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Tima nigroannulata (Cnidaria: Hydrozoa: Eirenidae), a New Species of Hydrozoan from Japan

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Tima nigroannulata sp. nov. is described from medusae collected in shallow waters of four prefectures on the Pacific coast of Japan (Miyagi, Fukushima, Kanagawa, and Miyazaki), as well as from cultures maintained at two aguaria (Enoshima Aguarium, Kanagawa Prefecture; Tsuruoka City Kamo Aquarium, Yamagata Prefecture). Adult medusae differ from those of other known species of the genus Tima Eschscholtz, 1829 in the following combination of characters: (1) umbrella usually hemispherical or higher, (2) marginal tentacles up to 50 or more in number; and (3) black pigment granules form a ring around the umbrella rim, and sometimes extend onto the tentacles and radial canals. Their hydroids, from aquarium cultures, have stolonal colonies with pedicels of varied length, vestigial hydrothecae, slender and vase- to club-shaped hydranths, and a whorl of about 20 filiform tentacles with an intertentacular web basally. Medusa buds develop singly within gonothecae that arise from the hydrothecal pedicels. The cnidomes of both hydroid and medusa stages comprise heteronemes, provisionally identified as microbasic mastigophores. Medusae of T. nigroannulata are confirmed as a unique, cohesive lineage by comparing mtDNA COI sequence fragments with those from two congeners, resulting in three well-supported reciprocally monophyletic clades, one representing each species. Records of the western Atlantic medusa Tima formosa L. Agassiz, 1862 from Japan overlap those of T. nigroannulata, and are believed to have been based on the new species described herein.

Key words: Hydroidolina, hydromedusae, Leptothecata, marine biology, marine invertebrates, Medusozoa, plankton, taxonomy

INTRODUCTION

Hydromedusae comprise a diverse faunal group in the marine plankton of Japan (Kubota and Gravili, 2007). In the leptothecate family Eirenidae Haeckel, 1879 alone, seven described and one incompletely identified species have been reported from coastal waters of the country: *Eirene hexanemalis* (Goette, 1886) from Amakusa, *Eirene lacteoides* Kubota and Horita, 1992 from Toba Aquarium, *Eirene menoni* Kramp, 1953 from Amakusa, *Eutima japonica* Uchida, 1925 from off Hokkaido, *Eutonia indicans* (Romanes, 1876) from the north of Japan to Onagawa Bay, *Tima formosa* L. Agassiz, 1862 from Hokkaido to Onagawa Bay, *Eugymnanthea japonica* Kubota, 1979 from central Japan (see Kubota and Horita, 1992), and *Helgicirrha* sp. from an unstated location (Kubota and Gravili, 2007). *Tima saghalinensis* Bigelow, 1913 was originally described from waters off a sector of Saghalin Island (= Sakhalin Island) then occupied by Japan (as Karafuto Prefecture) but assimilated into Russia after 1945. As for *T. formosa*, the species was originally described from Massachusetts Bay, USA (Agassiz L, 1862), with a distribution likely restricted to northeastern North America (Petersen, 1962: p. 107). Records of this hydrozoan from Japan (and China) were considered by Petersen to be "very doubtful".

This study was undertaken to establish the identity of medusae from Japan, earlier assigned to *Tima formosa*, by examining specimens from four locations in the country. These medusae were compared with accounts of the species from Japan (Uchida, 1925, 1938), and with descriptions

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of *T. formosa* from the northeastern United States (Agassiz L, 1862; Agassiz A, 1865; Mayer, 1910; Petersen, 1962). Molecular analyses were undertaken to complement morphological studies in establishing the identity, distinctness, and genetic affinities of the species.

In being distinct from *T. formosa*, and from all other known species of the genus, the Japanese hydromedusa population studied here is recognized and described as a new species. Also described for the first time is its hydroid stage. Hydroids referable to *Tima* are otherwise very poorly known, with the only previous account being a brief description of the hydroid of *T. formosa* from Massachusetts, USA, by A. Agassiz (1865).

MATERIALS AND METHODS

Collecting and culturing

Hydromedusae examined here were collected using a dipnet, or a submerged open-top container on a stick, from shallow waters (0–1 m) at four locations on the Pacific coast of Japan (Fig. 1; Table 1). Specimens were cultured either in a pseudokreisel or in kreisel aquaria (Hamner, 1990; Raskoff et al., 2003) at both the Enoshima Aquarium and the Tsuruoka City Kamo Aquarium. Monoculture holding tanks at the Kamo Aquarium were maintained for multiple generations utilizing hydroid cultures established from known medusae, collected at Iwaki, Fukushima Prefecture, in 2005. Hydroids at the Enoshima Aquarium and the Kamo Aquarium were fed a Vietnamese strain of *Artemia* nauplii once a day. Water quality was maintained with weekly water changes for hydroids, and medusae were housed in open system separate aquaria. Hydroids and medusae were maintained at an average temperature of 20°C and at salinities of 30–35%. Specimens for morphological and nematocyst studies were preserved in 3–4% formalin. Those for molecular analyses were preserved in > 85% ethanol.

Morphology and systematics

Preserved specimens from four locations in Japan were examined to characterize their morphology and to establish their identity. Medusae of *Tima* from the country had earlier been assigned to *T. formosa*, a species with its type locality in Massachusetts Bay on the Atlantic coast of the United States (Agassiz L, 1862). Medusae from the collections were compared morphologically with descriptions of those assigned to the species from Japan (Uchida, 1925, 1938) and with original accounts of *T. formosa* from the eastern USA (Agassiz L, 1862; Agassiz A, 1865; Mayer, 1910; Petersen, 1962). Specimens of the hydroid stage of the species, from cultures at the Kamo Aquarium, were examined and described. Characters employed in descriptions of medusae and hydroids follow those listed in Bouillon et al. (2006: p. 83–88).

Type specimens, and others, have been deposited in collections within the Section of Invertebrate Zoology, Department of Natural History, Royal Ontario Museum, Toronto, Canada. Collection numbers have been assigned the prefix ROMIZ (Royal Ontario Museum, Invertebrate Zoology).

Illustrations of nematocysts herein, all to the same magnification, are from photomicrographs originally taken at \times 1000 using a Zeiss Axioscop microscope. Nematocyst classification follows Weill (1934) and Östman (1979a, b, 1982, 1999).

All cited references have been examined, unless otherwise



Fig. 1. Map of Japan, showing historical distributional records of medusae identified as ?*Tima formosa* (TFJ), and sampling localities of *Tima nigroannulata* (TNJ). ▲ TFJ = ?*Tima formosa* (literature records of medusae, Japanese waters): **TFJ1** - Oshoro, Hokkaido (Uchida, 1925), collected in 1922 (43.3°N, 140.7°E); **TFJ2** - Onagawa Bay, Mangoku-ura (Uchida, 1938), collected in 1936 (38°N, 141°E); **TFJ3** - Oarai Fishing Port, Ibaraki (Saito and Shiba, 2008), collected 2007 (36°18′N, 140°34′E); **TFJ4** - Between Katsura-Jima Island and Mahahashi-Jima Island (Kubota and Yamazaki, 2007), collected in 1931 (34.5°N, 139°E); **TFJ5** - Lake Hamana (brackish), Shizuoka (Okamoto et al., 2016), (34°44′N, 137°34′E). ● TNJ = *Tima nigroannulata* (medusae from this study, Japanese waters): **TNJ1** – Sendai, Miyagi Prefecture (38°16′24.9″N, 141°00′19.7″E); **TNJ2** – Iwaki, Fukushima Prefecture (36.9°N, 140.9°E); **TNJ3** – Fujisawa, Kanagawa Prefecture (35°18′24.3″N, 139°28′53.8″E); **TNJ4** - Miyazaki, Miyazaki Prefecture (31°54′29″N, 131°27′29.3″E).

specified, and illustrations are based on specimens examined as part of his study.

DNA extraction, PCR amplification, and DNA sequencing

Whole specimens of hydromedusae from Japan (Table 2) were removed from the ethanol storage buffer and rinsed in deionized distilled autoclaved water. Genomic DNAs were extracted from each whole organism using a Macherey-Nagel NucleoSpin[®] Mini kit for DNA from cells and tissue (Takara Bio, Inc. USA, 1290 Terra Bella Ave., Mountain View, CA 94043, USA; Reference #740952.50) according to the manufacturer's protocol. Genomic DNA was eluted in 200 μ l of deionized autoclaved water and stored at -20° C.

Fragments of 613 basepairs (bp) of the mitochondrial DNA (mtDNA) cytochrome *c* oxidase I (COI) gene were amplified by polymerase chain reaction (PCR) using the primers LCO1490/ HCO2198 (Folmer et al., 1994). Target fragments were amplified using a MyCyclerTM Thermal Cycler (Bio-Rad, Hercules, CA, USA) with Conquest PCR Master Mix Optimizing Pack reagents and buffers from Lamda Biotech (catalog # D911-Mix1234). PCRs were conducted at Hawaii Pacific University's Oceanic Institute, in individual volumes of 20 µl each under the following conditions: 4 min at 94°C, 33 cycles of 94°C for 40 s, 52°C for 1 min and 72°C for 90 s, with a final 72°C extension for 5 min.

PCR fragments were purified with Macherey-Nagel NucleoSpin[®] Gel and PCR Clean-up columns (Takara Bio, Inc. USA, 1290 Terra Bella Ave., Mountain View, CA 94043, USA; Reference #740609.50), per the manufacturer's protocol, and visualized via agarose minigel electrophoresis. Amplified mtDNA fragments were sequenced using the same primer pair as the initial PCRs. DNA sequencing was performed at the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at the University of Hawaii, Manoa.

Phylogenetic analysis

RAxML (Randomized Axelerated Maximum Likelihood) (Stamatakis, 2014) and MEGA X (Molecular Evolutionary Analysis) (Kumar et al., 2018) were used for phylogenetic reconstruction; statistical support was assessed with 1000 bootstrap replicates. We compared topologies among maximum parsimony, minimum evolution, and maximum likelihood. In instances where different methods resulted in identical tree topologies, we have high confidence that the results are representative of the evolutionary history of the sequences comprising the data set (Holland and Hadfield, 2004), whereas differences in results indicate that more than one interpretation is possible. Tree topologies were extremely consistent, well resolved, and well supported.

RESULTS

Taxonomic account

Phylum **Cnidaria** Verrill, 1865 Subphylum **Medusozoa** Petersen, 1979 Class **Hydrozoa** Owen, 1843 Subclass **Hydroidolina** Collins, 2000 Order **Leptothecata** Cornelius, 1992 Family **Eirenidae** Haeckel, 1879

Irenidae Haeckel, 1879, p. 199 [emended to Eireninae by Mayer (1910)].

Diagnosis (after Kramp, 1961; Calder, 1991; Cornelius, 1995; Bouillon et al., 2006).

Hydroids typically forming stolonal or erect colonies arising from a creeping hydrorhiza, or occurring as simple, solitary commensals; one unusual species (*Eirene hexanemalis*) planktonic, without a hydrorhiza, with hydroid metamorphosing directly into a medusa. Hydrothecae when present pedicellate with a basal diaphragm; comprising either a vestigial collar at hydranth base or with cylindrical walls and an operculum of folded flaps indistinctly separated from hydrothecal rim, later reduced to a collar surrounding hydranth base. Hydranths extensile, with a whorl of amphicoronate filiform tentacles having an intertentacular web; hydranths of commensal species sessile, naked, usually solitary, attached to host by a basal disc.

 Table 1.
 Collection data for specimens of *Tima nigroannulata* sp. nov. examined here.

Location	Prefecture	Geographic Coordinates	Date	Collector	Depth	Туре
Miyazaki	Miyazaki	31°54′29″N, 131°27′29.3″E	29-Apr-20	Y Kawagoe	0–1 m	wild
Fujisawa	Kanagawa	35°18'24.3"N, 139°28'53.8"E	29-Apr-20	G Yamamoto	0–1 m	wild
Iwaki	Fukushima	36.9°N, 140.9°E	2005	S Mizutani	cultured	aquarium
Sendai	Miyagi	38°16'24.9"N, 141°00'19.7"E	12-Jan-20	S Ikeda	0–1 m	wild

Gonophores free medusae or medusoids, arising from hydranth, hydrocaulus, hydranth pedicel, or hydrorhiza; gonothecae present or absent. Medusae with small or smallish manubrium at tip of a distinct gastric peduncle; radial canals simple, four-six in number; marginal

Table 2. Taxa included in phylogenetic analyses, with GenBank accession numbers, sampling locations, and GPS coordinates.

ID #	Species	GenBank Accession #	Location	Geographic Coordinates
TBS1	Tima bairdii	MF00509	Fanafjord, Norway	60.2°N, 5.2°E
TBS2	Tima bairdii	MG935038	Skagerrak, Sweden	58.3°N, 10.5°E
TFC1	?Tima formosa	JQ716166	Changjiang River Estuary, China	31.5°N, 122.15°E
TFC2	?Tima formosa	JQ716168	Jiaozhou Bay, China	36.1°N, 120.25°E
TFC3	?Tima formosa	JQ716169	Jiaozhou Bay, China	36.1°N, 120.25°E
TFC4	?Tima formosa	JQ716170	Jiaozhou Bay, China	36.1°N, 120.25°E
TNJ1	Tima nigroannulata, sp. nov.	MW490678	Sendai, Japan	38°N, 141°E
TNJ2	Tima nigroannulata, sp. nov.	MW490677	Fukushima, Japan	36.9°N, 140.9°E
TNJ3	Tima nigroannulata, sp. nov.	MW490679	Fukushima, Japan	36.9°N, 140.9°E
AA	Alatina alata	KM200330	Waikiki Beach, Hawaii	21.276°N, 157.827°W

Note: Sequences obtained in this study are in bold. Hydrozoans identified as ?Tima formosa are of questionable specific identity.

tentacles hollow; bases of tentacles with or without excretory pores; marginal cirri and marginal warts present or absent; statocysts closed, eight or more in number; gonads on radial canals, not extending onto manubrium. Ocelli lacking.

Remarks. Hydrozoans of the family Eirenidae Haeckel, 1879 are much better known from the medusa stages than from their hydroids. The group is typically distinguished by having (1) medusae with a typically well-developed gastric peduncle; gonads occurring along the entire length of foursix simple radial canals; eight to many epidermal closed statocysts; hollow marginal tentacles; (2) hydroids with diaphanous gonothecae and reduced to vestigial hydrothecae (Cornelius, 1995; Bouillon et al., 2006). Following Bouillon (1985), the family name Eutimidae Haeckel, 1879 is currently included as a synonym of Eirenidae (e.g., Cornelius, 1995; Bouillon et al., 2006; Schuchert, 2021). However, Eirenidae as presently constituted has been shown to be polyphyletic, with two groups, "Eirenids I" and "Eirenids II", distinguished in molecular analyses by Maronna et al. (2016). Further refinement of eirenid systematics is warranted. See also remarks below.

Genus Tima Eschscholtz, 1829

Tima Eschscholtz, 1829, p. 103.

Type species. *Tima flavilabris* Eschscholtz, 1829, by monotypy.

Diagnosis (after Kramp, 1961; Cornelius, 1995; Bouillon et al., 2006). Hydroids inadequately described; where known, occurring as stolonal colonies with characters of other free-living species of the family.

Gonophores free medusae. Where known, gonothecae pedicellate, clavate, with thin, filmy perisarc, arising from hydrothecal pedicel. Medusae with characters of the family, with a well-developed gastric peduncle, four radial canals, and numerous (> eight) marginal vesicles and marginal warts; without lateral and marginal cirri and excretory pores; tentacle bases and tentacles with an abaxial longitudinal furrow; gonads extending along entire length of radial canals.

Remarks. Four species are currently assigned to Tima Eschscholtz, 1829 besides T. nigroannulata sp. nov. from Japan. Their type localities and reported distributions are as follows. (1) Tima flavilabris Eschscholtz, 1829, type species of the genus (type locality: North Atlantic Ocean northeast of the Azores); reported from the central and northeast North Atlantic (Petersen, 1957, 1962; Winkler, 1982; Bleeker and van der Spoel, 1988). (2) Tima bairdii (Johnston, 1833) (type locality: Berwick Bay, UK, surface); reported from the North Sea and vicinity from southern Norway to Belgium, and eastwards to the Baltic Sea off Germany (Cornelius, 1995). (3) Tima formosa L. Agassiz, 1862 (type locality: Massachusetts Bay, USA); reported from the Atlantic coast of

North America from southern New England (Petersen, 1962) to Nova Scotia (Stafford, 1907); as noted earlier, records from Japan and China have been considered doubtful (Petersen, 1962), as is a record from Bermuda (Fewkes, 1883). (4) *Tima saghalinensis* Bigelow, 1913 (type locality: off Sakhalin Island, Russia); reported from the Sea of Okhotsk (Naumov, 1960; Petersen, 1962; Zavolokin, 2010, as *Tima sachalinensis*). Medusae identified as *T. formosa* from China may be different from both the Atlantic species of that name and from the Japanese *T. nigroannulata*.

Conflicting results exist in previous phylogenetic studies regarding whether Tima should be assigned to "Eirenids I" or "Eirenids II" of Maronna et al. (2016). Zheng et al. (2014) included a medusa identified as T. formosa from China in a clade with Eirene kambara Agassiz and Mayer, 1899 and Eirene hexanemalis (Goette, 1886), species included by Maronna et al. (2016) in "Eirenids I". Schuchert et al. (2017) included T. bairdii in a clade with Eucheilota menoni Kramp, 1953, listed in "Eirenids II" by Maronna et al. (2016). The phylogenetic tree presented herein includes the cubozoan outgroup Alatina alata (Reynaud, 1830), and consists of three distinct, reciprocally monophyletic clades each with 100% bootstrap support (1000 replicates) (Fig. 2). The COI sequences for T. bairdii and T. nigroannulata also form a well-supported clade, suggesting that these two lineages are sister species. A combined molecular systematic and





morphological comparison of the species included here, together with *T. flavilabris* Eschscholtz, 1829 (type species of *Tima*) and species assigned to both *Tima* and *Eirene* Eschscholtz, 1829, would be taxonomically informative. Published tree topologies present conflicting results in the generic assignment of species within this group.

Medusae of the genus sometimes occur in significant numbers. Blooms of *T. bairdii* have been reported to interfere at times with fishing efforts in the Skagerrak, Scandinavia, and the southern Baltic Sea (Russell, 1970; Cornelius, 1995), and they occasionally strand on British shores in substantial numbers (Russell, 1953). According to Mayer (1910), *T. formosa* is common in Buzzards Bay, Massachusetts, and Newport Harbor, Rhode Island, USA, in April and May. *Tima saghalinensis* was found to be a dominant medusa in the central Sea of Okhotsk, Russia (Zavolokin, 2010).

Because of their large size, species of *Tima* have been used in exhibits at aquaria worldwide. Juveniles examined here were from cultures maintained at both the Tsuruoka City Kamo Aquarium and the Enoshima Aquarium, Japan.

Tima nigroannulata sp. nov. Figs. 3–9

?Tima formosa: Uchida (1925), p. 92; Uchida (1938), p. 53; Uchida (1940), p. 281; Kubota (1997), pp. 496, 534, figs.
4-40; Kubota and Yamazaki (2007), p. 6, pl. 2, fig. 5; Kubota and Gravili (2007), p. 194; Saito and Shiba (2008), p. 8; Murakami (2016), p. 71; Okamoto et al. (2016), p. 32; Yamamori et al. (2017), p. 175 [not *Tima formosa* L. Agassiz, 1862].

Type locality. Japan: Kyushu, Miyazaki Prefecture, Miyazaki, 31°54′29.0″N, 131°27′29.3″E, surface (0–1 m), < 1 m from shore.

Material examined. *Holotype*. ROMIZ B5390, Japan, Kyushu, Miyazaki Prefecture, Miyazaki, 31°54′29.0″N, 131°27′29.3″E, surface (0–1 m), < 1 m from shore, 29 April 2020, coll. Yoshiki Kawagoe, one medusa: umbrella 4.5 cm wide, 3 cm high, with 53 marginal tentacles, adult female.

Paratypes (13 medusae and several colonies of hydroids, in five lots). ROMIZ B5391, Japan, Miyazaki Prefecture, Miyazaki, 31°54'29.0"N, 131°27'29.3"E, surface (0-1 m), < 1 m from shore, 29 April 2020, coll. Yoshiki Kawagoe, three medusae: (1) umbrella 4.6 cm wide, 3.8 cm high, with 45 marginal tentacles, adult male; (2) umbrella 3.6 cm wide, 2.1 cm high, with 44 marginal tentacles, adult male; (3) umbrella 2.3 cm wide, 1.2 cm high, with 30 marginal tentacles, adult female. ROMIZ B5392, Japan, Kanagawa Prefecture, Fujisawa, 35°18'24.3"N, 139°28'53.8"E, surface (0-1 m), < 1 m from shore, 15.1°C, 29 April 2020, coll. Gaku Yamamoto, five medusae: (1) umbrella 4.5 cm wide, 2.5 cm high, with 38 marginal tentacles, adult male; (2) umbrella 2.9 cm wide, 1.5 cm high, with 30 marginal tentacles, adult male; (3) umbrella 2.0 cm wide, 1.2 cm high, with 22 marginal tentacles, juvenile; (4) umbrella 5.0 cm wide, 2.0 cm high, with 42 marginal tentacles, adult female; (5) umbrella 3.8 cm wide, 1.8 cm high, with 34 marginal tentacles, adult male. ROMIZ B5393, Japan, Fukushima Prefecture, Iwaki, 36.9°N, 140.9°E, in cultures at Kamo Aquarium, preserved 11 June 2020, cultures originating from medusae coll. 2005 by S. Mizutani, five medusae: (1) umbrella 2.5 cm wide, 1.5 cm high, with 36 marginal tentacles, juvenile; (2) umbrella 2.7 cm wide, 1.6 cm high, with 34 marginal tentacles, juvenile; (3) umbrella 2.6 cm wide, 1.4 cm high, with 36 marginal tentacles, juvenile; (4) umbrella 2.5 cm wide, 1.5 cm high, with 36 marginal tentacles, juvenile; (5) umbrella 2.1 cm wide, 1.5 cm high, with 38 marginal tentacles, juvenile. ROMIZ B5395, Japan, Kanagawa Prefecture, Fujisawa, 35°18'24.3"N, 139°28'53.8"E, fragments of a hydroid colony or colonies, with one gonophore, raised at Enoshima Aquarium from medusae collected 29 April 2020; coll. Gaku Yamamoto. ROMIZ B5396, Japan, Miyazaki Prefecture, Miyazaki, 31°54'29.0"N, 131°27'29.3"E, dense hydroid colony or colonies, with gonophores, raised at Enoshima Aquarium from medusae collected 29 April 2020, coll. Yoshiki Kawagoe.

Other material (four medusae and several colonies of hydroids, in five lots). ROMIZ B5394, Japan, Fukushima Prefecture, Iwaki, 36.9°N, 140.9°E, in cultures at Kamo Aquarium, cultures originating from medusae coll. 2005 by S. Mizutani, four medusae: (1) umbrella 2.3 cm wide, 1.7 cm high, with 33 marginal tentacles, juvenile; (2) umbrella 2.2 cm wide, 1.2 cm high, with 38 marginal tentacles, juvenile; (3) umbrella 2.0 cm wide, 1.4 cm high, with 41 marginal tentacles, juvenile; (4) umbrella 2.3 cm wide, 1.4 cm high, medusa fragmentary and missing tentacles, juvenile. ROMIZ B5397, Japan, Fukushima Prefecture, Iwaki, in cultures at Kamo Aquarium, cultures originating from medusae coll. 2005 by S. Mizutani, several hydroid colonies or colony fragments, to 1.2 mm high, without gonothecae.

Etymology. The specific name is an adjective derived from the Latin words *niger* (black) and *annulus* (ring), in reference to the ring of black pigment occurring around the periphery of the umbrella of this species.

Common name. Elegant jellyfish (Fig. 3), a name applied to medusae of the species in Japan; giyaman-kurage in Japanese.

Description of advanced juvenile medusae (based



Fig. 3. *Tima nigroannulata*, adult medusa (non-type). Drawn from a photograph (by S. Ikeda) of a specimen collected 12 January 2020 at Sendai, Japan (38°16′24.9″N, 141°00′17.9″E).

on paratype material, ROMIZ B5393, and non-type material, ROMIZ B5394. Fig. 4, non-type; Fig. 5A, B, non-type). Umbrella higher than hemisphere, with regular curvature, diameter at margin 20–27 mm, height 12–17 mm, mesoglea thick at apex, gradually thinning towards periphery. Gastric peduncle well-developed, conical, gradually tapering, 6–11 mm long, 9–15 mm in diameter at base, varied in length but usually extending beyond subumbrellar cavity. Tentacles varying from 33–41 in number, with four of them perradial



Fig. 4. *Tima nigroannulata*, advanced juvenile medusa (non-type), raised at the Enoshima Aquarium (photograph by G. Yamamoto).

and given off immediately below bases of radial canals, each with median abaxial groove; proximal ends of tentacles oblong-oval, elongated, fleshy, laterally compressed, projecting inwards, unevenly spaced, usually separated but occasionally arising side-by-side, outer edges partly adhering to exumbrella; part of tentacles beyond base varying greatly in length but usually guite long, wrinkled, thread-like, widest basally, thinnest at tip, irregularly segmented, densely armed with nematocysts throughout. Lateral cirri and marginal cirri lacking; excretory pores lacking. Marginal warts conical with rounded tips, varying in size and number, with zero-five between tentacles, appearing to increase in number with increasing size of medusa, occasional larger ones appearing to develop tentacles. Statocysts ectodermal, closed, oval to almost spherical, varied in size, 65-90 in number but numbers appearing to increase with increasing size of medusae, usually alternating with tentacles and marginal warts, but their spacing uneven; number of statoliths indeterminable in preserved specimens. Manubrium located at tip of gastric peduncle, short in preserved specimens, 1.5-3 mm long, cruciform in cross section, with bulbous pouches extending onto bases of radial canals, hanging partly or completely below velar opening; lips four, perradial, long, folded, curtain-like, with frilly edges. Radial canals four, slender, straight, widening slightly at insertion with manubrium, elsewhere nearly equal in diameter. Circular (ring) canal distinct. Velum thin, with wide velar opening. Gonads lacking. Color of tentacles, marginal warts, radial canals, manubrium, and lips whitish in preserved specimens; umbrella margin in all medusae with prominent ring of black pigment granules, extending over bases of tentacles and sometimes continuing outwards onto tentacles, pigmentation of ring becoming denser towards periphery; umbrella, gastric peduncle, and velum glassy and transparent. Living medusae from cultures with tan tentacular bases, with color likely influenced by digested prey.

Cnidome on tentacles (undischarged), Fig. 6A–C; nontype, ROMIZ B5394. Large microbasic mastigophores 16.4– 20.2 µm long \times 2.6–2.9 µm wide (n = 6); medium microbasic mastigophores 12.0–15.0 µm long \times 2.1–2.6 µm wide (n = 10); small microbasic mastigophores 9.0–10.8 µm long \times 1.7–2.1 µm wide (n = 10).

Cnidome on oral arms (undischarged), Fig. 6D; non-



Fig. 5. *Tima nigroannulata.* (A, B) oral view of part of rim of umbrella (non-type, ROMIZ B5394), showing bases of tentacles (tb), circular canal (cc), incipient marginal wart (mw), statocysts (sc), pigment particles (pp), and part of velum (ve), lwaki, Fukushima Prefecture, Japan.



Fig. 6. *Tima nigroannulata*, nematocysts of juvenile and adult medusae, and hydroids, all to the same magnification. (A) large microbasic mastigophore, juvenile medusa (non-type, ROMIZ B5394), tentacle, Fukushima Prefecture, Japan. (B) medium microbasic mastigophore, juvenile medusa (non-type, ROMIZ B5394), tentacle, Fukushima Prefecture, Japan. (C) small microbasic mastigophore, juvenile medusa (non-type, ROMIZ B5394), tentacle, Fukushima Prefecture, Japan. (C) small microbasic mastigophore, juvenile medusa (non-type, ROMIZ B5394), tentacle, Fukushima Prefecture, Japan. (D) small microbasic mastigophore, juvenile medusa (non-type, ROMIZ B5394), oral arm, Fukushima Prefecture, Japan. (E) large microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (F) medium microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (G) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, adult medusa (paratype, ROMIZ B5391), tentacle, Miyazaki Prefecture, Japan. (H) small microbasic mastigophore, hydranth of hydroid (paratype, ROMIZ B5396), Miyazaki Prefecture, Japan.

type, ROMIZ B5394. Small microbasic mastigophores 10.6– 11.8 μ m long \times 1.9–2.5 μ m wide (n = 10).

Description of adult medusae (based on holotype, ROMIZ B5390, and paratype material, ROMIZ B5391, ROMIZ B5392. Fig. 3, non-type). Umbrella usually higher than hemisphere, with regular curvature; diameter at margin 23–46 mm, height 12–38 mm, mesoglea thick at apex, gradually thinning towards periphery. Gastric peduncle welldeveloped, conical, gradually tapering, 7–17 mm long, 11–21 mm in diameter at base, of varied length but usually extending to or beyond umbrella margin. Tentacles varying in number from 30 to 53, with four of them perradial and given off immediately below bases of radial canals, each with median abaxial groove; proximal ends of tentacles oblong-oval, elongated, fleshy, laterally compressed, projecting inwards, unevenly spaced, usually separated but occasionally arising side-by-side, outer edges partly adhering to exumbrella; part of tentacles beyond base varying greatly in length but usually quite long, wrinkled, thread-like, appearing irregularly segmented, widest basally, thinnest near tip, densely armed with nematocysts throughout. Lateral cirri and marginal cirri lacking; excretory pores lacking. Marginal warts conical with rounded tips, varying considerably in size, 42–115 in number, with zero-five between tentacles, increasing in number with increasing size of medusa, occasional larger ones appearing to develop tentacles. Statocysts ectodermal, closed, varied in size, oval to almost spherical, 71–141 in number, tending to increase in number with increasing medusa size, varying in number between tentacles and marginal warts, spacing uneven, occasionally with two side-by-side; number of statoliths indeterminable in preserved material. Manubrium located at tip of gastric peduncle, short and wide, cruciform in cross section, with bulbous pouches extending onto bases of radial canals, hanging partly or completely below velar opening; mouth cruciform, with four perradial channels leading into lips; lips four, perradial, 8-13 mm long, folded, curtain-like, with frilly edges. Radial canals four, slender, straight to partly zigzag due to gonadal folds, widening slightly at insertion with manubrium, elsewhere nearly equal in diameter. Circular canal narrow, distinct. Velum thin, with wide velar opening. Gonads ribbon-like, nearly straight to tightly folded, occurring continuously along radial canals from tip of peduncle to circular canal; medusae dioecious. Color of tentacles, marginal warts, radial canals, manubrium, and lips whitish in preserved specimens; umbrella margin with prominent ring of black pigment granules, extending along and sometimes over bases of tentacles and occasionally continuing outwards onto tentacles, pigmentation of ring becoming denser towards periphery, black pigment also usually present along

edge of gonads next to radial canals; umbrella, gastric

peduncle, and velum glassy and transparent. When exposed to black light, bases of tentacles glow light blue.

Cnidome on tentacles (undischarged), Fig. 6E–G; paratype, ROMIZ B5391. Large microbasic mastigophores 17.0– 23.3 µm long \times 2.7–3.4 µm wide (n = 10); medium microbasic mastigophores 13.0–16.7 µm long \times 2.3–2.8 µm wide (n = 10); small microbasic mastigophores 8.7–12.5 µm long \times 1.7–2.5 µm wide (n = 10).

Cnidome on oral arms (undischarged), Fig. 6H; paratype, ROMIZ B5391. Small microbasic mastigophores 10.0– 12.2 μ m long × 1.9–2.7 μ m wide (n = 10).

Description of hydroids and newly liberated medusae (based on paratype material, ROMIZ B5396. Fig. 7A, D, non-types; Fig. 7B, C, paratypes; Fig. 8A, B, paratypes; Fig. 9A–C, non-types). Colonies stolonal, arising from creeping hydrorhiza. Hydrorhizal stolons long, slender, smooth or with occasional wrinkles, giving rise to hydrothecal pedicels of varied length, most of them quite short. Hydrothecal pedicels usually with a few irregular annulations or wrinkles at base, otherwise partly to mostly smooth, branched only when supporting pedicellate gonotheca below base of



Fig. 7. (A) hydroid colony, Fukushima Prefecture, Japan, raised at the Kamo Aquarium (photograph by S. Ikeda). (B) hydroid, Miyazaki Prefecture, Japan (paratype, ROMIZ B5396), raised at the Enoshima Aquarium (photograph by D. Calder). (C) Hydroid hydranth and gonotheca with a medusa bud, Miyazaki Prefecture (paratype, ROMIZ B5396), raised at the Enoshima Aquarium (photograph by D. Calder). (D) Medusa bud, 1.22 mm high, 1.32 mm wide, with four tentacles, about to be released from hydroid, specimen from Enoshima Aquarium (photograph by G. Yamamoto).

hydranth; perisarc of hydroid colonies very thin. Hydrotheca reduced to short, irregular, filmy collar enveloping only extreme base of hydranth, proximal end with distinct diaphragm below ring of desmocytes; operculum lacking. Hydranths quite large, naked except at proximal end, narrowest at base, gastric region typically vase- to club-shaped, constricted short distance below tentacular whorl, constriction usually pronounced but sometimes indistinct, hydranth expanding again at distal end, here becoming subspherical to knob-shaped, supporting whorl of tentacles; hypostome of varied shape, sometimes flaring but usually curved and flatter than hemisphere, with central, round mouth. Tentacles filiform, in one whorl, about 20 in number, their bases with intertentacular web. Nematophores absent.

Gonophores free medusae. Gonothecae long, slender, filmy, with rounded distal end, gradually tapering towards proximal end, arising from hydranth pedicels by gonothecal pedicels, each gonotheca with single medusa bud. Advanced medusa buds filling much of gonotheca, each bud with four large tentacles, four radial canals, circular canal, and tubular manubrium. Medusa bud nearing release higher than a hemisphere, 1.2 mm high, 1.3 mm wide, tentacles four, perradial, tentacular bases tan-coloured with black pigment spots (apparent in color photographs); manubrium short, without peduncle (Fig. 7D). Newly liberated medusae higher than a hemisphere; umbrella 1.2 mm high, 1.4 mm wide; perradial tentacles four, well-developed; interradial tentacles four, some little more than marginal warts; radial canals four; circular canal present, peduncle undeveloped;



Fig. 8. Tima nigroannulata, hydroid stages (paratypes, ROMIZ B5396), Miyazaki, raised at the Enoshima Aquarium from a medusa collected at Miyazaki, April 2020. (A) hydranth, and gonotheca with a medusa bud. (B) chitinous exoskeletons, showing stolons, hydrothecal pedicels, and vestigial hydrothecae.

manubrium short, not reaching velar opening; lips four, perradial, distinct, with frilly edges; velum well-developed, with radiating folds, velar opening small; statocysts with statoliths present (Fig. 9A-C).

Cnidome on hydranths of hydroid, including tentacles (undischarged), Fig. 6I; paratype, ROMIZ B5396. Small microbasic mastigophores 7.0–7.9 μ m long \times 1.5–1.8 μ m wide (n = 10).

Remarks. Medusae of T. nigroannulata sp. nov. differ from those of other described species of the genus (Mayer, 1910; Kramp, 1959, 1961; Petersen, 1962), as summarized in the following key:

- 1. Umbrella distinctly flatter than a hemisphere 2
- 2. Umbrella much flattened, resembling a watch glass; tentacles as many as 250-300, gastric peduncle short..... T. saghalinensis Umbrella less flattened but not hemispherical; tentacles 60–80, gastric peduncle relatively long
- 3. Tentacles normally ca. 16; umbrella diameter 50-65 mm; warts ca. 12 between tentacles; marginal vesicles ca. six between tentacles.....T. bairdii Tentacle number many more than 16 4
- 4. Tentacles ca. 32, sometimes up to 40; marginal warts usually 96; statocysts ca. 128; umbrella diameter 63-100 mm; without ring of black pigment around umbrella marginT. formosa Tentacles 30-53; marginal warts 40-115; statocysts

71-141; umbrella diameter at least 20-46 mm; margin with ring of black pigment particles..... T. nigroannulata

Medusae identified as T. formosa have been reported from several locations in Japan (Fig. 1), including Oshoro, Hokkaido Prefecture, collected 1922 (Uchida, 1925); Onagawa Bay, Miyagi Prefecture, collected 1936 (Uchida, 1938, 1940); between Katsura-jima Island and Mahanashi-jima Island, Miyagi Prefecture, collected 1931 (Kubota and Yamazaki, 2007); Oarai Fishing Port, Ibaraki Prefecture, collected 2007 (Saito and Shiba, 2008); Lake Hamana (a semi-enclosed brackish water bay), Shizuoka Prefecture, collected sometime between 2005-2015 (Okamoto et al., 2016). Studies undertaken on the species at the Tsuruoka City Kamo Aquarium by Murakami (2016) and Yamamori et al. (2017) were likely based on cultures of medusae from Iwaki, Fukushima Prefecture, established in 2005. In our opinion, these medusae were likely all conspecific with T. nigroannulata rather than



Fig. 9. *Tima nigroannulata*, newly liberated medusae, from hydroids raised at the Enoshima Aquarium from a medusa collected at Miyazaki, April 2020. (A) lateral view. (B) aboral view. (C) oral view (photographs by G. Yamamoto).

the northeast Atlantic *T. formosa*, and the records of them have been included with question in the synonymy list above. In this study, medusae of *T. nigroannulata* are reported from Sendai, Miyagi Prefecture; Iwaki, Fukushima Prefecture; Fujisawa, Kanagawa Prefecture; and Miyazaki, Miyazaki Prefecture (Table 1). Excluded from the synonymy list are records of *T. formosa* from China (e.g., Kao et al., 1958; Chow and Huang, 1958; Zhang, 1982; Xu and He, 1996; Xu et al., 2014; Zheng et al., 2014; Zuo et al., 2016). Based on the phylogenetic tree (Fig. 2), these records were not based on medusae of *T. nigroannulata*.

Medusae of *Tima* Eschscholtz, 1829 are a group of particularly large hydromedusae, accounting for part of their public appeal at aquaria. Those of *T. nigroannulata* available for study ranged from 2.0–4.6 cm in umbrella diameter, although most were juveniles below 3.0 cm. The species likely attains a considerably larger size, with specimens from Oshoro identified as *T. formosa* by Uchida (1925) being 6.0 cm in diameter. In other species of the genus, *T. flavilabris* reaches a diameter of about 8.0 cm (Kramp, 1961), *T. bairdii* a diameter of about 6.5 cm (Kramp, 1961; Cornelius, 1995), *T. formosa* a diameter of 10.0 cm (Mayer, 1910; Petersen, 1962), and *T. saghalinensis* a diameter of 10.0 cm (Kramp, 1961).

Bioluminescence is known to occur in *T. bairdii* (Johnston, 1833: 321; Evans and Ashworth, 1909: 305), and it may occur in others of the genus. No evidence yet indicates that *T. nigroannulata* is bioluminescent (Aya Adachi and Gaku Yamamoto, personal observations), but under black light, bases of the tentacles were shown to emit fluorescence (Fig. 10).

The cnidome of both medusa and hydroid stages of *T. nigroannulata* comprised heteronemes, provisionally identified here as microbasic mastigophores (Fig. 6A–I). No discharged nematocysts were observed in examined material, even after treatment with sodium hypochlorite. Tentacles of the medusa were densely armed with these nematocysts. Their capsules were distinguished as three size classes (small, medium, large), although they approached being a continuum in their size variations. Those of the oral arms, and those of the hydranths, were quite small and much more uniform in length. Nematocysts of the adult medusae appeared to be slightly larger than those of juveniles.

The hydroid stages of *Tima* are poorly known. Besides the hydroid of *T. nigroannulata*, reported herein, that of only



Fig. 10. *Tima nigroannulata*, juvenile medusa (5 mm in diameter), showing fluorescence of tentacle bases illuminated by black light. Specimen liberated from a hydroid raised at the Enoshima Aquarium from a medusa collected at Miyazaki, April 2020 (photograph by G. Yamamoto).

one other species has been described. Alexander Agassiz (1865: p. 115, fig. 172) provided a brief description and an illustration of a hydroid raised for 6 months from planulae of the medusa *T. formosa* in Massachusetts, USA. Its hydranth was slender with a reduced hydrotheca, and it bore a distal whorl of about 14 long, slender tentacles. An intertentacular web was present basally. Colonies of the species grew in small tufts on the side of a glass culture vessel. The account by A. Agassiz (1865) generally corresponds with the hydroid of *T. nigroannulata*.

In the shape of their hydranths, in possessing an intertentacular web, and in having hydrothecae reduced to a basal collar, hydroids of *T. nigroannulata* resemble those of several other eirenid species, including *Eirene viridula* (Péron and Lesueur, 1810), *Eirene lactea* (Mayer, 1900), *Eutima gracilis* (Forbes and Goodsir, 1853) and *Eutima gegenbauri* (Haeckel, 1864) (see Russell, 1970: pp. 259, 260; Brinckmann-Voss, 1973; Hündgen, 1978; Cornelius, 1995: pp. 227, 229). In these characters, eirenid hydroids thus appear to possess a distinctive morphology.

It is of nomenclatural and systematic significance that the hydroid Campanulina tenuis Van Beneden, 1847 is of the same type as those noted above (see Van Beneden, 1847: p. 457, fig. 6). That hydroid is the type species of the genus Campanulina Van Beneden, 1847 (not Campanulina sensu Allman, 1864 and Hincks, 1869), the type genus of the family Campanulinidae Hincks, 1869. Indeed, the family Campanulinidae, when based on its true type genus, is taxonomically distinct from the long-misdiagnosed and polyphyletic collective group currently assigned that name. That misclassified assemblage is currently a cache of diverse operculate species, many of uncertain identity, rather than being based on a hydroid with a reduced hydrotheca like those of the socalled eirenids (Rees, 1939; Calder, 1991). Nevertheless, current usage of the family name Eirenidae has been maintained here even though Campanulinidae would have been nomenclaturally correct. As knowledge of these hydrozoans advances, rectifications in the taxonomy of the group may be expected.

DNA analysis

The cytochrome *c* oxidase (COI) sequence fragments were aligned by eye and using Clustal X version 2.0 (Larkin et al., 2007). The NCBI GenBank Blastn Suite was used for initial taxonomic confirmation. We tested maximum likelihood fit of 24 nucleotide substitution models for this aligned dataset, and found that GTR + I (general time reversable + invariant sites) was the best fit. Therefore, this model was used to reconstruct phylogenetic trees. Tree topologies remained consistent, with three well-supported ingroup clades regardless of the phylogenetic algorithm used (Fig. 2). Bootstrap support for the three main ingroup clades was strong, ranging from 98% to 100%, depending on the optimality criterion used.

The maximum likelihood molecular phylogenetic reconstruction presented used a single cubozoan outgroup, *Alatina alata* (Reynaud, 1830), and resulted in three ingroup clades, representing three distinct lineages, each with bootstrap support of 100% (Fig. 2). Of the resulting 613 nucleotides per sequence, excluding the outgroup, there were 149 variable positions, 145 of which were parsimony informative and four of which were singleton sites. Including the outgroup, there were 214 variable sites. The overall mean genetic distance was 0.16 or 16%.

Tree topology provides strong evidence for the presence of three distinct, genetically cohesive evolutionary lineages. Clade 1 is comprised of three closely related COI sequences from three individuals sampled along the coast of Japan. These three sequences represent two haplotypes, TNJ2 and TNJ3 from Fukushima, which are identical to one another and differ from TNJ1 from Sendai by six base pairs (less than 1%), forming a strongly supported sister group to Clade 2 (95% bootstrap support). Clade 2 is made up of two sequences from *Tima bairdii*, TBS1 and TBS2, collected in Norway and Sweden, representing two haplotypes that differ by a single basepair. Clade 3 includes four sequences (TFC1–TFC4) that comprise a single haplotype, from samples collected off of coastal China, identified as ?*T. formosa* (Fig. 2; Table 2). The four specimens comprising Clade 3 form the basal ingroup clade; COI sequences were obtained from GenBank (Table 2). Sequences for *T. nigroannulata* were submitted to GenBank to obtain accession numbers (Table 2).

Biogeography

The Japanese Archipelago as we know it today is about 15–20 million years old, extends some 3500 km in length, and is 170 km from the continental mainland of Asia (Barnes, 2003; Neall and Trewick, 2008). The coastline of west-central Japan is influenced by cool waters from the Sea of Japan, ranging from 11–15°C during winter and spring (Fujikura et al., 2010). By contrast, surface water temperatures on the east-central Pacific side of the country are warmer depending on the path of the north-flowing Kuroshio Current, ranging from 13–28°C during winter and spring (Fujikura et al., 2010). Cool water temperatures in the Sea of Japan, and the deep-water basin separating Japan and the continental Asian mainland, may limit the natural dispersal of *T. nigroannulata* beyond its current geographic distribution.

Biogeographic distributions of the two fully documented species of Tima reported from the North Pacific Ocean are distinct. Tima saghalinensis is a predominantly offshore boreal species, restricted to Russian waters off Sakhalin Island and the Sea of Okhotsk. It has typically been collected at latitudes between 47-59°N, and in water temperatures of 3-15°C (Zavolokin, 2010). Tima nigroannulata is a temperate coastal species occurring on the Pacific coast of Japan. Specimens have been collected at four sites from Sendai to Miyazaki, between latitudes 31-37°N, and at a coastal surface water temperature in nature at Enoshima of 15°C. If the record of Tima formosa by Uchida (1925) was based on this species, its range extends northwards to southern Hokkaido. Medusae of the species have been maintained in a healthy reproductive state, at 20°C, in cultures at both the Enoshima and Kamo aquaria.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

DRC wrote the section on systematics, prepared line drawings and photomicrographs, organized the References, edited the manuscript, and approved the final draft. GLC conceived the project, coordinated different parts of it, obtained specimens, authored or reviewed drafts of the paper including a section on biogeography, and approved the final draft. SI, AA, and GY provided specimens and images for study, and approved the final draft. AH generated molecular data. BSH prepared the map, aligned and edited DNA sequences, performed analyses on the molecular results, and approved the final draft.

REFERENCES

- Agassiz A (1865) Illustrated Catalogue of the Museum of Comparative Zoölogy at Harvard College. No. II. North American Acalephae, Sever & Francis, Cambridge, Massachusetts
- Agassiz A, Mayer AG (1899) Acalephs from the Fiji Islands. Bull Mus Comp Zoöl Harvard College 22: 157–189
- Agassiz L (1862) Contributions to the Natural History of the United States of America. Vol. IV. Little, Brown and Company, Boston
- Allman GJ (1864) On the construction and limitation of genera among the Hydroida. Ann Mag Nat Hist 3rd Ser 13: 345–380
- Barnes GL (2003) Origins of the Japanese Islands: the new "big picture". Jpn Rev 15: 3–50
- Bigelow HB (1913) Medusae and Siphonophorae collected by the U. S. Fisheries Steamer "Albatross" in the northwestern Pacific, 1906. Proc US Natl Mus 44: 1–119
- Bleeker J, van der Spoel S (1988) Medusae of the Amsterdam Mid North Atlantic Plankton Expeditions (1980–1983) with description of two new species. Bijdr Dierk 58: 227–258
- Bouillon J (1985) Essai de classification des hydropolypeshydromeduses (Hydrozoa-Cnidaria). Indo-Malayan Zool 1: 29–243
- Bouillon J, Gravili C, Pagès F, Gili J-M, Boero F (2006) An introduction to Hydrozoa. Mém Mus Natl Hist Nat 194: 1–591
- Brinckmann-Voss A (1973) The life-cycle of *Eirene lactea* (Mayer, 1900) and *Helgicirrha schulzei* Hartlaub, 1909 (phylum Cnidaria, class Hydrozoa, order Leptomedusae, family Eirenidae). Pub Seto Mar Biol Lab 20: 63–72
- Calder DR (1991) Shallow-water hydroids of Bermuda. The Thecatae, exclusive of Plumularioidea. R Ontario Mus, Life Sci Contrib 154: 1–140
- Chow T-H, Huang M-C (1958) A study on hydromedusae of Chefoo. Acta Zool Sinica 10: 173–197
- Collins AG (2000) Towards understanding the phylogenetic history of Hydrozoa: hypothesis testing with 18S gene sequence data. Sci Mar 64 (Suppl 1): 5–22
- Cornelius PFS (1992) Medusa loss in leptolid Hydrozoa (Cnidaria), hydroid rafting, and abbreviated life-cycles among their remote-island faunae: an interim review. Sci Mar 56: 245–261
- Cornelius PFS (1995) North-west European thecate hydroids and their medusae. Part 1. Introduction: Laodiceidae to Haleciidae. Syn Brit Fauna New Series 50: 1–347
- Eschscholtz F (1829) System der Acalephen. Eine ausfürliche Beschreibung aller medusenartigen Strahltiere. Ferdinand Dümmler, Berlin
- Evans W, Ashworth JH (1909) Some medusae and ctenophores from the Firth of Forth. Proc Roy Phys Soc Edinb 17: 300–311
- Fewkes JW (1883) Exploration of the surface fauna of the Gulf Stream, under the auspices of the United States Coast Survey, by Alexander Agassiz. IV. On a few medusae from the Bermudas. Bull Mus Comp Zoöl Harvard College 11: 79–90

- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294–299
- Forbes E, Goodsir J (1853) On some remarkable marine Invertebrata new to the British seas. Trans R Soc Edin 20: 307–315
- Fujikura K, Lindsay D, Kitazato H, Nishida S, Shirayama Y (2010) Marine biodiversity in Japanese waters. PLOS ONE 5: e11836
- Goette A (1886) Verzeichniss der Medusen, welche von Dr Sander, Stabsarzt auf S.M.S. "Prinz Adalbert" gesammelt wurden. Sitzber K Preuss Aka Berlin 39: 831–837
- Haeckel E (1864) Beschreibung neuer craspedoter Medusen aus dem Golfe von Nizza. Jena Z Med Naturw 1: 325–342
- Haeckel E (1879) Das System der Medusen. Erster Theil einer Monographie der Medusen. Denkschr Med-Naturw Ges Jena 1: 1–360
- Hamner WM (1990) Design developments in the planktonkreisel, a plankton aquarium for ships at sea. J Plankton Res 12: 397– 402
- Hincks T (1869) A History of the British Hydroid Zoophytes. John van Voorst, London
- Holland BS, Hadfield MG (2004) Origin and diversification of the endemic Hawaiian tree snails (Achatinellidae: Achatinellinae) based on molecular evidence. Mol Phylo Evol 32: 588–600
- Hündgen M (1978) The biology of colonial hydroids. I. The morphology of the polyp of *Eirene viridula* (Thecata: Campanulinidae). Mar Biol 45: 79–92
- Johnston G (1833) Illustrations in British zoology. Mag Natur Hist J Zool Bot Min Geo Met 6: 320–324
- Kao C-S, Li F-L, Chang Ü-M, Li H-L (1958) On the hydromedusae from the Shantung coast. J Shandong Univ Nat Sci 1: 75–118 (in Chinese, with English summary)
- Kramp PL (1953) Hydromedusae. Sci Rep Great Barrier Reef Exped 1928–29 6: 259–322
- Kramp PL (1959) The hydromedusae of the Atlantic Ocean and adjacent waters. Dana Rep 46: 1–283
- Kramp PL (1961) Synopsis of the medusae of the world. J Mar Biol Assoc UK 40: 1–409
- Kubota S (1979) Occurrence of a commensal hydroid Eugymnanthea inquilina Palombi from Japan. J Fac Sci Hokkaido Univ Ser VI Zool 21: 396–406
- Kubota S (1997) Anthomedusae, Leptomedusae, Limnomedusae, Laingiomedusae, Ctenophora. In "An Illustrated Guide to Marine Plankton in Japan" Ed by M Chihara, M Murano, Tokai University Press, Tokyo, Japan. pp 485–500, 514–523, 531– 535, 555–566, 1498–1499
- Kubota S, Gravili C (2007) A list of hydromedusae (excluding Siphonophora, Milleporidae and Actinulidae) in Japan. The Nanki Seibutu, Nanki Biol Soc 49: 189–204 (in Japanese)
- Kubota S, Horita T (1992) A new hydromedusa of the genus *Eirene* (Leptomedusae; Eirenidae) from Toba, Japan. Zool Sci 9: 413– 421
- Kubota S, Yamazaki Y (2007) List of cnidarian medusae deposited in the Saito Ho-on Kai Museum of Natural History, with special reference to the presence of "large"-form immortal medusae *Turritopsis nutricula*. Saito Ho-on Kai Mus Nat Hist, Res Bull 72: 1–13
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35: 1547–1549
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948
- Maronna MM, Miranda TP, Peña Cantero ÁL, Barbeitos MS, Marques AC (2016) Towards a phylogenetic classification of Leptothecata (Cnidaria, Hydrozoa). Sci Rep 6: 18075
- Mayer AG (1900) Some medusae from the Tortugas, Florida. Bull

Mus Comp Zoöl Harvard Coll 37: 13-82

- Mayer AG (1910) Medusae of the World. Volume II. The Hydromedusae. Carnegie Inst Wash Pub 109: 231–498
- Murakami T (2016) History and introduction of the Kamo Aquarium. Zool Garten 85: 64–73
- Naumov DV (1960) Gidroidy i gidromeduzy morskikh, solonovatovodnykh i presnovodnykh basseinov SSSR. Akad Nauk SSSR, Opred Faune SSSR 70: 1–626
- Neall VE, Trewick SA (2008) The age and origin of the Pacific Islands: a geological overview. Phil Trans R Soc B 363: 3293– 3308
- Okamoto K, Sugimura T, Ohtake J, Toh H, Katoh O (2016) A checklist of the gelatinous zooplankton collected in Lake Hamana. Bull Shizuoka Pref Res Inst Fish 49: 31–33 (in Japanese with English abstract)
- Östman C (1979a) Two types of nematocysts in Campanulariidae (Cnidaria, Hydrozoa) studied by light and scanning electron microscopy. Zool Scr 8: 5–12
- Östman C (1979b) Nematocysts in the *Phialidium* medusae of *Clytia hemisphaerica* (Hydrozoa, Campanulariidae) studied by light and scanning electron microscopy. Zoon 7: 125–142
- Östman C (1982) Nematocysts and taxonomy in *Laomedea*, *Gonothyraea* and *Obelia* (Hydrozoa, Campanulariidae). Zool Scr 11: 227–241
- Östman C (1999) Nematocysts and their value as taxonomic parameters within the Campanulariidae (Hydrozoa). A review based on light and scanning electron microscopy. Zoosyst Ross, Suppl 1: 17–28
- Owen R (1843) Lectures on the Comparative Anatomy and Physiology of the Invertebrate Animals: Delivered at the Royal College of Surgeons, in 1843. Longman, Brown, Green, & Longmans, London
- Péron F, Lesueur CA (1810) Tableau des caractères génériques et spécifiques de toutes les espèces de méduses connues jusqu'à ce jour. Ann Mus Hist Nat 14: 325–366
- Petersen KW (1957) On some medusae from the North Atlantic. In "Papers from the 'Dana' Oceanographical Collection, Nr. 48". Vidensk Medd fra Dansk naturh Foren 119: 25–45
- Petersen KW (1962) A discussion of the genus *Tima* (Leptomedusae, Hydrozoa). Vidensk Medd fra Dansk naturh Foren 124: 101–113
- Petersen KW (1979) Development of coloniality in Hydrozoa. In "Biology and Systematics of Colonial Animals" Ed by G Larwood, BR Rosen, Academic Press, New York, pp 105–139
- Raskoff KA, Sommer FA, Hamner WM, Cross KM (2003) Collection and culture techniques for gelatinous zooplankton. Biol Bull 204: 68–80
- Rees WJ (1939) A revision of the genus *Campanulina* Van Beneden, 1847. Ann Mag Nat Hist, Ser II, 3: 433–447
- Reynaud M (1830) La Carybdée (méduse) ailee. *Carybdea* (*Medusa*) *alata*, Reyn. In: "Centurie Zoologique" Ed by RP Lesson, Levrault, Paris, p 95
- Romanes GJ (1876) An account of some new species, varieties, and monstrous forms of medusae. J Linn Soc Zool 12: 524– 531
- Russell FS (1953) The Medusae of the British Isles. Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae. Cambridge Univ Press, Cambridge
- Russell FS (1970) The Medusae of the British Isles. II. Pelagic Scyphozoa with a Supplement to the First Volume on Hydromedu-

sae. Cambridge Univ Press, Cambridge

- Saito S, Shiba Y (2008) The first records of six hydromedusae species (Cnidaria, Hydrozoa), collected in the coastal area of Ibaraki Prefecture, Japan. Bull Ibaraki Nat Mus 11: 7–10 (in Japanese)
- Schuchert P (2021) World Hydrozoa Database. Eutimidae Haeckel, 1879. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id= 196236 Accessed 1 February 2021
- Schuchert P, Hosia A, Leclère L (2017) Identification of the polyp stage of three leptomedusa species using DNA barcoding. Rev Suisse Zool 124: 167–182
- Stafford J (1907) On the fauna of the Atlantic coast of Canada. An introductory report. Contrib Can Biol Fish 2: 31–36
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313
- Uchida T (1925) Some hydromedusae from northern Japan. Jpn J Zool 1: 77–100
- Uchida T (1938) Medusae in Onagawa Bay and its vicinity. Sci Rep Tôhoku Imp Univ Ser 4 Biol 13: 47–58
- Uchida T (1940) The fauna of Akkeshi Bay. XI. Medusae. Contrib Akkeshi Mar Biol Sta 34: 277–297
- Van Beneden P-J (1847) Un note sur le mode de reproduction des animaux inférieurs. Bull Acad R Sci Lettr Beaux-Arts Belg 14: 448–462
- Verrill AE (1865) Classification of polyps: (extract condensed from a synopsis of the Polypi of the North Pacific Exploring Expedition, under Captains Ringgold and Rodgers, U.S.N.). Proc Essex Inst 4: 145–152
- Weill R (1934) Contribution à l'étude des cnidaires et de leurs nématocystes. II. Valeur taxonomique du cnidome. Trav Sta Zool Wimereux 11: 351–701
- Winkler JTh (1982) The hydromedusae of the Amsterdam Mid North Atlantic Plankton Expedition, 1980 (Coelenterata, Hydrozoa). Beaufortia 32: 27–56
- Xu R, He Z (1996) On hydromedusae from Dalian coast, China. Acta Zool Sinica 43: 19–21 (in Chinese with English abstract)
- Xu Z-Z, Huang J-Q, Lin M, Guo D-H, Wang C-G (2014) The Superclass Hydrozoa of the Phylum Cnidaria in China. Vol. II. China Ocean Press, Beijing
- Yamamori L, Okuizumi K, Sato C, Ikeda S, Toyohara H (2017) Comparison of the inducing effect of indole compounds on medusa formation in different classes of Medusozoa. Zool Sci 34: 173– 178
- Zavolokin AV (2010) Jellyfish of the Far Eastern seas of Russia. i. Species composition and spatial distribution. Izv TINRO 163: 45–66
- Zhang JB (1982) Notes on the hydromedusae fauna of the China Sea areas. Acta Oceanol Sinica 1: 126–135
- Zheng LM, He JR, Lin YS, Cao WQ, Zhang WJ (2014) 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. Acta Oceanol Sinica 33: 55–76
- Zuo T, Wang J, Wu Q, Yuan W, Luan Q-S (2016) Spatial distribution and biomass of large jellyfish in the Yellow Sea and northern part of the East China Sea in May 2015. Oceanol Limnol Sinica 47: 195–204

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