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Orchistoma integrale sp. nov. (Hydrozoa, Orchistomatidae), a new species of hydromedusa with the first record of the genus from the Northwest Pacific

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Abstract: Four species belonging to the genus *Orchistoma* in the monotypic family Orchistomatidae are currently recognized from several localities around the world, but none of these have been reported from the Northwest Pacific, including Japan. In this study, juvenile medusae collected in Tanabe Bay, Wakayama Prefecture were raised to full maturity and used to describe a new species, *Orchistoma integrale* sp. nov., based on integrated analysis of morphological and molecular data. *Orchistoma integrale* can be distinguished from its congeners by several morphological features, including prominently curled gonads and scattered nematocysts on the umbrella. Our molecular characterization of this species, connected to its type material, will allow the species to be detected through emerging genetic techniques involving eDNA derived from environmental samples. This is the first record of this family in Japan. We also describe medusa development from juvenile to adult.

Key words: development, laboratory rearing, Leptothecata, phylogenetics, Tsunashirazu-port

Introduction

The medusa stage of *Orchistoma* Haeckel, 1879 (Leptothecata, Orchistomatidae), a hydrozoan genus, is characterized by a combination of features: a very short manubrium; large gastric peduncle; mouth with 8–30 sinuous or crenulated lips; eight or more radial canals, simple, ramified, or in clusters of 4; up to 64 marginal tentacles, laterally compressed; no marginal cirri, but numerous short, cordyli-like, tentaculiform structures devoid of marginal bulbs, contractile, arising from the circular canal, in each intertentacular space; gonads usually on proximal parts of radial canals; numerous (up to 800) adaxial ocelli; and no statocysts, excretory pores or papillae (cf. Bouillon et al. 2006). The genus includes four accepted species (Schuchert 2023). The polyp stage is unknown for any species of *Orchistoma*.

The type species of *Orchistoma*, *O. pileus* (Lesson, 1843) was originally described as *Mesonema pileus* (Lesson 1843), as part of the no-longer-accepted genus *Mesonema* Eschscholtz, 1829 (=*Aequora* Péron & Lesueur, 1810). Later, Haeckel (1879) proposed the genus *Orchistoma* and reclassified *M. pileus* into this new genus. Bouillon 1984a thereafter erected the family Orchistomatidae to contain those species with a gastric peduncle, but without true cordyli and without statocysts and Bouillon 1984b described two new species within the genus (Bouillon 1984a, 1984b). Since that time, nine species have been described as part of the genus, but three of these have been synonymized with *O. pileus*, while two have been transferred to the poorly defined genus *Orchistomella* (Schuchert 2023), leaving four accepted species (but see below): *O. pileus, O. manam* Bouillon, 1984, *O. nubiae* Bouillon, 1984, and, *O. mauropoda* Gershwin, Zeidler & Davie, 2010 (Mayer 1900, Bouillon 1984a, Gershwin et al. 2010, Schuchert 2023).

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Taxonomic challenges related to the genus raise the possibility that additional species are still in need of characterization. For example, Haeckel (1879) added a second nominal species, *O. steenstrupii* Haeckel, 1879 when he established the genus *Orchistoma*. Haeckel (1879) distinguished the two species based on tentacle number: 32 in *O. pileus*, 64 in *O. steenstrupii*. However, Mayer (1910) synonymized the two species, and Kramp (1955) followed Mayer after reexamining Haeckel's specimens. Gershwin et al. (2010) mentioned that though both species possess 32 radial canals, they could be distinguished by the ratio of radial canals to tentacles (1 : 1 in *O. pileus*, 1 : 2 in *O. steenstrupii*), but Schuchert & Collins (2021) suggested that *O. steenstrupii* is a synonym of *O. pileus*. Likewise, two subsequently described species, *O. agariciforme* Keller, 1884 and *O. collapsum* (Mayer, 1900) are similar in many ways. Bouillon (1984a) said that *O. collapsum* could be distinguished from *O. agariciforme* in that *O. collapsum* has radial canals that remain clustered clearly into four groups even at maturity, and a longer, more distinct but short-lipped manubrium (Bouillon 1984a).

Finally, despite uncovering genetic evidence of two distinct species of *Orchistoma* in their study of Florida hydromedusae, Schuchert & Collins (2021) were unable to identify morphological characters (other than color, which was discarded) to distinguish the two lineages. As a result, the authors chose to synonymize both *O. agariciforme* and *O. collapsum* within *O. pileus* (Schuchert & Collins 2021). Thus, further examination of the genus and the delineation of its species is needed.

Medusae of *Orchistoma* have been described from West Africa, Papua New Guinea, Antilles, Florida, Italy, and Australia but none have previously been reported from the Northwest Pacific, including Japan. In 2018–2019, one of the authors (RS) collected specimens referable to new species of *Orchistoma* from Tanabe Bay, Wakayama Prefecture, Japan. The present report constitutes the first evidence of the genus *Orchistoma* and the family Orchistomatidae in Japanese waters and more broadly in the northwest Pacific.

Materials and Methods

Collection of specimens

Several dozen medusae of *Orchistoma* specimens were collected from 0–3 m depth at Tsunashirazu-port in Tanabe Bay (Fig. 1), Wakayama Prefecture, eastern Japan during 15–17th July 2018 and 14th July 2019. The medusae were captured with a dip net (mesh size about 0.5 mm). Collected medusae were reared in containers filled with 200–1000 mL seawater until 7–8 weeks after collection, when they reached up to about 15 mm in diameter. After that, medusae were reared in a tank (JellyCube RC-02; MicroBase) (except for paratype 1 because it was not large enough throughout its lifetime). They were kept in artificial seawater (Instant Ocean; NAPQO) at 27°C and at a salinity of about 32. Medusae were offered a variety of potential prey items, including *Artemia* nauplii, diced *Aurelia coerulea* von Lendenfeld, 1884, *Cladonema pacificum* Naumov, 1955, *Neomysis* sp., juvenile fish, chopped shrimp, or artificial food (ff18; Charm). About two weeks after collection, several medusae collected each year were separated from other individuals to more closely observe their development. During these periods, part of or all of the following features of each individual were measured irregularly: the size or shape of the umbrella, peduncle, gonads, stomach, and lips; the number of radial canals, tentacles, tentacle bulbs, tentaculiform structures between adjacent tentacle bulbs, and ocelli between adjacent tentacle bulbs. Three of them (two medusae collected in 2018 and one medusa collected in 2019) survived to full maturity, when their gonads and peduncles were fully developed. After they reached full maturity, two specimens collected in 2018 and 2019, respectively, were fixed in 3% formalin seawater after anesthetizing with magnesium chloride. The holotype (NSMT-Co 1900) and one formalin-preserved paratype (NSMT-Co 1901) were deposited in the National Museum of Nature and Science, Tsukuba, Japan (NSMT), and one tentacle of the holotype and one whole specimen collected in 2018, also herein designated a paratype (USNM 1517422) was fixed in 100% ethanol, after morphological characterization, and sent to the Smithsonian National Museum of Natural History for molecular analysis.

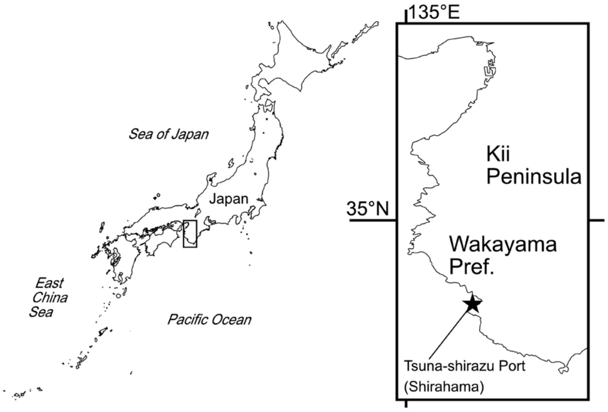

Fig. 1. Map of Japan showing the area where *Orchistoma integrale* sp. nov. was found. The black star shows the type locality of *O. integrale* sp. nov.

Morphological observation

The specimens were observed using a stereoscopic microscope (VCT-VBL2e; SHIMADZU), and macroscopically photographed with a camera (TG-4 Tough; OLYMPUS). The size, shape, and/or number of the umbrella, peduncle, manubrium lips, stomach, radial canals, gonads, tentacles, tentacle bulbs, tentaculiform structures and ocelli, and the color of each part of the body were measured.

Molecular characterization

DNA was extracted from pieces of tissue composed of tentacles and bell margin using an extraction kit (Qiagen Inc., Valencia, CA), following the manufacturer's protocol for animal tissues. Two mitochondrial markers, regions of 16S and COI, were amplified by thermocycling—initial denaturation at 95°C for 5 min., 35 cycles of denaturation at 95°C for 30 sec., annealing at 52°C for 30 sec., extension at 72°C for 60 sec., and final extension for 10 min. at 72°C—with forward and reverse primers for 16S (Lawley et al. 2016) and COI (Geller et al. 2013). PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP; USB Corp., Cleveland, OH). Cycle sequencing was accomplished using the same primers as those used in PCRs with fluorescently labeled dideoxy terminators and were visualized after clean up, using Sephadex columns on an Applied Biosystems (Thermo Fisher Scientific, Waltham, MA) 3730xl Genetic Analyzer. Sequences were submitted to GenBank under accession numbers PP713065 (16S), PP713066 (16S), and PP715637 (COI).

16S and COI sequences were aligned with all exemplars of Orchistomatidae and selected taxa of families Laodiceidae and Hebellidae as outgroups, following Schuchert & Collins (2021) using MAFFT (Katoh et al. 2002), as implemented in Geneious Prime (v. 2024; https://www.geneious.com). Maximum likelihood (ML) topologies were inferred for the 16S and COI alignments using PhyML v3.3 (Guindon et al. 2010), assuming the general time reversible (GTR) model of nucleotide evolution, with a gamma parameter for site heterogeneity with four rate categories. Nodal support was assessed through ML analyses of 400 bootstrap replicate datasets. Pair-wise patristic distances, the sum of the lengths of the branches connecting two nodes, were calculated based on the resulting ML topologies and their underlying alignments.

Results

Description

Phylum Cnidaria Verrill, 1865
Class Hydrozoa Owen, 1843
Order Leptothecata Cornelius, 1992
Family Orchistomatidae Bouillon, 1984
(New Japanese name: Ōkisutōma-kurage-ka)
Genus *Orchistoma* Haeckel, 1879
(New Japanese name: Ōkisutōma-kurage-zoku)

***Orchistoma integrale* sp. nov. Sugimoto, Izumi and Collins, 2025**
(New Japanese name: integuraru-kurage)
(English Common name: integral jellyfish)
Fig. 2–8

Material examined

Holotype: NSMT-Co 1900: whole specimen preserved in 3% formalin-seawater solution; Tanabe Bay, Wakayama Prefecture, Japan (between 33°41′13.7″N 135°21′25.8″E and 33°41′13.9″N 135°21′27.3″E); 0–3 m depth; 15–17th July 2018; female, umbrella diameter 23 mm, radial canals 23, tentacles 42; collectors: Ryoya Sugimoto, Takuya Sugimoto, and Karen Sugimoto.

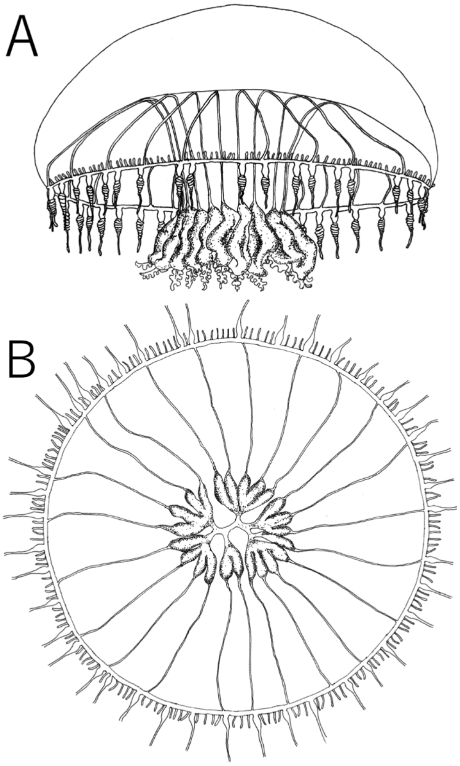

Fig. 2. Line drawings of holotype (NSMT-Co 1900) of *Orchistoma integrale* sp. nov. (A) Lateral view. (B) Apical view.

Paratype 1: USNM 1517422: whole specimen preserved in 99% ethanol; 0–3 m depth; 15–17th July 2018; female, umbrella diameter 12 mm; radial canals 19; tentacles 31; same locality and collectors as the holotype.

Paratype 2: NSMT-Co 1901: Whole specimen preserved in 3% formalin-seawater solution; 0–3 m depth; 14th July 2019; female, umbrella diameter 18 mm, radial canals 31; tentacles 32; same locality and collectors as the holotype.

Diagnosis

Orchistoma integrale sp. nov. is defined by a combination of features: umbrella hemispherical, without vertical sides; peduncle bell-shaped (spread at the base); gonads on the most proximal part of radial canals (connected to lips); tentacles rounded cord-like, very long when elongated; radial canals not bifurcated or bifurcated only just above gonads, not in four groups; manubrium very short, without point symmetry; lips shorter, sinuous frilled crape myrtle petal-like, without point symmetry; tentaculiform structures adnate to the edge of the umbrella for none–1/2 of their length; irregular number of radial canals and tentacles; stomach bifurcated irregularly, smaller when immature; brown endodermal cores in tentacle bulbs; scattered nematocysts on the exumbrella (Table 1).

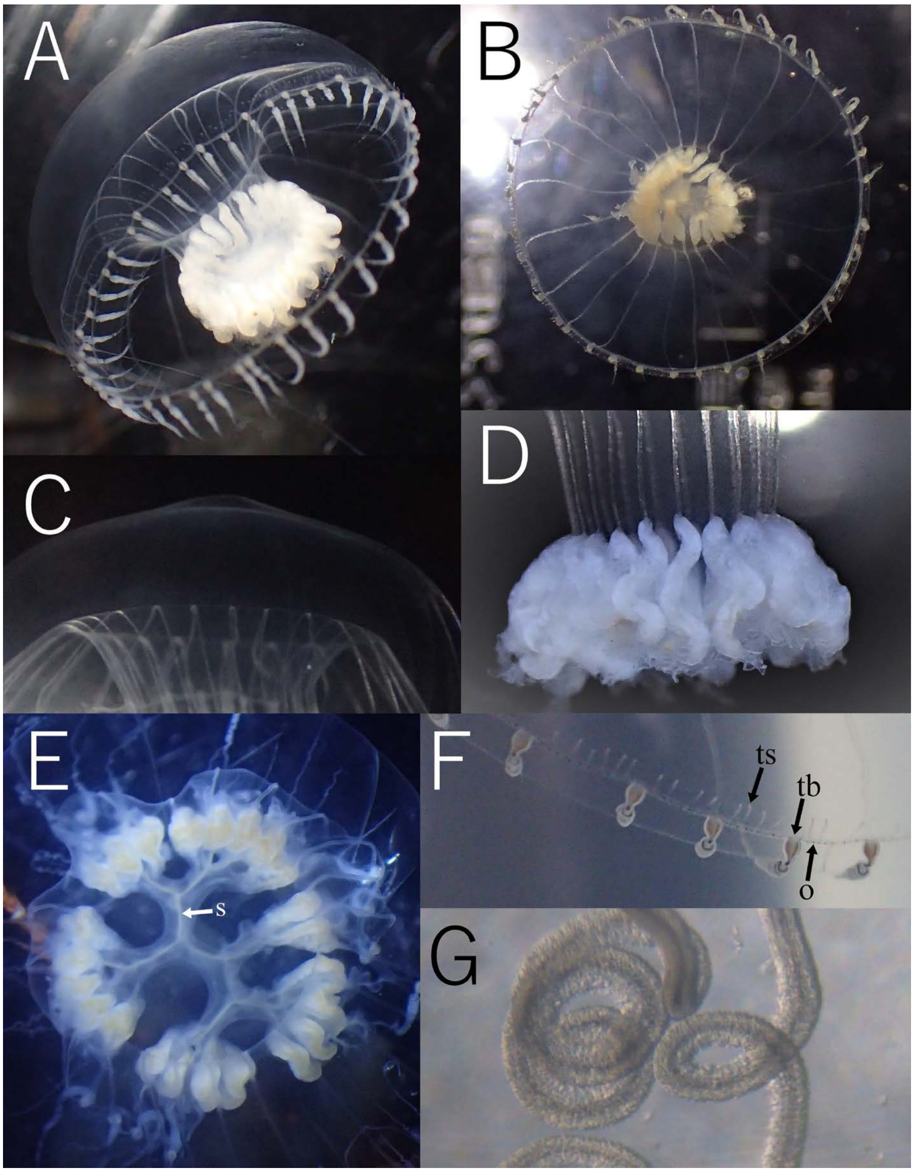

Fig. 3. Habitus of matured holotype (NSMT-Co 1900) of *Orchistoma integrale* sp. nov. (A) Diagonally above (photographed on 2nd October 2018). (B) Oral view (photographed on 23rd September 2018). (C) Tubercle on the top of the umbrella (photographed on 2nd October 2018). (D) Lateral view of matured gonads (photographed on 7th October 2018). (E) Dorsal view of stomach and gonads (photographed on 29th September 2018; s: stomach). (F) Edge of the umbrella (photographed on 1st October 2018; ts: tentaculiform structures; tb: tentacle bulbs; o: ocelli). (G) Enlarged tentacle (photographed on 3rd October 2018).

Description of Holotype (NSMT-Co 1900; Fig. 2, 3)

Umbrella diameter and height approximately 21 mm and 10.5 mm, respectively. Umbrella hemispherical (Fig. 2A, 3A), without vertical sides (Fig. 2A, 3A), with a tubercle at the apex (Fig. 3C). Mesoglea, half of umbrella height in the center of umbrella, thickest in the center of umbrella and tapering towards the edge of umbrella (Fig. 2A, 3A). Exumbrella transparent (Fig. 3A–C), with scattered nematocysts. Peduncle height changeable because of its stretchability, approximately 8 mm. Peduncle massive and broad (Fig. 2A, 3A, C, D), slightly protruding below the umbrella margin (Fig. 2A), about 1/2 of umbrella diameter at the base (Fig. 2A, 3A), bell-shaped (spread at the base) (Fig. 2A, 3A, C), with scattered nematocysts. Manubrium very short, along the stomach and without point symmetry. Stomach translucent whitish (Fig. 3E), without point symmetry (Fig. 2B, 3E), branching dendritically in the center (Fig. 2B, 3E), number and place of bifurcations irregular (Fig. 2B, 3E), then each branch subdividing into several diverticula (Fig. 2B, 3E), each diverticulum leading to one radial canal and gonad (Fig. 2B, 3E). Diverticula 23 in total, equal to the number of gonads and radial canals. Radial canals 23, irregular in number, extending towards the ring canal on the edge of the umbrella (Fig. 2B, 3B), leading to diverticula (Fig. 2B, 3B), simple, not bifurcated, narrow, straight (Fig. 2A, B, 3A, B), containing the gonads (Fig. 3D). Each of the 23 radial canals is usually connected to a single tentacle bulb (Fig. 2A, B, 3A, B, F). The number of radial canals is not proportional to the number of tentaculiform structures. Gonads 23, on proximal portion of radial canals (connected to lips) (Fig. 3A, B, D, E), covering about 1/4 of the peduncle, bilamellar, closely packed with oval eggs on both sides of radial canals (Fig. 3D), not the same length (ca. 2.2–3.4 mm) (Fig. 3D), strongly curled like integral (∫) or Arabic "ى" (Fig. 3D), slightly whitish to coral (Fig. 2A, B, 3A, B, D, E). Lips approximately 3 mm long, sinuous frilled crape myrtle petal-like, along the manubrium and without point symmetry, slightly translucent whitish. Tentacles 42, irregular in number, rounded cord-like (Fig. 2A, 3G), about 10 times longer than umbrella height when elongated, covered with nematocysts (Fig. 3G), with black endodermal core (Fig. 3F, G), tightly coiled during constriction (Fig. 2A, 3F), slightly translucent whitish (Fig. 3A, B, F, G). Tentacle bulbs 42, irregular in number, drop-like in shape (Fig. 3F), on umbrella margin (Fig. 2A, B, 3A, B, F), having two slender brown endodermal cores inside (Fig. 3F), not located at equal intervals, many in total (Fig. 2A, B, 3A, B), usually one at terminus of each radial canal, and 0–2 per interradial space (Fig. 2A, B, 3A, B, F), not proportional to tentaculiform structures and ocelli. Tentaculiform structures 1–9 (average 4) between adjacent tentacles, uneven in number between adjacent tentacle bulbs and intervals among them (Fig. 3A, B, F), filiform (Fig. 3F), cordyli-like (Fig. 3F), slightly translucent whitish (Fig. 3F), arising directly from ring canal, lacking tentacular bulbs (Fig. 3F), adnate to the edge of the umbrella for none–1/2 of their length, more nematocysts at the tip. Ocelli 4–11 (average 8) between adjacent tentacles, uneven in number between adjacent tentacles and intervals among them (Fig. 3F), on ring canal, small pin-prick-sized, black (Fig. 3F), the presence of a lens is unknown, no correspondence with the place and the number of tentaculiform structures (Fig. 3F). No statocysts. Measurements were made on 2nd October 2018.

Description of Paratype 1 (USNM 1517422; Fig. 4A–D)

Malformed. Umbrella diameter and height approximately 12 mm and 3.5 mm, respectively. Umbrella flat arched (Fig. 4A). Peduncle height approximately 3.3 mm. Diverticula 19 in total. Radial canals 19 in total; nine of them connected to the stomach, reaching the ring canal, not touching other radial canals; five of them connected to the stomach, not reaching the ring canal, not touching other radial canals (Fig. 4Ba); three of them adjacent and connected to the stomach, meeting halfway, reaching the ring canal (Fig. 4Bb); two of them adjacent and crossing, not reaching the ring canal (Fig. 4C). Gonads 19, covering about the 1/4 of peduncle (Fig. 3A), not as variable in length and not as sinuous as the holotype's (Fig. 3A). Lips approximately 1 mm. Tentacles 31, observed about five times longer than umbrella height when elongated (they were likely not fully extended because of the rearing condition without water flow). Tentacle bulbs 32. Tentaculiform structures 1–5 (average 3) between adjacent tentacles. Ocelli 1–10 (average 3) between adjacent tentacles. Measurements were made on 28th September 2018. Note that the features differing from the holotype were recorded.

Description of Paratype 2 (NSMT-Co 1901; Fig. 4C, D)

Umbrella diameter and height approximately 18 mm and 3 mm respectively (distorted because of anesthesia; see 25th September in the development section for the normal value). Umbrella flat arched (hemispherical before anesthesia: Fig. 4E). Peduncle height not measured. Diverticula 22, no correspondence with the number of radial canals. Radial canals 31 in total; 19 of them connected to the stomach, not bifurcated, reaching the ring canal; one of them connected to the stomach, not bifurcated, not reaching the ring canal (Fig. 4Ga); eight of them not connected to the stomach, not bifurcated, not reaching the ring canal (Fig. 4Gb); three of them connected to the stomach, bifurcated just above the gonads (Fig. 4Gc), one branch reaching the ring canal, the other not. Gonads 22, not as variable in length and not as sinuous as the holotype's (Fig. 4E, F, H), no correspondence with the total number of radial canals. Tentacles 32, about 10 times longer than umbrella height when elongated. Tentacle bulbs 34, two of them globular and having no tentacle (Fig. 4F), the others flask-shaped and having one tentacle (Fig. 4E, F). Tentaculiform structures 4–9 (average 6) between adjacent tentacles. Ocelli 5–12 (average 7) between adjacent tentacles. Lips approximately 1 mm long. Measurements were made after anesthesia on 28th September 2018. Note that the features differing from the holotype were recorded.

Description of juvenile medusae (Fig. 5A–D)

Umbrella diameter and height approximately 1.5 mm and 1 mm respectively. Mesoglea 1/5–1/4 as thin at the apex as umbrella height (Fig. 5A–C). Exumbrella transparent (Fig. 5A–D), with scattered nematocysts (Fig. 5D). No peduncle or gonads (Fig. 5A–C). Manubrium 1/3–1/4 as long as umbrella height (Fig. 5A–C), with a process on the opposite side of lips (Fig. 5A–C). Stomach unbranched, brown (Fig. 5B, D). Radial canals 4–6, extending towards the ring canal on the edge of the umbrella (Fig. 5A–D), and leading to the stomach at the other end (Fig. 5A–C), narrow (Fig. 5A–D), mostly simple and straight but occasionally branching into two (Fig. 5A) or winding (Fig. 5C), as many as tentacle bulbs. Tentacles only observed as constricted and tightly coiled (Fig. 5A–D), covered with nematocysts, slightly translucent whitish (Fig. 5D). Tentacle bulbs 4–6, globular (Fig. 5A–D), on umbrella margin (Fig. 5A–D), mostly located at equal intervals (Fig. 5A–C), brown (Fig. 5). Tentaculiform structures 6–12 between adjacent tentacles, uneven in number between adjacent tentacles, short (Fig. 5A, D), cordyli-like (Fig. 5A, D), lacking bulbs (Fig. 5A, D), translucent (Fig. 5A, D). Ocelli 2–7

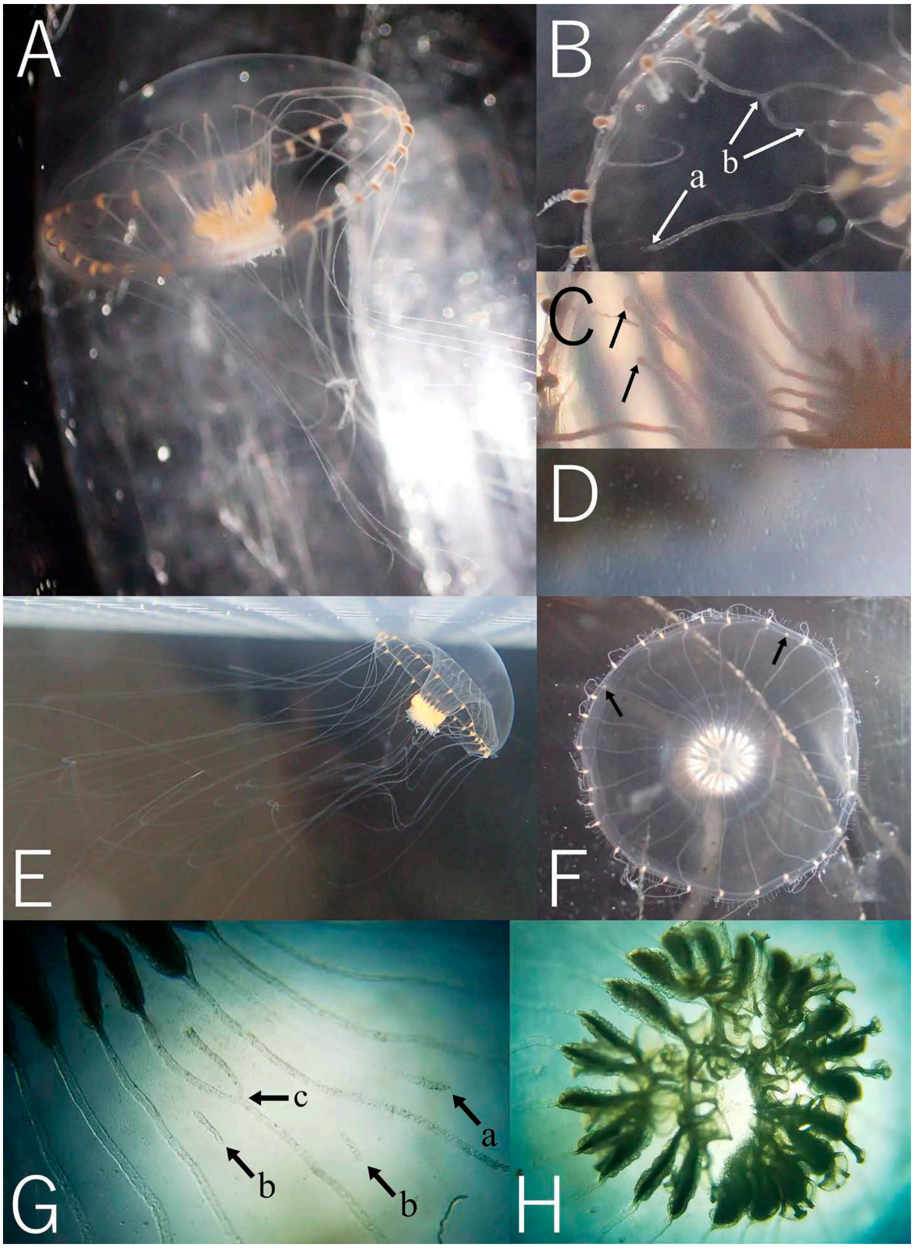

Fig. 4. Habitus of matured paratypes. (A) Lateral view of paratype 1 (USNM 1517422; photographed on 27th September 2018). (B) Radial canals of paratype 1 (photographed on 27th September 2018; a: connected to the stomach, not reaching the ring canal, not touching other radial canals; b: adjacent, connected to the stomach, meeting halfway, reaching the ring canal). (C) Radial canals of paratype 1 (photographed on 27th September 2018; arrows indicate distal ends of adjacent crossing radial canals). (D) Scattered nematocysts on the exumbrella of paratype 1 (photographed on 30th September 2018). (E) Lateral view of paratype 2 (NSMT-Co 1901; photographed on 21st September 2019). (F) Apical view of paratype 2 after anesthesia before preservation (photographed on 27th September 2019; arrows indicate tentacle bulbs globular and having no tentacle). (G) Radial canals of paratype 2 (photographed on 28th September 2019; a: connected to the stomach, not reaching the ring canal, not bifurcated; b: not connected to the stomach or the ring canal, not bifurcated c: connected to the stomach, bifurcated just above the gonads, one branch reaching the ring canal, the other not). (H) Oral view of gonads, stomach, and lips of paratype 2 (photographed on 28th September 2019).

between adjacent tentacles, on ring canal, small pin-prick-sized (Fig. 5D), black (Fig. 5D). Lips corolla daffodil-like (Fig. 5A–C), translucent brownish (Fig. 5A–D). No statocysts. Measurements were made on 15th July 2018 and 14th July 2019, soon after collection.

Development

Holotype (Fig. 6A–J) 3rd August 2018: umbrella diameter and height approximately 6 mm and 3.5 mm respectively, radial canals 12, tentacles 11, tentacle bulbs 12, tentaculiform structures 5.89 between adjacent tentacle bulbs on average, ocelli 3.54 between adjacent tentacle

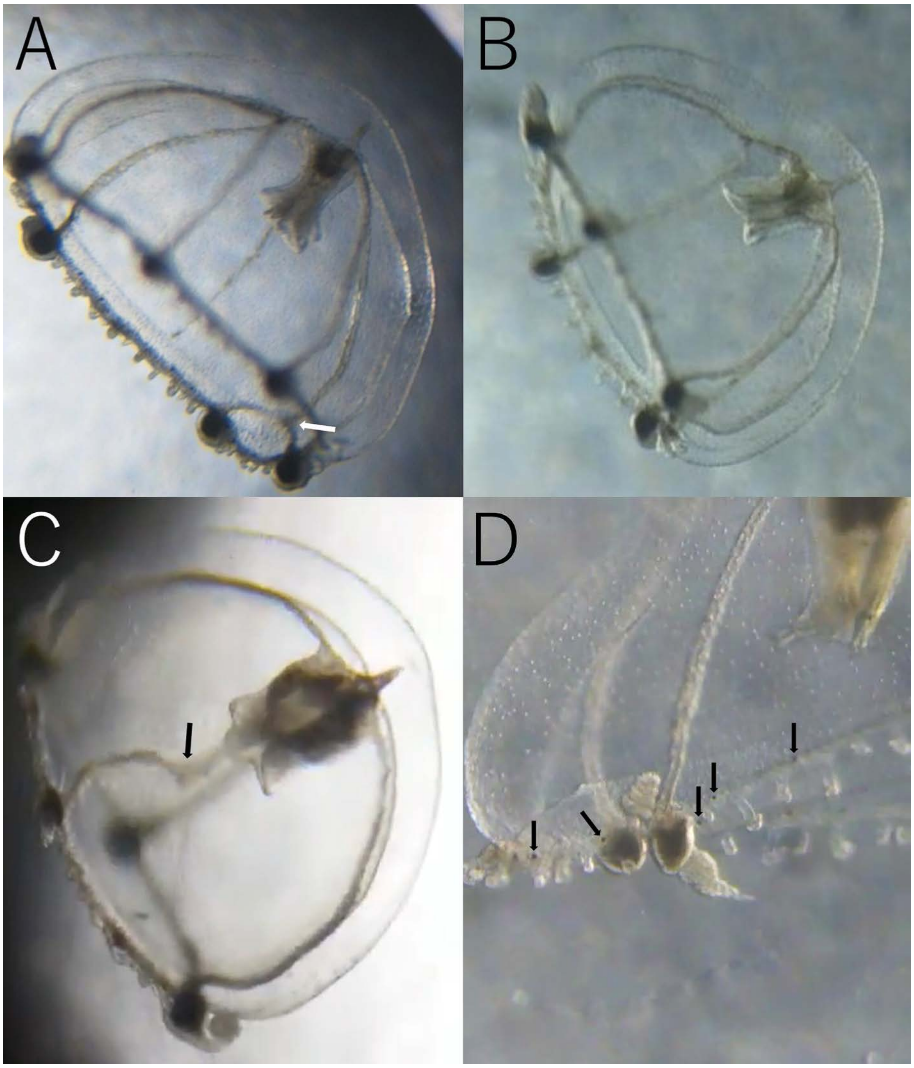

Fig. 5. Habitus of juvenile medusae of *Orchistoma integrale* sp. nov. just after collection (note that they are not necessarily identical to type specimens). (A) Lateral view (collected and photographed on 15th July 2018; the arrow indicates the bifurcation of the radial canals). (B) Lateral view (collected and photographed on 15th July 2018). (C) Lateral view (collected and photographed on 14th July 2019; the arrow indicates the winding radial canal). (D) Edge of the umbrella (collected and photographed on 15th July; arrows indicate the ocelli).

bulbs on average. Peduncle had begun to form (Fig. 6A). Lips sinuous sheet-like (Fig. 6A, B). No manubrium or gonads (Fig. 6A).

6th August: gonads had begun to form (Fig. 6C). Stomach bifurcating irregularly, H-shaped in the center (Fig. 6D).

13th August: peduncle cone-shaped (Fig. 6E).

19th September: umbrella diameter and height approximately 20 mm and 8.5 mm respectively, radial canals 23, tentacles 40, tentacle bulbs 40, tentaculiform structures 3.04 between adjacent tentacle bulbs on average, ocelli 3 between adjacent tentacle bulbs on average. Peduncle bell-shaped and fully developed (Fig. 6G), but gonads not as large or sinuous as matured (Fig. 6F–H). Bifurcations of the stomach are almost same as matured (Fig. 6G). Lips sinuous frilled crape myrtle petal-like (Fig. 6F–H).

2nd October: see description.

3rd October: the umbrella developed a hole at the apex (Fig. 6I).

After that, the hole gradually became larger. The umbrella was flattened accordingly, i.e. umbrella diameter and height approximately 23 mm and 7 mm respectively on 7th October, when it was fixed in formalin (the other features not changed) (Fig. 6J).

Paratype 1 (Fig. 7A–C) 11th September 2018: gonads very small (Fig. 7A). Peduncle bell-shaped (Fig. 7A), about 1/5 of the umbrella diameter at the base (Fig. 7A). Lips simple and slightly sinuous sheet-like (Fig. 7A). No manubrium (Fig. 7A).

17th September: umbrella diameter and height approximately 9.5 mm and 3 mm respectively, radial canals 18, tentacles 24, tentacle bulbs 28, tentaculiform structures 1–4 (average 3) between adjacent tentacle bulbs, ocelli 1–5 between adjacent tentacle bulbs. Peduncle about 1/4 of the

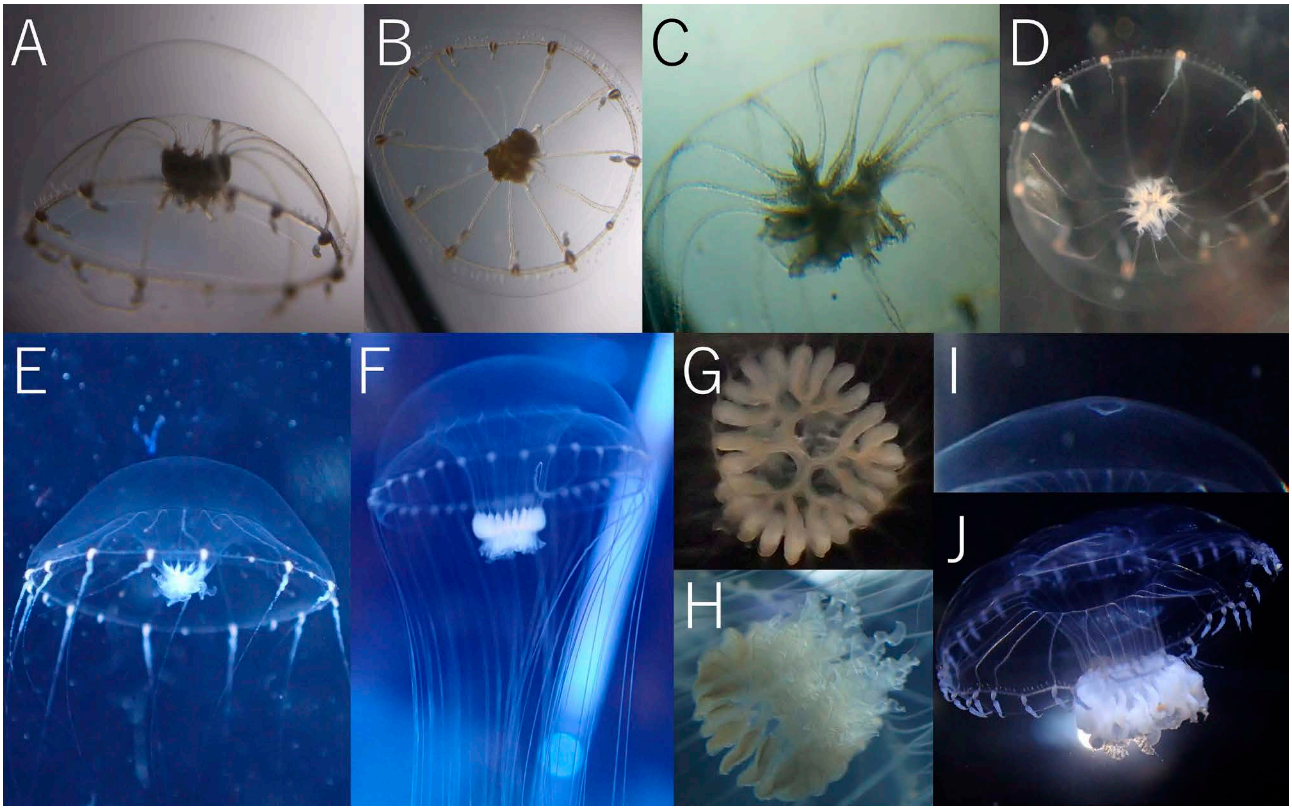

Fig. 6. Morphological transition of holotype (NSMT-Co 1900) of *Orchistoma integrale* sp. nov. (A) Lateral view (photographed on 4th August 2018). (B) Oral view (photographed on 4th August 2018). (C) peduncle, gonads, and lips (photographed on 6th August 2018). (D) Apical view (photographed on 8th August 2018). (E) Lateral view (photographed on 13th August 2018). (F) Lateral view (photographed on 21st August 2018). (G) Dorsal view of gonads and stomach (photographed on 21st August 2018). (H) Oral view of gonads and lips (photographed on 21st August 2018). (I) Hole at the apex of the umbrella (photographed on 4th October 2018). (J) Lateral view on the day of preservation (photographed on 7th October 2018).

umbrella diameter at the base (Fig. 7B), lips sinuous, sheet-like (Fig. 7B).

20th September: radial canals 19, tentacles 26, tentacle bulbs 29. Peduncle, gonads, lips almost same as on 17th September.

28th September: see description.

The specimen grew weak on 29th September (umbrella shrunk to 9.3 mm in diameter) (Fig. 7C) and was fixed in ethanol on 30th September.

Paratype 2 (Fig. 7D, E) 31st July 2019: umbrella diameter and height approximately 7 mm and 3 mm respectively, radial canals 21, tentacles 13, tentacle bulbs 22, tentaculiform structures 1–9 between adjacent tentacle bulbs, ocelli 1–6 between adjacent tentacle bulbs. Gonads very small (Fig. 7D). Peduncle height about 1/4 of the umbrella height. Lips sinuous sheet-like (Fig. 7D).

6th September: umbrella diameter and height approximately 13 mm and 5.2 mm respectively, radial canals 22, tentacles 32, tentacle bulbs 32, tentaculiform structures 2–5 between adjacent tentacle bulbs, ocelli 6–8 between adjacent tentacle bulbs. Peduncle bell-shaped, stomach bifurcated irregularly. Lips sinuous frilled crape myrtle petal-like.

25th September: umbrella diameter and height approximately 16 mm and 7 mm respectively, radial canals 22, tentacles 32, tentacle bulbs 33. Gonads and peduncle have been fully developed. Peduncle approximately 5 mm in height.

27th September: the umbrella developed a hole at the apex (Fig. 7E).

28th September: The umbrella flattened after anesthetizing, i.e. umbrella diameter and height approximately 18 mm and 3 mm respectively, when it was fixed in formalin (see description for other features).

Etymology

The specific epithet "*integrale*" is derived from a mathematic sign, integral (∫), referring to their prominently curled gonads. We are using the noun in apposition. The Japanese name "integuraru" comes from the same word.

Electronic Registration: This published work and the nomenclatural acts it contains have been registered in ZooBank under LSID: urn:lsid:zoobank.org:pub:976BA107-1664-4F7F-8B3B-B2FED38C626B. All the figures and additional supplemental figures are publicly available on Zenodo (https://zenodo.org/records/11094108).

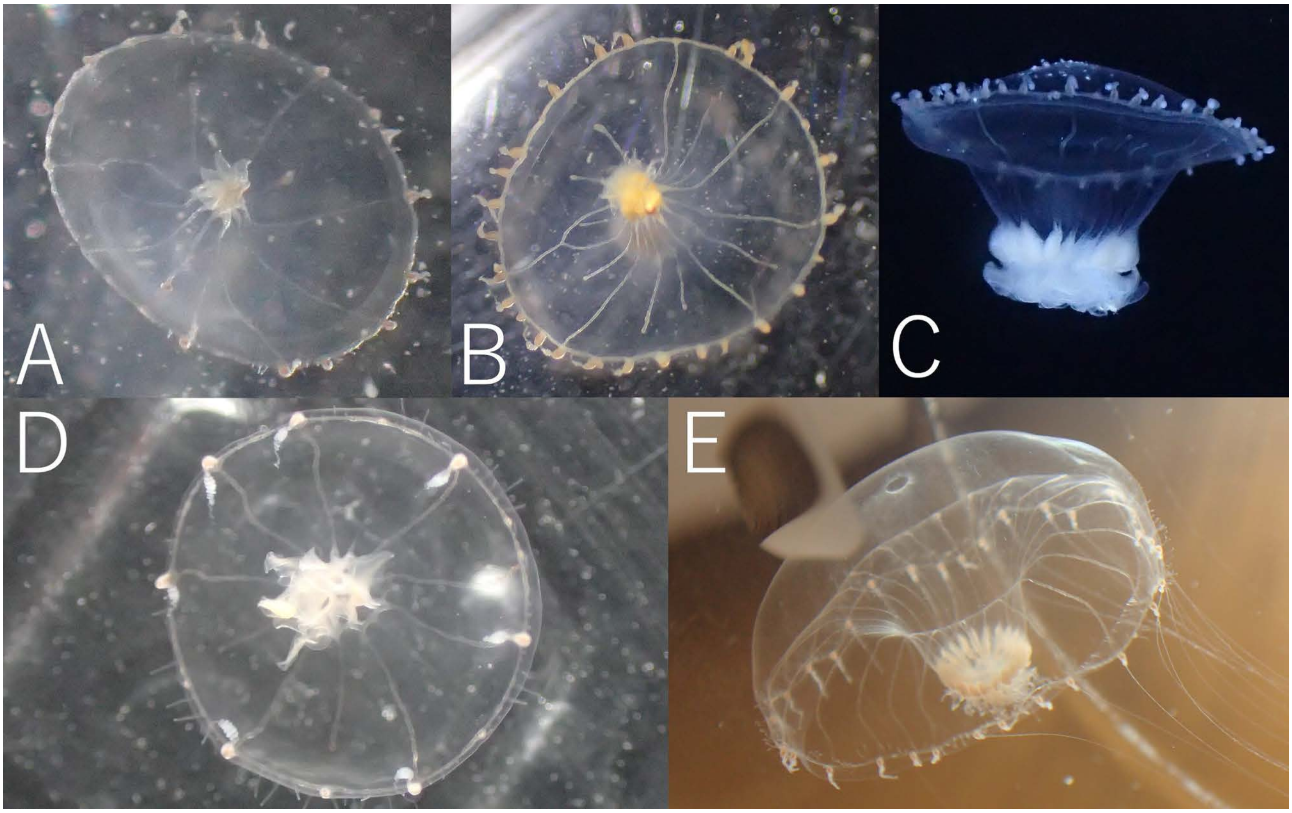

Fig. 7. Morphological transitions of paratypes of *Orchistoma integrale* sp. nov. (A) Oral view of paratype 1 (USNM 1517422; photographed on 11th September 2018). (B) Oral view of paratype 1 (photographed on 11th September 2018) (C) Lateral view of paratype 1 (photographed on 30th September 2018). (D) Apical view of paratype 2 (NSMT-Co 1901; photographed on 28th July 2019). (E) Lateral view of paratype 2 with a hole at the apex (photographed on 27th September 2019).

Genetic data

Two 16S and one COI sequence were successfully derived. The 16S and COI sequences were similar to, but distinct from, two lineages (gonads and manubria brownish-yellow versus white) labelled *Orchistoma pileus* in the works of Schuchert & Collins (2021, 2024). Pair-wise patristic distances for 16S and COI were 0.000–0.017 and 0.005 within the three *Orchistoma* lineages, respectively (Table 2). Interspecific patristic distances between *O. integrale* sp. nov. and the lineage characterized by brownish-yellow manubria and gonads were 0.120–0.138 and 0.243 in 16S and COI, respectively, and from the species with white manubria and gonads by 0.045–0.060 and 0.082–0.085 in 16S and COI. In our phylogenetic trees based on both markers, *O. integrale* sp. nov. is revealed as sister to the white variant of *O. pileus*, with the brownish-yellow variant diverging earlier (Fig. 8). The clade uniting all samples of *Orchistoma* has high support but there are conflicting results about which other species of Laodiceidae and Hebellidae might be the most closely related to *Orchistoma*. Increased taxonomic coverage and markers surveyed are needed to robustly address the phylogenetic position of Orchistomatidae within Leptothecata.

Discussion

The new species of *Orchistoma* we describe from Japanese waters fits the diagnosis of the genus *Orchistoma* (see introduction) except for the rounded tentacles, and the molecular evidence also corroborates this. Based on our morphological investigation, the present species differs from all other species of the genus *Orchistoma* (Table 1). *Orchistoma integrale* sp. nov. can be distinguished from *O. pileus* mainly by the hemispherical umbrella (without vertical sides), tentacles very long when elongated, sinuous frilled crape myrtle petal-like lips; from *O. agariciforme* mainly by the peduncle being bell-shaped (spread at the base), lips shorter, without point symmetry, manubrium without point symmetry, smaller stomach when both species are immature, adnate to the edge of the umbrella for none–1/2 of their length; from *O. collapsum* mainly by the hemispherical umbrella (without vertical sides), peduncle being bell-shaped (spread at the base), gonads connected to the lips, radial canals not in four groups, irregular number of radial canals and tentacles, very short manubrium; from *O. nubiae* mainly by peduncle being bell-shaped (spread at the base), gonads connected to the lips, tentacles rounded cord-like, very long when elongated, radial canals not bifurcated or bifurcated only just above gonads,

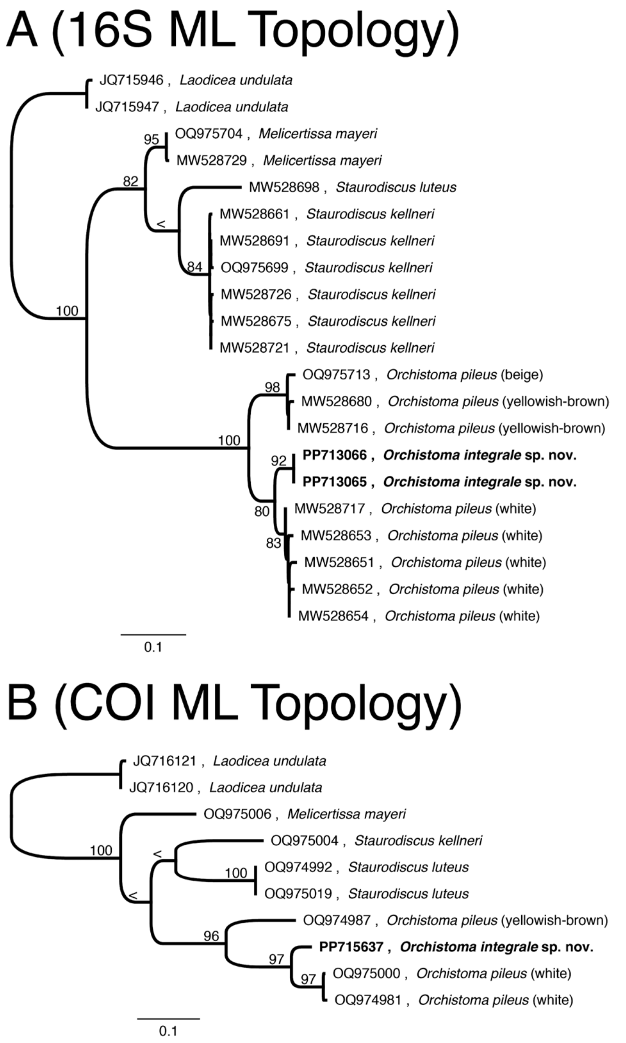

Fig. 8. Maximum likelihood topologies of *Orchistoma integrale* sp. nov. among exemplar taxa from the families Orchistomatidae, Laodiceidae and Hebellidae. Bootstrap indices shown at nodes; "<" indicates a bootstrap index <70. (A) 16S topology. (B) COI topology.

lips shorter; from *O. manam* mainly by the gonads on the most proximal part of the radial canals (connected to lips), the manubrium very short, peduncle being bell-shaped (spread at the base) tentaculiform structures adnate to the edge of the umbrella for none–1/2 of their length; and from *O. mauropoda* mainly by the brown endodermal cores in the tentacle bulbs, scattered nematocysts on the exumbrella, tentaculiform structures adnate to the edge of the umbrella for none–1/2 of their length. Therefore, we conclude that these jellyfish represent an undescribed species and herein provide them with a new name, *Orchistoma integrale* sp. nov.

Based on our genetic investigation, *O. integrale* sp. nov. is distinct from both of the lineages that Schuchert & Collins (2021, 2024) identified as *O. pileus*. The 16S and COI of the two lineages sampled by Schuchert & Collins (2021, 2024) from Florida differed from each other by 0.117–0.141 and 0.264–0.267, respectively (Table 2). These represent considerable genetic divergences, far exceeding what is typically observed for intraspecific variation for leptothecate hydrozoans (e.g. Moura et al. 2018). However, there is no agreed upon divergence threshold that can be applied to 16S or COI sequences to know with certainty that one is dealing with distinct species. Because the only morphological difference that Schuchert & Collins (2021) noted to correspond to the two Florida lineages was the color of the manubrium and gonads, brownish-yellow versus white, they chose not to put different names on the two lineages. In these specimens, umbrella diameter, the number of tentacles, radial canals and lips, and length of lips were variable among the individuals or different developmental stages, suggesting that these features might not be appropriate for delineating species of *Orchistoma*. In their 2024 paper, Schuchert & Collins (2024) noted that one of the specimens belonging to the brownish-yellow clade was beige, more closely resembling the coloration of the white, casting doubt on the reliability of this feature. Another confounding issue for these authors was that it was not clear which of the divergent lineages might be the "true" *O. pileus*, as exemplars from neither lineage consistently matched other morphologically similar nominal species of *Orchistoma* (i.e., *O. collapsum*, *O. agariciforme, O. steenstrupii*). In the case of *O. integrale* sp. nov., it can be differentiated from both lineages labeled *O. pileus* by Schuchert & Collins (2021) by its shorter lips and tentaculiform structures adnate to the edge of the umbrella for none–1/2 of their length, as well as by its considerable divergence in the 16S and COI markers (Table 2). We predict that further work on the *O. pileus* lineages from Florida will confirm them to be evolutionarily distinct species that should be known by two different names. At present, these two putative species are united under a single name provided by expert taxonomists, a situation which is by no means isolated, especially when it comes to hydrozoans and other taxa that contain a fraction of species that have proven to be very difficult to delimit (e.g., Cunha et al. 2020). This poses a serious challenge for marine biodiversity science because genetic data are increasingly being used to identify the presence of species in environmental samples, e.g., gut contents, water samples, etc. to address critical societal questions relating to the function and health of diverse aquatic ecosystems (e.g., Kelly et al. 2024, Suzuki et al. 2024). Information about varied aspects of species is linked through scientific names, making it an essential aspect to global biodiversity efforts to name and describe species as quickly as possible using approaches that integrate morphological and genetic characterization.

Orchistoma pileus, *O. agariciforme*, and *O. collapsum* have very similar morphologies. Lesson (1843) provided a simple description of *O. pileus*, noting numerous radial canals and especially the distinctively long mouth lips, and suggested that it inhabited the Red Sea ("Habite les mers d'Afrique?"). *Orchistoma agariciforme* was first de-

Table 1. (part 1) Comparison of morphological features between *Orchistoma integrale* sp. nov. and the other species in the genus. The underlined parts are not described in the original TEXT descriptions, but were determined from photographs and sketches. Bold type denotes major morphological features that differ between *O. integrale* sp. nov. and the remaining species (Abbreviations: UD, umbrella diameter; UH, umbrella height).

Species	Reference	Locality	Umbrella	Peduncle	Gonads	Radial canals	Lips
O. pileus	Lesson (1843)	West coast of Africa?	Hemispherical, **with vertical sides**	Not described (almost same as *O. integrale* sp. nov.)	On proximal portion of radial canals	Numerous (about 30?)	**Ciliar**
O. pileus	Mayer (1910)	Bahamas/Florida, USA	UD 30–40 mm; **flat-topped with vertical sides**	**Cone-shaped (not spread at the base)**	swollen, linear; upon each radial canal very near its point of juncture with the stomach (connected with lips)	32	32; **long (about 1/3 of UD),** complexly crenulated
O. pileus	Schuchert & Collins (2021)	Florida, USA	UD 10–34 mm; almost hemispherical or somewhat shallower	Tapering	**Brownish-yellow or whitish**, on proximal portion of radial canals (connected with lips)	Some individuals have irregular or reticulate radial canals likely due to a healed damage	10–16, **long, many are longer than gonads both in the mature and in the immature stage**
O. agariciforme	Keller (1884) (holotype)	Naples, Italy	UD 20 mm, UH 10 mm; very curved and completely hemispherical	**Shape of a truncated cone (not spread at the base)**	On proximal portion of radial canals (connected with lips)	20	2–3 mm in length; 7; pinnate
O. agariciforme	Keller (1884) (immature stage)	Collections at Stazione Zoologica Anton Dohrn	Not described	Almost undeveloped	Not existing	16	7
O. agariciforme	Bouillon (1984)	Laing Island, Papua New Guinea	UD 15 mm, UH 5 mm in the largest specimens; flatter than they are hemispherical	**Shape of a funnel (not spread at the base)**	On proximal portion of radial canals (connected with lips)	16–20; separated in four groupes, more rarely of 3 or 5	about 3 mm; 4; **very long (about 1/6 of UD)**, **cruciform**
O. collapsum	Mayer (1900b)	Florida, USA	UD 7 mm; **about as high as broad.** The top is dome-shaped, and **the side walls are vertical**	**Hemispherical (not spread at the base)**	Very near to the point where they branch off from the proboscis **(without contact with lips)**	16, **most likely doubling in number as they grow**; arranged in **four groups of four each**	8; slightly crenulated
O. nubiae	Bouillon (1984)	Laing Island, Papua New Guinea	UD 25–30 mm, UH 7 mm; more flattened than hemispherical	**Conical (not spread at the base)**	Narrow, elongated; on proximal portion of radial canals **(without contact with lips)**	33–38; branching into two **or three at the junction to manubrium,** many radial canals are more bifurcated on their way to the circular canal	7 mm in length; 32; **very long (about 1/4 of UD)**, with crenellated marginal edges
O. manam	Bouillon (1984)	Laing Island, Papua New Guinea	UD 5 mm; hemispherical	**Conical (not spread at the base)**	The form of pockets; **on the median part of the radial canals, without contact with stomach**	8	8; sinuous
O. mauropoda	Gershwin, et al. (2010)	Queensland, Australia	UD 7.06 mm (holotype)	Not described (almost same as *O. integrale* sp. nov.)	On proximal portion of radial canals (connected with lips)	16–18; each corresponding to a tentacle; simple (holotype)	Not well defined
O. integrale sp. nov.	Present study	Wakayama Prefecture, Japan	UD 21 mm and UH 10.5 mm in holotype on 2nd October 2018; hemispherical, without vertical sides	Bell-shaped (spread at the base)	On the most proximal part of radial canals (connected to lips)	23 in holotype on 2nd October 2018; irregular in number, not bifurcated or bifurcated only just above gonads, not in four groups	Short, sinuous frilled crape myrtle petal-like, without point symmetry

Table 1. (part 2)

Species	Tentaculiform structures	Stomachs	Tentacles	Ocelli	Tentacle bulbs	Manubrium	Nematocysts on the exumbrella
O. pileus	Not described	Not described	**Very short**, about 30?	Not described	About 30 ?	Very short	Not described
O. pileus	About 100; from the side of exumbrella **at a short distance above the bel margin**	Not described	64, **shorter than the bell-radius**; **flat and ribbon-like**	About 400	In the male, dull blue-gray (color in males is not observed in *O. integrale* sp. nov.)	Very short	Not described
O. pileus	4–12, usually 5–6 between adjacent tentacles; **proximal half adnate to or embedded in umbrella**	Base in centre H- or cross-shaped then subdividing dichotomously or irregularly into elongate diverticula attached to the distal part of the gastric peduncle	16–29, not in phase with the radial canals	Numerous; not correlated with tentacle positions	Ovoid, tapering rapidly into tentales	Brownish-yellow or whitish, very short	Not described
O. agariciforme	Average 6–8 between adjacent tentacles	Not described	20	400 in total, average 20 between adjacent tentacles	Heart-shaped	Very short	Not described
O. agariciforme	Not described	4.5 mm wide**; well-developed (about 1/3 of UD),** drawn out into numerous lobes, from which centrifugal single radial canal sprouting	16	Not described	23	Very short	Not described
O. agariciforme	8–10 intertentacular space; **adnate to the edge of the umbrella over half of their length**	Not described	16–27, long	16–20 between adjacent tentacles	Long, conical	Particularly short, flattened, **clearly cruciform**	Not described
O. collapsum	112 in total	Not described	16, **most likely doubling in number as they grow**; carried tightly coiled in close helices	not described (described as pigments at the base of each tentalce?)	Not described	**Prominent**, with an entoderm green in some specimens, pearly-white or yellowish in others	Not described
O. nubiae	2–3 between adjacent tentacles	Not described	Less than twice as many tentacles as radial canals (up to 64); **15 mm**; **compressed laterally over part of their length**	600–800	Elongated	Wide and exceptionnally short, flattened	Not described
O. manam	16–24 between adjacent tentacles; **adnate to the umbrella for half of their length**	Not described	4	36–48 between adjacent tentacles (usually 20 per octant, about twice as many as tentaculiform structures)	Voluminous and rounded	**Large**, low	Not described
O. mauropoda	About 12 between adjacent tentacles; **adnate to the umbrella**, with free end just above bell margin (holotype)	18 (holotype); branching irregularly into four major lobes, each branching dendritically into four to six lobes, leading to radial canals	16–20	16–20 bewtween adjacent tentacles (holotype); one or more at the base of each tentaculiform structures	Flask-shaped; with **blackish** endodermal core; one at terminusof each radial canal, plus a couple not corresponding to radial canals (holotype)	Very short	**Apparently lacking (holotype)**
O. integrale sp. nov.	1–9 (average 4) between adjacent tentacles in holotype on 2nd October 2018; adnate to the edge of the umbrella for none–1/2 of their length	Without point symmetry, bifurcated irregularly, small when immature	42 in holotype on 2nd October 2018; irregular in number, rounded cord-like, very long (about 10 times longer than the umbrella height in holotype) when elongated	4–11 (average 8) between adjacent tentacles in holotype on 2nd October 2018; no correspondence with the place and the number of tentaculiform structures	42 in holotype on 2nd October 2018; having brown endodermal cores inside	Very short, without point symmetry	Scattered

Table 2. Summary of pair-wise patristic distances among mitochondrial 16S and COI for exemplars of *Orchistoma.*

	O. integrale sp. nov.	*O. pileus* (brownish-yellow)	*O. pileus* (white)
16S			
O. integrale sp. nov.	0.000	—	—
O. pileus (brownish-yellow)	0.12–0.138	0.006–0.017	—
O. pileus (white)	0.045–0.060	0.117–0.141	0.006–0.017
COI			
O. integrale sp. nov.	NA	—	—
O. pileus (brownish-yellow)	0.243	NA	—
O. pileus (white)	0.082–0.085	0.264–0.267	0.005

scribed by Keller (1884) and distinguished from *O. pileus* by having fewer lips. Bouillon (1984a), who collected *O. agariciforme* from the Mediterranean said that it also can be distinguished by the number of radial canals and tentacles and lips, as well as by umbrella diameter. Further, he suggested that *O. agariciforme* and *O. collapsum* are different in that *O. collapsum* has radial canals separated in four clear groups even at maturity, with a longer manubrium and more distinct but shorter mouth lips. More recently, Schuchert & Collins (2021) decided to synonymize *O. agariciforme* and *O. collapsum* with *O. pileus*, arguing that there is a lack of reliable characters to distinguish them.

We disagree with this conclusion because there are notable morphological differences among the three species. Notably, Lesson's *O. pileus* was so unusual in terms of its long, filiform lips (described as "cils" by Lesson) that none of the *Orchistoma* specimens collected later, including those of Schuchert & Collins (2021), conform with this feature. Further, *O. agariciforme* and *O. collapsum* appear to be distinguishable (see Table 1).

In Mayer's *O. collapsum*, tentacles and radial canals are numbered regularly. The youngest medusa in Mayer's sample had four tentacles and four radial canals. In the next stage, they had 16 radial canals (according to his sketch, eight tentacles exist although he did not mention the tentacle number at this stage). The holotype of *O. collapsum* was described as being an adult medusa with 16 tentacles and 16 radial canals. Thus, Mayer's work suggests that tentacles and radial canals of *O. collapsum* double, i.e., 4→8→16. In contrast, the numbers of tentacles and radial canals of Keller's *O. agariciforme*, as well as the specimens of Schuchert & Collins (2021) do not follow the same regularity as *O. collapsum*. Further, Keller's *O. agariciforme* possesses an *Aequorea*-like stomach at an early stage of development without gonads, whereas the stomach of juvenile *O. collapsum* is inconspicuous. Moreover, the gonads of *O. collapsum* are located very near the point where the radial canals branch off from the manubrium (proboscis), not on the oral end of the radial canals, and the manubrium of *O. collapsum* appears from the original illustrations to be more conspicuous than that of *O. agariciforme*. Thus, the gonads of *O. collapsum* are separated from the lips, whereas in *O. agariciforme*, the gonads touch the lips. In light of these differences, we consider *O. pileus*, *O. agariciforme* and *O. collapsum* to be distinct and valid species until further evidence, and that the specimens of Schuchert & Collins (2021) are likely not correctly identified as *O. pileus*.

In this study, many juvenile medusae of *O. integrale* sp. nov. were observed at the type locality. Therefore, it seems likely that there is a population of polyps, which are still unknown for the genus, living in the vicinity. All of the three specimens collected in this study (holotype, paratype 1, and paratype 2) are female. When raised to maturity, the medusae exhibited variability in the shape of the umbrella, gonads and radial canals (bifurcated or not), the number of tentacles, and radial canals (see description for details). Variability in morphology was also evident across developmental stages. In the early stages of development, the gonads were small and simple, whereas they later become large and curled. Moreover, they have no peduncle just after being released but a peduncle develops and becomes quite large when mature. Also, at the mature stage, they have about eight times as many tentacles as just released medusae. In summary, there are large differences according to each individual or developmental stage in the morphology of species of Orchistomatidae, which further complicates their identification. Nonetheless they have sometimes been described and differentiated by these variable characters (Schuchert & Collins, 2021; see Introduction part). When a new species of Leptothecata with medusa stages is described, it would be ideal for it to be diagnosed by structural features that are not highly variable among adults and, when possible, relatively stable after the earliest developmental stages. That said, we recognize that it is difficult to accurately discern interspecific and individual differences, and we show all features that may be associated with a species, such as the shape of the stomach and the peduncle, which were variable among the developmental stages.

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