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Systematic affinities of Zygophylacidae (Cnidaria: Hydrozoa: Macrocolonia) with descriptions of 15 deep-sea species

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Hydrozoans (Cnidaria) are distributed worldwide and exhibit alternating benthic polyp (hydroid) and pelagic medusan life-history forms. Zygophylacidae are a hydrozoan family with an exclusive hydroid stage throughout their life cycle. Within Hydrozoa, they are unusual in that a relatively large proportion (50%) of species occur in deep waters, but their validity and systematic affinities have been controversial for more than a century. Here, 97 deep-sea specimens, collected by manned submersible or bottom dredging, were investigated using an integrative taxonomical approach. Molecular data concatenating the 16S, 18S and 28S rRNA genes support the validity of Zygophylacidae and their placement within Macrocolonia, instead of Lafoeida. Fourteen zygophylacids and one relevant lafoeid are described or re-described, including three newly proposed species: ***Zygophylax lighti*** **sp. nov.** and ***Z. tankahkeei*** **sp. nov.** from the South China Sea, ***Z. pseudosibogae*** **sp. nov.** from Portugal and five species introduced by Eberhard Stechow (1883–1959) through the re-examination of type material. Four candidate generic diagnoses are discussed for future Zygophylacidae fine systematics by integrating a taxonomic review of all 74 zygophylacids. Morphological characters such as long hydrothecae and complex protective gonothecal structures mostly present in the deep-sea zygophylacids are heuristic for future functional morphology and evolutionary studies.

ADDITIONAL KEYWORDS: hydroid – Leptothecata – molecular phylogeny – new species – species redescription – *Zygophylax*.

INTRODUCTION

Marine benthic hydroids (Hydrozoa) are frequent colonizers of hard substrates in both shallow and deep waters worldwide. Within Hydrozoa, Leptothecata are the most species-rich order (Bouillon *et al.*, 2006), with more than 2100 accepted (nominal) species (Schuchert, 2021). The family Zygophylacidae Quelch, 1885 are distributed worldwide, mostly in waters of the deep sea (Cornelius, 1995; Vervoort & Watson, 2003). At present, Zygophylacidae comprise 74 valid species (Bouillon *et al.*, 2006; Schuchert, 2021; this study), grouped into three nominal genera: *Abietinella* Levinsen, 1913, *Cryptolaria* Busk, 1857 and *Zygophylax* Quelch, 1885. However, the validity and systematic affinities of Zygophylacidae have been controversial for more than a century and are still unclear at present.

Initially, the presence of a pair of nematophores at the base of the hydrotheca was proposed as a diagnostic character for Zygophylacidae (Quelch, 1885). Later, Stechow (1921) treated them as a subfamily, Zygophylacinae, and assigned them to the family Lafoeidae Hincks, 1869, together with three other subfamilies: Bonneviellinae, Hebellinae and Oswaldariinae, based on a single shared character,

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their tubular hydrothecae. This classification was broadly accepted for a long time, including the studies by Bouillon *et al*. (1985, 2006), Rees & Vervoort (1987), Vervoort (1987), Calder (1991), Hirohito (1995), Calder & Vervoort (1998), Schuchert (2001) and Marques *et al.* (2006). Bouillon *et al.* (2006) even disregarded the subfamily Zygophylacinae and incorporated their genera into the family Lafoeidae. Nevertheless, Stepanjants (1979) and Antsulevich (1988) treated Zygophylacidae as a valid family due to the deep hydrothecae and the presence of hydrothecal diaphragms.

Recent molecular phylogenetic analyses, solely using the 16S marker, pointed out that Lafoeinae and Zygophylacinae might represent clades belonging to different families (Moura *et al.*, 2008, 2012, Peña Cantero *et al.*, 2010, Galea & Schuchert, 2019), with so far unclear phylogenetic positions within the Macrocolonia clade (Maronna *et al.*, 2016). However, the clade stability of Zygophylacidae in previous molecular trees based on the single 16S rRNA (Moura *et al.*, 2008, 2012; Peña Cantero *et al.*, 2010; Maronna *et al.*, 2016) was not well supported, with the exception of a tree contributed by Galea & Schuchert (2019) that only included three zygophylacids. This is mainly attributable to the limited resolution of the 16S rRNA gene (Song *et al.*, 2016a) and the low number of relevant taxa considered in previous analyses (Moura *et al.*, 2008; Peña Cantero *et al.*, 2010; Maronna *et al.*, 2016; Galea & Schuchert, 2019). According to verification by the molecular cloning method, the full DNA lengths of 18S and 28S genes amplified in some Macrocolonia hydrozoans are 1789–1837 bp and 3175–3381 bp, respectively (Song *et al.*, 2016b), while the full length of the 16S rRNA amplified by the commonly used primers SHA and SHB (Cunningham & Buss, 1993) is only 628–637 bp (Song *et al.*, 2016b). The use of 18S and 28S markers is preferable to investigate systematic relations above the genus level. In Hydrozoa, the concatenation of the 16S, 18S and 28S rRNA genes has been widely applied and showed high resolution for the systematics of order, family or genus levels (e.g. Cartwright *et al.*, 2008; Leclère *et al.*, 2009; Maronna *et al.*, 2016, Song *et al.*, 2018, 2019).

In the present study, the validity, systematic position and phylogenetic relationships of Zygophylacidae are resolved using both morphological and molecular approaches. A combination of 16S, 18S and 28S rRNA sequences derived from 56 species has revealed detailed tree topologies. Specimens belonging to 14 deep-sea zygophylacids and one lafoeid are morphologically described and illustrated. The present work investigates 14 *Zygophylax* species, including the descriptions of three new *Zygophylax* species and the redescriptions of five poorly known additional congeners based on type material.

MATERIAL AND METHODS

Specimens examined

In the present study, 97 specimens belonging to 14 zygophylacids and one lafoeid were investigated. The recently gathered specimens were collected either by the manned submersible *Shenhaiyongshi* or by bottom dredgings in the western Pacific (Japan, South China Sea), Indian, Atlantic and Southern Oceans (Fig. 1). All species, except for specimens of *Zygophylax pacifica* Stechow, 1920 (coastal water of Japan), originated from deep waters. Inspected specimens include: (1) syntypes of *Z. abyssicola* (Stechow, 1926), *Z. africana* Stechow, 1923, *Z. curvitheca* Stechow, 1913, *Z. pacifica* and *Z. valdiviae* Stechow, 1923 deposited in the Zoologische Staatssammlung München (with registration prefix ZSM, collected from Japan and the Indian and Atlantic Oceans, 178–1644 m depth); (2) the holotype and paratype of the new species *Z. lighti* (37 specimens, collected in the South China Sea, at 921–1816 m) and *Z. tankahkeei* (two specimens, collected in the South China Sea, at 2359 m) deposited in the Collection of Benthos, Museum of Marine Science and Technology, Xiamen University (with registration prefix XMUB); (3) specimens of six deep-sea species sampled from the north-eastern Atlantic, at depths of 526–1419 m (Moura *et al.*, 2008, 2012) and deposited in the Department of Biology, University of Aveiro (with registration prefix DBUA; several colonies for each species illustrated and re-assessed herein were simultaneously deposited and re-registered in the ZSM) including *Z. biarmata* Billard, 1905, *Z. brownei* Billard, 1924, *Z. leloupi* Ramil & Vervoort, 1992, *Z. levinseni* (Saemundsson, 1911), *Z.* cf. *pseudafricanus* Vervoort & Watson, 2003 (previously identified as *Z. sagamiensis* Hirohito, 1983 by Moura *et al.*, 2012) and the new species *Z. pseudosibogae* (previously identified as *Z. sibogae* Billard, 1918 by Moura *et al.*, 2012); (4) specimens of *Z. pacifica* recently collected from Shimoda, Japan (XMUB, 86–91 m depth); (5) specimens of *Z.* cf. *sagamiensis* collected in the South China Sea (XMUB, 1322–1426 m depth); (6) specimens of *Lafoea dumosa* (Fleming, 1820) from Maxwell Bay, Antarctic (XMUB, 260–420 m depth). The investigation of *L. dumosa* serves to compare the difference between zygophylacids and lafoeids. Detailed collection information is presented in the Supporting Information, Table S1. A map showing the collection locations was prepared with Ocean Data View v. 4.5.3 (Fig. 1).

Morphological examinations

Photography and light microscopy

At Xiamen University, images of hydroid colonies were taken using a Canon 6D camera provided

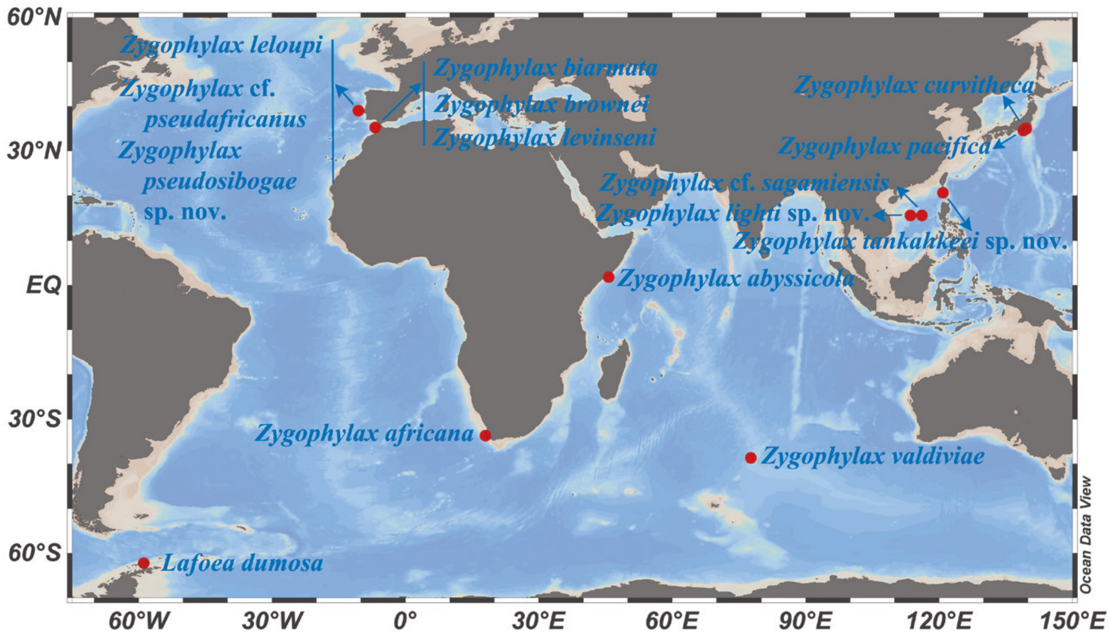

Figure 1. The collecting localities of the *Zygophylax* and *Lafoea* specimens examined herein.

with a 100 mm macro-lens; photomicrographs were obtained using a Leica DFC495 camera connected to a stereomicroscope (Leica MDG41), using the LEICA APPLICATION SUITE 4.5.0.418 software. At the ZSM (Stechow's hydroid collection), images of larger hydroid colonies were taken using a Sony Alpha 6000 camera provided with a 60 mm macro-lens; photomicrographs were taken with a Nikon 1 V1 camera attached to either a Leica DMRBE compound microscope or a Leica Z16 APO macroscope. Most high-magnification images were produced by stacking a z-series of 5–20 images using the software HELICON FOCUS 7.6.1.

Scanning electron microscopy (SEM)

Hydrocladia and coppiniae selected for SEM imaging were dried at 60 °C for 20 min, mounted on double-sided carbon NEMTAPE tape and sputter-coated with gold for 90 s. Images were captured on a Gemini Zeiss SEM (Suppa 55 Sapphire, 20 kV, Xiamen University).

Measurements and morphological plates

Sixteen selected morphological characters of *Zygophylax abyssicola*, *Z. lighti*, *Z. pseudosibogae*, *Z. tankahkeei* and related species were measured (Table 1). Nineteen morphological plates were prepared for all species using the ADOBE PHOTOSHOP CC 2019 software (Figs 2–20). All original images that support this study have been deposited in MorphoBank (https://morphobank.org/permalink/?P4108).

Morphological comparisons

For detailed morphological comparisons and character evolution analyses, 34 characters from all 74 nominal species of Zygophylacidae (Bouillon *et al.*, 2006; Schuchert, 2021; this study) were reviewed based on specimens examined in this study and in literature data (Supporting Information, Table S2). For the validation of these three new species, described herein, and the detection of potential synonyms of *Zygophylax abyssicola*, six key diagnostic characters of these species were compared with other morphologically similar taxa (Table 2).

LECTOTYPE DESIGNATIONS

To provide an objective standard of reference for *Zygophylax abyssicola*, *Z. africana*, *Z. curvitheca*, *Z. pacifica* and *Z. valdiviae* (material in the ZSM), the largest colony portion of each species preserved in ethanol was chosen as the lectotype. The remaining syntypes were chosen as paralectotypes. See details in the Systematic Account.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Non-destructive DNA extraction

Selected hydroid fragments (hydrocauli, hydrocladia or coppiniae) were detached from the corresponding colonies, immersed directly in 1.5-mL centrifuge tubes containing 100 µL manufacturer's LB14 buffer and 20 µL Proteinase K and shaken slowly (120 rotation

Table 1. Measurements of selected *Zygophylax* species based on light microscopy data (in mm).

Measurements	Group 1							Group 2			
	Z. abyssicola	*Z. bifurcata*	*Z. africana*	*Z. curvitheca*	*Z. pacifica*	*Z. pacifica*	*Z. valdiviae*	*Z. lighti*	*Z. pseudosibogae*	*Z. sibogae*	*Z. tankahkeei*
Specimen	Type material	Type material	Type material	Type material	Type material	Non-type, Shimoda, Japan	Type material	Type material	Type material	Type locality, Kei Islands, Indonesia	Type material
Water depth (m)	1644	828	178	600	250	86–91	672	921–1816	1294	310	2359
Colony length	50	55	18	80	35.8	75	2.27	20	38	Up to several cm	20
Hydrocaulus											
- Maximal diameter	1	1.5	0.5	2	1.33	1.25	0.05	0.2	0.77	Unknown	0.5
- Distance between two successive hydrocladia of a pair	/	Unknown	0.42–0.67	1–1.33	0.5–0.67	0.65–0.77	/	0.6–0.8	0.63–1	Unknown	0.69–0.86
- Distance between two successive hydrocladia on same side	/	Unknown	1.81–2.00	3–3.33	2.83–3.17	2.54–2.73	/	2.5–2.95	3.25–3.75	Unknown	2.15–3.6
Hydrocladium											
- Length of internode	0.86–1.06	Unknown	/	/	/	/	/	1.2–1.4	0.5–0.75	/	2.18–2.91
- Distance between two successive hydrothecae	0.74–0.98	Unknown	0.25–0.35	0.58–1.16	0.44–0.56	0.51–0.64	0.36–0.42	0.6–0.8	0.42–1	Unknown	0.5–1
Hydrotheca											
- Pedicel length (insertion-diaphragm)	0.27–0.36	0.46–0.57	0.06–0.1	0.22–0.30	0.13–0.22	0.13–0.15	0.04–0.10	0.35–0.45	0.40–0.58	0.35–0.5	0.27–0.95
- Diameter of pedicel	0.07–0.09	Unknown	0.06–0.09	0.10–0.11	0.1–0.13	0.08–0.1	0.03	0.05–0.06	0.05–0.06	0.04–0.05	0.05–0.12
- Length of hydrotheca (diaphragm-aperture)	0.40–0.49	0.36–0.39	0.25–0.35	0.72–0.96	0.6–0.7	0.53–0.59	0.22–0.30	0.4–0.5	0.42–0.58	0.3–0.35	0.52–1.14

Measurements	Group 1							Group 2			
	Z. abyssicola	*Z. bifurcata*	*Z. africana*	*Z. curvitheca*	*Z. pacifica*	*Z. pacifica*	*Z. valdiviae*	*Z. lighti*	*Z. pseudosibogae*	*Z. sibogae*	*Z. tankahkeei*
- Diameter of diaphragm	0.07–0.09	Unknown	0.04–0.05	0.13–0.15	0.13–0.15	0.09–0.10	0.03–0.04	0.07–0.1	0.10–0.12	0.05–0.06	0.09–0.14
- Diameter of aperture	0.17–0.19	0.16–0.19	0.06–0.08	0.26–0.33	0.27–0.33	0.23–0.25	0.08–0.09	0.1–0.12	0.19–0.24	0.15	0.1–0.2
Nematotheca											
- Length	0.16	0.14–0.16	0.07–0.18	0.23–0.26	0.1–0.13	0.09–0.14	0.04–0.07	0.08–0.28	0.05–0.22	0.08–0.09	0.05–0.16
- Diameter of aperture	0.04	0.05–0.06	0.03–0.05	0.02–0.03	0.05	0.05	0.02	0.02–0.03	0.03–0.04	0.02	0.03–0.04
Gonotheca											
- Length	0.82–0.98	1.38	/	/	/	/	/	0.7–0.73	0.7–0.8	/	1.1–1.2
- Maximal width	0.40–0.48	0.34–0.36	/	/	/	/	/	0.77–0.8	0.65–0.9	/	1.2–1.4
- Diameter of distal aperture	0.13–0.16	0.11–0.18	/	/	/	/	/	0.06–0.1	0.07–0.16	/	0.07–0.18
Reference	This study	Billard (1942); Rees & Vervoort (1987)	This study	This study	This study	This study	This study	This study	This study	Schuchert (2003)	This study

Notes: Group 1 is mainly for taxa revised in the present study; Group 2 is for *Zygophylax sibogae* and the new proposed species.

Table 2. Comparative characters of *Zygophylax abyssicola*, *Z. lighti*, *Z. pseudosibogae*, *Z. tankahkeei* and species with close morphological similarities

Species	Hydrocladium		Hydrotheca		Gonothecal apertures		References
	Branching	Internode	Twisted angle	Indentation*	Tubes	Number	
Group 1							
Z. abyssicola	Dichotomous	Regular	0°–15°	Absent	Straight	2	Stechow (1926); this study
Z. bifurcata	Dichotomous	Irregular	0°	Absent	Slightly twisted	2	Billard (1942); Rees & Vervoort (1987)
Z. flexilis	Dichotomous	Absent	0°	Absent	Unknown	Unknown	Pictet & Bedot (1900)
Z. geniculata	Dichotomous	Absent	0°	Absent	Unknown	Unknown	Clarke (1894)
Z. leloupi	Alternate	Absent	25°–30°	Absent	Twisted	2–3	Ramil & Vervoort (1992)
Z. levinseni	Alternate	Irregular	0°–30°	Absent	Twisted	2	Saemundsson (1911)
Z. stechowi	Alternate	Absent	0°–15°	Absent	Twisted	2	Jäderholm (1919); Hirohito (1995)
Z. tottoni	Subalternate	Irregular	0°–45°	Absent	Unknown	Unknown	Rees & Vervoort (1987)
Z. unilateralis	Alternate	Irregular	0°–30°	Absent	Straight	3	Totton (1930); Ralph (1958); Rees & Vervoort (1987)
Group 2							
Z. concinna	Subalternate	Irregular	30°–45°	Absent	Unknown	Unknown	Ritchie (1911)
Z. convallaria	Alternate	Unknown	15°–45°	Absent	Twisted	2	Allman (1877); Hirohito (1995)
Z. elongata	Alternate	Irregular	45°–60°	Absent	Straight	2–3	Ramil & Vervoort (1992)
Z. infundibulum	Alternate	Irregular	80°–90°	Absent	Twisted	2	Millard (1958); Rees & Vervoort (1987)
Z. kakaiba	Subopposite	Absent	80°–90°	Absent	Unknown	Unknown	Campos *et al.* (2016)
*Z. lighti***	Alternate	Irregular	60°	Present	Twisted	2	This study
*Z. pseudosibogae***	Alternate	Irregular	90°	Absent	Twisted	2	This study
Z. sibogae	Alternate	Absent	90°–120°	Absent	Twisted	2–3	Billard (1918); Hirohito (1995); Schuchert (2003)
*Z. tankahkeei***	Alternate	Irregular	60°	Present	Twisted	2–3	This study
Z. tizardensis	Alternate	Absent	90°	Present	Unknown	Unknown	Kirkpatrick (1890)

Notes: Group 1 comprises taxa relevant to *Zygophylax abyssicola*; Group 2 comprises taxa relevant to the new proposed species in this study.
*Kirkpatrick (1890) interpreted the indentation as a half-ring at the upper dorsal part of hydrotheca in *Zygophylax tizardensis*.
**Morphological comparisons in detail for all 74 zygophylacids, including the new proposed species, are given in the Supporting Information, Table S2.

per minute) at 55 °C for 6–12 h, depending on their size. The dissolved solution was used for further DNA extraction according to the manufacturer's protocol (Micro Genomic DNA EE181Kit, Transgen, Beijing, China). The remaining hydroid chitin skeletons were washed twice with deionized water, then dried at 50 °C for 1 h and finally kept in 1.5-mL centrifuge tubes for museum deposition. Some newly dried hydroid fragments were directly used for SEM mounting and morphological analyses.

DNA amplification and sequencing

DNA amplification and sequencing of the 16S rRNA and *COI* genes were performed as described by Song *et al.* (2016b), except for the direct sequencing of purified polymerase chain reaction (PCR) products (done by Thermo Fisher Scientific, Guangzhou, China). For the 18S and 28S rRNA genes, primers designed by Song *et al.* (2018) were used to amplify several overlapping short fragments, considering possible DNA degradation in older specimens. Thirty-two new sequences in the course of this study were deposited in GenBank with the accession numbers MT261928–MT261948, MT262560–MT262562, MZ680504, MZ680505, MZ686450–MZ686455 (Supporting Information, Table S3).

Phylogenetic analyses

Construction of phylogenetic trees

Three maximum likelihood (ML) and Bayesian phylogenetic trees were constructed (Figs 21, 22; Supporting Information, Fig. S1) based on three datasets (Supporting Information, Datasets S1–S3). The concatenated tree (16S + 18S + 28S) was designed to determine the phylogenetic position of Zygophylacidae within the order Leptothecata (Fig. 21), whereas the 16S tree was designed to reveal phylogenetic relations between the zygophylacids with available 16S rRNA data (Fig. 22). The *COI* tree was prepared to test the validity of the two new species (*Zygophylax lighti* and *Z. tankahkeei*) in this study (Supporting Information, Fig. S1). In addition to the 32 new sequences contributed by the present study, public sequences (Cunningham, 2004, direct submission; Govindarajan *et al.*, 2006; Leclère *et al.*, 2007, 2009; Cartwright *et al.*, 2008; Moura *et al.*, 2008, 2012; Peña Cantero *et al.*, 2010; Maronna *et al.*, 2016; Song *et al.*, 2016b, 2018, 2019; Boissin *et al.*, 2018; Galea & Schuchert, 2019) of the representative taxa of the main clades of Leptothecata, as well as selected available sequences (Moura *et al.*, 2008, 2012; Peña Cantero *et al.*, 2010; Boissin *et al.*, 2018; Galea & Schuchert, 2019) of all zygophylacids, were extracted from GenBank as reference. Type species were prioritized for taxa selection. Due to the absence of reference sequences, the *COI* tree was constructed using only the five new sequences contributed by the present study. *Hydractinia symbiolongicarpus* Buss & Yund, 1989 or *Clytia hemisphaerica* (Linnaeus, 1767) were chosen as outgroups.

The protocols for phylogenetic analyses are described in detail by Song *et al.* (2016b). The final sequence length after alignment for the concatenated, 16S and *COI* trees were 5446 bp (16S: 486 bp; 18S: 1661 bp; 28S: 3299 bp), 548 bp and 762 bp, respectively. According to the Akaike information criterion, GTR+I+G, TIM2+I+G and TIM2+I were chosen as the optimal probabilistic evolution models for the concatenated, 16S and *COI* trees, respectively. The Bayesian analyses for the concatenated and *COI* trees were integrated in the ML tree as they shared almost the same topologies (Fig. 21; Supporting Information, Fig. S1). The Bayesian results for the 16S tree were shown and annotated separately, as their topologies were slightly different from the ML tree (Fig. 22).

Genetic distances

Pairwise Kimura 2-Parameter (K2P) genetic distances for inter- and intraspecific within Zygophylacidae were calculated as specified in Song *et al.* (2016b) in order to detect cryptic or potential new species. Available 16S rRNA sequences of 14 zygophylacids from GenBank (Moura *et al.*, 2008, 2012; Peña Cantero *et al.*, 2010; Boissin *et al.*, 2018; Galea & Schuchert, 2019) and sequences contributed by this study were calculated. For calculating the interspecific genetic distance, only one representative sequence, which takes up the middle position within its species clade on the 16S tree (Fig. 22), was selected to simplify the calculation (Table 3). Five *COI* sequences of *Zygophylax* contributed by this study were also used for the genetic distance analysis (Table 4). To determine the intraspecific genetic distance, all available 16S sequences of several selected species, including *Cryptolaria pectinata* (Allman, 1888), *Z. biarmata*, *Z. levinseni*, *Z. niger* Galea, 2019 and *Z. rufa* (Bale, 1884) (Moura *et al.*, 2008, 2012; Boissin *et al.*, 2018; Galea & Schuchert, 2019), were calculated (Table 5).

RESULTS

Molecular phylogenetics

Affinities of the monophyletic Zygophylacidae within Macrocolonia

The monophyly of the Macrocolonia clade within Leptothecata is well supported by the concatenated tree (16S + 18S + 28S) (Fig. 21) with high clade stability values of ML bootstrap (98) and Bayesian posterior probability (1). The ML bootstrap and

Bayesian posterior probability values for the entire monophyletic Zygophylacidae clade are 93 and 1, respectively. The Zygophylacidae clade clusters with the nearest monophyletic clade of Plumularioidea within Macrocolonia (clade stability values: 97, 1). Additionally, both ML and Bayesian analyses, solely with the 16S rRNA (Fig. 22), also highly support the monophyly of Zygophylacidae (clade stability values: 96, 1). All these results provide solid support for the validity of the family Zygophylacidae and its placement within Macrocolonia instead of Lafoeida.

Inter- and intraspecific genetic distances

Based on all available Zygophylacidae molecular data, the interspecific K2P genetic distances of the 16S rRNA and *COI* genes and the intraspecific K2P genetic distances of the 16S rRNA gene for zygophylacids were calculated (Tables 3–5). The 16S rRNA interspecific genetic distances within zygophylacids are 0.010–0.181. The distances between *Zygophylax lighti* and *Z. tankahkeei* is 0.010, lower than the distance between *Z. pseudosibogae* and *Z. lighti* (0.022), and *Z. pseudosibogae* and *Z. tankahkeei* (0.022). This result is consistent with the two phylogenetic trees (Figs 21, 22): *Zygophylax lighti* clusters with *Z. tankahkeei* first (having the closest phylogenetic relationship) and both cluster with *Z. pseudosibogae.* The intraspecific K2P genetic distances of the 16S rRNA also reveal that both *Cryptolaria pectinata* and *Z. rufa* have large intraspecific genetic distances, 0.000–0.028 and 0.000–0.038, respectively.

The *COI* interspecific genetic distances within five zygophylacids are 0.041–0.159. The *COI* interspecific K2P distances are higher than the corresponding 16S interspecific K2P distances between every two species (Tables 3, 4), indicating that the 16S gene is more conservative than the *COI* gene in the Zygophylacidae clade. The *COI* distances between *Zygophylax lighti* and *Z. tankahkeei* is 0.041, higher than that for the 16S rRNA (0.010), providing a genetic basis for species distinction.

Systematic account

Class Hydrozoa Owen, 1843

Subclass Leptothecata Cornelius, 1992

Order Lafoeida Bouillon, 1984

Family Lafoeidae Hincks, 1869

Genus *Lafoea* Lamouroux, 1821

Lafoea dumosa (Fleming, 1820)

(Fig. 2)

Lafoea dumosa: Cornelius, 1995: 261–263, fig. 60 (synonymy).

Type locality: Arbroath, Scotland.

Specimens examined: Ten infertile colonies from the Antarctic; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA (Cunningham, 2004, direct submission; Peña Cantero *et al.*, 2010), 18S rRNA (this study) and 28S rRNA (Cartwright *et al.*, 2008;

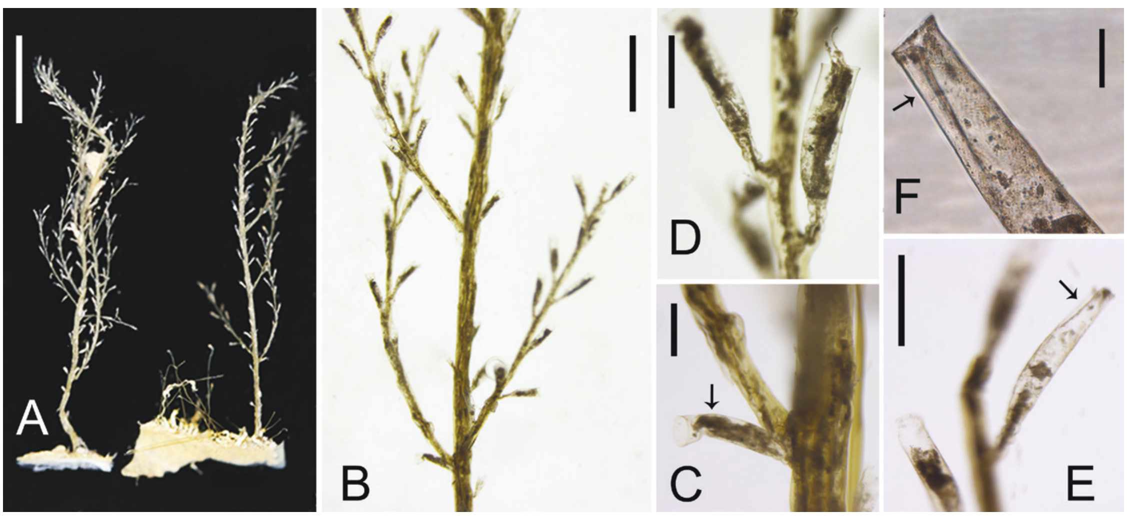

Figure 2. *Lafoea dumosa* (Fleming, 1820). A, colonies; B, a polysiphonic hydrocaulus with three polysiphonic hydrocladia; C, magnification of a cauline hydrotheca (arrow); D, two hydrothecae; E, F, two collapsed hydrothecae (arrows); A–D: XMUB6498; E, F: XMUB6468; all from Maxwell Bay, Antarctica. A–F, light microscope images. Scale bars: A = 5 mm; B = 2 mm; C–E = 500 µm; F = 100 µm.

this study) genes; see sequence details in Supporting Information, Table S3.

Measurements: See details in Supporting Information, Table S2.

Description: Trophosome. Colonies erect, about 2 cm in height (Fig. 2A). Hydrocaulus straight, polysiphonic, branched, unsegmented, with almost verticillate hydrothecae (Fig. 2B). Hydrocladia forming angles of about 45° with hydrocaulus, without axillary hydrotheca; almost verticillate, straight, polysiphonic except distally, unsegmented (Fig. 2B). Hydrothecae also verticillate, form three longitudinal rows, the adjacent two rows forming an angle of about 120° (Fig. 2B, D); borne on short pedicels, the distal part of hydrotheca straight or forming the angle of about 30° with the proximal part, without diaphragm, renovation or nematotheca (Fig. 2C–F), pedicels with one to four twists (Fig. 2D).

Gonosome: Absent.

Distribution: Widely distributed in the Antarctic, Pacific, north-eastern Atlantic and Indian Oceans (Fleming, 1820; Cornelius, 1995; Schuchert, 2001; this study).

Remarks: Schuchert (2001) found variations in the hydrothecal length and the presence or absence of a hydrothecal pedicel in sympatric specimens of *Lafoea dumosa* from Greenland and Iceland. Moura *et al.* (2012) found three distinct 16S rRNA lineages corresponding to specimens collected from Iceland and Antarctica, and suspected the existence of possible cryptic species. The current identification of *L. dumosa* in Antarctica relies on the original description of *L. dumosa* and Antarctic records by Peña Cantero *et al.* (1995) and molecular data obtained in the present study. The morphology of the investigated Antarctic specimens is also consistent with European records (Cornelius, 1995). *Lafoea dumosa* resembles the investigated Zygophylacidae species through its tubular hydrothecae (see below). However, the latter have one or two hydrothecal diaphragms and numerous nematothecae, while *L. dumosa* has none (Bouillon *et al.*, 2006).

Order Macrocolonia Leclère *et al.*, 2009

Family Zygophylacidae Quelch, 1885

Diagnosis: Solitary hydrothecae arising from hydrorhiza directly, or erect, pinnate or flabellate colonies, with polysiphonic hydrocauli in most species, otherwise monosiphonic. Hydrocladia mostly arranged in one plane, alternate to subopposite. Hydrothecae mostly tubular, seldom campanulate or subuliform, with short to long pedicel, with one or two diaphragms, with or without indentation (intrathecal projections) at distal part of the hydrothecae, and with one to numerous nematothecae on hydrocauli, hydrocladia and/or apophyses of hydrocladia. Gonothecae congregate to form coppiniae on stem or its hydrocladia; coppiniae with or without defensive tubes, the latter occasionally provided with hydrothecae or nematothecae. Individual gonothecae solitary or adnate, generally elongate or ovoid, seldom flask- or carrot-shaped, apices either with a circular aperture or with one to three straight or curved short tubes.

Remarks: Molecular data contributed by the present study support the genetic distinction and validity of the family Zygophylacidae (Figs 21, 22). Based on specimens examined in this study and morphological diagnoses proposed by Rees & Vervoort (1987), Vervoort (1987) and Cornelius (1995), both zygophylacids and lafoeids have similar tubular hydrothecae. However, they differ in the fact that nematothecae and hydrothecal diaphragms are commonly present in Zygophylacidae, but are absent in Lafoeidae (Bouillon *et al.* 2006; this study).

Genus *Zygophylax* Quelch, 1885

Zygophylax abyssicola (Stechow, 1926)

(Figs 3, 4, 5A–F)

Lictorella abyssicola Stechow, 1926: 99; Ruthensteiner *et al.*, 2008: 19.

Zygophylax abyssicola: Rees & Vervoort, 1987: 76; Galea & Schuchert, 2019: 59.

?*Zygophylax bifurcata* Billard, 1942: 34–35, figs 1–3.

Type locality: Somalia, near Mogadishu, north-west Indian Ocean; 1644 m.

Specimens examined: Eight syntypes from the Indian Ocean, four of them with gonothecae; see details in Supporting Information, Table S1.

Lectotype designation: Lectotype, a fertile, formalin-fixed then ethanol-preserved colony, ZSM 20040197 (Fig. 3A, arrow); paralectotypes, the remaining colonies from sample ZSM 20040197 (Fig. 3A, the left colony, G), ZSM 20040726 (Fig. 3A, the right colony, D), formalin-fixed then ethanol stored, ZSM 20043537–20043542 (Figs 3B, C, E, F, H, I, 5A–F), microslides.

Measurements: See Table 1.

Description: Trophosome. Colonies erect, up to 5 cm high (Fig. 3A, B). Hydrocauli upright, branched,

Table 3. Interspecific K2P genetic distances for zygophylacids with available 16S rRNA data

No.	Species	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13
1	*Abietinella operculata*													
2	*Cryptolaria pectinata*	0.029												
3	*Zygophylax biarmata*	0.037	0.042											
4	*Zygophylax brownei*	0.037	0.044	0.050										
5	*Zygophylax leloupi*	0.037	0.041	0.046	0.020									
6	*Zygophylax levinseni*	0.037	0.046	0.050	0.048	0.046								
7	*Zygophylax lighti*	0.039	0.048	0.048	0.044	0.046	0.050							
8	*Zygophylax niger*	0.131	0.137	0.137	0.128	0.138	0.122	0.136						
9	*Zygophylax pacifica*	0.075	0.087	0.081	0.094	0.105	0.095	0.071	0.164					
10	*Zygophylax* cf. *pseudafricanus*	0.016	0.022	0.029	0.039	0.035	0.033	0.039	0.131	0.080				
11	*Zygophylax rufa*	0.088	0.107	0.088	0.103	0.111	0.101	0.092	0.181	0.069	0.091			
12	*Zygophylax* cf. *sagamiensis*	0.022	0.035	0.035	0.039	0.044	0.033	0.031	0.125	0.072	0.014	0.088		
13	*Zygophylax pseudosibogae*	0.028	0.037	0.039	0.033	0.035	0.048	0.022	0.136	0.084	0.031	0.095	0.033	
14	*Zygophylax tankahkeei*	0.035	0.044	0.048	0.039	0.041	0.046	0.010	0.136	0.076	0.035	0.098	0.031	0.022

Notes: 16S sequence length after alignment is 522 bp. The 16S GenBank accession numbers of all taxa (No. 1–14) are FN424136, JN714656, JN714683, **MT261944**, JN714681, AM888344, **MZ686452**, MK073107, **MT261948**, **MT261945**, MH108228, **MZ686453**, **MT261946**, **MT261947**, respectively. Accession numbers in bold were contributed by this study. Information on sequences see Supporting Information, Table S3.

Table 4. Interspecific K2P genetic distances for zygophylacids with available *COI* data

No.	Species	No. 1	No. 2	No. 3	No. 4
1	*Zygophylax brownei*				
2	*Zygophylax pacifica*	0.138			
3	*Zygophylax* cf. *sagamiensis*	0.128	0.139		
4	*Zygophylax lighti*	0.110	0.141	0.134	
5	*Zygophylax tankahkeei*	0.119	0.159	0.136	0.041

Notes: *COI* sequence length after alignment is 761 bp, the *COI* GenBank accession numbers of all taxa (No. 1–5), contributed by this study, are MT262560, MT262562, MZ680505, MZ680504, MT262561, respectively. Information on sequences see Supporting Information, Table S3.

polysiphonic (Fig. 3C), nodes not observed, with alternate hydrothecae (Fig. 3B). Hydrocladia forming an angle of 60°–90° with the hydrocauli (or their higher-order counterparts) (Fig. 3B), with one axilliary hydrotheca per branching site (Figs 3E, 4B); coplanar, dichotomously branched, straight or slightly geniculate, polysiphonic proximally, monosiphonic distally (Fig. 3B, C), monosiphonic part divided by regular nodes, each internode with one or two hydrothecae (Figs 3D, 4A). Hydrothecae arranged alternately, forming angles of about 60° with the corresponding hydrocladium (Fig. 3B); borne on well-defined pedicels, tubular distally, tapering proximally, the distal part of hydrotheca straight or forming angles of about 15° with their proximal part; diaphragm straight (at junction with pedicel), with or without renovations. Only one tubular nematotheca observed on the hydrothecal pedicel (Figs 3F, 4C).

Gonosome: Female gonothecae observed. Gonothecae aggregated into coppiniae encircling both hydrocauli and hydrocladia (Fig. 3G); coppiniae with numerous dichotomous protective branches, without nematothecae (Figs 3I, 4E). Individual gonothecae free, urn-shaped, distal end with two lateral short tubes provided with circular apertures (Figs 3H, 4D).

Distribution: Only recorded from the type locality, off East Africa (Stechow, 1926; this study).

Remarks: The original description of *Zygophylax abyssicola* does not contain any morphological illustrations (Stechow, 1926). It is known exclusively from the type locality, with no additional records in the literature (Stechow, 1926; Rees & Vervoort, 1987; Ruthensteiner *et al.*, 2008; Galea & Schuchert, 2019). In the present study, we find that *Z. bifurcata* Billard, 1942, another deep-sea species (type locality: Malay Archipelago), may be a synonym of *Z. abyssicola*. Both species branch dichotomously, have straight, tubular, similarly sized hydrothecae (Table 1) and their gonothecae have two apertures (Table 2). They differ in the number of internodes on the hydrocladia: abundant internodes were observed in the type material of *Z. abyssicola*, while internodes of *Z. bifurcata* are rare, according to the original description (Billard, 1942). The geographic distance between the type localities of both species is noteworthy (about 9000 km). Consequently, whether *Z. bifurcata* is a synonym of *Z. abyssicola* needs further confirmation by molecular data. *Zygophylax elongata* Ramil & Vervoort, 1992 is

Table 5. Intraspecific K2P genetic distances for zygophylacids with available 16S rRNA data

Species	Haplotype numbers	Genetic distances	Sequence length (aligned)	Sequence source
Cryptolaria pectinata	7	0.000–0.028	521 bp	Moura *et al.* (2008, 2012)
Zygophylax biarmata	6	0.000–0.008	514 bp	Moura *et al.* (2008, 2012)
Z. levinseni	2	0.014	519 bp	Moura *et al.* (2008, 2012)
Z. niger	2	0.014	603 bp	Galea & Schuchert (2019)
Z. rufa	10	0.000–0.038	589 bp	Boissin *et al.* (2018)

Notes: See GenBank accession numbers for analysed sequences in Supporting Information, Table S3.

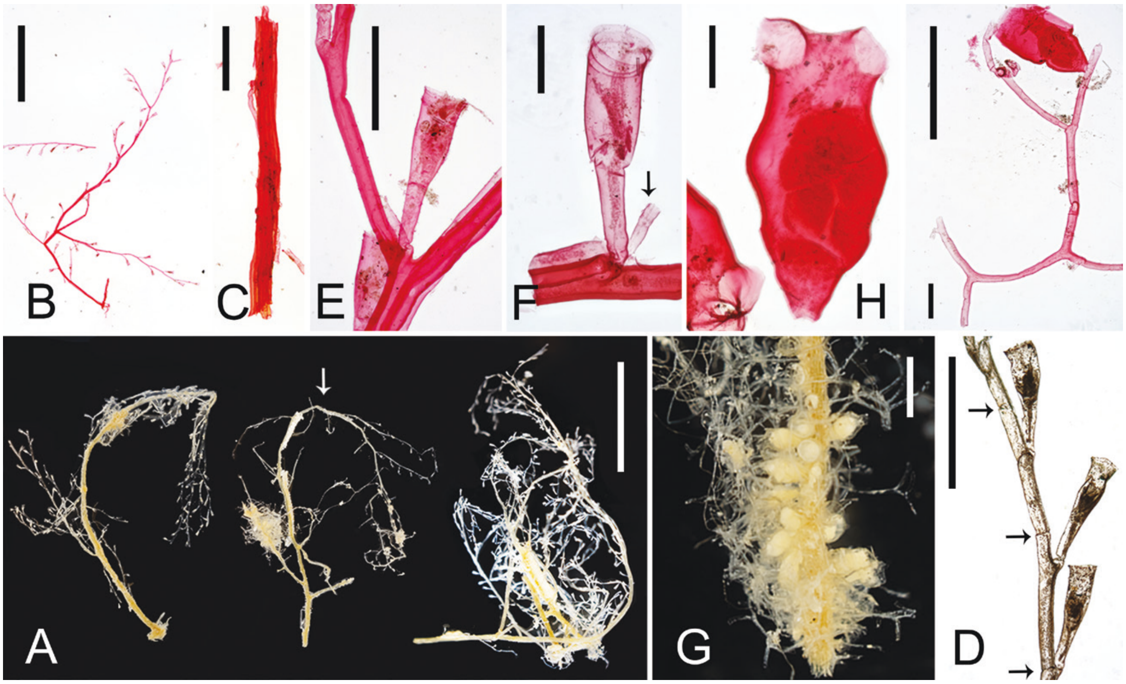

Figure 3. Type material of *Zygophylax abyssicola* (Stechow, 1926). A, B, colonies with dichotomous branching patterns, the arrow in A indicates the lectotype; C, a polysiphonic hydrocaulus; D, three successive nodes (arrows) on a hydrocladium, each internode bearing a hydrotheca; E, an axillary hydrotheca; F, a hydrotheca with diaphragm and renovations, and a nematotheca (arrow) near the hydrotheca; G, a coppinia; H, an isolated gonotheca, whose apex bears two apertures; I, a gonotheca with dichotomously branched defensive tubes. From samples: ZSM 20040197, paralectotype [A (left colony), G]; ZSM 20040197, lectotype [A (middle colony)]; ZSM 20040726, paralectotype [A (right colony), D]; ZSM 20043538, paralectotype (B, E); ZSM 20043539, paralectotype (C, F); ZSM 20043542, paralectotype (H, I). A–I, Indian Ocean. A–I, light microscope images. Scale bars: A = 1 cm; B = 0.5 cm; C, D, G, I = 1 mm; E = 0.5 mm; F, H = 200 μm.

also similar to *Z. abyssicola,* but the hydrothecal length of *Z. elongata* is about twice that of the investigated *Z. abyssicola* specimens. Furthermore, the former species has more curved hydrothecae.

ZYGOPHYLAX AFRICANA STECHOW, 1923

(FIGS 5G, H, 6)

Zygophylax africana Stechow, 1923a: 106–107; 1925: 445–446, fig. 18; Millard, 1964: 15–18, fig. 4A–F; 1968: 263; 1973: 28, fig. 4B; 1975: 189–190, fig. 62A–E; 1977: 106; 1980: 131; Gravier-Bonnet, 1979: 29; Rees & Vervoort, 1987: 75; Bouillon *et al.*, 2006: 341; Ruthensteiner *et al.*, 2008: 25; Altuna, 2012: 5–8, figs 2, 3; Campos *et al.*, 2020: 540–543, pl. 2A–G.

?*Zygophylax africana:* Hirohito, 1983: 22–24, fig. 6; 1995: 136–138, fig. 40a–e.

Zygophylax africanus: Vervoort & Watson, 2003: 69 [incorrect subsequent spelling].

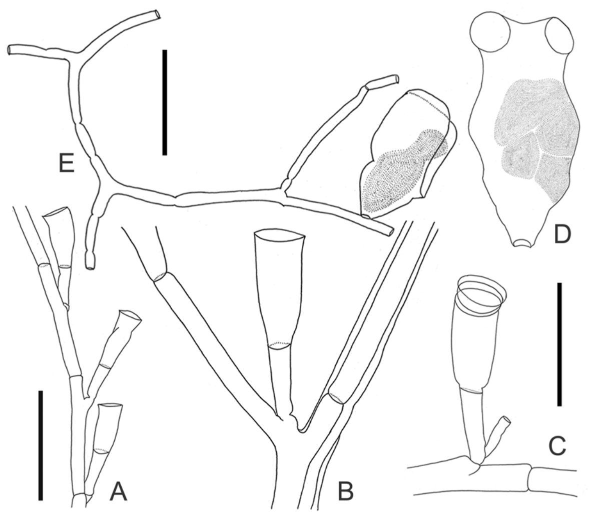

Figure 4. Line drawings of *Zygophylax abyssicola* (Stechow, 1926). A, three successive nodes on a hydrocladium, each internode bearing a hydrotheca; B, an axillary hydrotheca; C, a hydrotheca, showing its diaphragm and renovations, and a nematotheca; D, an isolated gonotheca, whose apex bears two apertures; E, a gonotheca with dichotomously branched defensive tubes. Scale bars: A = 1 mm; B–D = 500 µm; E = 500 µm.

Type locality: South Africa, off Cape Town, north of Agulhas Bank; 178 m.

Specimens examined: Three infertile syntypes from South Africa; see details in Supporting Information, Table S1.

Lectotype designation: Lectotype, an infertile, formalin-fixed then ethanol-preserved colony, ZSM 20040731 (Fig. 6A, arrow); paralectotypes, the remaining colonies from ZSM 20040731 (Fig. 6A), ZSM 20041574, ZSM 20043579 (Figs 5G, H, 6B–D), all as microslides.

Measurements: See Table 1.

Description: Trophosome. Incipient colonies composed of solitary hydrothecae arising directly from the hydrorhiza, well-developed colonies erect, about 1.8 cm high, pinnate (Fig. 6A). Hydrocaulus straight, polysiphonic, tapering upwards, unsegmented, bearing alternate hydrothecae and hydrocladia (Fig. 6A, B). Hydrocladia coplanar, straight, monosiphonic, undivided, forming angles of about 75° with the hydrocaulus, each branching site with an axillary hydrotheca (Fig. 6B). There are one or three hydrothecae between two successive pairs of hydrocladia. Hydrothecae forming angles of about 45° with the longitudinal axes of the corresponding hydrocladia (Fig. 6B, C), borne on short pedicels, tubular, distal part curved abaxially and forming angles of 15°–45° with the proximal part, diaphragm straight to oblique, margin slightly everted, with or without renovations (Fig. 6C). Nematothecae on hydrocladia, hydrocauli and apophyses of hydrocladia; tubular, with basal diaphragm (Fig. 6D).

Gonosome: Absent.

Distribution: South Africa (Stechow, 1923a; Millard, 1964, 1975; this study); Iberian Peninsula (Altuna, 2012); Réunion Island (Campos *et al.*, 2020).

Remarks: Campos *et al.* (2020) proposed designating the ethanol material and microslides as the holotype and paratypes, respectively, but did not examine the type material deposited in the ZSM. In the present study, lectotypes were designated with details following their proposal (Campos *et al.*, 2020). Millard (1964, 1975) provided accounts on the morphology of the gonothecae based on fertile specimens from the type locality. The Japanese records by Hirohito (1983, 1995) are questionable because his specimens have three to six hydrothecae between two successive hydrocladia, while the type material usually exhibits two of these.

ZYGOPHYLAX BIARMATA BILLARD, 1905

(FIG. 7)

Zygophylax biarmata: Ramil & Vervoort, 1992: 59–65, figs 11e–h, 12a–i, 13e, f (synonymy).

Type locality: Bay of Biscay, north-east Atlantic; 411 m.

Specimens examined: Three colonies from the Gulf of Cadiz, one fertile; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA gene (Moura *et al.*, 2008, 2012); see sequence details in Supporting Information, Table S3.

Measurements: See Supporting Information, Table S2.

Description: Trophosome. Colonies upright, about 0.9 cm high (Fig. 7A). Hydrocaulus geniculate, polysiphonic, unsegmented, branching regularly (Fig. 7A, B). Hydrocladia coplanar, forming angles of 60°–90° with the hydrocaulus (Fig. 7B), dichotomously branched, mono- or polysiphonic, with irregular nodes (Fig. 7B, C), axillary hydrothecae present at the

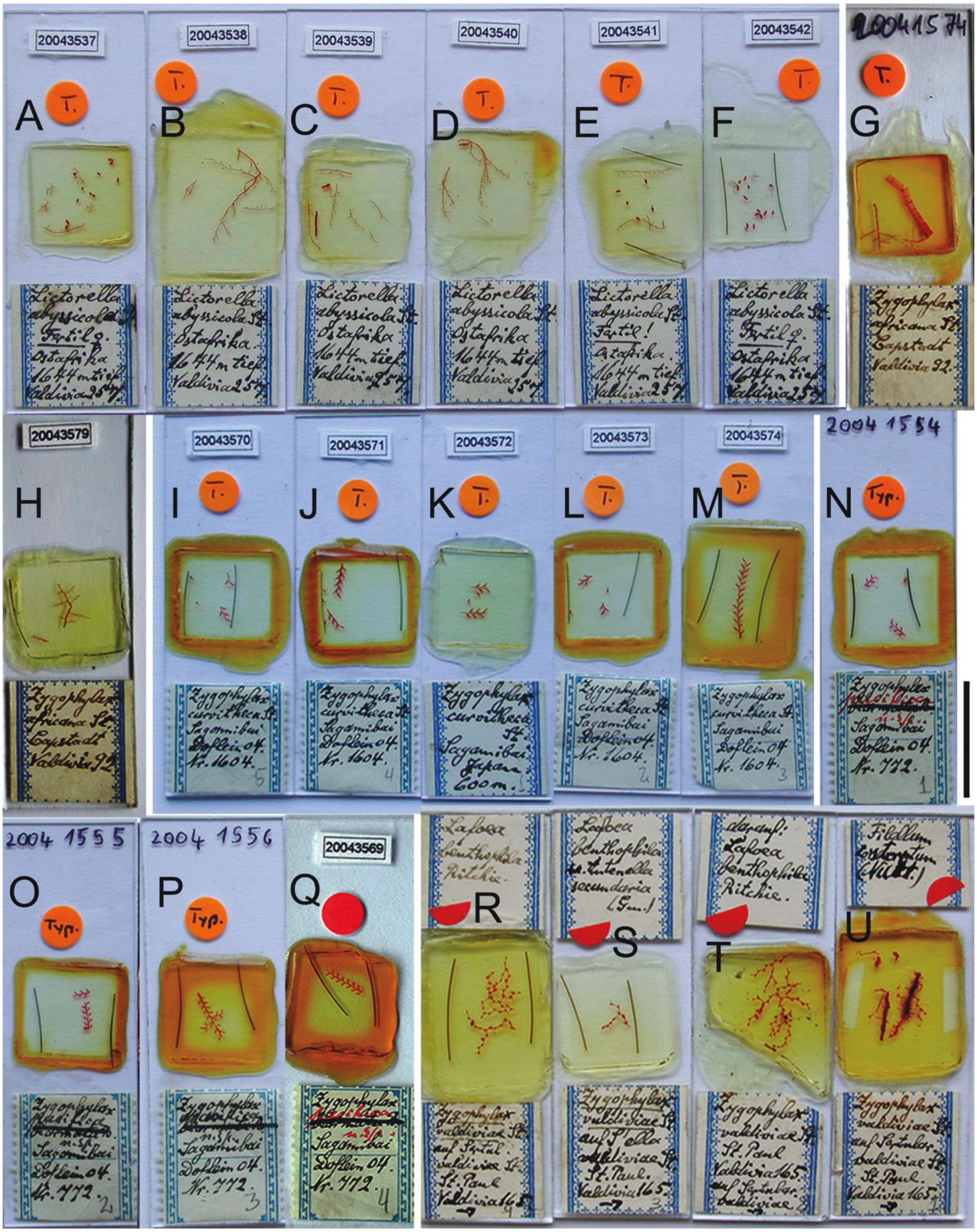

Figure 5. Type materials (all paralectotypes) of five *Zygophylax* species deposited in the Zoologische Staatssammlung München. A–F, *Z. abyssicola* (Stechow, 1926), ZSM 20043537–ZSM 20043542; G, H, *Z. africana* Stechow, 1923, ZSM 20041574, ZSM 20043579; I–M, *Z. curvitheca* Stechow, 1913, ZSM 20043570–ZSM 20043574; N–Q, *Z. pacifica* Stechow, 1920, ZSM 20041554–ZSM 20041556, ZSM 20043569; R–U, *Z. valdiviae* Stechow, 1923, ZSM 20041566–ZSM 20041569. Scale bars: A–U = 2 cm.

branching sites (Fig. 7D). Hydrothecae alternating regularly in one plane, forming angles of 60°–90° with the longitudinal axes of the corresponding hydrocladia (Fig. 7C), short-pedicellate, tubular, the distal part straight or curved away from the internode and forming angles of about 30° with the proximal part; diaphragm

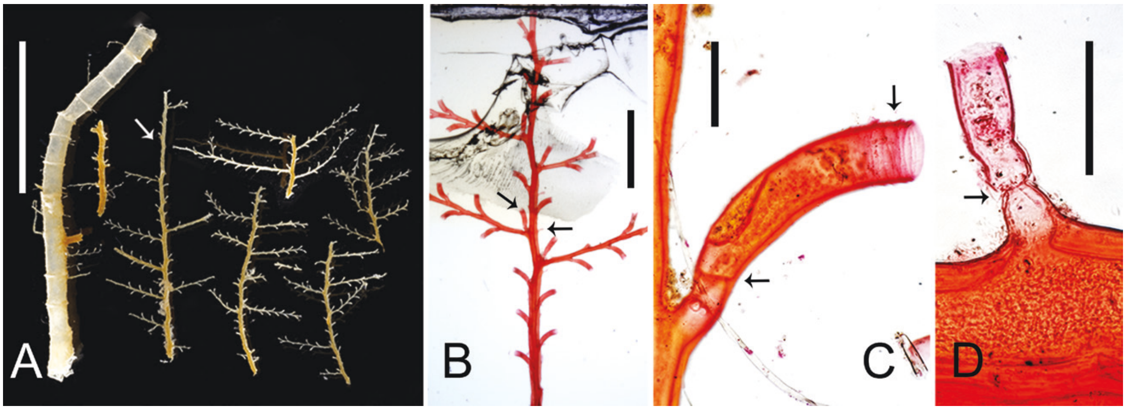

Figure 6. Type material of *Zygophylax africana* Stechow, 1923. A, various colonies; B, portion of polysiphonic hydrocaulus with four monosiphonic hydrocladia, the left and the right arrows indicate an axillary hydrotheca and a nematotheca, respectively; C, hydrothecal diaphragm (lower arrow) and renovations (upper arrow); D, magnification of the nematotheca in B, the arrow indicates a diaphragm within the nematotheca. From samples: A, ZSM 20040731, the arrow indicates the lectotype, other colonies are parelectotypes; B–D, ZSM 20041574, paralectotype. A–D, near Cape Town, South Africa. A–D, light microscope images. Scale bars: A = 1 cm; B = 1 mm; C, D = 100 µm.

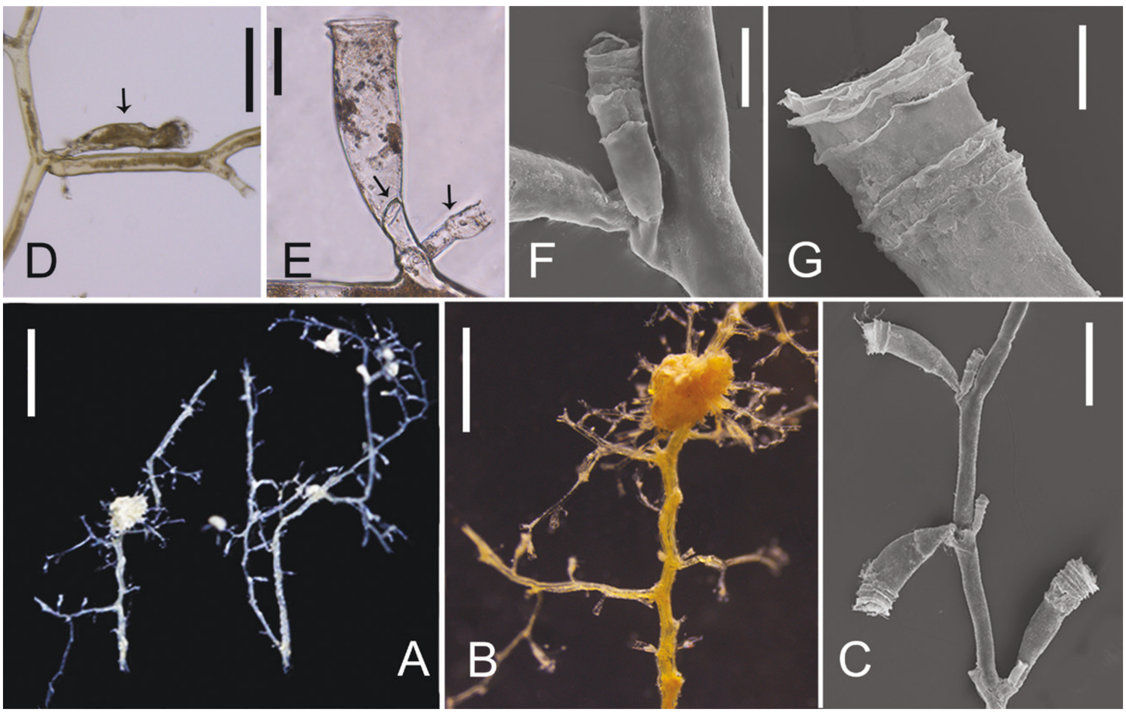

Figure 7. *Zygophylax biarmata* Billard, 1905. A, two colonies; B, hydrocaulus with coppinia; C, hydrothecal arrangement along a hydrocladium; D, an axillary hydrotheca (arrow); E, a hydrotheca with diaphragm (left arrow) and basal nematotheca (right arrow); F, magnification of a nematotheca; G, distal part of a hydrotheca showing multiple renovations. From samples: A–G: ZSM 20220294, Gulf of Cadiz. A, B, D, E, light microscope images; C, F, G, SEM images. Scale bars: A, B = 2 mm; C = 200 µm; D = 500 µm; E = 100 µm; F, G = 50 µm.

oblique, margin slightly everted, with two to seven renovations (Fig. 7C, E, G). A tubular nematotheca present at apophyses of hydrocladia, with one to six renovations (Fig. 7C, E, F).

Gonosome: Gonothecae clustering into coppiniae on hydrocaulus (Fig. 7A, B); coppiniae with dichotomously branched defensive tubes and hydrothecae. The morphology of individual gonothecae could not be established properly without destructive methods, and the coppiniae in the present material are obscured by adhering particles, not allowing a detailed examination to be done.

Distribution: Gulf of Cadiz (Moura *et al.*, 2012; this study); Bay of Biscay north-west of Spain; the Azores; Madeira; the Canary Islands; the east coast of South Africa; the Madagascar area; the Zanzibar area (See Ramil & Vervoort, 1992).

ZYGOPHYLAX BROWNEI BILLARD, 1924

(FIG. 8)

Zygophylax brownei: Ramil & Vervoort, 1992: 65–70, figs 13a–d, 14a–c (synonymy).

Type locality: Bay of Biscay, north-east Atlantic; 186 m.

Specimens examined: Three colonies from the Gulf of Cadiz, one of them fertile; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA, 18S rRNA, 28S rRNA and *COI* genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See Supporting Information, Table S2.

Description: Trophosome. Colonies erect, about 2.5 cm high. Hydrocaulus straight, polysiphonic, branched regularly (Fig. 8A). Hydrocladia coplanar, forming angles of 30°–60° with the hydrocaulus (Fig. 8A, B), axillary hydrothecae present at the branching sites (Fig. 8C), dichotomously branched, straight, polysiphonic except towards the distal ends, with irregular nodes (Fig. 8D). Hydrothecae arranged alternately along the hydrocladia, forming angles of 45°–60° with their longitudinal axes; borne on moderately long pedicels, tubular, distal parts straight or slightly curved, with a slightly oblique diaphragm basally and one or two renovations of their apertures, with or without annulations on the proximal part of the pedicel (Fig. 8C, D).

Gonosome: Gonothecae clustering into coppiniae, encircling the hydrocaulus halfway (Fig. 8B), without defensive tubes. Individual gonothecae solitary or adnate, elliptical in shape, tapering basally into an indistinct pedicel, apices with two or three rounded apertures mounted on short necks (Fig. 8E, F).

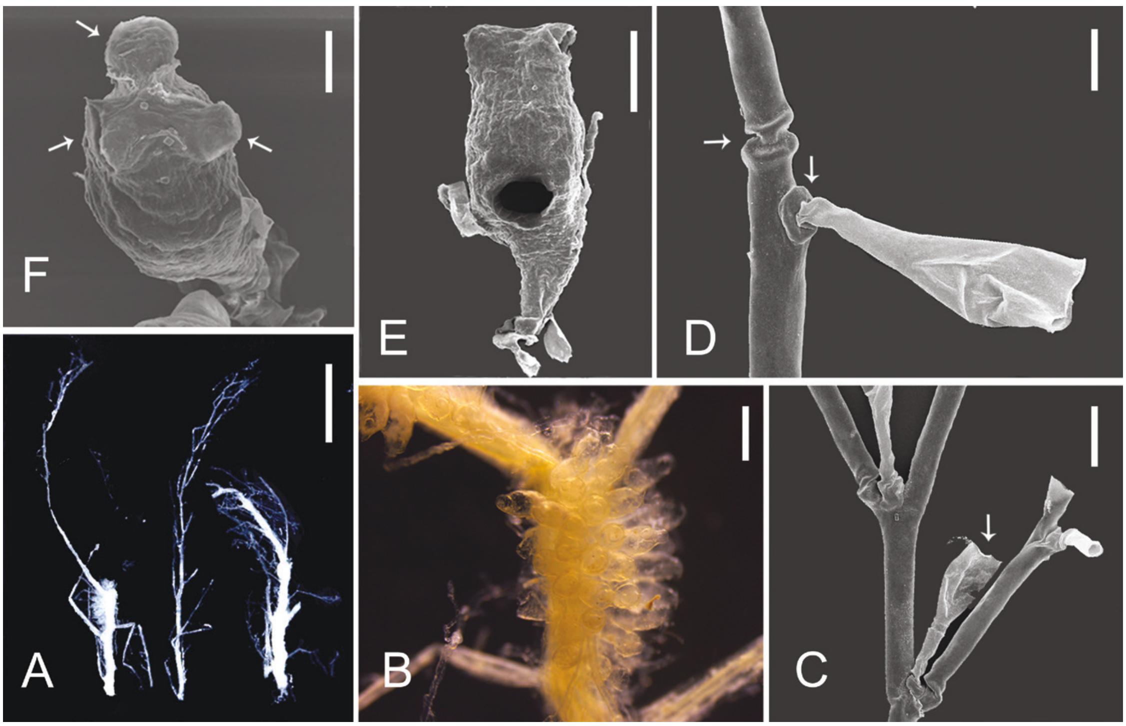

Figure 8. *Zygophylax brownei* Billard, 1924. A, three colonies; B, portion of hydrocaulus with coppinia; C, axillary hydrotheca (arrow); D, magnification of the node (left arrow) and the apophysis of hydrocladia (right arrow); E, a gonotheca; F, a gonotheca with three apical apertures (arrows). From samples: A–F: ZSM 20220295, Gulf of Cadiz. A, B, light microscope images; C–F, SEM images. Scale bars: A = 5 mm; B = 1 mm; C, E = 200 μm; D, F = 100 μm.

Distribution: Gulf of Cadiz (Moura *et al.*, 2012; this study); Bay of Biscay (see: Ramil & Vervoort, 1992).

ZYGOPHYLAX CURVITHECA STECHOW, 1913 (FIGS 5I–M, 9)

Zygophylax curvitheca Stechow, 1913a: 139–140; 1913b: 11, 116–117, fig. 89; 1923b: 10; Nutting, 1927: 212–213, pl. 41, fig. 3; Yamada, 1959: 47–48; Rees & Thursfield, 1965:78, 201; Smaldon *et al.*, 1976: 16; Rees & Vervoort, 1987: 72; Hirohito, 1995: 142, fig. 43a–d, pl. 9, fig. A; Ruthensteiner *et al.*, 2008: 25.

Type locality: Sagami Bay, Japan; 600 m.

Specimens examined: Six infertile syntypes from Japan; see details in Supporting Information, Table S1.

Lectotype designation: Lectotype, a fertile, formalin-fixed then ethanol-preserved colony, ZSM 20040199 (Fig. 9A–C); paralectotypes, ZSM 20043570–ZSM 20043574 (Figs 5I–M, 9D–F), microslides.

Measurements: See Table 1.

Description: Trophosome. Colony erect, up to 8 cm high, pinnate (Fig. 9A). Hydrocaulus straight, polysiphonic, unsegmented, with numerous nematothecae and alternate hydrothecae, branching (Fig. 9A, B). Hydrocladia coplanar, forming angles of 60°–80° with the hydrocaulus, each branching site with one axillary hydrotheca (Fig. 9B); straight, polysiphonic except towards the distal ends, unsegmented. Hydrothecae arranged alternately, forming angles of 30°–45° with the hydrocladia (Fig. 9B); tubular, short-pedicellate, distal part strongly curved abaxially, forming angles of 60°–90° with their proximal part, basally a straight diaphragm, margin slightly everted, with or without renovations (Fig. 9B, D, E). Nematothecae slender and tubular, present on hydrocauli, hydrocladia and the base of hydrothecal pedicels. (Fig. 9D–F).

Gonosome: An incipient coppinia with only defensive tubes but no gonothecae.

Distribution: Japan (Stechow, 1913a, b; Hirohito, 1995; this study); Philippines (Nutting, 1927).

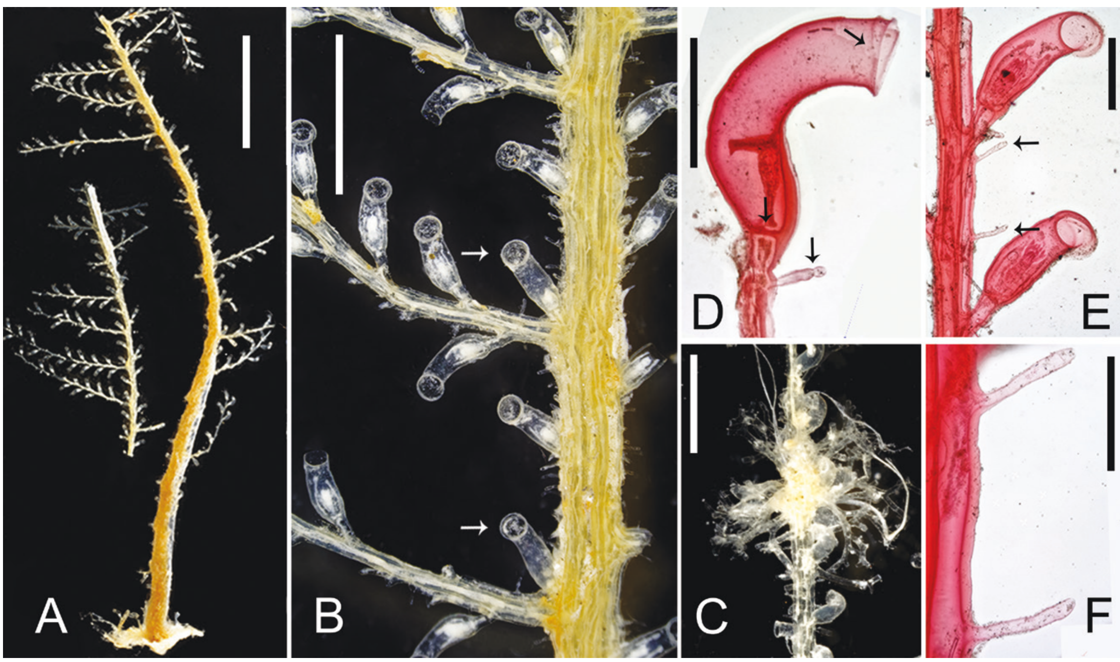

Figure 9. Type material of *Zygophylax curvitheca* Stechow, 1913. A, a colony; B, portion of a polysiphonic hydrocaulus with several basally polysiphonic hydrocladia, the arrows indicate two axillary hydrothecae; C, an incipient coppinia (with only defensive tubes but no gonothecae) on hydrocladium; D, a hydrotheca showing marginal renovations (upper arrow), diaphragm (middle arrow) and basal nematotheca (lower arrow); E, two hydrothecae and nematothecae (arrows) on a hydrocladium; F, magnification of the nematothecae in E. From samples: A–C: ZSM 20040199, lectotype; D: ZSM 20043572, paralectotype; E, F: ZSM 20043573, paralectotype. A–F, Sagami Bay, Japan. A–F, light microscope images. Scale bars: A = 1 cm; B, C = 2 mm; D, E = 500 μm; F = 250 μm.

Remarks: There are nematothecae on the defensive tubes of the coppiniae in the specimens collected from the type locality (Hirohito, 1995). Such nematothecae are absent in the Philippine material examined by Nutting (1927) and it can be assumed that they may have been overlooked.

ZYGOPHYLAX LELOUPI RAMIL & VERVOORT, 1992
(FIG. 10)

Zygophylax leloupi: Vervoort, 2006: 239–240, fig. 15 (synonymy).

Type locality: off Rabat, Marocco; 890 m.

Specimens examined: One infertile colony from Portugal; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA (Moura *et al.*, 2012) and 28S rRNA genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See Supporting Information, Table S2.

Description: Trophosome. Colony erect, about 1.3 cm in height (Fig. 10A). Hydrocaulus straight, polysiphonic, unsegmented, branched regularly. Hydrocladia coplanar, alternate, straight, monosiphonic (Fig. 10A, C), forming angles of 45°–120° with the hydrocaulus (Fig. 10A), axillary hydrothecae present at branching sites (Fig. 10E), with irregular nodes (Fig. 10D). Hydrothecae arranged alternately in two planes forming an angle of about 60° (Fig. 10C); borne on moderately long pedicels, tubular, distal part straight or slightly curved, diaphragm oblique, with one to four marginal renovations (Fig. 10E–G), with or without annulations at basal part of the pedicel (Fig. 10D). Only one nematotheca observed on an accessory tube of the hydrocaulus (Fig. 10B).

Gonosome: Absent.

Distribution: Near the Azores (Leloup, 1940); off Rabat, Morocco (Ramil & Vervoort, 1992); the Mid-Atlantic Ridge (Calder & Vervoort, 1998); Mound near Lisbon, Portugal (Moura *et al.*, 2012; this study).

ZYGOPHYLAX LEVINSENI (SAEMUNDSSON, 1911)
(FIG. 11)

Zygophylax levinseni: Vervoort, 2006: 240–242, fig. 16 (synonymy).

Type locality: Near the Vestmannaeyjar Archipelago, Iceland; 510 m.

Specimens examined: Five colonies from the Gulf of Cadiz, two fertile; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA (Moura *et al.*, 2008, 2012); 18S rRNA and 28S rRNA genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See details in Supporting Information, Table S2.

Description: Trophosome. Colonies erect, up to 4.5 cm in height (Fig. 11A). Hydrocaulus straight, polysiphonic, unsegmented, branching. Hydrocladia coplanar, dichotomously branched, straight, monosiphonic (Figs 11A, B), with irregular nodes (Fig. 11E), forming angles of 30°–60° with the hydrocaulus (Fig. 11A), axillary hydrothecae present at branching points (Fig. 11E). Hydrothecae arranged alternately in two planes forming angles of 30°–60° (Fig. 11B); borne on moderately long pedicels, tubular, distal part straight or slightly curved, one or two oblique diaphragms basally (Fig. 11D), margin slightly everted, with one to three renovations (Fig. 11D–F). One nematotheca present on apophyses of hydrocladia (Fig. 11F, H).

Gonosome: Gonothecae clustering into coppiniae encircling the hydrocaulus (Fig. 11C), with abundant protective tubes, one nematotheca observed on the base of one protective tube (Fig. 11G, I). Gonothecae free from one another, elliptical, tapering basally, apices with two lateral, curved tubes (Fig. 11C, G).

Distribution: Gulf of Cadiz (Moura *et al.*, 2008; this study); south of Iceland (Saemundsson, 1911); the Azores region (Leloup, 1940); near the coast of Rabat, Morocco (Ramil & Vervoort, 1992); one tropical Atlantic locality, south of Cape Verde Islands (Vervoort, 2006); Madeira (Moura *et al.*, 2012).

Remarks: *Zygophylax tottoni* Rees & Vervoort, 1987 resembles *Z. leviseni* by the hydrothecal morphology, but *Z. leviseni* has comparatively more numerous and smaller nematothecae; see details in Supporting Information, Table S2.

ZYGOPHYLAX LIGHTI GU & SONG **SP. NOV.**
(FIGS 12, 13A, B, 14A)

Zoobank registration: urn:lsid:zoobank.org:act:536BAF1F-9924-41D1-AF33-6612D85F206A.

Type locality: South China Sea; 921–1816 m.

Etymology: The specific name honours Professor Sol Felty Light (1886–1947) who served as the chairman of the Department of Zoology at Xiamen University from 1922 to 1924, and memorizes the centenary celebration of the biological disciplines in Xiamen University. Light's systematic studies on marine invertebrates

Figure 10. *Zygophylax leloupi* Ramil & Vervoort, 1992. A, fragmentary colony; B, a nematotheca (arrow) on an accessory tube of hydrocaulus; C, hydrothecal arrangement along a hydrocladium; D, annulations of the hydrothecal pedicel (upper arrow) and a node on a hydrocladium (lower arrow); E, axillary hydrotheca; F, a hydrotheca, showing its diaphragm (arrow); G, hydrothecal renovations. From samples: A–G: ZSM 20220296, Lisbon, Portugal. A, C–F, light microscope images; B, G, SEM images. Scale bars: A = 5 mm; B, G = 100 µm; C = 2 mm; D–F = 500 µm.

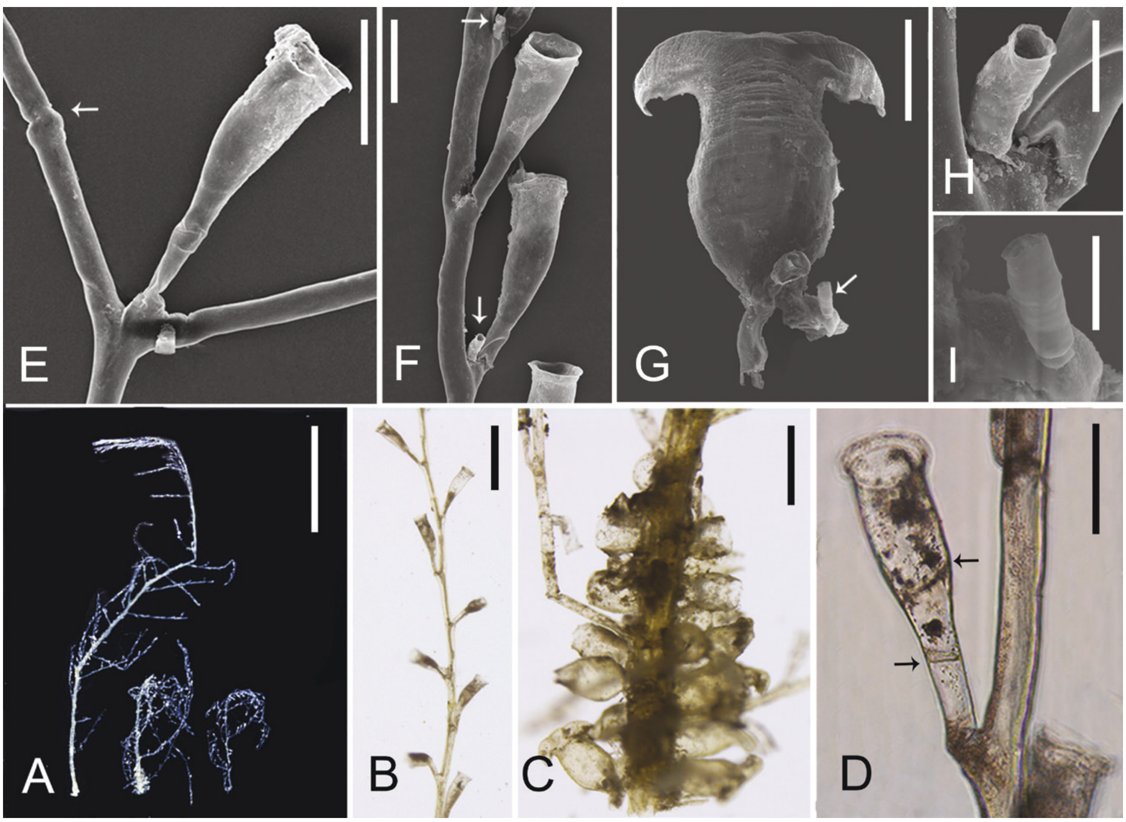

Figure 11. *Zygophylax levinseni* (Saemundsson, 1911). A, three colonies; B, hydrothecal arrangement along a hydrocladium; C, a coppinia; D, two hydrothecal diaphragms (arrows); E, axillary hydrotheca and a node (arrow); F, two hydrothecae and two clearly identifiable nematothecae (arrows); G, a gonotheca and one nematotheca on an accessory tube (arrow); H, magnification of nematothecae on apophysis of hydrocladia (in F, lower arrow); I, magnification of nematothecae on the accessory tubes (in G). From samples: A–I: ZSM 20220297, Gulf of Cadiz. A–D, light microscope images; E–I, SEM images. Scale bars: A = 1 cm; B, C = 1 mm; D–G = 200 µm; H, I = 50 µm.

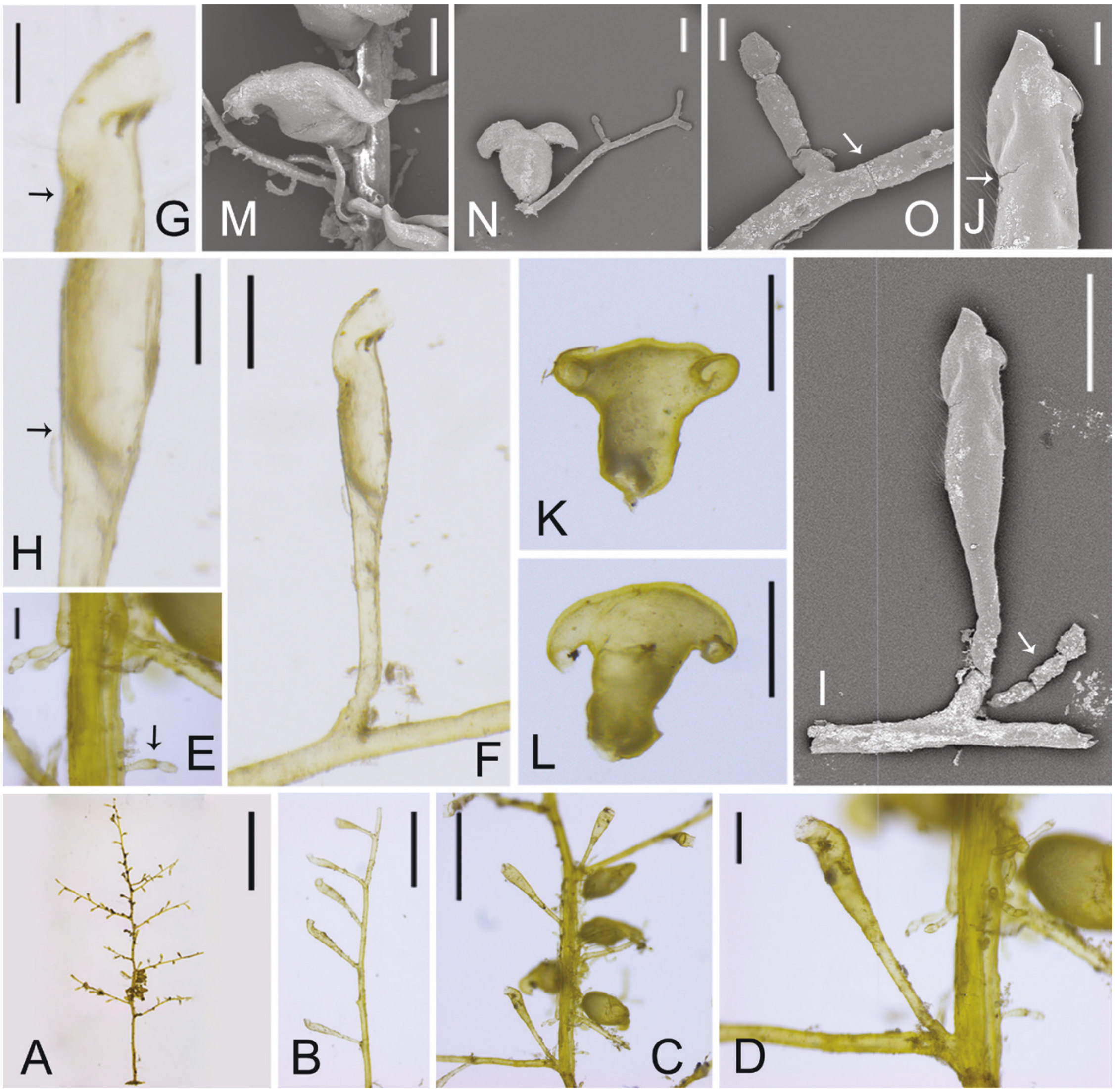

Figure 12. Type material of *Zygophylax lighti* sp. nov. A, a colony; B, hydrothecal arrangement; C, two axillary hydrothecae and four gonothecae; D, magnification of an axillary hydrotheca; E, magnification of nematothecae (arrow); F, I, hydrotheca, the arrow in I indicates a nematotheca; G, J, magnification of the indentation (arrows); H, magnification of an oblique hydrothecal diaphragm (arrow); K–M, gonothecae; N, gonotheca with dichotomous defensive tubes; O, magnification of a node (arrow) on the defensive tubes. A–O: XMUB7904, holotype, South China Sea. A–H, K, L, light microscope images; I, J, M–O, SEM images. Scale bars: A = 5 mm; B, C = 1 mm, D, F, I, M, N = 200 μm; E, G, H = 100 μm; J, O = 50 μm; K, L = 500 μm.

initiated the early development of marine science at the Xiamen University.

Type material: Holotype, XUMB7904, fertile; paratype, XUMB7905, fertile; see Supporting Information, Table S1.

Other material: Thirty-five infertile colonies deposited separately, each specimen containing one colony (XMUB7863, XMUB7869, XMUB7906–XMUB7938), also collected from South China Sea. See details in Supporting Information, Table S1.

Measurements: See Table 1.

Molecular sequences: 16S rRNA, 18S rRNA, 28S rRNA and *COI* genes (this study); see sequence details in Supporting Information, Table S3.

Description: Trophosome. Colonies erect, about 2 cm in height, pinnate (Fig. 12A). Hydrocaulus slightly geniculate, polysiphonic basally grading to monosiphonic distally, unsegmented, one to three alternate hydrothecae between successive hydrocladia.

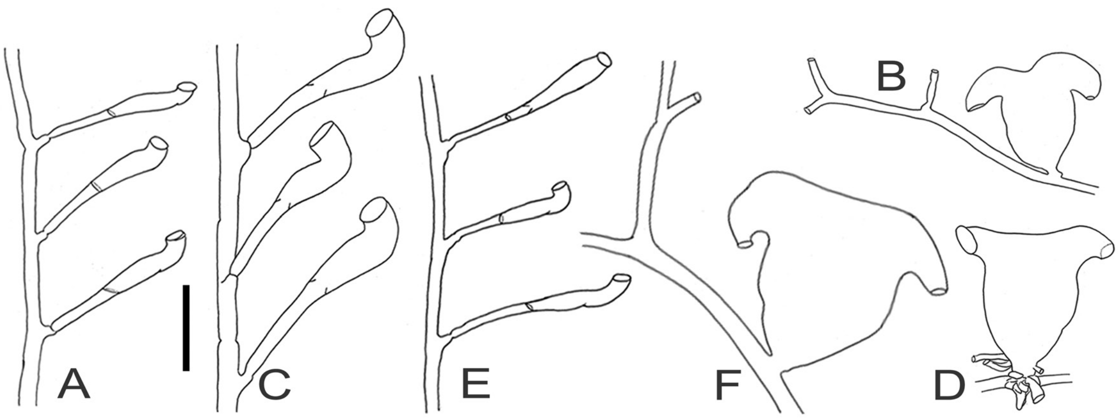

Figure 13. Hydrothecal arrangement and gonothecae of three new species. A, B, *Zygophylax lighti* sp. nov.; C, D, *Z. pseudosibogae* sp. nov.; E, F, *Z. tankahkeei* sp. nov. A, C, E, hydrothecal arrangement, the bases of hydrothecal pedicels are unilateral arranged, and the distal hydrothecal ends alternately turn left and right, respectively; B, D, F, gonothecae (with defensive tubes). Scale bars: A–F = 500 µm.

Hydrocladia coplanar, alternate, forming angles of 60°–80° with the hydrocaulus, with one hydrotheca per axil (Fig. 12C, D); straight, monosiphonic, with irregular nodes. Hydrothecae with long pedicels, the pedicels borne on apophyses of hydrocladia, unilaterally arranged, while the distal hydrothecal ends alternately turn left and right, respectively (Figs 12B, 13A), pedicels forming angles of 60°–90° with the hydrocladia; tubular (Fig. 12F, I), all the hydrothecae are distributed on the same side of the plane of the hydrocladia, distal part curved away and forming an angle of about 60° with the proximal part, with an oblique or convex (inverted funnel-shaped) diaphragm (Fig. 12H), with or without renovations, a conspicuous, internal, abaxial projection (indentation) below the curved part of the hydrotheca (Figs 12G, 14A). Nematothecae present singly on the hydrocaulus and apophyses of hydrocladia (Figs 12E, I, 14A), tubular, slender, with circular aperture, up to three annulations in the middle part (Fig. 12I).

Gonosome: Gonothecae form coppiniae on the lower part of the hydrocaulus (Fig. 12C), with dichotomously branched, disseminate defensive tubes (Figs 12N, 13B). Nematothecae present on defensive tubes, and on the accessory tubes of the stem near the coppinia. Individual gonothecae urn-shaped, tapering abruptly basally, two short, curved tubes present apically, each bearing a circular aperture (Figs 12K–N, 13B).

Distribution: Known only from the type locality, in the South China Sea (this study).

Remarks: Four species, namely *Zygophylax lighti*, *Z. pseudosibogae*, *Z. sibogae* and *Z. tankahkeei*, are highly relevant based upon both morphological (Table 2) and molecular data (Figs 21, 22; Supporting Information, Fig. S1). They can be distinguished from other congeners through the following shared characters: the bases of hydrothecal pedicels are unilaterally arranged, while the distal hydrothecal ends alternately point left and right; long hydrothecal pedicels; much-curved hydrothecae; twisted tubes on gonothecal apices.

Within Zygophylacidae, distinctly curved hydrothecae are also present in *Zygophylax infundibulum* Millard, 1958, *Z. kakaiba* Campos *et al.*, 2016, *Z. convallaria* (Allman, 1877), *Z. curvitheca*, *Z. pseudoabietinella* Peña Cantero, 2020 and *Z. tizardensis* Kirkpatrick, 1890. The curved hydrothecae of these zygophylacids are illustrated in Figure 14, and could be thus separated morphologically. They are also different in the following characters (Supporting Information, Table S2): *Z. curvitheca*, *Z. kakaiba* and *Z. tizardensis* have much shorter pedicels; *Z. pseudoabietinella* has a hydrothecal operculum; *Z. infundibulum*, *Z. kakaiba*, *Z. convallaria* and *Z. sibogae* do not have hydrothecal indentations. A similar hydrothecal indentation was also found in the original description of *Z. tizardensis* (Table 2) and was described as a half-ring (Kirkpatrick, 1890).

These four species can be easily separated from each other. *Zygophylax lighti* and *Z. tankahkeei* differ from the other two in the following characters: the distal end of their hydrothecae is shifted at about 60°, and not perpendicularly to the lower hydrothecal axis; their hydrothecae have indentations. Differences between *Z. pseudosibogae* and *Z. sibogae* can be found in the remarks of *Z. pseudosibogae*.

The two new species proposed, *Zygophylax lighti* and *Z. tankahkeei*, are similar in the following four characters:

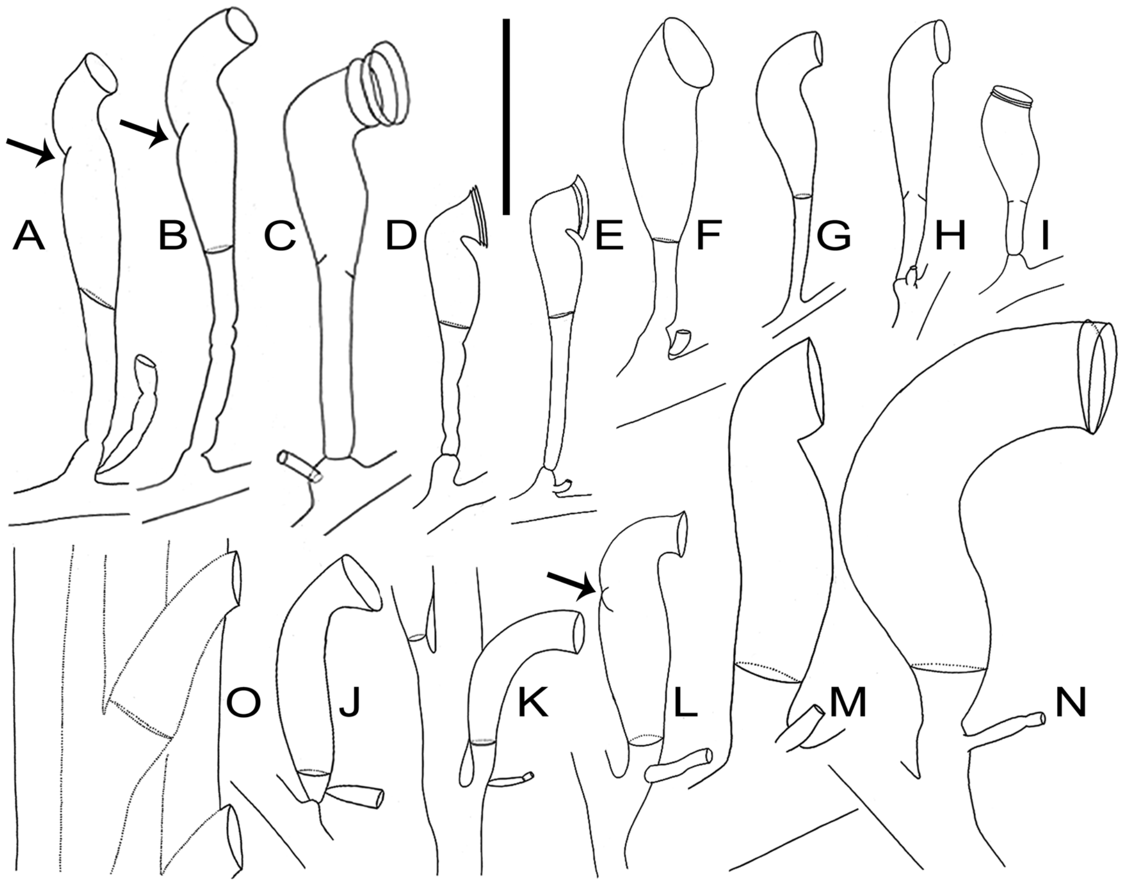

Figure 14. Morphological comparisons of selected zygophylacids with curved hydrothecae. A, *Zygophylax lighti* sp. nov.; B, *Z. tankahkeei* sp. nov.; C, *Z. pseudosibogae* sp. nov.; D, E, *Z. sibogae* Billard, 1918; F, *Z. unilateralis* Totton, 1930; G, *Z. convallaria* (Allman, 1877); H, *Z. infundibulum* Millard, 1958; I, *Z. concinna* (Ritchie, 1911); J, *Z. reflexa* (Fraser, 1948); K, *Z. adhaerens* (Fraser, 1938), hydrothecal basal portion is adherent on the hydrocladium; L, *Z. tizardensis* Kirkpatrick, 1890; M, *Z. kakaiba* Campos, Marques, Puce & Pérez, 2016; N, *Z. curvitheca* Stechow, 1913; O, *Cryptolaria prima* Busk, 1858, the hydrotheca is almost completely immersed in the hydrocladium. A, XMUB7904, holotype; B, XMUB6200-1, holotype; C, ZSM 20220300, holotype; N, ZSM 20043572, paralectotype. The following images were redrawn from the related original descriptions: D, Schuchert (2003); E, Billard (1918); F, Totton (1930); G, Allman (1877); H, Millard (1958); I, Ritchie (1911); J, Fraser (1948); K, Fraser (1938); L, Kirkpatrick (1890), Hirohito (1995); M, Campos *et al.* (2016); O, Busk (1858), Ralph (1958). All arrows indicate hydrothecal indentations. Scale bars: A–D, F–I, L–O = 500 µm; E, J, K, unknown.

(1) the curvature angle of their hydrothecae is about 60°; (2) the hydrothecal pedicels are slender; (3) there are obvious hydrothecal indentations; and (4) the gonothecae are urn-shaped, with curved, short tubes on their apices. Conversely, they differ in the following five characters: (1) when examining the fertile type material of both species in the same Petri dish, the general appearance of the colonies of *Z. tankahkeei* is robust, and slender in *Z. lighti*; the colony color of *Z. tankahkeei* is deep brown, while *Z. lighti* is light yellow; (2) the hydrothecal proportions of *Z. tankahkeei* are about 1.3 times bigger than in *Z. lighti*; (3) *Zygophylax lighti* has more numerous and much larger (three to five times) nematothecae than *Z. tankahkeei*; (4) the gonothecae of *Z. lighti* are arranged in two rows along the accessory tubes of the stem, while in *Z. tankahkeei* they are arranged radially; and (5) *Zygophylax lighti* has long, tubular nematothecae around the coppiniae, a situation not met with in *Z. tankahkeei*.

ZYGOPHYLAX PACIFICA STECHOW, 1920
(FIGS 5N–Q, 15)

Zygophylax biarmata: Stechow, 1913b: 114–115, fig. 88.
Zygophylax pacifica Stechow, 1920: 11; 1923b: 10; 1923c: 141–142; Vervoort, 1941: 198–199; Yamada, 1959: 48; Hirohito, 1983: 29, fig. 10; 1995:142–144, fig. 43e, f,

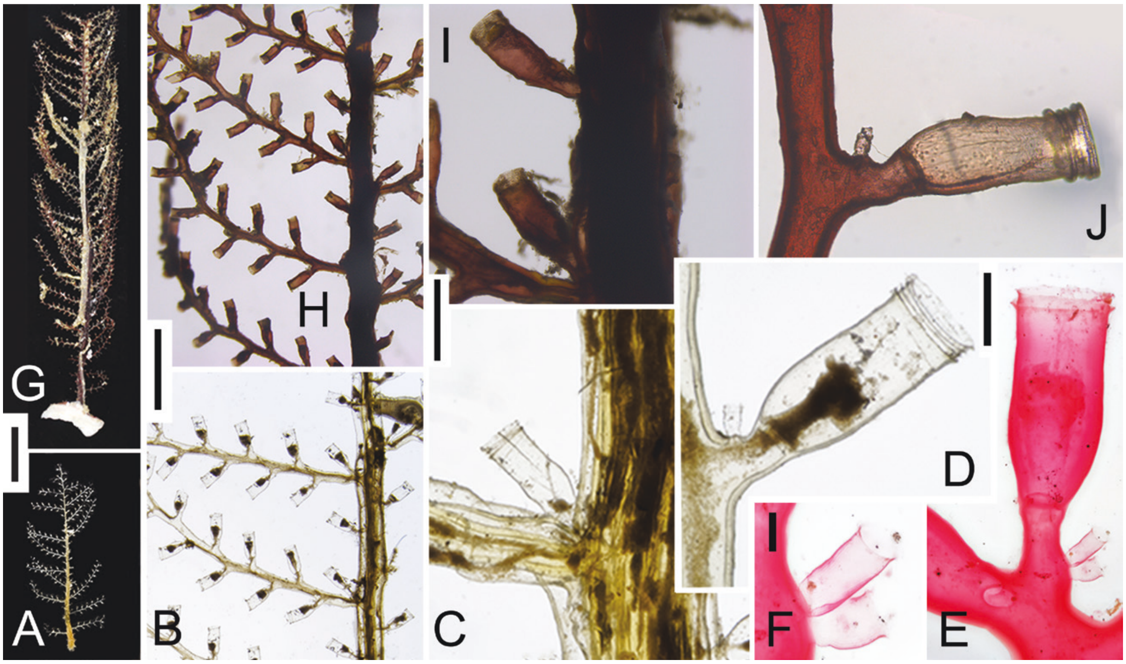

Figure 15. Type material and recent material of *Zygophylax pacifica* Stechow, 1920. A, G, colonies; B, H, polysiphonic portions of hydrocauli with alternate, monosiphonic hydrocladia; C, I, two axillary hydrothecae; D, E, J, three hydrothecae, showing diaphragms, renovations and basal nematothecae [D and J showing single nematothecae, E and F (magnification) showing a pair of nematothecae]. From samples: A–D: ZSM 20040198; E, F: ZSM 20041556; G–J: XMUB6610. A–D, lectotype; E, F, paralectotype; G–J, materials in this study. A–F, Sagami Bay; G–J, Shimoda, Japan. A–J, light microscope images. Scale bars: A, G = 1 cm; B, H = 2 mm; C, I = 500 µm; D, E, J = 200 µm; F = 50 µm.

pl. 9, fig. B; Rees & Vervoort, 1987: 74; Ruthensteiner *et al.*, 2008: 25.
?*Zygophylax pacifica:* Leloup, 1938: 10.

Type locality: Sagami Bay, Japan; 250 m.

Specimens examined: Five syntypes and two colonies from Japan, all the specimens are infertile; see details in Supporting Information, Table S1.

Lectotype designation: Lectotype, an infertile, formalin-fixed then ethanol-preserved colony ZSM 20040198 (Fig. 15A–D); paralectotypes, ZSM 20041554–ZSM 20041556, ZSM 20043569 (Figs 5N–Q, 15E, F), microslides.

Measurements: See Table 1.

Molecular sequences: 16S rRNA, 18S rRNA, 28S rRNA and *COI* genes (this study); see sequence details in Supporting Information, Table S3.

Description: Trophosome. Colonies erect, up to 7.5 cm in height (Fig. 15G). Hydrocaulus straight, polysiphonic except distally, one to three alternate hydrothecae present between two successive hydrocladia, unsegmented, branching (Fig. 15A, B, G, H). Hydrocladia forming angles of 60°–80° with the hydrocaulus (Fig. 15B, H), each branching point with an axillary hydrotheca (Fig. 15C, I). Hydrocladia arranged alternately and distributed in one plane, straight, basal part polysiphonic or monosiphonic, distal part monosiphonic, unsegmented (Fig. 15B, H). Hydrothecae arranged alternately, forming angles of 45°–60° with the corresponding hydrocladia (Fig. 15B, H); borne on short pedicels, campanulate, straight-sided or nearly so, with a straight diaphragm and two to six marginal renovations, rim slightly everted. One or two tubular nematothecae present at base of each apophysis of hydrocladia (Fig. 15D–F, J).

Gonosome: Absent.

Distribution: Japan (Stechow, 1913b, 1923c; Hirohito, 1983, 1995; this study); the Philippines (Vervoort, 1941).

Remarks: *Zygophylax pacifica* and *Z. rufa* are similar in hydrothecal morphology. They form a monophyletic group with high support in the phylogenetic trees (Figs

21, 22). They are different in the presence or absence of nematothecae attached to apophyses of hydrocladia. Nearly each apophysis of *Z. pacifica* has one tubular nematotheca, although in some rare cases there are two of these. Conversely, the hydrothecal pedicels of *Z. rufa* are devoid of nematothecae on apophyses of hydrocladia (Supporting Information, Table S2).

Schuchert (2015) proposed *Zygophylax pacifica* as a junior synonym of *Z. cyathifera* (Allman, 1888), because he thought that the only difference between the two species, namely the presence of a distinct node in *Z. cyathifera* separating the basal part of the hydrotheca from the apophysis (Rees & Vervoort, 1987), may not be reliable enough for species distinction. By re-examinating the type material of *Z. pacifica*, we found that distinct nodes were absent from the hydrocladia.

In this study, the sequences of the 16S rRNA, 18S rRNA, 28S rRNA and *COI* genes of *Zygophylax pacifica* are provided. Molecular data of *Z. cyathifera* from the type locality (New Hebrides, Vanuatu) are still required to clarify its relationship with *Z. pacifica*.

ZYGOPHYLAX CF. *PSEUDAFRICANUS* VERVOORT & WATSON, 2003

(FIG. 16)

Zygophylax pseudafricanus Vervoort & Watson, 2003: 78–80, fig. 13A–F.

Type locality: South-west Pacific; 520 m.

Specimens examined: Two infertile colonies from Portugal; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA, 18S rRNA and 28S rRNA genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See details in Supporting Information, Table S2.

Description: Trophosome. Colonies erect, about 1.3 cm in height (Fig. 16A). Hydrocaulus straight, polysiphonic, unsegmented, with alternate hydrothecae, branching

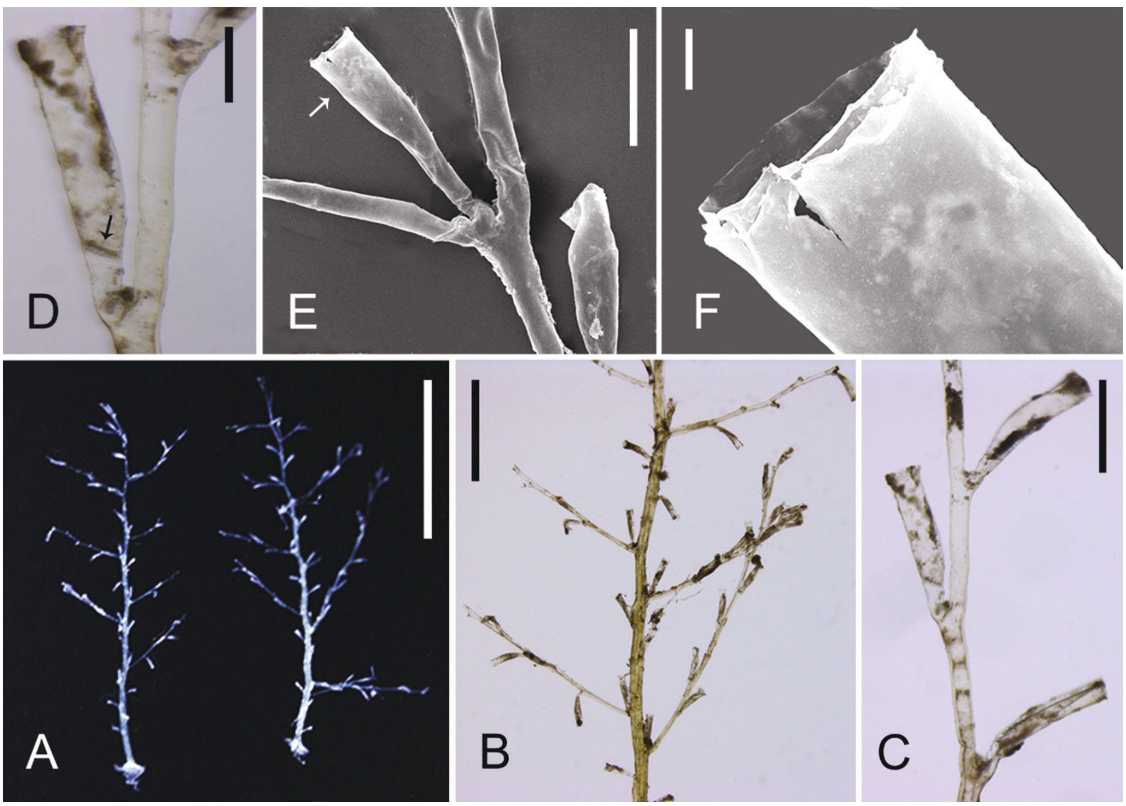

Figure 16. *Zygophylax* cf. *pseudafricanus* Vervoort & Watson, 2003. A, two colonies; B, portion of polysiphonic hydrocaulus with several monosiphonic hydrocladia; C, hydrothecal arrangement; D, hydrothecal diaphragm (arrow); E, an axillary hydrotheca (arrow); F, magnification of the hydrothecal margin, showing renovations. From samples: A–F: ZSM 20220299, Lisbon, Portugal. A–D, light microscope images; E, F, SEM images. Scale bars: A = 5 mm; B = 2 mm; C = 500 μm; D, E = 200 μm; F = 20 μm.

(Figs 16A, B). Hydrocladia forming angles of about 60° with the hydrocaulus (Fig. 16B), axillary hydrothecae present at branching points (Fig. 16E). Hydrocladia alternate, coplanar, straight, basally polysiphonic, distally monosiphonic (Fig. 16B), with irregular nodes (Fig. 16C). Hydrothecae arranged alternately in one plane, the distal part straight or slightly curved (Fig. 16C); borne on short pedicels, with one oblique diaphragm (Fig. 16D), margin slightly everted, with one or two renovations (Fig. 16D–F), only one nematotheca present on the apophysis of hydrocladium.

Gonosome: Absent.

Distribution: Southwest Pacific (Vervoort & Watson, 2003); Lisbon, Portugal (Moura *et al.*, 2012; this study).

Remarks: The investigated specimens mostly resemble *Zygophylax pseudafricanus* and *Z. sagamiensis*. The latter species has more abundant nematothecae (Supporting Information, Table S2). The present specimens were provisionally assigned to *Z.* cf. *pseudafricanus* because only a few nematothecae were found. The investigated specimens also resemble *Z. cervicornis* (Nutting, 1906), *Z. dispersa* Peña Cantero, 2020, *Z. echinata* Calder & Vervoort, 1998 and *Z. recta* Jarvis, 1922, but these species have differences: the distal part of *Z. cervicornis* is curved, while in *Z.* cf. *pseudafricanus* it is straight; the hydrothecal length in *Z.* cf. *pseudafricanus* (about 600 µm) is twice that of *Z. dispersa* and *Z. echinata* (both about 300 µm); the distal part of the hydrothecae of *Z. recta* is distinctly expanded, while in *Z.* cf. *pseudafricanus* it is not.

ZYGOPHYLAX PSEUDOSIBOGAE GU, MOURA & SONG SP. NOV.

(Figs 13C, D, 14C, 17)

Zoobank registration: urn:lsid:zoobank.org:act:A687734F-7329-41B6-993C-7BEA45F1C2E3.

Type locality: Off Lisbon, Portugal; 1294 m.

Etymology: The specific name *pseudosibogae* illustrates its close similarity to *Zygophylax sibogae* Billard, 1918.

Type material: Holotype, ZSM 20220300, fertile; paratype 1, ZSM 20220301, fertile; paratype 2, ZSM 20220302, fertile; paratype 3, DBUA1303.01, fertile; see Supporting Information, Table S1.

Molecular sequences: 16S rRNA, 18S rRNA and 28S rRNA genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See Table 1.

Description: Trophosome. Colonies erect, up to 3.8 cm in height (Fig. 17A). Hydrocaulus straight, polysiphonic, unsegmented, branching, one to three alternate hydrothecae present between two successive hydrocladia (Fig. 17A–C). Hydrocladia forming angles of about 60° (Fig. 17A, B) with the hydrocaulus, axillary hydrothecae present at branching points (Fig. 17C, D). Hydrocladia distributed alternately in one plane, straight, polysiphonic except distally, with irregular nodes (Fig. 17D, F). Hydrothecae forming angles of about 60° with the hydrocladium (Fig. 17B–D); borne on long pedicels, the base of hydrothecal pedicels unilateral arranged, while the distal hydrothecal ends alternately turn left and right (Fig. 13B); tubular, distal part curved adaxially, the bent forming angles of about 90°, with an oblique diaphragm, margin everted, with or without renovations (Figs 14C, 17C–F). Tubular nematothecae present on hydrocauli, hydrocladia and apophyses of hydrocladia (Fig. 17C, D, F, H, I).

Gonosome: Gonothecae clustering into coppiniae (Fig. 17A, B), the latter spherical, with numerous dichotomous defensive tubes (Figs 13D, 17B, G) bearing tubular nematothecae (Fig. 17G, J). Individual gonothecae urn-shaped, tapering downwards into the base, apex with two lateral, short, curved tubes with circular apertures (Fig. 17G).

Distribution: Lisbon, Portugal (Moura *et al.*, 2012; this study).

Remarks: Within the known Zygophylacidae species, the morphology of *Zygophylax pseudosibogae* mostly resembles that of *Z. sibogae*. The most reliable records of *Z. sibogae* are those dealt with in the original account (Billard, 1918) and specimens from the type locality, Kei Islands, Indonesia (Schuchert, 2003). However, all this material was infertile. Nevertheless, *Z. pseudosibogae* and *Z. sibogae* could be easily distinguished, even based only on the hydrothecal morphology: (1) the hydrothecal dimensions of *Z. pseudosibogae* are about 1.5 times bigger compared to *Z. sibogae*; (2) the distal end of hydrotheca of *Z. sibogae* is twisted and apparently much extended. Moreover, the geographic distance between the type localities of both species is considerable, and their bathymetric distributing in their original descriptions also varies much. *Zygophylax sibogae* was originally described from Indonesia, at a water depth of 310 m, while *Z. pseudosibogae* originates from Portugal, at 1294 m. Although molecular data for *Z. sibogae* from the type locality are still unavailable, the above morphological, geographic and bathymetric data support proposing the Portuguese samples as a new species. For morphological comparisons of *Z. pseudosibogae* and other zygophylacids also with

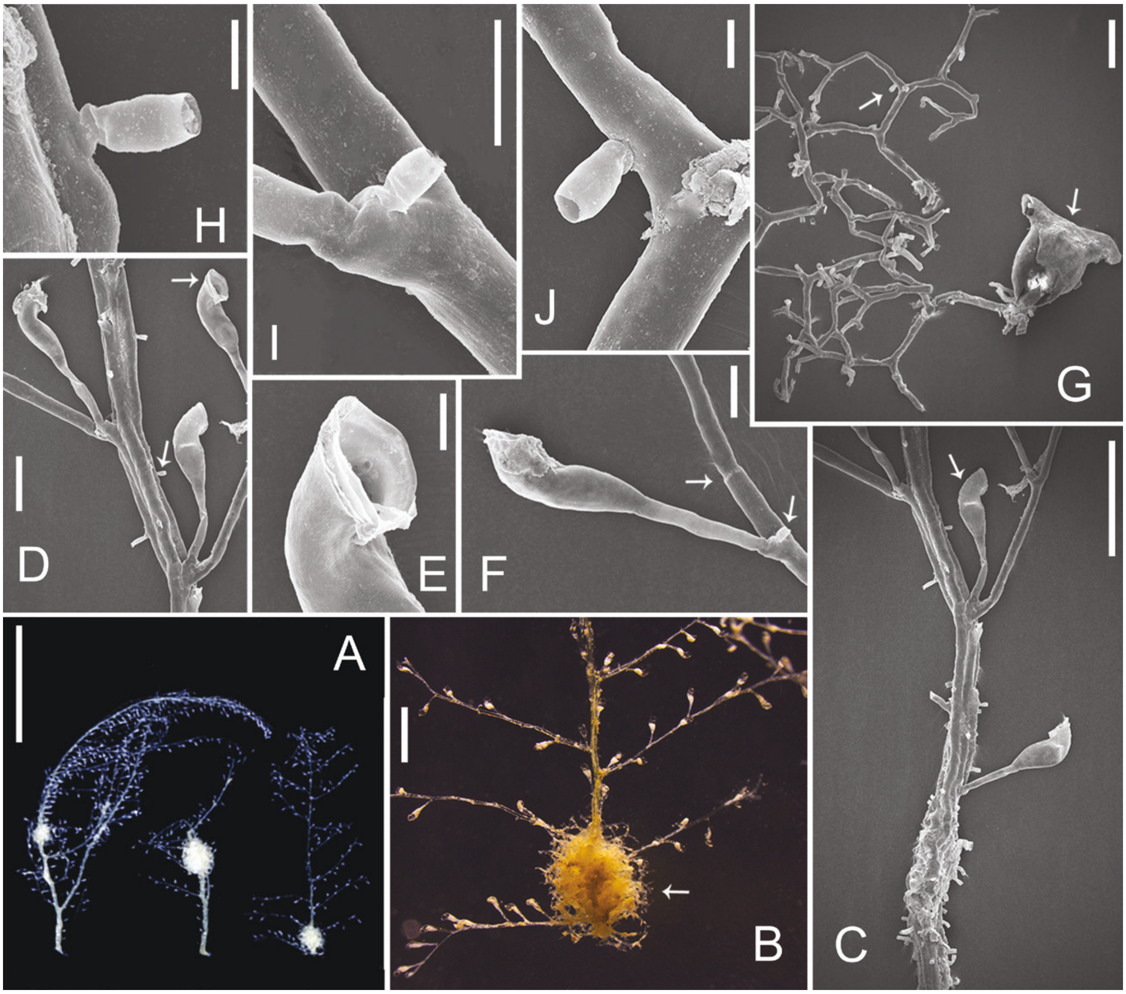

Figure 17. Type material of *Zygophylax pseudosibogae* sp. nov. A, colonies; B, portion of hydrocaulus with a coppinia (arrow); C, portion of a polysiphonic hydrocaulus with an axillary hydrotheca (arrow) and abundant nematothecae; D, nematothecae on the hydrocladium (lower arrow) and hydrothecal renovations (upper arrow); E, magnification of the hydrothecal margin, showing renovations; F, a node (left arrow) and a nematotheca (right arrow) from a hydrocladium; G, isolated gonotheca (right arrow) and dichotomously branched defensive tubes, the left arrow indicates a nematotheca on the defensive tubes; H–J, magnification of the nematothecae on hydrocladium (H), apophysis (I) and defensive tubes (J), respectively. From samples: ZSM 20220300, holotype [A (the left colony), C–F, H, I]; ZSM 20220301, paratype 1 [A (the right colony), B, G, J]; ZSM 20220302, paratype 2 [A (the middle colony)]. A–J, Lisbon, Portugal. A, B, light microscope images; C–J, SEM images. Scale bars: A = 1 cm; B = 2 mm; C = 500 µm; D, G = 200 µm; E, I = 50 µm; F = 100 µm; H, J = 20 µm.

curved hydrothecae, see details in the remarks of *Z. lighti* and Figure 14.

Some records previously identified as *Zygophylax sibogae*, but distributed far from its type locality, seem to be questionable due to apparent morphological differences. For example, nearly half of the whole hydrothecae are curved in the record collected from New Zealand (Vervoort & Watson, 2003) and Sagami Bay (Hirohito, 1995). The hydrothecal aperture recorded by Vervoort (2006) does not expand obviously. A comprehensive revision of material identified as *Z. sibogae* from several localities mentioned above is still needed, including the corresponding barcodes, in order to shed more light on this issue, to detect potential cryptic complex and to subsequently describe potential new species.

ZYGOPHYLAX CF. *SAGAMIENSIS* HIROHITO, 1983 (FIG. 18)

Zygophylax sagamiensis Hirohito, 1983: 6, 30–31, fig. 11; Rees & Vervoort, 1987: 85–86; Hirohito, 1995: 144, fig. 44a–e, pl. 9, fig. C; Vervoort & Watson, 2003: 69;

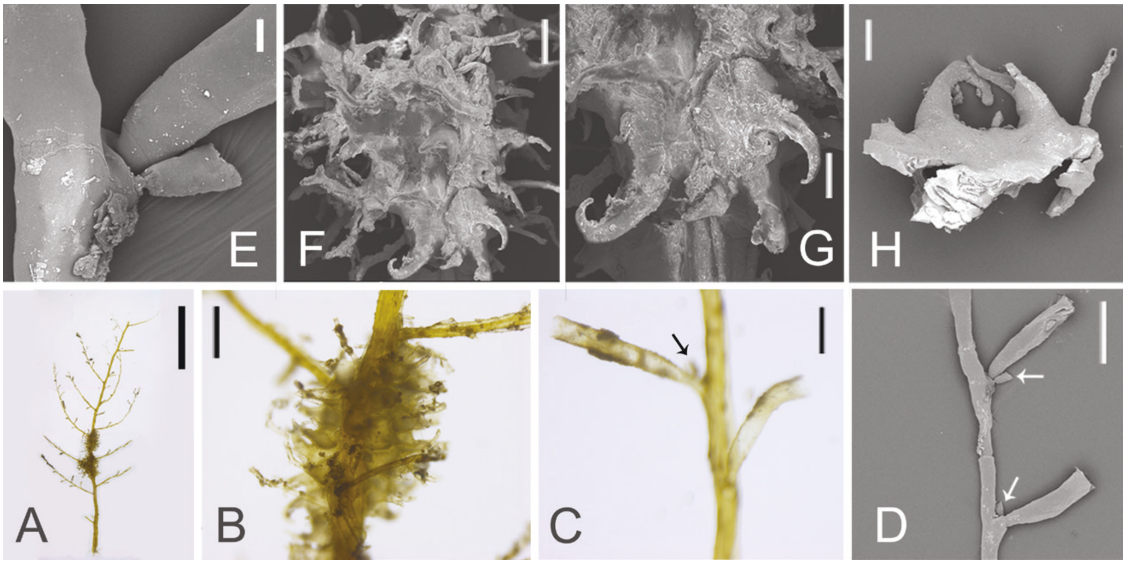

Figure 18. *Zygophylax* cf. *sagamiensis* Hirohito, 1983. A, a colony; B, F, G, images of the same coppinia; C, alternate hydrothecae with one nematotheca (arrow); D, hydrothecae and nematothecae (arrows); E, magnification of a nematotheca; H, two (fused) gonothecae. From samples: A–H: XMUB8109, South China Sea. A–C, light microscope images; D–H, SEM images. Scale bars: A = 5 mm; B = 500 μm; C, D, F = 200 μm; E = 20 μm; G, H = 100 μm.

Watson, 2003: 160–161, fig. 10A–F; 2006: 245–247, fig. 20.

Type locality: Sagami Bay, Japan; 250–300 m.

Specimens examined: Two fertile and 10 infertile colonies from South China Sea; see details in Supporting Information, Table S1.

Molecular sequences: 16S rRNA, 18S rRNA and *COI* genes (this study); see sequence details in Supporting Information, Table S3.

Measurements: See Supporting Information, Table S2.

Description: Trophosome. Colonies erect, about 2.5 cm in height (Fig. 18A). Hydrocaulus straight, polysiphonic, unsegmented, with alternate hydrothecae, branched alternately, axillary hydrothecae present at branching points. Hydrocladia forming angles of about 60° with the hydrocaulus (Fig. 18A); distributed alternately in one plane, straight, polysiphonic except distally, with irregular nodes. Hydrothecae arranged alternately in two planes forming an angle of about 60°, the distal part of hydrotheca straight or slightly curved ad- or abaxially (Fig. 18C, D). Hydrothecae borne on short pedicels, with a straight diaphragm, margin slightly everted, with renovations, at most one nematotheca present on each apophysis of hydrocladia (Fig. 18C–E).

Gonosome: Gonothecae aggregated to form coppiniae (Fig. 18B, F, G), the latter surrounding the hydrocaulus, and provided with protective tubes bearing hydrothecae. Individual gonothecae piriform, provided with one or two slender tips on the top (Fig. 18H).

Distribution: Sagami Bay (Hirohito, 1995); Macquarie Island (Watson, 2003); Atlantic Ocean (Vervoort, 2006); South China Sea (this study).

Remarks: The investigated specimens from the South China Sea are provisionally identified as *Zygophylax sagamiensis* based on morphological similarity (Supporting Information, Table S2). They differ from the original description of *Z. sagamiensis* (Hirohito, 1983) by the hydrothecal arrangement: the hydrothecae are arranged in two planes forming an angle of about 60°, while in the type material they are coplanar (Hirohito, 1983).

ZYGOPHYLAX TANKAHKEEI GU & SONG SP. NOV. (FIGS 13E, F, 14B, 19)

Zoobank registration: urn:lsid:zoobank.org:act:A5BD0B58-428E-473F-A080-206CCAC77CDE.

Type locality: South China Sea; 2359 m.

Etymology: The specific name honours the founder of Xiamen University, Tan Kah Kee, as well as the

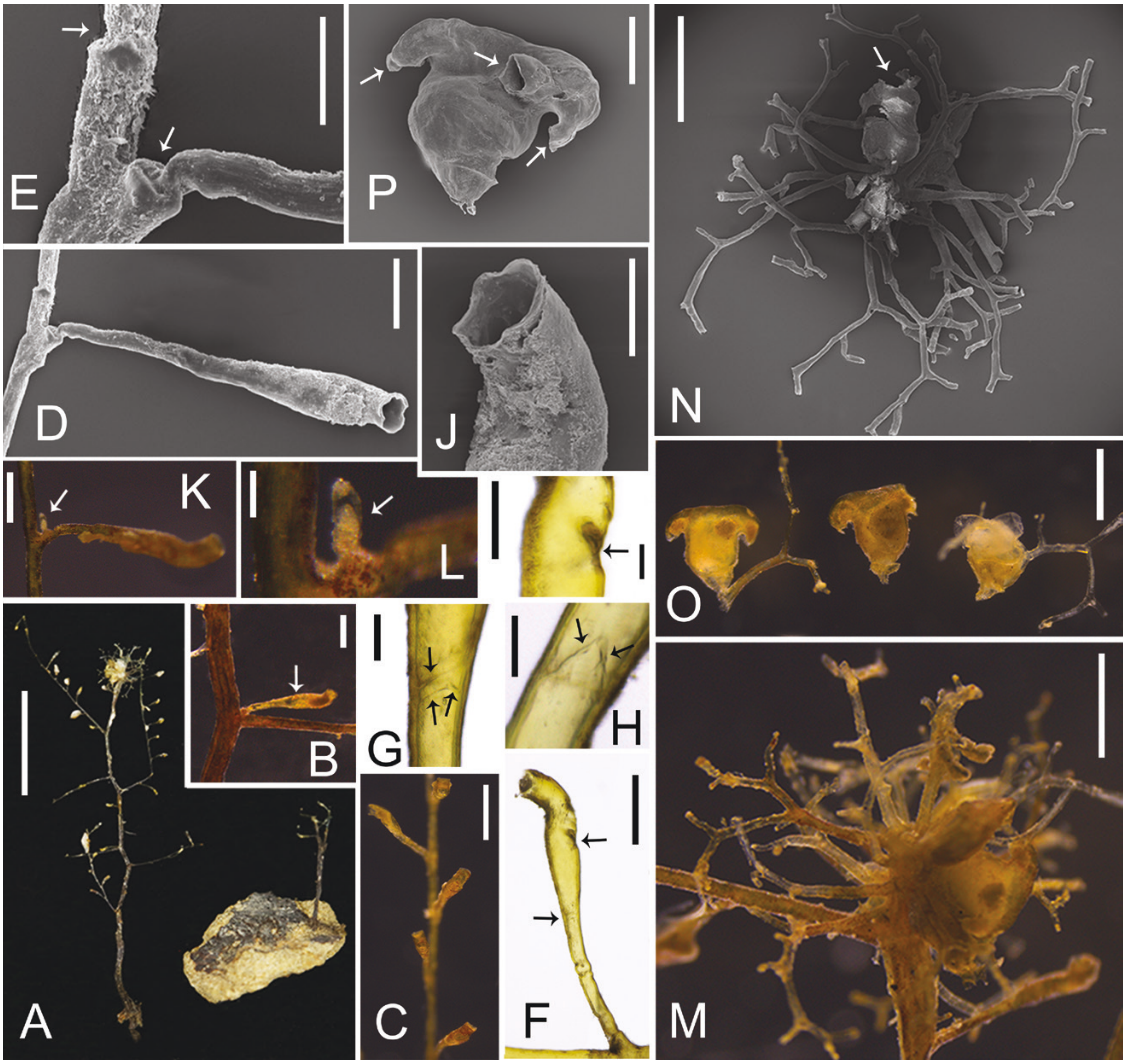

Figure 19. Type material of *Zygophylax tankahkeei* sp. nov. A, two colonies, holotype (left), paratype (right); B, portion of the polysiphonic hydrocaulus and a monosiphonic hydrocladium, and an axillary hydrotheca (arrow); C, arrangement of the hydrothecae in two different planes; D, a hydrotheca; E, magnification of a node (upper arrow) and apophysis (lower arrow); F–J, hydrothecal diaphragms (lower arrow in F, arrows in G, H), perisarc projection (upper arrow in F, and arrow in I) and distal renovations (J); K, L, the same nematotheca at different magnifications (arrows); M, coppinia; N, gonothecae (arrow) with repeatedly, dichotomously branched defensive tubes; O, P, isolated gonothecae, the arrows in P indicate three apertures. From samples: A, the left colony, XMUB6200-1, holotype; the right colony, XMUB6200-2, paratype; B–P: XMUB6200-1, holotype. A–P, South China Sea. A–C, F–I, K–M, O, light microscope images; D, E, J, N, P, SEM images. Scale bars: A = 5 mm; B, F, K, N = 500 μm; C, M, O = 1 mm; D, I, P = 200 μm; E, G, H, J, L = 100 μm.

research vessel *Tan Kah Kee* (served Xiamen University since 2016) that collected the type material of this species, and in memory of the University's 100th and 101st Anniversary Celebration (1921–2022).

Type material: Holotype, XUMB6200-1, with one coppinia; paratype, XUMB6200-2, infertile; see Supporting Information, Table S1.

Measurements: See Table 1.

Molecular sequences: 16S rRNA, 18S rRNA, 28S rRNA and *COI* genes (this study); see sequence details in Supporting Information, Table S3.

Description: Trophosome. Colonies erect, about 2 cm in height (Fig. 19A). Hydrocaulus geniculate, polysiphonic, unsegmented, one to three alternate hydrothecae present between two successive hydrocladia, branching (Fig. 19A). Hydrocladia forming angles of 60°–90° with the hydrocaulus (Fig. 19A), the whole holotype with two axillary hydrothecae present at the branching points (one hydrotheca shown in Fig. 19B). Hydrocladia distributed alternately in two planes forming an angle of 120°–150° (Fig. 19A); straight to geniculate, monosiphonic, with irregular nodes (Fig. 19E). Hydrothecae with long pedicels, borne on apophyses of hydrocladia, the pedicels unilaterally arranged, while the distal hydrothecal ends alternately turn left and right, respectively, forming angles of about 45° (Figs 13E, 19C); tubular, distal part curved and forming angles of about 60° with the proximal part (Figs 13E, 14B, 19D, F); diaphragm oblique and/or a convex above (Fig. 19G, H), with or without marginal renovations (Fig. 19J); an internal, conspicuous perisarc projection (indentation) present opposite to the curved direction on the distal part of hydrotheca (Figs 14B, 19F, I). Five nematothecae observed on accessory polysiphonic tubes of the hydrocaulus; four nematothecae on four apophyses of hydrocladia (Fig. 19K, L).

Gonosome: Three gonothecae aggregated into a coppinia on top of the hydrocaulus of holotype material (Fig. 19M), surrounded by numerous dichotomous defensive tubes (Figs 13F, 19N, O), the latter without nematothecae. Individual gonothecae urn-shaped, narrowing at the base, two or three short, curved tubes present on the apex (Figs 19O, P).

Distribution: The type locality, South China Sea (this study).

Remarks: See details in the remarks of *Zygophylax lighti*.

ZYGOPHYLAX VALDIVIAE STECHOW, 1923
(FIGS 5R–U, 20)

Zygophylax valdiviae Stechow, 1923d: 6–7; Stechow, 1925: 446–447, fig. 19; Rees & Vervoort, 1987: 75; Ruthensteiner *et al.*, 2008: 25.

Type locality: 7 km south of St. Paul Island, southern Indian Ocean; 672 m.

Specimens examined: Five infertile syntypes from the Indian Ocean; see details in Supporting Information, Table S1.

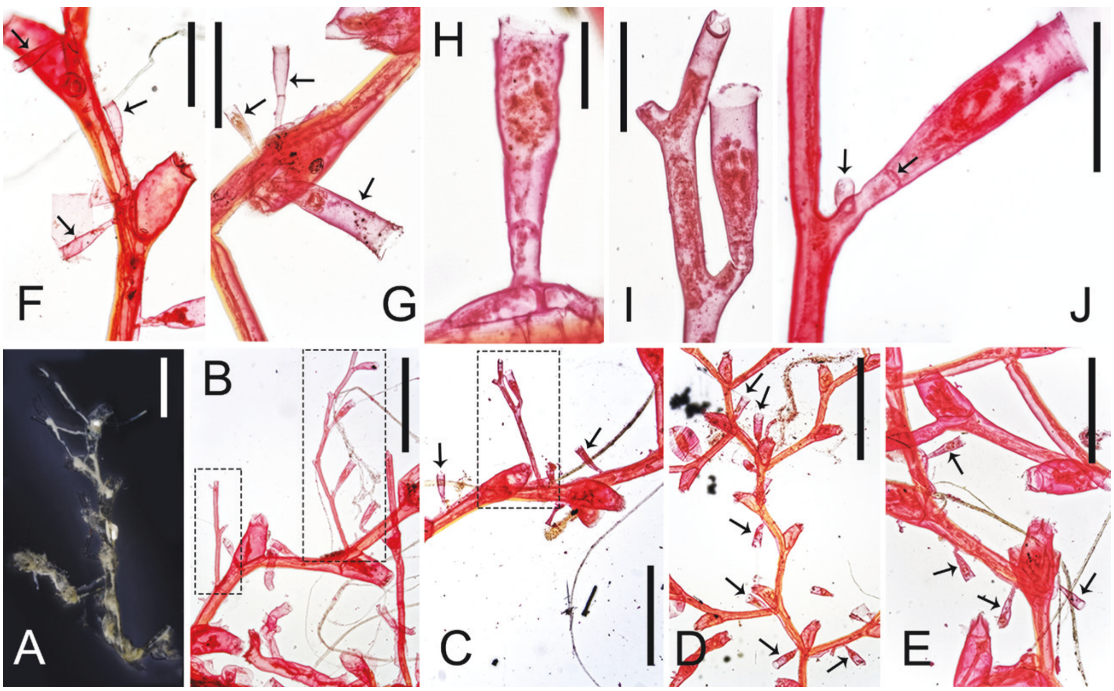

Figure 20. Type material of *Zygophylax valdiviae* Stechow, 1923. A–C, colonies (in dotted rectangles) growing on *Sertularella valdiviae* Stechow, 1923; the arrows in C indicate two solitary hydrothecae; D–G, solitary hydrothecae (arrows); H–J, magnification of various hydrothecae, the arrows in J indicate a nematotheca (left arrow) and the straight diaphragm (right arrow). From samples: A: ZSM 20040930, lectotype; B, J: ZSM 20041566, paralectotype; C, E, H, I: ZSM 20041569, paralectotype; D, F, G: ZSM 20041568, paralectotype. A–J, Indian Ocean. A–J, light microscope images. Scale bars: A, D = 2 mm; B, C, E = 1 mm; F, G = 500 μm; H = 100 μm; I, J = 200 μm.

Lectotype designation: Lectotype, an infertile, formalin-fixed then ethanol-preserved colony ZSM 20040930 (Fig. 20A); paralectotypes, microslides ZSM 20041566–ZSM 20041569 (Figs 5R–U, 20B–J).

Measurements: See Table 1.

Description: Trophosome. Hydrorhiza creeping on *Sertularella valdiviae* Stechow, 1923, giving off either short, erect hydrocauli or solitary hydrothecae. Hydrocauli monosiphonic, up to 0.23 cm high, unbranched, divided by transverse nodes, each internode with one or two hydrothecae. Hydrothecae forming angles of 30°–60° with the corresponding hydrocauli (Fig. 20A–C); arranged alternately in one plane, borne on short pedicels, tubular, straight, basally a straight diaphragm, margin slightly everted, with or without renovations (Fig. 20D–J). Nematothecae present on apophyses of hydrocauli or directly on the hydrorhiza (Fig. 20J).

Gonosome: Absent.

Distribution: Only recorded from the type locality, south of St. Paul Island, Southern Indian Ocean (Stechow, 1923d; this study).

DISCUSSION

Integrative analysis of Zygophylacidae

The newly obtained molecular phylogenetic trees cover all available sequences for 14 zygophylacids (Figs 21, 22; Supporting Information, Table S3). The combined tree and the 16S tree support the monophyly of Zygophylacidae that closely clustered with Plumularioidea in Macrocolonia. This supports the validity of the family Zygophylacidae. However, the fine systematics of Zygophylacidae is controversial. The monophyly of the genus *Zygophylax* is not supported due to the inclusion of members of the genera *Abietinella* and *Cryptolaria*. This corresponds to the findings by Moura *et al.* (2008, 2012) and Peña Cantero *et al.* (2010) based on fewer taxa. Given that *Abietinella* and *Cryptolaria* represent valid taxa, the topologies of the concatenated and 16S trees generated in the present study (Figs 21, 22) indicate that the circumscription of the genus *Zygophylax* has to be reassessed or split up.

On the combined and 16S trees (Figs 21, 22), the phylogenetic position of the genus *Abietinella* is represented by its type species *A. operculata* (Jäderholm, 1903), but the position of the genus *Cryptolaria* is still doubtful because molecular sequences for its type species (*C. prima* Busk, 1858) are still missing. The position is provisionally provided by the only species available with molecular data, *C. pectinata*. It should be noted that the morphology of *C. pectinata* seems to be different from the type species of that genus. The hydrothecae of the latter are completely immersed into the hydrocladia, while those of *C. pectinata* are only partially adnate. This difference might be related to the age of the colonies and the degree of hydrocladium polysiphony. Hence, molecular data of the type species of *Cryptolaria* are still required to clarify the phylogenetic position of that genus.

Potential generic diagnoses for Zygophylacidae

Obvious morphological variations between different ‘groups’ of species within Zygophylacidae hint towards dividing *Zygophylax* into several genera, and the polyphyly of *Zygophylax* was already found previously (Moura *et al.*, 2008, 2012; Peña Cantero *et al.*, 2010). These ‘groups’ were annotated manually on the 16S tree (Fig. 22). For instance, clades C1 and C7 with straight hydrothecae (e.g. *Z. pacifica*), clade C9 with obvious curved hydrothecae (e.g. *Z. pseudosibogae*); clades C1, C3, C4, C6 and C7 with one gonothecal aperture (e.g. *Z. pacifica*) and C2, C8 and C9 with two gonothecal apertures (e.g. *Z. tankahkeei*) (Fig. 22; Supporting Information, Table S2). An integrative analysis combining morphological comparisons of all nominal zygophylacids and molecular data (Figs 21, 22; Supporting Information, Table S2) suggests four candidate characters for future generic diagnoses of Zygophylacidae. These candidates are only provisionally discussed here, providing a reference for further taxonomic revisions of this family.

1. Presence or absence of a hydrothecal operculum: this has been treated as a generic diagnostic character in Zygophylacidae. It has been observed only in *Abietinella operculata* and *Zygophylax pseudoabietinella*. One of the different characters of these two species is the obviously larger hydrothecae in the former species (Peña Cantero, 2020); huge differences are seen in many other genera and families. Whether the latter species should be transferred to the genus *Abietinella* should be verified by additional molecular data.
2. Hydrotheca free or adnate: *Zygophylax adhaerens* (Fraser, 1938) and the present *Cryptolaria* species have adnate hydrothecae. They also share other similar features, such as the curved hydrothecae and the long cylindrical nematothecae (Supporting Information, Table S2). Thus, *Z. adhaerens* could be moved to a genus together with *Cryptolaria* species if also supported by molecular data.
3. Hydrotheca obviously straight or curved: among the species for which molecular data are available,

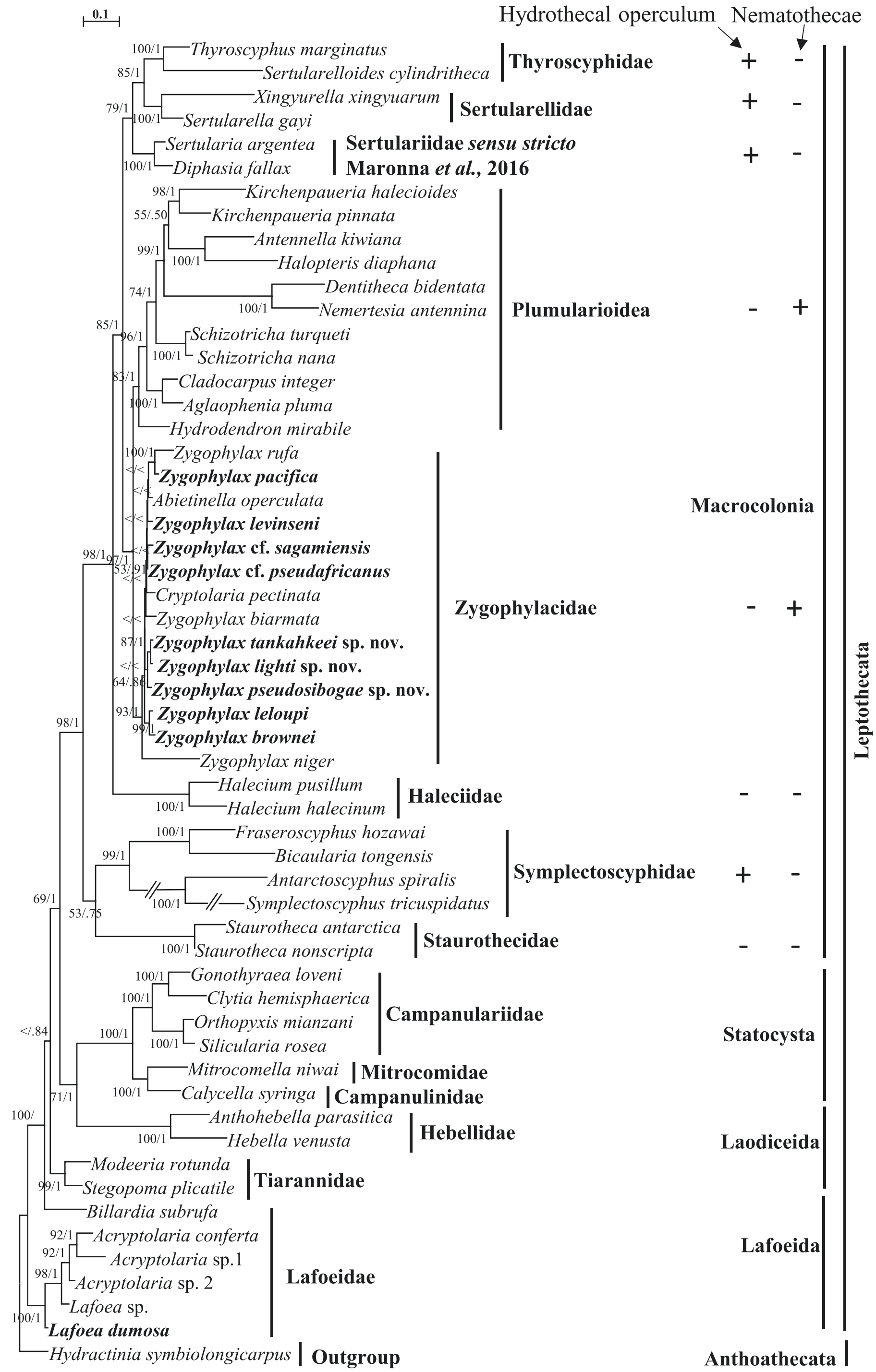

Figure 21. Maximum likelihood phylogenetic analyses with concatenated partial sequences of the 16S, 18S and 28S rRNA genes (5446 bp after multiple alignments) using the GTR+I+G model. Numbers near the nodes indicate bootstrap and Bayesian posterior probability values. Values lower than 50 or 0.5 were omitted and labelled with ‘<’. The scale bar represents 0.1 substitutions per site. Bayesian analyses using the GTR+I+G model. Sequences of species in bold were contributed by this study. ‘+’ (or ‘-’) indicates the families, or superfamily, use (or not) of opercular flaps or nematothecae as the protective strategies.

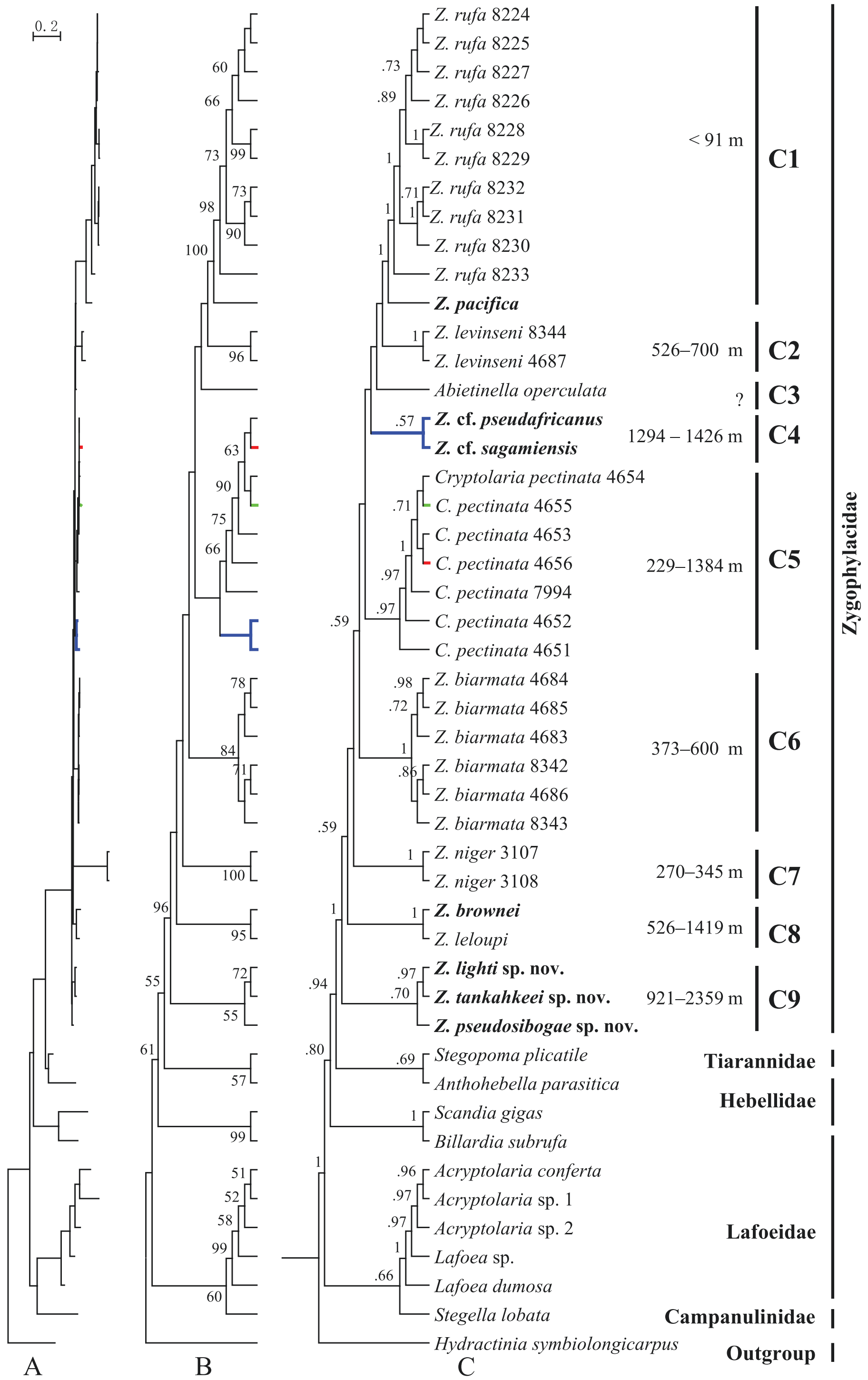

Figure 22. Phylogenetic analyses derived from partial sequences of the 16S rRNA gene (548 bp after multiple alignments). A, B, maximum likelihood analyses using the TIM2+I+G model; C, Bayesian analyses using the GTR+I+G model. A, with phylogram topology, the scale bar represents 0.2 substitutions per site; B, C, with transform cladogram topology. Numbers near the nodes indicate bootstrap or Bayesian posterior probability values, values lower than 50 or 0.5 were omitted; numbers alongside the taxa names indicate the last four digits of the GenBank accession numbers. Different topologies between different phylogenetic analyses in B and C are shown in colour. Sequences of taxa in bold are contributed by this study.

three species, *Zygophylax pacifica*, *Z. rufa* and *Z. niger*, have decidedly straight hydrothecae and form two monophyletic groups (C1 and C7 on Fig. 22), and three other species, *Z. pseudosibogae*, *Z. tankahkeei* and *Z. lighti*, have decidedly curved hydrothecae and form another clade (C9 on Fig. 22) (Supporting Information, Table S2). Species within the 'straight or curved hydrothecae' groups can also be further separated into several groups. For example, the three species with straight hydrothecae take up two distinct clades on the trees (Figs 21, 22): the former two species form a monophyletic group (C1 on Fig. 22) and the third species takes up a single clade (C7 on Fig. 22). These two clades could be separated by the length of the hydrothecal pedicel. The hydrothecal pedicles of the former clade (C1) are short, while those of the other clade (C7) are much longer. Similarly, species with curved hydrothecae (e.g. *Zygophylax pseudosibogae*) could also be further distinguished into several groups by the degree of curvature, the proportion of the curved portion relative to the whole hydrotheca, and the pedicel length. Clade C9, comprising *Z. pseudosibogae*, *Z. tankahkeei* and *Z. lighti* (Fig. 22) provides a typical example for species of this group with longer pedicels (see Systematics). In contrast, *Z. kakaiba* and *Z. tizardensis* represent species of this group with a shorter pedicle (Supporting Information, Table S2).

4. Number of gonothecal apertures: according to the Supporting Information, Table S2, 20 zygophylacids with one gonothecal aperture (e.g. *Zygophylax pacifica*) and 25 species with two or three gonothecal apertures (e.g. *Z. pseudosibogae*). *Zygophylax pseudosibogae*, *Z. tankahkeei*, *Z. lighti* (C9 on Fig. 22), *Z. leloupi* and *Z. brownei* (C8 on Fig. 22) could be assigned to a single group with two gonothecal apertures. In clades C8 and C9, the terminal tubes are straight or curved; clade C8 includes *Z. leloupi* and *Z. brownei* with short and straight terminal tubes, while the nearby clade C9 includes *Z. tankahkeei*, *Z. lighti* and *Z. pseudosibogae* with longer curved terminal tubes (Supporting Information, Table S2).

Protective structures in Zygophylacidae

Nematophores, hydrothecal opercula, chitinous perisarc plugs, cysts, bracts and gonothecae (coppiniae, glomulus, scapus, phylactocarps and corbulae) are diverse protective structures in Hydrozoa (Bouillon *et al.*, 2006). Within Macrocolonia, nematothecae are only present in the family Zygophylacidae and the superfamily Plumularioidea that form a clade on the concatenated tree (Fig. 21). Zygophylacids have tubular nematothecae that are distributed on the hydrocauli, hydrocladia, apophyses of hydrocladia and/or reproductive organs (Supporting Information, Table S2). Nematothecae seem to be lost in at least four zygophylacids, including *Zygophylax flexilis* (Pictet & Bedot, 1900), *Z. geniculata* (Clarke, 1894), *Z. niger* and *Z. stechowi* (Jäderholm, 1919) (Supporting Information, Table S2). It would be interesting to know if the species without nematotheca form a clade (i.e. if nematothecae were lost once in the evolution of the group). The loss or absence of hydrothecal operculum was also found in the Plumularioidea, Zygophylacidae, Haleciidae and Starurothecidae within the Macrocolonia (Fig. 21).

Additional protective structures for gonophores are present in the coppiniae of Zygophylacidae, or the phylactocarps and corbulae in Plumularioidae. The gonothecae of 46 zygophylacids aggregate into coppiniae, while for 28 species their gonothecal structures are still unknown (Supporting Information, Table S2). Secondary defensive tubules are found in the coppiniae of 30 zygophylacids (Supporting Information, Table S2).

According to the statistics provided by the present study, 45 species have been reported in the deep sea, 21 species were reported in shallow water and nine species have been reported both in shallow and deep water (Supporting Information, Table S2). This large portion of deep-water species is relatively unusual among hydrozoan taxa, though *Gonaxia* Vervoort, 1993, *Solenoscyphus* Galea, 2015 and *Streptocaulus* Allman, 1883 are also deep-water genera; the last two have only four and nine species, respectively. Based on the original descriptions of all zygophylacids (Supporting Information, Table S2), when compared to its shallow-water species, the deep-sea zygophylacids seem to have longer hydrothecae and nematothecae and mostly have complex coppiniae with secondary protective tubes. These may be due to the deep-water environment (e.g. temperature, salinity and food source), and they may be more stable than in shallow water. The number of nematothecae in zygophylacids has no obvious correlation to water depth. The above characters shared by the deep-sea zygophylacids may be valuable for future functional morphology studies combined with modern genomic approaches.

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AUTHOR CONTRIBUTIONS

XS designed the study. ZG and XS prepared the manuscript drafts. ZG, XS, RZ and LL conducted the morphological and molecular analyses. BR examined the ZSM material. ZG, RZ, CM and BR examined the COBI-DBUA material. BR and CM revised the paper. All authors wrote the paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Molecular data contributed by this study are available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with the accession numbers MT261928–MT261948, MT262560–MT262562, MZ680504, MZ680505, MZ686450–MZ686455. All original images that support this study have been deposited in MorphoBank (https://morphobank.org/permalink/?P4108).

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SUPPORTING INFORMATION

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

Table S1. Specimens examined in this study.
Table S2. Comparison of the 34 morphological characteristics of all zygophylacids.
Table S3. Information on molecular sequences used in this study.
Figure S1. Maximum likelihood phylogenetic analyses with partial sequences of the *COI* gene
Dataset S1. Dataset (16S + 18S + 28S) for phylogenetic analyses in the present study.
Dataset S2. Dataset (16S) for phylogenetic analyses in the present study.
Dataset S3. Dataset (*COI*) for phylogenetic analyses in the present study.