteraeolidia* host more than one *Symbiodinium* genotype simultaneously (Loh *et al*., 2006) there is the possibility of 'shuffling' of symbionts, potentially in favour of symbionts better-adapted to new environmental conditions. Additionally, symbiont acquisition in *Pteraeolidia* is horizontal (in contrast to many coral species) which gives this taxon the flexibility to possibly switch to more suitable symbiont types each generation.

An important contrast exists when looking at deeper temporal scales; the evolution of *Symbiodinium* appears tightly linked to specialisation within a particular host (Thornhill *et al*., 2014). In many cnidarian-*Symbiodinium* relationships, changes in the genetic identity of one partner indicated correlated changes in the other (Santos *et al*., 2004; Thornhill *et al*., 2010, 2013; Pinzon & LaJeunesse, 2011). Previous studies have shown that species interactions such as symbioses are an important driver of evolutionary change and may lead to enhanced diversification through niche expansion (Bordenstein, 2003; McGovern & Hellberg, 2003; Wägele *et al*., 2010; Joy, 2013).

Unravelling the full extent of the potential cryptic diversity within *Pteraeolidia* will take much more extensive sampling throughout its range. Characterizing the symbionts in standardised ways will also assist in understanding the role that *Symbiodinium* has played in this evolutionary arena. It may not be possible to unequivocally fix the type species *P. semperi* to a subclade, given that multiple subclades are found in the type locality. However, the identity of *P. ianthina* is clear, and only applicable to the subclade known from temperate New South Wales, Australia.

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REFERENCES

Angas GF. 1864. Description d'especes nouvelles appartenant a plusiers genres de Mollusques Nudibranches des environs de Port-Jackson (Nouvelle-Galles du Sud), accompagnee de dessins faits d'apres nature (GFA & H Crosse). *Journal de Conchyliologie* **12:** 43–70. pls. 4–6.

Apprill AM, Gates RD. 2007. Recognizing diversity in coral symbiotic dinoflagellate communities. *Molecular Ecology* **16:** 1127–1134.

Baba K. 1949. *Opisthobranchia of Sagami Bay collected by His Majesty the Emperor of Japan*. Tokyo: Iwanami Shoten.

Baker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics* **34:** 661–689.

Bergh LSR. 1870. Malacologische Untersuchungen. In: Semper C, ed. *Reisen im Archipel der Philippinen, Vol. 1 Section 2*, (I): 1–30, pls. 1–8.

Bergh LSR. 1875. Beitrage zur Kenntniss der Aeolidiaden 3. *Verhandlungen der königlich kaiserlich zoologisch-botanischen Gesellschaft in Wien* **25:** 633–658.

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

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22:** 148–155.

Booth DJ, Figueira WF, Gregson MA, Brown L, Beretta G. 2007. Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine, Coastal and Shelf Science* **72:** 102–114.

Bordenstein SR. 2003. Symbiosis and the origin of species. In: Bourtzis K, Miller T, eds. *Insect symbiosis*. New York: CRC Press, 283–304.

Brodie GD, Calado G. 2006. *Dendrodoris arborescens* (Collingwood, 1881) (Mollusca: Nudibranchia): larval characteristics reveal a masked porostome species. *Records of the Western Australian Museum, Supplement* **69:** 119–126.

Buddemeier RW, Fautin DG. 1993. Coral bleaching as an adaptive mechanism: a testable hypothesis. *BioScience* **43:** 320–326.

Burghardt I, Stemmer K, Wägele H. 2008. Symbiosis between *Symbiodinium* (Dinophyceae) and different taxa of Nudibranchia (Mollusca: Gastropoda) with analyses of long-term retention. *Organisms, Diversity and Evolution* **4:** 66–76.

Burn R. 1965. A centennial commentary and zoogeographical remarks on Angas' Sydney nudibranchs. *Journal de Conchyliologie* **104:** 85–93.

Burn R. 1989. Opisthobranchs (Subclass Opisthobranchia). In: Shepherd SA, Thomas IM, eds. *Marine invertebrates of Southern Australia. Part II*. Adelaide: South Australian Government Printing Division, 725–788.

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.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1660.

Eliot CNE. 1903. On some nudibranchs from East Africa and Zanzibar. Part II. *Proceedings of the Zoological Society, London* **1903:** 250–257.

Eliot CNE. 1909. Report on the nudibranchs collected by Mr James Hornell at Okhamandal in Kattiawar in 1905-6. In: Hornell J, ed. *Report of the Government of Baroda on the marine zoology of Okhamandal*. London: Williams and Norgate, 137–145.

Eliot CNE. 1913. Japanese nudibranchs. *The Journal of the College of Science, Imperial University of Tokiyo* **35:** 1–47, 2 pls.

FitzPatrick SK, Liberatore KL, Garcia JR, Burghardt I, Colman DR, Moquin SA, Takacs-Vesbach CD, Shepherd UL. 2012. *Symbiodinium* diversity in the soft coral *Heteroxenia* sp. and its nudibranch predator *Phyllodesmium lizardensis*. *Coral Reefs* **31:** 895–905.

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.

Gosliner TM. 1980. [for 1979]. The systematics of the Aeolidacea (Nudibranchia: Mollusca) of the Hawaiian Islands, with descriptions of two new species. *Pacific Science* **33:** 37–77.

Hadfield MG. 1976. Molluscs associated with living tropical corals. *Micronesia* **12:** 133–148.

Hansen EP. 1999. (Aug 8) *Pteraeolidia* from Sydney. Message in: *Sea Slug Forum*. Australian Museum, Sydney. Available at: http://www.seaslugforum.net/find/1152

Hebert PDN, Cywinska A, Ball SL. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270:** 313–321.

Hoegh-Guldberg O, Hinde R. 1986. Studies on a nudibranch that contains zooxanthellae I. Photosynthesis, respiration and the translocation of newly fixed carbon by zooxanthellae in *Pteraeolidia ianthina*. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **228:** 493–509.

Hoegh-Guldberg O, Hinde R, Muscatine L. 1986. Studies on a nudibranch that contains zooxanthellae II. Contribution of zooxanthellae to animal respiration (CZAR) in *Pteraeolidia ianthina* with high and low densities of zooxanthellae. *Proceedings of the Royal Society of London, Series B. Biological Sciences* **228:** 511–521.

Ishikura M, Hagiwara K, Takishita K, Haga M, Iwai K, Maruyama T. 2004. Isolation of new *Symbiodinium* strains from Tridacnid giant clam (*Tridacna crocea*) and sea slug (*Pteraeolidia ianthina*) using culture medium containing giant clam tissue homogenate. *Marine Biotechnology* **6:** 378–385.

Johnson S, Boucher LM. 1983. Notes on some opisthobranchs (Mollusca: Gastropoda) from the Marshall Islands, including 57 new records. *Pacific Science* **37:** 251–291.

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.

Joy JB. 2013. Symbiosis catalyses niche expansion and diversification. *Proceedings of the Royal Society B: Biological Sciences* **280:** 20122820.

Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Kempf SC. 1984. Symbiosis between the zooxanthella *Symbiodinium* (*Gymnodinium*) *microadriaticum* (Freudenthal) and four species of nudibranchs. *Biological Bulletin* **166:** 110–126.

Knowlton N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* **24:** 189–216.

Loh WK, Cowlishaw M, Wilson NG. 2006. Diversity of *Symbiodinium* dinoflagellate symbionts from the Indo-Pacific sea slug *Pteraeolidia ianthina* (Gastropoda: Mollusca). *Marine Ecology Progress Series* **320:** 177–184.

Marcus E. 1965. Some Opisthobranchia from Micronesia. *Malacologia* **3:** 235–262.

Marcus E, Marcus E. 1960. Opisthobranchia aus dem Roten Meer von den Malediven. Akademie der Wissenschaften und der Literatur; in Kommission bei F. *Steiner, Wiesbaden* **1959:** 871–934.

Marcus E, Marcus E. 1970. Some gastropods from Madagascar and West Mexico. *Malacologia* **10:** 181–223.

McGovern TM, Hellberg ME. 2003. Cryptic species, cryptic endosymbionts, and geographical variation in chemical defences in the bryozoan *Bugula neritina*. *Molecular Ecology* **12:** 1207–1215.

Mieog JC, van Oppen MJH, Cantin NE, Stam WT, Olsen JL. 2007. Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs* **26:** 449–457.

Moritz C, Agudo R. 2013. The future of species under climate change: resilience or decline? *Science* **341:** 504–508.

Palumbi SR, Martin AP, Romano SL, McMillan WO, Stice L, Grabowsky CI. 1991. *The simple fool's guide to PCR*. Honolulu: Special Publication of the Dept. of Zoology, University of Hawaii.

Pinzon JH, LaJeunesse TC. 2011. Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology* **20:** 311–325.

Pola M, Camacho-Garcia YE, Gosliner TM. 2012. Molecular data illuminate cryptic nudibranch species: the evolution of the Scyllaeidae (Nudibranchia: Dendronotina) with a revision of *Notobryon*. *Zoological Journal of the Linnean Society* **165:** 311–336.

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

Rambaut A. 1996-2002. *Se-Al: sequence alignment editor. V2.0a11*. Oxford: University of Oxford.

Ratnasingham S, Hebert PDN. 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes* **7:** 355–364.

Risbec J. 1928. Contribution it l'étude des nudibranches Nouvelle Calédoniens. *Faune des Colonies Françaises* **2:** 1–460.

Risbec J. 1953. Mollusques nudibranches de la Nouvelle Calédonie. *Faune de la Union Française, Paris* **15:** 1–189.

Risbec J. 1956. Nudibranches du Viet-nam. 9th Memoire de l'Institute Oceanographique de Nhatrang, Viet-nam. *Extrait des Archives du Muséum National d'Histoire Naturelle de Paris, Series* **7:** 1–34.

Rodriguez-Lanetty M, Hoegh-Guldberg O. 2002. The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the Western Pacific. *Molecular Ecology* **11:** 1177–1189.

Rose RA. 1985. The spawn and embryonic development of colour variants of *Dendrodoris nigra* Stimpson (Mollusca: Nudibranchia). *Journal of the Malacological Society of Australia* **7:** 75–88.

Rose RA, Hoegh-Guldberg O. 1982. A brood-protecting nudibranch with pelagic lecithotrophic development. *Journal of Molluscan Studies* **48:** 231–232.

Rudman WB. 1982. The taxonomy and biology of further aeolidacean and arminacean nudibranch molluscs with symbiotic zooxanthellae. *Zoological Journal of the Linnean Society* **74:** 147–196.

Santos SR, Shearer TL, Hannes AR, Coffroth MA. 2004. Fine- scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean. *Molecular Ecology* **13:** 459–469.

Silvestro D, Michalak I. 2011. raxMLGUI: a graphical front-end for RAxML. *Organisms, Diversity and Evolution* **12:** 335–337.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Thornhill DJ, Doubleday K, Kemp DW, Santos SR. 2010. Host hybridization affects specificity of cnidarian-dinoflagellate associations. *Marine Ecology Progress Series* **420:** 113–123.

Thornhill DJ, Lewis AM, Wham DC, LaJeunesse TC. 2014. Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution* **68:** 352–367.

Thornhill DJ, Xiang Y, Pettay DT, Zhong M, Santos SR. 2013. Population genetic data of a model symbiotic cnidarian system reveal remarkable symbiotic specificity and vectored introductions across ocean basins. *Molecular Ecology* **22:** 4499–4515.

Toonen RJ, Andrews KR, Baums IB, Bird CE, Concepcion GT, Daly-Engel TS, Eble JA, Faucci A, Gaither MR, Iacchei M, Puritz JB, Schultz JK, Skillings DJ, Timmers MA, Bowen BW. 2011. Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago. *Journal of Marine Biology* **460173:** 1–13.

Wägele H. 2004. Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation. *Organisms, Diversity and Evolution* **4:** 175–188.

Wägele H, Johnsen G. 2001. Observations on the histology and photosynthetic performance of "solar-powered" opisthobranchs (Mollusca, Gastropoda, Opisthobranchia) containing symbiotic chloroplasts or zooxanthellae. *Organisms, Diversity and Evolution* **1:** 193–210.

Wägele H, Raupach MJ, Burghardt I, Grzymbowski Y, Händeler K. 2010. Solar powered seaslugs (Opisthobranchia, Gastropoda, Mollusca): incorporation of photosynthetic units: a key character enhancing radiation? In: Glaubrecht M, ed. *Evolution in action. Case studies in adaptive radiation, speciation and the origin of biodiversity*. Special volume from the SPP 1127 'Radiations – Genesis of Biological diversity' of the DFG, Heidelberg: Springer, 263–282.

Willan RC. 1989. Field observations on feeding and antagonistic behavior by *Pteraeolidia ianthina* (Nudibranchia: Aeolidoidea). *Veliger* **32:** 228–229.

Wilson NG, Schrödl M, Halanych KM. 2009. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology* **18:** 965–984.

Yorifuji M, Takeshima H, Mabuchi K, Nishida M. 2012. Hidden diversity in a reef-dwelling sea slug, *Pteraeolidia ianthina* (Nudibranchia, Aeolidina), in the Northwestern Pacific. *Zoological Science* **29:** 359–367.

SUPPORTING INFORMATION

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

Figure S1. Maximum-likelihood topology of *Pteraeolidia* phylogeny based on COI, with bootstrap support assessed with 1000 'thorough' bootstrap replicates in RAxML. Intraclade bootstrap support is usually not shown, and bootstrap support less than 50 is not shown.

Figure S2. Maximum-likelihood topology of *Pteraeolidia* phylogeny based on 16S, with bootstrap support assessed with 1000 'thorough' bootstrap replicates in RAxML. Intraclade bootstrap support is usually not shown, and bootstrap support less than 50 is not shown.

Figure S3. Maximum-likelihood topology of *Pteraeolidia* phylogeny based on 16S, with areas of ambiguous alignment removed by Gblocks (Castresana, 2000). Bootstrap support assessed with 1000 'thorough' bootstrap replicates in RAxML. Intraclade bootstrap support is usually not shown, and bootstrap support less than 50 is not shown.