



Caltsacoryne setouchiensis (Hydrozoa, Anthoathecata) a new genus and species of hydrozoan jellyfish from Japan

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Abstract

We report a new genus and species of hydrozoan jellyfish belonging to the order Anthoathecata collected from the Seto Inland Sea, western Japan. *Caltsacoryne setouchiensis* **gen. et sp. n.** can be distinguished from other species of Corynidae based on the following combination of morphological characters: number of tentacles, cnidocyst pads, manubrium length, and the shape of the gonad and tentacles. A table comparing the primary diagnostic characters of this new genus of Corynidae is presented.

Key words: Anthomedusae, Corynidae, hydroid, medusa, Seto Inland Sea

Introduction

The hydrozoan family Corynidae Johnston, 1836 contains approximately 50 species in 13 genera (Nawrocki *et al.* 2009). Species of Corynidae are widely distributed in the shallow waters of the Atlantic, Pacific, and Indian oceans (Mayer 1910; Kramp 1959), a majority of which are characterized by an alternation of generations, with life cycles comprising asexual benthic hydroids and sexual planktonic medusae (Petersen 1990; Brinckmann-Voss 2000). Typically, free-swimming medusae are liberated by the budding of hydroids, although some corynids lack medusae and produce fixed gonophores (Schuchert 2001).

Historically, the taxonomy of the family Corynidae has been problematic due to the limited differences in morphological characters among genera. Johnston (1836) erected the family Corynidae, the first genus of which was *Coryne* Gaertner 1774. *Coryne muscoides* (Linnaeus, 1761), originally described as *Tubularia muscoides* Linnaeus, 1761, was the first identified species. Until the 1970s, the genera, *Coryne*, *Sarsia* Lesson, 1843, and *Dipurena* McCrady, 1859, were the major corynid taxa (Millard 1975). Bouillon (1985) later incorporated three pre-existing genera into the family, namely, *Bibrachium* Stechow, 1919, *Dicodonium* Haeckel, 1879, and *Sarsiella* Lesson, 1843. However, subsequent morphological analyses failed to establish any convincing synapomorphies for the family Corynidae, thereby indicating that it may be polyphyletic (Schuchert 2001). Recent molecular phylogenetic studies lend support to this hypothesis and furthermore provide evidence to indicate that the three major corynid genera *Sarsia*, *Dipurena*, and *Coryne* are polyphyletic (Collins *et al.* 2005; Nawrocki *et al.* 2010). Moreover, Nawrocki *et al.* (2010) placed the family Polyorchidae, which includes *Polyorchis* A. Agassiz, 1862 and *Scrippsia* Torrey, 1909, among the genera of Corynidae.

To date, eight species of Corynidae have been reported from Japanese waters (Table 1), and in present study, we collected seven specimens of a hitherto unidentified species of Corynidae from the Seto Inland Sea, western Japan. On the basis our morphological and molecular phylogenetic analyses of these specimens, we propose that this species of Anthoathecata should be recognized as a newly erected genus and a new species within the family Corynidae.

TABLE 1. List of Japanese Corynidae: a, Kubota 2014; b, Kubota & Gravili 2007.

Scientific name	Japanese name	Reference
<i>Cladosarsia capitata</i> Bouillon, 1978	Edawakare-sarusia-kurage	a
<i>Coryne polyocellata</i> (Uchida, 1927)	Itsutsume-sarusia-kurage	b
<i>Polyorchis karafutoensis</i> Kishinouye, 1910	Kitakami-kurage	b
<i>Sarsia tubulosa</i> (M. Sars, 1835)	Sarusia-kurage	b
<i>Sarsia princeps</i> (Haeckel, 1879)	Osarusia-kurage	b
<i>Spirocodon saltatrix</i> (Tilesius, 1818)	Kami-kurage	b
<i>Stauridiosarsia ophiogaster</i> (Haeckel, 1879)	Jyuzu-kurage	a, b
<i>Stauridiosarsia nipponica</i> (Uchida, 1927)	Yamato-sarusia-kurage	a

Methods

Collection and fixing. Specimens were collected from Okikamuro Island, Suo-Oshima, Yamaguchi Prefecture, western Japan, between April 24, 2016 and April 18, 2018 (Fig. 1). The seven medusae were taken using a scoop (170 mm in diameter), among which, five individuals were fixed in 5% formalin in seawater and subsequently deposited at the National Museum of Nature and Science, Tsukuba, Japan (NSMT), whereas the remaining two specimens were preserved in 99.5% ethanol for DNA extraction.

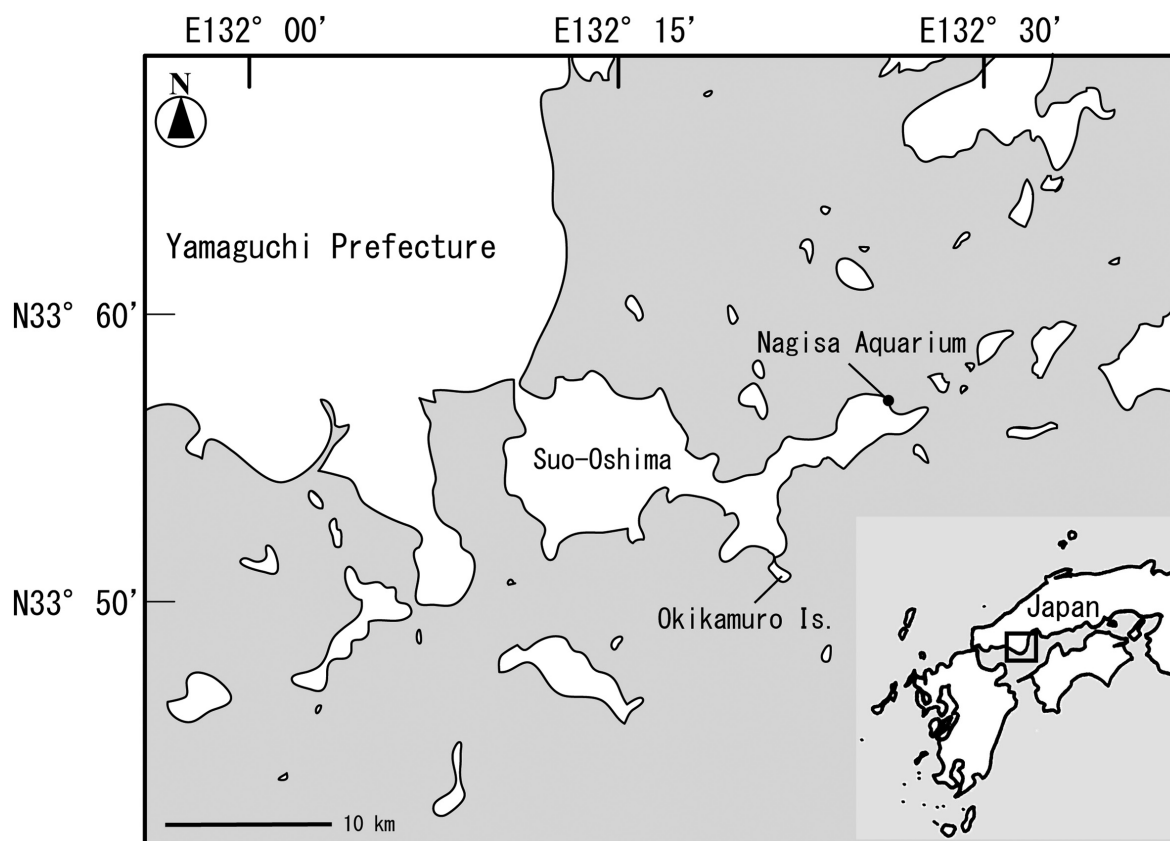


FIGURE 1. Map of the sampling sites.

Molecular phylogenetic analysis. For the purposes of molecular phylogenetic analysis, we used near complete sequences of 16S rDNA. Genomic DNA was extracted from the 99.5% ethanol-preserved tissue of two specimens using a DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. Mitochondrial 16S rDNA (approximately 600 bp) was PCR amplified and sequenced with the primer pair fwd: TCGACTGTT-TACCAAAAACATAGC and rev: ACGGAATGAACTCAAATCATGTAAG (Cunningham & Buss 1993), using the following PCR profile: an initial denaturation at 94°C/5 min; five cycles of 94°C/50 s, 45°C/50 s, and 72°C/60

s; 30 cycles of 94°C/50 s, 50°C/50 s, and 72°C/60 s; and a final elongation at 72°C/5 min (Collins *et al.* 2008). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced in both directions using an ABI 3730 automated sequencer (Applied Biosystems). The sequences thus obtained were aligned using MEGA 6 with built-in ClustalW (Tamura *et al.* 2013). Phylogenetic analysis and pairwise distance measurements were performed using the maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980) with 1000 bootstrap replications in MEGA 6. Two sequences have been deposited in GenBank under accession numbers LC635086 and LC635087 for the new genus (Table 2).

TABLE 2. Taxa included in the phylogenetic analyses and GenBank accession numbers for sequences: Sequences obtained in this study are in bold.

Family	Species	Accessioned name	Accession No.
Corynidae	<i>Caltsacoryne setouchiensis</i> n. sp.	<i>Caltsacoryne setouchiensis</i> n. sp.	LC635086
Corynidae	<i>Caltsacoryne setouchiensis</i> n. sp.	<i>Caltsacoryne setouchiensis</i> n. sp.	LC635087
Corynidae	<i>Codonium prolifera</i>	<i>Coryne prolifera</i>	GQ395318
Corynidae	<i>Stauridiosarsia cliffordi</i>	<i>Coryne cliffordi</i>	GQ395313
Corynidae	<i>Coryne epizoica</i>	<i>Coryne epizoica</i>	GQ395314
Corynidae	<i>Coryne epizoica</i>	<i>Coryne epizoica</i>	KX355418
Corynidae	<i>Coryne eximia</i>	<i>Coryne eximia</i>	AJ878712
Corynidae	<i>Coryne eximia</i>	<i>Coryne eximia</i>	KM402029
Corynidae	<i>Coryne fucicola</i>	<i>Coryne fucicola</i>	AM084259
Corynidae	<i>Coryne muscoides</i>	<i>Coryne muscoides</i>	EU876546
Corynidae	<i>Coryne muscoides</i>	<i>Coryne muscoides</i>	GQ395315
Corynidae	<i>Coryne pintneri</i>	<i>Coryne pintneri</i>	AJ878717
Corynidae	<i>Coryne pintneri</i>	<i>Coryne pintneri</i>	AJ878718
Corynidae	<i>Coryne pusilla</i>	<i>Coryne pusilla</i>	KP776759
Corynidae	<i>Coryne pusilla</i>	<i>Coryne pusilla</i>	KP776762
Corynidae	<i>Coryne uchidai</i>	<i>Coryne uchidai</i>	GQ395320
Corynidae	<i>Coryne uchidai</i>	<i>Coryne uchidai</i>	KP776809
Corynidae	<i>Polyorchis penicillatus</i>	<i>Polyorchis penicillatus</i>	KX355411
Corynidae	<i>Polyorchis penicillatus</i>	<i>Polyorchis penicillatus</i>	KX355412
Corynidae	<i>Sarsia apicula</i>	<i>Sarsia apicula</i>	GQ395330
Corynidae	<i>Sarsia lovenii</i>	<i>Sarsia lovenii</i>	AJ608796
Corynidae	<i>Sarsia lovenii</i>	<i>Sarsia lovenii</i>	AY787876
Corynidae	<i>Sarsia striata</i>	<i>Sarsia striata</i>	GQ395328
Corynidae	<i>Sarsia striata</i>	<i>Sarsia striata</i>	KX355408
Corynidae	<i>Sarsia tubulosa</i>	<i>Sarsia tubulosa</i>	AJ878720
Corynidae	<i>Sarsia tubulosa</i>	<i>Sarsia tubulosa</i>	AY512545
Corynidae	<i>Scrippisia pacifica</i>	<i>Scrippisia pacifica</i>	KX355419
Corynidae	<i>Slabberia halterata</i>	<i>Dipurena halterata</i>	AM084261
Corynidae	<i>Stauridiosarsia cliffordi</i>	<i>Stauridiosarsia cliffordi</i>	GQ395313
Corynidae	<i>Stauridiosarsia gemmifera</i>	<i>Dipurena gemmifera</i>	EU876547
Corynidae	<i>Stauridiosarsia marii</i>	<i>Stauridiosarsia marii</i>	AY512544
Corynidae	<i>Stauridiosarsia nipponica</i>	<i>Coryne nipponica</i>	GQ395316
Corynidae	<i>Stauridiosarsia nipponica</i>	<i>Coryne nipponica</i>	GQ395333
Corynidae	<i>Stauridiosarsia ophiogaster</i>	<i>Dipurena ophiogaster</i>	AJ878721
Corynidae	<i>Stauridiosarsia producta</i>	<i>Coryne producta</i>	AY512543
Corynidae	<i>Stauridiosarsia producta</i>	<i>Coryne producta</i>	GQ395317
Corynidae	<i>Stauridiosarsia reesi</i>	<i>Dipurena reesi</i>	GQ395321
Corynidae	<i>Stauridiosarsia</i> sp.	<i>Dipurena</i> sp.	GQ395331
Cladonematidae	<i>Cladonema radiatum</i>	<i>Cladonema radiatum</i>	AM088482

Morphological analyses. Taxonomic observations and measurements were performed using living or preserved specimens (Fig. 2). Measurements to the nearest 0.1 mm were made using ImageJ software (Rasband 2021). Nematocysts in the medusae were identified according to Kubota (1991) and Östman (2000) using squashes prepared from fresh tissues examined under a BX 53 compound microscope (Olympus, Japan). To determine the abundance of different nematocyst types, we examined approximately 300 nematocysts from cultured specimens, which were identified, measured, and counted.

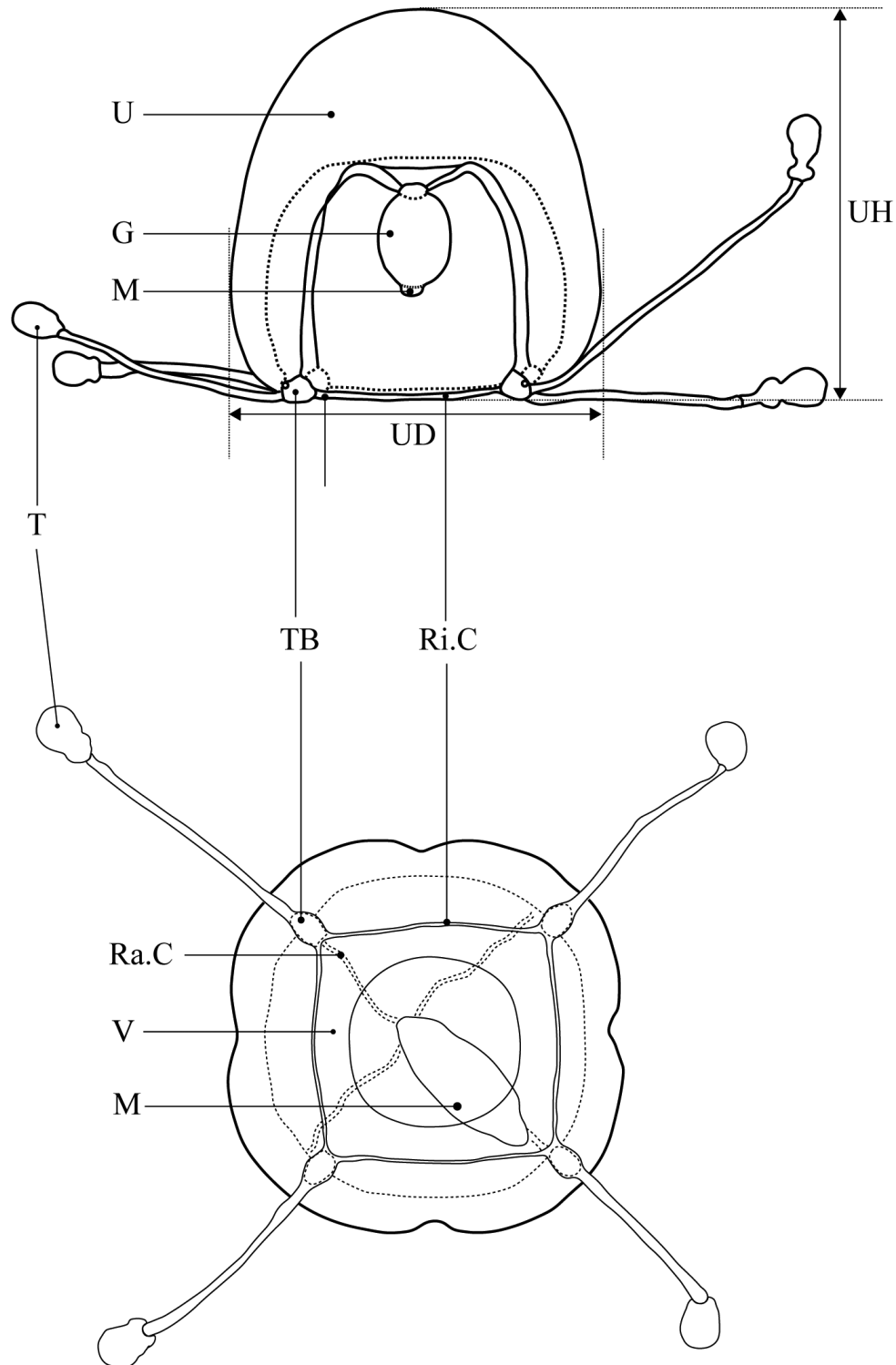


FIGURE 2. Key characters for identification and measurement of parts of the Corynidae. G = gonad; M = manubrium; Ra.C = radial canal; Ri.C = ring canal; T = tentacle; TB = tentacle bulb; U = umbrella; UD = umbrella diameter; UH = umbrella height; V = velum.

Results

Molecular phylogenetics. The maximum likelihood tree constructed for the family Corynidae based on 16S rDNA sequences (Fig. 3) was found to comprise two major clades, one of which contained *Codonium*, *Coryne*, *Scrippsia*, *Polyorchis*, the unidentified Corynidae, *Slabberia*, and *Sarsia*, whereas the other comprised the single genus *Stauridiosarsia*. The monophyly of the unidentified Corynidae from Japan was evident in the 16S phylogenetic tree (as indicated by the high bootstrap value of 92%), thereby supporting the validity of designating this taxon as a new genus. The Kimura 2-parameter distances between the unidentified Corynidae and other genera ranged from 0.06 to 0.18 (Table 3). The K2P distance factor between *Sarsia* and *Slabberia* was found to be comparatively low, ranging from 0.03 to 0.04, whereas intergeneric distances for *Sarsia*, *Stauridiosarsia*, and *Coryne* were 0.01–0.02, 0.05–0.16, and 0.01–0.19, respectively. Accordingly, the K2P divergence factor of between 0.06 and 0.19 could be considered as a threshold for discriminating corynid genera.

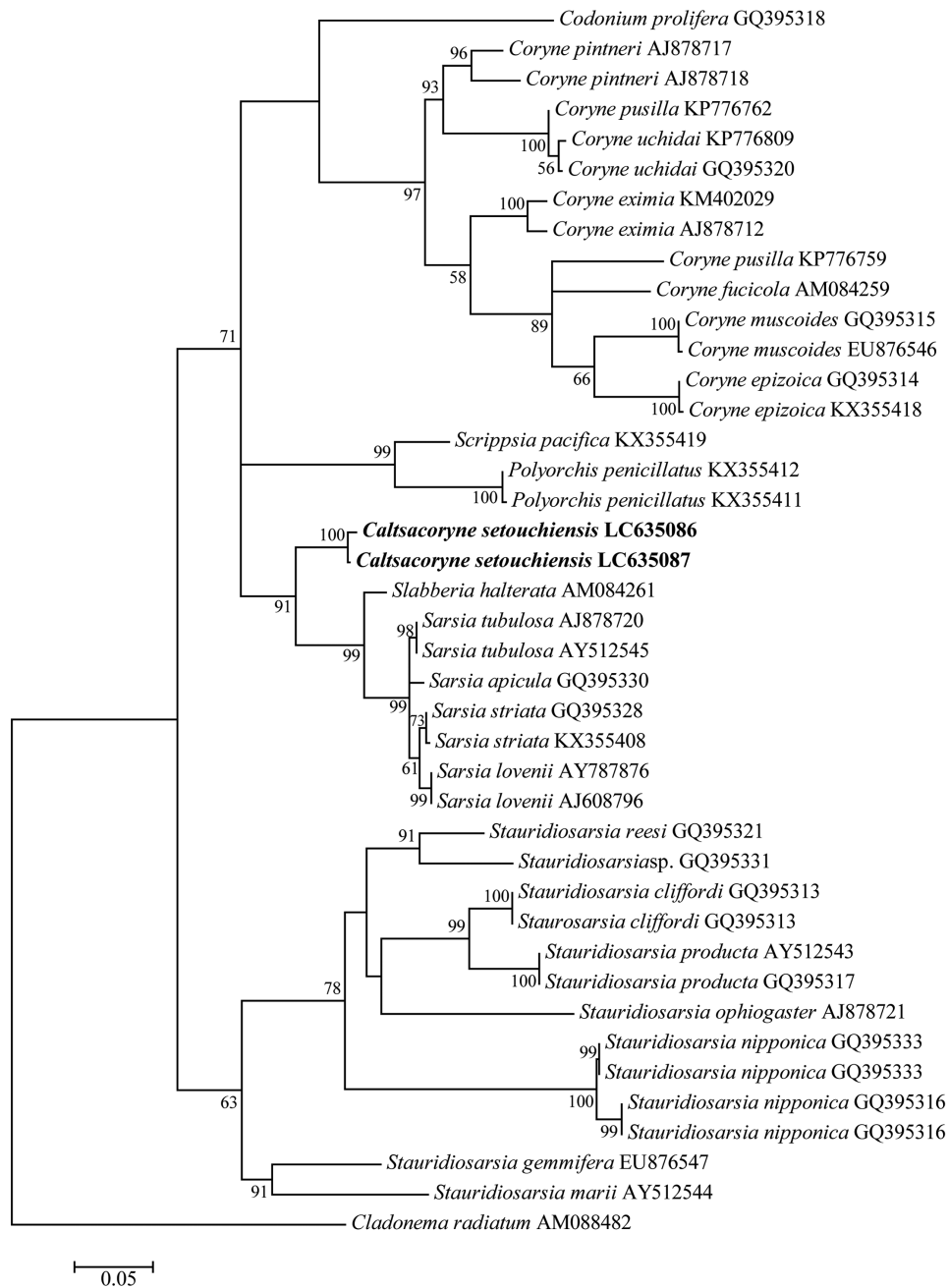


FIGURE 3. Nuclear 16S rDNA Maximum-likelihood tree for 25 anthoathcata taxa based on the General Time Reversible model: Scale bar indicates branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value; only values >50% are shown.

TABLE 3. Pair wise genetic distances (K2P) based on 570 positions of 16S sequences among Corynidae: The analysis involved 38 sequences.

No.	1	2	3	4	5	6	7	8	9	10
<i>Caltsacoryne setouchiensis</i> n. sp. LC635086										
<i>Caltsacoryne setouchiensis</i> n. sp. LC635087	0.01									
<i>Codonium prolifera</i> GQ395318	0.12	0.12								
<i>Coryne epizoica</i> GQ395314	0.11	0.11	0.14							
<i>Coryne epizoica</i> KX355418	0.11	0.11	0.14	0.00						
<i>Coryne eximia</i> AJ878712	0.11	0.11	0.12	0.09	0.09					
<i>Coryne eximia</i> KM402029	0.12	0.11	0.12	0.09	0.09	0.02				
<i>Coryne fucicola</i> AM084259	0.13	0.13	0.14	0.08	0.08	0.08	0.08			
<i>Coryne muscoides</i> EU876546	0.13	0.13	0.13	0.07	0.07	0.10	0.10	0.07		
<i>Coryne muscoides</i> GQ395315	0.13	0.13	0.13	0.07	0.07	0.10	0.10	0.07	0.00	
<i>Coryne pintneri</i> AJ878717	0.10	0.10	0.12	0.09	0.09	0.08	0.08	0.08	0.09	0.09
<i>Coryne pintneri</i> AJ878718	0.12	0.11	0.13	0.09	0.09	0.07	0.07	0.09	0.10	0.10
<i>Coryne pusilla</i> KP776759	0.13	0.13	0.13	0.08	0.08	0.09	0.09	0.08	0.08	0.08
<i>Coryne pusilla</i> KP776762	0.13	0.13	0.15	0.11	0.11	0.09	0.09	0.11	0.10	0.10
<i>Coryne uchidai</i> GQ395320	0.13	0.12	0.14	0.11	0.11	0.09	0.09	0.10	0.10	0.10
<i>Coryne uchidai</i> KP776809	0.13	0.13	0.14	0.11	0.11	0.09	0.09	0.11	0.11	0.11
<i>Polyorchis penicillatus</i> KX355411	0.09	0.09	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16
<i>Polyorchis penicillatus</i> KX355412	0.09	0.09	0.15	0.14	0.14	0.15	0.15	0.16	0.16	0.16
<i>Sarsia apicula</i> GQ395330	0.07	0.07	0.12	0.14	0.14	0.13	0.14	0.15	0.15	0.15
<i>Sarsia lovenii</i> AJ608796	0.06	0.06	0.12	0.13	0.13	0.13	0.13	0.15	0.15	0.15
<i>Sarsia lovenii</i> AY787876	0.06	0.06	0.12	0.13	0.13	0.13	0.13	0.15	0.15	0.15
<i>Sarsia striata</i> GQ395328	0.06	0.06	0.12	0.13	0.13	0.12	0.13	0.14	0.14	0.14
<i>Sarsia striata</i> KX355408	0.06	0.06	0.11	0.13	0.13	0.12	0.13	0.14	0.15	0.15
<i>Sarsia tubulosa</i> AJ878720	0.06	0.07	0.11	0.14	0.14	0.13	0.13	0.15	0.15	0.15
<i>Sarsia tubulosa</i> AY512545	0.06	0.07	0.11	0.14	0.14	0.13	0.13	0.15	0.15	0.15
<i>Scrippisia pacifica</i> KX355419	0.10	0.10	0.15	0.14	0.14	0.13	0.12	0.15	0.16	0.16
<i>Slabberia halterata</i> AM084261	0.06	0.06	0.10	0.12	0.12	0.11	0.11	0.13	0.13	0.13
<i>Stauridiosarsia cliffordi</i> GQ395313	0.14	0.14	0.17	0.18	0.18	0.14	0.15	0.18	0.19	0.19
<i>Stauridiosarsia gemmifera</i> EU876547	0.12	0.12	0.17	0.14	0.14	0.14	0.15	0.16	0.16	0.16
<i>Stauridiosarsia marii</i> AY512544	0.13	0.13	0.17	0.15	0.15	0.14	0.14	0.16	0.16	0.16
<i>Stauridiosarsia nipponica</i> GQ395316	0.17	0.17	0.17	0.18	0.17	0.17	0.17	0.17	0.18	0.18
<i>Stauridiosarsia nipponica</i> GQ395333	0.17	0.17	0.17	0.18	0.18	0.17	0.17	0.17	0.19	0.19
<i>Stauridiosarsia ophiogaster</i> AJ878721	0.18	0.17	0.17	0.19	0.19	0.16	0.17	0.21	0.20	0.20
<i>Stauridiosarsia producta</i> AY512543	0.12	0.12	0.17	0.17	0.17	0.15	0.15	0.18	0.20	0.20
<i>Stauridiosarsia producta</i> GQ395317	0.12	0.12	0.17	0.17	0.17	0.15	0.15	0.18	0.20	0.20
<i>Stauridiosarsia reesi</i> GQ395321	0.13	0.13	0.16	0.16	0.16	0.15	0.15	0.17	0.17	0.17
<i>Stauridiosarsia</i> sp. GQ395331	0.12	0.12	0.16	0.15	0.15	0.15	0.15	0.17	0.18	0.18
<i>Cladonema radiatum</i> AM088482	0.17	0.16	0.17	0.17	0.17	0.15	0.15	0.15	0.17	0.17

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TABLE 3. (continued)

No.	11	12	13	14	15	16	17	18	19	20
<i>Caltsacoryne setouchiensis</i> n. sp. LC635086										
<i>Caltsacoryne setouchiensis</i> n. sp. LC635087										
<i>Codonium prolifera</i> GQ395318										
<i>Coryne epizoica</i> GQ395314										
<i>Coryne epizoica</i> KX355418										
<i>Coryne eximia</i> AJ878712										
<i>Coryne eximia</i> KM402029										
<i>Coryne fucicola</i> AM084259										
<i>Coryne muscoides</i> EU876546										
<i>Coryne muscoides</i> GQ395315										
<i>Coryne pintneri</i> AJ878717										
<i>Coryne pintneri</i> AJ878718	0.03									
<i>Coryne pusilla</i> KP776759	0.09	0.09								
<i>Coryne pusilla</i> KP776762	0.06	0.07	0.11							
<i>Coryne uchidai</i> GQ395320	0.06	0.07	0.11	0.01						
<i>Coryne uchidai</i> KP776809	0.06	0.07	0.11	0.01	0.01					
<i>Polyorchis penicillatus</i> KX355411	0.12	0.15	0.17	0.15	0.14	0.15				
<i>Polyorchis penicillatus</i> KX355412	0.12	0.14	0.16	0.15	0.14	0.15	0.00			
<i>Sarsia apicula</i> GQ395330	0.12	0.13	0.15	0.13	0.12	0.12	0.12	0.12		
<i>Sarsia lovenii</i> AJ608796	0.12	0.12	0.15	0.13	0.13	0.13	0.12	0.12	0.02	
<i>Sarsia lovenii</i> AY787876	0.12	0.12	0.15	0.13	0.13	0.13	0.12	0.12	0.02	0.00
<i>Sarsia striata</i> GQ395328	0.12	0.12	0.14	0.12	0.12	0.12	0.11	0.11	0.01	0.01
<i>Sarsia striata</i> KX355408	0.12	0.12	0.14	0.12	0.12	0.12	0.11	0.11	0.01	0.01
<i>Sarsia tubulosa</i> AJ878720	0.12	0.12	0.15	0.13	0.12	0.12	0.12	0.12	0.01	0.01
<i>Sarsia tubulosa</i> AY512545	0.12	0.12	0.15	0.13	0.12	0.12	0.12	0.12	0.01	0.01
<i>Scrippsia pacifica</i> KX355419	0.13	0.14	0.16	0.14	0.14	0.14	0.07	0.07	0.12	0.13
<i>Slabberia halterata</i> AM084261	0.10	0.11	0.13	0.12	0.11	0.11	0.11	0.11	0.03	0.04
<i>Stauridiosarsia cliffordi</i> GQ395313	0.15	0.16	0.18	0.18	0.18	0.18	0.16	0.15	0.15	0.15
<i>Stauridiosarsia gemmifera</i> EU876547	0.11	0.12	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14
<i>Stauridiosarsia marii</i> AY512544	0.12	0.13	0.16	0.13	0.13	0.14	0.16	0.15	0.14	0.14
<i>Stauridiosarsia nipponica</i> GQ395316	0.15	0.15	0.17	0.17	0.17	0.17	0.18	0.17	0.17	0.17
<i>Stauridiosarsia nipponica</i> GQ395333	0.15	0.15	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16
<i>Stauridiosarsia ophiogaster</i> AJ878721	0.15	0.16	0.17	0.18	0.18	0.19	0.19	0.19	0.18	0.19
<i>Stauridiosarsia producta</i> AY512543	0.15	0.16	0.18	0.18	0.18	0.19	0.15	0.15	0.15	0.14
<i>Stauridiosarsia producta</i> GQ395317	0.15	0.16	0.18	0.18	0.18	0.19	0.15	0.15	0.15	0.14
<i>Stauridiosarsia reesi</i> GQ395321	0.15	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.13	0.13
<i>Stauridiosarsia</i> sp. GQ395331	0.14	0.15	0.17	0.16	0.16	0.16	0.13	0.13	0.13	0.13
<i>Cladonema radiatum</i> AM088482	0.15	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.18

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TABLE 3. (continued)

No.	21	22	23	24	25	26	27	28	29	30
<i>Caltsacoryne setouchiensis</i> n. sp. LC635086										
<i>Caltsacoryne setouchiensis</i> n. sp. LC635087										
<i>Codonium prolifera</i> GQ395318										
<i>Coryne epizoica</i> GQ395314										
<i>Coryne epizoica</i> KX355418										
<i>Coryne eximia</i> AJ878712										
<i>Coryne eximia</i> KM402029										
<i>Coryne fucicola</i> AM084259										
<i>Coryne muscoides</i> EU876546										
<i>Coryne muscoides</i> GQ395315										
<i>Coryne pintneri</i> AJ878717										
<i>Coryne pintneri</i> AJ878718										
<i>Coryne pusilla</i> KP776759										
<i>Coryne pusilla</i> KP776762										
<i>Coryne uchidai</i> GQ395320										
<i>Coryne uchidai</i> KP776809										
<i>Polyorchis penicillatus</i> KX355411										
<i>Polyorchis penicillatus</i> KX355412										
<i>Sarsia apicula</i> GQ395330										
<i>Sarsia lovenii</i> AJ608796										
<i>Sarsia lovenii</i> AY787876										
<i>Sarsia striata</i> GQ395328	0.01									
<i>Sarsia striata</i> KX355408	0.01	0.00								
<i>Sarsia tubulosa</i> AJ878720	0.01	0.01	0.01							
<i>Sarsia tubulosa</i> AY512545	0.01	0.01	0.01	0.00						
<i>Scrippisia pacifica</i> KX355419	0.13	0.12	0.11	0.12	0.12					
<i>Slabberia halterata</i> AM084261	0.04	0.04	0.04	0.03	0.03	0.11				
<i>Stauridiosarsia cliffordi</i> GQ395313	0.15	0.14	0.14	0.14	0.14	0.15	0.13			
<i>Stauridiosarsia gemmifera</i> EU876547	0.14	0.14	0.14	0.14	0.14	0.13	0.12	0.12		
<i>Stauridiosarsia marii</i> AY512544	0.14	0.14	0.13	0.14	0.14	0.14	0.11	0.12	0.09	
<i>Stauridiosarsia nipponica</i> GQ395316	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.14	0.14	0.15
<i>Stauridiosarsia nipponica</i> GQ395333	0.16	0.16	0.16	0.16	0.16	0.18	0.17	0.14	0.14	0.15
<i>Stauridiosarsia ophiogaster</i> AJ878721	0.19	0.19	0.19	0.18	0.18	0.19	0.17	0.12	0.12	0.16
<i>Stauridiosarsia producta</i> AY512543	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.05	0.13	0.12
<i>Stauridiosarsia producta</i> GQ395317	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.05	0.13	0.12
<i>Stauridiosarsia reesi</i> GQ395321	0.13	0.13	0.13	0.13	0.13	0.16	0.13	0.08	0.13	0.12
<i>Stauridiosarsia</i> sp. GQ395331	0.13	0.12	0.12	0.12	0.12	0.15	0.12	0.10	0.12	0.12
<i>Cladonema radiatum</i> AM088482	0.18	0.17	0.17	0.18	0.18	0.16	0.16	0.15	0.19	0.16

...continued on the next page

TABLE 3. (continued)

No.	31	32	33	34	35	36	37	38
<i>Caltsacoryne setouchiensis</i> n. sp. LC635086								
<i>Caltsacoryne setouchiensis</i> n. sp. LC635087								
<i>Codonium prolifera</i> GQ395318								
<i>Coryne epizoica</i> GQ395314								
<i>Coryne epizoica</i> KX355418								
<i>Coryne eximia</i> AJ878712								
<i>Coryne eximia</i> KM402029								
<i>Coryne fucicola</i> AM084259								
<i>Coryne muscoides</i> EU876546								
<i>Coryne muscoides</i> GQ395315								
<i>Coryne pintneri</i> AJ878717								
<i>Coryne pintneri</i> AJ878718								
<i>Coryne pusilla</i> KP776759								
<i>Coryne pusilla</i> KP776762								
<i>Coryne uchidai</i> GQ395320								
<i>Coryne uchidai</i> KP776809								
<i>Polyorchis penicillatus</i> KX355411								
<i>Polyorchis penicillatus</i> KX355412								
<i>Sarsia apicula</i> GQ395330								
<i>Sarsia lovenii</i> AJ608796								
<i>Sarsia lovenii</i> AY787876								
<i>Sarsia striata</i> GQ395328								
<i>Sarsia striata</i> KX355408								
<i>Sarsia tubulosa</i> AJ878720								
<i>Sarsia tubulosa</i> AY512545								
<i>Scrippsia pacifica</i> KX355419								
<i>Slabberia halterata</i> AM084261								
<i>Stauridiosarsia cliffordi</i> GQ395313								
<i>Stauridiosarsia gemmifera</i> EU876547								
<i>Stauridiosarsia marii</i> AY512544								
<i>Stauridiosarsia nipponica</i> GQ395316								
<i>Stauridiosarsia nipponica</i> GQ395333	0.01							
<i>Stauridiosarsia ophiogaster</i> AJ878721	0.13	0.13						
<i>Stauridiosarsia producta</i> AY512543	0.14	0.14	0.11					
<i>Stauridiosarsia producta</i> GQ395317	0.14	0.14	0.11	0.00				
<i>Stauridiosarsia reesi</i> GQ395321	0.12	0.12	0.12	0.09	0.09			
<i>Stauridiosarsia</i> sp. GQ395331	0.12	0.11	0.12	0.10	0.10	0.06		
<i>Cladonema radiatum</i> AM088482	0.16	0.16	0.19	0.16	0.16	0.16	0.16	

Taxonomy

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Petersen, 1979

Class Hydrozoa Owen, 1843

Subclass Hydroidolina Collins, 2000

Order Anthoathecata Cornelius, 1992

Suborder Capitata Kühn, 1913

Family Corynidae Johnston, 1836

Genus *Caltsacoryne* gen. n.

New Japanese name: Shitouzu-kurage-zoku

Genus diagnosis. Corynidae have a bell-shaped umbrella with four radial canals, and a ring canal. Tentacle bulbs swollen, each with an abaxial ocellus. Manubrium short, not extending beyond umbrella margin and without thin proximal part. Gonads undivided, and encircle manubrium for nearly its entire length, leaving manubrium visible only at the top and near the mouth. Four tentacles arising from bulbs with a single egg-shaped nematocyst swelling at tips.

Type species. *Caltsacoryne setouchiensis*

Etymology. The genus name *Caltsacoryne* is taken from the Greek words “kaltsa” and “koryne,” meaning “sock” and “club,” respectively. The gender is feminine. The name reflects the illusion that the medusa appears to be wearing socks on the tentacle tips.

Caltsacoryne setouchiensis sp. n.

New Japanese name: Shitouzu-kurage

Figures 4–7

Material examined. Holotype: NSMT-Co 1749. Suo-Oshima, Okikamuro Island, Yamaguchi Prefecture, western Japan, April 24, 2016, collector: Sho Toshino, one adult female medusa. Paratypes. NSMT-Co 1750. Same locality and date as the holotype, collectors: Yoshimi Hamatsu, one adult male. NSMT-Co1751–1753. Same locality as the holotype, April 18, 2018, collector: Hiroaki Uchida, two adult males and one adult female.

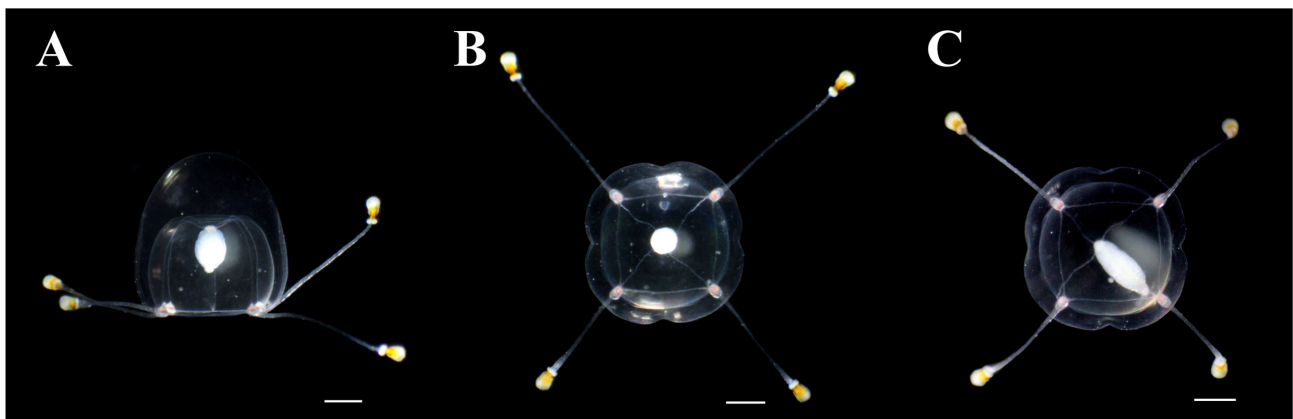


FIGURE 4. *Caltsacoryne setouchiensis* sp. n., live: A, lateral view; B, apical view; C, oral view. All bars represent 1 mm.

Description. Mature medusae with transparent, thick but soft bell-shaped umbrella (Fig. 4A–C, 5A–C). Umbrella height (UH) and diameter (UD) approximately 7 mm and 4 mm, respectively (Table 4). Umbrella apex thickened, tapered. Exumbrella smooth, nematocyst sparsely scattered. Bell cavity small. Four radial canals and single straight ring canal. Canals narrow, almost with same diameter. Tentacle bulbs swollen, reddish purple in color, each with abaxial ocellus (Fig. 6A–C). Tentacle arising from bulbs, short and terminating in single large egg-shaped swelling covered with nematocysts (Fig. 6D, E). Orange band present on middle part of swelling. Mouth simple and circular, whitish in color (Fig. 6F, G). Manubrium hanging in bell cavity, large and flask-shaped; rounded at apical side and furnished with small proboscis in distal portion. Extended manubrium length approximately 3 mm and approximately half umbrella height (almost full length of subumbrella). Gonads encircling entire surface of manubrium, with exception of apical and distal portions (Fig. 6D). Velum narrow (Fig. 4C, 5C).

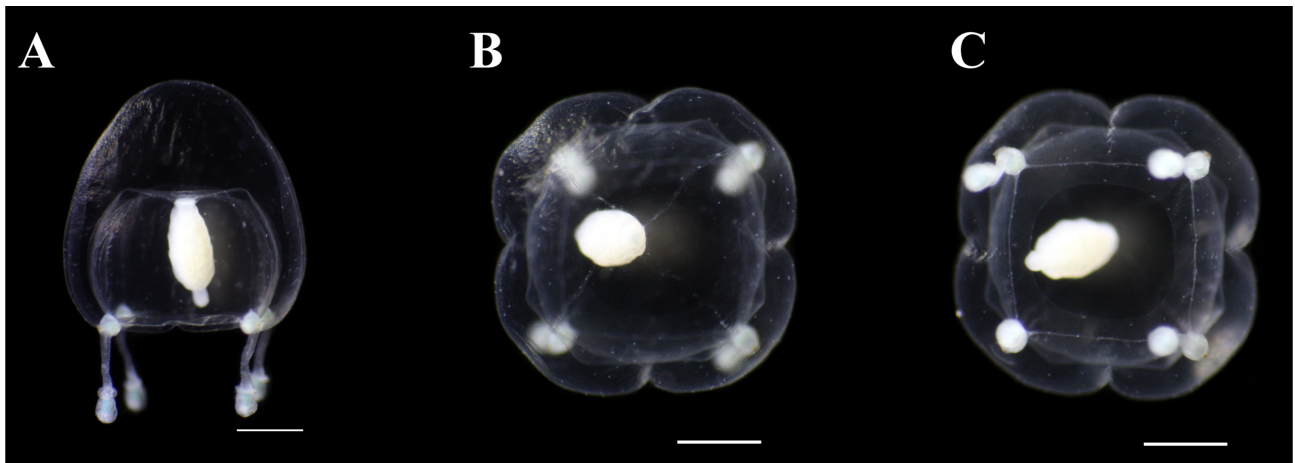


FIGURE 5. *Caltsacoryne setouchiensis* sp. n., fixed, holotype: A, lateral view; B, apical view; C, oral view. All bars represent 1 mm.

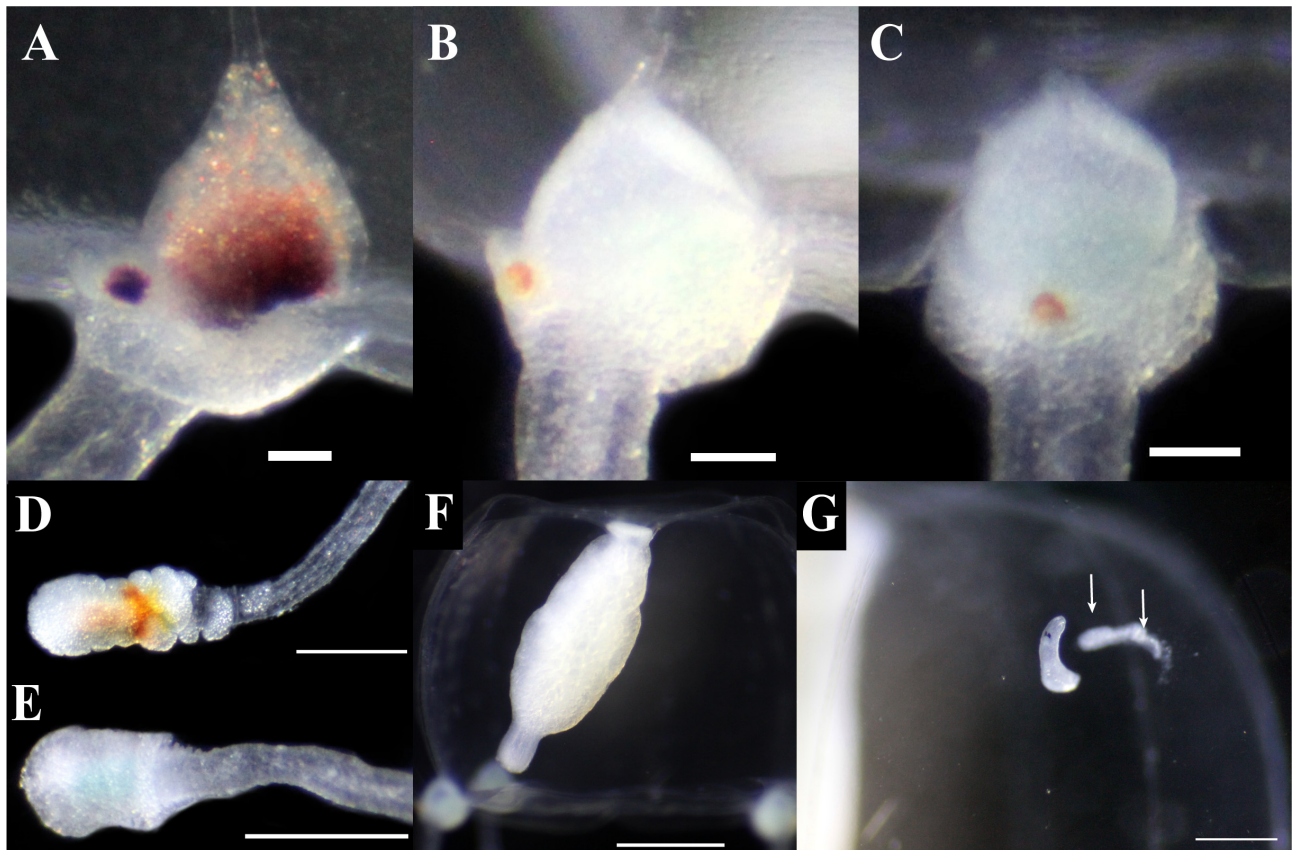


FIGURE 6. *Caltsacoryne setouchiensis*, sp. n.: A–C, tentacle bulb. Lateral view, live (A), lateral view, fixed (B); abaxial view, fixed (C); D–E, tentacle. Live (D), fixed (E); F, manubrium and gonad; G, exumbrella. Arrows indicate flukes;. Scale bars: 0.1 mm (A–C), 0.5 mm (D–G).

TABLE 4. Size (mm) of *Caltsacoryne setouchiensis*: * The holotype. Nos. NSMT-Co1750-1753 are paratypes. ML = manubrium length; UH = umbrella height; UD = umbrella width.

Specimen No.	UH	UD	ML	Sex	Date
NSMT-Co 1749*	3.7	2.8	1.8	Female	2016/4/24
NSMT-Co 1750	3.7	2.5	2.3	Male	2016/4/24
NSMT-Co 1751	6.7	3.2	2.5	Male	2018/4/18
NSMT-Co 1752	7.3	4.0	3.6	Male	2018/4/18
NSMT-Co 1753	6.5	3.9	2.6	Female	2018/4/18

Smallest young medusa with UH of 2.5 mm and UD of 2.6 mm (Fig. 7A–C). Umbrella spherical with few ex-umbrellar nematocysts. Mesoglea on apex of exumbrella thinner than that of adults. Manubrium thin, translucent to whitish, length approximately half that of umbrella. Mouth simple and circular. Gonad immature. Four radial canals and singular circular canal. Velum narrow. Tentacle bulbs swollen, reddish purple, each with dark brown abaxial ocellus. Four tentacles, terminating in single large spherical swelling. Orange band present on middle part of swelling.

TABLE 5. Cnidomes of *Caltsacoryne setouchiensis*: D, L represent capsule diameter and length, respectively, in μm .

Part	Type		Min	Max	Mean	SD	N
Tentacular bulb	Desmonema	D	5.7	8.7	7.8	0.6	50
		L	14.6	20.3	16.9	1.1	
	Stenotele (Large)	D	14.0	22.1	17.5	2.7	31
		L	21.0	29.0	24.1	2.2	
Tentacle tip	Desmonema	D	5.0	7.2	5.9	0.7	17
		L	8.9	15.3	11.5	1.7	
	Stenotele (Large)	D	16.0	20.3	18.1	1.0	50
		L	21.5	26.2	23.7	1.0	
Manubrium	Desmonema	D	5.1	9.4	7.2	0.8	49
		L	9.6	17.2	14.4	1.3	
	Stenotele (Large)	D	15.2	20.8	18.0	1.9	17
		L	20.8	27.8	24.8	2.1	

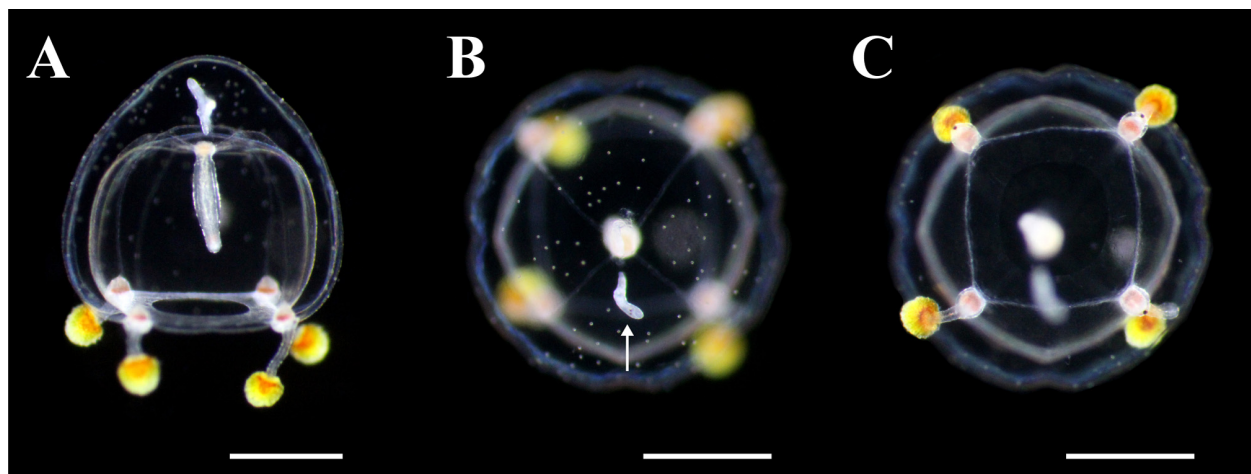


FIGURE 7. Young medusae of *Caltsacoryne setouchiensis* sp. n., live: A, lateral view; B, apical view; C, oral view. Arrow indicates fluke. All bars represent 1 mm.

TABLE 6. Morphology of Corynidae medusae in previous and the present studies: All bars represent lacking data. a, Schuchert 2001; b, Bouillion *et al.* 2006; c, Xu & Huang 2006; d, Huang *et al.* 2008; e, Forbes 1848; f, Huang *et al.* 2011; g, Minemizu *et al.* 2015; h, Torrey 1909.

Genus	Tentacle				Gonad	
	No. of tentacles	Adaxial pads on marginal bulb	Branching pattern	Shape	Shape	Cover region
<i>Caltsacoryne</i> gen. nov.	4	lacking	unbranched	capitate	undivided	Most of length
<i>Bicorona</i> Millard, 1966	-	-	-	-	-	-
<i>Cladosarsia</i> Bouillion, 1978	4	present	branched	capitate	undivided	Most of length or only its oral half
<i>Codonium</i> Haeckel, 1879	4	lacking	unbranched	filiiform	undivided	Most of length
<i>Coryne</i> Gaertner, 1774	2-4	lacking	unbranched	filiiform or pearl-string like	undivided	Most of length
<i>Dicycloctoryne</i> Annandale, 1915	4	lacking	unbranched	capitate	N/A	N/A
<i>Dipurenella</i> Huang, Xu & Guo, 2011	4	present	branched	capitate	divided	distal, swollen stomach region
<i>Nannocoryne</i> Bouillion & Grohmann, 1994	-	-	-	-	-	-
<i>Polyorchis</i> A. Agassiz, 1862	ca. 100	lacking	unbranched	filiiform	sausage-shaped on peduncular manubrium pouches, pendulous	-
<i>Sarsia</i> Lesson, 1843	4	lacking	unbranched	filiiform	undivided	thin part of manubrium
<i>Scrippisia</i> Torrey, 1909	over 100	lacking	unbranched	filiiform	sausage-shaped on peduncular manubrium pouches, pendulous	-
<i>Slabberia</i> Forbes, 1846	4	lacking	unbranched	capitate	divided	distal, swollen stomach region
<i>Spirocodon</i> Haeckel, 1880	over 100	lacking	unbranched	filiiform	spirally twisted on peduncular manubrium pouches	-
<i>Stauridiosarsia</i> Mayer, 1910	4	lacking	unbranched	filiiform or pearl-string like	divided or undivided	Most of length

.....continued on the next page

TABLE 6. (Continued)

Genus	Manubrium		Other distinguishing features	References
	Length	Thin proximal part		
<i>Calsacoryne</i> gen. nov.	not extending	absent	-	This study
<i>Bicorona</i> Millard, 1966	-	-	No medusae	a
<i>Cladosarsia</i> Bouillon, 1978	Extending or not extending beyond umbrella margin	absent	-	a, b, c, d
<i>Codonium</i> Haeckel, 1879	not extending	absent	Budding medusa buds on their manubrium and tentacle bulb	e
<i>Coryne</i> Gaertner, 1774	not extending	absent	-	a, b
<i>Dicylocoryne</i> Annandale, 1915	not extending	absent	newly liberated medusae, no ocelli	a,b
<i>Dipurenella</i> Huang, Xu & Guo, 2011	extending	absent	-	f
<i>Nannocoryne</i> Bouillon & Grohmann, 1994	-	-	No medusae	b
<i>Polyorchis</i> A. Agassiz, 1862	not extending	absent	referred as Polyorchidae	g
<i>Sarsia</i> Lesson, 1843	extending	present	-	b
<i>Scrippisia</i> Torrey, 1909	not extending	absent	referred as Polyorchidae	h
<i>Slabberia</i> Forbes, 1846	extending	absent	referred as <i>Dipurena</i>	b
<i>Spirocodon</i> Haeckel, 1880	not extending	absent	referred as Polyorchidae	g
<i>Stauridiosarsia</i> Mayer, 1910	not extending	absent	-	a

Cnidome. Two different types of nematocyst were identified and measured in the adult medusa (Table 5, Fig. 8), and two sizes of stenoteles (Fig. 8A, B) and desmonemes (Fig. 8C, D) were observed on tentacle tips, tentacle bulbs, and manubrium, although small stenoteles were absent from the manubrium. Stenoteles were also found on the bell; however, these could not be measured.



FIGURE 8. Nematocysts of *Caltsacoryne setouchiensis*, sp. n.: A–B, stenoteles. Intact (A), discharged (B); C–D, desmonemes. Intact (C), discharged (D). All bars represent 10 μ m.

Habitat and ecology. The medusae of *C. setouchiensis* collected in the present study appeared at the water surface in a shallow area (water depth 3–5 m) during the daytime around the coast of Suo-Oshima, the Seto Inland Sea, in April. They are not active swimmers but drift with the current using their extended tentacles. The species appears to be neritic. In some specimens, mesoglea at the apical part were found to be infested by flukes (Fig. 6G, 7B). Stinging events attributable to *C. setouchiensis* are currently unknown.

Etymology. The specific name “*setouchiensis*” refers to the Setouchi Region, which includes the type locality in which the species was found.

Differential diagnosis. A comparison of the key features of Corynidae species is presented in Table 6. *Caltsacoryne setouchiensis* can be distinguished from the species of other genera by the following combination of morphological characters: number of tentacles, cnidocyst pads, manubrium length, and shape of the gonads and tentacles in adult medusae. All species in the family Corynidae have four radial canals, circular canals, and marginal tentacle bulbs. *Caltsacoryne* bears four tentacles, whereas species in the genera *Polyorchis*, *Scrippsia*, and *Spirocodon* typically have more than 100. Whereas most corynids have unbranched filiform tentacles, those in *Caltsacoryne*, *Dicyclacoryne*, and *Slabberia* are unbranched capitate, and *Cladosarsia* and *Dipurenella* have branched

capitate tentacles. *Caltsacoryne* lacks adaxial cnidocyst pads on the marginal bulbs, whereas these pads are present in *Cladosarsia* and *Dipurenella*. Furthermore, unlike species of *Cladosarsia*, *Dipurenella*, *Sarsia* and *Slabberia*, the manubrium in *Caltsacoryne* does not extend beyond the umbrella margin, and in contrast to *Dipurenella*, *Slabberia*, and some species of *Stauridiosarsia*, in which the gonads are divided, those in *Caltsacoryne* are divided

Discussion and conclusions

The morphological characters of *C. setouchiensis* tend to resemble those of *Coryne inabai* (Uchida, 1933) and *Plotocnida borealis* Wagner, 1885. *Coryne inabai* was originally described by Uchida (1933) as *Sarsia inabai* collected from the western coast of Kamchatka. However, Kramp (1942; 1968) doubted the validity of this assignment and referred it to *Plotocnida borealis* Wagner, 1885, despite the lack of ocelli in the marginal tentacle bulbs. Bouillon (1978) reported a mature 1.3 mm medusa of *C. inabai* (as *Sarsia inabai*) from the Seychelles. However, Schuchert (2001) pointed out that the specimen described by Bouillon is probably a different species, given that Uchida (1933) originally described the medusae of *C. inabai* as maturing at 3–8.5 mm; moreover, it also has a slight peduncle. Schuchert (2001) also re-examined a medusa collected by Bouillon (1985b) from Papua New Guinea, and identified the specimen as *Coryne inabai*. Unfortunately, it is likely that the material investigated for the original description of *C. inabai* has been lost and thus cannot be re-examined.

The specimens of *Caltsacoryne setouchiensis* were collected from the Seto Inland Sea, western Japan, whereas *C. inabai* has been reported from the cold northern Pacific (Uchida 1933) and in the tropical Pacific and Indian oceans (Bouillon 1978; 1985; Schuchert 2001), and thus the distribution of these two species would not appear to overlap.

The morphological and molecular phylogenetic analyses undertaken in the present study provide convincing evidence that the Corynidae collected from the Seto Inland Sea is a new genus and new species. Currently, however, the development, seasonal distribution, and stings of this species have yet to be determined. Accordingly, further investigations will be necessary to gain an insight into the ecology of *C. setouchiensis*.

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