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Polyphyly of the genus *Zanclea* and family Zancleidae (Hydrozoa, Capitata) revealed by the integrative analysis of two bryozoan-associated species

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Abstract

The Zancleidae is a hydrozoan family that currently comprises three genera and 42 nominal species. The validity of numerous taxa in this family still needs to be assessed with integrative analyses and complete life cycle descriptions. The vast majority of its species live symbiotically with other organisms, among which cheilostomate bryozoans are the most common hosts. These bryozoan-associated zancleids are host-specific and encompass all species of the genera *Halocoryne* and *Zanclella*, as well as several species in the genus *Zanclea*. Zancleids show variable morphologies, including highly reduced polyps and medusae. Their phylogenetic history is uncertain due to the often intergrading morphologies and the shortage of molecular data. In the present study, two species of *Zanclea* from the Indian Ocean and the Red Sea are analysed, using morphological and DNA-based approaches. Morphological analyses of the polyp and medusa stages show that, despite a general resemblance with each other and with *Zanclella diabolica*, the two species differ in some characters, and this is supported also by molecular investigations. The DNA analyses show that the two species are monophyletic and closely related, but divergent from other *Zanclea* lineages. This newly recovered clade may correspond with the genus *Zanclella* or with a cryptic genus. The lack of both morphological and molecular data for several zancleid species did not allow to address this issue. Additionally, the single- and multi-locus phylogeny reconstructions reveal that both the family Zancleidae and the genus *Zanclea* are polyphyletic taxa, since they are composed of at least three divergent lineages. Most zancleid species have polyps and medusae similar to other closely related taxa, and this conserved general morphology poses a challenge in the delimitation of species, genera and even families in this group. Consequently, further conjunct morphological and molecular efforts are strongly needed to clarify the diversity and evolution of the family Zancleida as a whole.

Contents

Introduction

The Zancleidae (Russel, 1953) is a family of capitate anthoathecate hydrozoans with a complex and confusing taxonomy. Throughout the years, various authors have defined *Zanclea* species using conservative approaches, in some cases including multiple species into a unique 'variable' taxon (Russel, 1953) and in other cases describing several new species (Boero *et al*., 2000) and new genera (Boero and Hewitt, 1992). The family currently comprises 42 nominal species grouped in three genera: *Zanclea* Gegenbaur, 1856, *Halocoryne* Hadzi, 1917, and *Zanclella* Boero & Hewitt, 1992. However, Schuchert (1996, 2010) argued against the separation of these three taxa based on morphology alone, since the characters used to discriminate them often intergrade. According to the family diagnosis given in Bouillon *et al*. (2006), all species in the Zancleidae are colonial, stolonal, with creeping hydrorhiza, and with or without a perisarc sheet covering the hydrocauli and hydrorhizae. Polyps can be monomorphic or polymorphic, and in some

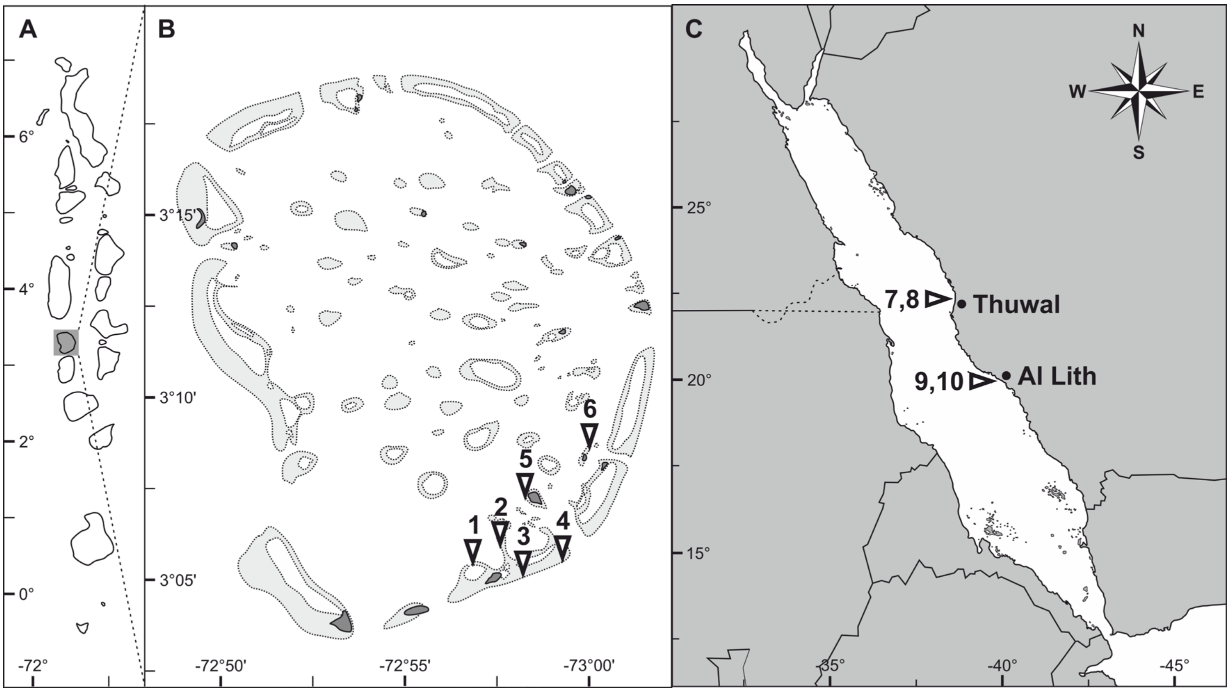

Figure 1. Maps of the investigated localities. **A:** Maldivian archipelago; **B:** Faafu Atoll; C**:** Red Sea. Numbers denote sampling sites. 1: Kika Reef; 2: Blue Cove; 3: Route 66 Reef; 4: Dhigu Reef; 5: Wall Street Reef; 6: Sunny Reef; 7: Tahla Reef; 8: Abu Gishaa Reef; 9: Abu Latt Island; 10: Sofia's Reef.

cases highly reduced. Medusae generally have a bell-shaped umbrella and zero, two, or four tentacles. Tentacles, when present, are armed with cnidophores, and gonads are usually interradial. Some species of *Zanclella* and *Halocoryne* have undergone reduction in the polyp and/or medusa stages (Boero *et al*., 2000). Petersen (1990) observed that reductions in the medusa stage are common in anthoathecate hydrozoans (*e.g*. in the family Corynidae) but these do not qualify as valid generic characters, since they have likely occurred independently in different species lineages after the synapomorphies defining genera evolved. For instance, all known *Halocoryne* polyps lack tentacles, but only the reproductive stage of *Halocoryne epizoica* Hadzi, 1917 is represented by a short-living eumedusoid, whereas all other species show a completely developed medusa stage (Piraino *et al*., 1992; Boero *et al*., 2000). *Zanclella* has polyps with one or two tentacles, with the exception of *Zanclella diabolica* Boero, Bouillon & Gravili 2000, which shows multiple oral and aboral tentacles, and the type species *Zanclella bryozoophila* Boero & Hewitt, 1992 reproduces through an eumedusoid, contrarily to its congeners (Boero and Hewitt, 1992; Boero *et al*., 2000). *Zanclella* is separated from *Zanclea* based on the reduced number of tentacles of the gastrozooids and the laterally compressed appearance of the umbrella of the medusa (Bouillon *et al*., 2006), but these features cannot be applied reliably to all *Zanclella* species, and, as suggested by Schuchert (1996, 2010), *Zanclella* should be regarded as a synonym of *Zanclea*. Similarly, Schuchert (2010) also highlighted that an evaluation of the relationships of *Halocoryne* species with other Zancleidae is impeded by the reduction of both the polyp and medusa stages. A proper molecular phylogeny of the three genera may therefore help resolving this uncertainty. Indeed, the taxonomic confusion is exacerbated by the fact that DNA sequences are available only for a few *Zanclea* species, whereas no molecular data have been generated so far for any *Zanclella* and *Halocoryne* species.

The majority of zancleid species live in strict association with other organisms, and the relationships with their hosts span from a presumable mutualism (Osman and Haugsness, 1981; Ristedt and Schuhmacher, 1985; Montano *et al*., 2017a) to parasitism (Piraino *et al*., 1992). Among the 30 species with a described benthic polyp stage, 16 are specifically associated with bryozoans (Boero *et al*., 2000; Puce *et* *al*., 2002; Pica *et al*., 2017), four with scleractinians (Boero *et al*., 2000; Pantos and Bythell, 2010; Hirose and Hirose, 2011; Montano *et al*., 2015a), two with octocorals (Puce *et al*., 2008; Varela, 2012), two with bivalves (Boero *et al*., 2000), two with algae (Boero *et al*., 2000; Galea, 2008), while four other show no substrate specificity (Agassiz, 1862; Schuchert, 2010; Peña Cantero *et al*., 2013). In particular, *Halocoryne* and *Zanclella* species live symbiotically with bryozoans and are thought to have derived from *Zanclea* species associated with bryozoans and with a perisarc-free hydrorhiza protected by the skeleton of the host (Puce *et al*., 2002). Indeed, all species in these two genera have their naked hydrorhizae embedded in the skeleton of the host (Boero *et al*., 2000), and this aspect, together with the presence of highly modified polyps and medusae, and the behavioural integration with the bryozoan (Boero and Hewitt, 1992; Piraino *et al*., 1992), are considered as derived traits among the Zancleidae (Boero *et al*., 2000; Puce *et al*., 2002). Overall, several aspects of this enigmatic family need to be investigated further in more detail, particularly regarding the evaluation of its diversity, systematics and evolution.

The current work aims to characterise two bryozoan-associated zancleid species similar to *Zanclella diabolica* based on specimens collected in the Indian Ocean and Red Sea. The aim is to investigate their placement within the superfamily Zancleida and present an updated phylogenetic assessment of the family Zancleidae.

Material and methods

Specimen sampling was conducted between December 2015 and May 2017 in Faafu Atoll (Maldives) and in reefs surrounding Thuwal and Al Lith (Red Sea, Saudi Arabia) (Figure 1). The presence of hydroids on bryozoans was recorded qualitatively *in situ*, and small fragments of bryozoans hosting hydrozoans were collected with hammer and chisel. After anesthetisation with menthol crystals, single hydrozoan polyps were carefully collected one by one using syringe needles, precision forceps, and micropipettes, directly from a bowl filled with seawater that was placed under a stereomicroscope. Afterwards, they were preserved in 95% ethanol for molecular analyses and 10% formalin for morphological studies. Additional portions of colonies were cultured and fed *Artemia* nauplii in small bowls at the MaRHE Center (Magoodhoo Island, Faafu Atoll, Maldives) in order to observe the release of medusae. Thereafter, the medusae were maintained in small bowls, and the sea water was replaced every day. The reared medusae were observed on a daily basis and some of them were fixed in 10% formalin. Morphological observations and pictures of polyps, medusae, and nematocysts were mainly performed on living specimens, whereas measurements were taken on formalin-fixed material. Photomicrographs of hydroids, medusae, and nematocysts were taken using a Leica EZ4 D stereomicroscope and a Zeiss Axioskop 40 compound microscope, both equipped with a Nikon AW 100 camera. Measurements were taken with a Nikon Eclipse E600 microscope using the NIS-Elements Viewer 4.30 software, and up to 20 specimens per species, and 50 nematocysts per type for each life stage of each species, were measured.

Tables including all available diagnostic characters for polyp and medusa stages of all the nominal zancleid species and the two species herein analysed (Supplementary tables S1, S2) were compiled by retrieving data from all the relevant literature (Agassiz, 1862; Haeckel, 1879; Browne, 1916; Hastings, 1930; Kramp, 1959; Uchida and Sugiura, 1976; Calder, 1988; Hirohito, 1988; Xu *et al.,* 1991, 2008; Boero and Hewitt, 1992; Piraino *et al.,* 1992; Gravili *et al*., 1996; Migotto, 1996; Cerrano *et al.,* 1997; Schuchert, 1996, 2010; Boero *et al.,* 2000; Puce *et al.,* 2002, 2008; Gershwin and Zeidler, 2003; Galea, 2008; Pantos and Bythell, 2010; Hirose and Hirose, 2011; Varela, 2012; Altuna, 2013, 2016; Peña Cantero *et al.,* 2013; Montano *et al.,* 2015a; Pica et al., 2017).

Total genomic DNA of ethanol-fixed samples was extracted following a protocol modified from Zietara *et al*. (2000). Four different molecular markers were amplified: i) a ~600 bp portion of the mitochondrial 16S ribosomal DNA gene (*16S rRNA*), ii) a ~700 bp portion of the mitochondrial cytochrome *c* oxidase subunit I gene (*COX1*), iii) a ~1700 bp portion of the nuclear 18S ribosomal DNA gene (*18S rRNA*), iv) a ~1700 bp portion of the nuclear 28S ribosomal DNA gene (*28S rRNA*). *16S rRNA* and *28S rRNA* loci were amplified using hydrozoan-specific primers following the protocols described in Cunningham and Buss (1993) and Maggioni *et al*. (2016). *COX1* and *18S rRNA* genes were amplified using metazoan universal primers and the protocols proposed by Folmer *et al*. (1994) and Medlin *et al*. (1988), respectively. All PCR products were purified with Illustra ExoStar (GE Healthcare) at 37° C for

Table 1. GenBank accession numbers of the sequences included in the analyses. Newly obtained sequences are in bold.

Family	**Genus**	**GenBank Accession Numbers**			
		16S rRNA	**COX1**	**18S rRNA**	**28S rRNA**
Asyncorynidae	*Asyncoryne ryniensis*	EU876552	-	EU876578	GQ424289
Cladocorynidae	*Cladocoryne haddoni*	LT593865	LT593890	LT593875	LT593870
	Pteroclava krempfi	LT158199	LT158209	LT593876	LT222039
Hydrocorynidae	*Hydrocoryne miurensis*	GQ395326	-	-	GQ424313
Milleporidae	*Millepora alcicornis*	EU876551	-	AF358088	EU879950
Moerisiidae	*Moerisia inkermanica*	KT266626	-	KT722408	KT757161
	Odessia maeotica	GQ395324	-	GQ424341	GQ424314
Pennariidae	*Pennaria disticha*	KF962510	KF962169	KF962290	KF962370
Porpitidae	*Porpita porpita*	AY935322	GQ120060	GQ424319	EU883551
	Velella velella	EU305487	KC706685	EU876576	EU879949
Solanderiidae	*Solanderia secunda*	EU305484	JX121599	EU305502	EU305533
Sphaerocorynidae	*Astrocoryne cabela*	LT714176	-	LT714177	LT714178
	Heterocoryne caribbensis	LT714185	**MH248019**	LT714186	LT714187
	Sphaerocoryne sp.	LT714182	**MH248018**	LT714183	LT714184
Zancleidae	*Zanclea costata*	AY512531	-	EU876579	EU879951
	Zanclea sango	LN714107	LT593889	LT593874	LT593869
	Zanclea sp. (DNA-XMZS)	KF962532	KF962188	KF962298	KF962373
	Zanclea prolifera	EU305488	-	EU272639	EU272598
	Zanclea sp. 1	**MH244086,** **MH244087,** **MH244090,** **MH244092,** **MH244094,** **MH244097**	**MH244068,** **MH244069,** **MH244072,** **MH244074,** **MH244076,** **MH244079**	**MH244050,** **MH244051,** **MH244054,** **MH244056,** **MH244058,** **MH244061**	**MH244104,** **MH244105,** **MH244108,** **MH244110,** **MH244112,** **MH244115**
	Zanclea sp. 2	**MH244080-085,** **MH244088,** **MH244089,** **MH244091,** **MH244093,** **MH244095,** **MH244096**	**MH244062-067,** **MH244070,** **MH244071,** **MH244073,** **MH244075,** **MH244077,** **MH244078**	**MH244044-049,** **MH244052,** **MH244053,** **MH244055,** **MH244057,** **MH244059,** **MH244060**	**MH244098-103,** **MH244106,** **MH244107,** **MH244109,** **MH244111,** **MH244113,** **MH244114**
Outgroups	*Coryne uchidai*	GQ395320	KT981912	GQ424332	GQ424306
	Candelabrum cocksii	AY512520	GU812438	EU876556	EU879928
Sequences used for the 16S analysis only					
Zancleidae	*Zanclea sessilis*	AY512532, FN687557-558, KP776747, KX355442			
	Zanclea migottoi	MF538731			
	Zanclea implexa	KX355448			
	Zanclea sp. (DNA-977)	KP776810			
	Zanclea sp. (DNA-XMZS)	KF962533-536			
	Zanclea costata	EU876553, FN687559			
	Zanclea giancarloi	FN687560-562, KP776811			
	Zanclea spp. (coral-associated)	LN714050, LN714059, LN714064, LN714095, LN714107, LN714110, LT607009			
Milleporidae	*Millepora* spp.	KP776777-779, KT891227, KT891230, KT891239, KT891291, KX670777			
Solanderiidae	*Solanderia ericopsis*	AY787881			

60 min, followed by 85° C for 15 min, and then directly sequenced in forward and reverse directions using an ABI 3730xl DNA Analyzer (Applied Biosystems). The obtained chromatograms were visually checked and assembled using Sequencher 4.1.4 (Gene Codes). Additionally, *COX1* sequences were translated using Geneious 6.1.6 (Drummond *et al*., 2010), in order to check for the presence of stop codons. The sequences obtained were deposited with EMBL (GenBank accession numbers: MH244044-MH244115, MH248018, MH248019), including information on specimen vouchers, collection dates, localities, and coordinates. Other available sequences belonging to representatives of the superfamily Zancleida and outgroups (Table 1) were downloaded from GenBank and added to each dataset. Sequences of each marker were aligned with MAFFT 7.110 (Katoh and Standley, 2013) using the *E-INS-i* option. Two datasets were generated for subsequent analyses. First, all markers were concatenated using Mesquite 3.2 (Maddison and Maddison, 2006), and appropriate partition schemes and models were determined using PartitionFinder 1.1.1 (Lanfear *et al*., 2012) by means of the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). A second dataset was generated through inclusion of all *16S rRNA* sequences belonging to the family Zancleidae and closely related taxa, since for most zancleid species only this marker was available in GenBank. Phylogenetic inference analyses were performed for the concatenated and *16S rRNA* datasets using Bayesian inference (BI) and maximum likelihood (ML). BI analyses were performed using MrBayes 3.2.6 (Ronquist *et al*., 2012): four parallel Markov Chain Monte Carlo runs (MCMC) were run for 10^7 generations, trees were sampled every 100th generation, and burn-in was set to 25%. Maximum likelihood trees were built with RAxML 8.2.9 (Stamatakis, 2014) using 1000 bootstrap replicates. Both BI and ML analyses were run on the CIPRES server (Miller *et al*., 2010). Genetic distances (uncorrected *p*-distance, 1000 bootstrap) within and among Zancleidae species were computed for the *16S rRNA* dataset using MEGA 6 (Tamura *et al*., 2013).

Table 2. Polyp measurements of *Zanclea* sp. 1 and *Zanclea* sp. 2. X: absent. Measurements (in µm) are rounded to the nearest unit and are shown as mean values ± standard deviations (range).

	***Zanclea* sp. 1**	***Zanclea* sp. 2**
Hydrorhiza	Projecting out in roughly spherical clusters, with euryteles and rarely stenoteles	Projecting out for some of its length, with euryteles and rarely stenoteles
Polyp length	1227 ± 242 (803-1498)	2135 ± 478 (1442-2930)
Polyp diameter (proximal)	75 ± 14 (55-80)	125 ± 20 (108-156)
Polyp diameter (distal)	113 ± 19 (92-142)	158 ± 13 (106-147)
N° of oral tentacles	4-5	4-5
N° of aboral tentacles	16-21	23-30
Oral capitula diameter	84 ± 8 (74-93)	104 ± 6 (97-117)
Aboral capitula diameter	48 ± 4 (41-56)	86 ± 9 (73-104)
Proximal capitula diameter	36 ± 2 (32-40)	57 ± 5 (52-65)
Medusa buds	1-5	1-6
Large stenoteles length	16 ± 1 (15-18)	20 ± 1 (18-22)
Large stenoteles width	13 ± 1 (12-14)	16 ± 2 (12-17)
Medium-sized stenoteles length	X	17 ± 1 (15-18)
Medium-sized stenoteles width	X	14 ± 1 (13-15)
Small stenoteles length	6 ± 0 (6-7)	7 ± 0 (6-8)
Small stenoteles width	5 ± 1 (4-6)	5 ± 0 (4-6)
Euryteles length	28 ± 1 (24-29)	20 ± 1 (19-21)
Euryteles width	15 ± 1 (11-16)	12 ± 1 (11-14)
Euryteles shaft length	92 ± 5 (84-95)	138 ± 5 (134-143)

Table 3. Medusa measurements of *Zanclea* sp. 1 and *Zanclea* sp. 2. X: absent. Measurements (in µm) are rounded to the nearest unit and are shown as mean values ± standard deviations (range).

	***Zanclea* sp. 1**	***Zanclea* sp. 2**
Height	154 ± 6 (152-162)	174 ± 6 (168-180)
Width	180 ± 7 (172-191)	208 ± 6 (202-215)
Manubrium length	86 ± 6 (79-97)	137 ± 8 (126-146)
Manubrium width	43 ± 9 (33-52)	50 ± 5 (47-57)
Bulbs length	85 ± 9 (72-99)	77 ± 13 (56-88)
Oral arms length	6 ±1 (4-7)	5 ± 1 (4-6)
Tentacles length	694 ± 118 (561-935)	510 ± 33 (480-560)
Cnidophoral pedicels length	44 ± 15 (30-70)	31 ± 6 (17-38)
N° of cnidophores	10-13	11-17
Cnidophores length	19 ± 1 (17-21)	20 ± 2 (15-25)
Cnidophores width	13 ± 2 (10-17)	17 ± 1 (14-20)
Mastigophores length	8 ± 1 (7-9)	8 ± 1 (7-10)
Mastigophores width	7 ± 0 (6-7)	7 ± 1 (6-8)
Mastigophores shaft length	35 ± 3 (30-37)	36 ± 1 (36-37)
Euryteles length	6 ± 1 (5-7)	7 ± 0 (6-8)
Euryteles width	5 ± 0 (4-6)	5 ± 0 (4-5)
Euryteles shaft length	34 ± 4 (33-39)	38 ± 1 (37-40)

Results

Morphology

Morphological analyses revealed that the collected samples belong to two *Zanclea* species (here called *Zanclea* sp. 1 and *Zanclea* sp. 2) showing several affinities with *Zanclella diabolica*. Both species were found in the Maldives, whereas *Zanclea* sp. 2 was also found in the Red Sea. These species live in association with cheilostomate bryozoans and are similar to each other, but morphological and morphometrical analyses revealed differences in the polyp, medusa, and cnidome (Tables 2, 3; Figure 2), as shown in the 'Systematics' section in the Appendix. Specifically, both *Zanclea* sp. 1 and *Zanclea* sp. 2 have a perisarc-free hydrorhiza growing under the bryozoan skeleton, projecting out in clusters or for some of its length, and containing macrobasic holotrichous euryteles and stenoteles. Polyps are colonial, monomorphic, and with oral and aboral capitate tentacles with stenoteles of two or three size classes. Medusa buds are borne in clusters on short blastostyles arising directly form the hydrorhiza, and develop in minute medusae. Newly released medusae are characterised by a globular umbrella with several macrobasic holotrichous mastigophores, a manubrium with small oral arms, no exumbrellar nematocyst pouches, two radial canals, and two bulbs bearing tentacles equipped with cnidophores containing macrobasic apotrichous euryteles. The main differences between *Zanclea* sp. 1 and *Zanclea* sp. 2 are represented by the size and colour of gastrozooids, the number of aboral tentacles, the type of hydrorhizal projections, the size of euryteles and stenoteles found in the polyp stages, and the length of the manubrium of the medusa stages. These species differ from other *Zanclea* polyps by the combination of different morphological characters (Supplementary Table S1), including the association with specific organisms, the monomorphic state of the colony, the absence of a perisarc sheet surrounding the hydrorhiza, the position of medusa buds, and the presence, type, and distribution of macrobasic euryteles. They also differ from the highly reduced polyps of *Halocoryne* and *Zanclella*, which show zero, one, or two tentacles, with the only exception of the *Zanclea*-like polyps of *Zanclella diabolica*. The newly released medusae of *Zanclea* sp. 1 and *Zanclea* sp. 2 are very similar to those of *Zanclella diabolica* and *Zanclella glomboides* Boero, Bouillon

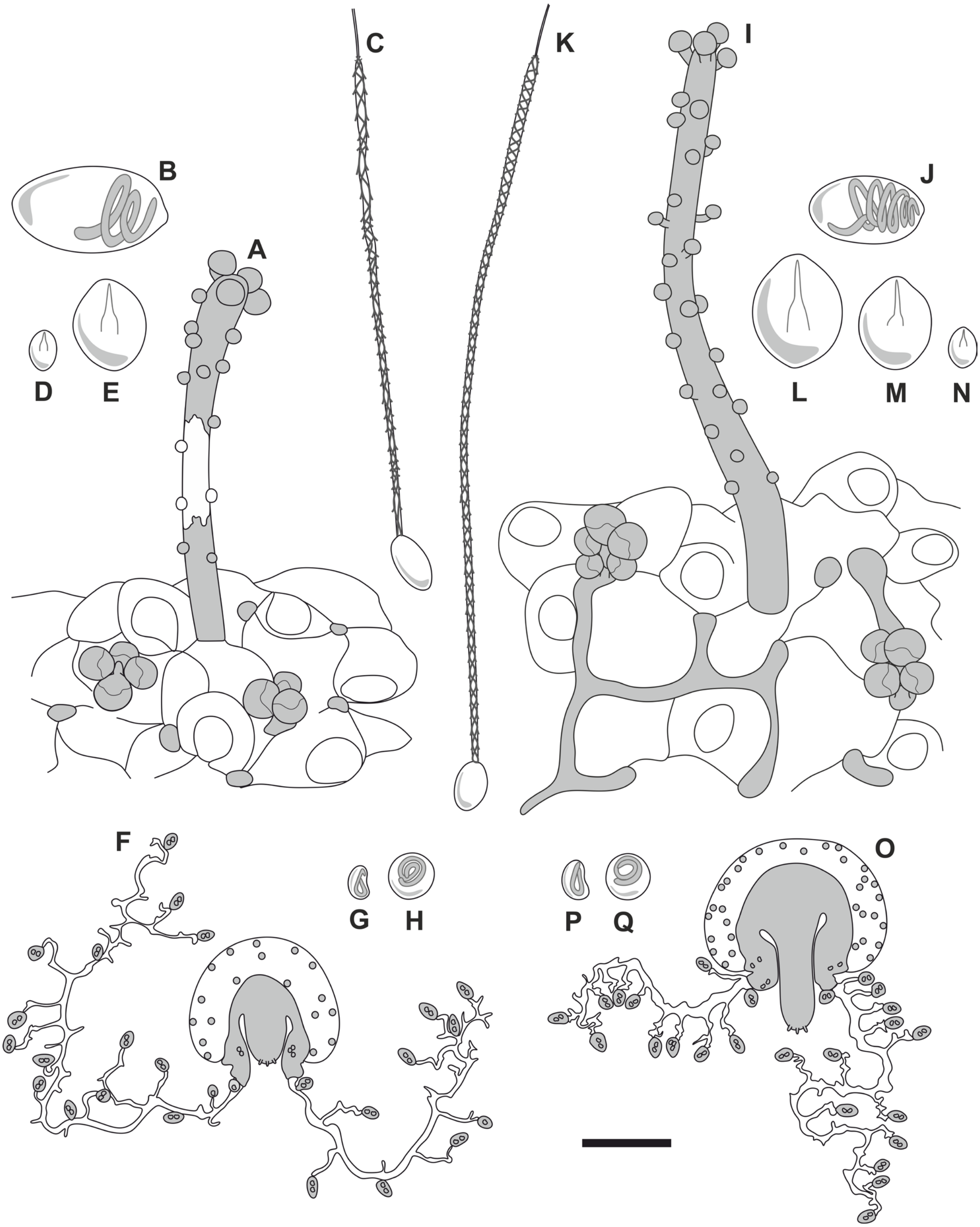

Figure 2. Polyps, medusae, and nematocysts of *Zanclea* sp. 1 (A-H) and *Zanclea* sp. 2 (I-Q). *Zanclea* sp. 1. **A:** Fertile colony; **B, C**: Undischarged and discharged macrobasic holotrichous eurytele of the polyp; **D, E:** Stenoteles of two size classes of the polyp; **F:** Newly released medusa; **G:** Macrobasic apotrichous eurytele of the medusa; **H:** Macrobasic holotrichous mastigophore of the medusa. *Zanclea* sp. 2. **I:** Fertile colony; **J, K:** Undischarged and discharged macrobasic holotrichous eurytele of the polyp; **L-N:** Stenoteles of three size classes of the polyp; **O:** Newly released medusa; **P:** Macrobasic apotrichous eurytele of the medusa; **Q:** Macrobasic holotrichous mastigophore of the medusa. Scale bar: A, I: ~250 µm; F, O: ~100 µm; C, K: ~30 µm; B, D, E, G, H, L-N, P, Q: ~15 µm.

& Gravili 2000, and highly differ from those known for all other zancleid species (Supplementary Table S2). Comparisons were nevertheless not possible with 12 zancleid species for which only the mature medusa is known (Supplementary Table S2). Therefore it was impossible to identify these specimens to species level and to formally describe *Zanclea* sp. 1 and *Zanclea* sp. 2.

Phylogeny and genetic diversity

The total genomic DNA was successfully extracted and four loci were amplified for all samples. The total alignments of the *16S rRNA*, *COX1*, *18S rRNA*, *28S rRNA*, and concatenated datasets were 569, 607, 1682, 1629, and 4487 bp long, respectively. PartitionFinder found similar partition schemes and models under the AIC and the BIC. AIC: 16S (GTR+G+I), 18S (GTR+G+I), 28S (GTR+G+I), COX1_pos1 (GTR+G), COX1_pos2 (GTR), COX1_pos3 (GTR+G+I). BIC: 16S (GTR+G+I), 18S (GTR+G+I), 28S (SYM+G+I), COX1_pos1 (GTR+G), COX1_pos2 (F81), COX1_pos3 (GTR+G). The general topology of the phylogenetic trees based on BI and ML analyses, and computed under both AIC and BIC models and partitions, were similar, and only the Bayesian topologies are shown in Figures 3 and 4. The phylogram of the superfamily Zancleida (Figure 3) is mostly concordant with previous works (Maggioni *et al*., 2016, 2017a). The superfamily Zancleida is well supported (Bayesian posterior probability - BPP = 1, Maximum likelihood bootstrap support - BS = 77), but the relationships within this group are, in some cases, still uncertain. The less resolved branch of the phylogenetic tree is that composed of the families Asyncorynidae Kramp, 1949, Milleporidae Fleming, 1828, Solanderiidae Marshall, 1892, and Zancleidae. The family Zancleidae appears to be polyphyletic and is split in three divergent lineages, highlighted by stars in Figure 3. A first clade (BPP = 1, BS =

Table 4. Pairwise intra- and inter specific genetic distances (uncorrected *p*-distances in %) of *Zanclea* species based on the *16S rRNA*. Standard deviations are in parentheses. n.c.: not calculated.

		1	2	3	4	5	6	7	8	9	10	11	12
1.	*Zanclea* sp. 1	0.5 (0.2)											
2.	*Zanclea* sp. 2	10.5 (1.2)	0.4 (0.1)										
3.	*Zanclea costata*	12.4 (1.3)	12.1 (1.3)	3.7 (0.7)									
4.	*Zanclea divergens*	12.7 (1.4)	13.6 (1.4)	13.1 (1.3)	n.c.								
5.	*Zanclea giancarloi*	11.7 (1.3)	11.7 (1.2)	7.1 (1.0)	11.2 (1.2)	1.0 (0.3)							
6.	*Zanclea implexa*	11.2 (1.3)	12.4 (1.3)	7.0 (1.0)	11.0 (1.2)	5.0 (0.8)	n.c.						
7.	*Zanclea migottoi*	12.7 (1.3)	12.9 (1.3)	9.1 (1.1)	11.7 (1.3)	7.0 (1.0)	7.6 (1.0)	n.c.					
8.	*Zanclea prolifera*	13.7 (1.4)	12.4 (1.3)	13.9 (1.3)	12.7 (1.4)	12.7 (1.3)	13.2 (1.3)	13.2 (1.3)	n.c.				
9.	*Zanclea sessilis*	11.7 (1.3)	12.9 (1.3)	8.0 (1.1)	11.0 (1.2)	4.8 (0.8)	3.6 (0.7)	7.7 (0.7)	13.5 (1.3)	1.3 (0.3)			
10.	*Zanclea* sp. (DNA-XMZS)	12.2 (1.4)	13.9 (1.4)	10.5 (1.2)	13.7 (1.5)	8.5 (1.2)	9.9 (1.2)	8.2 (1.2)	13.3 (1.4)	9.4 (1.2)	0.0 (0.0)		
11.	*Zanclea* sp. (DNA-977)	10.7 (1.3)	12.4 (1.3)	7.9 (1.1)	10.0 (1.2)	5.0 (0.9)	3.4 (0.7)	8.4 (0.7)	12.8 (1.3)	3.9 (0.7)	9.3 (1.2)	n.c.	
12.	Coral-associated *Zanclea*	13.3 (1.3)	14.1 (1.3)	11.8 (1.2)	11.9 (1.3)	9.0 (1.0)	10.5 (1.1)	9.1 (1.1)	15.1 (1.4)	9.9 (1.1)	10.3 (1.2)	9.8 (1.1)	4.4 (0.6)

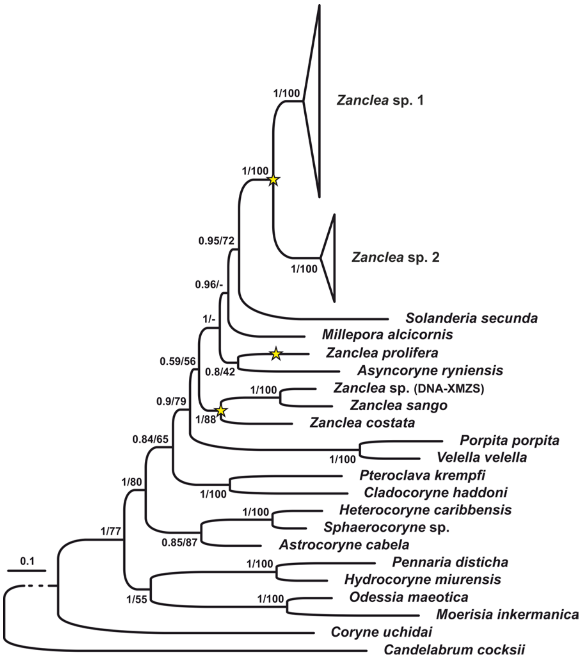

Figure 3. Multi-locus Bayesian phylogram based on the concatenated dataset *16S rRNA-COX1-18S rRNA-28S rRNA* of the Zancleida, with *Coryne uchidai* and *Candelabrum cocksii* as outgroups. Numbers at nodes show the Bayesian posterior probabilities and the maximum likelihood bootstrapping values, respectively. '-' indicates that a node is not present in BI or ML analyses. Zancleidae lineages are indicated by stars.

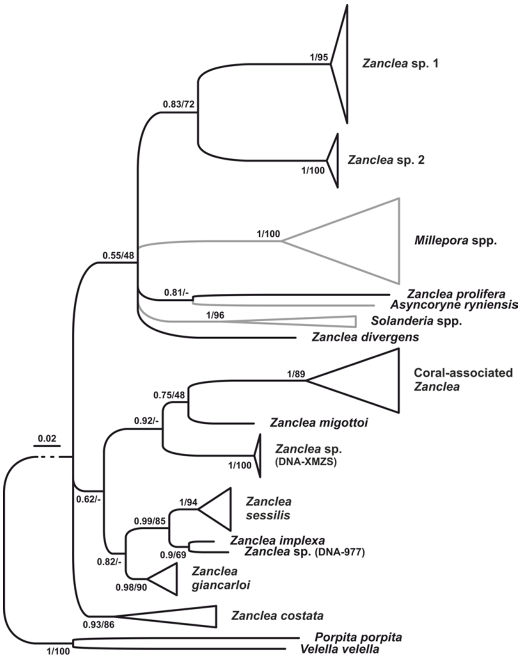

Figure 4. *16S rRNA* phylogram of the Zancleidae with *Porpita porpita* and *Velella velella* as outgroups. Numbers at nodes show the Bayesian posterior probabilities and the maximum likelihood bootstrapping values, respectively. '-' indicates that a node is not present in BI or ML analyses. Non-Zancleidae taxa are in grey.

88) is composed of the type species *Zanclea costata* Gegenbaur, 1857, *Zanclea* sp. (DNA-XMZS) from the China Sea, and coral-associated *Zanclea* (*Zanclea gallii* Montano, Maggioni & Puce, 2015, *Zanclea sango* Hirose & Hirose, 2011, and *Zanclea* spp.). A second clade includes *Zanclea prolifera* Uchida & Sugiura, 1976, and is likely to be closely related to *Asyncoryne ryniensis* Warren, 1908. A third clade (BPP = 1, BS = 100) is represented by the two *Zanclea* species analysed in this work. They are monophyletic and together form a fully supported cluster. In the phylogenetic hypothesis, they are more closely related to *Solanderia secunda* (Inaba, 1892) (BPP = 0.92, BS = 72), followed by *Millepora alcicornis* Linnaeus 1758 (BPP = 0.96, BS = not supported - n. s.) than to the remaining zancleids, even if these latter relationships are generally less supported by ML analysis. The *16S rRNA* phylogram including all available sequences of the Zancleidae (Figure 4) shows a similar situation, although with low overall statistical supports. The two species of *Zanclea* herein analysed are monophyletic, and together form a clade with BPP = 0.83 and BS = 72 that falls within a poorly supported group together with *Asyncoryne ryniensis*, *Millepora* spp., *Solanderia* spp., *Zanclea prolifera*, and *Zanclea divergens* Boero, Bouillon & Gravili, 2000 (BPP = 0.55, BS = 48). Coral-associated *Zanclea* species cluster with *Zanclea* sp. (DNA-XMZS), as shown also in Figure 4, and with *Zanclea migottoi* Galea, 2008 (BPP = 0.92, BS = n. s.). The remaining *Zanclea* species included in the tree are the temperate water *Zanclea sessilis* (Gosse, 1853), *Zanclea giancarloi* Boero, Bouillon & Gravili, 2000, *Zanclea implexa* (Alder, 1856), and *Zanclea* sp. (DNA-977), which cluster together (BPP = 0.82, BS = n. s.), and *Zanclea costata*. Inter-specific genetic distances are high among all species (Table 4). The lowest values are found among the *Zanclea* species in the temperate water cluster (3.4-5 %), whereas in all other pairwise comparisons values are higher than 7%. Intra-group distances are generally low, with the exception of *Zanclea costata* (3.7 %) and coral-associated *Zanclea* (4.4 %), the latter being nevertheless composed of multiple species (Montano *et al*., 2015b, Maggioni *et al*., 2017).

Discussion

As anticipated by Boero *et al*. (2000), the family Zancleidae is constantly being enlarged as a result of the descriptions of new species and cryptic taxa (Maggioni *et al*., 2017b; Pica *et al*., 2017). Indeed, the integration of in-depth morphological and molecular assessments is helping re-evaluate the diversity of both Zancleidae and closely related families, taking their geographical distributions and host relationships into account if relevant (Montano *et al*., 2015a, 2015b, 2015c, 2017b; Maggioni *et al*., 2016, 2017a, 2017b; Takama *et al*., 2018). Moreover, ecological studies are widening the knowledge of these poorly known organisms (Montano *et al*., 2016; Dubé *et al*., 2017a, 2017b). However, the amount of undescribed or cryptic species is probably still high, due to the paucity of diagnostic morphological characters, the often intergrading morphologies (Schuchert, 2010), the incomplete knowledge of life cycles, and the specific associations or behaviour that zancleids often establish and that could, in some cases, make them difficult to spot (Boero and Hewitt, 1992; Boero *et al*., 2000). Similarly, the outcomes of the symbiotic relationships involving zancleids and the degree of integration with their hosts are still unexplored in most species, although recent studies unveiled patterns of host-specificity (Fontana *et al*., 2012; Montano *et al*., 2015b), characterised the anatomy of the interactions (Pantos and Hoegh-Guldberg, 2011), and discovered previously unsuspected relationships (Montano *et al*., 2015c, 2017a). Most of the studies have focused on the widespread and more common genus *Zanclea*, whereas the enigmatic genera *Halocoryne* and *Zanclella* have been less investigated, with the latter dealt with in only two earlier works (Boero and Hewitt, 1992; Boero *et al*., 2000).

In the present study, we analysed two species whose morphology is remarkably similar to *Zanclella diabolica*. This latter species has typical *Zanclea* gastrozooids but was originally placed in the genus *Zanclella* by Boero *et al*. (2000) because of the peculiar bilateral symmetric bell of the newly released medusa. Indeed, this character is shared with another *Zanclella* species, *Zanclella glomboides*, which has nevertheless highly reduced polyps (Boero *et al*. 2000). The gastrozooids of the type species *Zanclella bryozoophila* are reduced in the same way, but the sexual stage is completely different from those of the other two *Zanclella* species, being reduced to eumedusoid (Boero and Hewitt, 1992). This inconsistent intra-generic morphological variability does not allow a secure diagnosis of *Zanclella* and therefore supports the synonymisation of the genus with *Zanclea,* at least until a robust phylogenetic hypothesis will clarify this issue. The general reduction of tentacles in *Zanclella* and *Halocoryne* gastrozooids may be linked to their behavioural integration with the bryozoan hosts. For instance, the one-tentacled polyps of *Zanclella bryozoophila* were observed feeding on food particles taken from the host lophophores (Boero and Hewitt, 1992). Furthermore, the atentaculate polyps of *H. epizoica* were demonstrated to feed on the lophophoral tentacles of the host (Piraino *et al*., 1992). The convergent evolution of these active and highly specialised feeding mechanisms may have promoted a paralleled progressive reduction of the tentacular structures, making both the reduction of medusae (Petersen, 1990) and polyps ambiguous characters to be used in generic diagnoses. On the other hand, the absence of such an extreme polyp reduction in the two *Zanclea* species analysed herein, as well as in *Zanclella diabolica* and all other symbiotic *Zanclea* species, may reflect a less specialised feeding behaviour of the gastrozooids, which are likely passive predators, as shown, for instance, for *Zanclea sessilis* and *Zanclea giancarloi* (Miglietta *et al*., 2000).

Zanclea sp. 1 and *Zanclea* sp. 2 are monomorphic, but have a distinctive feature, which is the hydrorhiza projecting out of the bryozoan skeleton. This characteristic is found in a few other zancleids, namely *Zanclella diabolica, Zanclea divergens*, and *Zanclea eilatensis* Pica, Bastari & Puce, 2017. Another species, *Zanclea exposita* Puce, Cerrano, Boyer, Ferretti & Bavestrello, 2002, exhibits a perisarc-free hydrorhiza not surrounded by the bryozoan and running above the skeleton of the host (Puce *et al*., 2002). With the exception of *Zanclea eilatensis*, these species share the presence of macrobasic holotrichous euryteles in the hydrorhiza, but *Zanclea exposita* and *Zanclea divergens* have euryteles also in the gastrozooids, and the latter species produces a typical *Zanclea* medusa. According to the *16S rRNA* phylogram, *Zanclea divergens, Zanclea* sp. 1 and *Zanclea* sp. 2 seem to belong to a clade diverging from other *Zanclea* species, and the presence of exposed portions of the perisarc-free hydrorhiza may therefore have taxonomic relevance. The hydrorhizal clusters armed with nematocyst batteries were interpreted by Boero *et al*. (2000) as a potential first step towards colony polymorphism. If this is true, polymorphism may have arisen independently in divergent lineages within the Zancleidae. For instance, *Zanclea divergens*, the polymorphic coral-associated *Zanclea* (Montano *et al*., 2015b), and the facultatively polymorphic *Zanclea sessilis* (Altuna, 2016) are highly divergent from each other.

The phylogenetic hypotheses presented in this work are concordant in recovering the family Zancleidae and the genus *Zanclea* as polyphyletic taxa, even if in some cases the nodes are still not fully supported by both Bayesian inference and maximum likelihood analyses. Single- and multi-locus phylogenies show that *Zanclea prolifera* is more closely related to *Asyncoryne ryniensis* than to its nominal congeners, even with low statistical support, confirming the findings of previous works (Nawrocki *et al*., 2010; Maggioni *et al*., 2017a). *Zanclea costata*, the type species of the genus *Zanclea*, clusters with coral-associated *Zanclea* and *Zanclea* sp. (DNA-XMZS) from the China Sea in the multi-locus phylogeny, suggesting that these species belong to the 'real' family Zancleidae and to the genus *Zanclea*. The phylogenetic position of *Zanclea costata* is still dubious in the single-locus tree, and the placement in *Zanclea* of *Z. giancarloi*, *Z. sessilis*, *Z. migottoi*, *Z. implexa,* and *Zanclea* sp. (DNA-977) needs to be confirmed with further multi-locus analyses. Similarly, the position of *Zanclea divergens* is not fully resolved in the *16S rRNA* phylogram, even if it seems more closely related to *Zanclea prolifera*, *Asyncoryne ryniensis*, *Millepora* spp., *Solanderia* spp., *Zanclea* sp. 1 and *Zanclea* sp. 2. The two latter species are monophyletic and closely related, as suggested by their striking morphological similarities. They form a third, divergent clade of *Zanclea* in the concatenated analysis, with *Solanderia secunda* as a sister taxon, demonstrating a further polyphyly of both *Zanclea* and the Zancleidae.

According to these results, *Zanclea* sp. 1 and *Zanclea* sp. 2 may belong to *Zanclella* or to a new cryptic genus, and even to a new family in the superfamily Zancleida. However, we cautiously refrain from assigning them to *Zanclella* or from erecting new taxa, at least until genetic data of *Zanclella* and *Halocoryne* species will be produced and included in the analyses. Overall, the chaotic taxonomy of the Zancleidae is likely due to the general appearance of the polyps and medusae, which are often similar to those of related taxa (*e.g*. the gastrozooids are very similar to those of many *Solanderia*, *Millepora* and Corynidae species, while the medusae resemble those of *Asyncoryne*), and this could have contributed to the misplacement of some species and even genera.

Another relevant source of confusion is illustrated by the fact that the complete life cycle is not known for most of the 42 zancleid species. Indeed, 11 species are known only from their adult medusa stage, six from their polyp stage, and 12 from their polyp and newly released medusa stages. This is mostly due to the fact that medusa-based descriptions do not provide any information about the polyp stage and to the difficulty to find fertile colonies and rear the newly released medusa to maturity. These difficulties, along with the prior fragmentary knowledge, the rarity of some species, and the lack of DNA sequences or material suitable for molecular analyses for most taxa, pose a challenge to the resolution of the evolutionary relationships and the systematics of the Zancleidae. Therefore, we stress the need for filling the previous gaps in order to allow a further clarification of the diversity of this enigmatic family.

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Online supplementary material

S1. Polyp stage characteristics of all nominal zancleid species, *Zanclea* sp. 1, and *Zanclea* sp. 2. * Data obtained from drawings in original descriptions.

S2. Medusa stage characteristics of all nominal zancleid species, *Zanclea* sp. 1, and *Zanclea* sp. 2. * Data obtained from drawings in original descriptions.

S3. Polyp stage characteristics of all nominal zancleid species, Zanclea sp. 1, and Zanclea sp. 2. * Data obtained from drawings in original descriptions.

S4. Medusa stage characteristics of all nominal zancleid species, Zanclea sp. 1, and Zanclea sp. 2. * Data obtained from drawings in original descriptions.

Appendix

Systematics

Class Hydrozoa Owen, 1843
Order Anthoathecata Cornelius, 1992
Suborder Capitata Kühn, 1913
Family Zancleidae Russel, 1953
Genus *Zanclea* Gegenbaur, 1856

Zanclea sp. 1

Material examined. Polyp stage. MA0216061: Wall Street Reef, Faafu Atoll, Maldives (3.12061°N, 73.09581°E), 10/02/2016, 19 m depth. MA0316014: Blue Cove, Faafu Atoll, Maldives (3.09383°N, 72.96651°E), 09/03/2016, 20 m depth. MA0316101: Route 66 Reef, Faafu Atoll, Maldives (3.07717°N, 72.97328°E), 21/03/2016, 8 m depth. MA0316171: Blue Cove, Faafu Atoll, Maldives (3.09383°N, 72.96651°E), 22/03/2016, 20 m depth. MA1016034: Wall Street Reef, Faafu Atoll, Maldives (3.12061°N, 73.09581°E), 16/10/2016, 17 m depth. MA0117058: Sunny Reef, Faafu Atoll, Maldives (3.14294°N, 73.01206°E), 04/02/2017, 20 m depth. MA0117174: Route 66 Reef, Faafu Atoll, Maldives (3.07717°N, 72.97328°E), 21/02/2017, 5 m depth. Medusa stage. MA021661B: 2-day-old medusae released from sample MA0216061.

Diagnosis. Polyps monomorphic, with 4-5 oral and 16-21 aboral capitate tentacles and a typical white band in the middle or in proximal part of the column. Hydrorhiza projecting out of the bryozoan skeleton with clusters of nematocysts. At release, medusa laterally compressed, with two tentacular bulbs bearing opposite tentacles; manubrium reaching the velar opening, mouth with oral arms.

Description of the polyp. Colony stolonal, growing in association with cheilostomate bryozoans (Figures 2A, 5A). Hydrorhiza devoid of a perisarc sheet, reticular, crawling under the bryozoan skeleton, often projecting out at the corners of zooeciae with clusters of nematocysts (Figures 2A, 5B). Polyps monomorphic. Gastrozooids tubular, up to 1.5 mm long, 55-80 µm wide proximally and 92-142 µm distally. Mouth distal and circular, surrounded by 4-5 short oral tentacles; 16-21 additional, aboral tentacles scattered irregularly-spirally over 2/3rd of the polyp column, comparatively shorter than their oral counterparts, occasionally represented by sessile capitula only (Figures 2A, 5C-D). Capitula with nematocysts and with inclusions (Figure 5E), bigger in oral tentacles (74-93 µm), and decreasing in size proximally (32-56 µm) (Figures 2A, 5C-D). Living polyps transparent, with a whitish mouth, an orange hypostome, and a typical white band in the middle or proximal half of the polyp (Figures 5A, C-D), which disappears in fixed material. Medusa buds minute, originating from the protruding hydrorhiza, and grouped in clusters of 1-5 (Figures 2A, 5F). Cnidome composed of stenoteles of two size classes (Figures 2D-E, 5G) and macrobasic holotrichous euryteles (Figures 2B-C, 5H-I). Large stenoteles (14.5-17.5 x 11.5-14 µm) in oral capitula and rarely in aboral capitula, hydrorhiza, and nematocyst clusters. Small stenoteles (5.5-7.5 x 4-6.5 µm) in all capitula, and rarely in hydrorhiza. Macrobasic holotrichous euryteles with shaft coiling along the short axis (24.5-29 x 11-16.5 µm, discharged shaft: 84-95 µm) abundant in hydrorhiza and in its projections.

Description of the newly released medusa. When released, medusae motionless for several hours. After about 10 hours, tentacles become projected outside the bell cavity. Newly released medusae small, globular, 152-162 µm high and 172-191 µm wide (Figures 2F, 5J-K). Several nematocysts scattered over the exumbrella. Manubrium 79-97 µm long and 33-52 µm wide, reaching the velar opening, with a terminal circular mouth with 3-4 arms 4-7 µm long (Figures 2F, 5L). Two opposite tentacular bulbs, initially projecting inside the bell cavity, and everted after two days. Each bulb bearing a tentacle up to 935 µm long, armed with 10-15 oval, hairy cnidophores (17-21 x 10-17 µm) borne on 30-70 µm long pedicels (Figures 2F, 5M). Each cnidophore containing 2 nematocysts. Living medusae transparent, with an orange manubrium basally, white distally (Figure 5K). After one week of cultivation, medusae with slightly longer tentacles but not displaying other differences. Adult, mature medusae not observed. Cnidome composed of macrobasic holotrichous mastigophores (Figures 2H, 5N-O) and macrobasic apotrichous euryteles (Figures 2G, 5P-Q). Macrobasic holotrichous mastigophores (7-8.5 x 6-7.5 µm, discharged shaft: 30.5-36.5 µm) scattered over the exumbrella; macrobasic apotrichous euryteles (5-7 x 3.5-6 µm, discharged shaft: 33-39 µm) in cnidophores and tentacular bulbs.

Distribution. Known from Faafu Atoll, Maldives.

Taxonomic remarks. *Zanclea* sp. 1 is strikingly similar to *Zanclella diabolica*, but it nevertheless

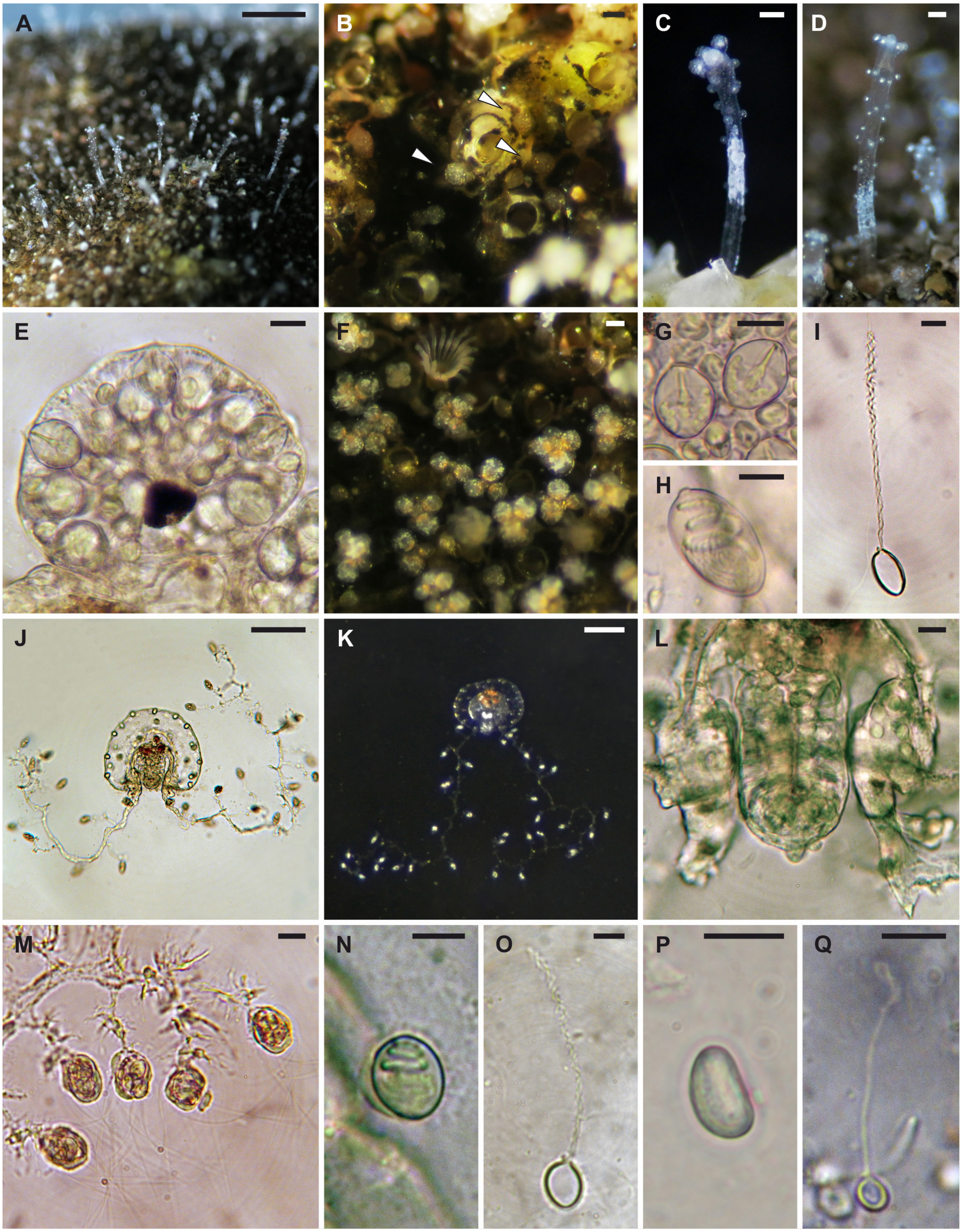

Figure 5. Polyp (A-I) and medusa (J-Q) of *Zanclea* sp. 1. **A:** General aspect of a colony; **B:** Clusters of nematocysts projecting out of the bryozoan skeleton (arrowheads); **C, D:** Gastrozooids; **E:** Aboral capitulum showing nematocysts and a central inclusion; **F:** Medusa buds arising from hydrorhiza; G: Stenoteles of two size classes; **H, I:** Undischarged and discharged macrobasic holotrichous euryteles, respectively; **J, K:** Newly released medusae; **L:** Manubrium ending in a terminal mouth with oral arms; **M:** Cnidophores; **N, O:** Undischarged and discharged macrobasic holotrichous mastigophores, respectively; **P, Q:** Undischarged and discharged macrobasic apotrichous euryteles, respectively. Scale bars: A: 1.5 mm; B-D, F, J, K: 100 µm; E, G-I, L, M: 10 µm; N-Q: 5 µm.

shows some distinguishing features (Tables 2, 3, Supplementary Tables S1, S2). In particular, the polyps of *Zanclea* sp. 1 have a higher number of aboral tentacles, a distinct white band on their column, nematocyst clusters containing euryteles and rarely stenoteles, in contrast with *Zanclella diabolica*, which contains three stenoteles according to Boero *et al.* (2000). Additionally, all nematocysts in the polyp stage of *Zanclea* sp. 1 are smaller than in *Zanclella diabolica*, and the mouth of the medusa is equipped with oral arms. No other zancleid medusae show this latter feature, which could be a synapomorphy shared by *Zanclea* sp. 1 and *Zanclea* sp. 2. Alternatively, the oral arms may have gone unnoticed in other zancleid species due to their extremely small size. At this stage, it is not clear whether the morphological differences between *Zanclea* sp. 1 and *Zanclella diabolica* represent intra- or inter-specific divergence, and only a thorough re-analysis of material from the type locality and its inclusion in molecular analyses will address this issue.

Zanclea sp. 2

Material examined. Polyp stage. FB002, FB015, FB018: Abu Latt Island, Al Lith, Saudi Arabia (19.94617°N, 40.15102°E), 30/04/2017, 3 m depth. FB476: Sofia's Reef, Al Lith, Saudi Arabia (19.79325°N, 40.40007°E), 07/05/2017, 15 m depth. KA119: Tahla Reef, Thuwal, Saudi Arabia (22.27501°N, 39.04715°E), 14/12/2015, 11 m depth. KA133: Abu Gishaa Reef, Thuwal, Saudi Arabia (22.42706°N, 39.05111°E), 16/12/2015, 17 m depth. MA0216066: Dighu Reef, Faafu Atoll, Maldives (3.08025°N, 72.98269°E), 13/02/2016, 20 m depth. MA1016036: Wall Street Reef, Faafu Atoll, Maldives (3.12061°N, 73.09581°E), 16/10/2016, 19 m depth. MA0117130, MA0117144: Kika Reef, Faafu Atoll, Maldives (3.08702°N, 72.95581°E), 13/02/2017, 14 m depth. Medusa stage. MA0216066B: 2-day-old medusae released from sample MA0216066.

Diagnosis. Polyps monomorphic, with 4-5 oral and 23-30 aboral capitate tentacles. Hydrorhiza projecting out of the bryozoan skeleton for some of its length. At release, medusa laterally compressed, with two tentacular bulbs bearing opposite tentacles; manubrium long, protruding from the velar opening, mouth with oral arms.

Description of the polyp. Colony stolonal, monomorphic, living in association with cheilostomate bryozoans (Figures 2I, 6A). Hydrorhiza naked, reticular, partially crawling under the bryozoan skeleton and partially projecting out for some of its length at the borders of zooeciae (Figures 2I, 6B). Gastrozooids cylindrical, up to 3 mm long, 106-147 µm wide at base and 108-156 µm wide distally, with an apical circular mouth, 4-5 oral tentacles and 23-30 aboral tentacles arranged spirally over the distal 3/4th of the polyp (Figures 2I, 6C). Tentacles short and reduced to sessile capitula in proximal part, their terminal capitations with nematocysts and inclusions (Figure 6.D). Oral tentacles with larger capitula (97-117 µm) and aboral tentacles with comparatively smaller capitula (52-104 µm), decreasing in size towards base of polyp (Figures 2I, 6C). Living polyps transparent (Figures 6A, C). Medusa buds minute, borne on short blastostyles arising directly from the projected hydrorhiza and grouped in clusters of up to 6 buds (Figures 2I, 6E). Cnidome composed of stenoteles of three size classes (Figures 2L-N, 6F) and macrobasic holotrichous euryteles (Figures 2J-K, 6G-H). Large stenoteles (18.5-21.5 x 12-17 µm), medium-sized stenoteles (15-18 x 12.5-14.5 µm) and small stenoteles (6-7.5 x 4-6 µm) in capitula and rarely in hydrorhiza; macrobasic holotrichous euryteles with shaft coiling along the short axis (18.5-21 x 11.5-14 µm, discharged shaft: 134-143 µm) abundant in hydrorhiza.

Description of the newly released medusa. At release, medusae motionless for several hours and projecting tentacles outside the bell cavity after about 10 hours. Newly released medusae small, globular, 168-180 µm high and 202-215 µm wide (Figures 2O, 6I-J). Exumbrella with several scattered nematocysts. Manubrium 126-146 µm long and 47-57 µm wide, protruding from the bell cavity, with a terminal circular mouth with 4-5 arms 4-6 µm long (Figures 2O, 6K). Two opposite bulbs everted from the bell cavity after two days and bearing two tentacles up to 560 µm long, armed with 11-17 oval and hairy cnidophores (15-25 x 14-20 µm) borne on 17-38 µm long pedicels (Figures 2O, 6L). Each cnidophore with 1-3 nematocysts. Living medusae transparent, with manubrium orange proximally, and whitish for most of its length (Figure 6J). After one week of cultivation, medusae with slightly longer tentacles and no other differences. Adult, mature medusae not observed. Cnidome composed of macrobasic holotrichous mastigophores (Figures 2Q, 6M-N) and macrobasic apotrichous euryteles (Figures 2P, 6O-P). Macrobasic holotrichous mastigophores (7-10 x 6-8.5 µm, discharged shaft: 35.5-37 µm) scattered over the exumbrella; macrobasic apotrichous euryteles

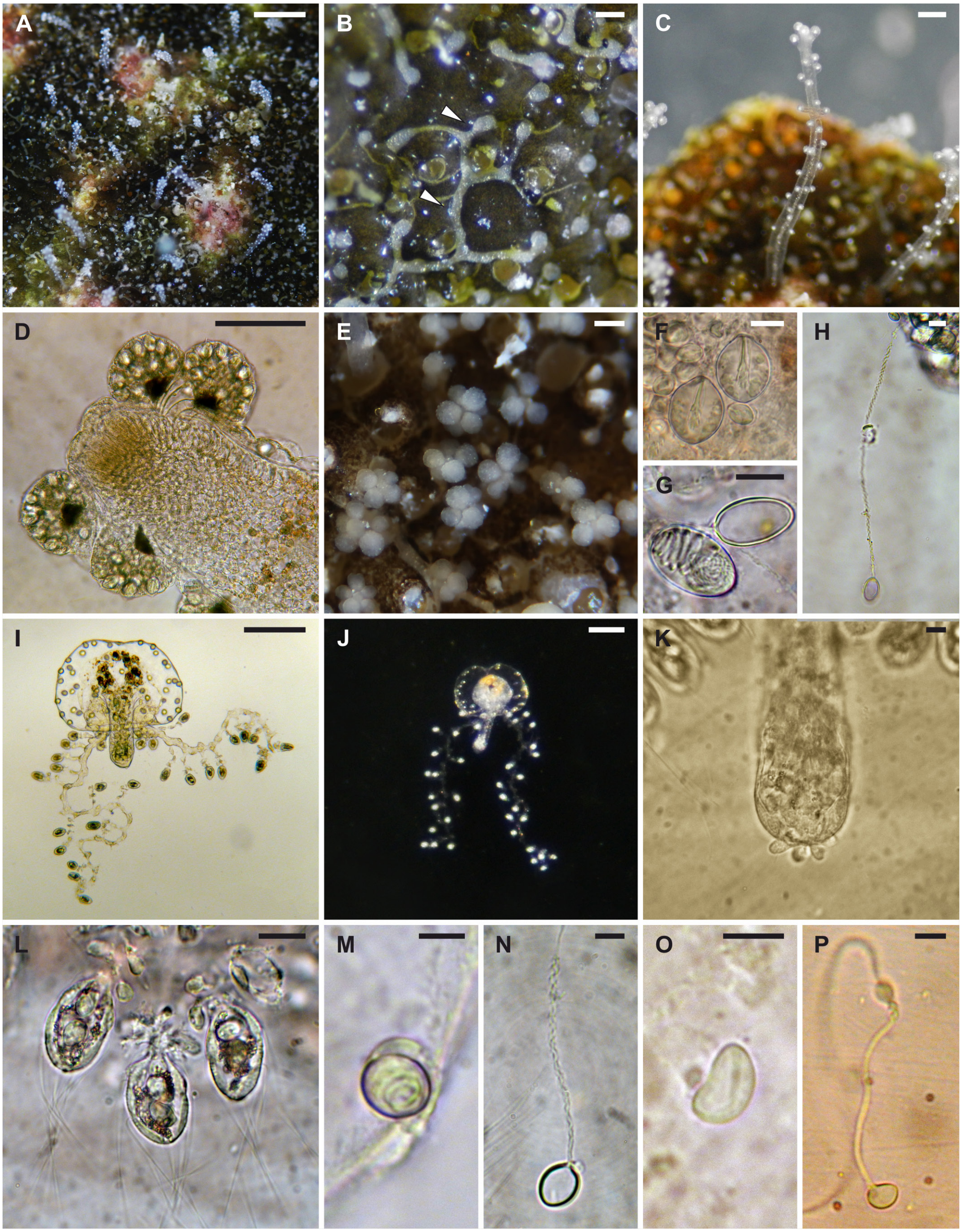

Figure 6. Polyp (A-H) and medusa (I-P) of *Zanclea* sp. 2. **A:** General aspect of a colony; **B.** Hydrorhiza partially extruding at the surface of the bryozoan host (arrowheads); **C.** Gastrozooids; **D.** Apical portion of a gastrozooid, showing four oral tentacles with nematocysts and central inclusions; **E:** Medusa buds arising from the exposed hydrorhiza; **F:** Large and small stenoteles; **G, H:** Undischarged and discharged macrobasic holotrichous euryteles, respectively; **I, J:** Newly released medusae; **K:** Terminal part of the manubrium showing a mouth with five arms; **L:** Cnidophores; **M, N:** Undischarged and discharged macrobasic holotrichous mastigophores, respectively; **O, P:** Undischarged and discharged macrobasic apotrichous euryteles, respectively. Scale bars: A: 1.5 mm; B, C: 200 µm; D, E, I, J: 100 µm; F, G, H, K, L: 10 µm; M-P: 5 µm.

(6-8 x 4-5.5 µm, discharged shaft: 37.5-39.5 µm) in cnidophores and tentacular bulbs.

Distribution. Known from Faafu Atoll (Maldives), Thuwal and Al Lith (Red Sea, Saudi Arabia).

Taxonomic remarks. *Zanclea* sp. 2 and *Zanclea* sp. 1 are similar to *Zanclella diabolica* in both the polyp and medusa stages. These species have *Zanclea*-like polyps, hydrorhizae projecting out of the bryozoan skeleton, minute medusa buds borne on small pedicels given off from the hydrorhiza, newly released medusae small, globular, and a similar cnidome. However, some diagnostic differences are found in both the polyp and medusa stages (Tables 2, 3, Supplementary Tables S1, S2). The hydrorhizae in *Zanclella diabolica* and *Zanclea* sp. 1 show nematocyst clusters with a more regular and approximately spherical shape, whereas in *Zanclea* sp. 2 the hydrorhiza projects out of the bryozoan skeleton in a more irregular way and for some of its length. Moreover, the polyps in *Zanclea* sp. 2 are longer and have generally more tentacles than the other two species. Other differences are found in the size of the polyp nematocysts, as *Zanclea* sp. 2 has stenoteles of three size classes (instead of two) and smaller euryteles with a shaft length in-between the other two species. Also, the medusa of *Zanclea* sp. 2 has a longer manubrium extending further out of the velar aperture. The morphological differences between *Zanclea* sp. 1 and *Zanclea* sp. 2 are well supported by phylogenetic and genetic distance analyses, since these two species form two fully supported monophyletic clusters.