Journal of the Marine Biological Association of the United Kingdom, 2013, 93(8), 2075–2088.
doi:10.1017/S0025315413000969

Detection of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) with DNA barcoding and life cycle analyses

KONGLIN ZHOU[1], LIANMING ZHENG[1,2,3], JINRU HE[1], YUANSHAO LIN[1,2], WENQING CAO[1,2] AND WENJING ZHANG[1,2]

[1]College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China, [2]Marine Biodiversity and Global Change Research Center, Xiamen University, Xiamen 361005, China, [3]Fujian Provincial Key Laboratory for Coastal Ecology and Environmental Studies (CEES), Xiamen University, Xiamen 361005, China

The genus Clytia *is distributed worldwide, but most accepted species in this genus have been examined either only at the hydroid or medusa stage. The challenge in identifying* Clytia *species reflects their complex life cycles and phenotypic plasticity. In this study, molecular and morphological investigations of* Clytia *specimens from the coastal waters of China revealed an as yet unreported species, designated* C. xiamenensis *sp. nov., that was considered as conspecific to two nearly cosmopolitan species,* C. hemisphaerica *and* C. gracilis. *DNA barcoding based on partial mitochondrial cytochrome c oxidase subunit I (COI) and large subunit ribosomal RNA gene (16S) confirmed the highly distinct lineage of* C. xiamenensis *sp. nov. These results were corroborated by the detailed observations of its mature medusae and its colonies, which showed that* C. xiamenensis *sp. nov. was morphologically distinct from other species of* Clytia. *Thus, based on our findings, the nearly cosmopolitan distribution attributed to some species of* Clytia *might rather be due to the misidentification, and it is necessary to elucidate their whole life cycle in order to establish the systematic validity of all species within the genus* Clytia.

Keywords: *Clytia*, new species, DNA barcoding, life cycle

Submitted 22 January 2013; accepted 20 June 2013; first published online 7 August 2013

INTRODUCTION

Clytia (Lamouroux, 1812) is the genus within Campanulariidae (Cnidaria, Leptomedusae) with the highest number of species and it is widely distributed throughout the world. Approximately 60 species of *Clytia* are currently recognized (Bouillon & Boero, 2000; Calder *et al.*, 2003; Schuchert, 2003, 2012; Bouillon *et al.*, 2006; Govindarajan *et al.*, 2006; Lindner *et al.*, 2011), and because they are usually the most abundant hydromedusae, they play an important ecological role in the plankton and shallow-water benthic environments, acting as both competitor and predator (Madin *et al.*, 1996; Avent *et al.*, 2001; Boero *et al.*, 2005; Adamík *et al.*, 2006). Furthermore, the ubiquitous *Clytia hemisphaerica* (Linnaeus, 1767) has emerged as an important model organism in developmental biology and evolutionary studies (Amiel *et al.*, 2010; Houliston *et al.*, 2010).

Clytia has a life history consisting of medusa and hydroid stages. *Clytia* medusae are commonly found in coastal waters, and their hydroids are either benthic or free-living planktonic colonies (Cornelius, 1995; Lindner *et al.*, 2011). However, taxonomy is particularly challenging in *Clytia* because of the phenotypic plasticity exhibited by its species. At either the medusa or hydroid level, most of the morphological characters used to distinguish the different species, have little or no taxonomic value because these characters have been observed to vary within a single species (Bouillon & Boero, 2000). Furthermore, even among the few taxonomically relevant characters, it is unclear whether the differences are environmentally or genetically based, which presents a major limitation to their use in the identification of new species (Cornelius, 1990). An additional obstacle is that most species of *Clytia* have been examined only at the hydroid or medusa stage (1982, 1987; Calder, 1991) and potentially distinguishing features of other life cycle stages are not yet recognized. In fact, specific identification is also hindered because the description of species from the plankton may have been based on developmental stages of other species (Lindner & Migotto, 2002). Consequently, taxonomic homonyms and synonyms are common within *Clytia*, in particular for the nearly cosmopolitan *C. hemisphaerica* and *C. gracilis* (Sars, 1850). Elucidation of the complete life cycles of the known *Clytia* species is therefore important as it is likely to bring about important revisions (Bouillon & Boero, 2000). Yet, for most *Clytia* species, life cycle studies may also be problematic since the developmental relationship between polyp and medusa stages is poorly understood. As reviewed by Lindner *et al.* (2011), only ten species have had their life cycles investigated.

Most species of *Clytia* inhabiting the coastal waters of China have been described solely on the basis of their medusae (Huang, 2008), with only a few reports in which hydroids were studied (Gao, 1956). Recently, we used DNA

Corresponding author:
L. Zheng
Email: zhlm@xmu.edu.cn

barcoding based on two partial mitochondrial genes sequences, those of cytochrome c oxidase subunit I (COI) and the large subunit of ribosomal RNA (16S) (Zheng *et al.*, unpublished data), to examine hydromedusae from the coastal waters of China. These efforts led to the identification of highly distinct lineages for a species of *Clytia* presented in the plankton samples from Xiamen Bay. Although this species is usually identified as *C. hemisphaerica* (Xu *et al.*, 1985; Cheng *et al.*, 2012), in the phylogenetic tree it was determined to be the same species as *Clytia* cf. *gracilis* sp., from the coastal north-eastern United States (see Lindner *et al.*, 2011). We therefore re-examined the morphology of the Xiamen Bay species and investigated its complete life cycle. The results showed that it differed from both *C. hemisphaerica* and *C. gracilis* based on the distinct morphological characters of its hydroid and medusae stages. Molecular and morphological evidence supported the valid placement of this species within the genus *Clytia*, and the name *C. xiamenensis* sp. nov. was chosen.

MATERIALS AND METHODS

Molecular methods

Total DNA was extracted from whole medusae (four field individuals and four individuals released from colonies) by SDS-proteinase K/phenol-chloroform extraction (Zheng *et al.*, 2009). The DNA was preserved in TE buffer and stored at −20 °C. The COI was amplified using the procedure and primers described by Zheng *et al.* (2009). 16S was partially amplified using the published primers (16S-L 5′GAC TGT TTA CCA AAA ACA TA3′ and 16S-H 5′CAT AAT TCA ACA TCG AGG3′) (Ender *et al.*, 2003). Amplification for 16S was achieved with five cycles of 94°C/1 min, 45°C/50 s, 72°C/1 min, followed by 30 cycles of 94°C/50 s, 50°C/1 min, 72°C/1 min, and a final elongation step at 72°C for 5 min. All PCR products were sequenced directly in both directions on an ABI PRISM 3730 Genetic Analyzer using BigDye® Terminator v.3.1 (Shanghai Sangon Biological Engineering Technology & Service Co., Ltd, China).

The COI and 16S sequences were aligned using ClustalX v.2 (Larkin *et al.*, 2007) and then edited manually, using EditSeq v.7.1, to ensure the correct alignment and placement of insertion/deletion events. GenBank BLAST searches were performed to confirm the accuracy and validity of all sequences, which were then deposited in GenBank (Accession numbers for field samples: JQ716198–JQ716201 for COI and JQ716037–JQ716040 for 16S; Accession numbers for sequences derived from medusae liberated from the colonies: JQ7161202–JQ716205 for COI and JQ716041–JQ716044 for 16S). These sequences along with the sequences of *Clytia folleata* (McCrady, 1859) collected from the coastal waters of China (COI: JQ716211; 16S: JQ716051-JQ716055), and other species in Campanulariidae which have already been deposited in Genbank (Table S1, Supplementary Information) were used to infer phylogenetic trees using neighbour-joining (NJ, based on K2P model) and maximum-likelihood (ML, based on GTR + G model) algorithms in MEGA V5 (Tamura *et al.*, 2011). Node support for the two approaches was inferred with a bootstrap analysis (1000 replicates). Two Leptothecata species were chosen as outgroups: *Aequorea conica* (Browne, 1905) and *Gangliostoma guangdongensis* (Xu, 1983).

Sample collection

Medusae of *Clytia xiamenensis* sp. nov. were collected using a plankton net (mesh size: 500 μm) from Xiamen Bay, China, in March 2011 (Figure 1). Individuals used in DNA barcoding studies were acclimated in filtered seawater (FSW) for at least 24 h before they were photographed, and then preserved in 95% ethanol. The remaining individuals were kept alive and cultured together in FSW for morphology and life cycle observations.

Culturing and morphological description

Medusae collected from the sea were reared in covered glass vessels with a roughened glass slide placed at the bottom which facilitates colony settlement. Zygotes were collected

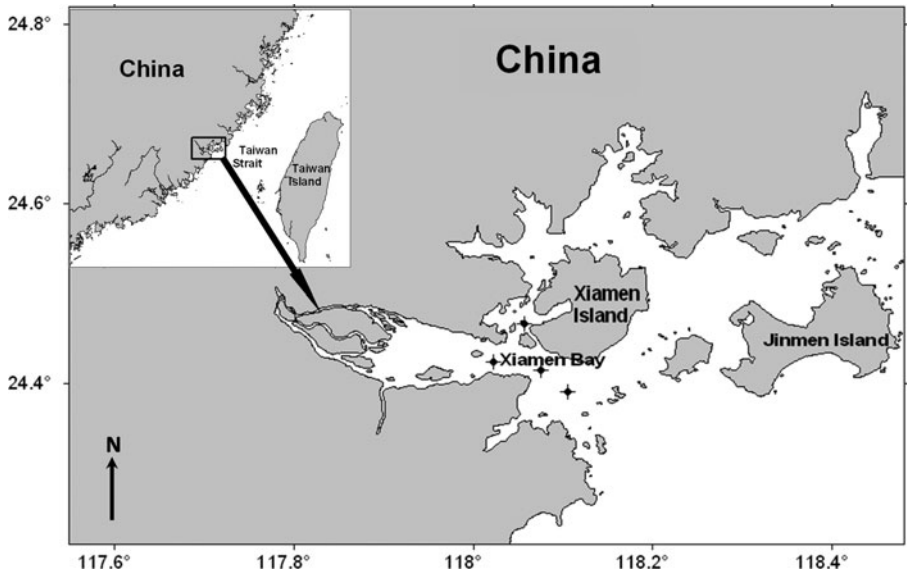

Fig. 1. Collecting sites of *Clytia xiamenensis* sp. nov.

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

and cultured in small crystallizing dishes to observe embryonic development. The glass slides with the newly settled colonies of *C. xiamenensis* sp. nov. were subsequently transferred to crystallizing dishes for further observation. Newly released medusae were collected and reared through maturity in the laboratory for morphological and DNA barcoding analyses. The hydroids and medusae were fed 2–3 times per day with *Artemia* sp. nauplii; the FSW was changed every second day. The temperature of the water was kept below room temperature (17–25°C) and the salinity was 30–32‰.

Planulae, hydroids, and medusae were examined under a stereomicroscope and by biological microscopy (Zeiss V12 and Nikon SMZ1000). Prior to their observation, hydranths and medusae were acclimated in a 10% $MgCl_2$ solution. *Artemia* sp. nauplii juice and distilled water were used to induce the nematocysts to discharge in squash preparation (Östman, 1987; Wang & Xu, 1990). Nematocyst types and their distribution were determined using a light microscope equipped with differential-interference-contrast optics (Olympus BX51). Nematocyst nomenclature followed that of Weill (1934), Mariscal (1974) and Östman (1979a, b, 1999).

RESULTS

DNA barcoding

The COI and the 16S trees strongly supported *Clytia xiamenensis* sp. nov. as a member of the genus *Clytia* (Figures 2 & 3). In both trees, *C. xiamenensis* sp. nov. formed a strongly supported cluster with '*C. hemisphaerica*' from the China Sea (Wand *et al.*, 2010, sequence obtained from GenBank) and '*Clytia* cf. *gracilis* sp. A' from the coastal north-eastern United States (see Lindner *et al.*, 2011). All three were clearly separated from other *Clytia* species. For both the partial COI and the 16S sequences, genetic divergence was

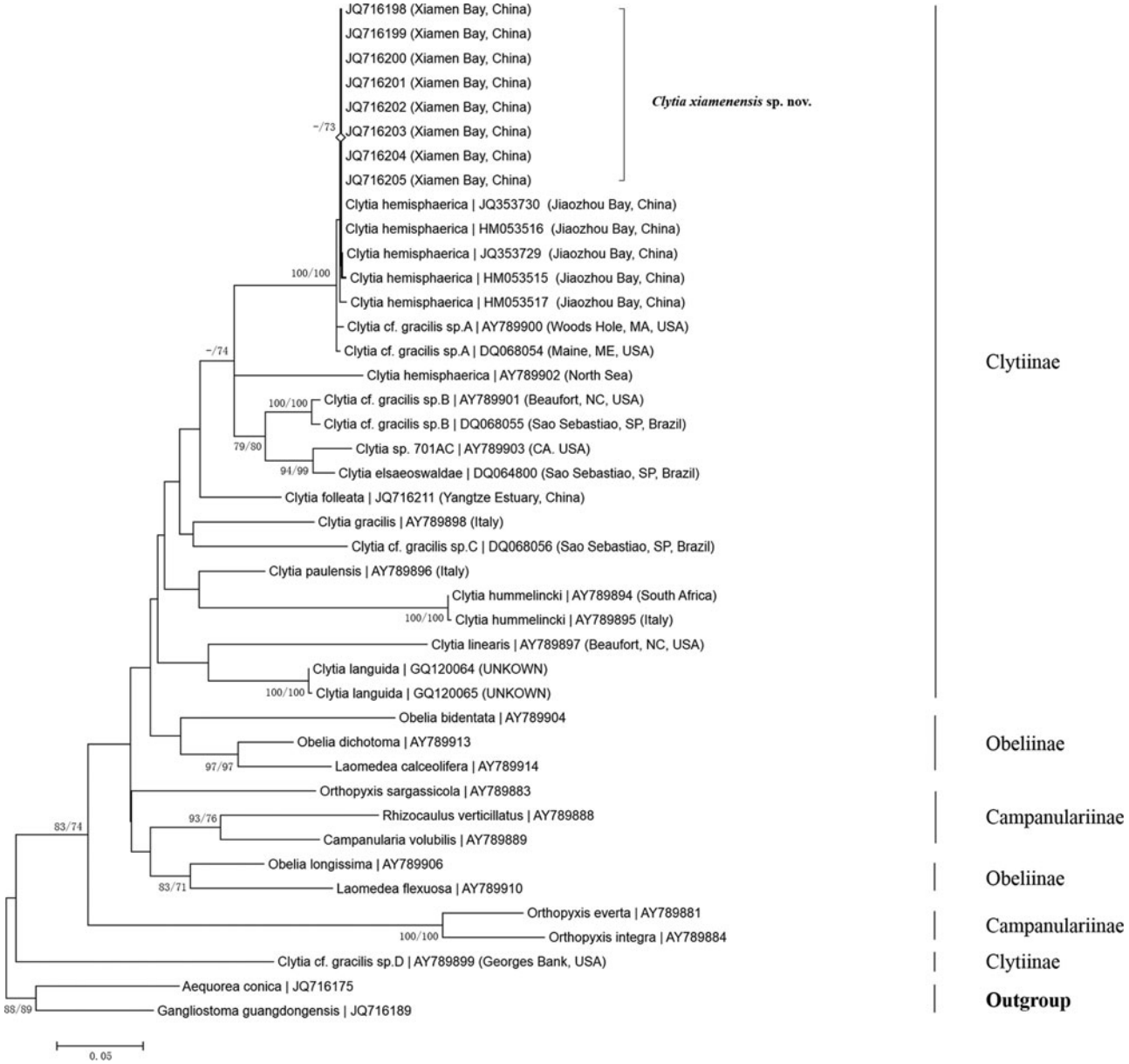

Fig. 2. Maximum-likelihood (ML) topology based on mtCOI. Bootstrap values higher than 70 were shown above the branches. First number along the branches refers to ML bootstrap values, second number refers to neighbour-joining bootstrap values, *Clytia xiamenensis* sp. nov. and subfamily lineages are indicated.

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

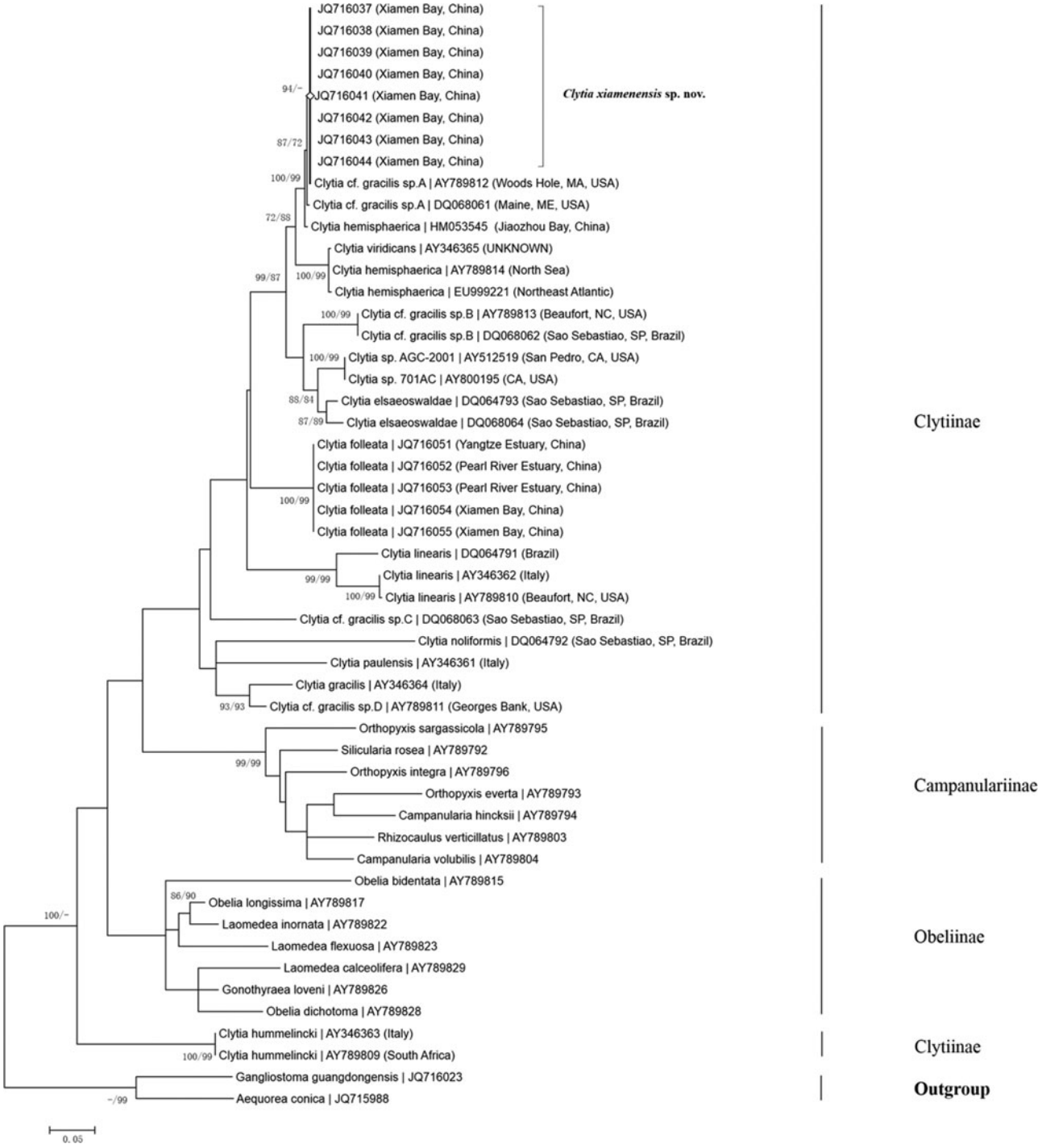

Fig. 3. Maximum-likelihood (ML) topology based on 16S rRNA. Bootstrap values higher than 70 were shown above the branches. First number along the branches refers to ML bootstrap values, second number refers to neighbour-joining bootstrap values, *Clytia xiamenensis* sp. nov. and subfamily lineages are indicated.

zero within individuals of *C. xiamenensis* sp. nov., and low within the clade comprising *C. xiamenensis* sp. nov., '*C. hemisphaerica*' (from the China Sea), and '*Clytia* cf. *gracilis* sp. A' (COI: 0–0.007; 16S: 0–0.006) (Tables S2 & S3; Supplementary Information), which supported the common lineage of its members. And there were obvious genetic divergences between *C. xiamenensis* sp. nov. and other species of *Clytia* (COI/16S: 0.093-0.315/0.055-0.241). According to the tree-based identification, *C. xiamenensis* sp. nov. is most closely related to the clade containing *C. hemisphaerica* (two individuals, collected from the North Sea and the north-east Atlantic; see Lindner *et al.*, 2011 and Licandro *et al.*, 2010, respectively).

RESULTS

SYSTEMATICS

Order LEPTOTHECATA Cornelius, 1992
Family CAMPANULARIIDAE Johnston, 1836
Genus *Clytia* Lamouroux, 1812
Clytia xiamenensis sp. nov.
(Figures 4–7; Tables 1–3)

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

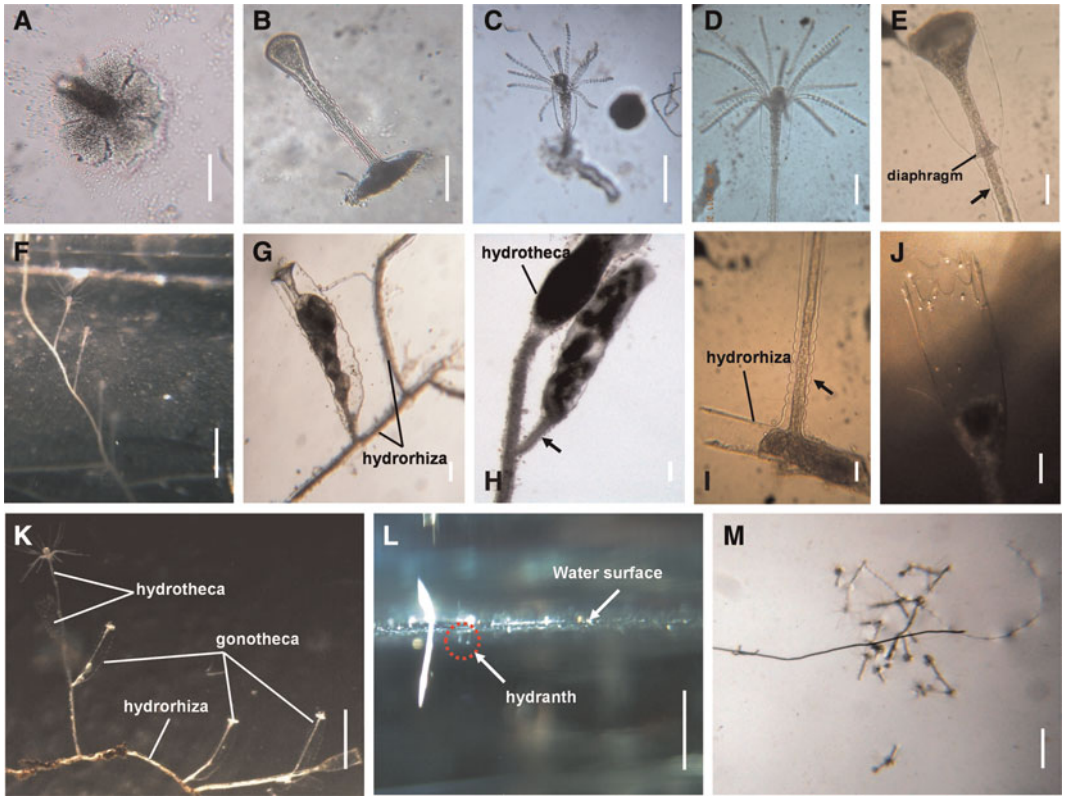

Fig. 4. The hydranth of *Clytia xiamenensis* sp. nov.: (A–C) primary hydranth from the settled planulae; (D) distal portion of hydranth; (E) hydrotheca, arrow: annuli; (F) unbranched stem; (G–H) gonangium with medusa buds (G) gonothecae on the stolon; (H) gonothecae on the hydrocaulus, arrow: a short stalk with annuli; (I) pedicle with annuli (arrow); (J) hydrothecal margin with cusps; (K) a part of colony with a branch and gonothecae; (L–M) the floating colony. Scale bars: A,B,E,G–J, 100 μm; C, 250 μm; D, 125 μm; F,K,M, 1 mm; L, 2 mm

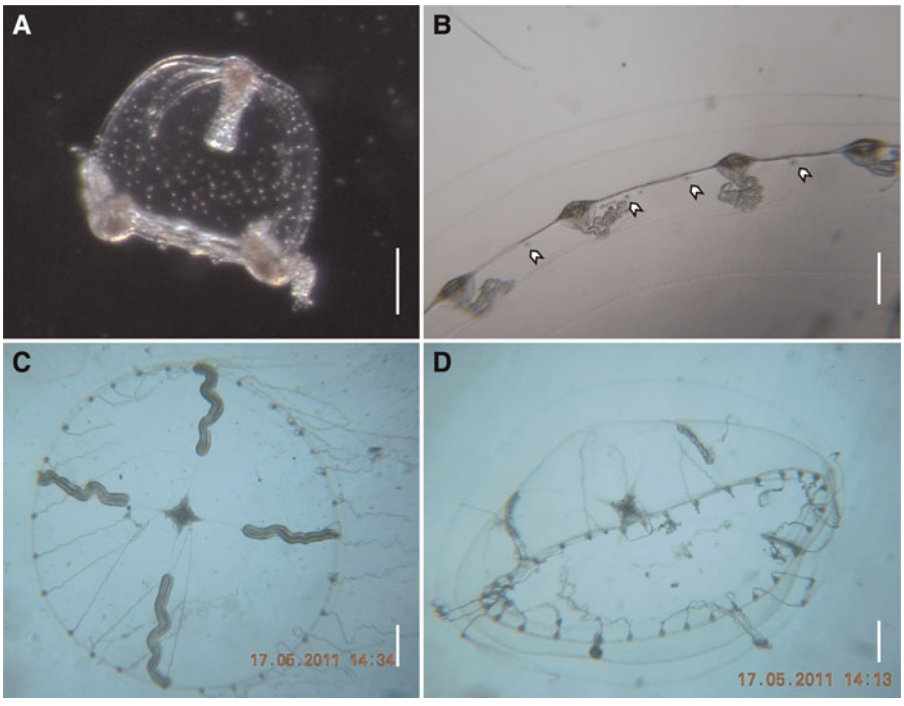

Fig. 5. The medusae of *Clytia xiamenensis* sp. nov.: (A) newly liberated medusa; (B–D) mature medusa: (B) marginal structure of mature medusa; (C) male species; (D) female species. Note: Swallow-tailed arrow: the statocysts between tentacle (or marginal wart). Scale bars: A, 100 μm; B, 300 μm; C–D, 1 mm.

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

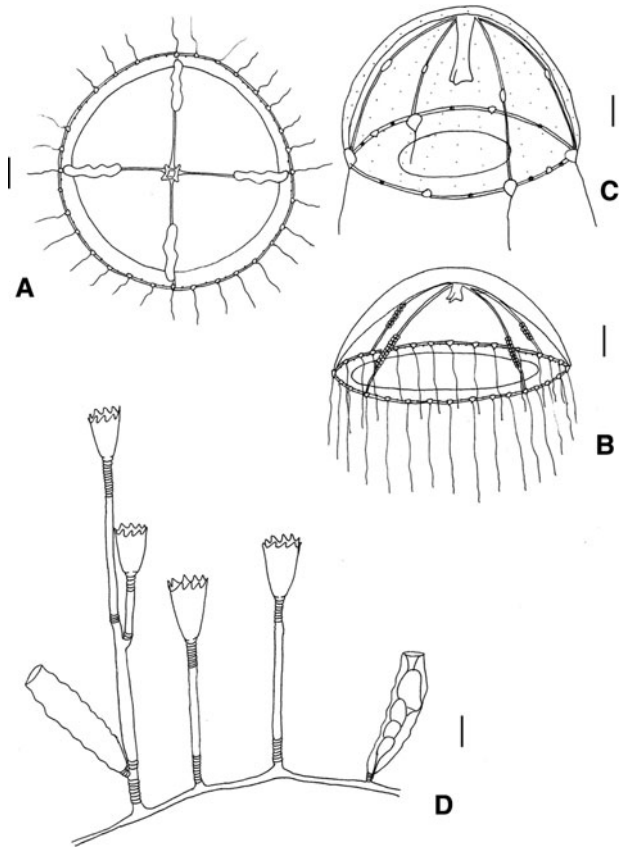

Fig. 6. The line drawings of *Clytia xiamenensis* sp. nov.: (A) mature male individual; (B) mature female individual; (C) newly liberated medusa; (D) a part of colony with a branch and gonothecae. Scale bars: A,B, 1 mm; C,D, 100 μm.

MATERIAL EXAMINED

Holotype: CXXB 11, female, 9.0 mm.

Paratype: CXXB 12, male, 8.6 mm. Both were collected in Xiamen Bay, China, 24.3871°N 118.1430°E, March 2011. The type specimens are deposited in the Department of Oceanography, Xiamen University, China.

ETYMOLOGY

Clytia xiamenensis sp. nov. is named after Xiamen Bay where the species was collected.

DIAGNOSIS

Clytia xiamenensis sp. nov. is distinguished from its congeners and other Campanulariidae based on a combination of the following characters:

Polyp: the stem is monosiphonic, stolonal, branching rarely. Hydrothecal cusps usually incline to one side. Gonothecae are borne on both hydrorhiza and pedicel, with a short annulated pedicel and undulated walls. B-type microbasic mastigophores are 9.5–11.2 μm long *in vivo*.

Adult medusa: the bell is 5.7–9.1 mm in its marginal diameter, with up to 44 tentacles and 0–3, but usually 1, statocysts between the hollow tentacles. In male medusae, green pigments are present in gonads, tentacle bulbs, and warts. LA-type microbasic mastigophores (9.5–12.4 μm, it shared similar shape with A-type, but much larger than A-type, so named it as LA-type) were also observed.

Hydroid

Colonies are stolonal, and the pedicels branch only rarely (Figures 4F, K & 6D). The pedicel is short (0.26–1.5 mm), with 4–29 distal and 7–14 proximal annuli (Figure 4D, E, I); several pedicels are thoroughly covered with annuli.

The hydrotheca is campanulate in shape, with 6–12 triangular, pointed cusps inclining to one side (Figures 4J & 6D), and variable in size: 0.26–0.47 mm long and 0.14–0.23 mm wide at the margins (Figure 4D, E). The hydrothecal diaphragm is thin, transverse, and located at the base of the hydrotheca (Figure 4E); the basal chamber is cup-like. The hydranth is elastic, reaching a height of up to 0.88 mm; the pedunculated hypostome is spherical in oral view. There are 12–18 filiform tentacles and two amphicoronate whorls, with an average length of 0.49 mm (Figure 4D). Nematocysts distribute on tentacles circularly. Hydrothecal length:width ratio: 1.5–2.5 (Table 1).

The gonothecae arise on stolons, sometimes on the pedicel or directly on a branch (Figures 4G, H, K & 6D). They have distinctly undulated walls (Figure 4G) and short pedicels, each with 2–6 annuli (Figure 4H); 4–7 buds are present (Figure 4G, H). Gonothecae are cylindrical, 0.89–1.4 mm long and 0.2–0.3 mm wide at their margins. Their length: width ratio is 3.8–6.5 (Table 2).

Most areas of the hydroid body contain A-type nematocysts with a spindle-shaped capsule (Figure 7F, G; details in Table 3). The tube is discharged from the tip in a direction almost coinciding with that of the long axis of the capsule (Figure 7G). B-type microbasic mastigophore nematocysts are 10.2 ± 0.5 μm (9.5–11.2 μm, N = 26) long and 2.8 ± 0.3 μm (2.1–3.3 μm, N = 26) wide *in vivo*. They are along coenosarcs and are much larger and more curved than other types of nematocysts (Figure 7H, I; details in Table 3).

Newly released medusa

The umbrella is bell-shaped, 0.55–1.2 mm wide and 0.39–0.78 mm in height. The jelly is comparatively thin. D-type nematocysts are scattered over the exumbrella (Figures 5A & 6C). There are four radial canals and one circular canal. The stomach is small and the manubrium is quadrate, with four simple lips. The ovate and transparent gonads are located in the middle of the radial canals. The velum is broad. Four hollow tentacles contain A- and C-type nematocysts. Additional features: four developing marginal warts; eight adradial statocysts, with closed marginal vesicles, containing one statolith (Figures 5A & 6C).

Development

Medusae attained up to eight tentacles within 3 d and 16 tentacles within about 10 d. During this period, D-type nematocysts on the exumbrella disappeared; the umbrella became flat and the lips recurved (details in Table 2).

Mature medusa

When the medusae are 25 d old, the umbrella is flattened, 5.7–9.1 mm wide and 1.8–3.0 mm in height (Figures 5C, D & 6A, B). The stomach is small; the manubrium is short, with undulated lips. There are four radial canals. The gonads are usually elongated and may be slightly wavy, about 0.9–1.5 mm long and 0.30–0.40 mm wide, occupying the distal part of the radial canal. The velum is narrow. There may be 19–44 well-developed hollow tentacles, but generally about 30. The tentacle bulbs are oval or triangular,

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

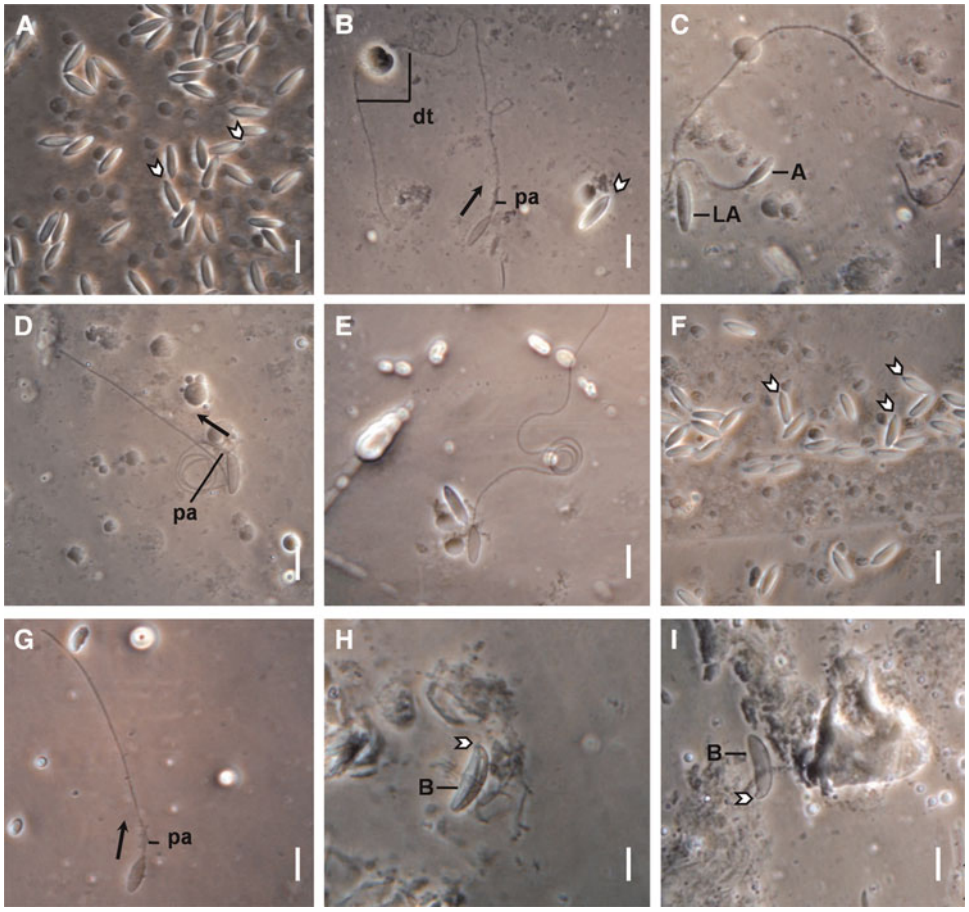

Fig. 7. Interference contrast micrographs of the nematocyst from *Clytia xiamenensis* sp. nov.: (A–E) nematocysts form medusa: (A–B) A-type; (C) LA-type; (D) C-type; (E) isorhizas; (F–I) nematocysts form hydroid: (F–G) A-type; (H–I) B-type. Note: swallow-tailed arrow: the small protruding tip; arrow: the direction of discharge in nematocyst; pa: the proximal armature; dt: the distal tube; A: A-type; LA: LA-type; B: B-type. Scale bars: 5 μm.

without papilla. There are 10–43 statocysts in a medusa but the number is usually less than that of the total tentacles. Between 0 and 3 statocysts, but usually only one, are located between successive tentacles and margin warts. The statocysts contain one statolith, rarely two (Figure 5B). In male medusae, green pigments are present in the gonads, tentacle bulbs, and warts (Figure 5C).

A-type nematocysts are scattered throughout most of the body (Figure 7A, B). The nematocysts of mature medusae are larger than those of immature forms (Table 3). LA-type

Table 1. Measurements (mean ± SD (range)) of colonies of *Clytia xiamenensis* sp. nov. (N = 30 unless otherwise mentioned).

Hydrothecae	Shape	Campanulate
	Length (mm)	0.35 ± 0.063 (0.26–0.47)
	Width at margin (mm)	0.17 ± 0.027 (0.14–0.23)
	Length:Width	2.0 ± 0.23 (1.5–2.5)
	Shape of margin	Pointed cusps, inclined to one side
	Number of cusps	6.6 ± 0.9 (6–12)
Hydrothecal pedicel	Length (mm)	0.78 ± 0.28 (0.26–1.50)
	Annuli at distal end	9.4 ± 6.0 (4–29)
	Annuli at proximal end	10 ± 1.8 (7–14)
Tentacle	Length (mm)	0.49 ± 0.11 (0.18–0.78)
	Number	14.4 ± 1.6 (12–18)
Gonothecae (N = 28)	Shape	Cylindrical
	Wall	Undulated
	Number of medusae	4–7
	Length (mm)	1.1 ± 0.11 (0.89–1.40)
	Width at margin (mm)	0.24 ± 0.023 (0.20–0.30)
	Length:width	4.7 ± 0.68 (3.8–6.5)
	Annuli at proximal end	3.4 ± 0.96 (2–6)

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

Table 2. Comparison of morphology (mean ± SD (range)) of *Clytia xiamenensis* sp. nov. at successive developing medusae stages, in mm (N = 25).

		New released stage	8 Tentacles stage	16 Tentacles stage	Mature stage
Age (day)		0	3	10	25
Umbrella	Shape	Bell	Bell	Lower than a hemisphere	Lower than a hemisphere
	Diameter	0.76 ± 0.15 (0.55–1.20)	1.50 ± 0.35 (0.98–2.20)	6.0 ± 1.2 (4.0–8.7)	7.4 ± 1.3 (5.7–9.1)
	Height	0.54 ± 0.08 (0.39–0.78)	0.96 ± 0.18 (0.70–1.4)	2.3 ± 0.55 (1.2–3.3)	2.6 ± 0.41 (1.8–3.0)
	DU/HU	1.4	1.6	2.7	2.9
Gonad	Shape	Oval	Oval	Linear, little waved	Linear or waved
	Position	Middle	Middle	Distal part	Distal part
	Length	0.035 ± 0.008 (0.013–0.052)	0.083 ± 0.029 (0.050–0.150)	0.86 ± 0.34 (0.30–1.50)	1.1 ± 0.19 (0.9–1.5)
	Width	0.021 ± 0.008 (0.013–0.039)	0.061 ± 0.020 (0.050–0.100)	0.29 ± 0.082 (0.12–0.40)	0.32 ± 0.05 (0.30–0.40)
Shape of mouth and lips		Simple, square	Simple, cross	Simple, cross	Simple, cross
Manubrium	Height	0.22 ± 0.05 (0.13–0.35)	0.33 ± 0.057 (0.20–0.40)	0.53 ± 0.14 (0.20–0.80)	0.51 ± 0.099 (0.40–0.70)
	HM/HU	2/5	1/3	1/4	1/5
Tentacle	Number	4.0 ± 0.3(4–5)	7.8 ± 1.7 (5–12)	15.8 ± 3.6 (9–24)	30.1 ± 4.4 (19–44)
	SB	Oval	Triangular	Triangular	Triangular
Statocyst	Sum	8	9 ± 2 (6–14)	26.4 ± 4.0 (20–34)	29.4 ± 9.3 (10–43)
	NSSB	1	0–1	0–3, usually 1	0–3, usually 1

DU/HU, diameter of umbrella/height of umbrella; HM, height of manubrium; SB, shape of bulbs; NSSB, number of statocysts between successive bulbs.

Table 3. Measurements (mean ± SD (range)) of microbasic mastigophore nematocysts of *Clytia xiamenensis* sp. nov., in μm.

		N	Length	Width
A-type				
	Hydroid			
	from tentacle	30	6.3 ± 0.5 (5.4–7.4)	1.8 ± 0.2 (1.2–2.2)
	from hypostome	7	6.9 ± 0.5 (6.0–7.5)	1.9 ± 0.1 (1.7–2.1)
	from coelenteron	30	7.0 ± 0.5 (5.4–8.2)	2.0 ± 0.2 (1.7–2.3)
	from pedicel	11	5.7 ± 0.4 (4.9–6.2)	2.0 ± 0.2 (1.8–2.2)
	from stolon	10	6.0 ± 0.2 (5.8–6.4)	1.7 ± 0.2 (1.4–1.8)
	Medusa			
	4-tentacle stage			
	from tentacle	4	6.9 ± 0.1 (6.7–7.1)	1.7 ± 0.3 (1.4–2.0)
	from lip	10	6.8 ± 0.4 (6.0–7.1)	1.6 ± 0.2 (1.4–1.9)
	whole medusa	11	7.1 ± 0.5 (6.2–7.9)	2.0 ± 0.2 (1.7–2.6)
	mature stage			
	from tentacle	30	7.7 ± 0.5 (6.6–8.7)	2.1 ± 0.2 (1.8–2.9)
	from lip	30	7.9 ± 0.4 (7.4–8.8)	2.1 ± 0.2 (1.7–2.7)
LA-type				
	Medusa			
	from the circular canal	15	11.2 ± 0.8 (9.5–12.4)	2.5 ± 0.2 (2.1–2.9)
B-type				
	Hydroid			
	from coenosarc	26	10.2 ± 0.5 (9.5–11.2)	2.8 ± 0.3 (2.1–3.3)
Isorhizas				
	4-tentacle stage			
	whole medusa	9	6.2 ± 0.5 (5.4–7.0)	2.0 ± 0.2 (1.8–2.5)
	mature stage			
	from tentacle	30	7.6 ± 0.5 (6.6–8.7)	2.1 ± 0.4 (1.7–2.9)
	from lip	30	7.2 ± 0.4 (6.6–7.9)	2.0 ± 0.1 (1.7–2.2)

microbasic mastigophores are 11.2 ± 0.8 μm (9.5–12.4 μm, N = 15) long and 2.5 ± 0.2 μm (2.1–2.9 μm, N = 15) wide *in vivo* and are much larger than A-type (Figure 7C), scattering only at the level of the circular canal. C-type nematocysts are found only in the tentacles of the medusae, are relatively few in number, and their tubules roughly form a right angle with the long axis of the capsule when discharged (Figure 7D). Atrichous isorhiza nematocysts are present in the tentacles (Figure 7E) and in the lips (Table 3).

Biological notes

The mature medusae released eggs and sperm mainly at dawn, although occasionally at other times of the day. The eggs were variable in diameter, 158 ± 12.8 μm (130–182 μm), but the sperm were much smaller. The head of the sperm was 3.3 ± 0.28 μm (2.5–3.8 μm) long and 2.0 ± 0.25 μm (1.7–2.5 μm) wide. Embryonic development lasted only for several hours. Fertilized eggs developed into blastula within 3–4 h; the blastula became free-swimming hollow planulae after 1–3 h and then solid planulae during the following 3 h.

Planulae are small, milky white, 0.14–0.36 mm long and 0.060–0.12 mm wide. They can change direction by rotation during swimming. The planulae observed in this study settled mainly along the bottom and walls of the rearing jars, where they underwent typical development into hydroids (Figure 4A–C). However, some planulae settled on the underside of the surface film of the water, where they gave rise to floating colonies that developed for a few days (Figure 4L, M) before sinking to the bottom of the jar, where they continued to develop. We did not observe any morphological differences between free and attached hydranths.

The hydroids were kept at 17–25°C, as higher temperatures were lethal. The formation of a considerable number of gonothecae was observed in spring (17–20°C), while fewer than 20 gonothecae were counted in summer (24–25°C).

DISCUSSION

The similarities in shape and size of medusae of *Clytia* species, especially immature medusae, are greater than those of the polyps of this genus (e.g. Table 4). As a result, it is difficult to describe species unequivocally based on medusae collected in the plankton. This has frequently led to the synonymization of species within the genus *Clytia* at the medusa level (Bouillon & Boero, 2000). For example, Millard (1966) combined *C. johnstoni* (Alder, 1856) and *C. gracilis* and established *C. hemisphaerica* based on the morphology of the medusae. Cornelius (1982) also synonymized *C. gracilis* with the cosmopolitan *C. hemisphaerica*, but the former was subsequently shown to be distinct on the basis of its hydroid and nematocysts (Östman, 1979a, 1987). In plankton collected from the China Sea, specimens assigned to *C. hemisphaerica* were reviewed and subsequently divided into two types by Xu *et al.* (1985) based on field medusae samples. Although with its 19–44 tentacles and linear or slightly wavy gonads *C. xiamenensis* sp. nov. resembles the larger of these two types, which is detected in the China Sea from March to April, the molecular and morphological analyses presented below strongly suggest that it is a distinct species.

Overall colony form and hydrothecal characteristics are frequently used as diagnostic characters in the

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

Table 4. General features of some similar species of *Clytia*.

		C. xiamenensis	*C. hemisphaerica*	*C. gracilis*	*C. elsaeoswaldae*	*C. linearis*	*C. viridicans*	*C. uchidai*
Hydroid	Colony shape	Stolonal, branch rarely	Stolonal, branch rarely	Branching, dichotomous	Stolonal or branching, dichotomous	Branching, sympodial	Stolonal	–
	Shape of cusps	Pointed, asymmetrical, inclined to one side	Rounded-triangular, symmetrical	Pointed, asymmetrical, inclined to one side	Pointed, asymmetrical, inclined to one side	Each with a pleat projecting into the hydrothecal cavity and extending from the apex of the cusp towards the middle of the hydrotheca	Rounded-triangular, symmetrical	–
Gonothecae	Wall	Undulated	Markedly corrugated	Smooth	Smooth, with undulated wall	Smooth	Smooth, with undulated wall	–
	Location	Hydrorhiza or hydrocaulus	Hydrorhiza or hydrocaulus	Hydrorhiza	Hydrorhiza	Hydrorhiza or hydrocaulus	Hydrorhiza	–
Newly released medusa	Gonad	Present	Present	Present	Present	Present	Absent	Absent
Adult medusa	Diameter (mm)	5.7–9.1	Up to 20	6–8	3.6–5.5	2.5–3.6	6	Reaching 10
	Bell shape	Flatter than a hemisphere	Flatter than a hemisphere	Flatter than a hemisphere	Saucer-shaped	Saucer-shaped to flattened	Flatter than a hemisphere	Low-dome-like
	Gonad	Linear or waved, on the diatal part radial canal	Elongate-oval, on distal 1/3 of radial canal	Oval	Oval	Oval, on the distal half of radial canals	Oval, on the distal half of radial canals	Oblong
	Number of tentacles	19–44, generally about 30	Up to 32	Up to 16	Up to 16	20–29	14–16	16–28
	NSSB	0–3, usually 1	1–3, usually 2	1–2, usually 2	1–2	1–2, usually 1	–	1–2, usually 2
	Green pigment	Present, only in male	Absent	Absent	Absent	Absent	Present	Absent
Source		Present study	Cornelius, 1995	Cornelius, 1995	Linder *et al.*, 2011	Lindner & Migotto, 2002	Pagliara *et al.*, 2000	T. Uchida, 1947

NSSB, number of statocysts between successive bulbs.

Campanulariidae (Cornelius, 1982). However, for either of these there may be considerable phenotypic plasticity (Ralph, 1956; Cornelius, 1982), which presents a major obstacle to their taxonomic utility. In fact, variations in these characters have been reported for many species, such as *C. gregaria* (L. Agassiz, 1862) (Roosen-Runge, 1970), *C. attenuate* (Calkins, 1899) (West & Renshaw, 1970), *C. edwardsi* (Nutting, 1901) (Kubota, 1978a), *C. linearis* (Thorneley, 1900), and *C. noliformis* (McCrady, 1859) (Lindner & Migotto, 2002). For *C. xiamenensis* sp. nov., despite variations in some of the characteristics of the hydrothecae, such as their dimensions, the number of tentacles, and the annulations on the pedicels, others, including the shapes of the hydrothecal cusps and gonothecae, are relatively consistent, at least according to our observations (Table 1). The absence of variability in these features was also reported for *C. attenuate* (West & Renshaw, 1970). Alternatively, for many groups of hydroids, including Campanulariidae, the morphological characteristics of the nematocysts are taxonomically useful (Östman, 1979a, b, 1982, 1987); but again, some of these characters may be plastic (Östman *et al.*, 1987).

In addition to the morphological similarity among species, difficulty in determining species boundaries within *Clytia* is also due to the lack of information on the life cycles of its member species and on intraspecific morphological variation. These and related problems are further aggravated by the absence of sufficient morphological data, which prevents the recognition of cryptic species (Lindner *et al.*, 2011). Thus, at least in the case of *Clytia*, additional diagnostic characters should be added to the species description. DNA barcoding has enabled species tagging for the purpose of identification (Hebert *et al.*, 2003, 2004). For hydrozoans, both COI and 16S have been recommended as valuable DNA barcodes that allow the differentiation of morphologically undistinguishable, nominal, and cryptic or pseudo-cryptic species, including undescribed taxa (Govindarajan *et al.*, 2005; Schuchert & Reiswig, 2006; Moura *et al.*, 2008, 2011; Bucklin *et al.*, 2011). Recently, Lindner *et al.* (2011) determined that a Brazilian '*C. gracilis*' was instead a distinct species based on a detailed life cycle study and molecular analysis, and they resurrected the name *C. elsaoswaldae* (Stechow, 1914). In the present work, we obtained morphological and molecular evidence supporting *C. xiamenensis* sp. nov., previously regarded as conspecific to *C. hemisphaerica* or *Clytia* cf. *gracilis* sp., as a valid species belonging to the genus *Clytia*.

In defining the genus *Clytia*, Hincks (1868) stated that one of its main characteristics was the production of medusae with four radial canals, four marginal tentacles, and eight lithocysts. Nutting (1915) expanded the definition by describing the trophosome: 'Colony often simple but always consisting of a creeping rootstock from which spring pedicels which are not regularly branched as a rule. Hydrothecae campanulate, hydranths with trumpet-shaped proboscis'. Years later, Bouillon & Boero (2000) emphasized the 'hydrothecal rim sinuous or deeply indented, with clefts between the round to sharply-pointed cusps; hydrothecae with a true hydrothecal diaphragm'. All of these characteristics were observed in *C. xiamenensis* sp. nov. (Figures 4–6; Tables 1 & 2), and the phylogenetic-tree-based identification also strongly supported this species as belonging to the genus *Clytia* (Figures 2 & 3).

Mature medusae of both *C. xiamenensis* sp. nov. and *C. hemisphaerica* have more than 30 tentacles, four oral lips, and oval or linear gonads close to the circular canal (Table 4). These similarities make it difficult to distinguish between members of these two species at the medusa stage. However, *C. xiamenensis* sp. nov. differs from *C. hemisphaerica* in that the former usually has one statocyst between successive bulbs whereas the latter species usually has two (Mayer, 1910; Gao *et al.*, 1958; Kramp, 1968). In addition, the gonads, tentacle bulbs, and warts of adult male medusae of *C. xiamenensis* sp. nov. contain green pigments but this has never been reported for *C. hemisphaerica*. Medusae of *C. hemisphaerica* collected in the plankton are also much larger (up to 20 mm in diameter; Russell, 1953; Cornelius, 1995) than those of *C. xiamenensis* sp. nov. (up to 9.1 mm in diameter). Another similarity between *C. xiamenensis* sp. nov. and *C. hemisphaerica* is that of their hydroids, as stolonal growth and usually a few branched stems are seen in both; however, *C. xiamenensis* sp. nov. differs from *C. hemisphaerica* in that its hydrothecae have pointed cusps and are usually inclined to one side (rather than rounded and not inclined; Cornelius, 1995). Another difference is that the 'B-type' nematocysts (9.5–11.2 × 2.1–3.3 μm) in the hydroid of *C. xiamenensis* sp. nov. are smaller than those of *C. hemisphaerica* hydroids [(10.5–13.5 × 2.5–4.0 μm (Sweden), 21.0–24.0 × 4.0–4.5 μm (Italy), 15.5–17.0 × 3.5–4.0 μm (Brazil); Östman, 1999)]. Moreover, only *C. xiamenensis* sp. nov. contains LA-type nematocysts scattered at the level of the circular canal, further corroborating that it is not conspecific with *C. hemisphaerica*. Finally, there were obvious genetic divergences between *C. xiamenensis* sp. nov. and *C. hemispherica* (COI/16S:0.093/0.055). *Clytia xiamenensis* sp. nov. formed a strongly supported lineage in the COI and 16S trees that was clearly separate from the lineage of *C. hemisphaerica* (two individuals, collected from North Sea and north-east Atlantic, see Lindner *et al.*, 2011 and Licandro *et al.*, 2010, respectively), although the two species were closely related (Figures 2 & 3). This phylogenetic evidence corroborates that obtained from the life cycle and morphological studies. Here, we need to mention that the distinct mitochondrial lineages are only suggestive, a nuclear marker is essential to detect the relationship between the species of *Clytia*.

Thus, according to the phylogenetic-tree-based identification, *C. xiamenensis* sp. nov., '*Clytia* cf. *gracilis* sp. A' from the coastal north-eastern United States (Lindner *et al.*, 2011), and '*C. hemisphaerica*' from the China Sea (Cheng *et al.*, 2012) are the same species, with *C. xiamenensis* sp. nov. clearly distinct from other species assigned to *C. gracilis* or *Clytia* cf. *gracilis* (genetic divergences of COI/16S: 0.096-0.315/0.071-0.152; Figures 2 & 3). Based on the evidence discussed above, these '*C. hemisphaerica*' from the China Sea were misidentified. Although *C. xiamenensis* sp. nov. is similar to *C. gracilis* in having inclined hydrothecal cusps (Cornelius, 1995), it differs in that it has mostly stolonal (rather than mostly branching) colonies and its gonothecae have markedly undulated (rather than smooth) walls (Cornelius, 1995) (Table 4). Although gonothecal shape may vary in some species of *Clytia* (Kubota, 1978a; Lindner & Migotto, 2002), *C. gracilis* has never been collected on the coast of China (Huang, 2008), suggesting that it is not present in this region and thus has a more limited distribution. Moreover, the 'B-type' nematocysts in the hydroid of *C. xiamenensis* sp. nov. are much smaller (9.5–11.2 × 2.1–3.3 μm) than those of *C. gracilis* (12.0–18.0 × 3.0–5.5 μm (Sweden); Östman, 1999). Mature medusae of *C. gracilis* are

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press

unfortunately still unknown. If they are indeed 6–8 mm in diameter and bear 16 tentacles, as hypothesized by Cornelius (1995), then they have fewer tentacles than *C. xiamenensis* sp. nov. Moreover, LA-type nematocysts and green pigments have never been reported for *C. gracilis*. Thus, *C. xiamenensis* sp. nov. is not a cryptic species of *C. gracilis* and a careful review of specimens assigned to *C. gracilis* is necessary.

Clytia xiamenensis sp. nov. and *C. viridicans* (Leuckart, 1856) are similar in that both have green pigments in the manubrium, gonads, and marginal bulbs, but they differ in that *C. xiamenensis* sp. nov. has inclined cusps (rather than rounded and not inclined), its newly liberated medusae bear gonads (rather than absent thereof), its mature medusae have 19–44 tentacles (rather than 16 tentacles), and bright green pigmentation is seen only in mature male medusae (rather than beginning in the newly released medusae and persisting in mature medusae, both male and female; Pagliara *et al.*, 2000) (Table 4). Green pigmentation was also reported by Mayer (1910) in a study of *C. folleata* from the west Atlantic.

Clytia folleata is also found in Xiamen Bay, China, and it resembles *C. xiamenensis* sp. nov. with respect to the shape of its medusae. However, its mature medusae have only 16 tentacles and, based on our observations, the gonads differ in shape, as those of the former are usually oval rather than linear. Although information on the life cycle of *C. folleata* is still lacking, our DNA-based identification showed that this species formed a distinct clade which was well separated from *C. xiamenensis* sp. nov. (Figures 2 & 3).

In addition to *C. gracilis* and *C. xiamenensis* sp. nov., other species of *Clytia* are characterized by hydrothecae with inclined cusps, i.e. *C. linearis*, *C. delicatula* (Thornely, 1904), and *C. elsaeoswaldae* (Table 4). However, in *C. linearis* the hydrothecal margin has 10–14 sharp cusps, each with a pleat projecting into the hydrothecal cavity and extending from the apex of the cusp towards the middle of the hydrotheca. In addition, the gonothecae of *C. linearis* are smooth (Lindner & Migotto, 2002) and the slightly compressed bell of its medusae may be unique. Both *C. delicatula* and *C. elsaeoswaldae* differ from *C. xiamenensis* sp. nov. in having smooth gonothecal walls. Also, newly liberated medusae of *C. delicatula* lack gonads (Kubota, 1978b) and the gonothecae of *C. elsaeoswaldae* develop exclusively in the hydrorhiza, not on branches (Lindner *et al.*, 2011). Although both *C. uchidai* and *C. xiamenensis* sp. nov. have type localities in the same biogeographic zone, they differ in that *C. xiamenensis* sp. nov. has one statocyst between successive bulbs (rather than usually two), its newly liberated medusae bear gonads (rather than absent thereof), its mature medusae have up to 44 tentacles (rather than 28 tentacles; Uchida, 1947) (Table 4). In addition, green pigmentation has never been reported for *C. uchidai*.

Interestingly, planktonic hydroids were observed for *C. xiamenensis* sp. nov. These free hydroids were produced by the planula and then settled on the underside of the surface film of the water, eventually giving rise to floating colonies that developed over a period of a few days (Figure 4L, M), as has been observed for *C. viridicans* (Pagliara *et al.*, 2000). This planula behaviour in culture may be a cultivation artefact, or represent its natural behaviour in nature. In fact, although there are no direct observations in which planktonic hydroids are produced by planula, huge numbers of floating colonies of *C. gracilis* have been found in Georges Bank (Madin *et al.*, 1996). In most hydrozoan species, medusae and the other floating organisms are generally the main agents of species dispersal. Yet, in *C. xiamenensis* sp. nov. and *C. viridicans* the behaviour of their planulae suggests the important contribution of this life cycle stage to dispersal. While the ecological role of planktonic hydroids in the field is not well understood, they could prey upon young copepods as well as on the eggs and larvae of commercially important fish species (Madin *et al.*, 1996; Adamík *et al.*, 2006), in addition to being a food source for these fish (Avent *et al.*, 2001).

The morphological and molecular analyses described above support *C. xiamenensis* sp. nov. as a valid species of the genus *Clytia*. Our findings further suggest that currently recognized species of *Clytia*, in particular *C. hemisphaerica* and *C. gracilis*, may represent complexes of several species. Since most *Clytia* studies have not considered this possibility or have been based only on a single life cycle stage (i.e. polyp or medusa), a careful review of those *Clytia* species regarded as nearly cosmopolitan in shallow waters is necessary. Furthermore, our approach demonstrates that life cycle studies in which *Clytia* species described based on their polyps are linked to those described based on their medusae could correct misidentifications, resulting in more accurate species descriptions. However, additional species-level studies would still be needed to assess diversity. Finally, it is important to emphasize that studies based on the life cycle and DNA, especially the nuclear DNA, of the organism in question are effective and efficient for species identification and both should be adopted as standard taxonomic approaches in future revisions, as shown here for the genus *Clytia*.

DNA barcoding offers great help to understand the extent of biodiversity by providing a simple and quick way to identify species (Hebert *et al.*, 2003; Hebert & Gregory, 2005). A challenge remains, however, in its accuracy and efficiency (Krishnamurthy & Francis, 2012). And another point of contention is that regions of DNA used for barcoding often present limited information for higher phylogenetic resolution (Moritz & Cicero, 2004). The results from our study established two facts indicating the barcode potential of COI and 16S for *Clytia*, even for the Campanulariidae. First, both COI and 16S could identify *C. xiamenensis* sp. nov. and other Campanulariidae species efficiently and accurately using the distance-based (K2P distance) approach. Second, 16S was shown to be a better phylogenetic marker for Campanulariidae at the subfamily level, Campanulariinae and Obeliinae appeared a monophyletic clade respectively and all but one species (*Clytia hummelincki*) of Clytiinae were monophyletic in the 16S tree (Figure 3). And we believe that increased taxon sampling will increase the better understanding of the phylogenetic relationships of the members of Campanulariidae in the coastal water of China based on 16S data.

ACKNOWLEDGEMENTS

We appreciate that Dr Carina Östman kindly mailed us her papers about nematocysts, Dr Senjie Lin provided valuable comments on an earlier draft of the manuscript, and Professors Jiaqi Huang and Zhenzu Xu helped to identify species. We are grateful to Open and Sharing Platform of

Equipment and Technology (OSPET) of COE of Xiamen University for providing equipment and technical support.

FINANCIAL SUPPORT

This work was supported by the National Natural Science Foundation of China (Grant No. 41006078), the Fundamental Research Funds for the Central Universities (Grant No. 2010121037), the Public Science and Technology Research Funds Projects of Ocean (Grant No. 201005015-5), the Natural Science Foundation of Fujian Province of China (Grant No. 2011J05116).

Supplementary materials and methods

The supplementary material referred to in this article can be found online at journals.cambridge.org/mbi.

REFERENCES

Adamík P., Gallager S.M., Horgan E., Madin L.P., McGillis W.R., Govindarajan A. and Alatalo P. (2006) Effects of turbulence on the feeding rate of a pelagic predator: the planktonic hydroid *Clytia gracilis*. *Journal of Experimental Marine Biology Ecology* 333, 159–165.

Amiel A., Chang P., Momose T. and Houliston E. (2010) *Clytia hemisphaerica*: a cnidarian model for studying oogenesis. In Verlhac M.H. and Villeneuve A. (eds) *Oogenesis: the universal process*. Chichester: John Wiley & Sons, pp. 81–102.

Avent S.R., Bollens S.M., Butler M., Horgan E. and Rountree R. (2001) Planktonic hydroids on Georges Bank: ingestion and selection by predatory fishes. *Deep-Sea Research Part II: Topical Studies in Oceanography* 48, 673–684.

Boero F., Camillo C.D. and Gravili C. (2005) Aquatic invasions: phantom aliens in Mediterranean waters. *MarBEF Newsletter* 3, 21–22.

Bouillon J. and Boero F. (2000) Synopsis of the families and genera of the Hydromedusae of the world, with a list of the worldwide species. *Thalassia Salentina* 24, 47–296.

Bouillon J., Gravili C., Pagés F., Gili J.M. and Boero, F. (2006) *An introduction to Hydrozoa*. Paris: Publications Scientifiques du Muséum, 591pp.

Bucklin A., Steinke D. and Blanco-Bercial L. (2011) DNA barcoding of marine metazoa. *Annual Review of Marine Science* 3, 471–508.

Calder D.R. (1991) *Shallow-water hydroids of Bermuda: the Thecatae, exclusive of Plumularioidea*. Toronto: Royal Ontario Museum.

Calder D.R., Mallinson J.J., Collins K. and Hickman C.P. (2003) Additions to the hydroids (Cnidaria) of the Galápagos, with a list of species from the islands. *Journal of Natural History* 37, 1173–1218.

Cheng F.P., Wang M.X., Wang Y.T., Zhang F., Li C.L. and Sun S. (2012) DNA barcoding of common medusozoa in northern China based on mtCOI sequence. *Oceanologia et Limnologia Sinica* 43, 451–459. [In Chinese.]

Cornelius P.F.S. (1982) Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of the genera. *Bulletin of the British Museum (Natural History): Zoology* 42, 37–148.

Cornelius P.F.S. (1987) The hydranths of *Clytia linearis* (Cnidaria, Hydrozoa) and related species. In Bouillon J., Boero F., Cicogna F. and Cornelius P.F.S. (eds) *Modern trends in the systematics, ecology and evolution of Hydroids and Hydromedusae*. Oxford: Clarendon Press, pp. 291–297.

Cornelius P.F.S. (1990) European *Obelia* (Cnidaria, Hydroida): systematics and identification. *Journal of Natural History* 24, 535–578.

Cornelius P.F.S. (1995) North-west European thecate hydroids and their medusa, part 2, Sertulariidae to Campanulariidae. In Barnes R.S.K. and Crothers J.H. (eds) *Synopses of the British fauna (new series)*, No. 50. London: Linnean Society of London.

Ender A. and Schierwater B. (2003) Placozoa are not derived cnidarians: evidence from molecular morphology. *Molecular Biology and Evolution* 20, 130–134.

Gao C.S. (1956) On the hydroids along Shantung coast. *Journal of Shandong University* 4, 70–103. [In Chinese.]

Gao C.S., Li F.L., Chang U.M. and Li H.L. (1958) On the Hydromedusae from the Shantung coast. *Journal of Shandong University* 1, 75–118. [In Chinese.]

Govindarajan A.F., Halanych K.M. and Cunningham C.W. (2005) Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). *Marine Biology* 146, 213–222.

Govindarajan A.F., Boero F. and Halanych K.M. (2006) Phylogenetic analysis with multiple markers indicates repeated loss of the adult medusa stage in Campanulariidae (Hydrozoa, Cnidaria). *Molecular Phylogenetics and Evolution* 38, 820–834.

Hebert P.D.N. and Gregory T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology* 54, 852–859.

Hebert P.D.N., Ratnasingham S. and deWaard J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society*, B 270, S96–S99.

Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H. and Hallwachs W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Science of the United States of America* 101, 14812–14817.

Hincks T. (1868) *A history of the British hydroid zoophytes*. Volume 1. London: John van Voorst, 112 pp.

Houliston E., Momose T. and Manuel M. (2010) *Clytia hemisphaerica*: a jellyfish cousin joins the laboratory. *Trends in Genetics* 26, 159–167.

Huang Z.G. (2008) *Marine species and their distributions in China's Seas*. Beijing: China Ocean Press. [In Chinese.]

Kramp P.L. (1968) *The hydromedusae of the Pacific and Indian Oceans: Sections II and III*. Copenhagen: A.F. Host.

Krishnamurthy P.K. and Francis R.A. (2012) A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and Conservation* 21, 1901–1919.

Kubota S. (1978a) The life-history of *Clytia edwardsi* (Hydrozoa; Campanulariidae) in Hokkaido, Japan. *Journal of the Faculty of Science Hokkaido University Series VI. Zoology* 21, 317–354.

Kubota S. (1978b) Notes on *Clytia* and *Phialidium* (Hydrozoa; Campanulariidae) from Shimoda, Japan. *Proceeding of the Japanese Society of Systematic Zoology* 15, 1–7.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. and Higgins D.G. (2007) Clustal W and clustal X version 2.0. *Bioinformatics* 23, 2947–2948.

Licandro P., Conway D.V.P., Yahia M.N.D., de Puelles M.L.F., Gasparini S., Hecq J.H., Tranter P. and Kirby R.R. (2010) A

blooming jellyfish in the northeast Atlantic and Mediterranean. *Biology Letters* 6, 688–691.

Lindner A. and Migotto A.E. (2002) The life cycle of *Clytia linearis* and *Clytia noliformis*: metagenic campanulariids (Cnidaria: Hydrozoa) with contrasting polyp and medusa stages. *Journal of the Marine Biological Association of the United Kingdom* 82, 541–553.

Lindner A., Govindarajan A.F. and Migotto A.E. (2011) Cryptic species, life cycles, and the phylogeny of *Clytia* (Cnidaria: Hydrozoa: Campanulariidae). *Zootaxa* 2980, 23–36.

Madin L.P., Bollens S.M., Horgan E., Butler M., Runge J., Sullivan B.K., Klein-Macphee G., Durbin E., van Keuren D., Plourde S., Bucklin A. and Clarke M.E. (1996) Voracious planktonic hydroids: unexpected predatory impact on a costal marine ecosystem. *Deep-Sea Research Part II: Topical Studies in Oceanography* 43, 1823–1829.

Mariscal R.N. (1974) Nematocysts. In Muscatine L. and Lenhoff H.M. (eds) *Coelenterate biology: reviews and new perspectives*. New York: Academic Press, pp. 129–178.

Mayer A.G. (1910) *Medusae of the world. The Hydromedusae*. Washington, DC: Carnegie Institution of Washington, 498 pp.

Millard N.A.H. (1966) The Hydrozoa of the south and west coasts of South Africa. Part III. The Gymnoblastea and small families of Calyptoblastea. *Annals of the South African Museum* 48, 426–487.

Moritz C. and Cicero C. (2004) DNA barcoding: promise and pitfalls. *Plos Biology* 2, 1529–1531.

Moura C.J., Cunha M.R., Porteiro F.M. and Rogers A.D. (2011) Polyphyly and cryptic diversity in the hydrozoan families Lafoeidae and Hebellidae (Cnidaria: Hydrozoa). *Invertebrate Systematics* 25, 454–470.

Moura C.J., Harris D.J., Cunha M.R. and Rogers A.D. (2008) DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zoologica Scripta* 37, 93–108.

Nutting C.C. (1915) *American hydroids. Part III. The Campanularidae and the Bonneviellidae*. Washington, DC: Smithsonian Institution, Special Bulletin 4.

Östman C. (1979a) Two types of nematocysts in Campanulariidae (Cnidaria, Hydrozoa) studied by light and scanning electron microscopy. *Zoologica Scripta* 8, 5–12.

Östman C. (1979b) Nematocysts in the *Phialidium* medusae of *Clytia hemisphaerica* (Hydrozoa, Campanulariidae) studied by light and scanning electron microscopy. *Zoon* 7, 125–142.

Östman C. (1982) Nematocysts and taxonomy in *Laomedea, Gonothyraea* and *Obelia* (Hydrozoa, Campanulariidae). *Zoologica Scripta* 11, 227–241.

Östman C. (1987) New techniques and old problems in hydrozoan systematics. In Bouilon J., Boero F., Cicogna F. and Cornelius P.F.S. (eds) *Modern trends in the systematics, ecology and evolution of Hydroids and Hydromedusae*. Oxford: Clarendon Press, pp. 67–82.

Östman C. (1999) Nematocysts and their value as taxonomic parameters within the Campanulariidae (Hydrozoa). A review based on light and scanning electron microscopy. In Stepanjants S.D. (ed.) Obelia *(Cnidaria, Hydrozoa): phenomenon, aspects of investigations, perspectives of employment*. St Petersburg: Russian Academy of Sciences, pp. 17–28.

Östman C., Piraino S. and Roca I. (1987) Nematocyst comparisons between some Mediterranean and Scandinavian campanulariids (Cnidaria, Hydrozoa). In Bouilon J., Boero F., Cicogna F. and Cornelius P.F.S. (eds) *Modern trends in the systematics, ecology and evolution of Hydroids and Hydromedusae*. Oxford: Clarendon Press, pp. 299–310.

Pagliara P., Bouillon J. and Boero F. (2000) Photosynthetic planulae and planktonic hydroids contrasting strategies of propagule survival. *Scientia Marina* 64, 173–178.

Ralph P.M. (1956) Variation in *Obelia geniculata* (Linnaeus, 1758) and *Silcularia bilabiata* (Coughtrey, 1875) (Hydroida, F. Campanulariidae). *Transactions of the Royal Society of New Zealand* 84, 279–296.

Roosen-Runge E.C. (1970) Life cycle of the Hydromedusa *Phialidium gregarium* (A. Agassiz, 1862) in the laboratory. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 139, 203–221.

Russell F.S. (1953) *The medusae of the British Isles Vol. I: Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae*. Cambridge: Cambridge University Press.

Schuchert P. (2003) Hydroids (Cnidaria, Hydrozoa) of the Danish expedition to the Kei Islands. *Steenstrupia* 27, 137–256.

Schuchert P. (2012) *World Hydrozoa database*. Available at: http://www.marinespecies.org/hydrozoa/aphia.php?p=taxdetails&id=117030 (accessed 2 July 2013).

Schuchert P. and Reiswig H.M. (2006) *Brinckmannia hexactinellidophila*, n. genera, n. sp. a hydroid living in tissues of glass sponges of the reefs, fjords, and seamounts of Pacific Canada and Alaska. *Canadian Journal of Zoology* 84, 564–572.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Uchida T. (1947) Some medusa from the central pacific. *Journal of the Faculty of Science. Hokkaido University* 9, 297–319.

Wang W.Q. and Xu Z.Z. (1990) Nematocysts of some species of Siphonophores and Scyphomedusae in Xiamen Harbour. *Journal of Xiamen University (Natural Science)* 29, 85–88. [In Chinese.]

Weill R. (1934) Contribution à l'étude des cnidaries et leurs nématocysts. I, II. *Travaux de la Station Zoologique de Wimereux*, 10/11, 1–701.

West D.L. and Renshaw R.W. (1970) The life cycle of *Clytia attenuata* (Calyptoblastea: Campanulariidae). *Marine Biology* 7, 332–339.

Xu Z.Z., Huang J.Q. and Wang W.Q. (1985) On new species and records of Hydromedusae from the Jiulong river estuary of Fujian, China. *Journal of Xiamen University (Natural Science)* 24, 102–110. [In Chinese.]

and

Zheng L.M., Lin Y.S., Li S.J., Cao W.Q., Xu Z.Z. and Huang J.Q. (2009) *Aequorea taiwanensis* n. sp. (Hydrozoa, Leptomedusae) and mtCOI sequence analysis for the genus *Aequorea*. *Acta Oceanologica Sinica* 28, 109–115.

Correspondence should be addressed to:
L. Zheng
College of Ocean and Earth Sciences, Xiamen University
Xiamen 361005, China
email: zhlm@xmu.edu.cn.

https://doi.org/10.1017/S0025315413000969 Published online by Cambridge University Press