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Description of a New Species of the Marine Flatworm *Prosthiostomum* (Platyhelminthes: Polycladida) and its Three Known Congeners from Misaki, Japan, with Inference of Their Phylogenetic Positions within Prosthiostomidae

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The present study provides morphological descriptions of four species of *Prosthiostomum* (Polycladida, Prosthiostomidae)—*P. auratum* Kato, 1937; *P. hibana* sp. n.; *P.* cf. *ostreae* Kato, 1937; and *P. vulgare* Kato, 1938—based on specimens collected among branching coralline algae and kelp holdfasts in Misaki, Japan. The new species *P. hibana* sp. n. is characterized by i) the dorsal surface of the body covered with numerous orange maculae, some of which coalesce together to form larger ones; ii) a pair of linear cerebral-eyespot clusters, each consisting of relatively few (7–9) cerebral eyespots; iii) 3–4 pairs of ventral eyespots embedded in parenchyma: iv) the inner wall of the male atrium deeply ruffled; v) the lumen of the seminal vesicle being narrow and elongated in shape; and vi) a large sucker situated in the center of the body. We remark on some morphological characters that were not mentioned in the original description of *P. auratum*. We infer the phylogenetic positions of these four species within Prosthiostomidae using the maximum-likelihood analysis based on partial 28S rRNA and *COI* gene sequences determined *de novo*, in addition to those that are currently available in public databases. In the resulting tree, the four species—*P. auratum*, *P. hibana* sp. n., *P.* cf. *ostreae*, and *P. vulgare*—were nested in a clade that was composed of all the other *Prosthiostomum* species included in the analysis.

Key words: Cotylea, Phylogeny, *Species inquirenda*, Systematics, Taxonomy.

BACKGROUND

The order Polycladida consists of more than 800 species of marine flatworms (Tyler et al. 2006–2020) that live in variety of marine habitats, including tide pool, coral reefs, mud flat, and deep sea (Prudhoe 1985). Some species are known to associate with other invertebrates, such as corals (*e.g.*, Rawlinson et al. 2011), hermit crabs (*e.g.*, Lytwyn and McDermott 1976), innkeeper worms (Anker et al. 2005), and mollusks (*e.g.*, Fujiwara et al. 2016). Of the about 150 species of Polycladida known to inhabit Japanese waters (Kato 1944), 130 were originally described from this area; ~100 of the latter are poorly known and thus can be regarded *species inquirendae*, or species of questionable taxonomic status. They were mostly established over 70

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years ago (*e.g.*, Kato 1944) and have not been recorded since. As for the species that were either redescribed or originally described since the 1990s (Hagiya 1992; Oya and Kajihara 2017 2019a b; Oya et al. 2019; Tsuyuki et al. 2019; Oya et al. 2020; Tsuyuki and Kajihara 2020), morphological characters important for generic assignment have been clearly documented and/or illustrated. However, because such key characters became incorporated to taxonomic revisions during the 1980s (Faubel 1984; Prudhoe 1985), it is no surprise that these features were scarcely mentioned in original taxonomic descriptions before that period (*e.g.*, Stimpson 1857; Yeri and Kaburaki 1918 1920; Kato 1944) with some notable exceptions (*e.g.*, Bock 1922 1923 1924).

The genus affiliations of these species remain questionable, and they cannot be confirmed based on their name-bearing type specimens because those specimens are entirely non-existent. Stimpson's material is said to have been destroyed during the Great Chicago Fire in 1871 (*e.g.*, Evans 1967; Deiss and Manning 1981). The material used by Yeri and/or Kaburaki has not been found, likely because it was lost during the Great Kanto Earthquake and a subsequent disastrous fire in 1923 (cf. Kato 2018). Kato's material was destroyed during the Bombing of Tokyo in 1945 (Kawakatsu 2004). Therefore, to precisely understand the polyclad biodiversity and systematics in not only Japanese waters but also the Northeast Pacific, morphological and molecular information is needed on these *species inquirendae* based on newly collected specimens, preferably from type localities.

The cotylean polyclad family Prosthiostomidae Lang, 1884 is characterized by i) an elongated body with a ventral sucker posterior to the female gonopore, ii) a plicate tubular pharynx, and iii) paired prostatic vesicles. Monophyly of this family has been supported in previous molecular phylogenetic studies based on partial sequences of the 28S rRNA gene alone (Bahia et al. 2017; Tsunashima et al. 2017; Litavaitis et al. 2019) or in combination with the 18S rRNA gene (Dittmann et al. 2019). Aguado et al. (2017) argued that Prosthiostomidae is not monophyletic, but this is probably due to the fast-evolving gene markers that they utilized (*i.e.*, the mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I (*COI*) genes). Prosthiostomidae currently includes five genera: *Enchiridium* Bock, 1913; *Enterogonimus* Hallez, 1911; *Euprosthiostomum* Bock, 1925; *Lurymare* Du Bois-Reymond Marcus and Marcus, 1968; and *Prosthiostomum* Quatrefages, 1845 (Faubel 1984). Of these, *Lurymare* may be a junior synonym of *Prosthiostomum* (cf. Dittmann et al. 2019; Litvaitis et al. 2019; Tsuyuki et al. 2019), because the alleged morphological distinction between the two genera is based on a character that can vary ontogenetically (Prudhoe 1989), namely the presence/absence of a muscle bulb surrounding the seminal and prostatic vesicles (Faubel 1984). Indeed, Litvaitis et al. (2019) transferred two species formerly placed in *Lurymare* into *Prosthiostomum* primarily because their phylogenetic positions were nested within a clade comprising *Prosthistomum* species. While the separation of *Lurymare* from *Prosthiostomum* based on this character alone (*i.e.*, presence/absence of a muscle bulb) appears systematically unsubstantiated, a definitive taxonomic act to formally synonymize *Lurymare* with *Prosthiostomum* should not proceed until an analysis is performed using reliably identified prosthiostomid specimens, including those representing the type species of the two genera (*Prosthiostomum drygalskii* Bock, 1931 for *Lurymare*; *Planaria siphunculus* Delle Chiaje, 1828 for *Prosthiostomum*).

For the sake of conciseness, this *Prosthiostomum*–*Lurymare* complex is simply referred to as the genus *Prosthiostomum* in this paper. It currently contains about 60 species worldwide, which are characterized by i) the seminal and prostatic vesicles that are occasionally (but usually not) surrounded by a common muscle bulb, ii) the main intestine accompanied with a frontal branch over the pharynx, and iii) the penis armed with a pointed tubular stylet (cf. Faubel 1984). Congeners are distinguished chiefly based on body color pattern and eyespot arrangement (Bock 1913; Hyman 1939b). In Japan, 22 species of *Prosthiostomum* have been reported (Kato 1944; Tsuyuki et al. 2019). Twenty of these—all except for *P. purum* Kato, 1937b (Litvaitis et al. 2019 [Israel]) and *P. trilineatum* Yeri and Kaburaki, 1920 (Newman and Cannon 2003 [Australia]; Pitale et al. 2014 [India]; Litvaitis et al. 2019 [Guam])—have so far been found exclusively along the Japanese coasts, and thus may be endemic to this area.

Misaki, situated on the western coast of the Miura Peninsula of Honshu, Japan, is one of the faunistically best-studied areas in terms of not only polyclads but also other marine organisms along Japanese coastal regions (Kajihara and Kakui 2017). So far, 29 polyclad species have been originally described based on specimens collected in shallow water around Misaki (Yeri and Kaburaki 1918; Bock 1922 1923 1924; Kato 1937b). Of these, five represent the genus *Prosthiostomum*: *P. auratum* Kato, 1937b; *P. ostreae* Kato, 1937b; *P. purum* Kato, 1937b; *P. rubropunctatum* Yeri and Kaburaki, 1918; and *P. yerii* Kato, 1937b. These five species are *species inquirendae* and have not been reported from Misaki since their original descriptions, although distribution of *P. auratum* and *P. purum* in localities other than Misaki have been reported (*P. auratum* from

other Japanese coastal regions such as Asamushi, Noto, Shimoda, Shirahama, and Amakusa (Kato 1938a b 1939b 1944) and Manazuru (Hagiya and Gamo 1992); *P. purum* from the northern coast of the Red Sea (Litvaitis et al. 2019)).

A faunal survey was conducted in Misaki in the hope of collecting topotypes of *P. auratum*, *P. ostreae*, *P. purum*, *P. rubropunctatum*, and *P. yerii*, as well as revealing undiscovered polyclad diversity in this region. As a result, we obtained polyclad specimens representing four species of *Prosthiostomum*, one of which turned out to be new to science. The purposes of this paper are to i) describe the new species and provide morphological accounts of the other three species with taxonomic remarks and ii) examine the generic placements of these species through molecular phylogenetic analysis using sequences of the 28S rRNA and *COI* genes.

MATERIALS AND METHODS

Polyclad specimens were collected in Arai-hama, Misaki, Kanagawa, Japan; nine specimens were collected from kelp holdfasts at 2 m deep by SCUBA on February 19, 2019; the others were collected subtidally from branching coralline algae by snorkeling on March 25, 2019. Worms were anesthetized in seawater containing menthol before fixation. The relaxed worms were photographed with a Nikon D5600 digital camera with external strobe lightning provided by a pair of Morris Hikaru Komachi Di flash units. For DNA extraction, a posterior piece of the body was removed and stored in 99.5% ethanol. The rest of the body was fixed in Bouin's solution for 24 h and preserved in 70% ethanol for long-term storage.

For histological examination, tissues were dehydrated in an ethanol series, cleared in xylene, embedded in paraffin wax, and sectioned serially at a thickness of 7 µm on a sagittal plane using a microtome. Sections were stained with hematoxylin and eosin, mounted on glass slides in Entellan New (Merck, Germany), and then observed and photographed under an Olympus BX51 compound microscope. All slides were deposited into the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan. Other specimens were deposited in Aoi Tsuyuki's personal polyclad collection (AT). All graphical treatments were done with Adobe Photoshop CC. Illustrations were prepared with Adobe Illustrator CC.

Total DNA was extracted using a silica-based method (Boom et al. 1990) after specimens were incubated overnight at 55°C in 180 µl of ATL buffer (Qiagen, Germany) with 20 µl of proteinase K (> 700 U/ml; Kanto Chemical, Japan). A 585-bp fragment of the *COI* gene was amplified with primers Pros_*COI*F and Pros_*COI*R (Tsuyuki and Kajihara 2020). A fragment (ca. 1010 bp) of the 28S rRNA gene was amplified with primers fw1 and rev2 (Sonnenberg et al. 2007). Polymerase chain reaction (PCR) amplification conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s, 52.5°C (28S rRNA) or 50°C (*COI*) for 30 s, 72°C for 1.5 min (28S rRNA) or 1 min (*COI*); and 72°C for 7 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, California, USA). Following a protocol by Oya and Kajihara (2020), two internal primers—hrms_fw2 (Oya and Kajihara 2020) and rev4 (Sonnenberg et al. 2007)—were used in addition to fw1 and rev2. Sequences were checked and edited using MEGA ver. 7.0 (Kumar et al. 2016). In addition, the following were determined by the same methods described above: a 944-bp partial sequence of the 28S rRNA gene and a 553-bp partial sequence of the *COI* gene from a specimen of *P. grande* Stimpson, 1857 (ICHUM 6032), collected by A. Tsuyuki in Kagoshima, Japan, on July 25, 2018, as well as a 585-bp partial *COI* gene sequence from the holotype of *P. torquatum* Tsuyuki et al., 2019 (ICHUM 5563). All the edited sequences were deposited into DDBJ/EMBL/GenBank under accession numbers LC625886–LC625900 and LC635089.

For a phylogenetic analysis, a concatenated dataset comprised of partial 28S rRNA and *COI* gene sequences was prepared. We employed the 28S rRNA gene as one of the two markers because it is the most commonly used for determining members of Polycladida (Bahia et al. 2017; Tsunashima et al. 2017; Dittmann et al. 2019; Litvaitis et al. 2019). In addition to the sequences determined for the four species from Misaki and for *P. grande* (see above), 24 other partial 28S rRNA gene sequences from 19 prosthiostomid species downloaded from GenBank were used in the analysis; multiple sequences derived from individuals representing geographically different local populations were used for *Enchiridium periommatum* Bock, 1913; *Enchiridium* sp. 2 of Litvaitis et al. (2019); and *P. siphunculus* (Table 1). The 28S rRNA gene sequences were aligned using MAFFT ver. 7.427 (Katoh et al. 2017), with the L-INS-i strategy selected by the "Auto" option; ambiguous sites were removed with Gblocks ver. 0.91b (Castresana 2002) using options for a less stringent selection. The *COI* sequences of the four species collected in Misaki were used, plus *P. grande* and *P. torquatum* (see above) and *E. daidai* Tsuyuki and Kajihara, 2020 (holotype, ICHUM 5993) (Table 1). *COI* was aligned manually with MEGA ver. 7.0 (Kumar et al. 2016). A concatenated dataset (1524 bp in total length, consisting

of 939-bp 28S rRNA and 585-bp *COI*) was prepared, also with MEGA ver. 7.0.

The phylogenetic analysis was performed with the maximum likelihood (ML) method using RAxML-NG ver. 0.9.0 (Kozlov et al. 2019) under a partition model. The optimal substitution models selected with Partitionfinder ver. 2.1.1 (Lanfear et al. 2016) under the Akaike Information Criterion (AIC) (Akaike 1974) using the greedy algorithm (Lanfear et al. 2012) were GTR+I+G (28S rRNA, second codon position in *COI*) and GTR+G (first and third codon positions in *COI*). *Prostheceraeus crozieri* (Hyman, 1939a) (Eurylepti dae) and *Pseudobiceros splendidus* (Lang, 1884) (Pseudocerotidae) were selected as outgroups (Tsuyuki and Kajihara 2020) (Table 1). Nodal support within the ML tree was assessed by analyzing 100 bootstrap pseudoreplicates (Felsenstein 1985). We considered ML bootstrap (BS) values ≥ 70% as indicating clade support.

Table 1. List of species used for the molecular phylogenetic analysis, sample locations, DDBJ/EMBL/GenBank accession numbers, and references

Species	Location	Accession number		Reference
		28S rRNA	*COI*	
Enchiridium daidai Tsuyuki and Kajihara, 2020	Bonotsu, Kagoshima, Japan	LC504235	LC504240	Tsuyuki and Kajihara (2020)
Enchiridium evelinae Marcus, 1949	Praia das Conchas, Rio de Janeiro, Brazil	KY263683.2	-	Bahia et al. (2017)
Enchiridium japonicum Kato, 1943	Eilat, Israel	MH700298	-	Litvaitis et al. (2019)
Enchiridium periommatum Bock, 1913	St. Ann's Bay, Jamaica	MH700299	-	Litvaitis et al. (2019)
Enchiridium periommatum Bock, 1913	Crawl Cay, Bocas del Toro, Panama	MH700300	-	Litvaitis et al. (2019)
Enchiridium periommatum Bock, 1913	Tavernier Key, Florida, USA	MH700301	-	Litvaitis et al. (2019)
Enchiridium sp. 1	Saint Helena Island, UK	KY263665	-	Bahia et al. (2017)
Enchiridium sp. 2	New South Wales, Australia	MH700302	-	Litvaitis et al. (2019)
Enchiridium sp. 2	Heron Island, Australia	MH700303	-	Litvaitis et al. (2019)
Enchiridium sp. 3	Lizard Island, Australia	MN384686	-	Dittmann et al. (2019)
Euprosthiostomum mortenseni Marcus, 1948	St. Ann's Parish, Jamaica	MH700304	-	Litvaitis et al. (2019)
Prosthiostomum acroporae (Rawlinson et al., 2011)	Victorville, California, USA	MH700370	-	Litvaitis et al. (2019)
Prosthiostomum auratum Kato, 1937b	Misaki, Kanagawa, Japan	LC625886	LC625892	this study
Prosthiostomum cynarium Marcus, 1950	St. John, US Virgin Islands	MH700371	-	Litvaitis et al. (2019)
Prosthiostomum grande Stimpson, 1857	Sakurajima, Kagoshima, Japan	LC635089	LC625900	this study
Prosthiostomum hibana sp. n.	Misaki, Kanagawa, Japan	LC625887	LC625894	this study
Prosthiostomum katoi Poulter, 1975	Lizard Island, Australia	MN384694	-	Dittmann et al. (2019)
Prosthiostomum lobatum Pearse, 1938	Missouri Key, Florida, USA	MH700372	-	Litvaitis et al. (2019)
Prosthiostomum milcum Du Bois-Reymond Marcus and Marcus, 1968	Long Key, Florida, USA	MH700373	-	Litvaitis et al. (2019)
Prosthiostomum cf. *ostreae* Kato, 1937b	Misaki, Kanagawa, Japan	LC625889	LC625896	this study
Prosthiostomum purum Kato, 1937b	Gulf of Aqaba, northern Red Sea	MH700374	-	Litvaitis et al. (2019)
Prosthiostomum siphunculus (Delle Chiaje, 1828)	Spain	HQ659012	-	Rawlinson et al. (2011)
Prosthiostomum siphunculus (Delle Chiaje, 1828)	Asturias, Spain	MN384697	-	Dittmann et al. (2019)
Prosthiostomum siphunculus (Delle Chiaje, 1828)	Barcelona, Spain	MN421934	-	Dittmann et al. (2019)
Prosthiostomum torquatum Tsuyuki et al., 2019	Shirahama, Wakayama, Japan	LC504234	LC625899	Tsuyuki and Kajihara (2020)
Prosthiostomum trilineatum Yeri and Kaburaki, 1920	Ritidian Point, Guam	MH700376	-	Litvaitis et al. (2019)
Prosthiostomum utarum Marcus, 1952	Piscadera Baai, Curaçao	MH700377	-	Litvaitis et al. (2019)
Prosthiostomum vulgare Kato, 1938b	Misaki, Kanagawa, Japan	LC625891	LC625898	this study
Prosthiostomum sp.	Eilat, Israel	MH700375	-	Litvaitis et al. (2019)
Outgroup				
Prostheceraeus crozieri (Hyman, 1939a)	Long Key, Florida, USA	HQ659013	-	Rawlinson et al. (2011)
Pseudobiceros splendidus (Lang, 1884)	North Heron Island, Australia	MH700388	-	Litvaitis et al. (2019)

RESULTS

TAXONOMY

Family Prosthiostomidae Lang, 1884
Genus *Prosthiostomum* Quatrefages, 1845

Prosthiostomum auratum Kato, 1937b
(Fig. 1)

Prosthiostomum auratum Kato, 1937b: 363–364, pl. 22, fig. 8, text-figs. 23–24; Kato 1938a: 572; Kato 1938b: 589, pl. 39, fig. 7; Kato 1939b: 152; Kato 1944: 307; Prudhoe 1985: 191; Hagiya and Gamo 1992: 18, pl. 1, fig. 9, pl. 2, fig. 9.
Euprosthiostomum auratum – Faubel 1984: 234.

Material examined: 10 specimens (2 series of sagittal sections [ICHUM 6149, 6150]; 8 unsectioned specimens [AT2019033110, 2019022104–2019022110]), all collected by T. Miura, K. Oguchi, and H. Kohtsuka in Arai-hama (35.1609°N, 139.6105°E), Misaki, Kanagawa, Japan. ICHUM 6149, 5 slides, March 25, 2019; ICHUM 6150, 4 slides, March 25, 2019; AT2019033110, 70% ethanol, March 25, 2019; AT2019022104–AT2019022110, 70% ethanol, February 19, 2019.

Type locality: Misaki, Kanagawa, Japan.

Description: Body elongated, tapered posteriorly, 7.3–12.0 mm long and 2.3–3.4 mm wide at its widest point while alive (n = 10); anterior margin rounded; mid-point of posterior margin acute. Tentacles absent. Dorsal surface smooth, uniformly golden-yellow except cerebral-eyespot area; yellowish in color, a little darker along midline; a few reddish-brown spots present in front of brain in one individual (Fig. 1A). Ventral surface translucent without color pattern (Fig. 1B, C). Pair of linear cerebral-eyespot clusters, each consisting of five to 10 eyespots (n = 10); anterior end of cluster located at distance of 0.80 mm posterior to anterior margin of body (Fig. 1D). About 12 marginal eyespots (n = 10) arranged in single row along frontal margin, extending anterior to brain (Fig. 1D, E). One pair of ventral eyespots present near front end of brain (Fig. 1E). Anterior branch of main intestine short, extending to position 0.33 mm posterior from anterior margin of body (Fig. 1F, G). Plicated pharynx tubular in shape, 1.46 mm in length (about one-third of body), located in anterior half of body (Fig. 1B, F). Mouth situated at anterior end of pharynx, located at 1.10 mm posterior from anterior margin of body (Fig. 1B, F). Male copulatory apparatus consisting of large seminal vesicle, pair of prostatic vesicles, and armed penis papilla, located immediately posterior to pharyngeal pocket (Fig. 1F). Pair of spermiducal vesicles forming single row on each side of midline, separately entering into seminal vesicle laterally (Fig. 1C, F). Ejaculatory duct with thick muscular layer, entering penis papilla. Prostatic ducts with thin muscular layer, separately connected to ejaculatory duct behind proximal end of penis papilla. Pair of spherical prostatic vesicles, each coated with 0.05-mm-thick, non-nucleated muscular wall, located on each side of ejaculatory duct. Seminal vesicle oval, coated with 0.04-mm-thick muscular wall. Diameter of prostatic vesicle (0.16 mm) as long as dorsoventral diameter of seminal vesicle (0.13 mm) (n = 1). Penis papilla armed with pointed tubular stylet (0.08 mm in length; n = 1), enclosed in penis pouch, protruding into male atrium (Fig. 1F, H). Male atrium elongated anteriorly from gonopore to penis pouch (3.1 mm in length; n = 1). Female gonopore situated at 0.25 mm behind male gonopore (n = 1) (Fig. 1B). Female copulatory apparatus posterior to male reproductive system. Female gonopore leading to vagina across cement pouch; proximal end of vagina anteriorly curved (Fig. 1F). Cement glands developed, concentrated around vagina and releasing their contents in cement pouch (Fig. 1H). Oviducts not observed. Sucker large (0.46 mm in diameter; n = 1), situated at center of body (0.32 mm length from female atrium; 4.10 mm length from posterior extremity; n = 1) (Fig. 1B).

Distribution: So far, this species has only been confirmed along Japanese coasts, from the northmost Honshu Island to the southwestern Kyushu: Yuno-shima near Asamushi, Aomori; Ohtsuchi, Iwate; Nanao, Noto, Ishikawa; Misaki, Kanagawa; Manazuru, Kanagawa; Suzaki, Shimoda, Shizuoka; Shirahama, Wakayama; and Tomioka, Amakusa, Kumamoto.

Habitat: In the original description, this species was found on the surfgrass *Phyllospadix* in Misaki (Kato 1937b). In Shirahama, numerous specimens were obtained under stones (Kato 1938b). Our specimens were collected from the seaweed Corallinales spp. and holdfasts of the kelp *Eisenia bicyclis* subtidally in Misaki.

Sequences: Partial 28S rRNA (1010 bp) and *COI* (585 bp) sequences from two individuals. LC625886 (28S rRNA) and LC625892 (*COI*) from AT2019033110; LC625893 (*COI*) from ICHUM 6149.

Remarks: Although *P. auratum* was once placed in *Euprosthiostomum* (Faubel 1984), our morphological examination of the present topotypes confirmed that it is part of *Prosthiostomum*, primarily due to the presence of a frontal branch of the main intestine, a character state that was not mentioned in the original description by Kato (1937b). Our specimens are consistent with the original description in that i) the dorsal body is colored uniformly golden-yellow, ii) each cerebral-eyespot cluster is formed in a linear shape, and iii) a pair of prostatic vesicles are moderately large. No

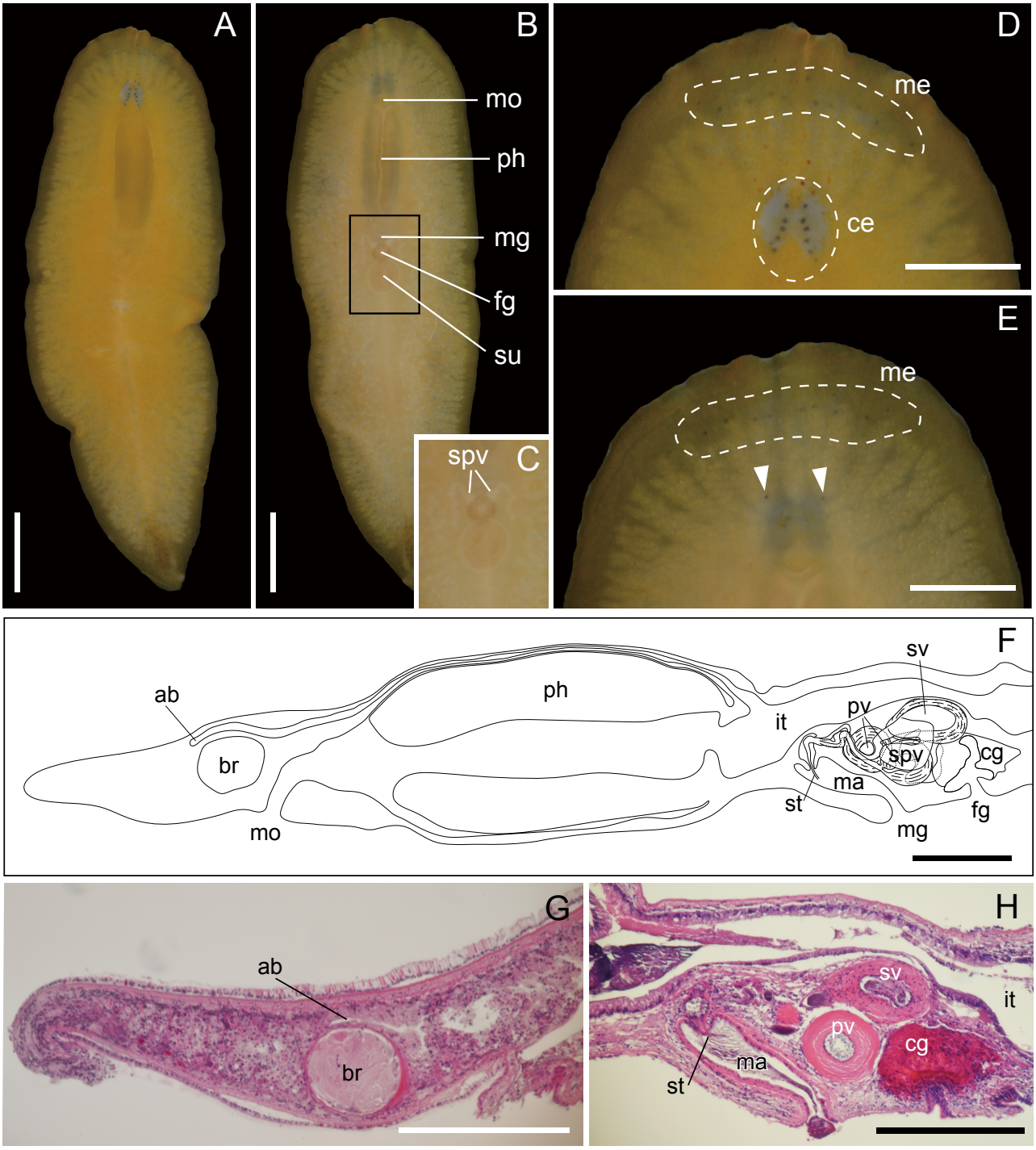

Fig. 1. *Prosthiostomum auratum* Kato, 1937b; photographs taken in life (ICHUM 6150) (A–E), schematic diagram (F), and photomicrographs of sagittal sections (anterior to the left) (ICHUM 6149) (G, H); A, entire body, dorsal view; B, entire body, ventral view; C, magnification of the black edged area on B; D, magnification of head, dorsal view; E, magnification of head, ventral view, showing ventral eyespots (arrowheads); F, anterior half of the body, lateral view, anterior to the left; G, anterior end of body; H, middle portion of body, showing male and female copulatory apparatuses. Abbreviations: ab, anterior branch of main intestine; br, brain; ce, cerebral eyespots; cg, cement glands; fg, female gonopore; it, intestine; ma, male atrium; me, marginal eyespots; mg, male gonopore; mo, mouth; ph, pharynx; pv, prostatic vesicle; spv, spermiducal vesicle; st, stylet; su, sucker; sv, seminal vesicle. Scale bars: A, B = 1 mm; D, E = 100 µm; F–H = 300 µm.

mention was made as to the ventral eyespots in the original description, but these were clearly present in a photograph of a specimen from Shirahama (Kato 1938b, pl. 39, fig. 7) as well as in our specimens (Fig. 1E).

As was shown in *P. auratum* based on topotypes, there is room for examination in the adequacy of classifying the other five species, *P. angustum* Bock, 1913, *P. bellum* Kato, 1939a, *P. laetum* Kato, 1938a, *P. matarazzoi* Marcus, 1950, and *P. pulchrum* Bock, 1913, in the genus *Euprosthiostomum*, because these were listed under the latter genus by Faubel (1984) without sound basis. The genus *Euprosthiostomum* was established by Bock (1925) based on *E. adhaerens* Bock, 1925, which was characterized by i) the location of the sucker relatively near the caudal end of the body and ii) the absence of a frontal median branch of the intestine (Bock 1925; Marcus 1948; Hyman 1953). Subsequently, *E. viscosum* Palombi, 1936, *E. mortenseni* Marcus, 1948, and *E. pakium* Du Bois-Reymond Marcus and Marcus, 1968 were established in this genus. Also, *P. molle* Freeman, 1930 was transferred to *Euprosthiostomum* by Hyman (1953). Later, Faubel (1984) proposed the presence/absence of the frontal median branch of the intestine as a determination key to distinguish *Prosthiostomum* (present) from *Euprosthiostomum* (absent). As a result, Faubel (1984) transferred *P. angustum*, *P. auratum*, *P. bellum*, *P. exiguum* Hyman, 1959, *P. laetum*, *P. matarazzoi* (= *Lurymare matarazzoi*), and *P. pulchrum* to *Euprosthiostomum*. In fact, however, in the original descriptions of *P. angustum*, *P. auratum*, *P. bellum*, *P. laetum*, *P. matarazzoi*, and *P. pulchrum*, the presence/absence of this branch was not clearly shown (Bock 1913; Kato 1937b 1938b 1939a; Marcus 1950), although Faubel (1984) apparently assumed as if the frontal median branch was absent in these species. In the same work, Faubel (1984) categorized those species for which the presence/absence of the frontal branch was unknown and placed them in *Prosthiostomum*. Among them, *P. matarazzoi* was redescribed based on freshly collected material (Bahia 2016); a lectotype was subsequently designated for this species (Bahia and Schrödl 2018). Still, the presence/absence of the frontal branch in *P. matarazzoi* was not mentioned in these works (Bahia 2016; Bahia and Schrödl 2018), although a common muscle bulb was confirmed to wrap up the prostatic and seminal vesicles, a character that was alleged to distinguish *Lurymare* from *Prosthiostomum* (Faubel 1984), but has been said to vary ontogenetically (Prudhoe 1989).

Prosthiostomum hibana **sp. n. Tsuyuki, Kohtsuka, and Kajihara**

[New Japanese name: hibana-hoso-hiramushi]
(Figs. 2–4)
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Material examined: Two specimens (ICHUM 6147, holotype, 6 slides; ICHUM 6148, paratype, 4 slides), both collected by T. Miura, K. Oguchi, and H. Kohtsuka in Arai-hama (35.1609°N, 139.6105°E), Misaki, Kanagawa, Japan, on March 25, 2019.

Etymology: The new specific name *hibana* is a Japanese noun, meaning fire sparks. It was named after the dorsal color pattern of the orange maculae, which look like sparks flying.

Type locality: Arai-hama, Misaki, Kanagawa, Japan.

Diagnosis: Body elongated; anterior margin rounded; dorsal surface translucent, covered with numerous orange maculae, some of which being agglutinated and forming larger maculae; pair of linear cerebral-eyespot clusters composed of relatively few eyespots; 3–4 pairs of ventral eyespots, embedded in parenchyma; marginal eyespots distributed antero-ventrally; inner wall of male atrium deeply ruffled; lumen of seminal vesicle narrow and elongated in shape; sucker large, occupying about 3% of body length, situated on body center.

Description of holotype: Body elongated, tapered posteriorly, 14 mm long and 3 mm wide at its widest point while alive (Fig. 2A); anterior margin rounded; mid-point of posterior margin acute. Tentacles absent. Dorsal surface smooth, translucent, uniformly covered with numerous orange maculae, some of which being agglutinated and forming larger maculae; the larger maculae scattered throughout (Fig. 2A); orange pigments more abundant medially. Ventral surface translucent, without color pattern (Fig. 2B, C). Pair of cerebral-eyespot clusters, each consisting of nine (left) and eight (right) eyespots; each cluster forming an antero-posteriorly elongated, curved line; anterior end of clusters located at distance of 0.85 mm posterior to anterior margin of body (Fig. 2D). About 20 marginal eyespots distributed antero-ventrally in front of brain (Fig. 2E, F). Four pairs of ventral eyespots, embedded in parenchyma (Fig. 2G); four eyespots on each side arranged at corner of parallelogram (Fig. 2E). Intestine highly branched, spreading all over body; anterior branch of main intestine extending to position 0.4 mm posterior from anterior margin of body. Plicated pharynx tubular in shape, 4.1 mm in length (about two-sevenths of body), located in anterior half of body (Fig. 2A, B). Mouth situated at anterior end of

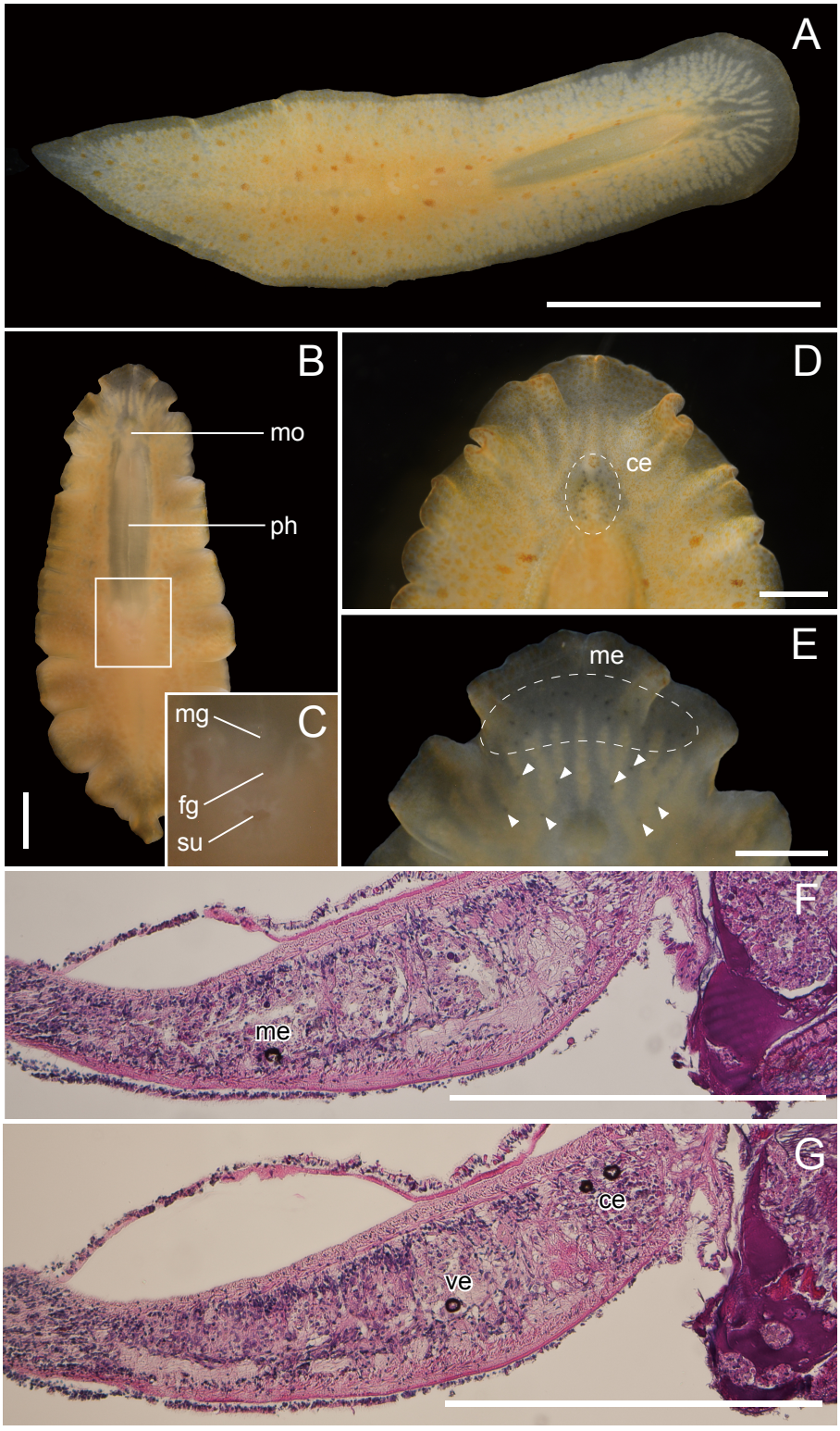

Fig. 2. *Prosthiostomum hibana* sp. n., ICHUM 6147 (holotype); photographs taken in life (A–E) and photomicrographs showing eyespots observed in sagittal sections (anterior to the left) (F, G); A, entire body, dorsal view; B, entire body, ventral view; C, magnification of the white edged area on B; D, magnification of head, dorsal view; E, magnification of head, ventral view (ventral eyespots indicated by arrowheads); F, anterior portion of body, showing marginal eyespot; G, anterior portion of body, showing cerebral and frontal eyespots. Abbreviations: ce, cerebral eyespots; fg, female gonopore; me, marginal eyespot(s); mg, male gonopore; mo, mouth; ph, pharynx; su, sucker; ve, ventral eyespot. Scale bars: A = 5 mm, B = 1 mm, D–G = 500 µm.

pharynx, located at 1.04 mm posterior from anterior margin of body (Fig. 2B). Male gonopore, female gonopore, and sucker closely set on body center (Fig. 2B, C); distance between male and female gonopores being 0.34 mm; distance between female gonopore and sucker being 0.39 mm. Male copulatory apparatus consisting of large seminal vesicle, pair of prostatic vesicles, and armed penis papilla, located immediately posterior to pharyngeal pocket (Fig. 3A). Spermiducal vesicles forming single row on each side of midline, each running from posterior to anterior, then bending posteriorly and separately entering into seminal vesicle. Ejaculatory duct wide, with thick muscular layer, entering penis papilla. Prostatic ducts with muscular layer, connected to ejaculatory duct separately at proximal end of penis papilla. Pair of spherical prostatic vesicles coated within 0.05-mm-thick, non-nucleated muscular wall, located on both sides of ejaculatory duct (Fig. 3A, B). Seminal vesicle oval, coated with 0.11-mm-thick muscular wall; its lumen narrow and elongated in shape (Fig. 3A, C). Without common muscular bulb enclosing male copulatory apparatus. Seminal vesicle (long axis 0.34 mm, short axis 0.23 mm) more than twice as large as prostatic vesicle (0.14 mm in diameter) (Fig. 3A, B). Penis papilla armed with pointed tubular stylet (0.14 mm in length), enclosed in penis pouch, protruding into male atrium (Fig. 3D). Penis sheath present between penis pouch and male atrium (Fig. 3A, B). Male atrium elongated anteriorly from male gonopore to penis pouch (0.40 mm in length); inner wall deeply ruffled, lined with ciliated and muscularized epithelium (Fig. 3A, B). Immature female reproductive system immediately posterior to male copulatory apparatus. Female gonopore leading to vagina across cement pouch (Fig. 3A, E); proximal end of vagina anteriorly curved (Fig. 3A). Cement glands and oviducts undeveloped and not observed. Lang's vesicle absent. Sucker large (0.40 mm in diameter; 2.9% of body length), situated immediately behind female gonopore (Fig. 3E), at 4.2 mm anterior from posterior margin of body.

Description of paratype: Body 7.8 mm long and 2.9 mm wide at its widest point when slightly contracted while alive. Body coloration almost same as holotype. Pair of cerebral-eyespot clusters, each consisting of seven (left) and eight (right) eyespots (Fig. 4A). About 20 marginal eyespots, distributed ventrally along anterior margin (Fig. 4B). Ventral eyespots, 3–4 pairs in number, embedded in parenchyma (Fig. 4B). Frontal branch of main intestine extending anterior to brain. Pharynx 2.82 mm in length. Male and female reproductive systems undeveloped. Sucker large (0.20 mm in diameter; 2.6% of body length), situated on body center (3.6 mm anterior from posterior margin of body).

Distribution: So far only from the type locality, Misaki, Kanagawa, Japan.

Habitat: Among branching coralline algae Corallinales spp.

Sequences: Partial 28S rRNA gene (1008 bp) and *COI* (585 bp) sequences from two individuals. LC625887 (28S rRNA) and LC625894 (*COI*) from the holotype (ICHUM 6147); LC625888 (28S rRNA) and LC625895 (*COI*) from the paratype (ICHUM 6148).

Remarks: Among ~60 species in *Prosthiostomum*, our new species is unique in having 3–4 pairs of ventral eyespots (Figs. 2E and 4B) and thus can easily be distinguished from the other congeners, where the ventral eyespots are mostly absent or at most single pair in number, if present. Only *P. bellum* has been known to possess two pairs of ventral eyespots (Kato 1939a), but it is quite different from *P. hibana* sp. n. in the body coloration (white background with numerous brown spots scattered over the body in *P. bellum*; translucent with orange maculae in *P. hibana* sp. n.) as well as the number of cerebral eyespots in each cluster (about 40 in *P. bellum*; 7–9 in *P. hibana* sp. n.).

Nine other congeners are known to show a similar character state to that in *P. hibana* sp. n. pertaining to either dorsal coloration or cerebral-eyespot arrangement (Table 2). *Prosthiostomum capense* Bock, 1931, *P. dohrnii* Lang, 1884, and *P. grande* resemble our new species in having yellow to orange maculae or spots scattered all over the body; *P. dohrnii* and *P. grande* are different from the new species in the number and distribution of the cerebral eyespots; *P. capense* is separated from *P. hibana* sp. n. by the size and position of the sucker (small, situated at four-fifths of the body in *P. capense*; large, situated at the middle of the body in *P. hibana* sp. n.) (Table 2). The five species *P. auratum*, *P. cynarium* Marcus, 1950, *P. purum* Kato, 1937b, *P. siphunculus*, and *P. vulgare* Kato, 1937b have cerebral-eyespot arrangements similar to that in our new species, *i.e.*, a pair of linear cerebral-eyespot clusters composed of relatively few (≤ 15) eyespots, but can be easily distinguished from *P. hibana* sp. n. by the dorsal coloration (Table 2). *Prosthiostomum parvicelis* Hyman, 1939b also has this type of cerebral-eyespot arrangement; although the dorsal coloration is not known for this species, it can be distinguished from *P. hibana* sp. n. by the pyriform lumen of the seminal vesicle (Hyman 1939b), whereas the seminal-vesicle lumen is narrow and elongated in *P. hibana* sp. n. (Fig. 3A, C).

Noticeably, in *P. hibana* sp. n., the inner wall of the male atrium is deeply ruffled (Fig. 3A, B). The morphology of the inner wall of the male atrium has so far attracted little attention as taxonomic features in

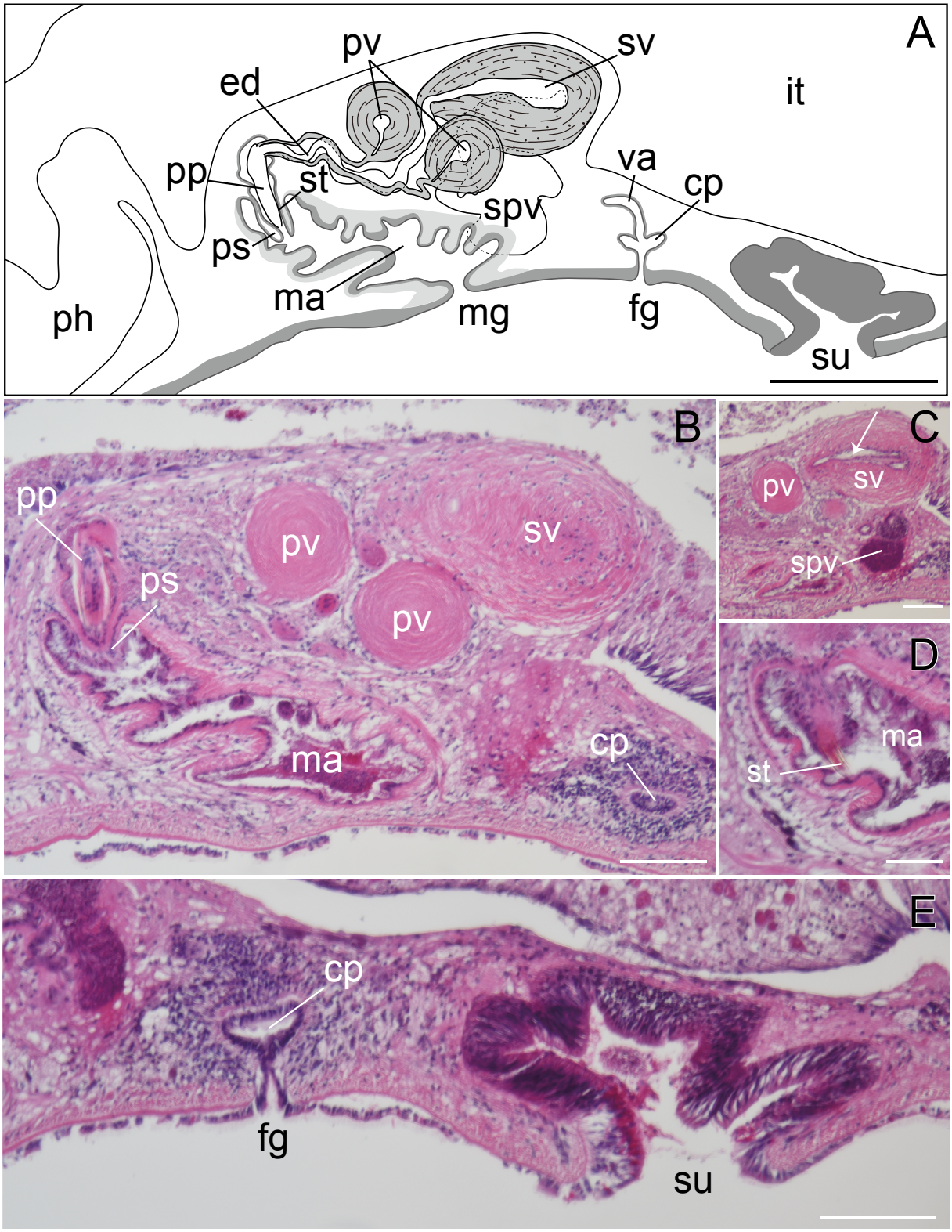

Fig. 3. *Prosthiostomum hibana* sp. n., ICHUM 6147 (holotype); schematic diagram (A) and photomicrographs of sagittal sections (anterior to the left) (B–D); A, copulatory complex and sucker; B, male copulatory apparatus through male atrium lumen; C, male copulatory apparatus through seminal vesicle lumen, indicated by arrow; D, proximal portion of male atrium; E, female gonopore and sucker. Abbreviations: cp, cement pouch; ed, ejaculatory duct; fg, female gonopore; it, intestine; ma, male atrium; mg, male gonopore; ph, pharynx; pp, penis papilla; ps, penis sheath; pv, prostatic vesicle; spv, spermiducal vesicle; st, stylet; su, sucker; sv, seminal vesicle; va, vagina. Scale bars: A = 300 µm; B, C, E = 100 µm; D = 50 µm.

Prosthiostomidae. This feature is not mentioned in the original descriptions or re-descriptions for most of the prosthiostomid species, but a slightly ruffled inner wall of the male atrium has been illustrated for *P. gilvum* Marcus, 1950, *P. latocelis* Hyman, 1953, and *P. ostreae* Kato, 1937b (Kato 1937b; Marcus 1950; Hyman 1953).

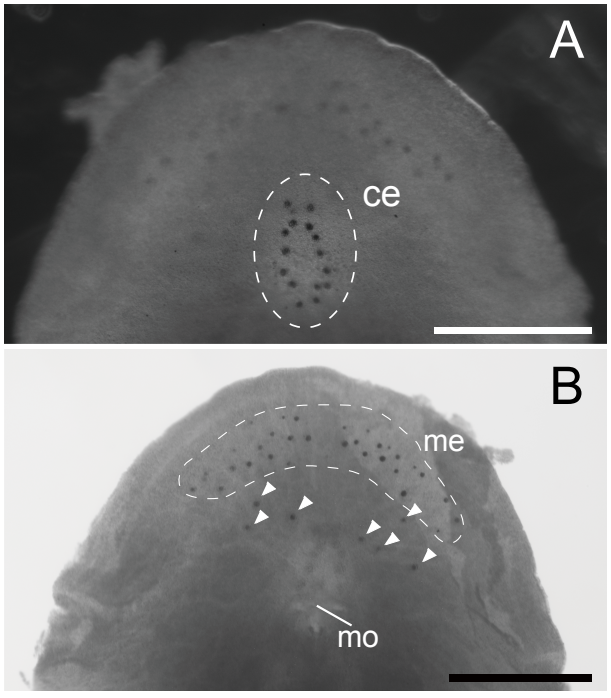

Fig. 4. *Prosthiostomum hibana* sp. n., ICHUM 6148 (paratype); photographs taken after being cleared in xylene; A, magnification of head, dorsal view; B, magnification of head, ventral view (ventral eyespots indicated by arrowheads). Abbreviations: ce, cerebral eyespots; me, marginal eyespots; mo, mouth. Scale bars: 500 μm.

***Prosthiostomum* cf. *ostreae* Kato, 1937b**
(Fig. 5)

?*Prosthiostomum ostreae* Kato, 1937b: 365–366, pl. 22, figs. 4–5, text-figs. 25–27; Kato 1944: 308; Faubel 1984: 232; Prudhoe 1985: 192.

Material examined: Three specimens, all collected by T. Miura, K. Oguchi, and H. Kohtsuka in Arai-hama (35.1609°N, 139.6105°E), Misaki, Kanagawa, Japan. ICHUM 6151, 4 slides, February 19, 2019; ICHUM 6152, 4 slides, March 25, 2019; ICHUM 6153, 8 slides, March 25, 2019.

Description: Body elongated, tapered posteriorly, 9.0–13.0 mm long and 2.4–3.3 mm wide at its widest point when slightly contracted while alive (n = 3); anterior margin rounded (Fig. 5A–D). Tentacles absent. Dorsal surface smooth, light brown, covered with numerous brown maculae (Fig. 5A). Brown pigments aggregating mid-dorsally to form posteriorly fading band, which runs from behind brain; non-pigmented specks forming line along midline on brown band (Fig. 5A). Body margin translucent. Ventral surface translucent, without color pattern (Fig. 5B). Pair of cerebral-eyespot clusters, each consisting of 20–22 eyespots (n = 3); cerebral-eyespot clusters medially approaching each other at three points; anterior end of clusters located at distance of 1.20 mm posterior to anterior margin of body (Fig. 5C, D). About 70 marginal eyespots irregularly scattered along anterior margin, extending to half position of brain (Fig. 5D). One pair of ventral eyespots present near front end of brain (Fig. 5D). Anterior branch of main intestine extending to position 0.52 mm posterior from anterior margin of brain. Plicated pharynx tubular in shape, 4.7 mm in

Table 2. Comparison of selected characters between *P. hibana* sp. n. and nine other species of *Prosthiostomum*, which share either body coloration or cerebral-eyespot arrangement; species for which body coloration in life is unknown are also listed

Species	*P. auratum* Kato, 1937b	*P. capense* Bock, 1931	*P. cynarium* Marcus, 1950	*P. dohrnii* Lang, 1884	*P. grande* (Stimpson, 1857)
Body size	7.3–12 mm in length; 2.3–3.4 mm in width	about 6 mm in length; 1–1.5 mm in width	5 mm in length	25 mm in length; 6 mm in width	22 mm in length; 5 mm in width
Dorsal coloration	uniformly golden yellow except for cerebral-eyespot area	with yellow or brown spots	ivory-colored or grayish	soft bright orange yellow, with darker orange-yellow spots scattered over the body especially denser along midline	buffy ground color, with numerous small spots of ochraceous color distributed all over body
Cerebral eyespots	single pair of linear clusters composed of 5–10 eyespots	single pair of roughly linear clusters composed of about 15 eyespots	single pair of linear clusters composed of 4–10 eyespots	single pair of oval clusters composed of numerous eyespots	single pair of wedge clusters composed of about 25 eyespots
Marginal eyespots	about 12 eyespots in single row along frontal margin; distributed anterior to brain	about 40 in number; distributed anterior to brain	5–15 in number; distributed anterior to brain	numerous; elongated to the level behind brain	two irregular rows along the anterior margin

Table 2. (Continued)

Species	*P. auratum* Kato, 1937b	*P. capense* Bock, 1931	*P. cynarium* Marcus, 1950	*P. dohrnii* Lang, 1884	*P. grande* (Stimpson, 1857)
Ventral eyespots	single pair	none	single pair	single pair	none
Male atrium	elongated; inner wall smooth	?	elongated; inner wall smooth	?	?
Seminal vesicle	oval; lumen oval	?	spherical; lumen spherical	?	spherical; lumen shape unknown
Position of the junction of the spermiducal vesicles into the seminal vesicle	at the anterior corner of the seminal vesicle	?	middle portion of the seminal vesicle	?	?
Sucker	0.46 mm in diameter; located on body center	0. 11 mm in diameter; located at the four-fifths of body	0.35 mm in diameter; located on body center	present; details not described	about 4% of body length in size; located slightly behind body center
Distribution	Japan (Honshu and Kyushu)	South Africa (Simons Bay, Cape Town)	Brazil (São Sebastião Island)	Italy (60–80 m depth, Secca di Gajola in the Gulf of Naples)	Japan (Noto, Misaki, Shimoda, Shirahama, Amakusa, Amami-Oushima)
Reference	Kato (1937b); this study	Bock (1931)	Marcus (1950); Bahia and Schrödl (2018)	Lang (1884)	Stimpson (1857); Yeri and Kaburaki (1918); Tsuyuki (personal observation)

Species	*P. hibana* sp. nov.	*P. parvicelis* Hyman, 1939b	*P. purum* Kato, 1937b	*P. siphunculus* Delle Chiaje, 1828	*P. vulgare* Kato, 1938a
Body size	14 mm in length; 3 mm in width	6 mm in length	15–20 mm in length; about 1 mm in width	8–11 mm in length (Lang 1884); 10–18 mm in length, 4–6 mm in width (Noreña et al. 2014)	6.8–8.8 mm in length; 1.4–2.1 mm in width
Dorsal coloration	translucent, uniformly covered with numerous orange maculae, some of which being agglutinated and forming larger maculae; the larger maculae scattered throughout	?	translucent milky white without any color pattern	dirty white, beige to yellow; without spots or dots	light buffy, cinnamon pigments medially abundant
Cerebral eyespots	single pair of linear clusters composed of 6–9 eyespots	single pair of linear clusters composed of 7–8 eyespots; a pair of eyespots, each located at the level of the anterior end of the cerebral cluster	single pair of linear clusters composed of 6–8 eyespots	single pair of linear clusters composed of 10–14 eyespots; a pair of eyespots, each located at the level of the anterior end of the cerebral cluster	single pair of linear clusters composed of 7 eyespots
Marginal eyespots	about 25 in number; distributed ventrally along anterior margin in front of the brain	obviously a few in number, elongated to the half position of the brain	about 40 in number; elongated to the half position of the brain	about 40 in number; distributed anterior to brain; eyeless on midline	about 40 in number; distributed anterior to brain
Ventral eyespots	3–4 pairs	none	none	none	single pair
Male atrium	elongated; inner wall deeply ruffled	?	elongated; inner wall smooth	elongated; inner wall smooth	elongated; inner wall smooth
Seminal vesicle	oval; lumen narrow and elongated	oval; lumen pyriform	oval; lumen oval	oval to elongated; lumen fusiform	oval; lumen oval
Position of the junction of the spermiducal vesicles into the seminal vesicle	middle portion of the seminal vesicle	middle portion of the seminal vesicle	middle portion of the seminal vesicle	at the posterior corner of the seminal vesicle	at the anterior corner of the seminal vesicle
Sucker	0.40 mm in diameter; located on body center	located on body center	small; located immediately posterior to female gonopore	located on body center	0.21 mm in diameter; located on body center
Distribution	Japan (Misaki, Kanagawa)	Galápagos Islands	Japan (Misaki, Kanagawa)	European Atlantic coasts, the Mediterranean Sea, the Tyrrhenian Sea, North and South Africa, Somalia, and Vietnam	Japan (Noto, Misaki, Susaki, Suga-shima Island, Shirahama, Amakusa)
Reference	this study	Hyman (1939b)	Kato (1937b)	Lang (1884); Noreña et al. (2014)	Kato (1938a); this study

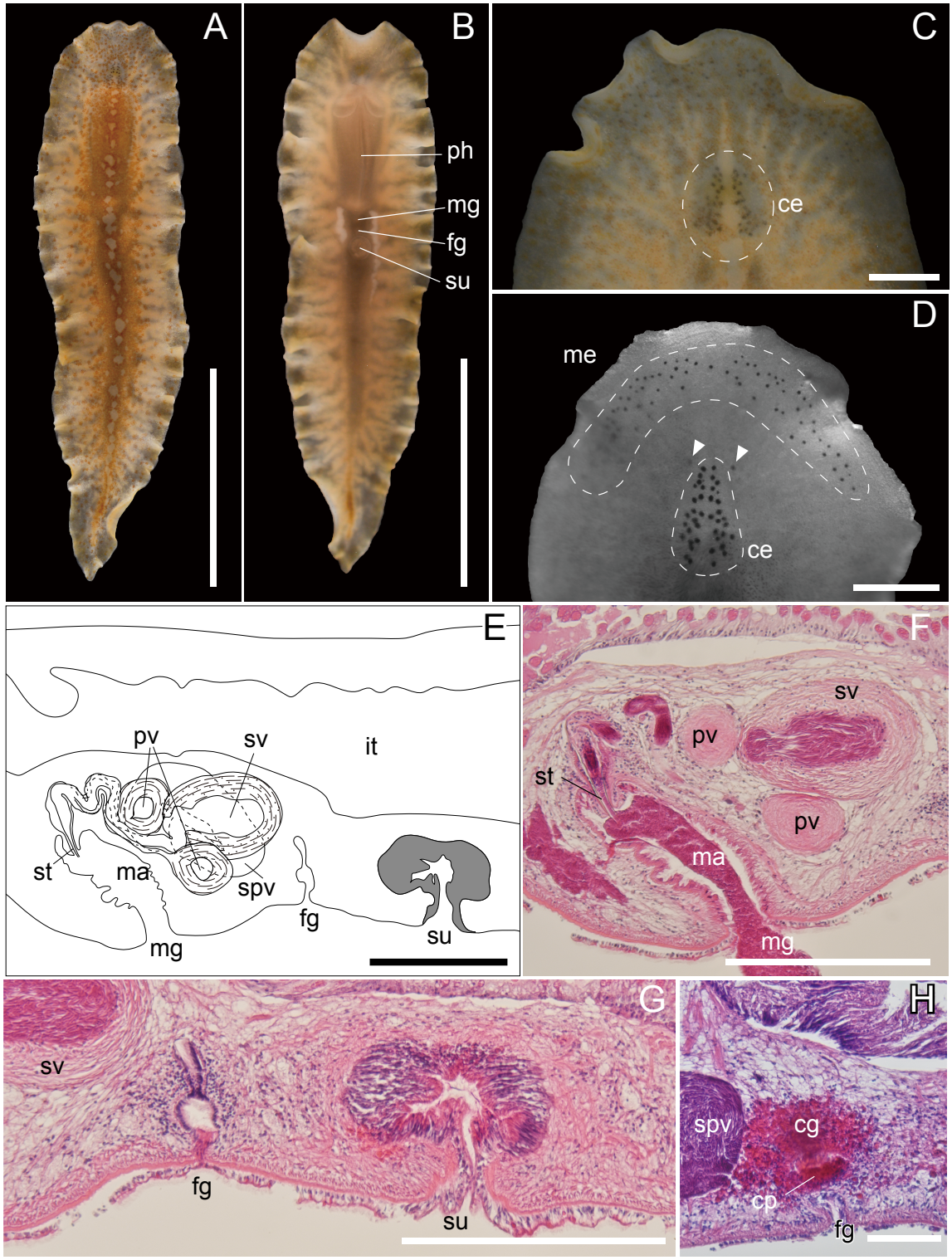

Fig. 5. *Prosthiostomum* cf. *ostreae* Kato, 1937b, ICHUM 6153 (A, B, H), ICHUM 6151 (C, E–G), ICHUM 6152 (D); photographs taken in life (A–C) and after being cleared in xylene (D), schematic diagram (E), and photomicrographs of sagittal sections (F–H) (anterior to the left); A, entire body, dorsal view; B, entire body, ventral view; C, magnification of head, dorsal view; D, magnification of head, dorsal view, showing ventral eyespots (arrowheads); E, copulatory organs and sucker; F, male copulatory apparatus; G, female gonopore and sucker; H, cement glands. Abbreviations: ce, cerebral eyespots; cg, cement glands; cp, cement pouch; fg, female gonopore; it, intestine; ma, male atrium; me, marginal eyespots; mg, male gonopore; ph, pharynx; pv, prostatic vesicle; spv, spermiducal vesicle; st, stylet; su, sucker; sv, seminal vesicle. Scale bars: A, B = 5 mm; C, D = 1 mm; E–G = 300 µm; H = 100 µm.

length (about one-third of body), located in anterior half of body (Fig. 5B). Mouth situated at anterior end of pharynx, located at 1.53 mm posterior from anterior margin of body. Male gonopore, female gonopore, and sucker closely set on body center (Fig. 5B); distance between male and female gonopores being 0.34 mm; distance between female gonopore and sucker being 0.31 mm. Male copulatory apparatus consisting of large seminal vesicle, pair of prostatic vesicles, and armed penis papilla, located immediately posterior to pharyngeal pocket. Pair of spermiducal vesicles, running on each side of midline and curving posteriorly behind penis to separately enter into anteroventral end of seminal vesicle (Fig. 5E). Ejaculatory duct with thin muscular layer, entering penis papilla. Prostatic ducts with thin muscular layer, connected to ejaculatory duct separately at proximal end of penis papilla. Pair of spherical prostatic vesicles coated with 0.04-mm-thick, non-nucleated muscular wall, located on both sides of ejaculatory duct (Fig. 5E, F). Seminal vesicle oval, coated with 0.04-mm-thick muscular wall (Fig. 5E, F). Seminal vesicle (long axis 0.26 mm, short axis 0.17 mm) more than twice as large as prostatic vesicle (0.12 mm in diameter) (Fig. 5E) (n = 1). Penis papilla armed with rather thick, pointed tubular stylet (0.13 mm in length; n = 1), enclosed in penis pouch, protruding into male atrium (Fig. 5E, F). Male atrium elongated anteriorly from gonopore to penis pouch (0.27 mm in length; n = 1); inner wall slightly ruffled, lined with ciliated and muscularized epithelium (Fig. 5F). Female copulatory apparatus posterior to male reproductive system (Fig. 5G, H). Cement glands concentrated around vagina and releasing their contents in cement pouch when developed (Fig. 5H). Oviducts not observed. Sucker large (0.26 mm in diameter; n = 1), situated immediately behind female reproductive system (Fig. 5G), at 7.4 mm anterior from posterior margin of body (n = 1).

Distribution: Arai-hama, Misaki, Kanagawa, Japan.

Habitat: Among branching coralline algae Corallinales spp. and holdfasts of the kelp *Eisenia bicyclis* subtidally in Misaki.

Sequences: Parital 28S rRNA (1012 bp) and *COI* gene sequences from two individuals. LC625889 (28S rRNA) and LC625896 (*COI*, 585 bp) from ICHUM 6151; LC625890 (28S rRNA) and LC625897 (*COI*, 553 bp) from ICHUM 6152.

Remarks: We tentatively identified our specimens as *P.* cf. *ostreae*. Kato (1937b) originally described *P. ostreae* based on three specimens found on cultivated oyster shells from Moroiso, Misaki, Kanagawa, Japan. Unfortunately, the type series of *P. ostreae* is not extant (Kawakatsu 2004), and therefore we could not compare our samples to the type series. The dorsal body color pattern, the arrangements of cerebral and marginal eyespots, and the form of male copulatory apparatus in our specimens are largely consistent with those given by Kato (1937b). However, our specimens differ from the original description of *P. ostreae* by i) the body-margin coloration (translucent in our specimens; partly lemon-yellow in the original description) and ii) the frontal eyespots (absent in our specimens; four eyespots present in the original description). In addition, the pair of the ventral eyespots were confirmed in our materials although they were not mentioned in the original description. Our materials were collected from coralline algae and kelp holdfasts whereas the original specimens were found on oyster shells. Additional data are needed to test whether the two (possibly three) morphological differences between our specimens and the original description represent interspecific or intraspecific ones stemming from the habitat difference.

Prosthiostomum vulgare **Kato, 1938b**

(Fig. 6)

Prosthiostomum vulgaris [sic] Kato, 1938b: 589–590, pl. 39, figs. 3–4; Kato 1938a: 578; Kato 1944: 308; Faubel 1984: 232; Prudhoe 1985: 192; Hagiya and Gamo 1992: 18, pl. 1, fig. 10, pl. 2, fig. 10; Tsunashima et al. 2017: fig. 2B.

Prosthiostomum siphunculus – Yeri and Kaburaki 1918: 41, pl. 2, fig. 13; Kato 1937a: 230.

Material examined: Three specimens (ICHUM 6036, 3 slides; ICHUM 6154, 4 slides; ICHUM 6155, 5 slides), all collected by T. Miura, K. Oguchi, and H. Kohtsuka in Arai-hama (35.1609°N, 139.6105°E), Misaki, Kanagawa, Japan, on March 25, 2019.

Type locality: Yuzaki, Shirahama, Wakayama, Japan.

Description: Body elongated, tapered posteriorly, 6.8–8.8 mm long and 1.4–2.1 mm wide at its widest point when slightly contracted while alive (n = 3); anterior margin rounded (Fig. 6A–D). Tentacles absent. Dorsal surface smooth, buffy; cinnamon pigments medially abundant, forming wide midline (Fig. 6A). Ventral surface translucent, without color pattern (Fig. 6B). Pair of cerebral-eyespot clusters, each consisting of seven eyespots (n = 3); each cluster forming antero-posteriorly elongated line; anterior end of clusters located at distance of 0.58 mm posterior to anterior margin of body (Fig. 6C). Marginal eyespots distributed along frontal margin rather irregularly but largely arranged into two or three rows, extending backward to half position of brain (Fig. 6C). One pair of ventral eyespots present near front end of brain (Fig. 6D). Anterior branch of main intestine extending anterior to brain. Plicated pharynx tubular in shape,

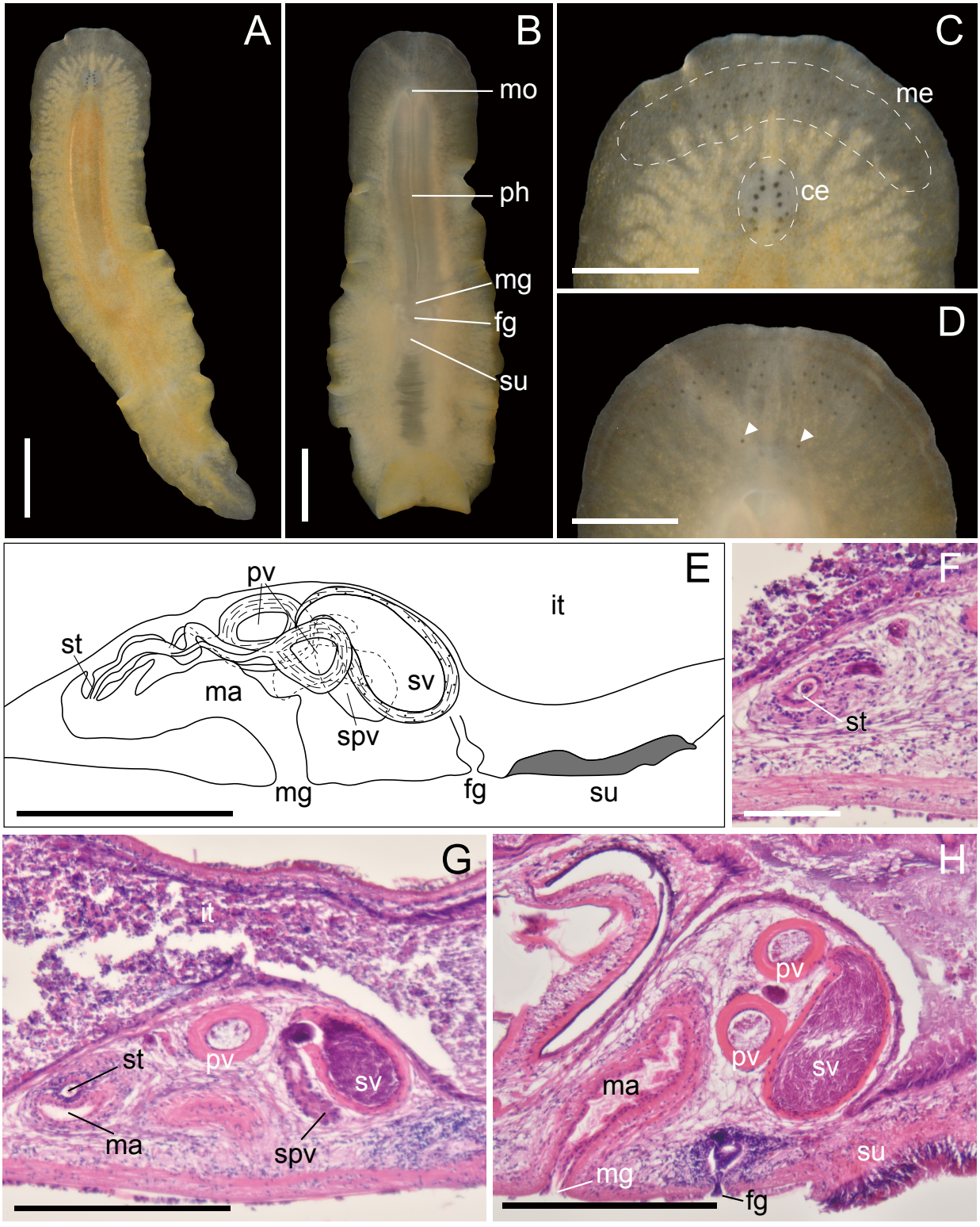

Fig. 6. *Prosthiostomum vulgare* Kato, 1938b, ICHUM 6036 (A, C), ICHUM 6154 (B, D, H), ICHUM 6155 (E–G); photographs taken in life (A–D), schematic diagram (E), and photomicrographs of sagittal sections (anterior to the left) (F–H); A, entire body, dorsal view; B, entire body, ventral view; C, magnification of head, dorsal view; D, magnification of head, ventral view, showing ventral eyespots (arrowheads); E, copulatory complex and sucker; F, stylet; G, male copulatory apparatus; H, copulatory complex and sucker. Abbreviations: ce, cerebral eyespots; fg, female gonopore; it, intestine; ma, male atrium; me, marginal eyespots; mg, male gonopore; mo, mouth; ph, pharynx; pv, prostatic vesicle; spv, spermiducal vesicle; st, stylet; su, sucker; sv, seminal vesicle. Scale bars: A, B = 1 mm; C, D = 0.5 mm; E, G, H = 300 µm; F = 100 µm.

3.0 mm in length (about one-third of body), located in anterior half of body (Fig. 6B). Mouth situated at distance of 0.81 mm posterior to anterior margin of body (Fig. 6B). Male copulatory apparatus consisting of large seminal vesicle, pair of prostatic vesicles, and armed penis papilla, located immediately posterior to pharyngeal pocket (Fig. 6E–H). Pair of spermiducal vesicles forming single row on each side of midline, separately entering into seminal vesicle at point being close to proximal end of ejaculatory duct (Fig. 6E, G). Ejaculatory duct with thick muscular layer, entering penis papilla. Prostatic ducts with thin muscular layer, connected to ejaculatory duct separately posterior to proximal end of penis papilla. Pair of spherical prostatic vesicles coated with 0.03-mm-thick, non-nucleated muscular wall, located on both sides of ejaculatory duct (Fig. 6E, G, H). Seminal vesicle oval, coated with 0.009-mm-thin muscular wall (Fig. 6G, H). Seminal vesicle (long axis 0.17 mm, short axis 0.09 mm) twice as large as prostatic vesicle (0.09 mm in diameter) (Fig. 6E) (n = 1). Penis papilla armed with pointed tubular stylet, enclosed in penis pouch, protruding into male atrium (Fig. 6F). Male atrium elongated, lined with ciliated and muscularized epithelium (Fig. 6H). Female copulatory apparatus immature; only female gonopore developed, located at distance of 0.20 mm behind male gonopore (Fig. 6E, H). Sucker large (0.21 mm in diameter; n = 1), situated immediately (0.19 mm in length; n = 1) behind female gonopore, at distance of 3.47 mm anterior to posterior margin of body (n = 1) (Fig. 6E, H).

Distribution: This species was confirmed along Japanese coasts, from the Noto Peninsula of Honshu Island to the southwestern Kyushu: Nozaki, Noto, Ishikawa; Misaki, Kanagawa; Manazuru, Kanagawa; Suzaki, Shimoda, Shizuoka; Suga-shima, Mie; Shirahama, Wakayama; and Tomioka, Amakusa, Kumamoto.

Habitat: The information about habitats of this species was not mentioned in Yeri and Kaburaki (1918), Kato (1937a 1938a b), or Hagiya and Gamo (1992). Our specimens were collected from branching coralline algae Corallinales spp. in Misaki, Kanagawa, Japan.

Sequence: Partial 1008-bp 28S rRNA (LC625891) and 585-bp *COI* (LC625898) gene sequences from ICHUM 6036.

Remarks: Kato (1938b) originally described this species from Shirahama, Wakayama, Japan. He also pointed out that the specimens from Misaki identified as *P. siphunculus* by Yeri and Kaburaki (1918) should represent *P. vulgare*, assuming that these would possess a pair of spermiducal vesicles that open into the seminal vesicle at its anterior part near the ejaculatory duct. With this character, Kato (1938b) speculated that *P. vulgare* could be differentiated from *P. siphunculus*. Our specimens are consistent with the original description by Kato (1938b) in this characteristic position of the junction of the spermiducal vesicles into the seminal vesicle (Fig. 6E, G) in addition to the body coloration and the arrangement of the cerebral-eyespot clusters. We were not able to compare our specimens with Yeri and Kaburaki's (1918) and Kato's (1937a 1938a b) specimens, which had been lost (Kawakatsu 2004).

Molecular phylogeny

The resulting tree (Fig. 7) showed the genus *Prosthiostomum* to be monophyletic (with 76% bootstrap [BS] support), with *P. lobatum* Pearse, 1938 being sister to all the other species of the genus included in this analysis. All remaining *Prosthiostomum* species except for *P. lobatum* formed a clade supported with a 90% BS value. Furthermore, except for the unidentified *Prosthiostomum* in Litvaitis et al. (2019), the remaining *Prosthiostomum* clade had 92% BS support. Included in this latter clade were all the species for which sequences were generated *de novo* in this study, *i.e.*, *P. auratum*, *P. grande*, *P. hibana* sp. n., *P.* cf. *ostreae*, *P. torquatum*, and *P. vulgare*. *Euprosthiostomum mortenseni*'s position as sister to the *Prosthiostomum* clade did not receive high nodal support (76% BS value), while all the *Enchiridium* species included in the analysis formed a highly supported clade (96% BS value).

DISCUSSION

All the four *Prosthiostomum* species for which we gave morphological accounts in this study—*P. auratum*, *P. hibana* sp. n., *P.* cf. *ostreae*, and *P. vulgare*—were nested in the *Prosthiostomum* clade (Fig. 7), corroborating our morphology-based generic assignments. The reconstructed tree based on 24 species of Prosthiostomidae was largely in accordance with that in Litvaitis et al. (2019). In terms of the entire polyclad phylogeny, the analysis by Litvaitis et al. (2019) represented the densest sampling of Prosthiostomidae taxa before our study (cf. Aguado et al. 2017; Bahia et al. 2017; Tsunashima et al 2017; Dittmann et al. 2019). While a different taxonomic view was once proposed in terms of the generic affiliation (see Remarks for *P. auratum*), *P. auratum* was more closely related to *P. siphunculus* (type species of *Prosthiostomum*) than to *Euprosthiostomum mortenseni* in our tree (Fig. 7). This result supports the placement of the species in *Prosthiostomum* based on our morphological observation, given that the *E. mortenseni* specimen sequenced by Litvaitis et al. (2019) was actually more

closely related to *E. adhaerens* Bock, 1925 (type species of *Euprosthiostomum*) than to *Prosthiostomum* species.

Prosthiostomum katoi Poulter, 1975 was nested in the *Prosthiostomum* clade (Fig. 7), rendering further support for the taxonomic view that *Lurymare* is indeed a junior synonym of *Prosthiostomum* (cf. Dittmann et al. 2019; Litvaitis et al. 2019; Tsuyuki et al. 2019). Being originally described as a member of the subgenus *Lurymare* within the genus *Prosthiostomum* (Poulter 1975), *P. katoi* was then transferred into the genus *Lurymare* by Faubel (1984). Since then, the generic affiliation of the species has been controversial in relation to the validity of *Lurymare* (Prudhoe 1985; Marquina et al. 2015; Dittmann et al. 2019).

Among the five species of *Prosthiostomum* for which Misaki being the type locality, only *P. auratum* was recovered in this study. Of the other four, *P. purum* has been reported from Israel (Gulf of Aqaba, northern Red Sea) along with a partial sequence of the 28S rRNA gene (Litvaitis et al. 2019). While the phylogenetic position of the *P. purum* of Litvaitis et al. (2019) undoubtedly indicates its reasonable genus assignment (Fig. 7), conspecificity should be confirmed in future studies using molecular techniques by comparing it with topotypic sequence(s), given that its geographic ranges in Israel and Japan are > 9,000 km apart. The other three—*P. ostreae*, *P. rubropunctatum*, and *P. yerii*—remain *species inquirendae* because their genus affiliation is open to question, due especially to the lack of information as to the presence/absence of a frontal branch of the main intestine over the pharynx.

CONCLUSIONS

In this study, we described the new species *P. hibana* sp. n. and presented morphological accounts on *P. auratum*, *P.* cf. *ostreae*, and *P. vulgare* based on specimens collected from branching coralline algae and kelp holdfasts in Misaki, Japan. Our examination of topotypic specimens of *P. auratum* suggested that this species should be placed in *Prosthiostomum*, not *Euprosthiostomum*, based on morphological and molecular phylogenetic evidence. The new species *P. hibana* sp. n. is characterized by i) the dorsal surface of the body covered with numerous orange maculae, some

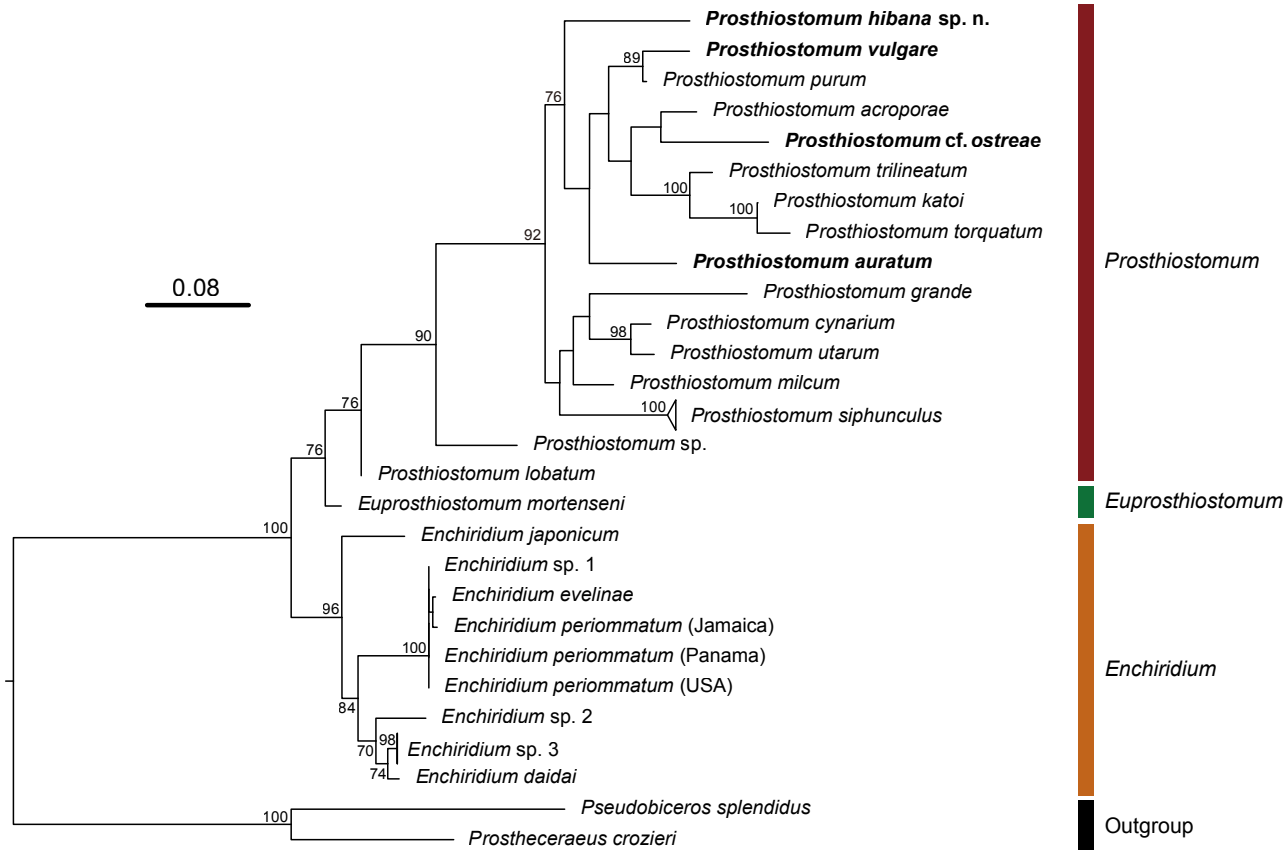

Fig. 7. Maximum likelihood phylogenetic tree based on the 28S rRNA (939 bp) and *COI* (585 bp) gene sequences. Numbers near nodes are the bootstrap values (≥ 70) (%). The names of species for which morphological description are provided in this study are indicated in boldface.

of which are agglutinated, ii) the pair of linear cerebral-eyespot clusters composed of relatively few eyespots, iii) 3–4 pairs of ventral eyespots, iv) the inner wall of the male atrium deeply ruffled, v) the lumen of the seminal vesicle narrow and elongated in shape, and vi) the large sucker situated on body center. This new species can be easily distinguished from other congeners by the unique ventral-eyespot number. In addition to the ventral-eyespot arrangement, the combination of the features in body coloration, cerebral-eyespot arrangement, and the size and position of the sucker allows separation of our new species from other similar-looking congeners (Table 2). Our specimens of *P.* cf. *ostreae* differed from the original description by the body-margin coloration and the frontal eyespots. In addition, our specimens had a pair of ventral eyespots, which might have been ignored in the original description. Further studies should test whether these two or three morphological differences are inter- or intraspecific. The reconstructed tree based on the partial 28S rRNA and *COI* gene sequences of 24 prosthiostomid species revealed that *P. auratum*, *P. hibana* sp. n., *P.* cf. *ostreae*, and *P. vulgare* are nested in the *Prosthiostomum* clade composed of the other 12 species, corresponding to their generic placements based on the morphological characteristics. The phylogenetic position of *P. katoi* in *Prosthiostomum* also supported a taxonomic view that *Lurymare* is indeed a junior synonym of *Prosthiostomum* (cf. Dittmann et al. 2019; Litvaitis et al. 2019; Tsuyuki et al. 2019).

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Authors' contributions: AT designed the present study. HKo collected materials. AT conducted the morphological observation and molecular phylogenetic analyses as well as drafted the manuscript. HKa and HKo contributed to improvement of the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no conflict of interests.

Availability of data and materials: Sequences determined in this study were deposited into the DNA Data Bank of Japan (DDBJ) database (accession numbers in manuscript).

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Ethics approval consent to participate: Not applicable.

REFERENCES

Aguado MT, Noreña C, Alcaraz L, Marquina D, Brusa F, Damborenea C, Almon B, Bleidorn C, Grande C. 2017. Phylogeny of Polycladida (Platyhelminthes) based on mtDNA data. Org Divers Evol **17:**767–778. doi:10.1007/s13127-017-0344-4.

Akaike H. 1974. A new look at the statistical model identification. IEEE Trans Autom Control **19:**716–723. doi:10.1109/TAC.1974.1100705.

Anker A, Murina GV, Lira C, Vera Caripe JA, Palmer AR, Jeng MS. 2005. Macrofauna associated with Echiuran burrows: a review with new observations of the innkeeper worm, *Ochetostoma erythrogrammon* Leuckart and Rüppel, in Venezuela. Zool Stud **44:**157–190.

Bahia J. 2016. First records of polyclads (Platyhelminthes, Polycladida) associated with *Nodipecten nodosus* (Linnaeus 1758) aquaculture. Mar Biodiv **46:**911–915. doi:10.1007/s12526-015-0425-6.

Bahia J, Padula V, Schrödl M. 2017. Polycladida phylogeny and evolution: integrating evidence from 28S rDNA and morphology. Org Divers Evol **17(3):**653–678. doi:10.1007/s13127-017-0327-5.

Bahia J, Schrödl M. 2018. Brazilian Polycladida (Rhabditophora: Platyhelminthes): rediscovery of Marcus' type material and general revision. Zootaxa **4490(1):**1–121. doi:10.11646/zootaxa.4490.1.1.

Bock S. 1913. Studien über Polycladen. Zool Bidr Uppsala **2:**31–344.

Bock S. 1922. Two new cotylean genera of polyclads from Japan and remarks on some other cotyleans. Ark Zool **14(13):**1–31. doi:10.5962/bhl.part.7726.

Bock S. 1923. Two new acotylean polyclads from Japan. Ark Zool **15(17):**1–39.

Bock S. 1924. Eine neue *Stylochoplana* aus Japan. Ark Zool **16(7):**1–24.

Bock S. 1925. Papers from Dr. Th. Mortensen's Pacific Expedition 1914–16. XXV. Planarians, Pts. I–III. Vidensk Medd Dansk Naturh Foren **79:**1–84.

Bock S. 1931. Die Polycladen. Dt Südpol Exped 20 (Zool) **12:**259–304.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol **28:**495–503. doi:10.1128/JCM.28.3.495-503.1990.

Castresana J. 2002. Gblocks, v. 0.91b. Online version. http://molevol.cmima.csic.es/castresana/Gblocks_server.html Accessed 11 Aug. 2020.

Deiss WA, Manning RB. 1981. The fate of the invertebrate collections of the North Pacific Exploring Expedition, 1853–1856. Arch Nat Hist **1981(1):**79–85.

Delle Chiaje S. 1828. Memorie sulla storia e notomia degli animali senza vertebre del regno di Napoli. Vol. III (1828): 1–232. Fratelli Fernandes, Napoli. doi:10.5962/bhl.title.10021.

Dittmann IL, Cuadrado D, Aguado MT, Noreña C, Egger B. 2019. Polyclad phylogeny persists to be problematic. Org Divers Evol **19:**585–608. doi:10.1007/s13127-019-00415-1.

Du Bois-Reymond Marcus E, Marcus E. 1968. Polycladida from Curaçao and faunistically related regions. Stud Fauna Curaçao **26:**1–106.

Evans AC. 1967. Syntypes of Decapoda described by William Stimpson and James Dana in the collections of the British Museum (Natural History). J Nat Hist **1:**399–411.

Faubel A. 1984. The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. Mitt Hamb Zool Mus Inst **81:**189–259.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791. doi:10.2307/2408678.

Freeman D. 1930. Three polyclads from the region of Point Firmin, San Pedro, California. Trans Am Microsc Soc **49:**334–341. doi:10.2307/3222162.

Fujiwara Y, Iwata T, Urabe J, Takeda S. 2016. Life history traits and ecological conditions influencing the symbiotic relationship between the flatworm *Stylochoplana pusilla* and host snail *Monodonta labio*. J Mar Bio Assoc UK **96(3):**667–672. doi:10.1017/S0025315415001009.

Hagiya M. 1992. A new polyclad turbellarian from northern Japan. Proc Jap Soc Syst Zool **47:**57–63.

Hagiya M, Gamo S. 1992. Polyclads (Platyhelminthes) collected from the intertidal rocky shore at Manazuru, Sagami Bay. Rep Manazuru Mar Lab for Sci Edu Fac Edu, Yokohama National Univ **8:**13–24. (in Japanese with English abstract)

Hallez P. 1911. Double fonction des ovaires de quelques polyclades. CR Acad Sci Paris **153:**141–143.

Hyman LH. 1939a. Acoel and polyclad Turbellaria from Bermuda and the Sargassum. Bull Bingham Oceanogr Coll **7(Art I):**1–26.

Hyman LH. 1939b. Polyclad worms collected on the Presidential cruise of 1938. Smithson Misc Coll **98(17):**1–13.

Hyman LH. 1953. The polyclad flatworms of the Pacific coast of North America. Bull Amer Mus Nat Hist **100:**269–391.

Hyman LH. 1959. A further study of Micronesian polyclad flatworms of the West Indian region. Pros US Natn Mus **108:**543–597.

Kajihara H, Kakui K. 2017. An overview of recent marine-biodiversity research in Japan. *In*: Motokawa M, Kajihara H (eds) Species diversity of animals in Japan. Springer Japan, Tokyo.

Kato K. 1937a. Polyclads collected in Idu, Japan. Jpn J Zool **7:**211–232.

Kato K. 1937b. Thirteen new polyclads from Misaki. Jpn J Zool **7:**347–371.

Kato K. 1938a. Polyclads from Amakusa, southern Japan. Jpn J Zool **7:**559–576.

Kato K. 1938b. Polyclads from Seto, middle Japan. Jpn J Zool **7:**577–593.

Kato K. 1939a. Polyclads in Onagawa and vicinity. Sci Rept Tôhoku Imp Univ (4) Biol **14:**65–79.

Kato K. 1939b. Report of the biological survey of Mutsu Bay. 34. The polyclads of Mutsu Bay. Sci Rept Tôhoku Imp Univ (4) Biol **14:**141–153.

Kato K. 1943. Polyclads from Formosa. Bull Biogeogr Soc Jpn **13:**69–77.

Kato K. 1944. Polycladida of Japan. Sigenkagaku Kenkyusyo (J Res Inst Nat Resources) **1:**257–319.

Kato M. 2018. Restoration of the collection information for an Australian mammal skeletal specimen stored in the National Museum of Nature and Science, Japan. Proc Jpn Soc Syst Zool **45:**61–72. (In Japanese with English abstract)

Katoh K, Rozewicki J, Yamada KD. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform **20(4):**1160–1166. doi:10.1093/bib/bbx108.

Kawakatsu M. 2004. [The status of management for type series of Japanese turbellarians (Platyhelminthes: "Turbellaria")—especially Tricladida]. *In*: Ohtaka A (ed) [Promotion of appropriate management of important specimens in the taxonomy of freshwater invertebrates in Japan]. Report from the River Foundation 16-1-10-1, Tokyo, pp. 5–6. (in Japanese)

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: a fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics **35(21):**4453–4455. doi:10.1093/bioinformatics/btz305.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol **33:**1870–1874. doi:10.1093/molbev/msw054.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol **29:**1695–1701. doi:10.1093/molbev/mss020.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol **34:**772–773. doi:10.1093/molbev/msw260.

Lang A. 1884. Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. Engelmann W, Leipzig. doi:10.5962/bhl.title.10545.

Litvaitis MK, Bolaños DM, Quiroga SY. 2019. Systematic congruence in Polycladida (Platyhelminthes, Rhabditophora): are DNA and morphology telling the same story? Zool J Linn Soc **186:**865–891. doi:10.1093/zoolinnean/zlz007.

Lytwyn MW, McDermott JJ. 1976. Incidence, reproduction and feeding of *Stylochus zebra*, a polyclad turbellarian symbiont of hermit crabs. Mar Biol **38:**365–372. doi:10.1007/BF00391376.

Marcus E. 1948. Turbellaria do Brasil. Bol Fac Fil Ci Let U São Paulo Zool **13:**111–243. doi:10.11606/issn.2526-4877.bsffclzoologia.1948.125311.

Marcus E. 1949. Turbellaria brasileiros (7). Bol Fac Fil Ci Let U São Paulo Zool **14:**7–155. doi:10.11606/issn.2526-4877.bsffclzoologia.1949.129106.

Marcus E. 1950. Turbellaria brasileiros (8). Bol Fac Fil Ci Let U São Paulo Zool **15:**5–191. doi:10.11606/issn.2526-4877.bsffclzoologia.1950.125192.

Marcus E. 1952. Turbellaria brasileiros (10). Bol Fac Fil Ci Let U São Paulo Zool **17:**5–187. doi:10.11606/issn.2526-4877.bsffclzoologia.1952.125189.

Marquina D, Aguado MT, Noreña C. 2015. New records of Cotylea (Polycladida, Platyhelminthes) and one new species from Lizard Island (Australia), with remarks on the distribution of the *Pseudoceros* Lang, 1884 and *Pseudobiceros* Faubel, 1984 species of the Indo-Pacific marine region. Zootaxa **4019(1):**354–377. doi:10.11646/zootaxa.4019.1.14.

Newman LJ, Cannon LRG. 2003. Marine flatworms: the world of polyclad flatworms. CSIRO Publishing, Collinwood.

Noreña C, Marquina D, Perez J, Almon B. 2014. First records of Cotylea (Polycladida, Platyhelminthes) for the Atlantic coast of the Iberian Peninsula. ZooKeys **404:**1–22. doi:10.3897/zookeys.404.7122.

Oya Y, Kajihara H. 2017. Description of a new *Notocomplana*

species (Platyhelminthes: Acotylea), new combination and new records of Polycladida from the northeastern Sea of Japan, with a comparison of two different barcoding markers. Zootaxa **4282(3):**526–542. doi:10.11646/zootaxa.4282.3.6.

Oya Y, Kajihara H. 2019a. A new bathyal species of *Cestoplana* (Polycladida: Cotylea) from the West Pacific Ocean. Mar Biodivers **49(2):**905–911. doi:10.1007/s12526-018-0875-8.

Oya Y, Kajihara H. 2019b. A new species of *Phaenoplana* (Platyhelminthes: Polycladida) from the Ogasawara Islands. Species Divers **24(1):**1–6. doi:10.12782/specdiv.24.1.

Oya Y, Kajihara H. 2020. Molecular phylogenetic analysis of Acotylea (Platyhelminthes: Polycladida). Zool Sci **37(3):**271–279. doi:10.2108/zs190136.

Oya Y, Kimura T, Kajihara H. 2019. Description of a new species of *Paraplehnia* (Polycladida, Stylochoidea) from Japan, with inference on the phylogenetic position of Plehniidae. ZooKeys **864:**1–13. doi:10.3897/zookeys.864.33955.

Oya Y, Tsuyuki A, Kajihara H. 2020. A new species of *Zygantroides* (Platyhelminthes: Polycladida) from Amakusa, Japan. Species Divers **25(2):**189–196. doi:10.12782/specdiv.25.189.

Palombi A. 1936. Policladi liberi e commensali raccoliti sulle coste del Sud Africa, della Florida e del golfo di Napoli. Arch Zool Italiano **23:**1–45.

Pearse AS. 1938. Polyclads of the East Coast of North America. Proc US Natn Mus **86:**67–97. doi:10.5479/si.00963801.86-3044.67.

Pitale R, Bhave V, Apte D. 2014. First record of family Prosthiostomidae and *Prosthiostomum trilineatum* (Platyhelminthes: Polycladida) from the west coast of India. Mar Biodivers Rec **7:**1–6. doi:10.1017/S1755267214000128.

Poulter JL. 1975. Hawaiian polyclad flatworms. Prosthiostomids. Pacific Sci **29:**317–339.

Prudhoe S. 1985. A monograph on polyclad Turbellaria. Oxford University Press, Oxford.

Prudhoe S. 1989. Polyclad turbellarians recorded from African waters. Bull British Mus Nat Hist **55:**47–96.

Quatrefages A de. 1845. Études sur les types inférieurs de l'embranchement des annelés: mémoire sur quelques planairées marines appartenant aux genres *Tricelis* (Ehr.), *Polycelis* (Ehr.), *Prosthiostomum* (Nob.), *Proceros* (Nob.), *Eolidiceros* (Nob.), et *Stylochus* (Ehr.). Annls Sci Nat (3) Zoologie **4:**129–184.

Rawlinson KA, Gillis JA, Billings RE, Borneman EH. 2011. Taxonomy and life history of the *Acropora*-eating flatworm *Amakusaplana acroporae* nov. sp. (Polycladida: Prosthiostomidae). Coral Reefs **30(3):**693. doi:10.1007/s00338-011-0745-3.

Sonnenberg R, Nolte AW, Tautz D. 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. Front Zool **4(1):**1–12. doi:10.1186/1742-9994-4-6.

Stimpson W. 1857. Prodromus descriptionis animalium evertebratorum quae in Expeditione ad Oceanum Pacificum Septentrionalem, Johanne Rodgers Duce a Republica Federata missa, observavit et descripsit. Pars. I. Tubellaria Dendrocoela. Proc Acad Nat Sci Philadelphia **9:**19–31.

Tsunashima T, Hagiya M, Yamada R, Koito T, Tsuyuki N, Izawa S, Kosoba K, Itoi S, Sugita H. 2017. A molecular framework for the taxonomy and systematics of Japanese marine turbellarian flatworms (Platyhelminthes, Polycladida). Aquat Biol **26:**159–167. doi:10.3354/ab00682.

Tsuyuki A, Kajihara H. 2020. A giant new species of *Enchiridium* (Polycladida, Prosthiostomidae) from southwestern Japan. ZooKeys **918:**15–28. doi:10.3897/zookeys.918.47061.

Tsuyuki A, Oya Y, Kajihara K. 2019. A new species of *Prosthiostomum* (Platyhelminthes: Polycladida) from Shirahama, Japan. Species Divers **24(2):**137–143. doi:10.12782/specdiv.24.1.

Tyler S, Schilling S, Hooge M, Bush LF. (comp.) 2006–2020. Turbellarian taxonomic database. Version 1.7 http://turbellaria.umaine.edu Accessed 7 Mar. 2021.

Yeri M, Kaburaki T. 1918. Description of some Japanese polyclad Turbellaria. J Coll Sci Imp Univ Tokyo **39(9):**1–54.

Yeri M, Kaburaki T. 1920. Notes on two new species of Japanese polyclads. Annot Zool Jpn **9:**591–598.