



# Identification of a Novel Species, *Cladonema digitatum* sp. nov. (Cnidaria: Hydrozoa: Cladonematidae), Using DNA Barcoding and Life Cycle Analyses

Xinyu Fang<sup>1,2</sup>, Shen Lin<sup>1</sup>, Yuting Zhang<sup>1</sup>, Zonghua Wang<sup>1</sup>, Konglin Zhou<sup>1\*</sup> and Jianming Chen<sup>1\*</sup>

<sup>1</sup>Fujian Key Laboratory on Conservation and Sustainable Utilization of Marine Biodiversity, Fuzhou Institute of Oceanography, Minjiang University, Fuzhou, China, <sup>2</sup>College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, China

## OPEN ACCESS

### Edited by:

Rachel Collin,  
Smithsonian Tropical Research  
Institute (SI), United States

### Reviewed by:

Maria Pia Miglietta,  
Texas A&M University at Galveston,  
United States  
Allen G. Collins,  
NOAA Fisheries, United States

### \*Correspondence:

Konglin Zhou  
zhoukl@mju.edu.cn  
Jianming Chen  
chenjm@mju.edu.cn

### Specialty section:

This article was submitted to  
Marine Biology,  
a section of the journal  
Frontiers in Marine Science

Received: 08 March 2022

Accepted: 16 June 2022

Published: 28 July 2022

### Citation:

Fang X, Lin S, Zhang Y,  
Wang Z, Zhou K and Chen J  
(2022) Identification of a Novel  
Species, *Cladonema digitatum*  
sp. nov. (Cnidaria: Hydrozoa:  
Cladonematidae), Using DNA  
Barcoding and Life Cycle Analyses.  
*Front. Mar. Sci.* 9:891998.  
doi: 10.3389/fmars.2022.891998

In this study, a new *Cladonema* species was identified in a laboratory aquarium in Fuzhou, China, and named *Cladonema digitatum* sp. nov. based on its morphological characteristics and DNA barcoding. It is distinct from other *Cladonema* medusae in having a manubrium with finger-like protuberances, radial canals with Y-shaped bifurcations, tentacles with 3–11 adhesive branches, and 3–7 stinging branches growing from the main branch as side branches. The validity of *C. digitatum* sp. nov. was supported by molecular phylogenetic analyses based on both mitochondrial cytochrome oxidase and mitochondrial 16S rRNA sequences. Similar to other *Cladonema* medusae, the adhesive and stinging branches of each tentacle, oral tentacle, manubrium, and gonads in *C. digitatum* displayed considerable phenotypic plasticity, thus making species identification based solely on morphology difficult. Although diagnostic characters such as filiform tentacles and medusa buds of hydroids and nematocysts are also useful for species identification in the genus *Cladonema*, related information is missing in some *Cladonema* species. Thus, information on the life cycle and DNA barcoding should be updated to describe new or cryptic species and to improve the taxonomy of the genus *Cladonema*.

**Keywords:** *Cladonema*, morphology, life cycle, DNA barcoding, 16S rRNA, COI, photosensitivity

## INTRODUCTION

Hydromedusae play an important role in marine ecosystems (Colin et al., 2003; Tewksbury et al., 2014). In recent years, increasing jellyfish blooms have exacerbated the adverse ecological effects of medusae as competitors and predators in coastal marine ecosystems (Gravili et al., 2008; Miglietta et al., 2008). Cladonematidae is a family of anthoathecate hydrozoans containing three genera: *Cladonema*, *Eleutheria*, and *Staurocladia* (Ghory et al., 2020). Although *Cladonema* species are distributed in coastal waters worldwide (Chou and Huang, 1958; Xu, 1993; Schuchert, 2006; Gershwin and Zeidler, 2008; Cedeno-Posso, 2014), they are not frequently caught in plankton nets because they prefer to remain in benthic zones (Schuchert, 2006). Thus, the distribution, abundance, and biodiversity of *Cladonema* species are likely underestimated (Zhou et al., 2022). *Cladonema* medusae can also make a difference in local habitats when they gather in Yantai waters during the

summer (Chou and Huang, 1958). Furthermore, *Cladonema pacificum* and *Cladonema radiatum* are used as model organisms in the fields of developmental (e.g., branching morphogenesis and eye development), regenerative, and evolutionary biology (Graziussi et al., 2012; Fujiki et al., 2019; Fujita et al., 2019; Fujita et al., 2021).

*Cladonema* medusae can be distinguished from other Cladonematidae by the presence of branched tentacles and two types of tentacle branches: (1) stinging branches, used for predation and self-defense with numerous bulbous nematocyst clusters and (2) adhesive branches, which end with a structure that can adhere to substrates (Schuchert, 2006; Farias et al., 2020). However, species identification is challenging in the genus *Cladonema*. Zhou et al. (2022) suggested several diagnostic characteristics of the genus, including the number of radial canals and adhesive branches, arrangement of stinging branches and gastric pouches in the manubrium during the medusa stage, existence of filiform tentacles, and the number and arrangement of medusa buds on the hydroids. The diagnostic characteristics often vary among individuals in the same population (Bouillon and Boero, 2000). Several rounds of lumping and splitting among *Cladonema* species, especially *C. radiatum* and its subspecies, have occurred because of the phenotypic plasticity of the medusae (Schuchert, 2006; Gershwin and Zeidler, 2008). Currently, the life cycles in only five *Cladonema* species have been investigated, increasing the difficulties in cryptic species identification in the genus *Cladonema* (Schuchert, 2006; Gershwin and Zeidler, 2008; Zhou et al., 2022). The use of mitochondrial cytochrome oxidase (COI) and mitochondrial 16S rRNA (16S) in DNA barcoding is an efficient and reliable tool for the identification of hydromedusae (Bucklin et al., 2010a; Bucklin et al., 2010b; Zheng et al., 2014; Schuchert, 2016) and detecting cryptic or new species (Lindner et al., 2011). The use of both morphological descriptions and molecular phylogenetic analyses may provide a more accurate way to describe new or cryptic hydromedusae (Zhou et al., 2013; He et al., 2015).

In this study, *Cladonema digitatum* sp. nov. was found in an *Oryzias melastigma* aquarium in our laboratory (Fuzhou, China) and was verified as a new *Cladonema* species through both morphological and molecular tests. In terms of morphological characteristics and molecular sequences, *C. digitatum* sp. nov. showed obvious differences from *Cladonema multiramum*, another *Cladonema* species previously found in the same aquarium (Zhou et al., 2022). We further revised the taxonomy within the genus *Cladonema* and the phylogenetics in Cladonematidae.

## MATERIALS AND METHODS

### Sample Collection

*Cladonema* species were found in an *O. melastigma* aquarium in Dr. Chen's laboratory (Fuzhou, China). Polyps were collected, and artificial seawater was prepared to keep the polyps alive for morphology and life cycle observation. Individuals prepared for the molecular tests were purified for at least 72 h in sterilized artificial seawater.

### Culturing and Morphological Description

The hydroids of *Cladonema* were kept in covered glass vessels (diameter: 125 mm) with a polyethylene Petri dish (diameter: 90 mm) placed at the bottom for hydroids to attach to. The culture conditions were maintained at room temperature (20–22°C) and 30–35 ppt salinity. The polyps were fed twice daily with *Artemia* sp. nauplii, and half of the seawater was changed after feeding to maintain a clean culture environment. Newly released medusae were collected and fed to maturity under the same culture conditions for morphological analysis. Before feeding, the hydroids and medusae were observed under a stereomicroscope (Leica S9D, Leica Microsystems, Wetzlar, Germany) to record their development.

Thirty hydroids and newly released and mature medusae were photographed and measured under a stereomicroscope (Leica M165FC, Leica Microsystems) after an anesthetic treatment with 10% MgSO<sub>4</sub>. Several hydroids and medusae were used to determine the type, size, and distribution of their nematocysts under a microscope (Leica DM2500, Leica Microsystems), following the methods of Östman (1987).

### Photosensitivity Experiment

Newly released (1 to 2 days old), young (7–10 days old), and mature (25–30 days old) medusae were collected to test their reaction to light. The experiments were performed as follows: 50 medusae from each developmental stage were randomly collected in a culture dish. Each group was exposed to strong white light (light phase) for 30 s, then the light source was removed (dark phase) for 5 s, and the group was exposed to light (light phase) again for 5 s. The light-to-dark switch was performed four times, and the number of medusae bouncing up was recorded. For each group, three parallel control groups were used to avoid random errors. The data were analyzed using Prism 6.0. A two-way ANOVA was conducted to test the effect of the developmental stages and light-to-dark switches on the photosensitivity of medusae. A Tukey's multiple-comparisons test was conducted when the effect was significant.

### Molecular Analyses

The DNA was extracted from a pool of five medusae from a single colony using a TIANamp Marine Animals DNA Kit (TIANGEN, Beijing, China), according to the manufacturer's instructions. In total, five colonies were sampled. Before DNA extraction, the medusae were examined under a stereoscopic microscope, cultured without food, and cleaned in sterile seawater for at least 72 h. Partial mitochondrial 16S (~0.55 kb) and COI (~0.70 kb) fragments were amplified using 2×Ex Taq Mastermix (Takara Bio, Kyoto, Japan) and the primers which are listed in **Table 1**. The PCR system and program were as described by Zhou et al. (2022). The 550- and 700-bp amplicons were visualized using 2% agarose gel electrophoresis (130 V, 30 min) and purified with a DNA Purification Kit (Omega, Georgia, USA). The target fragments were ligated into the pMD-19T vector (Takara Bio) and transformed into *Escherichia coli* JM109 competent cells (Takara Bio). *E. coli* was uniformly plated on Luria–Bertani agar plates



containing 50 µl/ml ampicillin and incubated at 37°C overnight. Colonies containing target DNA fragments were selected by colony PCR using programs and primers as previously described and then sent to Sangon Biotech Co. Ltd. (Shanghai, China) for sequencing using an ABI 3730 automatic DNA sequencer with the universal M13 primer and two sequences per fragment. All samples were sequenced in both directions to ensure sequence accuracy.

The sequences were examined based on chromatogram files. Target sequences were obtained using COI and 16S primers, aligned using the NCBI Nucleotide BLAST program to confirm their accuracy and validity, and deposited in GenBank (Table 1). Pairwise genetic distances between COI and 16S fragments in Cladonematidae species were calculated using MEGA X with the Kimura-2-Parameter (K2P) model. The normality and homogeneity of the data were tested, and the Kruskal–Wallis test was used to assess differences in the genetic distances among the different levels using SPSS 16.0. The sequences of the Cladonematidae species were used to establish the phylogenetic trees using the maximum likelihood (ML, the GTR + I model for COI, and the GTR + I + R model for 16S) and neighbor-joining (NJ, based on the K2P model) method using MEGA X (Tamura et al., 2011) with 1,000 bootstrap replicates. The Corynidae species *Coryne eximia* (AY512541 and AJ878713) and *Coryne pusilla* (AY512552 and AY787874) and *Sarsia tubulosa* (GQ395327 and EU876548) were chosen as outgroups for the phylogenetic tree of 16S (Nawrocki et al., 2010), whereas *Coryne eximia* (KT981902 and KT981909) was chosen as an outgroup for the phylogenetic tree of COI.

## RESULTS

### Systematics

Phylum Cnidaria Hatschek, 1888  
 Class Hydrozoa Owen, 1843  
 Subclass Hydroidolina Collins, 2000  
 Order Anthoathecata Cornelius, 1992  
 Suborder Capitata Kühn, 1913  
 Family Cladonematidae Gegenbaur, 1857  
 Genus *Cladonema* Dujardin, 1843  
*Cladonema digitatum* sp. nov.

### Material Examined

Holotype: MJU-HYD-6, male medusa, diameter: 2.92 mm, height: 2.56 mm. Paratypes: MJU-HYD-6, 30 mature medusae

(19 male and 11 female individuals), diameter: 2.22–3.75 mm, height: 1.62–3.17 mm; MJU-HYD-7, 30 newly released medusae, with a diameter of 0.67–1.08 mm and a height of 0.60–0.98 mm; MJU-HYD-8, polyps with trophosomes; MJU-HYD-9, polyps with immature medusa buds.

All specimens were sampled from individuals kept in the laboratory in October 2021 and were deposited at the Institute of Oceanography, College of Geography and Oceanography, Minjiang University, Fuzhou, China.

### Diagnosis

**Polyp:** Hydranth with one oral whorl of 3–6 capitate tentacles and one aboral whorl of 2–4 filiform tentacles; medusa buds naked, one to two per polyp, born on the hydranth body between capitate and filiform tentacles

**Adult medusa:** umbrella bell-shaped with an apical projection on top; manubrium with 4–7 finger-like protuberances and able to extend beyond the umbrella margin; with gonads around the upper manubrium; mouth with 3–6 bulbous nematocyst clusters; radial canals, simply straight or some with a Y-shaped bifurcation; tentacles 7–9, each bearing 3–7 stinging branches and 3–11 adhesive branches; stinging branches growing from the main branch as side branches. The tentacle bases were bent and cylindrical, each with a black abaxial ocellus.

### Description

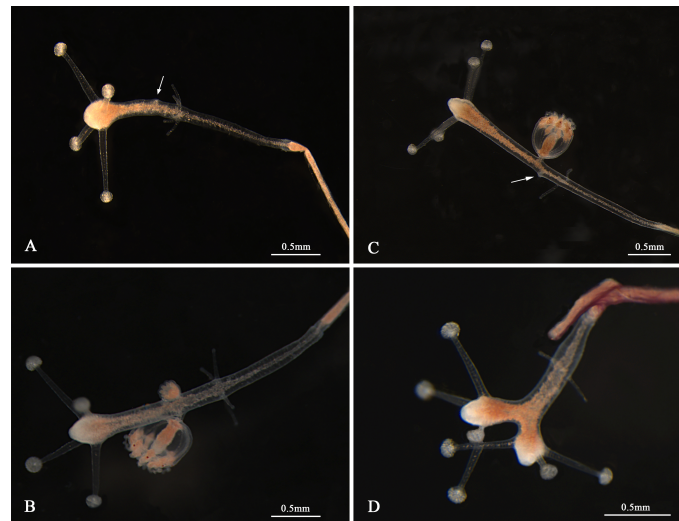
#### Hydroid

The morphological characteristics of the hydroids are shown in Figure 1 and Table 2. Hydroids are stolonial, are rarely branched (Figure 1D), and originate from ramified stolons. The hydranth was naked and fluctuant in size (1.15–3.05 mm in height and 0.16–0.25 mm in width). The hydrocaulus and stolons were covered with a smooth peri-arc. A hydranth with one oral whorl had 3–6 capitate tentacles (mainly four, 0.36–1.35 mm in length under anesthesia), with bulbous nematocyst clusters at the end. One aboral whorl of 2–4 (mainly four) slender filiform tentacles was located approximately 1/2–5/7 of the distance from the hyposome to the base. The medusa buds were bare, with 1 to 2 buds at different developmental stages per hydranth (up to three), located approximately 3/8–5/8 of the distance from the hypostome to the base (Figures 1B, C). The nematocysts can be described as follows:

a) stenoteles (10.55–25 × 6.23–14.83) µm

**TABLE 1 |** PCR primers and GenBank accession numbers of the sequences used in this study.

Gene	Primer name	Sequence (5' to 3')	Reference	GenBank accession numbers
16S	16S-L	GACTGTTTACCAAAAACATA	Ender and Schierwater, 2003	OK571354–OK571363
	16S-H	CATAATTCAACATCGAGG		
COI	LCO-1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994	OK562209–OK562218
	HCO-2198	TAACTTCAGGGTGACCAAAAATCA		



**FIGURE 1** | Hydroids of *Cladonema digitatum* sp. nov. **(A)** Hydranth. **(B, C)** Polyp with medusa buds. **(D)** Branches of a polyp. The arrows marked the early stage of medusa bud.

b) mastigophores, only in stolons ( $12.16\text{--}18.45 \times 3.12\text{--}7.57$ )  $\mu\text{m}$

### Newly Released Medusa

The morphological characteristics of the medusae are shown in **Figure 2** and **Table 3**. The newly released medusa has a bell-shaped umbrella, not fully extended, 0.67–1.08 mm in width, and 0.60–0.98 mm in height. The umbrella had a smooth surface, jelly-thin with an apical projection, and the velum was broad. The manubrium was columnar or spindle-shaped without pouches (0.11–0.21 mm in width and 0.49–0.75 mm in length, within the bell cavity). There was an immature mouth, without bulbous nematocyst clusters. Six to nine radial canals are issued directly from the manubrium, while nine radial canals reached the circular canal, simply straight or some with 1–3 Y-shaped bifurcations. The tentacle bulbs were mainly nine, one opposite each radial canal reaching the circular canal, hourglass-shaped, and with an abaxial red–brown ocellus on the tentacle base. One to three adhesive branches and stinging branches on each tentacle bulb were observed, respectively.

**TABLE 2** | Measurements [mean  $\pm$  SD (range)] of polyps of *Cladonema digitatum* sp. nov. ( $n = 30$ ).

Parts	Parameters	Results
Hydranth	Height (mm)	$2.09 \pm 0.49$ (1.15–3.05)
	Width (mm)	$0.19 \pm 0.03$ (0.16–0.25)
Oral tentacle	Type	Capitate
	Number	3–6, mainly 4
	Length (mm)	$0.66 \pm 0.16$ (0.36–1.35)
Aboral tentacles	Type	Filiform
	Number	2–4, mainly 4
Medusa buds	Position	Located about 3/8–5/8 of distance from the hypostome to the base
	Number	1–2
Hydrorhiza	Width (mm)	$0.10 \pm 0.01$ (0.07–0.12)

### Adult Medusa

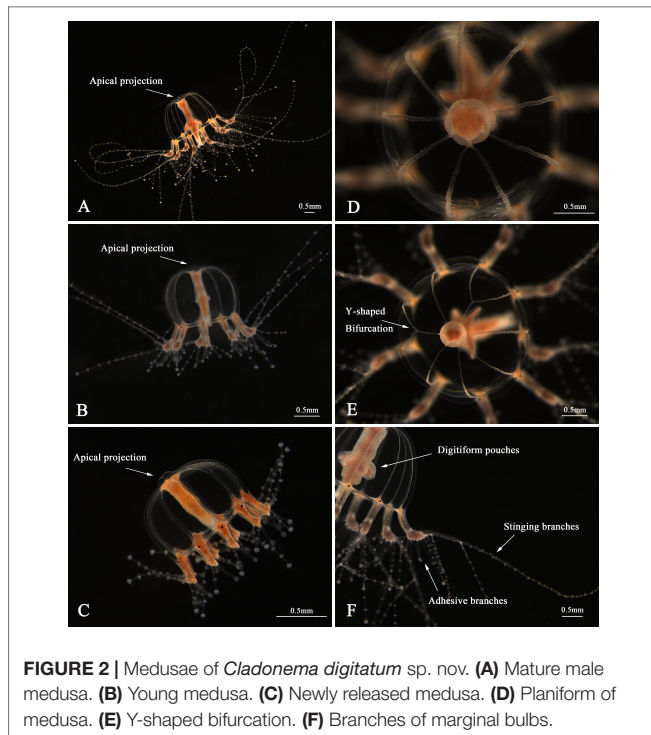
Bell-shaped umbrella, 2.22–3.75 mm in width and 1.62–3.17 mm in height, exumbrella surface smooth, jelly moderately thin with a weak apical projection (**Figure 2B**), velum broad; manubrium columnar or spindle-shaped, 0.73–1.95 mm in width and 1.79–2.89 mm in length, within bell cavity; manubrium with 4–7 (mainly six) finger-like protuberances in its middle region, with opalescent gonads around the upper 1/2–8/9 (mean 3/4). The mature female gonads were granular, whereas the male gonads were smooth; mouth with 3–6 bulbous nematocyst clusters, mainly six. Five to nine radial canals were issued directly from the manubrium, and seven to nine (mainly nine) canals reached the circular canal. The radial canals are simply straight or with 1–4 Y-shaped bifurcations of mostly two or three. The tentacle bases (7–9) are cylindrical and have one opposite radial canal. One black ocellus per bulb was positioned abaxially at the top of the tentacle base. The adhesive branches were 3–11 (mainly > 4) and the stinging branches 3–7 within each tentacle. The tentacle bases extended with an increase in the adhesive tentacles; stomach and tentacle- base orange. The medusae can swim freely but prefer to stick to bases using adhesive tentacles. The nematocysts can be described as follows:

a) stenoteles ( $6.03\text{--}22.38 \times 3.16\text{--}13.83$ )  $\mu\text{m}$ )

b) desmonemes ( $5.58\text{--}8.88 \times 3.09\text{--}4.93$ )  $\mu\text{m}$ )

### Distribution

This species was found only in *O. melastigma* aquaria in our laboratory in Fuzhou, China. *C. digitatum* may have originated from Hong Kong through the introduction of *O. melastigma* from the City University of Hong Kong or from a salt lake in Tibet along with *Artemia* sp. nauplii. Further regional distributions and seasonal variations remain unresolved.



**FIGURE 2 |** Medusae of *Cladonema digitatum* sp. nov. (A) Mature male medusa. (B) Young medusa. (C) Newly released medusa. (D) Planiform of medusa. (E) Y-shaped bifurcation. (F) Branches of marginal bulbs.

## Etymology

This species is named after the Latin term *digitatum* because of the finger-like protuberances along its manubrium, which are more obvious than those of other species in the genus *Cladonema*.

## Biological Notes

The hydroids of *C. digitatum* can be easily maintained at temperatures of 20–22°C and salinity of 30–35 ppt. Sometimes the hydranth fractured and separated from the stolon or hydrocaulus. The hydranth part can reattach to a new substrate

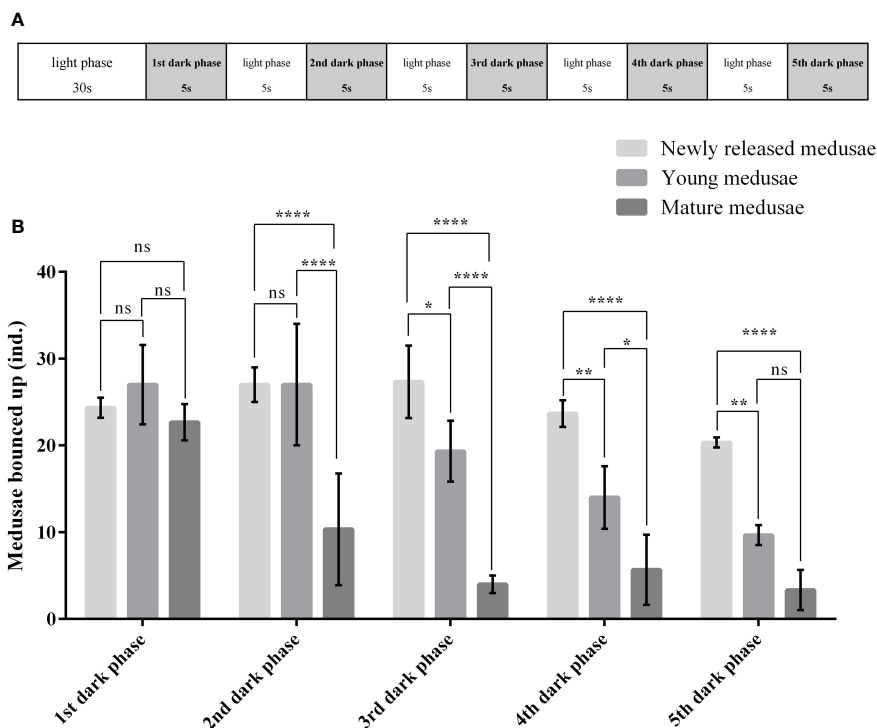
and develop into a new colony under good conditions. When fed twice a day, the hydroids grew quickly and produced abundant medusa buds within 1 month. The medusa buds took 5 or 6 days to complete their development and eventual release. The medusae matured after 20 days in the same breeding environment as the hydroids. During the developmental period of the medusae, both the stinging and adhesive branches increased with the elongation of the tentacle bases. The pouches on the manubrium gradually became apparent as the medusae developed. The adhesive branches were reduced, and the color of the manubrium became dim with the aging of the medusae.

## Photosensitivity

The responses of *C. digitatum* medusae to light are shown in the **Supplementary Video**, and the number of medusae bouncing up during each dark phase is shown in **Figure 3**. Most medusae preferred to stay at the bottom during the first 30 s of the light phase. However, some medusae bounced up when the light was shifted away (1st dark phase), and some medusae chased the moving light. The photosensitivity of medusae was influenced by developmental stages and the light-to-dark switch as well as the interaction between these two factors (**Table 4**). The newly released medusae were highly sensitive to photostimulation, and half of them bounced up when the light was removed. For the young and mature medusae, however, photosensitivity was significantly reduced from the fourth and second light-to-dark switch, respectively (**Figure 3B**; two-way ANOVA,  $F = 23.02$ ,  $P < 0.01$ ). In the first dark phase, the medusae of the three different stages showed similar responses to light changes (Tukey's multiple-comparisons test,  $P < 0.01$ ). However, as the experiment progressed, the number of young and mature medusae bouncing up became less than that of the newly released medusae. In summary, *C. digitatum* medusae had reduced photosensitivity as they grew up, and they became less sensitive to the light-to-dark switch as the number of trials increased.

**TABLE 3 |** Measurements [mean  $\pm$  SD (range)] of medusae of *Cladonema digitatum* sp. nov. ( $n = 30$ ).

Parts	Parameters	Newly released medusa	Mature medusa
Umbrella	Height (mm)	0.77 $\pm$ 0.10 (0.60–0.98)	2.56 $\pm$ 0.32 (1.62–3.17)
	Diameter (mm)	0.87 $\pm$ 0.11 (0.67–1.08)	2.92 $\pm$ 0.39 (2.22–3.75)
Apical projection	Height (mm)	0.07 $\pm$ 0.02 (0.03–0.13)	0.08 $\pm$ 0.06 (0–0.2)
	Radial canals	Number of radial canals out of manubrium	6–9
Marginal tentacles	Number of radial canals reaching circular canal	9	7–9, mainly 9
	Type	Simply straight or Y-shaped branch	Simply straight or Y-shaped branch
	Number	9	7–9, mainly 9
	Number of adhesive branches	1–3	3–11, mainly >4
Ocelli	Number of stinging branches	1–3, mainly 2	3–7
	Location	On bulbs	On bulbs
	Number	9	7–9
Pouches	Color	Red–brown	Black
	Number	Absent	4–7
Manubrium	Length (mm)	0.61 $\pm$ 0.07 (0.49–0.75)	2.33 $\pm$ 0.27 (1.79–2.89)
	Width (mm)	0.14 $\pm$ 0.03 (0.11–0.21)	1.22 $\pm$ 0.25 (0.73–1.95)
Oral tentacle	Number	Absent	3–6
Gonad	Length (mm)	Absent	1.77 $\pm$ 0.30 (0.95–2.43)
	Color	Absent	White



**FIGURE 3** | Photosensitivity of *Cladonema digitatum* medusae at different development stages. **(A)** Design of the photosensitivity experiment. **(B)** Number of medusae bounced up during each dark phase. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001

### DNA Barcoding

Among samples of *C. digitatum* sp. nov., the genetic divergences of both the 16S (558 bp) and COI (709 bp) sequences were 0–0.009 and 0–0.004, respectively. For the 16S rRNA, the pairwise intra-species K2P genetic distances (0–0.053) did not overlap with intra-genus distances in the genera *Cladonema* (0.087–0.187), *Staurocladia* (0.108–0.216), and *Eleutheria* (0.227–0.236), as shown in **Table 5**, indicating an obvious “barcoding gap” (**Table 5**; Kruskal–Wallis test, *p* < 0.01). This result was consistent with that of the phylogenetic tree. In Cladonematidae, *C. digitatum* sp. nov. displayed the least genetic divergence from *Cladonema radiatum* [0.133 ± 0.009 (0.117–0.145)], the second closest species was *Cladonema* sp. [MT709261:0.143 ± 0.002 (0.140–0.147)], the third closest species was *C. multiramosum* [0.153 ± 0.003 (0.150–0.161)], and the most distant species was *Staurocladia* sp. [MT709274:0.263 ± 0.002 (0.259–0.268)]. Without *Cladonema* sp. (AM088484), the intra-species distances [0.003 ± 0.003 (0–0.009)] were smaller than

the intra-genus distances [0.145 ± 0.032 (0.087–0.236), Kruskal–Wallis test, *p* < 0.01], which were also smaller than the intra-family distances [0.212 ± 0.046 (0.093–0.340), Kruskal–Wallis test, *p* < 0.01] in Cladonematidae. *Cladonema* sp. (AM088484) showed large genetic divergences with other Cladonematidae species [0.254 ± 0.016 (0.224–0.277)], which were larger than the intra-family distances mentioned above (Kruskal–Wallis test, *p* < 0.01).

The pairwise intraspecies K2P genetic distances for COI are listed in **Table 6**. *C. digitatum* sp. nov. showed the closest genetic distance to *Cladonema* sp. (KC706693, 0.036). Except *Cladonema* sp. (KC706693), the intra-species K2P genetic distances (0–0.004) did not intersect with the intra-genus distances in the genera *Cladonema* (0.168–0.241) and *Staurocladia* (0.128–0.153), suggesting an obvious “barcoding gap”. *C. digitatum* sp. nov. presented slightly larger distances with other *Cladonema* species [0.205 ± 0.010 (0.195–0.241)] than those with *Staurocladia* species [0.183 ± 0.002 (0.180–0.187); Kruskal–Wallis test, *p* < 0.01]. The intra-genus distances in the genus *Cladonema* [0.209 ± 0.014 (0.168–0.241)] were also larger than the intra-family distances [0.176 ± 0.017 (0.137–0.211), Kruskal–Wallis test, *p* < 0.01] in Cladonematidae.

The NJ and ML topologies of Cladonematidae based on both 16S rRNA and COI revealed an independent clade of *C. digitatum* sp. nov. with strong support in the genus *Cladonema* (**Figures 4, 5**). The genus *Cladonema* was recovered as monophyletic based on the 16S phylogenetic tree but was polyphyletic based on the COI phylogenetic

**TABLE 4** | Two-way ANOVA for different light to dark switches and development stages on the photosensitivity of medusae.

Source of variation	SS	df	MS	F	P-value
Light-to-dark switch	1,055	4	263.8	23.02	<0.0001
Development stage	1,828	2	913.8	51.85	0.0002
Interaction	530	8	66.26	5.784	0.0004
Residual	274.9	24	11.46		



**TABLE 5** | Pairwise Kimura 2 parameter genetic distances [mean  $\pm$  SD (range)] between species in the family Cladonematidae based on mitochondrial 16S rDNA.

	<i>Staurocladia wellingtoni</i>	<i>Staurocladia vallentini</i>	<i>Staurocladia oahuensis</i>	<i>Staurocladia bilateralis</i>	<i>Staurocladia sp. (MT70-9274)</i>	<i>Eleutheria clapa-redii</i>	<i>Eleutheria dichotoma</i>	<i>Cladonema pacificum</i>	<i>Cladonema radiatum</i>	<i>Cladonema sp. (AM0-88484)</i>	<i>Cladonema sp. (MT70-9261)</i>	<i>Cladonema multiramum</i>	<i>Cladonema digitatum sp.nov.</i>
<i>Staurocladia wellingtoni</i>	<b>0</b>												
<i>Staurocladia vallentini</i>	0.203 $\pm$ 0.008 (0.195–0.211)	<b>0.037</b>											
<i>Staurocladia oahuensis</i>	0.197	0.113 $\pm$ 0.005 (0.110–0.116)	-										
<i>Staurocladia bilateralis</i>	0.216 $\pm$ 0.001 (0.215–0.216)	0.118 $\pm$ 0.014 (0.108–0.128)	0.160	-									
<i>Staurocladia sp. (MT709274)</i>	0.199 $\pm$ 0.001 (0.199–0.200)	0.123 $\pm$ 0.013 (0.114–0.132)	0.158	0.135	-								
<i>Eleutheria claparedii</i>	0.193	0.224 $\pm$ 0.009 (0.218–0.231)	0.215	0.231	0.246	-							
<i>Eleutheria dichotoma</i>	0.201 $\pm$ 0.004 (0.195–0.206)	0.110 $\pm$ 0.012 (0.093–0.127)	0.136 $\pm$ 0.008 (0.130–0.145)	0.108 $\pm$ 0.007 (0.100–0.115)	0.125 $\pm$ 0.004 (0.119–0.128)	0.232 $\pm$ 0.004 (0.227–0.236)	<b>0.030 <math>\pm</math> 0.023 (0–0.053)</b>						
<i>Cladonema pacificum</i>	0.158 $\pm$ 0.001 (0.157–0.159)	0.219 $\pm$ 0.016 (0.203–0.239)	0.220 $\pm$ 0.007 (0.215–0.225)	0.258	0.219 $\pm$ 0.006 (0.215–0.223)	0.190 $\pm$ 0.004 (0.187–0.193)	0.241 $\pm$ 0.004 (0.234–0.245)	<b>0.002</b>					
<i>Cladonema radiatum</i>	0.133 $\pm$ 0.007 (0.127–0.144)	0.198 $\pm$ 0.009 (0.185–0.211)	0.184 $\pm$ 0.009 (0.176–0.196)	0.215 $\pm$ 0.008 (0.204–0.222)	0.207 $\pm$ 0.006 (0.201–0.216)	0.198 $\pm$ 0.012 (0.184–0.210)	0.201 $\pm$ 0.010 (0.188–0.214)	0.120 $\pm$ 0.006 (0.112–0.129)	<b>0.019 <math>\pm</math> 0.015 (0–0.033)</b>				
<i>Cladonema sp. (AM088484)</i>	0.239 $\pm$ 0.001 (0.239–0.240)	0.287 $\pm$ 0.004 (0.284–0.289)	0.283	0.340	0.299	0.272	0.315 $\pm$ 0.003 (0.312–0.320)	0.229 $\pm$ 0.007 (0.224–0.233)	0.267 $\pm$ 0.005 (0.261–0.274)	-			
<i>Cladonema sp. (MT709261)</i>	0.133	0.185 $\pm$ 0.005 (0.181–0.188)	0.195	0.199	0.197	0.205	0.191 $\pm$ 0.005 (0.184–0.196)	0.143 $\pm$ 0.004 (0.140–0.145)	0.104 $\pm$ 0.005 (0.100–0.111)	0.259	-		
<i>Cladonema multiramum</i>	0.174 $\pm$ 0.001 (0.173–0.177)	0.193 $\pm$ 0.002 (0.191–0.197)	0.209 $\pm$ 0.002 (0.206–0.211)	0.215 $\pm$ 0.001 (0.214–0.217)	0.213 $\pm$ 0.001 (0.212–0.215)	0.217	0.228 $\pm$ 0.013 (0.214–0.240)	0.142 $\pm$ 0.003 (0.138–0.147)	0.104 $\pm$ 0.004 (0.009–0.110)	0.275 $\pm$ 0.002 (0.274–0.277)	0.088 $\pm$ 0.001 (0.087–0.089)	<b>0.002 <math>\pm</math> 0.001 (0–0.004)</b>	
<i>Cladonema digitatum sp. nov.</i>	0.179 $\pm$ 0.002 (0.175–0.183)	0.254 $\pm$ 0.004 (0.248–0.261)	0.254 $\pm$ 0.002 (0.251–0.259)	0.253 $\pm$ 0.002 (0.250–0.258)	0.263 $\pm$ 0.002 (0.259–0.268)	0.235 $\pm$ 0.002 (0.232–0.240)	0.261 $\pm$ 0.003 (0.254–0.269)	0.183 $\pm$ 0.001 (0.180–0.187)	0.133 $\pm$ 0.009 (0.117–0.145)	0.243 $\pm$ 0.002 (0.240–0.248)	0.143 $\pm$ 0.002 (0.140–0.147)	0.153 $\pm$ 0.003 (0.150–0.161)	<b>0.003 <math>\pm</math> 0.003 (0–0.009)</b>

Bold values is highlighted intra-species relationships.

**TABLE 6 |** Pairwise Kimura 2 parameter genetic distances [mean  $\pm$  SD (range)] between species in the family Cladonematidae based on mitochondrial cytochrome oxidase.

	<i>Staurocladia wellingtoni</i>	<i>Staurocladia vallentini</i>	<i>Staurocladia charcoti</i>	<i>Cladonema californicum</i>	<i>Cladonema pacificum</i>	<i>Cladonema radiatum</i>	<i>Cladonema sp. (KC706693)</i>	<i>Cladonema multiramum</i>	<i>Cladonema digitatum sp. nov.</i>
<i>Staurocladia wellingtoni</i>	-								
<i>Staurocladia vallentini</i>	0.153	-							
<i>Staurocladia charcoti</i>	0.129 $\pm$ 0.002 (0.128–0.130)	0.144 $\pm$ 0.002 (0.143–0.146)	-						
<i>Cladonema californicum</i>	0.174 $\pm$ 0.001 (0.174–0.177)	0.172 $\pm$ 0.001 (0.172–0.176)	0.138 $\pm$ 0.002 (0.137–0.141)	0.001 $\pm$ 0.001 (0–0.003)					
<i>Cladonema pacificum</i>	0.165	0.181	0.158	0.169 $\pm$ 0.001 (0.168–0.172)	-				
<i>Cladonema radiatum</i>	0.185	0.185	0.178 $\pm$ 0.000 (0.178–0.179)	0.178 $\pm$ 0.001 (0.178–0.182)	0.194	-			
<i>Cladonema sp. (KC706693)</i>	0.209	0.210	0.192	0.197	0.230	0.192	-		
<i>Cladonema multiramum</i>	0.171	0.177 $\pm$ 0.001 (0.177–0.178)	0.195 $\pm$ 0.001 (0.193–0.196)	0.188 $\pm$ 0.002 (0.188–0.182)	0.208 $\pm$ 0.002 (0.207–0.211)	0.221 $\pm$ 0.002 (0.220–0.224)	0.201 $\pm$ 0.002 (0.201–0.205)	<b>0.001 <math>\pm</math> 0.001 (0–0.003)</b>	
<i>Cladonema digitatum sp. nov.</i>	0.180 $\pm$ 0.001 (0.180–0.182)	0.184 $\pm$ 0.001 (0.184–0.186)	0.184 $\pm$ 0.002 (0.182–0.187)	0.204 $\pm$ 0.002 (0.203–0.210)	0.239 $\pm$ 0.001 (0.237–0.241)	0.196 $\pm$ 0.001 (0.195–0.197)	0.036	0.198 $\pm$ 0.001 (0.197–0.203)	<b>0.001 <math>\pm</math> 0.001 (0–0.004)</b>

*Bold values is highlighted intra-species relationships.*

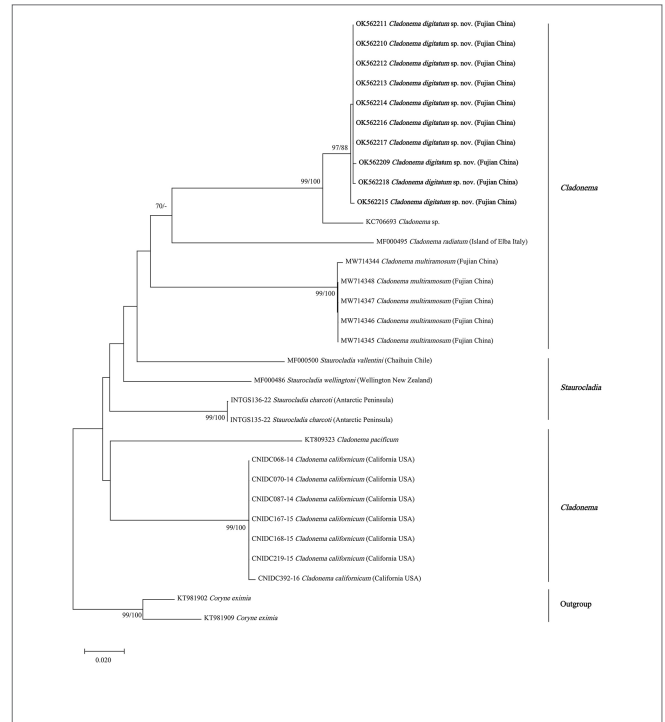
tree with weak support. In the 16S phylogenetic tree, the genera *Eleutheria* and *Staurocladia* were polyphyletic, and the *Eleutheria dichotoma* clade was nested within the *Staurocladia* clade with weak support.

### DISCUSSION

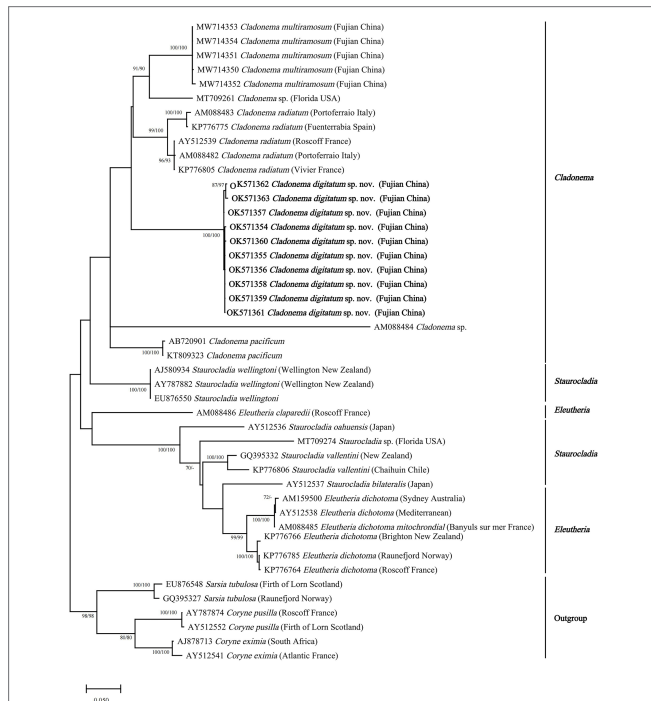
The medusa of *C. digitatum* sp. nov. is distinguishable from other nominal *Cladonema* species in having a combination of characteristics: a manubrium with finger-like protuberances, radial canals with Y-shaped bifurcations, tentacles with 3–11 adhesive branches, and 3–7 stinging branches growing from the main branch as side branches. The validity of *C. digitatum* sp. nov. was confirmed by both morphological and molecular analyses.

### Morphological Differences Among *Cladonema* Species

The morphological characteristics of *C. digitatum* sp. nov. were compared to those of other nominal *Cladonema* species (Tables 7–9). The hydroid of *C. digitatum* sp. nov. differs from those of *C. pacificum* and *C. myersi*, which lack filiform tentacles (Rees, 1949; Hirai, 1958; Takeda et al., 2018), whereas it is similar to those of *C. californicum*, *C. radiatum*, and *C. multiramisum*, which possess an oral whorl of filiform



**FIGURE 5 |** Neighbor-joining clustering of Cladonematidae based on mitochondrial cytochrome oxidase (COI). Bootstrap values of 1,000 pseudoreplicates higher than 70% were shown above the branches as node-support values. The first number along the branches refers to the maximum likelihood bootstrap values, while the second number refers to the neighbor-joining bootstrap values.



**FIGURE 4 |** Neighbor-joining clustering of Cladonematidae based on mitochondrial 16S rRNA. Bootstrap values of 1,000 pseudoreplicates higher than 70% were shown above the branches as node-support values. The first number along the branches refers to the maximum likelihood bootstrap values, while the second number refers to the neighbor-joining bootstrap values.

tentacles (Rees, 1979; Schuchert, 2006; Zhou et al., 2022). *C. digitatum* sp. nov. has fewer medusa buds per polyp (1 to 2, mainly one) than *C. californicum* (2 to 3) and *C. multiramisum* (1–5) (Rees, 1979; Zhou et al., 2022). In addition, the medusa buds of *C. digitatum* sp. nov. appeared dispersed on the hydranth, whereas those of *C. californicum* were arranged in a single whorl (Rees, 1979). These findings confirm the validity of the diagnostic characteristics of *Cladonema* polyps, such as filiform tentacles, and the number and arrangement of medusa buds (Zhou et al., 2022).

Compared with other *Cladonema* medusae, the main distinct features of *C. digitatum* sp. nov. are Y-shaped bifurcations in the upper radial canals, stinging branches growing from the main branch as side branches, and finger-like protuberances along the middle region of the manubrium (Table 8). Most *C. digitatum* sp. nov. have two or three Y-shaped bifurcations, more than those of *C. novaezelandiae* medusae, another species with a Y-shaped bifurcation in the genus *Cladonema* (Ralph, 1953). The other distinctive feature of *C. digitatum* sp. nov. and *C. novaezelandiae* is the arrangement of their stinging branches, where the stinging branches of *C. digitatum* sp. nov. grow from the main branch as side branches (Figure 2F), whereas those of *C. novaezelandiae* all emit directly from the tentacle bulb (Ralph, 1953). This is also the case for *C. californicum* (Hyman, 1947) and *C. timmsii* (Gershwin and Zeidler, 2008). Except for *C. pacificum*, female *C. timmsii*, and *C. myersi*, the other *Cladonema* medusae have gastric pouches

**TABLE 7** | Comparison of the morphology of polyps in the genus *Cladonema*.

Species	Capitate tentacles	Filiform tentacles	Medusae buds	Reference
<i>Cladonema californicum</i>	4	4 to 5	2 to 3, in a single whorl between capitate and filiform tentacles	Rees, 1979
<i>Cladonema radiatum</i>	4 to 5	4 to 5	1, above filiform tentacles	Schuchert, 2006
<i>Cladonema myersi</i>	4	None	1, below capitate tentacles	Rees, 1949
<i>Cladonema pacificum</i>	4 to 5	None	1, below capitate tentacles	Hirai, 1958; Rees, 1982; Takeda et al., 2018
<i>Cladonema novaezelandiae</i>	Not described	Not described	Not described	Ralph, 1953
<i>Cladonema timmsii</i>	Not described	Not described	Not described	Gershwin and Zeidler, 2008
<i>Cladonema multiramosum</i>	4–6, rarely 10	4–6, rarely 10	1–5, between capitate and filiform tentacles	Zhou et al., 2022
<i>Cladonema digitatum</i> sp. nov.	3–6, mainly 4	2–4	1 to 2, located about 3/8–5/8 of distance from the hypostome to the base	This study

(Table 8), and *C. digitatum* sp. nov. medusae possess the most obvious finger-like protuberances.

Most morphological differences among nominal *Cladonema* medusae vary (Schuchert, 2006; Zhou et al., 2022). Owing to the overlap in the numbers of radial canals, tentacles, adhesive and stinging branches per tentacle, pouches in the manubrium, and oral tentacles (Table 8), *C. digitatum* sp. nov. medusae could not be easily distinguished from other *Cladonema* medusae, except *C. californicum*, which has only one adhesive and one to two stinging branches per tentacle (Hirai, 1958; Rees, 1979). *C. digitatum* sp. nov. medusae have more adhesive branches (3–11, mainly > 4) than most *Cladonema* medusae, next only to *C. multiramosum* (Zhou et al., 2022). In addition, *C. digitatum* sp. nov. medusae have a flexible manubrium, and some lack an apical projection, reflecting the morphological plasticity of the medusae. Thus, the diagnostic criteria for mature *Cladonema* medusae should be a combination of characteristics, including the number of adhesive branches, stinging branches, tentacles, radial canals, the branch patterns of stinging branches and radial canals, and the presence of manubrium pouches.

However, the newly released medusae of *C. digitatum* sp. nov. differ from those of *C. pacificum* (Hirai, 1958), *C. californicum* (Rees, 1979), *C. radiatum* (Schuchert, 1996), *C. myersi* (Rees, 1949), and *C. multiramosum* (Zhou et al., 2022) in two stinging branches and Y-shaped bifurcations in the upper radial canals. The number of adhesive branches (1–3) in *C. digitatum* sp. nov. covers those of other *Cladonema* species (one or two). Moreover, with the development of *C. digitatum* sp. nov. medusae, finger-like protuberances formed on the manubrium and became increasingly obvious. Meanwhile, the number of stinging and adhesive branches increases during development, similar to other *Cladonema* medusae (Zhou et al., 2022).

As for the nematocysts, the medusae and polyps of *C. digitatum* sp. nov., *C. multiramosum*, *C. radiatum*, *C. californicum*, and *C. pacificum* all have stenoteles in variable sizes. Mastigophores only appeared in the stolons of the polyps of *C. digitatum* sp. nov., *C. multiramosum*, and *C. radiatum* and were absent in *C. californicum* and *C. pacificum* (Table 9). Desmonemes were only found in medusae, but in small numbers. Although the morphological features of nematocysts are taxonomically useful for many groups

of hydromedusae (Östman, 1979; Östman, 1982; Östman, 1987), they are not recommended for species identification in the genus *Cladonema* because of the polytropic size of nematocysts and time-consuming observation work (Rees, 1979; Rees, 1982; Zhou et al., 2022).

Except for two species without a description of the hydroid stage, *Cladonema* species could be divided into two groups based on the presence of filiform tentacles in hydroids and gastric pouches in medusae: *C. radiatum*-like medusae, including *C. radiatum*, *C. californicum*, *C. multiramosum*, and *C. digitatum* sp. nov. and *C. pacificum*-like medusae, including *C. myersi* and *C. pacificum*. Based on the version of Zhou et al. (2022), we added information on *C. digitatum* sp. nov. and updated the taxonomy of *Cladonema* based on the morphological characteristics of the mature medusae.

### Key to the accepted nominal species of the genus *Cladonema*:

- 1a With 7, rarely 5–6 marginal tentacles ..... *C. myersi*
- 1b With 8–11 marginal tentacles ..... 2
- 2a Stinging branches all emitting directly from the tentacle bulb ..... 3
- 2b Stinging branches growing from the main branch, in turn, as side branches ..... 5
- 3a Most radial canals straight and one-branched ..... *C. novaezelandiae*
- 3b Radial canals straight ..... 4
- 4a Tentacles with one adhesive branch and 1–2 stinging branches ..... *C. californicum*
- 4b Tentacles with 5–7 adhesive branches and 6–8 stinging branches ..... *C. timmsii*
- 5a Manubrium without pouches ..... *C. pacificum*
- 5b Manubrium with pouches ..... 6
- 6a Tentacles with 1–4 adhesive branches ..... *C. radiatum*
- 6b Tentacles with over four adhesive branches ..... 7
- 7a Tentacles with 8–24 adhesive branches (rarely 5–7), radial canals without Y-shaped bifurcation on the upper part ..... *C. multiramosum*
- 7b Tentacles with 3–11 adhesive branches (mainly >4), radial canals with 1–4 Y-shaped bifurcations in the upper part ..... *C. digitatum* sp. nov.



**TABLE 8** | Comparison of the morphology of mature medusae in the genus *Cladonema*.

Species	Synonym	Umbrella			Radial canals		Tentacles		Ocelli		Manubrium	Gonads	Oral tentacles	Locality	Reference
		BH	BD	AP	N	BP	Number	AB	SB						
<i>Cladonema radiatum</i> Dujardin, 1843	–	2–4.5	2–3	+/-	7–11	Straight or bifurcating near origin	8–10	1–4	3–10; AM I	1; red–brown, black, dark red	Not extending beyond bell margin	4–6 gastric pouches; encircling upper 2/3 of the manubrium	4–5	North-Eastern Atlantic; Mediterranean; Black Sea; coast of Brazil, Bermuda, New Zealand, Japan; northern Arabian Sea, Pakistan; Southeast coast of India	Prasad et al., 1961; Schuchert, 1996; 2006; Bouillon and Boero, 2000; Ghory et al., 2020; Farias et al., 2020; Ranjith et al., 2021
	<i>Cladonema allmani</i> Haeckel, 1879	2–4	2–3	ND	10	ND	10	ND	ND	ND	ND	5 pouches	5	European coasts	Haeckel, 1879; Gershwin and Zeidler, 2008
	<i>Cladonema dujardinii</i> Haeckel, 1879	2–4	2–3	ND	8	ND	8	ND	ND	ND	ND	5 pouches	5	European coasts	Haeckel, 1879; Gershwin and Zeidler, 2008
	<i>Cladonema gegenbaui</i> Haeckel, 1879	2–4	2–3	ND	8	ND	8	ND	ND	ND	ND	4 pouches	4	European coasts	Haeckel, 1879; Gershwin and Zeidler, 2008
	<i>Cladonema krohnii</i> Haeckel, 1879	2–4	2–3	ND	10	ND	10	ND	ND	ND	ND	4 pouches	4	European coasts	Haeckel, 1879; Gershwin and Zeidler, 2008
	<i>Cladonema mayeri</i> Perkins, 1906	2.5	ND	ND	9	Straight or bifurcating near origin	9	4	5–8	Red–brown	ND	6 pouches	6	Tortugas Florida, USA	Perkins, 1908; Gershwin and Zeidler, 2008
	<i>Cladonema perkinsii</i> Mayer, 1904	ND	Less than 2	ND	8	Straight, simple	8	ND	ND	Black	ND	Around the whole manubrium	5	Nassau Harbour, Bahamas	Gershwin and Zeidler, 2008
<i>Cladonema californicum</i> Hyman, 1947	–	Short than diameter	2–3	–	9, rarely 11	Unbranched	9–11	1	1–2; AM II	1; elongated; red	Beyond bell margin	6, rarely 7, elongated rounded protrusions	6	Northern California, USA	Hyman, 1947; Rees, 1979
<i>Cladonema myersi</i> Rees, 1949	–	ND	ND	–	7, rarely 5–6	Unbranched	7	3	7; AM I	1; reddish	Half of bell cavity	ND	6	Southern California, USA	Rees, 1949
<i>Cladonema novaezelandiae</i> Ralph, 1953	–	ND	1.5	ND	7–8	Most straight and 1 branched	9	≤7	≤10; AM II	1	ND	6 pouches	6	Wellington, New Zealand	Ralph, 1953; Schuchert, 1996; Gershwin and Zeidler, 2008

TABLE 8 | (Continued)

Species	Synonym	Umbrella			Radial canals		Tentacles			Ocelli		Manubrium	Gonads	Oral tentacles	Locality	Reference
		BH	BD	AP	N	BP	Number	AB	SB							
<i>Cladonema pacificum</i> Naumov, 1955	<i>Cladonema pacifica</i> Naumov, 1955	2	2.2	+	9	Straight or bifurcating near origin	9	6–8 branches in total; AM I		1; black	Extending beyond bell margin	Around the whole manubrium	6	Saghalin Island and Tohoku region, Japan	Gershwin and Zeidler, 2008; Takeda et al., 2018	
	<i>Cladonema uchidai</i> Hirai, 1958	Up to 3.5	ND	ND	6	Straight, bifurcating near origin	8–9	2–4	4–6; AM I	1; deep purple, nearly black	Extending beyond bell margin	Around the whole manubrium	6	Asamushi, Japan; San Francisco, USA; Coastal China and the USSR	Hirai, 1958; Rees, 1982	
<i>Cladonema timmsii</i> Gershwin and Zeidler, 2008	–	2	2	–	9	Unbranched	9	5–7	6–8; AM II	1; dark red	Not extending beyond bell margin	Female: encircling upper half of the manubrium, greatly swollen, without pouches; male: 6 radially arranged pouches	6	Penong, South Australia	Gershwin and Zeidler, 2008	
<i>Cladonema multiramsum</i> Zhou et al., 2022	–	1.36–1.95	1.57–2.26	+	8–11	Straight or bifurcating near origin	8–11	8–24, rarely 5–7	3–6; AM I	1, rarely 2; black	Extending beyond bell margin or not	5–8 gastric pouches, around upper 1/2–1 of the manubrium	5–8	Fuzhou, China	Zhou et al. 2022	
<i>Cladonema digitatum</i> sp. nov.	–	1.62–3.17	2.22–3.75	+/-	7–9, mainly 9	Straight, bifurcating near origin, or 1–4 Y-shaped bifurcations	7–9, mainly 9	3–11, mainly >4	3–7; AM I	1; black	Extending beyond bell margin	4–7 finger-like pouches; gonads around upper 1/2–8/9 of the manubrium	3–6, mainly 5 or 6	Fuzhou, China	This study	

BH, bell height; BD, bell diameter; AP, apical projection; N, number; BP, branch pattern; AB, adhesive branches; SB, stinging branches; ND, not described; +, present; –, absent; AM I, the arrangement mode of stinging branches that grow from the main branch, in turn, as side branches; AM II, the arrangement mode of stinging branches that are all emitting directly from the tentacle bulb.

**TABLE 9** | Nematocysts of *Cladonema* species.

Stage	Type	<i>Cladonema digitatum</i> sp. nov.	<i>Cladonema multiramosum</i>	<i>Cladonema radiatum</i>	<i>Cladonema californicum</i>	<i>Cladonema pacificum</i>
Polyp	Stenoteles	10.55–25 × 6.23–14.83	10.2–18.6 × 6.5–10.9	11–17 × 8–10	14–18.5 × 10–12 28 × 18	16.5–20 × 11.5–12 13–14 × 8
	Mastigophores	12.16–18.45 × 3.12–7.57	11.1–14.3 × 3.3–4.7	10–12 × 3.5–4	Absent	Absent
Medusa	Stenoteles	6.03–22.38 × 3.16–13.83	7.5–18.5 × 4.6–11.9	13–16 × 9–10 9.5–11 × 5–8.5	21–23 × 14–16 14–18 × 9.5–11	20.5–23 × 13.5–14 11.5–14 × 7–8
	Desmonemes	5.58–8.88 × 3.09–4.93	6.2–9.0 × 3.3–5.1	9–12 × 3.5–5	9–11 × 4.5–5	9–10 × 4
Reference		This study	Zhou et al., 2022	Schuchert, 1996	Rees, 1979	Rees, 1982

## Molecular Phylogenetic Analysis

The phylogenetic hypotheses based on 16S rRNA and COI sequences and within-species genetic diversity analyses supported *C. digitatum* sp. nov. as a distinct species. *C. digitatum* sp. nov. and *Cladonema* sp. (KC706693) shared the closest genetic distance (0.036) within the reported intra-species distance range for COI in hydrozoans (0–0.076) (Ortman et al., 2010; Zheng et al., 2014; Liu et al., 2018) and formed a robust clade in the COI phylogenetic tree (Figure 5). A short COI fragment (313 bp) of *Cladonema* sp. (KC706693) was found in the fish gut content of Moorea Island, French Polynesia (Leray et al., 2013), which might be a geographic population of *C. digitatum* sp. nov. The genetic distances between *C. digitatum* sp. nov. and other *Cladonema* species are almost within the reported intra-genus distances—for example, 0.062–0.236 for 16S and 0.045–0.243 for COI (Zheng et al., 2014).

With the exception of *Cladonema* sp., *C. radiatum*-like species (*C. digitatum* sp. nov., *C. radiatum*, and *C. multiramosum*) clustered together and were presented as a sister group of *C. pacificum* in both the 16S and COI phylogenetic trees (Figures 4, 5). Unlike the report by Zhou et al. (2022), *Cladonema* species formed a clade with weak support in the 16S phylogenetic tree of Cladonematidae after adding the data of *C. digitatum* sp. nov. (Figure 4). However, *Cladonema* was recovered as polyphyletic based on the COI phylogenetic tree with weak support, which might be attributed to data deficiency. Currently, the 16S sequences of only 10 valid species and the COI sequences of only eight valid species have been reported in Cladonematidae, accounting for approximately half of the valid species in this family (Schuchert, 2022). In addition, the 16S rRNA and COI data of *C. myersi*, *C. novazelandiae*, and *C. timmisii* have not been reported. Although the mature medusae of *C. digitatum* sp. nov. can be distinguished from *C. myersi*, *C. novazelandiae*, and *C. timmisii* in terms of morphological characteristics, synonyms might still exist among them owing to phenotypic plasticity. Therefore, further sequences are required to revise the phylogenetic tree in the future.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

## AUTHOR CONTRIBUTIONS

XF, SL, and YZ conducted the experiments. XF and KZ completed the manuscript. KZ, JC and ZW revised the manuscript and provided the funds and resource for this research. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the National Natural Science Foundation of China (no. 41806181), the Natural Science Foundation of Fujian Province of China (no. 2021J011042), the Program for Innovative Research Team in Science and Technology in Fujian Province University, and The Talent Introduction Pre-research Project of Minjiang University (no. MJY20017).

## ACKNOWLEDGMENTS

We thank the editors and the reviewers for their comments and suggestions on this manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.891998/full#supplementary-material>

## REFERENCES

- 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. doi: 10.1285/i15910725v24p47
- Bucklin, A., Hopcroft, R. R., Kosobokova, K. N., Nigro, L. M., Ortman, B. D., Jennings, R. M., et al. (2010a). DNA Barcoding of Arctic Ocean Holozooplankton for Species Identification and Recognition. *Deep Sea Res. Part II: Topical Stud. Oceanogr.* 57 (1), 40–48. doi: 10.1016/j.dsr2.2009.08.005
- Bucklin, A., Ortman, B. D., Jennings, R. M., Nigro, L. M., and Wiebe, P. H. (2010b). A “Rosetta Stone” for Metazoan Zooplankton: DNA Barcode Analysis of Species Diversity of the Sargasso Sea (Northwest Atlantic Ocean). *Deep Sea Res. Part II: Topical Stud. Oceanogr.* 57 (24), 2234–2247. doi: 10.1016/j.dsr2.2010.09.025
- Cedeno-Posso, C. (2014). First Record of the Genus *Cladonema* (Medusae and Polyps) in Colombia. *Zootaxa*. 3793 (5), 597–599. doi: 10.11646/zootaxa.3793.5.8
- Chou, T.-H. and Huang, M.-C. (1958). A Study on Hydromedusae of Chefoo. *Acta Zoologica Sin.* 10 (2), 173–191.
- Colin, S. P., Costello, J. H. and Klos, E. (2003). *In Situ* Swimming and Feeding Behavior of Eight Co-Occurring Hydromedusae. *Mar. Ecol. Prog. Series*. 253, 305–309. doi: 10.3354/meps253305
- Ender, A. and Schierwater, B. (2003). Placozoa Are Not Derived Cnidarians: Evidence From Molecular Morphology. *Mol. Biol. evolution*. 20 (1), 130–134. doi: 10.1093/molbev/msg018
- Farias, G. B., Leitão, S. N., Melo, P. A. M. D. C., Júnior, M. N., and Tosetto, E. G. (2020). First *In Situ* Record of the Medusa Stage of *Cladonema Radiatum* (Cnidaria: Anthoathecata) in the South Atlantic Ocean. *Ocean Coast. Res.* 68, e20349. doi: 10.1590/s2675-28242020068349
- Folmer, O., Black, M. B., Hoeh, W. R., et al. (1994). DNA Primers for Amplification of Mitochondrial Cytochrome C Oxidase Subunit I From Diverse Metazoan Invertebrates. *Mol. Mar. Biol. Biotechnol.* 3 (5), 294–299. doi: 10.4028/www.scientific.net/DDE7.460
- Fujiki, A., Hou, S., Nakamoto, A., and Kumano, G. (2019). Branching Pattern and Morphogenesis of Medusa Tentacles in the Jellyfish *Cladonema Pacificum* (Hydrozoa, Cnidaria). *Zoological Letters*. 5 (1), 12. doi: 10.1186/s40851-019-0124-4
- Fujita, S., Kuranaga, E. and Nakajima, Y.-I. (2019). Cell Proliferation Controls Body Size Growth, Tentacle Morphogenesis, and Regeneration in Hydrozoan Jellyfish. *Cladonema pacificum*. *PeerJ*. 7, e7579. doi: 10.7717/peerj.7579
- Fujita, S., Kuranaga, E. and Nakajima, Y.-I. (2021). Regeneration Potential of Jellyfish: Cellular Mechanisms and Molecular Insights. *Genes*. 12 (5), 758. doi: 10.3390/genes12050758
- Gershwin, L.-A. and Zeidler, W. (2008). *Cladonema Timmsii*, a New Species of Hydromedusa (Cnidaria: Hydrozoa) From a Salt Lake in South Australia. *Zootaxa*. 1826 (1), 59–68. doi: 10.11646/zootaxa.1826.1.4
- Ghory, F. S., Ali, Q. M. and Ahmed, Q. (2020). First Record of *Cladonema Radiatum* Dujardin 1843 (Hydrozoa: Cladonematidae) From Northern Arabian Sea, Pakistan. *Int. J. Fisheries Aquat. Res.* 5 (3), 14–16. doi: 15560/11.2.1609
- Gravili, C., D’ambrosio, P., Di Camillo, C., Renna, G., Bouillon, J., and Boero, F. (2008). *Clytia Hummelincki* (Hydrozoa: Leptomedusae) in the Mediterranean Sea. *J. Mar. Biol. Assoc. United Kingdom*. 88 (8), 1547–1553. doi: 10.1017/S0025315408001975
- Graziussi, D. F., Suga, H., Schmid, V., and Gehring, W. J. (2012). The “Eyes Absent” (Eya) Gene in the Eye-Bearing Hydrozoan Jellyfish *Cladonema Radiatum*: Conservation of the Retinal Determination Network. *J. Exp. Zoology Part B: Mol. Dev. Evolution*. 318 (4), 257–267. doi: 10.1002/jez.b.22442
- Haeckel, E. (1879). Das System der Medusen. Erster Teil einer Monographie der Medusen. Denkschriften der Medicinisch-Naturwissenschaftlichen Gesellschaft zu Jena, (1): XX+1-360, 320 plates.
- He, J., Zheng, L., Zhang, W., Lin, Y., and Cao, W. (2015). Morphology and Molecular Analyses of a New *Clytia* Species (Cnidaria: Hydrozoa: Campanulariidae) From the East China Sea. *J. Mar. Biol. Assoc. United Kingdom*. 95 (2), 289–300. doi: 10.1017/S0025315414000836
- Hirai, E. (1958). “On the Species of *Cladonema radiatum* var. *mayeri* Perkins”: in *Bulletin of the Marine Biological Station of Asamushi*, (Tohoku University) 9, 23–25.
- Hyman, L. H. (1947). Two New Hydromedusae From the California Coast. *Trans. Am. Microscopical Society*. 66 (3), 262–268. doi: 10.2307/3223391
- Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., et al. (2013). A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial COI Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents. *Front. zoology*. 10, 34. doi: 10.1186/1742-9994-10-34
- 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. doi: 10.11646/zootaxa.2980.1.2
- Liu, C., Gu, Z., Xing, M., Sun, Y., Chen, S., and Chen, Z. (2018). Sex Determination and Differentiation in *Aurelia* Sp.1: The Absence of Temperature Dependence. *J. Oceanol. Limnol.* 36 (2), 457–464. doi: 10.1007/s00343-017-6170-x
- Miglietta, M. P., Rossi, M. and Collin, R. (2008). Hydromedusa Blooms and Upwelling Events in the Bay of Panama, Tropical East Pacific. *J. Plankton Res.* 30 (7), 783–793. doi: 10.1093/plankt/fbn038
- Nawrocki, A. M., Schuchert, P. and Cartwright, P. (2010). Phylogenetics and Evolution of Capitata (Cnidaria: Hydrozoa), and the Systematics of Corynidae. *Zoologica Scripta*. 39 (3), 290–304. doi: 10.1111/j.1463-6409.2009.00419.x
- Ortman, B. D., Bucklin, A., Pagès, F., and Youngbluth, M. (2010). DNA Barcoding the Medusozoa Using mtCOI. *Deep Sea Res. Part II: Topical Stud. Oceanogr.* 57 (24–26), 2148–2156. doi: 10.1016/j.dsr2.2010.09.017
- Östman, C. (1979). Two Types of Nematocysts in Campanulariidae (Cnidaria, Hydrozoa) Studied by Light and Scanning Electron Microscopy. *Zoologica Scripta*. 8 (1–4), 5–12. doi: 10.1111/j.1463-6409.1979.tb00614.x
- Östman, C. (1982). Nematocysts and Taxonomy in *Laomedea*, *Gonothyrea* and *Obelia* (Hydrozoa, Campanulariidae). *Zoologica Scripta*. 11 (4), 227–241. doi: 10.1111/j.1463-6409.1982.tb00536.x
- Östman, C. (1987). “New Techniques and Old Problems in Hydrozoan Systematics,” in *Modern Trends in the Systematics, Ecology and Evolution of Hydrozoa and Hydromedusae*. Eds. Bouillon, J., Boero, F., Cicogna, F. and Cornelius, P. F. S. (Oxford: Clarendon Press), 67–82.
- Perkins, H. F. (1908). *Notes on Medusae of the Western Atlantic*. (Carnegie Institution of Washington), 1. 135–149.
- Prasad, R., Tampi, P. and Durve, V. (1961). Note on the Occurrence of the Anthomedusa *Cladonema* in the Indian Region. *J. Mar. Biol. Assoc. India*. 3 (1 & 2), 251–252.
- Ralph, P. M. (1953). A Guide to the Athecate (Gymnoblatic) Hydrozoa and Medusae of New Zealand. *Tuatara*. 5, 59–75.
- Ranjith, L., Kalidas, C., Saravanan, R., Gagadis, I., Prabu, D. L., Kavitha, M., et al. (2021). Observation on the Morphological and Gonadal Aspects of *Cladonema Radiatum* (Class: Hydrozoa) From Tuticorin Bay, Southeast Coast of India. *Zootaxa*. 4990 (3), 591–595. doi: 10.11646/zootaxa.4990.3.12
- Rees, W. J. (1949). On *Cladonema Myersi*, a New Species of Hydroid From the Californian Coast. *Proc. Zoological Soc. London*. 119 (4), 861–865. doi: 10.1111/j.1096-3642.1950.tb00912.x
- Rees, J. T. (1979). The Polyp and Immature Medusa Stages of *Cladonema Californicum*, Hyman 1947 (Anthomedusae: Cladonemidae) With Biological Notes and a Discussion of the Taxonomy of the Genus *Cladonema*. *J. Natural History*. 13 (3), 295–302. doi: 10.1080/00222937900770231
- Rees, J. T. (1982). The Hydrozoan *Cladonema* in California: A Possible Introduction From East Asia. *Pacific Science*. 36 (4), 439–444.
- Schuchert, P. (1996). The Marine Fauna of New Zealand: Athecate Hydrozoa and Their Medusae (Cnidaria: Hydrozoa). *Oceanogr. Lit. Review*. 1 (45), 1–159.
- Schuchert, P. (2006). The European Athecate Hydrozoa and Their Medusae (Hydrozoa, Cnidaria): Capitata Part 1. *Rev. suisse Zoologie*. 113 (2), 325–410. doi: 10.5962/bhl.part.80356
- Schuchert, P. (2016). The Polyps of *Oceania Armata* Identified by DNA Barcoding (Cnidaria, Hydrozoa). *Zootaxa*. 4175 (6), 539–555. doi: 10.11646/zootaxa.4175.6.3
- Schuchert, P. (2022) *World Hydrozoa Database. Cladonema Dujardin 1843*. Available at: <http://marinespecies.org/aphia.php?p=taxdetails&id=117049>.
- Takeda, N., Deguchi, R. and Itabashi, T. (2018). “Reproductive Strategies in Marine Hydrozoan Jellyfish: Sexual Medusae and Asexual Polyps,” in *Reproductive and Developmental Strategies* (Springer), 157–174.
- 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. *Mol. Biol. evolution*. 28 (10), 2731–2739. doi: 10.1093/molbev/msr121



- Tewksbury, J. J., Anderson, J. G. T., Bakker, J. D., Billo, T. J., Dunwiddie, P. W., Groom, M. J., et al. (2014). Natural History's Place in Science and Society. *BioScience*. 64 (4), 300–310. doi: 10.1093/biosci/biu032
- Xu, Z. (1993). Revisions of Nominal Species on the Hydromedusae of China Sea Areas. *J. Oceanogr. Taiwan Strait* 12 (3), 197–204.
- Zheng, L., He, J., Lin, Y., Cao, W., and Zhang, W. (2014). 16s rRNA is a Better Choice Than COI for DNA Barcoding Hydrozoans in the Coastal Waters of China. *Acta Oceanologica Sinica*. 33 (4), 55–76. doi: 10.1007/s13131-014-0415-8
- Zhou, K., Gu, Y., Wang, L., and Chen, J. (2022). Discovery of *Cladonema Multiramsum* Sp. Nov. (Cnidaria: Hydrozoa: Cladonematidae) Using DNA Barcoding and Life Cycle Analyses. *Acta Oceanologica Sin* 41, 44–52. doi: 10.1007/s13131-021-1900-5
- Zhou, K., Zheng, L., He, J., Lin, Y., Cao, W., and Zhang, W. (2013). Detection of a New *Clytia* Species (Cnidaria: Hydrozoa: Campanulariidae) With DNA Barcoding and Life Cycle Analyses. *J. Mar. Biol. Assoc. United Kingdom*. 93 (8), 2075–2088. doi: 10.1017/S0025315413000969

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Fang, Lin, Zhang, Wang, Zhou and Chen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

