Genetic diversity of the *Acropora*-associated hydrozoans: new insight from the Red Sea

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Abstract

To date, four nominal species and several other unidentified species of *Zanclaea* hydrozoans are known to live symbiotically with scleractinians, and recent surveys reported this association also in the Red Sea. Previous molecular studies showed that each coral genus involved in this association hosts only one species or molecular clade of *Zanclaea*, with the only exception being the genus *Acropora*, which hosts at least two *Zanclaea* species. Moreover, some of the detected genetic lineages were morphologically undistinguishable in the polyp stage, suggesting the presence of cryptic species. In this study, we investigated the morphology and genetic diversity of *Acropora*-associated *Zanclaea* specimens collected in previous studies in Egypt and Israel, as well as new samples collected in Saudi Arabia. Based on the current data, all the analysed samples were morphologically identical to *Zanclaea gallii*, a species associated with *Acropora* corals from the Maldives. However, molecular analyses separated the samples collected in the Red Sea from all other coral-associated hydroids. Therefore, phylogenetic reconstructions, haplotype networks, genetic distance analyses and distribution data allowed us to identify a previously unknown cryptic species of *Acropora*-associated hydroid, here named *Zanclaea gallii* IIa, following a recently proposed molecular nomenclature.

Keywords

Symbiosis

Zanclaea

Integrative taxonomy

Cryptic species

Biodiversity

Coral reefs

Molecular nomenclature

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Introduction

The Red Sea is a hotspot for endemism, hosting high percentages of endemic taxa (Guinot 1967; Campbell 1987; Randall 1994; Hughes et al. 2002; Shenkar 2012; DiBattista et al. 2016a, b) and outstanding biodiversity, with the list of marine organisms being continuously revised due to descriptions of new nominal and cryptic species (e.g. Tornabene et al. 2013; Terraneo et al. 2014; Alamaru et al. 2016; Arrigoni et al. 2016). Traditionally, coral reef research has predominantly focused on the most conspicuous organisms, such as fish and corals, and non-coral invertebrates have often been overlooked (Stella et al. 2011). Since the latter group is known to provide the greatest biodiversity to coral reef environments (Bouchet et al. 2002; Stella et al. 2011; Appeltans et al. 2012) and to harbour a high cryptic diversity (Knowlton 2000), the magnitude of the Red Sea fauna is likely to be underestimated in terms of species richness (Berumen et al. 2013). Among invertebrates, hydrozoans have been poorly investigated in the Red Sea and a total of 160 valid species of non-siphonophoran hydrozoans have been recorded so far (Gravili et al. 2013; Seveso et al. 2015; Pica et al. in press). Only one of these species is known to occur solely in the Red Sea, i.e. *Zanclaea eilatensis* Pica, Bastari & Puce living in association with bryozoans, whereas all other species are reported also from other localities of the Indo-Pacific. Recently, Montano et al. (2014) and Pica et al. (in press) reported the presence of coral-associated hydrozoans belonging to the genus *Zanclaea* Gegenbaur, 1856 in Dahab and Eilat, respectively, extending the geographic distribution and the host range of this symbiosis, and confirming the hypothesis that this association is more widespread than previously known (Millard and Bouillon 1974; Boero et al. 2000; Pantos and Bythell 2010; Hirose and Hirose 2012; Fontana et al. 2012; Montano et al. 2013, 2015a, b, c, 2016a). Molecular phylogenetic analyses have revealed that *Zanclaea* hydroids are associated with their coral hosts with a variable degree of specificity, resulting in either generalist interactions or genus-specific associations (Fontana et al. 2012; Montano et al. 2015b). Moreover, despite very low or even undetectable morphological variation, *Zanclaea* hydrozoans living in symbiosis with corals show a remarkable genetic diversity and some of the recovered lineages could represent independent species. This genetic diversity might be explained by host-specificity, since each molecular clade is associated with either a specific host genus or a group of genera (Montano et al. 2015b). Schuchert (2014) also found a similar strong genetic structure for the hydrozoan *Plumularia setacea*, likely due to geography, and highlighted that choosing different species concepts when discussing the species status of populations can result in opposite conclusions. For instance, different monophyletic clades identified with multiple markers and belonging to the same morphospecies constitute a

species complex following the genealogical species concept (Baum and Shaw 1995) or an extensive population subdivision according to morphology-based species definitions.

In the Maldives, 13 genera of corals were examined, and each was found to host its own unique genetic clade of *Zancklea* (Montano et al. 2015b). Contrarily, the species *Zancklea margaritae* Pantos & Bythell, 2010, from the Great Barrier Reef, and *Zancklea gallii* Montano, Maggioni & Puce, 2014, from the central Maldives and the northern Red Sea, are both known to live in strict association with the same coral genus, i.e. *Acropora* Linnaeus, 1758 (Pantos and Bythell 2010; Montano et al. 2015a; Pica et al. in press). Fontana et al. (2012) and Montano et al. (2014) also found *Zancklea* polyps associated with different *Acropora* species from Taiwan and Egypt (Dahab), respectively, but they did not perform detailed morphological analyses, leaving those *Zancklea* colonies unidentified. The two nominal *Acropora*-associated *Zancklea* species differ morphologically in the cnidome of the polyp and medusa stages, but no molecular data are available for *Z. margaritae*. DNA sequences are, so far, available only for *Z. gallii* from the Maldives and for *Zancklea* sp. from Taiwan, and molecular phylogenetic analyses have revealed that these two organisms are closely related but genetically distinct (Montano et al. 2015a). Due to the lack of morphological and molecular data, the real diversity of *Acropora*-associated hydrozoans is still unclear and the presence of other *Acropora*-associated *Zancklea* species cannot be excluded.

Recently, molecular analyses have also shown variable levels of cryptic diversity and host fidelity in other coral-associated invertebrates, and, in some cases, a cryptic radiation similar to *Zancklea* hydrozoans has been demonstrated (e.g. Gittenberger and Gittenberger 2011; van der Meij et al. 2015). For instance, coral endo-parasitic snails belonging to the genus *Leptoconchus* Rüppell, 1835 have a variable and irregular morphology, and closely related species associated with specific hosts could be detected only through molecular analyses. In this case, DNA sequence alignments were the basis of putative diagnostic analyses, but details of the diagnostic nucleotides were not explained (Gittenberger and Gittenberger 2011). Several other studies have included DNA information in taxonomic descriptions, but, in most cases, in a non-standardised way (reviewed by Goldstein and DeSalle 2011; Renner 2016). Many of the cryptic species detected with this approach still lack a formal description since molecular characters were not presented as diagnostic characters (Jörger and Schrödl 2013). Species delineation methods are now emerging as valuable, rapid tools for cryptic species detection, especially for organisms with few obvious diagnostic morphological characters (Fontaneto et al. 2015), but, when used alone, they do not qualify for character-based species descriptions, as requested by the International Code of Zoological Nomenclature (ICZN, 1999, Article 13.1.1). Character-based approaches, such as the Characteristic Attribute Organization System (CAOS), could, therefore, be used as tools to establish diagnostic characters from DNA sequences to be directly used in species descriptions (Sarkar et al. 2008; Zou et al. 2011; Jörger and Schrödl 2013). This approach has allowed taxonomists to formally describe some cryptic taxa identified with molecular methods at both the species and the genus levels (e.g. Churchill et al. 2014; Johnson et al. 2015; Shipman and Gosliner 2015; Zielske and Haase 2015; Scarpa et al. 2016).

However, in most cases, cryptic species remain nameless because of the lack of additional evidences other than molecular data and, when named, the use of DNA alone could potentially lead to the establishment of a parallel nomenclature, especially if only few molecular markers are investigated. To prevent that, Morard et al. (2016) recently proposed a novel nomenclatural system to be applied to taxa showing a structured genetic diversity occurring below the level of morphospecies, viz. cryptic species. This system allows to classify any genetic data associated to molecular operational taxonomic units (MOTUs) of any level; it should follow the rules of an interim taxonomic system, which are uniqueness of the name employed, stability and proper definition of the given ranks

(Schindel and Miller 2010); it should be flexible and facilitate the transfer of an MOTU into the formal nomenclature as soon as new relevant evidences are provided. The advantage of this method is that it allows a reliable association of genetic units to any other type of data without creating taxonomical confusion, and permitting their inclusion in future research, such as biodiversity assessments, endemism studies or conservation efforts (Morard et al. 2016).

Here, we provide an updated phylogenetic reconstruction of coral-associated *Zanclaea* hydrozoans, including both morphological and molecular analyses of *Acropora*-associated specimens collected from four localities in the Red Sea (utilising both existing and new materials). Based on the DNA sequence results, we demonstrate the presence of a cryptic species of coral-associated *Zanclaea* in the Red Sea, identify its diagnostic molecular characters and propose a nomenclature for the *Acropora*-associated clade based on the method described by Morard et al. (2016).

Materials and methods

Fragments of *Acropora* spp. bearing *Zanclaea* hydrozoans were collected during three separate field surveys conducted in Dahab, Egypt, in October 2013 (Montano et al. 2014), in Eilat, Israel, in May 2014 (Pica et al. in press) and in Al Lith and Thuwal, Saudi Arabia, in December 2015 (new material presented in this study) (Fig. 1). Corals were collected in situ by scuba diving. Fragments of corals bearing hydrozoans were carefully detached from the colony using a chisel, samples were placed into plastic bags underwater and were immediately placed in water tanks after the dive. In the lab, colonies were observed under a Leica EZ4 D stereo microscope and hydroids were carefully detached one by one using syringe needles and micropipettes. Samples were fixed in 4% formalin and in 99% ethanol (EtOH) for morphological and molecular characterisations, respectively. Morphology was studied using a Zeiss Axioskop 40 microscope and pictures were taken with a Nikon AW100 camera.

Fig. 1

Map of the Red Sea. The *arrowheads* indicate the four sampling localities for *Zanclaea* hydroid species

DNA from ethanol-preserved samples was extracted following a protocol modified from Zietara et al. (2000). Five molecular markers were amplified: a portion of the nuclear 28S rRNA (~1700 bp), 18S rRNA (~1700 bp) and ITS (~700 bp), and a portion of the mitochondrial 16S rRNA (~400 or ~600 bp) and COI (~700 bp). The polymerase chain reaction (PCR) assays were set up using the same protocols and primers described by Maggioni et al. (2016), Medlin et al. (1988), Fontana et al. (2012), Cunningham and Buss (1993) and Folmer et al. (1994), respectively. All PCR products were purified with Illustra ExoStar (GE Healthcare, Buckinghamshire) at 37° for 60 min, followed by 85° for 15 min, and directly sequenced in forward and reverse directions using an ABI 3730xl DNA Analyzer (Applied Biosystem, Foster City, CA, USA). The sequences were deposited in GenBank with related information on host, locality and voucher number (accession numbers: LN714109, LN714231, LT606967–LT607018). Sequences were aligned with other sequences of coral-associated *Zanclaea* and outgroups retrieved from GenBank and DRYAD (doi: 10.5061/dryad.g0b20), using MAFFT 7.110 (Katoh and Standley 2013) with the E-INS-i option. The sequences were combined in a concatenated dataset and PartitionFinder 1.1.1 (Lanfear et al. 2012) was used to determine the appropriate partition and molecular models (16S+COI: GTR+I+G; 28S+ITS: GTR+G; 18S: HKY). Bayesian inference (BI) and maximum likelihood (ML) were used to infer phylogenetic relationships for both single-locus and multi-locus datasets. BI analyses were performed using MrBayes 3.2 (Ronquist et al. 2012). Four parallel Markov chain Monte Carlo (MCMC) runs were run for 2×10^6 generations. Trees were sampled every 100th generation and burn-in was set to 25%. ML trees were built with Garli 2.01 (Zwickl 2006). Non-parametric bootstrap values were calculated from 1000 replicates, each based on five heuristic search replicates; the resulting trees were read into the SumTrees 4.0.0 program in the DendroPy 4.0.0 package (Sukumaran and Holder 2010) to obtain bootstrap support values and to map them on the best ML tree. The genetic distances (uncorrected p-distance, 1000 bootstraps) within and among the obtained clades were also estimated for each mitochondrial locus using MEGA 6 (Tamura et al. 2013). Finally, haplotype networks for mitochondrial loci were constructed in Network 4.6.1.2 (<http://www.fluxus-technology.com>) using the median-joining algorithm (Bandelt et al. 1999) and default settings. We decided to interpret the molecular results following the genealogical species concept (Baum and Shaw 1995), according to which a genealogical species is a monophyletic group recovered by multiple molecular markers (Hudson and Coyne 2002). In order to identify diagnostic nucleotides for the newly recovered species, we used the software CAOS (Sarkar et al. 2002, 2008; Bergmann et al. 2009), following the instructions of Jörger and Schrödl (2013, 2014). We considered as diagnostic characters only single pure character attributes, which are single nucleotides present in all members of a clade identified as a species, but absent in members of other clades (Jörger and Schrödl 2014). We then used the nomenclatural system recently proposed by Morard et al. (2016) in order to name the recovered clades. This method is based on the definition of basetypes and the use of their hierarchical phylogenetic structure to define levels of divergence below that of morphospecies. A basetype is defined as a specific DNA substitution pattern observed within a single marker gene, whereas a basegroup is a set of basetypes and constitute the lowest MOTU level. If a gene exists in a unique version, a basegroup will contain only one basetype, otherwise it will contain all pairs of basetypes observed to co-occur in one individual. The genetic variability between the basegroup and the morphospecies is then used to identify intermediate levels at different degrees of divergence, and any of these levels could be theoretically considered a species hypothesis. Similarly to Morard et al. (2016), we propose to define basetypes in hydrozoans by using sequence patterns in a widely used barcode gene in the group, namely the 16S rRNA.

Results

Morphological observation of Red Sea *Acropora*-associated hydroids revealed morphology

consistent with the description of *Zancklea gallii* in all specimens. No morphometric differences were found among all the examined polyps (see ‘Taxonomic section’). However, according to molecular analyses, specimens from the Red Sea formed a *Zancklea* clade divergent from the Indian Ocean clade and, based on these results, we characterise the Red Sea group as a cryptic species of *Zancklea gallii*.

Molecular results

The total alignments of the concatenated dataset was 5072 bp long and included representatives of all the coral-associated *Zancklea* clades detected by Fontana et al. (2012) and Montano et al. (2015a, b). According to the five-loci tree as well as all single-locus analyses, the coral-associated *Zancklea* clade **was** monophyletic, with high support values (Fig. 2a, Figs. S1–S5). Mitochondrial markers **showed** the highest resolution of molecular lineages within the scleractinian-associated *Zancklea* clade (Figs. S1 and S2), and the same clades as in Montano et al. (2015b) **were** recovered. All hydrozoans associated with *Acropora* corals from different localities **formed** a highly supported monophyletic lineage, which comprises **specimens** from the Maldives, Taiwan and the Red Sea (Fig. 2a). In addition, the *Acropora*-associated clade **showed** a further genetic diversification (Fig. 2a, b), and hydroids collected from the three different areas **formed** three distinct well-supported subclades.

Fig. 2

Molecular diversity of coral-associated *Zancklea*. **a** Bayesian phylogenetic hypothesis of coral-associated *Zancklea* hydrozoans based on the concatenated dataset of COI, 16S rRNA, 18S rRNA, 28S rRNA and ITS genes. Clade support values are posterior probabilities from Bayesian inference and bootstrap values from maximum likelihood analyses, respectively. Different clades of *Acropora*-associated hydrozoans are highlighted with different colours and are named according to the molecular nomenclature applied to the 16S rRNA tree. The node supporting the scleractinian-associated *Zancklea* clade is highlighted in *red*. **b, c** Most parsimonious median-joining networks of *Zancklea* associated with *Acropora* inferred from mitochondrial genes 16S rRNA (**b**) and COI (**c**). The size of the circles is proportional to the frequencies of specimens sharing the same haplotype. The colours of the circles refer to clades found in **a**

According to this genetic structure, we used the 16S rRNA tree to identify two levels of divergence below the morphospecies level (Fig. S1) and we used them to apply to the group the nomenclatural system proposed by Morard et al. (2016) (Fig. 2). Level one (indicated with Roman numerals) corresponds to a monophyletic cluster of intermediate-level MOTUs and likely represents at least the level of species; level two (indicated with lowercase letters) is a monophyletic cluster of basegroups and likely corresponds to the level of species or population.

Specimens growing on *Acropora* from the Maldives correspond to the type population of *Zanclea gallii* (*Zanclea gallii* Ia) and form the sister clade of *Zanclea* sp. from Taiwan, here named *Zanclea gallii* Ib. These two groups cluster in a monophyletic lineage and are separated by low genetic distances for 16S rRNA (Table 1), whereas there are no available COI sequences for Taiwanese samples. A third monophyletic clade formed by all the sequences from the Red Sea obtained in this study and corresponding to *Zanclea gallii* IIa **wasis** highly divergent from the other two *Acropora*-associated clades and the genetic distances between *Zanclea* from the Red Sea and *Zanclea* from the Maldives and Taiwan **wereare** high, with a mean of 4% for 16S rRNA and 8% for COI (Table 1). Conversely, intra-clade distances **whereare** generally low, ranging from 0.2% for 16S rRNA sequences of Maldivian specimens to 0.8% for COI sequences of samples from the Red Sea. A total of seven and 11 haplotypes for COI and 16S rRNA, respectively, were obtained from *Zanclea* associated with *Acropora*; median-joining networks for each marker are shown in Fig. 2b, c. Both networks **are were** congruent with the phylogenetic reconstruction and they **wereare** similar to each other, with the only exception being the absence of COI sequences of *Zanclea* from Taiwan. *Zanclea*

specimens from the Red Sea ~~were~~~~are~~ separated from other clades by 12 substitutions in the 16S rRNA network and 41 substitutions in the COI network, whereas Maldivian and Taiwanese specimens ~~were~~~~are~~ separated by two substitutions in 16S rRNA network. Interpreting these genetic results within the framework of the genealogical species concept, all the specimens of *Acropora*-associated *Zanclaea* hydrozoans from the Red Sea constitute an independent lineage ascribable to an independent species and CAOS analysis identified 16 diagnostic positions in the 16S rRNA and COI sequences for this group (Table 2).

Table 1

Pairwise comparisons of genetic distance (uncorrected p-distance in %) within and between *Zanclaea* clades associated with *Acropora*, based on the mitochondrial genes 16S rRNA and COI. Standard deviations are in parentheses

	<i>Zanclaea gallii</i> Ia		<i>Zanclaea gallii</i> Ib		<i>Zanclaea gallii</i> IIa	
	16S rRNA	COI	16S rRNA	COI	16S rRNA	COI
<i>Zanclaea gallii</i> Ia	0.2 (0.2)	0.3 (0.1)				
<i>Zanclaea gallii</i> Ib	1.1 (0.5)	n.c.	0.3 (0.3)	n. c.		
<i>Zanclaea gallii</i> IIa	4.1 (1.1)	8.1 (1.3)	4.4 (1.1)	n. c.	0.3 (0.2)	0.8 (0.2)

Table 2

Molecular diagnostic characters of *Zanclaea gallii* IIa

Marker	Diagnostic characters with position in alignment (in holotype -reference sequence)
COI	20 (48), G; 38 (66), G; 54 (82), T; 71 (99), T; 98 (126), G; 143 (171), C; 158 (186), G; 242 (270), C; 308 (336), T; 374 (402), G; 389 (417), G; 503 (531), C; 635 (663), G
16S rRNA	161 (297), T; 203 (339), G; 233 (369), G

Taxonomic section

Phylum Cnidaria Verrill, 1865

Class Hydrozoa Owen, 1843

Order Anthoathecata Cornelius, 1992

Family Zancleidae Russell, 1953

Genus *Zanclaea* Gegenbaur, 1856

Zanclaea gallii IIa

Materials examined Sample KA037, Abu Latt Island, Al Lith, Saudi Arabia (19.946 N, 40.151 E), depth: 8 m, 11 December 2015. Colony growing on *Acropora* sp. preserved in 99% EtOH and 6% formalin. GenBank accession numbers for reference DNA sequences are LT607016 for COI, LT607008 for 16S rRNA, LT606979 for 18S rRNA, LT606992 for 28S rRNA and LT607002 for ITS. - Sample KA074, Tahla Reef, Thuwal, Saudi Arabia (22.274 N, 39.047 E), depth: 14 m, 15 December 2015, preserved in 99% EtOH. - Sample KA148, Abu Geisha Reef, Thuwal, Saudi Arabia (22.427 N, 39.051 E), depth: 4 m, 16 December 2015, preserved in 99% EtOH. - Sample EA003

(DA001), Dahab, Egypt (28.467 N, 34.500 E), October 2013, preserved in 99% EtOH. - Sample EA004 (DA002), Dahab, Egypt (28.467 N, 34.500 E), October 2013, preserved in 99% EtOH. - Sample EAC1, Dahab, Egypt (28.467 N, 34.500 E), November 2013, preserved in 99% EtOH. - Sample HYD_57 (EI020), Eilat, Israel (29.502 N, 34.917 E), 12 May 2014, preserved in 99% EtOH.

Morphological description Colonies live in association with the scleractinians *Acropora* spp. (Fig. 3). Gastrozooids arise from coral tissues, mainly near corallites and sometimes between corallites (Fig. 3a). Coral tissues surround the base of hydranths with collar-like elevations (Fig. 3b). Perisarc around the polyp base was not observed. Gastrozooids are cylindrical and range from 0.7 to 1.4 mm in height (Fig. 3b, c). The hypostome is surrounded by five capitate tentacles (diameter of capitula 40–45 μ m), whereas the body of the hydranth is surrounded by 15–25 scattered capitate tentacles (diameter of capitula 30–35 μ m) (Fig. 3c). Up to two medusa buds at different stages of maturation grow at the base of gono-gastrozooids (Fig. 3c, d). The cnidome is composed of stenoteles of two sizes **classes** (10–11 $\times$ 9–10 μ m and 6–7 $\times$ 5–6 μ m), which are confined in the capitula (Fig. 3e). Living hydrants are transparent, with a white hypostome. Dactylozooids and medusae were not observed. CAOS analysis identified 13 diagnostic positions in COI and three diagnostic nucleotides in 16S rRNA (see Table 2 for the complete list of diagnostic positions).

Fig. 3

Zancklea gallii IIa. **a** In situ photograph of a colony associated with *Acropora* sp. (modified from Montano et al. 2014). **b** Gastrozoid showing the basal collar of tissue produced by the acroporid host. **c** Micrograph of a detached gono-gastrozoid (modified from Montano et al. 2014). **d** Close-up of two medusa buds at different stages of maturation. **e** Cnidome of the polyp, composed of stenoteles of two sizes. **f** Colony arising from a recently bleached *Acropora* sp. Scale bars: **a**, **f** 2 mm; **b**, **c** 100 μ m; **d** 50 μ m; **e** 5 μ m

Habitat, relationship with the host and distribution *Zancklea gallii* IIa was found at depths ranging from 5 to 27 m (this study; Pica et al. in press) and living in association with corals belonging to the genus *Acropora*. *Zancklea* colonies were found on both healthy and completely bleached corals

(Fig. 3f).

The known geographic range for this species extends from the northern tip of the Red Sea (Eilat, Israel, in the Gulf of Aqaba) to the south-central Red Sea (Al Lith).

Remarks Specimens from Dahab were previously identified as *Zancklea* polyps by Montano et al. (2014), whereas Pica et al. (in press) considered the specimens from Eilat as belonging to *Zancklea gallii*. Indeed, our description of the polyp stage of *Zancklea gallii* IIa is completely consistent with the description of *Z. gallii* Ia from the Maldives and both the general morphology and the morphometric measurements are almost identical in the two species. Slight differences are detectable in the size range of large stenoteles ($11\text{--}15 \times 10\text{--}14\text{ }\mu\text{m}$ in Maldivian *Z. gallii*) and in the diameter of capitula (about $10\text{ }\mu\text{m}$ bigger in Maldivian *Z. gallii*), but these minor variations are not enough to morphologically discriminate between the two species reliably. By contrast, polyps of the other *Acropora*-associated species from Australia, i.e. *Zancklea margaritae*, are easily recognisable thanks to the presence of isorhiza capsules. Dactylozooids in *Z. margaritae* are highly retractile and can retract almost completely in host tissues when disturbed (Pantos and Bythell 2010). In Maldivian *Z. gallii* Ia colonies, they are very rare and only in situ pictures are available to document their presence (Montano et al. 2015a). For these reasons, the fact that dactylozooids were not observed in *Z. gallii* IIa might be due to the cryptic nature of these specialised polyps and uncertainties remain about the polymorphic or monomorphic state of the species. *Zancklea gallii* Ia and *Z. margaritae* differ in the medusa stage for the presence of mastigophores in the exumbrella of the latter. Throughout our surveys, we never found mature medusa buds, so morphological observations of newly liberated medusa were, therefore, not possible for *Z. gallii* IIa. However, despite the conserved morphology and the missing medusa data, the distinction can be easily made based on distribution data and DNA sequences, and we, therefore, consider *Z. gallii* Ia from the Maldives and *Z. gallii* IIa from the Red Sea as independent species.

Discussion

Few hydroid species are found in strict association with other members of Cnidaria, and most of them are epibiont of octocorals (Puce et al. 2008a; Bo et al. 2011; Maggioni et al. 2016; Montano et al. 2016b). The hydrozoan genus *Zancklea* comprises species associated with a wide range of hosts, including both octocorals and hexacorals (Boero et al. 2000; Puce et al. 2008b). In particular, the species *Zancklea timida* Puce, Di Camillo & Bavestrello, 2008 is known to grow epibiotically on the octocoral *Paratelesto* sp. (Puce et al. 2008b), whereas *Zancklea gilii* Boero, Bouillon & Gravili, 2000, *Zancklea sango* Hirose & Hirose 2011, *Z. margaritae* and *Zancklea gallii* are symbionts of scleractinian corals (Boero et al. 2000; Pantos and Bythell 2010; Hirose and Hirose 2012; Montano et al. 2015a). Describing the first species of *Zancklea* associated with scleractinians, i.e. *Z. gilii*, Boero et al. (2000) hypothesised that coral-associated *Zancklea* hydroids might have had a radiation similar to bryozoan-associated *Zancklea* species, but they stated that they found just one species, despite inspecting several corals. Subsequent surveys confirmed this hypothesis, with the description of three other species associated with scleractinian corals from different localities (Pantos and Bythell 2010; Hirose and Hirose 2012; Montano et al. 2015a). Moreover, molecular analyses revealed the existence of several other lineages identified as *Zancklea* spp. and associated with 11 coral genera from central Maldives (Montano et al. 2015b). These lineages showed a great genetic diversity and might, therefore, be ascribable to independent species, some of which are morphologically indistinguishable, at least regarding the polyp stage (Montano et al. 2015b). Although these molecular lineages showed different levels of host-specificity, all clades were specifically associated with a single coral genus or group of genera, with no overlap of two or more lineages on the same coral host. Fontana et al. (2012) analysed the molecular diversity of *Zancklea*

hydroids associated with 14 *Acropora* species from Taiwan, finding a very low genetic diversity among all the *Zanclaea* specimens, thus hypothesising a genus-specific association of a single *Zanclaea* species. However, two coral-associated *Zanclaea* nominal species, i.e. *Z. margaritae* and *Z. gallii*, were described in association with the same acroporid host from other localities (Pantos and Bythell 2010; Montano et al. 2015a), suggesting a more complex situation for *Acropora*-associated hydroids. Both host-specificity and other factors, such as geography, might have, ~~therefore~~, shaped the genetic diversity of *Zanclaea* growing on *Acropora*, and this scenario is confirmed by the present detection of *Zanclaea gallii* IIa. Previously collected *Acropora*-associated *Zanclaea* from Dahab were formerly unidentified (Montano et al. 2014), and colonies from Eilat were identified as *Z. gallii* (Pica et al. in press) due to their resemblance with the latter, but according to our molecular analyses, they both belong to a divergent independent lineage. Even though molecular investigations clearly separated *Z. gallii* IIa and *Z. gallii* in two distinct and divergent clades, also supported by high mitochondrial genetic distances, our morphological and morphometric investigations of the polyps did not allow any distinction between them.

The three *Acropora*-associated species appear at this stage to have non-overlapping geographic distributions: *Z. gallii* Ia is found in Maldivian reefs, *Z. margaritae* in the Great Barrier Reef and *Z. gallii* IIa in the Red Sea. Sequences of *Zanclaea* growing on *Acropora* from Taiwan analysed by Fontana et al. (2012) are highly divergent from *Z. gallii* IIa but not from *Z. gallii* Ia, and they form a monophyletic clade with the latter. Comparing genetic distances and haplotype networks for the three *Zanclaea* groups, *Z. gallii* IIa was clearly separated from the other two, whereas the distinction between *Z. gallii* Ia and *Zanclaea gallii* Ib was not so apparent and doubts remain about their conspecificity. Since no exhaustive molecular data and no morphological data are available for Taiwanese *Zanclaea*, these specimens might constitute a divergent population of *Z. gallii* Ia, *Z. margaritae* or another independent species and further studies are needed to address this question. Moreover, the phylogenetic position of *Z. margaritae* is still to be assessed since it has not been included yet in molecular analyses, and future molecular studies might theoretically identify one of the already known genetic lineages with this species.

Although the cnidome composition separates *Z. margaritae* from all other coral-associated hydrozoans, this species shares several morphological traits with *Z. gallii* Ia and *Z. gallii* IIa, notably the absence of euryteles and possibly the loss of perisarc (Pantos and Bythell 2010; Pantos and Hoegh-Guldberg 2011; Montano et al. 2015a). These characteristics suggest a highly integrated and dynamic relationship between hydroids and their acroporid hosts, in particular because the absence of perisarc allows the direct anchoring of hydranths on coral skeleton through desmocytes (Puce et al. 2002; Pantos and Hoegh-Guldberg 2011). Conversely, in other coral-associated clades, the presence of the perisarc, the low host-specificity or both these conditions might indicate a less specialised host–symbiont interaction (Hirose and Hirose 2012; Montano et al. 2015b), but this scenario needs to be confirmed through a more comprehensive survey of all coral-associated clades.

Despite the hypothesised more intimate relationship between *Zanclaea* and *Acropora*, this coral genus is, so far, the only one found in association with more than one *Zanclaea* species or molecular clade. The high diversity of *Acropora*-associated *Zanclaea* might be explained by its biogeography, especially regarding *Z. gallii* IIa, since this species was found exclusively in the Red Sea, a biodiversity and endemism hotspot (Hughes et al. 2002). The Red Sea is isolated from the Indian Ocean by a narrow and shallow entrance (Eshel et al. 1994; Bailey 2010) and is characterised by heterogeneous environmental conditions of both inner and bordering waters, including salinity, temperature and productivity fluctuations (Sofianos and Johns 2003; Raitsos et al. 2013). These unusual environmental conditions, along with a peculiar geological history, likely contribute to the high number of endemic species (see DiBattista et al. 2016a, b for reviews) and might have played

a role also in the speciation of *Z. gallii* IIa, even though further investigations in the southern Red Sea, the Gulf of Aden and nearby areas are needed to fully assess the endemism of this species. Another possible explanation of the *Acropora*-related *Zanclus* diversity could refer to the ecological and evolutionary traits of *Acropora* corals. *Acropora* species are characterised by fast growth rates and are more prone to stressors; for example, *Acropora* are more susceptible to bleaching than slow-growing massive species (e.g. Marshall and Baird 2000; Loya et al. 2001; Montano et al. 2010), including within the Red Sea (Furby et al. 2013). However, this genus has been seen to constitute one of the major taxonomic components of recruits to settlement plates (Sammarco and Carleton 1981; Wallace and Bull 1982; Wallace 1983; Fisk and Harriott 1990; Harriott 1992), to dominate in some cases coral assemblages recovering from damage (Pearson 1981) and to regenerate from fragments (Wallace 1985). These characteristics suggest an important early successional role for this genus, with some species demonstrating high population turnover rates (e.g. Guzman et al. 2007). The ecological nature of the *Acropora* genus might have influenced the speciation rate of its specifically associated organisms, such as *Zanclus* hydroids.

Throughout our surveys, we found *Z. gallii* IIa on both healthy and bleached *Acropora* corals. Similar situations were found for *Z. margaritae* and *Z. gallii* Ia (Pantos and Bythell 2010; Montano et al. 2015a). Even if some hypotheses have been formulated, the nature of coral–*Zanclus* symbioses is still unclear, and different species might have different effects in both unaltered and stressed conditions. For these reasons, the clarification of the systematics of all coral-associated hydroids and the characterisation of morphological and ecological traits that could play a role in their interaction with scleractinian corals are fundamental steps to better understanding this symbiosis. In particular, given the lack of informative morphological characters at this stage, the use of diagnostic molecular characters and a flexible molecular nomenclature are efficient tools to refer to cryptic or pseudocryptic species. This allows to include in further studies taxa that could otherwise be hardly and confusingly considered, and make possible the transfer of knowledge across disciplines. Moreover, this nomenclatural system easily allows the transfer of a given genetic group to formal nomenclature, as soon as it is diagnosed with other lines of evidence (Morard et al. 2016), such as new morphological characters or ecological information. The detection and naming of cryptic species identified using molecular techniques is, therefore, an important step forward to better characterise the biodiversity, the level of endemism and the diversification processes of a given area, especially in case of remote and isolated areas, such as the Red Sea. Hence, further studies are needed in order to assess the diversity, evolution and ecology of coral-associated *Zanclus* from different localities and growing on different hosts, since the genetic diversification of these organisms has been shown here to be related to both host-specificity and biogeography.

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Electronic supplementary material

Below are the links to the electronic supplementary material.

Fig. S1

(PDF 19 kb)

Fig. S2

(PDF 17 kb)

Fig. S3

(PDF 16 kb)

Fig. S4

(PDF 17 kb)

Fig. S5

(PDF 16 kb)

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