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A New Species of *Zanclea innocens* and New Record of *Zanclea medusopolypata* (Hydrozoa, Anthoathecata) from Japan †

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Abstract: Cnidarian jellyfish (Medusozoa) comprise approximately 3800 known species which play important roles for marine ecosystem. This study aimed to understand the diversity of cnidarian jellyfish and symbiosis among marine organisms. The taxonomy of the family Zancleidae (Hydrozoa, Anthoathecata) has been problematic because of the limited differences in morphological characteristics between species. This family comprises approximately 40 species belonging to four genera: *Apatizanclea*, *Halocoryne*, *Zanclea*, and *Zanclella*. In this study, we describe a new species of hydromedusa belonging to the family Zancleidae found in Japanese waters. *Zanclea innocens* sp. nov. can be distinguished from other *Zanclea* species by the presence of nematocyst clusters on the exumbrella, slightly thickened apical projection, four narrow exumbrellar nematocyst pouches on ridges often reaching the umbrella apex, four marginal bulbs, and two tentacles. Additionally, *Zanclea medusopolypata* was recorded for the first time in Japanese waters. This paper provides taxonomic keys for the identification of species in the genus *Zanclea*.

Keywords: Anthomedusae; Cnidaria; hydroid; medusa; new species; hydromedusa; Zancleidae

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1. Introduction

The family Zancleidae is one of the major taxa in the order Anthoathecata, comprising approximately 40 species in four genera *Apatizanclea*, *Halocoryne*, *Zanclea*, and *Zanclella* [1–4]. Zancleidae species are widely distributed in the shallow waters of the Atlantic, Pacific, and Indian oceans [5,6], the majority of which are characterized by alternating generations with life cycles comprising asexual benthic polyps and sexual planktonic medusae [1]. Typically, free-swimming medusae are liberated by the budding of hydroids, although some Zancleidae species undergo reductions in the polyp and/or medusa stages [1]. The vast majority of this species live symbiotically with other organisms, among which cheilostomate bryozoans are the most common hosts [2,3].

The taxonomy of the family Zancleidae has been problematic because of the limited differences in morphological characteristics among species. The type genus *Zanclea* was established by Gegenbaur from the Mediterranean Sea and is associated with the Cladonematidae [7]. Secondarily, the genus *Halocoryne* was established based on the description of *Halocoryne epizoica* Hadzi, 1917 [8]. Russell classified *Zanclea* in the new family Zancleidae with a description of *Zanclea costata* from the British Islands [9]. Boero and Hewitt [10] established the third genus *Zanclella* based on the reduced number of tentacles in the

gastrozooids and the laterally compressed appearance of the umbrella of the medusa [11]. Morphological and molecular phylogenetic analyses have revealed that both the family Zancleidae and the genus *Zanclea* are polyphyletic taxa that comprise at least three divergent lineages [2]. Phylogenetic reconstructions by Maggioni et al. demonstrated that the lineage described here as *Apatizanclea* diverged from the genus *Zanclea*, forming a cohesive group and requiring the establishment of a new genus and combinations [3].

Zanclea is characterized as follows: Medusa umbrella bell-shaped, lateral walls evenly thin, mesoglea slightly thicker at the apex; 4 exumbrellar perradial cnidocyst patches or tracts, with stenoteles; mouth simple, circular; 4 radial canals; marginal tentacles 0, 2 or 4, with numerous abaxial extensile cnidophores with macrobasic euryteles; "gonads" interradial, no ocelli. Hydroid colonial, stolonal with creeping hydrorhiza; hydrocaulus unbranched; often associated with bryozoans, bivalves, and corals; monomorphic or polymorphic polyps; polymorphic colony with gastrozooids, dactylozooids, and gonozooids; gastrozooids on unbranched short pedicels, often almost sessile, elongated, cylindrical, or claviform with an oral whorl of capitate tentacles and numerous aboral capitate tentacles scattered or in several whorls over the body; gonozooids and dactylozooids, when present, vary in expression [11].

Several *Zanclea* species have been reported in Japanese waters. The medusa of *Zanclea* sp. was first recorded by Maas [12] and subsequently described as a new species, *Zanclea maasi* Uchida, 1925 [13]. However, Hartlaub [14] pointed out that this species is a young medusa of *Urashimea* and is now synonymous with *Urashimea globosa*. Two species, *Zanclea costata* Gegenbaur, 1857 and *Zanclea indopacifica* (Stechow, 1919 as *Halocharis indopacifica*), have been reported by Stechow [15,16], but the taxonomic validity of *Z. indopacifica* is uncertain. Uchida also reported *Zanclea* sp. and referred to *Zanclea gemmosa* McCrady, 1859; however, it remained unidentified [17]. Uchida and Sugiura [18] described a new species, *Zanclea prolifera* Uchida & Sugiura, 1976 in Misaki, Sagami Bay. Hirohito [19] reported polyps of three *Zanclea* species from Sagami Bay, one of which (*Zanclea* sp. I) was described as a new species, *Zanclea hirohitoi* Boero, Bouillon & Gravili, 2000 [1]. Hirose and Hirose [20] described a new species, *Zanclea sango* Hirose and Hirose, 2011 from Okinawa Island, Japan.

To date, four valid *Zanclea* species, *Z. costata*, *Z. hirohitoi*, *Z. prolifera*, and *Z. sango*, are known in Japanese waters [1,18,21–23]. In this study, unknown medusae of *Zanclea* species were collected from the Kanagawa, Nagasaki, Kochi, and Okinawa prefectures in Japan. Our morphological and molecular phylogenetic analyses suggest that this *Zanclea* species should be regarded as a new species and a new species record to Japan within the genus *Zanclea*.

2. Materials and Methods

2.1. Collection and Fixation

Thirty medusae of *Zanclea* specimens were collected near the water surface (within approximately 10 m) at ports in the Kanagawa, Kochi, and Okinawa prefectures between 27 April 2018, and 19 June 2023 (Tables 1 and 2, Figure 1). The medusae were captured using either a dipper (diameter, 17 cm; volume, 2000 mL) or a dip net (mesh size, approximately 0.5 mm). Eight specimens were fixed in 3% formalin-buffered seawater for taxonomic observation after anesthesia with a saturated aqueous solution of $MgCl_2$. Medusa specimens were deposited at the National Museum of Nature and Science (NSMT) in Tsukuba, Japan. Four *Zanclea* specimens from the Shizuoka and Kochi Prefectures were preserved in 99.5% ethanol for molecular analysis. One specimen was used to determine the abundance of each nematocyst type. Additionally, three specimens of *Zanclea prolifera* from Shizuoka Prefectures were used for molecular phylogenetic analyses [24–29].

Table 1. Collection details of *Zanclea innocens* sp. nov. and *Zanclea medusopolypata* in this study.

Specimen No.	Species	Date	Sampling site	Lat./Long.
NSMT-Co1903	*Zanclea innocens* sp. nov.	27 April 2018	Itoman Port, Itoman, Okinawa Prefecture, Japan	26°7′33.10″ N 127°39′47.54″ E
NSMT-Co1904	*Zanclea innocens* sp. nov.	30 May 2019	Iburi Port, Tosashimizu, Kochi Prefecture, Japan	32°48′8.27″ N 132°57′47.71″ E
NSMT-Co1905	*Zanclea innocens* sp. nov.	19 June 2023	Shonan Port, Fujisawa, Kanagawa Prefecture, Japan	35°17′52.4″ N 139°28′32.2″ E
Photograph-only specimen-1	*Zanclea innocens* sp. nov.	14 June 2020	Shonan Port, Fujisawa, Kanagawa Prefecture, Japan	35°17′52.4″ N 139°28′32.2″ E
Photograph-only specimen-2	*Zanclea innocens* sp. nov.	22 November 2019	Komame Port, Otsuki, Hata, Kochi Prefecture, Japan	32°47′22.81″ N 132°41′11.61″ E
Photograph-only specimen-3	*Zanclea innocens* sp. nov.	17 June 2020	Komame Port, Otsuki, Hata, Kochi Prefecture, Japan	32°47′22.81″ N 132°41′11.61″ E
Photograph-only specimen-4	*Zanclea innocens* sp. nov.	30 May 2020	Iburi Port, Tosashimizu, Kochi Prefecture, Japan	32°48′8.27″ N 132°57′47.71″ E
Photograph-only specimen-5	*Zanclea innocens* sp. nov.	27 June 2022	Iburi Port, Tosashimizu, Kochi Prefecture, Japan	32°48′8.27″ N 132°57′47.71″ E
NSMT-Co1906	*Zanclea medusopolypata*	30 November 2021	Iwachi, Matsuzaki, Kamo, Shizuoka Prefecture, Japan	34°44′17.3″ N 138°45′27.6″ E
NSMT-Co1907	*Zanclea medusopolypata*	23 August 2021	Tabira Port, Tabira, Hirado, Nagasaki Prefecture, Japan	33°21′43.4″ N 129°34′37.8″ E

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

Species	Accession No.	Locality (Origin)	Reference
***Zanclea innocens* sp. nov.**	**PV468597**	Japan: Iburi, Tosashimizu, Kochi	This study
***Zanclea innocens* sp. nov.**	**PV468598**	Japan: Iburi, Tosashimizu, Kochi	This study
Zanclea medusopolypata	**PV468599**	Japan: Iwachi, Matsuzaki, Shizuoka	This study
Zanclea medusopolypata	**PV468600**	Japan: Iwachi, Matsuzaki, Shizuoka	This study
Zanclea sango	LN714095	Maldives	[24]
Zanclea sango	LN714099	Maldives	[24]
Zanclea gallii	LK934472	Maldives: Magoodhoo Island, Faafu Atoll	[24]
Zanclea gallii	LK934473	Maldives: Magoodhoo Island, Faafu Atoll	[24]
Zanclea migottoi	MF538731	Mexico: Alacranes Reef, Gulf of Mexico	[25]
Zanclea sp.	KF962532	China Sea	[26]
Zanclea prolifera	EU305488	Unknown	[26]
Zanclea prolifera	**PV468601**	Japan: Iwachi, Matsuzaki, Shizuoka	This study
Zanclea prolifera	**PV468602**	Japan: Iwachi, Matsuzaki, Shizuoka	This study
Zanclea prolifera	**PV468603**	Japan: Iwachi, Matsuzaki, Shizuoka	This study
Zanclea giancarloi	FN687560	France: Banyuls-sur-Mer, below Village Catalan	Unpublished
Zanclea giancarloi	KP776811	France: Bay of Villefranche-sur-Mer	Unpublished
Zanclea sessilis	AY512532	Mallorca: Cala Murada	[27]
Zanclea sessilis	FN687557	France: Roscoff	Unpublished
Zanclea implexa	KX355448	Norway: Raunefjord, Vatlestraumen	Unpublished
Zanclea sp.	KP776810	France: Bay of Villefranche-sur-Mer	Unpublished
Zanclea costata	EU876553	France: Marie-de-la-Mer, Mediterranean	[28]
Zanclea costata	AY512531	France: Marie-de-la-Mer, Mediterranean	[27]
Cladocoryne floccosa	MG811590	France: Bay of Villefranche-sur-Mer	Unpublished

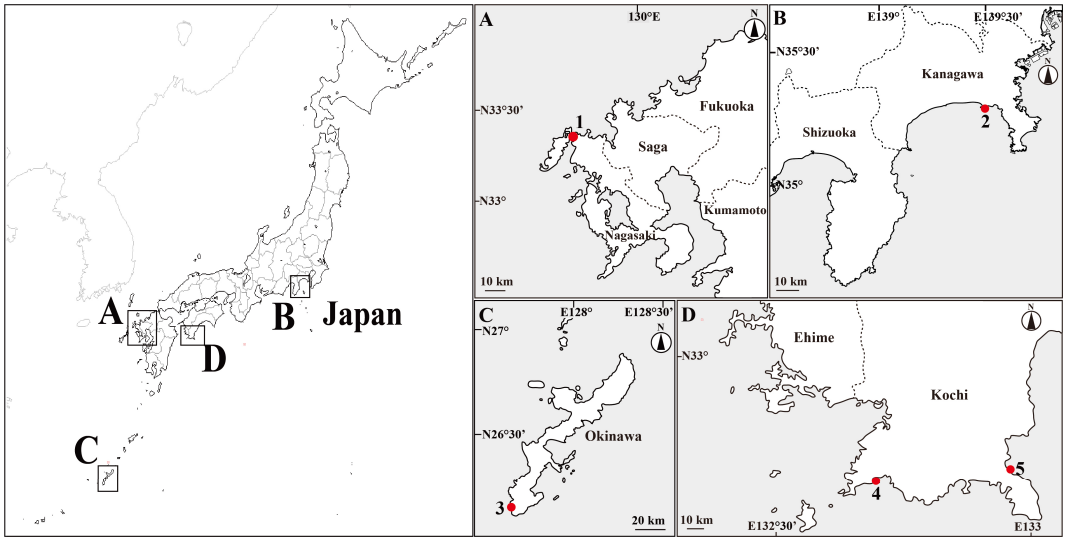

Figure 1. A map of the sampling sites. (**A**) Tabira Port, Tabira, Hirado, Nagasaki Prefecture (1); (**B**) Shonan Port, Fujisawa, Kanagawa Prefecture (2); (**C**) Itoman Port, Itoman, Okinawa Prefecture (3); (**D**) Komame Port, Otsuki, Hata, Kochi Prefecture (4); Iburi Port, Tosashimizu, Kochi Prefecture (5). Red closed circles are sampling sites.

2.2. Morphological Investigation

Taxonomic observations and measurements were conducted on both live and preserved specimens, according to Bouillon et al. and Schuchert & Collins [1,4,29] (Figure 2). The medusae were flattened on a glass dish (50 mm in diameter) filled with seawater. Umbrella height (UH) was measured from the apex of the umbrella to its margins. Umbrella diameter (UD) was measured across the exumbrella turnover. The specimens were photographed using a compound microscope (SZ61, OLYMPUS, Tokyo, Japan) equipped with an OLYMPUS OM-D E-M5 MarkII camera. Measurements were made to the nearest 0.01 mm using methods previously published [30] with ImageJ software (version 1.53k) [31].

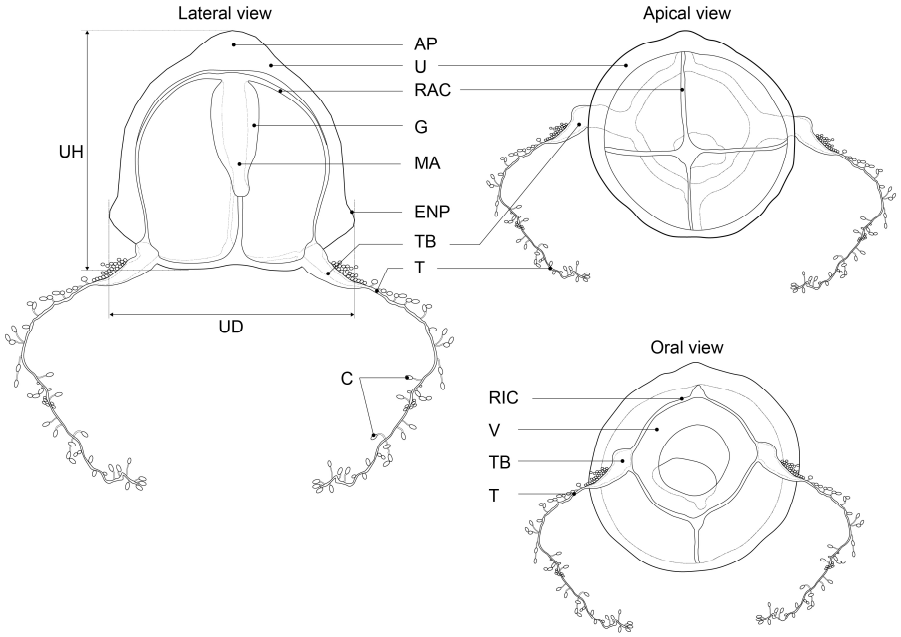

Figure 2. Key characters for identification and measurement of parts of *Zanclea*. AP = apical projection; C = cnidophore; ENP = exumbrellar nematocysts pouch; G = gonad; MA = manubrium; RAC = radial canal; RIC = ring canal; T = tentacle; TB = tentacle bulb; U = umbrella; UD = umbrella diameter; UH = umbrella height; V = velum.

For nematocyst identification in medusae, squash prepared from fresh tissue was examined under a compound microscope (ECLIPSE Ci; Nikon, Tokyo, Japan). Nematocysts were identified using a previously described method [32]. To determine the abundance of nematocyst types in medusae, approximately 60 nematocysts were identified, measured, and counted from unregistered specimens. Measurements were performed to the nearest 0.1 µm using ImageJ software.

2.3. Molecular Phylogenetic Analysis

Near-complete sequences of the mitochondrial 16S rDNA gene (approximately 600 bp) were used for molecular phylogenetic analyses. Genomic DNA was extracted from the ethanol-preserved tissue of the cultured specimens using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The 16S rDNA was PCR-amplified and sequenced with the forward and reverse primers primer 1: TCGACTGTTTACCAAAAACATAGC and primer 2: ACGGAATGAACTCAAATCATG-TAAG [33], using the following PCR profile: initial denaturation at 94 °C for 5 min; 5 cycles at 94 °C for 50 s, 45 °C for 50 s, and 72 °C for 60 s; 30 cycles at 94 °C for 50 s, 50 °C for 50 s, and 72 °C for 60 s; and final elongation at 72 °C for 5 min [27]. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN) and sequenced in both directions using an ABI 3730 automated sequencer (Applied Biosystems, Bedford, MA, USA). The new sequences were aligned using MEGAX with built-in ClustalW [34]. Substitution models and partitions were determined with MEGAX using the Akaike information criterion, resulting in partitioning by gene and codon (for protein-coding genes), and the selection of GTR + I + G for all partitions. Phylogenetic reconstruction was performed based on maximum likelihood. Genetic distances with and between *Zanclea* were calculated using MEGAX [35] as % uncorrected *p*-distances with 1000 nonparametric bootstrap replicates. Eight sequences of *Zanclea* species were deposited in GenBank under the accession numbers PV468597–468603 (Table 2).

3. Results

3.1. Taxonomy

Phylum Cnidaria Hatschek, 1888
Subphylum Medusozoa Petersen, 1979
Class Hydrozoa Owen, 1843
Subclass Hydroidolina Collins, 2000
Order Anthoathecata Cornelius, 1992
Suborder Capitata Kühn, 1913
Family Zancleidae Russell, 1953
Genus *Zanclea* Gegenbaur, 1856

3.2. Species Description

3.2.1. *Zanclea innocens* sp. nov. Toshino, Yamamoto, Nozoe and Akiyama, 2025

ZooBank LSD: urn:lsid:zoobank.org:pub:82DD4192-0FBB-49ED-B641-8BB0080168FC.
New Japanese name. Mizutama-suzufuri-kurage

Material examined. Holotype: NSMT-Co1903; Itoman Port, Itoman, Okinawa Prefecture, Japan; 26°7′33.10″ N 127°39′47.54″ E; 27 April 2018; collector: Sho Toshino.

Paratypes: NSMT-Co1904: Iburi Port, Tosashimizu, Kochi Prefecture, Japan; 32°48′8.27″ N 132°57′47.71″ E; 30 May 2019; collector: Sho Toshino. NSMT-Co1905: Shonan Port, Fujisawa, Kanagawa Prefecture, Japan; 35°17′52.4″ N 139°28′32.2″ E; 14 June 2020; collector: Gaku Yamamoto.

Photograph-only specimen-1: same locality as NSMT-Co1905; 19 June 2023; collector: Gaku Yamamoto. Photograph-only specimen-2: Komame Port, Otsuki, Hata, Kochi Prefecture, Japan; 32°47′22.81″ N; 132°41′11.61″ E; 22 November 2019; collector: Sho Toshino. Photograph-only specimen-3: same locality as Photograph-only specimen-2; 17 June 2020; collector: Sho Toshino. Photograph-only specimen-4: same locality as NSMT-Co1904; 30 May 2020; collector: Sho Toshino. Photograph-only specimen-5: same locality as NSMT-Co1904; 27 June 2022; collector: Sho Toshino.

Description. Mature medusae with bell-shaped umbrella, 1.2–1.5 mm (1.5 mm in the holotype) in height and 1.2–1.4 mm (1.4 mm in the holotype) in diameter (Figure 3A–D). The umbrella apex is slightly protruding with a thickened mesoglea. Exumbrella with white nematocyst clusters consisting of round warts dispersed over the middle part of the exumbrella (Figure 4A). Exumbrella with four perradial nematocyst pouches as narrow bands on ridges, often reaching umbrella apex. Manubrium is white or faintly pink and hangs in the umbrella cavity and the cylindrical bottom (Figure 4B). The extended manubrium length is approximately 1/2 as long as the umbrella height. The gonads cover the entire surface of the manubrium, except for the lip of the mouth. The mouth is simple and circular. Four narrow radial canals extend from the stomach to the umbrella margin (Figure 4C). The circular canal is white, simple, and narrow (Figure 4D). The velum is broad, with a velarial width of 20% of the umbrella diameter. Four marginal bulbs contained blackish or deep-purple pigment granules without apparent ocelli (Figure 4E). Two tentacles, with an abaxial row of 30–60 white cnidophores that are spherical with long contractile stalks (Figure 4F). The tentacles at the base are thick without bulb formation.

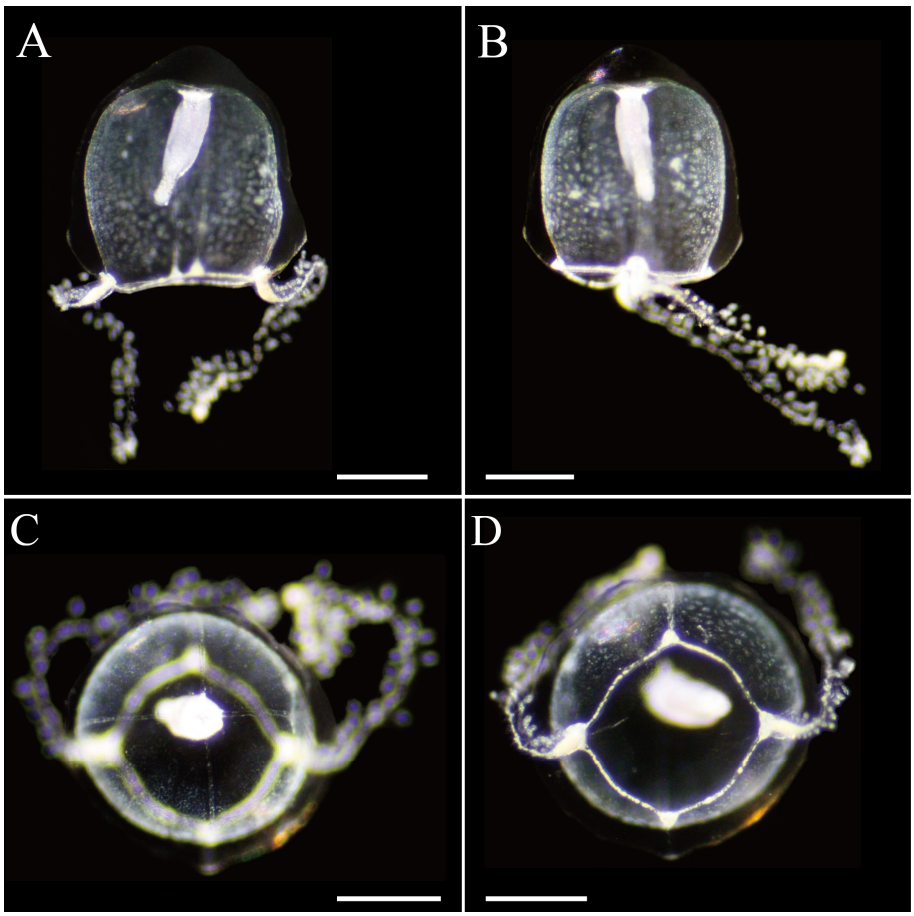

Figure 3. Live mature medusae of *Zanclea innocens* sp. nov. (NSMT-Co1903): (**A**,**B**) lateral, (**C**) apical, and (**D**) oral views. Scale bars represent 0.5 mm.

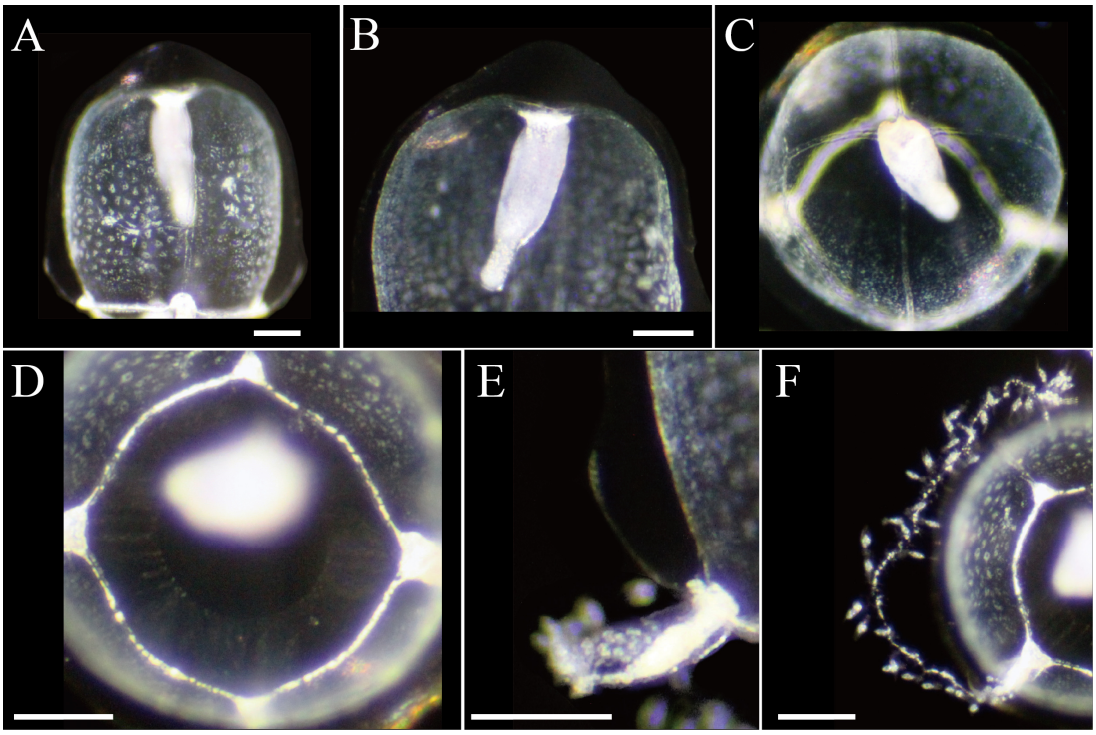

Figure 4. Live mature medusae of *Zanclea innocens* sp. nov. (NSMT-Co1903): (**A**) exumbrella, (**B**) manubrium, (**C**) umbrella, apical view, (**D**) velum, (**E**) tentacular bulb, (**F**) tentacle. Scale bars represent 0.2 mm.

Young medusae with bell-shaped umbrella, 0.6–0.8 mm (mean 0.7 mm; n = 5) in height and 0.7–0.9 mm (mean 0.7 mm; n = 5) in diameter (Figure 5A–D). The mesoglea of the exumbrella is thinner than that of adults. Umbrella apex is flat. The exumbrella almost spherical, with white nematocyst clusters consisting of round warts dispersed over the middle. Exumbrella with four perradial nematocyst pouches as narrow bands on ridges, often reaching umbrella apex. The manubrium is cylindrical, thinner than that of adults, and 1/2 of the umbrella height. The mouth is simple and circular. There are four radial canals, narrow. The circular canal is white, simple, and narrow. The velum is broad, with a velarial width of 20% of the umbrella diameter. Four marginal bulbs contained blackish or deep-purple pigment granules without apparent ocelli. Two tentacle, with an abaxial row of 30–60 white cnidophores that are spherical with long contractile stalks. The tentacles at the base are thick without bulb formation.

Cnidome. Two nematocyst types, macrobasic euryteles and stenoteles, were identified and measured in adult medusae (Table 3, Figure 6A–D).

Table 3. Cnidomes of *Zanclea innocens* sp. nov. D and L are capsule diameter and length, respectively, in μm.

Part	Type		Min	Max	Mean	SD	N
Exumbrella	Stenotele	D	9.9	11.4	10.9	0.7	6
		L	11.9	12.7	12.4	0.4	6
Manubrium	Stenotele	D	5.3	7.8	6.2	0.9	7
		L	7.4	9.2	8.0	0.6	7
Tentacle	Macrobasic eurytele	D	3.4	5.6	4.6	0.5	44
		L	6.2	10.4	8.5	0.9	44

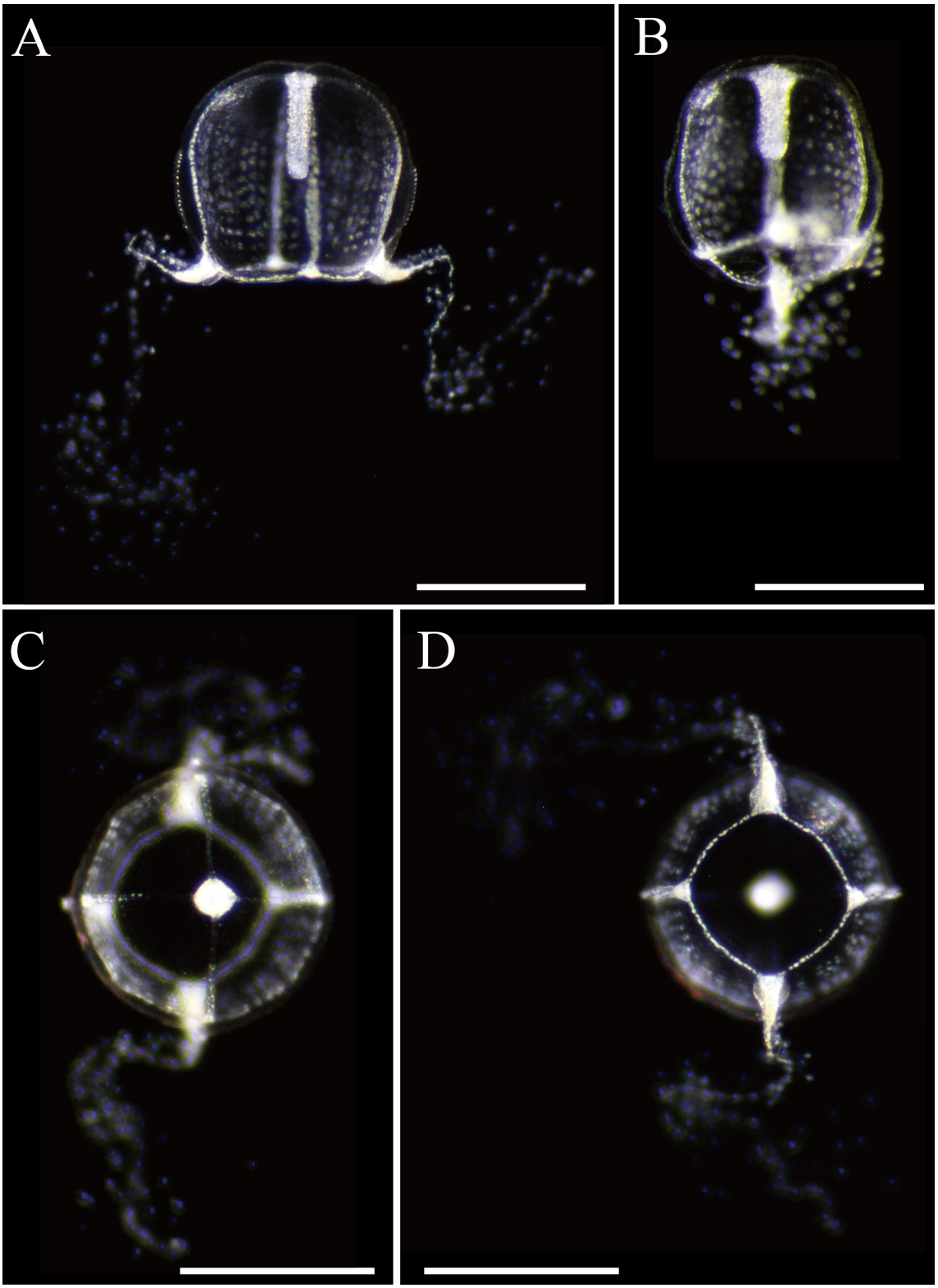

Figure 5. Live young medusa of *Zanclea innocens* sp. nov. (photograph-only specimen-4): (**A**,**B**) lateral, (**C**) apical, and (**D**) oral views. Scale bars represent 0.5 mm.

Habitat and ecology. Medusae of *Zanclea innocens* appeared on the surface of shallow waters (3–5 m in depth) in Shonan Port (eastern Japan) in June, Iburi Port (western Japan) from May to June, Komame Port (western Japan) from June to November, and Itoman Port (southern Japan) in April. The release of medusae seems to occur between spring and fall in Japanese waters. The early life cycles, including embryogenesis and polyps, are unknown.

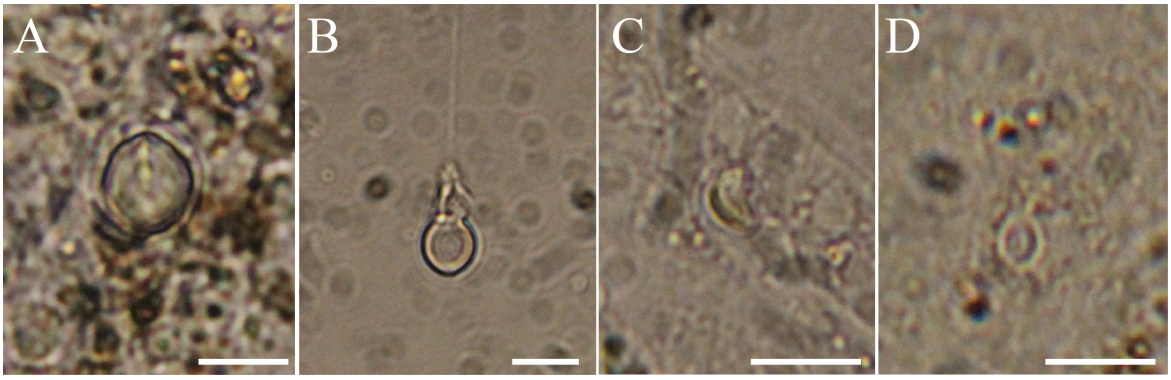

Figure 6. Nematocysts of *Zanclea innocens* sp. nov. (unregistered specimen): (**A**,**B**) stenoteles, (**C**,**D**) macrobasic euryteles. Scale bars represent 10 μm.

Etymology. The specific name '*innocens*' is a Latin adjective, referring to the white color of exumbrellar nematocyst clusters, manubrium, and tentacles of the new species.

3.2.2. *Zanclea medusopolypata* Boero, Bouillon and Gravili, 2000

New Japanese name: Kanzashi-suzufuri-kurage

Zanclea medusopolypata Boero, Bouillon and Gravili, 2000: 105, figs 10A,B.

Zanclea implexa Rees and Roa, 1966: 37–41, figs 1.

Material examined. NSMT-Co1906: Iwachi, Matsuzaki, Kamo, Shizuoka Prefecture, Japan; 34°44′17.3″ N 138°45′27.6″ E; 30 November 2021; collector: Gaku Yamamoto. NSMT-Co1907: Tabira Port, Tabira, Hirado, Nagasaki Prefecture, Japan; 33°21′43.4″ N 129°34′37.8″ E; 23 August 2021; collector: Hisashi Akiyama.

Description. Medusae with bell-shaped umbrella, 1.2–1.5 mm (mean 1.4 mm; n = 2) in height and 1.2–1.4 mm (mean 1.3 mm; n = 2) in diameter (Figure 7A–C). The umbrella apex is rounded with a slightly thickened mesoglea. Exumbrella has four perradial nematocyst pouches as narrow bands on ridges, often reaching the umbrella apex (Figure 8A). The manubrium is reddish or orange and hangs in the umbrella cavity; cylindrical bottom (Figure 8B). The extended manubrium length is 1/2 as long as umbrella height. A single hydranth is attached to the base of the manubrium. The gonads cover the entire surface of the manubrium, except for the lip of the mouth. The mouth is simple and circular. Four narrow radial canals extend from the stomach to the umbrella margin (Figure 8C). The circular canal is white, simple, and narrow (Figure 8D). The velum is broad, with a velarial width of 20% of the umbrella diameter. Two marginal bulbs contained reddish or orange pigment granules without apparent ocelli (Figure 8E). Two tentacles are present, with an abaxial row of 60–100 white cnidophores that are spherical with long contractile stalks (Figure 8F). The tentacles at the base are thick without bulb formation.

Cnidome. Not examined in this study. Two nematocyst types, stenoteles (two sizes in the exumbrellar pouches and around the mouth), and bean-shaped apotrichous macrobasic euryteles (cnidophores) were identified in the adult medusa [1].

Habitat and ecology. Medusae of *Zanclea medusopolypata* appeared on the surface of shallow waters (3 m in depth) in Shizuoka Prefecture (eastern Japan) in November, and in Nagasaki Prefecture (western Japan) in August. Polyps develop on their manubrium, whereas polyps settled on substrates have never been found in the wild. The production of polyps on their manubria may be an adaptation to drifting in the ocean.

Etymology. The specific name '*medusopolypata*' is taken from the Latin '*medusa*' and '*polyp*' The name indicates budding polyps in the manubrium of the medusa in the same individual [1].

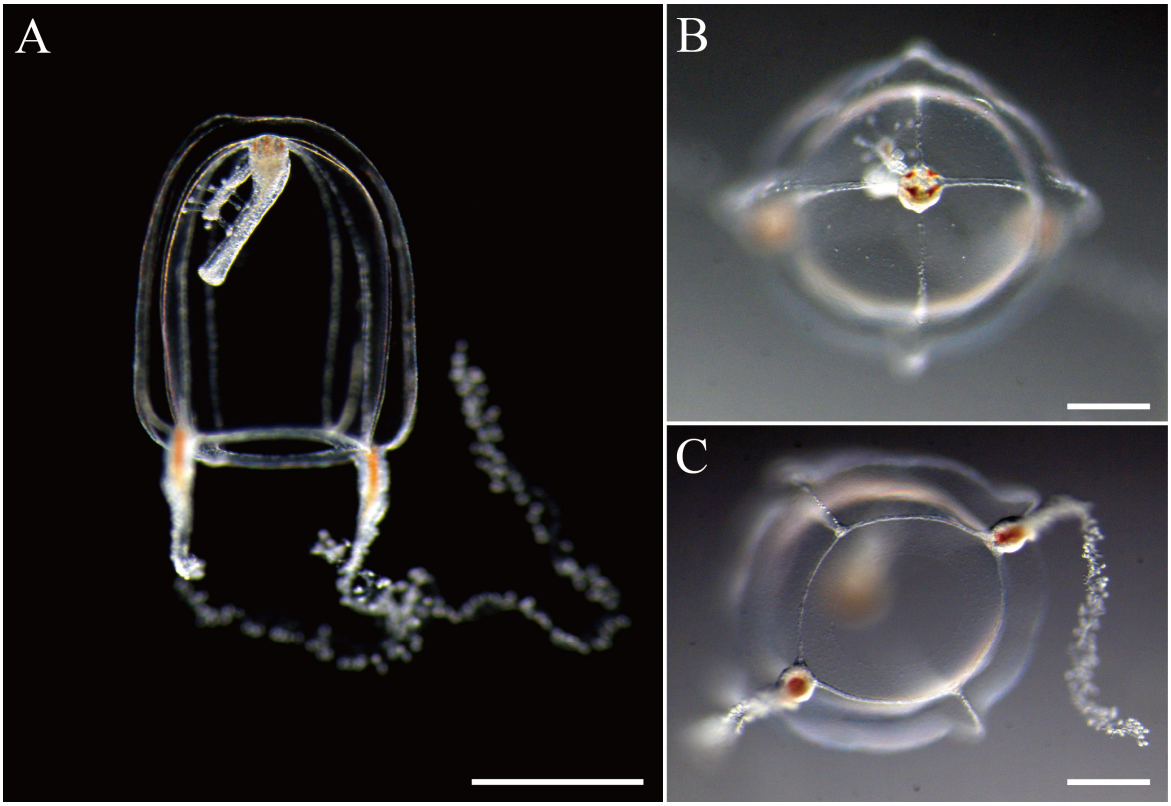

Figure 7. Live mature medusa of *Zanclea medusopolypata* (NSMT-Co1907): (**A**) lateral, (**B**) apical, and (**C**) oral view. Scale bars: (**A**). 0.5 mm, (**B**,**C**). 0.2 mm.

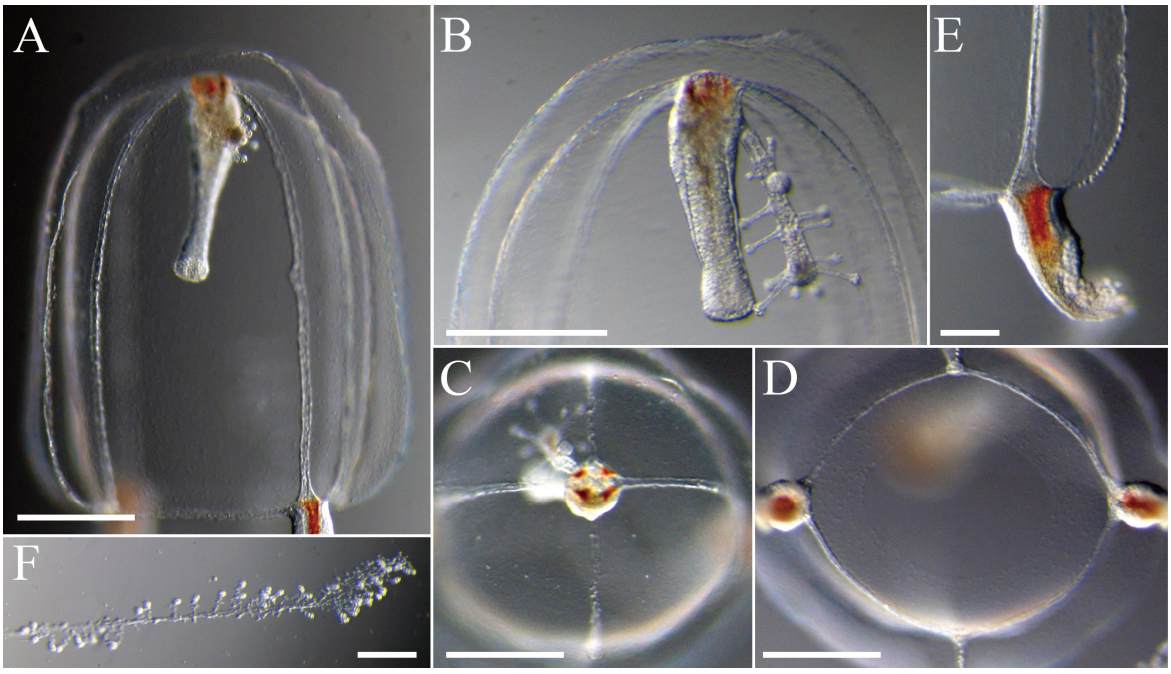

Figure 8. Live mature medusae of *Zanclea medusopolypata* (NSMT-Co1907): (**A**) exumbrella, (**B**) manubrium, (**C**) umbrella, apical view, (**D**) velum, (**E**) tentacular bulb, (**F**) tentacle. Scale bars: (**A**–**D**): 0.2 mm, (**E**,**F**): 0.1 mm.

3.3. Molecular Phylogenetics

We sequenced *Zanclea innocens*, *Z. medusopolypata*, *Z. prolifera*, and 10 Anthoathecata taxa for statistical analyses using 16S rDNA fragments. A maximum-likelihood tree was

constructed for the family Zancleidae based on the 16S rDNA sequences (Figure 9) comprising 10 major clades formed by the family Zancleidae: (1) *Z. sango* (Maldives), (2) *Z. gallii* (Maldives), (3) *Z. innocens* sp. nov., (4) *Z. medusopolypata* (Japan), (5) *Z. migottoi* (Gulf of Mexico) and *Z.* sp. (China Sea), (6) *Z. prolifera* (EU305488 and Japan), (7) *Z. giancarloi* (Mediterranean), (8) *Z. sessilis* (Roscoff and Mallorca), (9) *Z. implexa* (Norway) and *Z.* sp. (Mediterranean), and (10) *Z. costata* (Mediterranean). *Zanclea innocens* was most closely related to *Z. medusopolypata*. *Zanclea prolifera* from Japan clustered with *Z. prolifera* (EU305488; locality unknown).

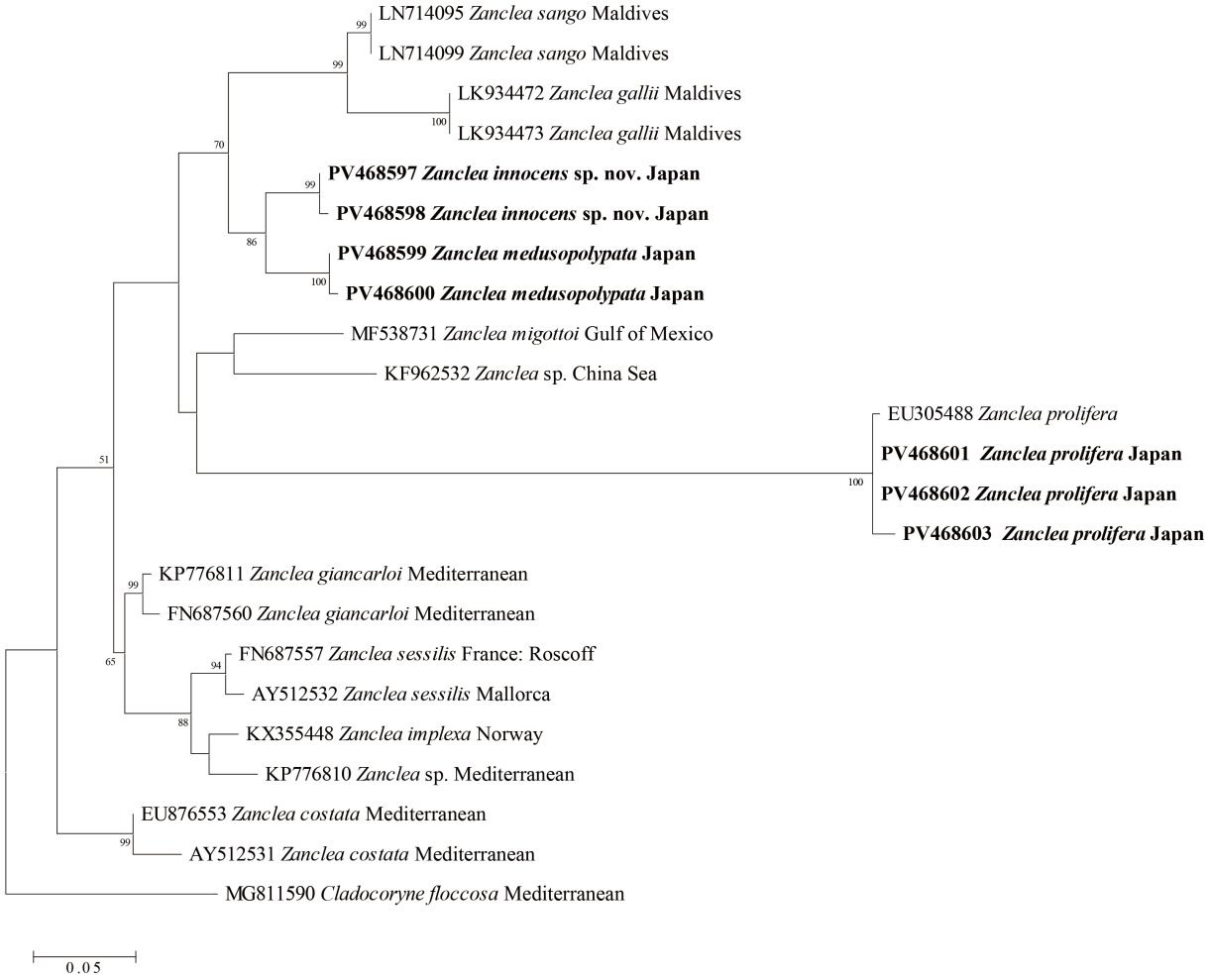

Figure 9. Maximum-likelihood tree for 12 anthomedusan taxa based on the nuclear 16S rDNA data set. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the maximum likelihood bootstrap value; only values > 50% are shown.

The Kimura two-parameter distance was 0.04–0.14 between *Zanclea innocens* n. sp. and other *Zanclea*, 0.04 between *Z. innocens* and *Z. medusopolypata*, 0.07–0.08 between *Z. innocens* and *Z. sango*, 0.08 between *Z. innocens* and Clade 5 (*Z. migottoi* and *Z.* sp.), 0.13–0.14 between *Z. innocens* and *Z. prolifera*, 0.07–0.08 between *Z. innocens* and *Z. sessilis*, 0.07 between *Z. innocens* and Clade 9 (*Z. implexa* and *Z.* sp. from Mediterranean), and 0.08–0.10 between *Z. innocens* and *Z. costata* (Table S1).

4. Discussion

4.1. Morphological Investigation

4.1.1. *Zanclea innocens* sp. nov.

A comparison of the key features of *Zanclea* species is presented in Table 4 [1,3,18,23,32,36–47]. All species of *Zanclea* have a bell-shaped umbrella, four radial canals, four exumbrellar perradial cnidocyst patches or tracts, interradial gonads, marginal tentacles with numerous abaxial extensile cnidophores, and no ocelli [11]. *Zanclea innocens* sp. nov. can be distinguished from other *Zanclea* species by exumbrella nematocyst clusters, apical projection, manubrium length, the shape of exumbrella nematocyst pouches, and the number of marginal bulbs and tentacles. Exumbrella nematocyst clusters consisting of round warts were dispersed over the middle part of the exumbrella in *Z. innocens* but sparsely dispersed in *Z. fanella*, *Z. giancarloi*, and *Z. hirohitoi* (newly released medusae). Apical projections are absent in most *Zanclea* species, but short in *Z. innocens*, *Z. bomala*, *Z. costata*, *Z. fanella*, long in *Z. apicata*, and hemisphere in *Z. macrocystae* (present in wild, absent in laboratory in *Z. polymorpha*). Manubrium length does not exceed the velum in most *Zanclea* species (ex. *Z. innocens* sp. nov., *Z. costata*, and *Z. gallii*) but not in *Z. sardii*. There are various types of exumbrella nematocyst pouches in *Zanclea* species. The exumbrella nematocyst pouches are round in *Z. alba*, *Z. bomala*, and *Z. giancarloi*. The pouches are narrow on the ridge, often reaching the umbrellar apex in *Z. innocens* and *Z. medusopolypata* and over half the UH in *Zanclea tipis*. The size of the pouches is different: two large, near tentacular bulbs on prominent apophyses, projecting transversely; two smaller, without apophyses, with short canals connected to marginal bulbs in *Z. apophysis*. Four marginal bulbs (ex. *Z. innocens* sp. nov., *Z. bomala*, and *Z. costata*), while two other species (ex. *Z. medusopolypata*, *Z. apophysis*, and *Z. gilii*). Most *Zanclea* species have two tentacles; however, *Z. bomala* and *Z.* sp. from Florida have four, whereas *Z. dubia* lacks one. On the basis of these morphological differences, the present species was identified as a new species.

Table 4. The morphology of *Zanclea* in previous and the present study. UD) umbrella diameter; UH) umbrella height.

Species	Umbrella (mm)	Apical Projection	Manubrium	Exumbrellar Nematocysts Pouches	Marginal Bulb	No. of Tentacle	No. of Cnidophore	Color	Distribution	Reference
Zanclea innocens sp. nov.	1.2–1.5 (UH) 1.2–1.4 (UD)	Present, slightly thickened (small or short)	Conical, 2/3 of subumbrellar cavity	4 Narrow, on ridge often reaching umbrellar apex	4	2	30–60	Transparent, with whitish brown in bulbs and manubrium	Japan: Kanagawa, Kochi, and Okinawa prefectures	This study
Zanclea medusopolypata	-	Absent	1/3 to 1/2	4 Narrow, on ridge often reaching umbrellar apex	2	2	Hundreds	Transparent, with reddish or orange in manubrium and marginal bulbs, white in circular canal	Atlantic Ocean (Brazil, Venezuela) (Navas-Pereira, 1984), Pacific Ocean (Bismarck Sea)	[1,44], This study
Zanclea alba	-	Absent	Unknown	4 Small, equal, near bell margin (in juvenile)	4 (in juvenile)	2 (in juvenile)	30–40	Unknown	Atlantic Ocean (Sargasso Sea)	[1,36]
Zanclea apicata	0.8–2.0 (UH) 0.6–1.5 (UD)	Present, large, long-rounded	Almost reaching velar opening	4 Ovaliform	2 opposite, perradial marginal bulbs, elongate conical	2	70–80	Unknown	China: Beibu Bay	[37]
Zanclea apophysis	1.0 (UH)	Absent	Pot-shaped, about 1/2 of subumbrellar cavity	4 Two big, near tentacular bulbs on prominent apophyses, projecting transverse, other 2 smaller, without apophyses, with short canal connected with marginal bulbs	2	2	More than 100	Unknown	China: Beibu Bay	[37]

Table 4. *Cont.*

Species	Umbrella (mm)	Apical Projection	Manubrium	Exumbrellar Nematocysts Pouches	Marginal Bulb	No. of Tentacle	No. of Cnidophore	Color	Distribution	Reference
Zanclea baudini	1.8 (UH) 1.6 (UD)	Absent	Reaching velum, mouth round, lacking peduncle	4 All on prominent, downward-facing apophyses	2 At base of tentacles with abaxial clasp	2	More than 100	Mostly white, with faint orange endoderm	Australia: Petrel Bay, St. Francis Island	[38]
Zanclea bomala	-	Present, small (short)	Reaching 2/3 of subumbrella; stomach wide	4 Round, small, on apophyses above tentacular bulbs	4	4	Hundreds	Transparent	USA: Bodega Harbor, Central California	[1]
Zanclea carinata	1.2 (UH) 1.4 (UD)	Absent	Mouth round, simple reaching 1/2 way to velum	4 Lacking in the proper sense, but crest of each paravane with narrow track	4 Two small at base of tentacles; other two about as large	2	About 50, abaxial, with about 20 adaxial papillae	White	Australia: Petrel Bay, St. Francis Island	[38]
Zanclea costata	3.0 (UH) 3.0 (UD)	Present	Tubular, 2/3 of bell cavity	4 Small, on projections, far from bell margin	4	2	Hundreds	Unknown	Mediterranean Sea, Japan	[1,39]
Zanclea cubensis	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Cuba	[40]
Zanclea dubia	1.5 mm (UH)	Absent	Long, protruding outside of subumbrella, with lips	4 Elongated, 1/3 of exumbrella	4 Two big and two small ones	Absent	Absent	Unknown	Java Sea; Indian Ocean (Bay of Bengal, Arabian Sea); Papua New Guinea (Bismarck Sea)	[1]
Zanclea exposita	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Indonesia: Bunaken Island	[41]
Zanclea fanella	-	Present, very small (in juvenile)	Conical, 2/3 of subumbrellar cavity (in juvenile)	4 Large, elongated, on 1/3 of umbrella, reaching margin (in juvenile)	2 (in juvenile)	2 (in juvenile)	50 (in juvenile)	Transparent	Pacific Ocean (Bismarck Sea)	[1]

Table 4. *Cont.*

Species	Umbrella (mm)	Apical Projection	Manubrium	Exumbrellar Nematocysts Pouches	Marginal Bulb	No. of Tentacle	No. of Cnidophore	Color	Distribution	Reference
Zanclea gallii	0.8–1.1 (UD) (in juvenile)	Absent	Cylindrical, approximately 1/4 to 1/3 of the subumbrellar cavity	4 Four perradial nematocyst pouches extend along the exumbrella. Two pouches are short and placed above non-tentaculate marginal bulbs. The two other pouches are elongated and are above large bulbs bearing tentacles	2 (in juvenile)	2 (in juvenile)	60 (in juvenile)	Unknown	Maldives	[24]
Zanclea giancarloi	0.5 (UH)	Absent	Mouth round 2/3 of subumbrella	4 Round, small, on apophyses above tentacular bulbs	4	2	About 50	Transparent	Mediterranean Sea	[1]
Zanclea gilii	-	Absent	3/4 of subumbrellar cavity	4 Two long ones, above tentacle bulbs, the other two small	2	2	Hundreds	Transparent, with white bulbs and oral region	Pacific Ocean (Bismarck Sea)	[1]
Zanclea hirohitoi	-	Absent	Cylindrical, about half of subumbrellar cavity	4 Two above the two perradial tentacular bulbs slightly larger than the other two	2 (in juvenile)	2 (in juvenile)	About 20	Transparent	Pacific Ocean Qapan; Papua New Guinea, Bismarck Sea)	[1,19]
Zanclea macrocystae	1.5 (UH) 1.1 (UD)	Present, hemisphere	2/5 of subumbrellar cavity	4 Two large pouches of cnidocyst upon two opposite marginal bulbs and two small pouches of cnidocyst upon two opposite rudimentary marginal bulbs	2	2	50–60	Unknown	Southern China: Minnan-Taiwan Bank fishing ground	[42]

Table 4. *Cont.*

Species	Umbrella (mm)	Apical Projection	Manubrium	Exumbrellar Nematocysts Pouches	Marginal Bulb	No. of Tentacle	No. of Cnidophore	Color	Distribution	Reference
Zanclea margarita	0.4–0.6 (UD) (in juvenile)	Absent	Conical, extend to the full length of the umbrella	4 Extending the length of the bell	4	2	Unknown	Tentacular bulb white, manubrium pink	Coral Gardens forereef site, Heron Island, Australia	[43]
Zanclea migottoi	1.8 (UH) 1.6 (UD)	Absent	Nematocysts around the mouth, short, smooth	4 Exumbrella with four perradial exumbrellar nematocyst tracks arising from bell margin and reaching to upper part of bell	2	2	Many	Manubrium and tentacle base reddish in the water	The Azores, Caribbean Sea, Brazil	[3,32,45,46]
Zanclea ngeriana	1.0 (UH)	Present, thickened	Simple, round month, reaching velum	4 Two tentacular about 2x the size of the other two	4 Two at base of tentacles short	2	About 20	White	Australia: Petrel Bay, St. Francis Island	[38]
Zanclea polymorpha	1.6 (UH) 1.6 (UD)	Present in wild, absent in laboratory reared medusae	Almost reaching velar opening	4 Reduced to narrow bands	4	2	About 70	Unknown	New Zealand (Wellington Harbour, Leigh Marine Reserve, South Island)	[1,47]
Zanclea prolifera	2.8–3.7 (UH) 3.2–3.5 (UD)	Absent in mature (Present, narrow in juvenile)	Tubular, beyond 1/3 of subumbrella cavity	4 Ellipsoidal or spoon-like	4	2	35–65	Tentacular bulb and manubrium pale brownish	Japan: Misaki, Sagami Bay	[18]
Zanclea retractilis	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Pacific Ocean (Bismarck Sea)	[1]
Zanclea sango	0.5–0.8 (UD) (in juvenile)	Absent	200 µm in length when extended	4 Perradial	4	2	About 30	The manubrium with white oral region	Japan: Okinawa; Maldives	[20,24]

Table 4. *Cont.*

Species	Umbrella (mm)	Apical Projection	Manubrium	Exumbrellar Nematocysts Pouches	Marginal Bulb	No. of Tentacle	No. of Cnidophore	Color	Distribution	Reference
Zanclea sardii	3.5 (UH) 2.6 (UD)	Absent	Protruding beyond velum	4 Two tentacular about 2x the size of the other two	4 Two fully developed. Plus two rudimentary	2	About 100	Tentacle bulbs purple, pink, orange	Australia: Petrel Bay, St. Francis Island	[38]
Zanclea sessilis	-	Absent	Reaching velar opening	4 Two long and two short, linear, above bulbs	4	2	Hundreds	Transparent at liberation greenish when adult	Mediterranean Sea	[1]
Zanclea tipis	0.5 (UD) (in juvenile)	Absent	Short, about one-third of the subumbrellar cavity	4 White very long, reach over half the umbrella height	2	2	About 40	Manubrium upper portion has little whitish spots	Indonesia: Bunaken Island	[41]

4.1.2. *Zanclea medusopolypata*

Zanclea medusopolypata from Laing Island, Papua New Guinea was described by Boero et al. [1]. The morphological inspection of *Z. medusopolypata* from Japan agreed well with the morphological description of the medusa stage by Rees and Roa [44] and Boero et al. [1]. This species bears medusa buds in clusters at the base of the hydranths. In the present study, medusa buds were observed in both the base and middle parts of the hydranths. However, the development of medusa buds is yet to be observed.

Budding of polyps from the base of manubrium has been observed in *Teissiera polypofera*, *Apatizanclea mayeri*, and *Zanclea* sp. off the coast of Florida. The medusae of *T. polypofera* have ocelli, which distinguish *Teissiera* medusae from those of *Zanclea*. *Apatizanclea mayeri* was previously described as belonging *Zanclea* [29]; however, it is now classified as *Apatizanclea* [3]. *Zanclea* sp. from Florida resembles *Z. medusopolypata*; however, *Zanclea* sp. has four tentacles [4].

4.1.3. Cnidomes

Cnidomes are one of the most important characteristics for the identification of Hydrozoa. Two types of nematocysts, namely macrobasic euryteles and stenoteles, were examined in medusa of *Z. innocens*. *Zanclea medusopolypata* has bean-shaped apotrichous macrobasic euryteles and stenoteles (two sizes) [44]. Apotrichous macrobasic euryteles were found in *Z. gilii* and *Z. hirohitoi*, whereas stenoteles were found in *Z. giancarloi* and *Z. sessilis* [1,19,24].

4.2. Molecular Phylogenetic Analysis

The paraphyly of *Zanclea*, including *Zanclea innocens* and nine other species (*Z. sango*, *Z. gallii*, *Z. medusopolypata*, *Z. migottoi*, *Z. prolifera*, *Z. giancarloi*, *Z. sessilis*, *Z. implexa*, and *Z. costata*) was evident in the 16S rDNA phylogenetic tree with high bootstrap values, supporting the validity of the new species.

Kimura two-parameter distance was 0.04–0.14 between different *Z. innocens* and other *Zanclea* species. In the class Hydrozoa, the interspecific and intraspecific variability were 0.06–0.64 and 0.00–0.02, respectively [48]. Given the results of the K2P distances in this study, *Z. innocens* is considered a genetically independent species in the genus *Zanclea*.

5. Conclusions

Our morphological and molecular phylogenetic analyses suggest that the specimens collected from the coasts of Kanagawa, Kochi, and Okinawa prefectures in Japan are new species belonging to the genus *Zanclea*. In addition, we report for the first time *Zanclea medusopolypata* from the Shizuoka and Nagasaki prefectures in Japan. The medusae of *Zanclea innocens* sp. nov. appeared in the shallow waters of Japan in April and November. However, polyps of this species have not yet been detected in the wild. Polyps of *Zanclea* species can be symbiotic with bryozoans, bivalves, or corals; however, few species are substrate specialists [1]. Further investigations, including those of polyps and their life cycles, are necessary to gain insights into the ecology of this new species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/taxonomy5020022/s1, Table S1: Pairwise genetic distances (K2P) based on 326 positions of 16S sequences among Anthomedusae.

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Data Availability Statement: All datasets collected and analyzed in the current study are available from the corresponding author upon request.

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