

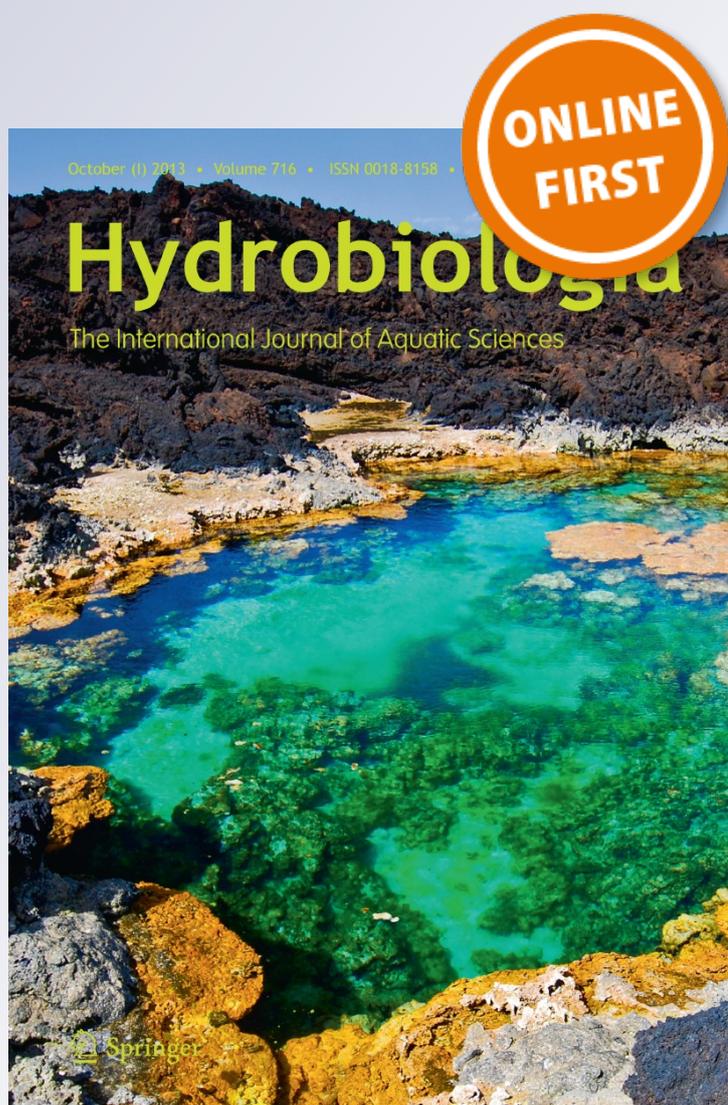
Molecular and morphological evidence for conspecificity of two common Indo-Pacific species of Palythoa (Cnidaria: Anthozoa)

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Hydrobiologia
The International Journal of Aquatic
Sciences

ISSN 0018-8158

Hydrobiologia
DOI 10.1007/s10750-013-1587-5



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Molecular and morphological evidence for conspecificity of two common Indo-Pacific species of *Palythoa* (Cnidaria: Anthozoa)

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Received: 24 February 2013 / Accepted: 2 June 2013
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Abstract Zoanths of the genus *Palythoa* are common in coral reef environments worldwide, particularly in the intertidal zone. However, their taxonomy remains problematic, resulting in an incomplete understanding of their diversity. *Palythoa caesia* Dana, 1846 is found in Fiji, Australia, and the Indian Ocean, while *P. tuberculosa* (Esper, 1805) has been reported from India, the Red Sea, Singapore, Madagascar, and Japan. The lack of obvious characters differentiating the two species, their wide distributions and high levels of intraspecific variation raise the

possibility that these species are in fact one. Based on specimens from Australia, the Red Sea, and Japan, we used three DNA markers (mitochondrial cytochrome oxidase I, 16S ribosomal DNA, and the nuclear internal transcribed spacer region of ribosomal DNA) combined with morphological analyses of tentacle numbers, and cnidae to re-examine the identity of these two taxa. Phylogenetic results showed sequences from all specimens for all markers formed one monophyly, and morphological results showed little differentiation between the two putative taxa. Overall, it is apparent these two taxa are the same species, and the senior synonym *P. tuberculosa* should be used for specimens for the entire Indo-Pacific region.

Electronic supplementary material The online version of this article (doi:[10.1007/s10750-013-1587-5](https://doi.org/10.1007/s10750-013-1587-5)) contains supplementary material, which is available to authorized users.

Guest editors: M. Tokeshi & H. T. Yap / Biodiversity in Changing Coastal Waters of Tropical and Subtropical Asia

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Keywords Hexacorallia · Synonymy ·
Taxonomy · Zoanthid

Introduction

Zoanths of the genus *Palythoa* Lamouroux, 1816 are among the most common anthozoans in shallow subtropical and tropical oceans. Like many other shallow water zoanths, the majority of species in this genus are zooxanthellate, hosting endosymbiotic dinoflagellate species of *Symbiodinium* Freudenthal, 1962 (Burnett, 2002; Reimer et al., 2006c). Typical of zoanths, *Palythoa* spp. have their tentacles arranged in two rows around their oral disk, and are encrusted

with sand and detritus. *Palythoa* spp. are particularly encrusted, with up to 65% of their body weight made up of foreign materials (Haywick & Mueller, 1997).

Historically, species of *Palythoa* have been described and identified based on the morphology (including size and coloration) of polyps and colonies, as well as on the development and shape of their sphincter muscles. Currently, there at least 107 nominal species of *Palythoa* mentioned in the literature (Reimer 2012). Many species of *Palythoa* were described in the twentieth century, primarily by Pax (30 species described between 1908 and 1957) and Carlgren (20 species described between 1900 and 1952) as both of them examined specimens from expeditions to various parts of the world (e.g., West Africa, Australia). However, it is likely that the true number of species is much lower due to high levels of morphological variation within species and inadvertent redescrptions (Burnett et al., 1997; Reimer et al., 2004). Asides from some research by Ryland and co-workers (e.g., Ryland & Lancaster, 2003), there has been no major taxonomic revision of this group since the first half of the twentieth century. A complete understanding of *Palythoa* spp. diversity is impossible until a comprehensive reassessment addresses the abnormally elevated “synonymy load” in this group (Low & Reimer, 2011).

Within *Palythoa*, two of the most commonly used binomens in the Indo-Pacific are *Palythoa tuberculosa* (Esper, 1805) and *Palythoa caesia* Dana, 1846 (Fig. 1; Table 1). Both species are zooxanthellate, have heavy sand encrustation and an “immersae” gross colony morphology with polyps deeply embedded in a well-developed coenenchyme (Pax, 1910). Different Indo-Pacific regions apply one of the two binomens, although there are some regions where both are used. No obvious morphological differences exist between the two species (Fig. 1; Table 1) and typify a common problem in zoanthid taxonomy, that is, the lack of accurate species level diagnostic characters (e.g., Swain, 2010).

Palythoa tuberculosa (Esper, 1805) was originally described as *Alcyonium tuberculosum* from specimens (since lost) collected in Tranquebar (now Tharangambadi), India, as colonies with a leather-like surface and ocher coloration. In 1834, Ehrenberg described two similar species, *P. flavoviridis* and *P. argus*, from the Red Sea. After this, Klunzinger (1877) redescrbed *P. tuberculosa* from specimens in the Red Sea, and

synonymized this species with *P. flavoviridis* Ehrenberg, 1834 and *P. argus* Ehrenberg, 1834. According to Klunzinger's (1877) redescrption, *P. tuberculosa* inhabits the surf zone, with colonies 5–20 mm in thickness and having an uneven, brown surface. Polyps possess 30–40 tentacles, are 1–6 mm in height and 5–7 mm in diameter, although it is not clear whether Klunzinger referred to dried, preserved, or live specimens. *P. tuberculosa* is currently used throughout much of the Indo-Pacific, and is mentioned in literature and guide books from the Red Sea (Erhardt & Knop, 2005; Polak et al., 2011), Oman (Erhardt & Knop, 2005), the Maldives (Erhardt & Knop, 2005), India (Esper, 1805), New Caledonia (Sinniger, 2006), Singapore (Reimer & Todd, 2009), Palau (Erhardt & Knop, 2005), Taiwan (Reimer et al., 2011), Japan (Yamazato et al., 1973; Reimer et al., 2006b, c, 2007b), Hawaii (Hoover, 1999), and the Galapagos (Reimer & Hickman, 2009).

Palythoa caesia was described from Fiji by Dana (1846) as having “immersae”, dark brown colonies with uneven surfaces, and many tentacles (the number was not specified). This binomen is most commonly applied in Australia (Burnett et al., 1994, 1997), and has also been used in the Indian Ocean (Burnett, 2002), Papua New Guinea (Erhardt & Knop, 2005), Guam (Paulay et al., 2003), and Hawaii (Hoover, 1999).

Examining the total descriptive data for *P. tuberculosa* from Esper (1805), Ehrenberg (1834), and Klunzinger (1877), and *P. caesia* from Dana (1846), it is impossible to clearly distinguish these two species except by locality (Table 1), and even this is confused in certain regions such as Hawaii, and in some publications (e.g. Fossa & Nilsen, 1998). This problem is specifically mentioned by Hoover (1999), who notes that due to identification difficulties, both binomens are used in Hawaii.

Past analyses of *Palythoa* spp. using molecular methods have indicated that individual species may encompass a wide variety of morphologies (Burnett et al., 1994, 1997; Reimer et al., 2006a) over a wide distribution range, raising the possibility that *P. tuberculosa* and *P. caesia* are in fact the same species (Sinniger, 2006). The two binomens have been in common use for over 150 years, and resolving their identity is of importance not only for the taxonomy of *Palythoa*, but also for understanding their diversity and distribution in the Indo-Pacific. Here, we examine specimens from the ranges of both species utilizing

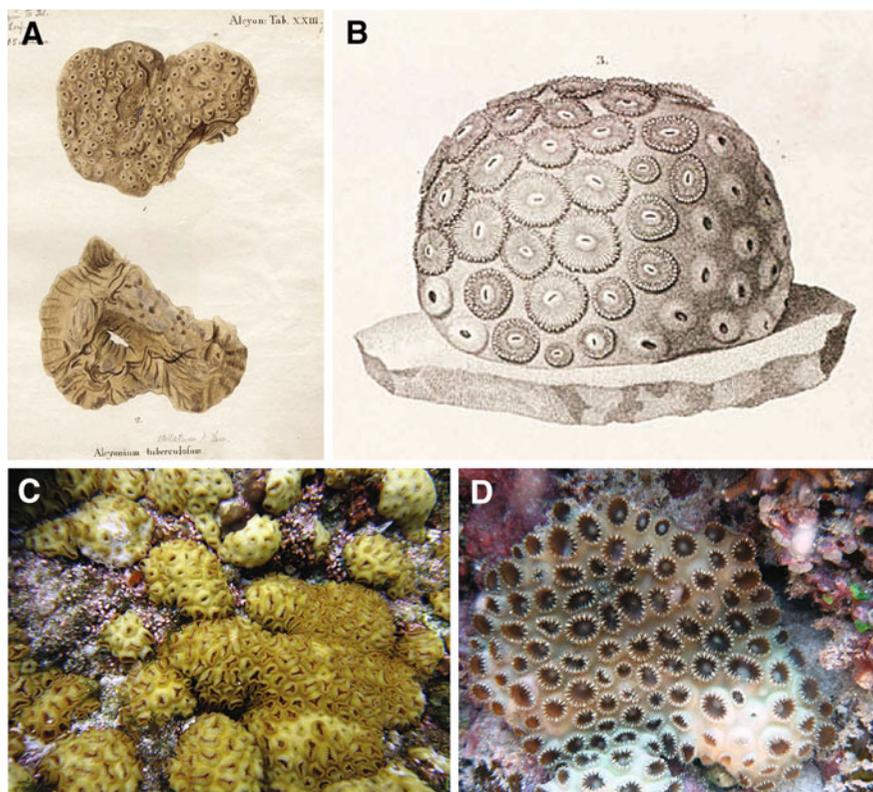


Fig. 1 Original description drawings and in situ images of **A**, **C** *Palythoa tuberculosa* (Esper, 1805) and **B**, **D** *P. caesia* Dana, 1846. **A** *P. tuberculosa* from original description in Esper (1805) (Table XXIII), and **B** drawing of *P. caesia* by Dana (1846) from original description (plate 30). **C** *P. tuberculosa* in the intertidal zone at Kabira Bay, Ishigaki, Japan (specimen not

collected), and **D** *P. caesia* specimen Heron91 from Broomfield Reef, near Heron Island, Great Barrier Reef, Australia, depth 2 m. Note both *P. tuberculosa* and *P. caesia* have large varieties of color variation (light tan to dark brown, sometimes with fluorescent yellow-green) and colony morphology (e.g., Burnett et al., 1997; Reimer et al., 2006b)

molecular techniques with additional, morphological data (tentacle number, cnidae) in an attempt to resolve the long-standing confusion surrounding these taxa.

Materials and methods

Specimen collection

A total of 75 *Palythoa* specimens from six locations in three regions were newly collected for this study (Table S1 in Supplementary Material). Specimens were collected by SCUBA or by snorkeling from depths ranging from the intertidal to 20 m. Digital in situ images with scale bars were taken for use in later morphological analyses. All specimens were preserved in 99% ethanol except for those from the Red Sea, which were initially preserved in 70% ethanol.

Palythoa tuberculosa specimens ($n = 7$ colonies) were collected from Okinawa, Japan from the following locations: Miyagi, Chatan ($26^{\circ}19'N$, $127^{\circ}44'E$) in March and September 2009 ($n = 5$ colonies), and Odo, Itoman ($26^{\circ}05'N$, $127^{\circ}42'E$) in July and August 2009 ($n = 2$ colonies) (Table S1 in Supplementary Material). Additional *P. tuberculosa* specimens were collected from Eilat, Israel, at the northern tip of the Gulf of Aqaba in the Red Sea ($29^{\circ}31'N$, $34^{\circ}57'E$) ($n = 12$ colonies) on May 30, 2011.

Specimens of *P. caesia* ($n = 56$ colonies) were collected from three areas in Australia: from sites around Heron Island ($23^{\circ}27'S$, $151^{\circ}57'E$) ($n = 30$ colonies) in November 2009; around Ningaloo Station/Reef ($22^{\circ}41'S$, $113^{\circ}41'E$) ($n = 14$ colonies) in May 2010; and around Lizard Island ($22^{\circ}50'S$, $113^{\circ}48'E$) ($n = 12$ colonies) in August 2010 (Table S1 in Supplementary Material) during the Census of Coral

Table 1 Comparison of original and re-descriptions of *Palythoa tuberculosa* (Esper, 1805) and *Palythoa caesia* Dana, 1846^a, with distribution information^b and results of this study

Character	<i>Palythoa tuberculosa</i>	<i>Palythoa caesia</i>	<i>P. tuberculosa</i> + <i>P. caesia</i> (this study)
Type locality	Tanqueray, India, and Red Sea	Fiji	
External color	Gray, rust, brown, yellow-green, or yellow-brown	Umber	White-dark brown, or yellow-green, may be mottled, may be fluorescent
Oral disc color	No data	Grayish violet	White, tan, cream, grayish-violet
Polyp size (mm)	5–10 mm (dried?)	10–15 mm lines broad when contracted	5–15 mm
Tentacle number, color	Numerous, 20–40, pale brown	30–40, umber	30–50, various colors
Distribution	India, Red Sea, Oman, Maldives, New Caledonia, Singapore, Palau, Taiwan, Japan, Hawaii, Galapagos	Fiji, Australia, Indian Ocean, Papua New Guinea, Guam, Hawaii	Subtropical + tropical Indo-Pacific

^a Data collated from Esper (1805) and Klunzinger (1877) for *P. tuberculosa*, from Dana (1846) for *P. caesia*. See also Fig. 1 for images

^b Collated from descriptions plus Burnett et al. (1994, 1997), Burnett (2002), Erhardt & Knop (2005), Hoover (1999), Paulay et al. (2003), Polak et al. (2011), Reimer & Hickman (2009), Reimer & Todd (2009), Reimer et al. (2006b, c, 2007b, 2011), Sinniger (2006), Yamazato et al. (1973)

Reef Ecosystems (Australian node), part of the Census of Marine Life (Yarincik & O'Dor, 2005).

Molecular analyses

DNA extraction and PCR amplification

DNA extraction was performed on the above specimens following the guanidine method as described in Sinniger et al. (2010). Additional molecular data from *P. tuberculosa* from previous publications are plentiful (Reimer et al., 2006b, 2007b; Reimer & Hickman, 2009; Reimer & Todd, 2009), and were also utilized in this study.

PCR amplification using template genomic DNA was performed using HotStarTaq Plus Master Mix Kit (Qiagen) following the manufacturer's instructions. Three DNA markers for *Palythoa* were targeted in this study: mitochondrial cytochrome oxidase subunit I (COI), 16S ribosomal DNA (mt 16S rDNA), and nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA), using primers and amplification conditions reported in Reimer et al. (2007a), Sinniger et al. (2010), and Reimer et al. (2007b), respectively.

All amplified products were visualized by 1.5% agarose gel electrophoresis and positive products were

treated with Shrimp Alkaline Phosphate (SAP; Takara) and Exonuclease I. Sequencing was performed by MacroGen Japan (Tokyo) or Fasmac (Tokyo).

Phylogenetic analyses

New sequences obtained in this study were deposited in GenBank (Accession Numbers KF499863–KF499987). Sequences of all three *Palythoa* DNA markers were aligned with previously reported sequences of Indo-Pacific *Palythoa* specimens (from Reimer et al., 2006b, 2007b; Reimer & Todd, 2009), including sequences from *P. tuberculosa*, *P. mutuki* Haddon and Shackleton, 1891, and *P. heliodiscus* (Ryland and Lancaster, 2003). For *P. tuberculosa*, sequences from specimens from several locations in Japan (listed in Reimer et al., 2006b, 2007b), as well as Israel, Saipan, Singapore, and Madagascar were utilized. For mitochondrial COI and 16S-rDNA alignments, sequences of *P. heliodiscus* were treated as outgroups, while for ITS-rDNA, sequences of *P. mutuki* were used as the outgroup (as in Reimer et al., 2007b).

The three *Palythoa* alignments were constructed as mentioned in Reimer et al. (2012a), following previous alignments as guides. All alignments were inspected by eye and ambiguous sites (ambiguous or double peaks,

<2 sites/alignment) were removed prior to analyses. Three alignment datasets were generated: (1) a COI alignment of 425 sites for 93 sequences, (2) a 16S-rDNA alignment of 703 sites for 117 sequences, and (3) an ITS-rDNA alignment of 603 sites for 64 sequences. All three alignments generated in this study are available from the corresponding author upon request.

For phylogenetic analyses of the three alignment datasets, the same methods were independently applied. Alignments were analyzed using Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian posterior probability methods. ML was performed using PhyML (Guindon & Gascuel, 2003) with an input tree generated by BIONJ with the general time-reversible model (Lanave et al., 1984) of nucleotide substitution incorporating a discrete gamma distribution (eight categories) (GTR+). The discrete gamma distribution and base frequencies of the model were estimated from the dataset. PhyML bootstrap trees (1,000 replicates) were constructed utilizing the same parameters as the individual ML tree. The distances were calculated using a Kimura's two-parameter model (Kimura, 1980). Support for NJ branches was tested by bootstrap analysis (Felsenstein, 1985) of 1,000 replicates. CLC Free Workbench 3.2.2 (Aarhus, Denmark) was used for NJ phylogenetic analyses (1,000 replicates).

Morphological analyses

Tentacle numbers

The descriptions of both *P. tuberculosa* and *P. caesia* include only limited information on tentacle number. Dana (1846) mentioned that *P. caesia* had "tentacles very numerous" and Klunzinger (1877) described *P. tuberculosa* as having 30–40 tentacles. In this study we examined tentacle numbers from in situ digital images taken just before specimen collection. Three to six polyps were examined for each colony, and averages and standard error were calculated for specimens from each sampling area. The average tentacle number for both *P. tuberculosa* and *P. caesia* between species were then compared by *t* test.

Cnidae

Cnidae have been used as diagnostic characteristics in some recent descriptions of *Palythoa* species (e.g., Ryland & Lancaster, 2003). Similar to Fujii & Reimer

(2011), in the present study ethanol preserved specimens were used for cnidae examination. Nematocyst nomenclature followed England (1991) and Ryland & Lancaster (2004). However, Schmidt (1974) and Hidaka et al. (1987) previously suggested basitrichs and mastigophores are in fact the same type of nematocyst, and in the present study these two types were treated as the same (basitrichs and b-mastigophores), unless they could be clearly distinguished from one another (basitrichs and p-mastigophores), in which case they were analyzed separately.

Cnidae examination procedures generally followed Reimer et al. (2012b). Undischarged nematocysts were measured from tentacles, pharynx, and mesenterial filaments of polyps (specimens examined $n = 7-9$ colonies per species). $\times 400$ digital images of the nematocysts were obtained via optical microscopy, and measured using the software ImageJ (National Institutes of Health, USA). For each type of nematocyst in each tissue, the maximum, minimum, and average dimensions (and standard error) were calculated. The average lengths of each nematocyst type (except small holotrichs—which were prone to measurement error) were plotted using Multiple Dimension Scaling (MDS). As some types of nematocyst were absent or rare in certain tissues, only the most abundant types of nematocyst in each examined tissue region were used in the MDS analyses. Therefore, the average length of spirocysts from tentacles, basitrichs from the actinopharynx, and large holotrichs and p-mastigophores from mesenterial filaments from three individuals of each population of the two species were used as input variables for the MDS. Analyses were conducted using Primer v6 (Clarke & Gorley, 2006).

Results

Phylogenetic results

Of the 89 *P. tuberculosa* and *P. caesia* COI sequences, 87 were completely identical, with two specimens' sequences from Ogasawara Islands having single base pair differences. All sequences formed a single large clade (ML = 61%, NJ = 82%, Bayes = 1.00) (Fig. 2a).

Of the 101 *P. tuberculosa* and *P. caesia* 16S rDNA sequences, 95 were completely identical over the entire 703 base pair length of the alignment. Only one sequence new to this study, from specimen *P. caesia* Ningaloo12, had one base pair difference from the

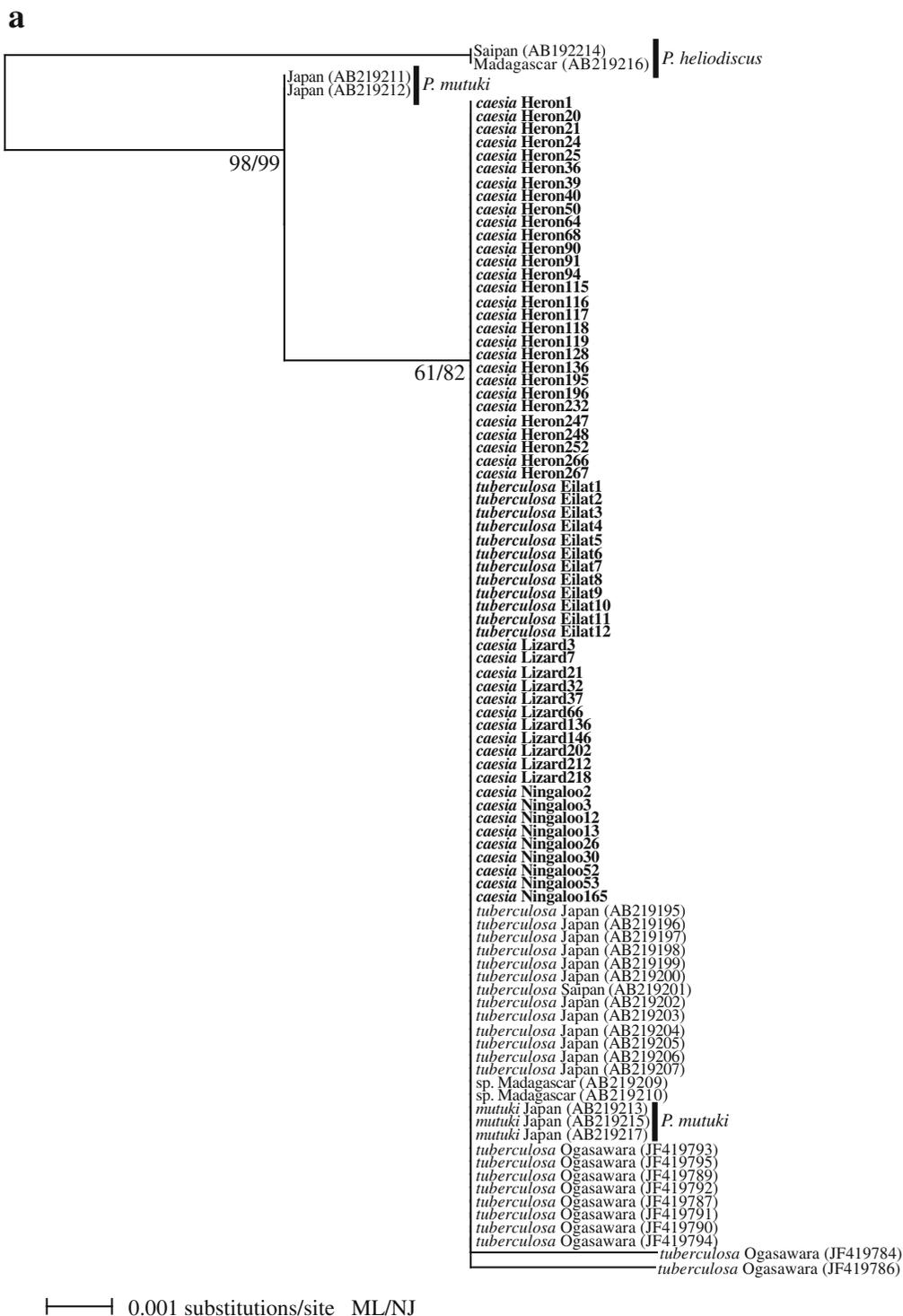
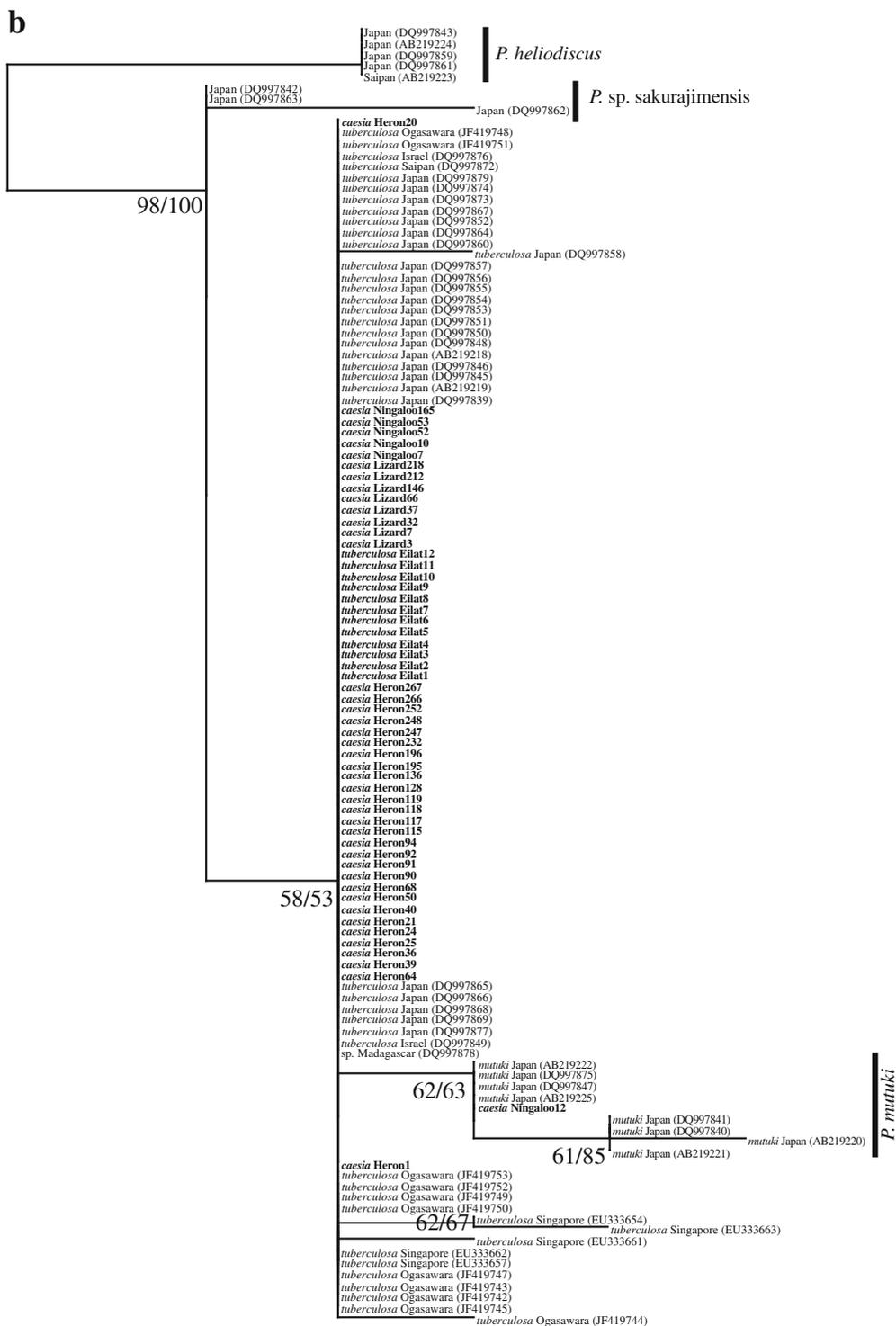


Fig. 2 Maximum likelihood (ML) trees of **a** mitochondrial cytochrome oxidase subunit I sequences, and **b** mitochondrial 16S ribosomal DNA sequences. Novel sequences from this study in **bold** (species, specimen number). Sequences (species,

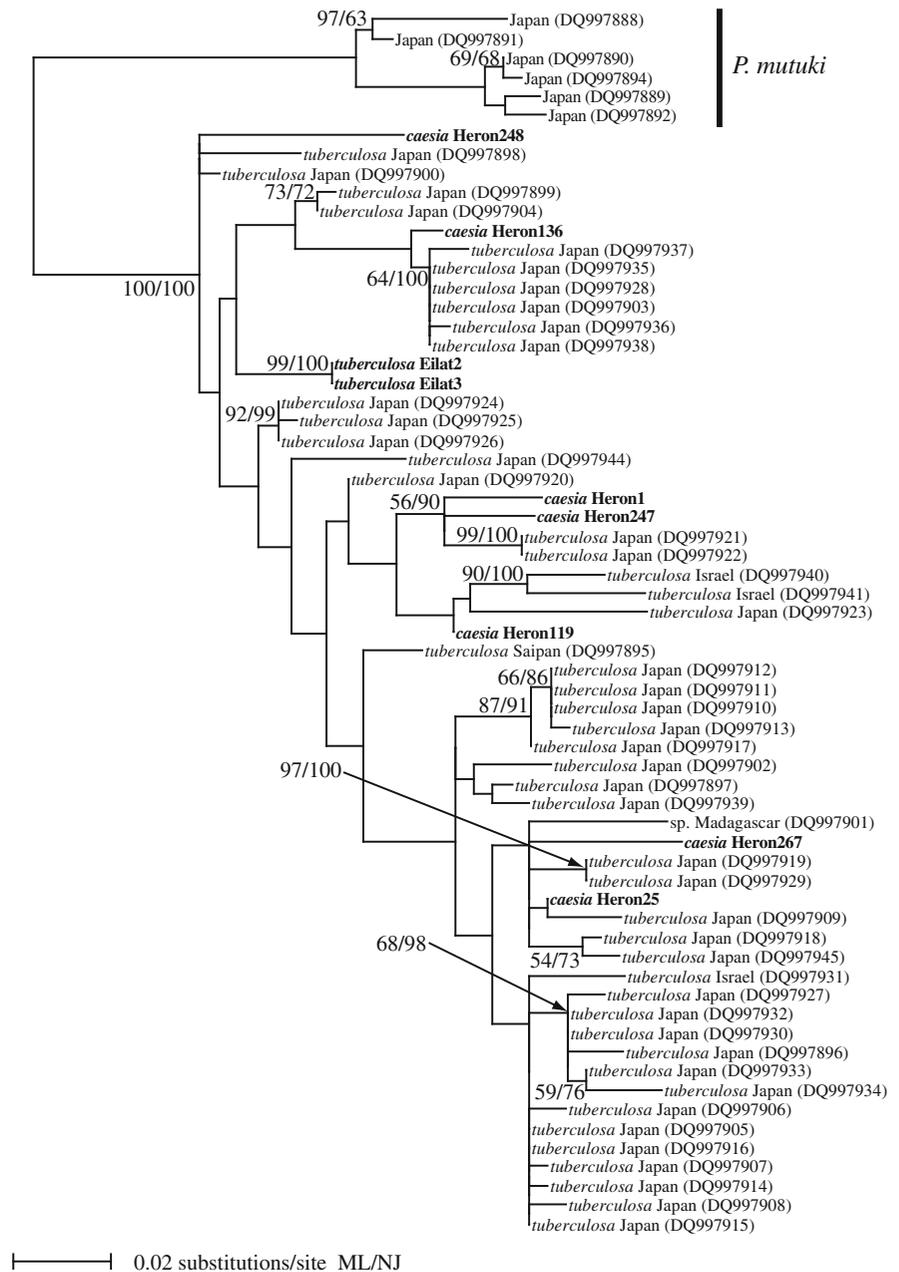
location, GenBank Accession Number) from previous studies in *regular font*. Values at nodes represent ML and neighbor-joining (NJ) values, respectively



—|— 0.001 substitutions/site ML/NJ

Fig. 2 continued

Fig. 3 Maximum likelihood (ML) trees of nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA) sequences. Novel sequences from this study in *bold* (species, specimen number). Sequences (species, location, GenBank Accession Number) from previous studies in *regular font*. Values at nodes represent ML and neighbor-joining (NJ) values, respectively



majority, and was instead identical to some *P. mutuki* sequences. All mt 16S-rDNA sequences from *P. caesia*, *P. tuberculosis*, and *P. mutuki* formed one large clade (ML = 58%, NJ = 53%) (Fig. 2b). The *P. mutuki* sequences (along with *P. caesia* Ningaloo12) were in a separate subclade within this larger clade (ML = 62%, NJ = 63%).

Despite the high percentage of variable sites seen in the ITS-1 and ITS-2 regions (ITS-1 47.4% variable sites, ITS-2 46.9% variable sites), all *P. caesia* and *P. tuberculosis* sequences formed one large clade (ML = 100%, NJ = 100%) (Fig. 3). Sequences from *P. caesia* did not form one subclade, but instead were intermingled with *P. tuberculosis* sequences.

Table 2 Average tentacle number of *Palythoa caesia* and *P. tuberculosa* from different populations

Species	Locality	<i>n</i>	Average \pm SD
<i>P. caesia</i>	Heron Island	26	41.4 \pm 5.1
	Lizard Island	15	44.1 \pm 3.2
	Ningaloo Reef	7	41.0 \pm 2.5
	Total	48	41.6 \pm 4.8
<i>P. tuberculosa</i>	Miyagi	28	34.7 \pm 4.7
	Odo	12	38.8 \pm 2.3
	Total	40	36.1 \pm 4.5

n Number of polyps examined/locality

Morphological analyses

Tentacle number

For tentacle number analyses, 11 specimens of *P. caesia* (Heron Island = 5, Lizard Island = 4, Ningaloo Reef = 2) and seven specimens of *P. tuberculosa* were examined. Eilat (Red Sea, Israel) specimens of *P. tuberculosa* could not be examined as no detailed in situ images of the collected specimens were available.

Average tentacle number (\pm standard error) for *P. caesia* was 41.6 ± 5.1 ($n = 48$ polyps), while for *P. tuberculosa* it was 36.1 ± 4.5 ($n = 40$) (Table 2). For *P. caesia*, averages for each site were as follows: Heron Island, 41.4 ± 5.1 ($n = 26$ polyps); Lizard Island, 44.1 ± 3.2 ($n = 15$); Ningaloo Reef, 41.0 ± 2.5 ($n = 7$) (Table 2). For *P. tuberculosa*, polyps from Miyagi averaged 34.7 ± 4.7 tentacles ($n = 28$ polyps), and Odo averaged 38.8 ± 2.3 ($n = 12$) (Table 2). Overall, *P. caesia* had significantly more tentacles than *P. tuberculosa* (*t* test, $P < 0.05$).

Cnidae

The cnidae of nine colonies of *P. caesia* (three from each population) and seven colonies of *P. tuberculosa* (Red Sea $n = 4$, Okinawa $n = 3$) were examined. Both *P. caesia* and *P. tuberculosa* had five types of cnidae; large holotrichs, small holotrichs, basitrichs, microbasic p-mastigophores, and spirocysts, and these were observed in all three examined areas of the polyps (tentacles, pharynx, mesenteries), albeit in variable densities.

Overall, large holotrichs and basitrichs were most abundant, particularly in the mesenteries. Small holotrichs and microbasic p-mastigophores were generally

rare, but most often observed in the mesenteries. Spirocysts were most common in the tentacles, and rare in other polyp parts. There were no obvious differences between types of cnidae between *P. caesia* and *P. tuberculosa*, or among different populations of the same species, excepting the absence of spirocysts from the mesenteries of Red Sea *P. tuberculosa* specimens. There was also no distinct grouping in the MDS results, with individuals of *P. caesia* and *P. tuberculosa* mixed together, although there were some within-location similarities (Fig. 4). *P. caesia* samples from Ningaloo Reef were closest to *P. tuberculosa* from Okinawa. *P. tuberculosa* samples from the Red Sea were mixed with those of *P. caesia* from Lizard Island. The Heron Island specimens were the only ones that separated out slightly from other sites' specimens.

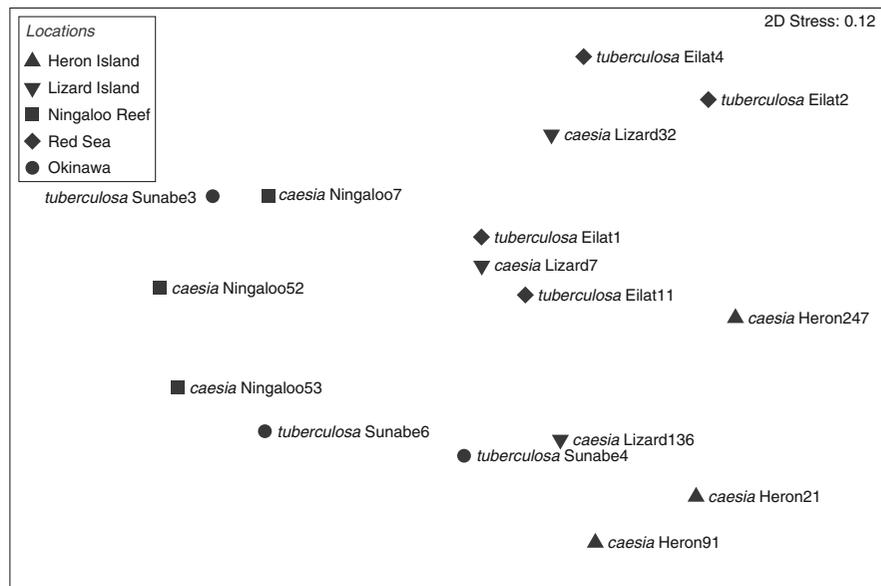
Discussion

Until now, no study has used molecular or morphological techniques to specifically compare *P. tuberculosa* and *P. caesia*. The phylogenetic results of our research match closely with those previously reported (Reimer et al., 2006b, 2007b) for *P. tuberculosa* from various Indo-Pacific locations, and all sequences formed one large clade to the exclusion of sequences from other known *Palythoa* congeners. Morphological analyses of cnidae also showed no clear distinction could be made between *P. tuberculosa* and *P. caesia*.

Within the slower-evolving mt DNA (Shearer et al., 2002; Huang et al., 2008) trees, no differences in sequences between the two putative species were observed. While these results alone may not indicate the two groups are a single species, they do show that they are phylogenetically even more closely related than *P. tuberculosa* and *P. mutuki*, which have very small (1 bp) 16S rDNA differences and are purported to have a reticulate evolutionary history (Reimer et al., 2007b; Shiroma & Reimer, 2010). The inclusion of specimen Ningaloo12 within *P. mutuki* in the mt 16S rDNA phylogenetic tree (Fig. 2b) may be due to such a history, as morphologically this specimen was 'immersae' in form, which is characteristic of both *P. tuberculosa* and *P. caesia*, and unlike the 'intermediae' or 'liberae' (=polyps less embedded in coenecium; [Pax, 1910]) form of *P. mutuki*.

Within the ITS-rDNA tree, sequences from specimens from the Red Sea, Japan, and Australia were

Fig. 4 Multiple dimension scaling results of the average lengths of primary nematocyst types (spirocysts from tentacles, basitrichs from actinopharynx, large holotrichs and p-mastigophores from mesenterial filaments) from five populations (3–4 colonies/population) of *Palythoa caesia* and *P. tuberculosa*



mixed together and, even though some small subclades were observed, there was no clear separation between *P. caesia* and *P. tuberculosa*. Another possibility, i.e., that the *P. caesia*–*P. tuberculosa* group contains several different species, is also not supported by our data, as specimens from each region were placed in various locations in the phylogenetic tree. The combined evidence from the mt DNA and ITS-rDNA strongly indicate the two putative species are in fact the same.

The morphological results show that *P. caesia* had significantly more tentacles than *P. tuberculosa*, and it would appear possible to discern between these two species based on this character. However, this is likely to be difficult in practice. One issue is that zoanthid polyps increase their tentacle number as they grow (Karlson, 1988) and it is often impossible to judge when a polyp is “fully grown”. Additionally, many zoanthid species have high levels of intraspecific morphological variation; for example, *Zoanthus sansibaricus* Carlgren, 1900 in Japan has tentacle numbers between 40 and 58 (Reimer et al., 2006a), and *Isozoanthus sulcatus* Gosse, 1859 from northern Europe has between 16 and 30 tentacles (Williams, 2000). In the present study, large variations in tentacle number were also observed (e.g., *P. caesia* from Heron and Lizard Islands 32–50 tentacles, Ningaloo Reef 36–44; *P. tuberculosa* 30–44 tentacles at Miyagi, 36–44 from Odo). Thus, as ranges overlap, assigning

individual unidentified specimens to either *P. caesia* or *P. tuberculosa* based tentacle count alone would, at best, be a challenge.

In recent research on *Palythoa*, the presence or absence of different types of cnidae in various tissues were used to discern between species. For example, Ryland & Lancaster (2004) examined differences in cnidae between *P. mutuki* and *P. heliodiscus*, and found that *P. heliodiscus* did not have basitrichs in its mesenteries or tentacles. In the current study, we observed five types of cnidae, but the only difference in populations was the lack of spirocysts in the mesenteries of *P. tuberculosa* specimens from the Red Sea. We could not distinguish between the two species based on the cnidae types in various tissues of specimens. In addition, the MDS results suggest that differences in cnidae morphology may be more related to individuals and populations; hence, the utility of cnidae sizes as accurate species-level diagnostic characters in zoanthids remains questionable. Acuña et al. (2003, 2007) have shown that cnidae sizes can vary among individuals in four species of anemones (Actiniaria), and Kamezaki et al. (2012) found that sizes of cnidae in the zoanthid *Zoanthus sansibaricus* can vary between populations separated by only a few kilometers. Ryland & Lancaster (2004) observed a similar result in *P. mutuki*, with microbasic p-mastigophore size varying by up to a factor of two among populations.

The morphological data, similar to the molecular data, indicate that *P. caesia* and *P. tuberculosa* constitute one species. The morphological variation observed within this *Palythoa* group (Burnett et al., 1997; this study) is a probably result of the ‘generalist’ nature of this species (Reimer et al., 2006c). In the very similar Atlantic sibling species *P. caribaeorum* (Duchassaing & Michelotti, 1860) morphological plasticity has been suggested to be an adaptive strategy to different environments (Costa et al., 2011). Recently, environmentally-induced changes in polyp-scale morphology have also been demonstrated in *P. tuberculosa* (Ong et al. 2013), although no work on cnidae plasticity has yet been undertaken.

Based on the molecular and morphological analyses conducted here, it is highly likely that the *P. caesia* and *P. tuberculosa* populations examined in this study are, in fact, part of one pan-Indo-Pacific species. The high dispersal potential of *P. tuberculosa* (Hirose et al., 2011), which have larvae that can survive up to 180 days (Polak et al., 2011), underlines the capacity of this species to have an extremely wide range. Other research showing *P. tuberculosa* to be a “generalist” found in a variety of environments (Reimer et al., 2006c; Ong et al. 2013) combined with this species’ high morphological variation and known plastic responses (Burnett et al., 1994, 1997; Ong et al. 2013; also Costa et al., 2011 with *P. caribaeorum*) all point towards *P. caesia* and *P. tuberculosa* being one and the same. It is even possible that many of the Indo-Pacific *Palythoa* are the same species, as first proposed by Burnett et al. (1994). Species diversity within *Palythoa* is probably much lower than has been believed in the literature, echoing recent results from the Atlantic (Reimer et al. 2012a).

For now, we strongly recommend that the binomen *P. tuberculosa* supercedes *P. caesia* in Australia, Fiji and other regions, as *P. tuberculosa* is the senior synonym, but we stop short of the formal taxonomic merging of the two species pending analyses of type specimens.

Acknowledgments The corresponding author was supported by the Rising Star Program, and the International Research Hub Project for Climate Change and Coral Reef/Island Dynamics at the University of the Ryukyus. In Australia, the Census of Coral Reef Ecosystems (CReefs) Australia Project, and in particular Dr. Julian Caley and Shawn Smith (both AIMS) are thanked for logistical support. Specimens from Australia were collected under Great Barrier Reef Marine Park Authority permit

#G32313.1 and Queensland Fisheries permit #9512, and from Ningaloo Reef under Western Australia’s Department of Environment and Conservation Permit #SF007428. Field work at Eilat (Red Sea) was supported by the Israel Cohen Chair in Environmental Zoology to YB and collection there complied with a permit issued by the Israel Nature and National Parks Protection Authority. YB would like to thank the staff of the Interuniversity Institute for Marine Science in Eilat (IUI) for their kind hospitality and facilities. Profs. Shoichiro Suda and Euichi Hirose reviewed an earlier version of this manuscript. Two anonymous reviewers’ comments greatly improved the manuscript.

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