The abyssal parasitic flatworm Fecampia cf. abyssicola: new records, anatomy, and molecular phylogeny, with a discussion on its systematic position

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The Abyssal Parasitic Flatworm *Fecampia cf. abyssicola*: New Records, Anatomy, and Molecular Phylogeny, with a Discussion on Its Systematic Position

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Abstract

The order Fecampiida, a group of parasitic turbellarians, has been poorly studied in terms of its species diversity, morphology, and ecology. Fecampiida is positioned within the monophyletic clade Adiaphanida, along with Tricladida and Prolecithophora, but their phylogenetic relationships are not well understood. Although the nervous and muscular systems of only two species in Fecampiida have been studied, recent research inferred morphological similarities between Fecampiida and Prolecithophora. In this study, we collected fecampiid cocoons and juveniles at depths of 1861–4438 m in Japanese waters. We identified the species on the basis of swimming juvenile specimens and by using histological and molecular methods, while we also examined its musculature and nervous system. Our study revealed a more complex nervous system than previously reported, with dorsal, lateral, and ventral pairs of longitudinal nerve cords connected through an anterior neuropile and posterior transverse commissures. While the nervous and muscular morphology suggested similarities with Prolecithophora, our phylogenetic analysis did not support a close relationship between Fecampiida and Prolecithophora.

Introduction

Turbellarians, a paraphyletic group within the phylum Platyhelminthes Claus, 1887 (Egger *et al.*, 2015), are primarily free-living carnivorous predators, with a few commensal and parasitic taxa (Jennings, 1971). Fecampiida Rohde, Luton, & Johnson, 1994, an order of parasitic turbellarians, has a diverse range of hosts, including fish, crustaceans, bivalves, gastropods, cephalopods, and polychaetes (Baylis,

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Abbreviations: BBT, phosphate-buffered saline with 0.1% Triton X-100 containing 1% bovine serum albumin; BI, Bayesian inference; bs, bootstrap; DAPI, 4′,6-diamidino-2-phenylindole; FSW, filtered seawater; HE, hematoxylin and eosin; ML, maximum likelihood; PBS, phosphate-buffered saline; PBT, phosphate-buffered saline with 0.1% Triton X-100; pp, posterior probability.
Online enhancements: supplemental video files.

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1949; Syromynatnikova, 1949; Christensen, 1981a; Bellon-Humbert, 1983; Blair and Williams, 1987; Robledo *et al.*, 1994; Sudo *et al.*, 2011; Gordeev *et al.*, 2022). Currently, Fecampiida comprises 14 species in seven genera and four families (Gordeev *et al.*, 2022). Phylogenetically, Fecampiida is placed within Adiaphanida Noren & Jondelius, 2002, along with Tricladida Lang, 1884 and Prolecithophora Karling, 1940, although relationships within this clade remain unclear (Laumer and Giribet, 2017).

All species in the family Fecampiidae Graff, 1903 produce species-specific cocoons, albeit the cocoon of *Glanduloderma* Jägersten, 1940 has not been reported in the literature. After reaching sexual maturity inside their host, the fecampiid parasites emerge and settle on hard substrates, where they enclose themselves in a cocoon. Within the cocoon, the parasite deposits numerous egg capsules, after which the individual worm starts to degenerate. From each capsule emerges a juvenile, or larva, which leaves the cocoon, in search of a new host specimen. The morphology of fecampiid cocoons is variable and generally species specific (Sluys and van Ginkel, 1989).

Fecampia abyssicola Christensen, 1981 is the only known fecampiid species that has been reported from abyssal depths (Christensen, 1981a, b; Sluys and van Ginkel, 1989). The species is characterized by its flask-shaped cocoon with a long neck (Christensen, 1981b; Sluys and van Ginkel, 1989). It was first described based on specimens and cocoons collected from the Atlantic, Indian, and eastern Pacific Oceans (Christensen, 1981b). Cocoon samples, some containing worms, were also reported from the Indian Ocean near Madagascar, the Kermadec Trench, and the eastern Atlantic (Christensen, 1981a, b; Sluys and van Ginkel, 1989). Recently, similar cocoons were found in the South China Sea, where they were attached to deep-sea plastic debris (Song *et al.*, 2021). Despite their relatively common occurrence among the bathyal and abyssal benthic fauna (Christensen, 1981b), new molecular, morphological, or ecological information on the species has not become available over the past 40 years.

Information on the organization and nature of the nervous and muscular systems may provide valuable data for systematic studies of the Platyhelminthes (*e.g.*, Raikova *et al.*, 2017; Grosbusch *et al.*, 2019, 2021). The main components of the nervous system in Platyhelminthes consist of the central nervous system (brain and nerve cords), peripheral nervous system, and additional minor nerves in the pharynx and genital organs (Reuter and Gustafsson, 1995; Kotikova *et al.*, 2002; Grosbusch *et al.*, 2021). The structure, number, appearance, and nature of these components vary across turbellarian taxa (Grosbusch *et al.*, 2021). Previous studies have attempted to identify synapomorphies for these features, thus providing data for systematic evaluations. Unfortunately, limited availability of morphological information for minor turbellarian taxa, including Fecampiida, makes such evaluations problematic. Furthermore, modern immunohistochemistry methods have visualized the detailed morphology of musculature and the nervous system more clearly than was possible with the traditional hematoxylin and eosin (HE) staining procedures (*e.g.*, Kotikova *et al.*, 2002). Currently, only two species of Fecampiida, *Urastoma cyprinae* (Graff, 1882) and *Notentera ivanovi* Joffe, Selivanova & Kornakova, 1997, have been studied with immunohistochemical procedures, the former for its musculature and the latter for both its musculature and cholinergic nervous system (Hooge and Tyler, 1999; Raikova *et al.*, 2017).

During the 2022–2023 deep-sea cruises aboard the R/V *Kaimei*, we collected fecampiid cocoons and living juvenile worms from four stations in Japanese waters, at depths ranging from 1861 to 4438 m (Fig. 1). We identified the specimens on the basis of their internal morphology, as observed on live specimens and serial histological sections, and also performed phylogenetic inference, using partial sequences of *28S* rRNA (*28S*) and *18S* rRNA (*18S*). In addition, we studied the musculature by using phalloidin labeling and the nervous system by using anti-FMRF-amide fluorescent immunostaining of whole-mounted specimens. This paper gives an account of our specimens and their identification and evaluates the data generated on molecular sequences and the immunocytochemical study of the musculature and nervous systems.

Materials and Methods

Sample collection

Specimens of Fecampiidae Graff, 1903 examined in the present study were collected in 2022–2023 during the two research cruises (KM22-02 and KM22-15, Leg. 2; R/V *Kaimei*, Japan Agency for Marine-Earth Science and Technology). The samples were collected at four localities in the northwestern Pacific by use of beam trawl at depths ranging between 1861 and 4438 m (Fig. 1; Table 1). Living juvenile worms were obtained by breaking a cocoon with a forceps. Photographs and videos of living specimens were taken on board using a Nikon D5600 digital SLR camera mounted on a stereomicroscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany). Cocoons were photographed and preserved in 70% or 99% ethanol for DNA extraction and also in 4% formaldehyde in filtered seawater (FSW) and in Bouin's fluid for anatomical studies. Materials (comprising cocoons and histological sections of worms) will be deposited in the National Museum of Nature and Science, Tsukuba, Japan, and are specified in Table 1.

Histology and immunocytochemistry

Our morphological study was based on juveniles (in the available literature frequently called larvae) that were collected from opened cocoons; these specimens were prepared for histological and immunocytochemical analyses. Before fixation, live worms were anesthetized with 3.5%

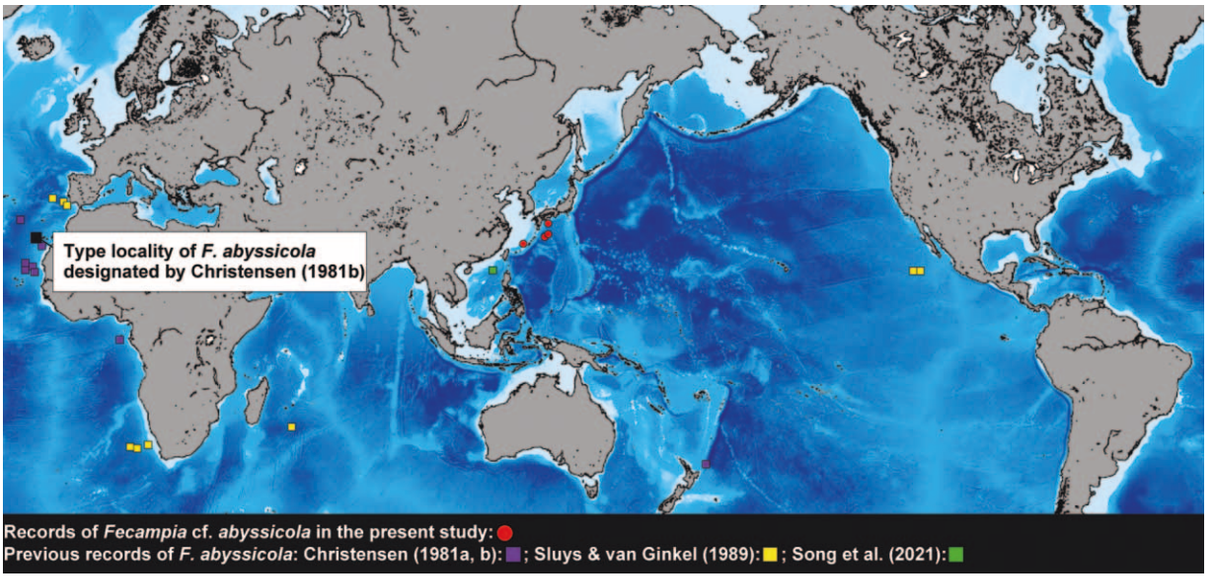

Figure 1. Geographical distribution of *Fecampia cf. abyssicola* (collection sites from the present study) and *Fecampia abyssicola* (from Christensen, 1981a, b; Sluys and van Ginkel 1989; Song *et al.*, 2021).

magnesium chloride hexahydrate in FSW. For histological studies, the relaxed worms were fixed in Bouin's fluid for 24 h and later transferred to 70% ethanol. The specimens were dehydrated in a graded ethanol series (70%, 80%, 90%, 95%, 99%), cleared three times for 30 min in xylene, and embedded in Surgipath Paraplast paraffin (Leica). Serial sections were made at intervals of 6 μm and stained with Mallory's trichrome method (Winsor and Sluys, 2018).

For immunocytochemistry, relaxed worms were fixed with 4% formaldehyde in FSW for 24 h at 4 °C. Fixed specimens were washed three times for 15 min in phosphate-buffered saline (PBS) and stored at 4 °C for 1 week until observation. Preserved specimens were permeabilized in PBS with 0.1% Triton X-100 (Nacarai Tesque, Kyoto, Japan; PBT) by three washes, then washed three times in PBS with 0.1% Triton X-100 containing 1% bovine serum albumin (BBT) for 15 min and subsequently blocked in BBT with 2% normal goat serum for 30 min. Immunofluorescence double staining, using mixtures of two primary antibodies (Table 2), was performed. The primary antibodies were removed by three washes in BBT for 15 min each and incubated in BBT with secondary antibodies. The secondary antibodies were removed by three 15-min washes in BBT and subsequently stained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St. Louis, MO; final concentration: 2 μg mL^{-1}) and rhodamine-phalloidin (Invitrogen, Carlsbad, CA; final concentration: 1 : 40) for 1 h at room temperature. After three 15-min washes in PBT, the specimens were mounted in Vectashield mounting medium (Vector Laboratories, Newark, CA) between long (24 mm × 60 mm) and short (24 mm × 24 mm) coverslips (Matsunami Glass, Osaka, Japan); the preparations were examined under a fluorescence digital microscope BZ-X810 (Keyence, Osaka, Japan) and analyzed with image processing software BZ-X Analyzer (Keyence, Osaka, Japan).

DNA extraction, polymerase chain reaction amplification, sequencing, and phylogenetic analyses

Live worms were preserved in 99% ethanol for DNA extraction. Total DNA was extracted using a DNeasy tissue kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) amplification was performed using KOD One PCR master mix (Toyobo, Osaka, Japan), following the manufacturer's

Table 1

Collection information of *Fecampia cf. abyssicola*

Collection location	Coordinates (lat., long)	Depth (m)	Collection date	NSMT number
Off Nansei Islands, Okinawa Trough	26°50.608′ N, 126°25.832′ E	1861	February 5, 2022	. . .
Shikoku Basin	32°2.097′ N, 133°58.744′ E	3640	December 27, 2022	. . .
Shikoku Basin	28°59.863′ N, 132°59.992′ E	4438	December 31, 2022	. . .
Shikoku Basin	29°1.184′ N, 133°59.436′ E	2793	January 2, 2023	NSMT-Pl 6456 (cocoons)
Shikoku Basin	29°1.184′ N, 133°59.436′ E	2793	January 2, 2023	NSMT-Pl 6457 (histological sections, 1 slide)

NSMT, National Museum of Nature and Science, Tsukuba.

Table 2

Immunofluorescence double-staining protocol used in the present study

Primary antibody	Secondary antibody	DAPI	Phalloidin
FMRF-amide (20091, ImmunoStar, Hudson, WI)	Alexa Fluor 488 donkey-anti-rabbit secondary antibody	Stained	Stained

instructions, with the following phosphorothioate primers: for *28S*, ZX-1 (5′-ACCCGCTGAATTTAA GCATAT-3′) (Van der Auwera *et al.*, 1994), 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) (Tkach *et al.*, 2003), LSU_300F (5′-CAAGTACCGTGAGGGAAAGTTG-3′), ECD2 (5′-CTTGGTCCGTGTTTCAAGACGGG-3′) (Littlewood *et al.*, 2000), 1090F (5′-TGAAACACGGACCAAGG-3′) (Littlewood *et al.*, 2008), and LSU_1200F (5′-CCCGAAA GATGGTGAACTATGC-3′) (Telford *et al.*, 2003). The region that we amplified corresponds to currently available sequences of other fecampiid species in GenBank. The PCR protocol was as follows: preheating at 94 °C for 2 min; 35 cycles of 94 °C for 40 s, 52 °C for 75 s, and 72 °C for 60 s; then a final extension at 72 °C for 7 min. The PCR products were purified using Exo-SAP-IT (ThermoFisher Scientific, Waltham, MA) and premixed with the primers mentioned above; for sequencing they were outsourced to Eurofins Genomics (Tokyo, Japan). We successfully obtained a *28S* partial sequence of 1076 bp from the specimen used in this study, while amplification for *18S* was unsuccessful with primer pairs 1F/9R (Giribet *et al.*, 1996).

Alignment of *28S* and *18S* sequences of selected platyhelminth species (Table 1) was performed using MAFFT version 7 (Katoh and Standley, 2013). Subsequently, ambiguous nucleotide sites were trimmed using Gblocks version 0.91b (Castresana, 2000), resulting in a concatenated dataset comprising *18S* (1594 bp) and *28S* (737 bp). Maximum-likelihood (ML) analyses were performed using RAxML-NG (Kozlov *et al.*, 2019) installed in raxmlGUI 2.0 (Edler *et al.*, 2021). The optimal substitution model, GTR+FC+G4m+BU, was selected for both *18S* and *28S* in ModelTest-NG (Darriba *et al.*, 2019). Nodal support values were obtained from 1000 bootstrap (bs) pseudoreplicates. Bayesian inference (BI) analyses were performed with MrBayes version 3.2.6 (Huelsenbeck and Ronquist, 2001) installed in Geneious Prime (Kearse *et al.*, 2012) based on the GTR+G+I model, launching two independent Metropolis-coupled analyses with four Markov chains for 10^6 generations and sampling every 100 generations from the chain. Convergence of runs was determined by examining in Geneious Prime that the average standard deviation of split frequencies did not fall below 0.01. The newly obtained sequences in this study were deposited in GenBank, and their accession numbers are listed in Table 3, along with the species used in the abovementioned analyses.

Results

Taxonomic account

Phylum—Platyhelminthes Claus, 1887

Order—Fecampiida Rohde, Luton, & Johnson, 1994

Family—Fecampiidae Graff, 1903

Genus—Fecampia *Giard, 1886*

Fecampia cf. abyssicola *Christensen, 1981*

Material examined. Collection information on specimens examined in the present study is summarized in Table 1.

Morphological description. The flask-shaped, pale-pink cocoons consist of an oval capsule with a long, tubelike neck (Fig. 2); empty capsules were pale to translucent (Fig. 2B). The capsule part measured 3.5–4.0 mm in length and 2.6–3.1 mm in width, while the neck measured 2.2–2.7 mm in length and 0.5 mm in diameter (Fig. 2B–D) ($n = 4$).

Body of juveniles unpigmented, linear-oblong, with a somewhat attenuated anterior portion, giving the impression of a kind of "neck." Anterior end rounded and posterior end obtusely pointed (Fig. 3A; Videos S1, S2 [Videos S1–S3 are available online],). After anesthetization, body of live juveniles approximately 150 μm in length and 75 μm at their maximum width (Fig. 3A). Body epithelium densely ciliated (Fig. 3A–C; Video S2). Eyes are absent.

Mouth opening to the exterior located at the anterior tip, leading to a curved duct, internally lined with cilia (Fig. 3A–C; Video S2), presumed to be the pharynx (Fig. 3D, E).

The muscular and nervous systems were studied on the basis of swimming juveniles obtained from opened cocoons that were prepared for these analyses (see above). The anterior part of the body was densely stained with DAPI (Fig. 4A). The musculature of the body wall in juveniles consists of longitudinal, circular, and diagonal muscle fibers (Fig. 4B). The longitudinal and diagonal muscle fibers are equally distributed throughout the body (Fig. 4B), while the circular muscle fibers are densely developed around the anterior and posterior parts of the body (Fig. 4B).

Molecular analysis. In both ML and BI analyses, the specimens were identified as members of the Fecampiida (Fig. 5). Although the nodal values within the Fecampiida clade were low, the tree topology was the same in the two analyses. Specifically, *F. cf. abyssicola* is sister to *Kronborgia cf. amphipodicola*, supported by 60% bs and 0.98 posterior probability (pp), with this clade sharing a sister-group relationship with a clade comprising *Octopoxenus antarcticus* and *Notentera ivanovi* (77% bs; 0.87 pp). In turn, this clade of four taxa is sister to a clade comprising the sister species *Kronborgia isopodicola* and *Ichthyophaga* sp. (99% bs; 1.00 pp).

Table 3

List of species used for phylogenetic analyses, with GenBank accession numbers

Order, species	*28S*	*18S*	Reference
Fecampiida			
Fecampia cf. abyssicola	OR677932	. . .	Present study
Ichthyophaga sp.	AY157166	AJ012512	Lockyer *et al.*, 2003
Kronborgia cf. amphipodicola	KY678761	KY678764	Laumer and Giribet, 2017
Kronborgia isopodicola	AY157168	AJ012513	Lockyer *et al.*, 2003
Notentera ivanovi	AY157167	AJ287546	Lockyer *et al.*, 2003
Octopoxenus antarcticus	MZ262534	. . .	Gordeev *et al.*, 2022
Octopoxenus antarcticus	MZ330691	. . .	Gordeev *et al.*, 2022
Genostomatida			
Genostoma kozloffi	KY678762	KY678763	Laumer and Giribet, 2017
Rhabdocoela			
Didymorchis sp.	AY157163	AY157182	Lockyer *et al.*, 2003
Pterastericola australis	AY157161	AJ012518	Lockyer *et al.*, 2003
Temnosewellia minor	AY157164	AY157183	Lockyer *et al.*, 2003
Prolecithophora			
Plicastoma cuticulata	AY157158	AF065422	Lockyer *et al.*, 2003
Reisingeria hexaoculata	AY157157	AF065426	Lockyer *et al.*, 2003
Tricladida			
Bdelloura candida	AY157154	Z99947	Lockyer *et al.*, 2003
Girardia tigrina	U78718	AF013157	Lockyer *et al.*, 2003
Hausera hauseri	MN719494	MN719501	Benítez-Álvarez *et al.*, 2020
Polycladida			
Amemiyaia pacifica	LC508203	LC508166	Oya and Kajihara, 2020
Notocomplana hagiyai	LC508129	LC508152	Oya and Kajihara, 2020
Prorhynchida			
Geocentrophora wagini	AY157156	AJ012509	Lockyer *et al.*, 2003
Proseriata			
Invenusta sp.	KY320152	KY320092	Scarpa *et al.*, 2017

The Fecampiida clade was found to share a sister-group relationship with the Genostomatida + Prolecithophora + Tricladida clade, supported by a 99% bs in ML and a 1.00 pp in BI. The remaining interorder relationships were also consistent between the ML and BI analyses (Fig. 5). In particular, the Fecampiida + Prolecithophora + Tricladida clade formed the sister group of the Rhabdocoela Ehrenberg, 1831 clade (Fig. 5).

Immunocytochemistry of the nervous system

Cells labeled with DAPI appeared to be arranged in paired clusters on the ventral side of the anterior neuropile (Fig. 4A, C). The immunoreactivity of FMRF-amide was expressed in longitudinal nerve cords and transverse commissures, including anterior neuropile (Fig. 4C–E; Video S3). Most conspicuous FMRF-amide immunoreactivity was expressed in the anteriorly located neuropile (Fig. 4C–E; Video S3). Anterior and posterior ganglia were connected to each other through the longitudinal nerve cords (Fig. 4D, E). Anterior longitudinal nerve cords extending from anterior ganglia were paired and situated dorsally (Fig. 4E, F). Anterior ventral nerve cords were not clearly discernible in our preparations. Longitudinal nerve cords immunoreactive to FMRF-amide comprise the paired dorsal, lateral, and ventral nerve cords (Fig. 4D–F). The lateral nerve cords were each connected *via* transverse commissures (Fig. 4D–F).

Ecology

Our samples revealed that the fecampiid cocoons were attached to pumice (Fig. 2A, B), wood debris (Fig. 2C), and metal debris (Fig. 2E). In laboratory cultures at 4 °C we observed that after the juveniles had emerged from their cocoons, they exhibited a swimming phase of at least 4 days, presumably corresponding to the host-searching phase. Although amphipods and shrimps were also collected during our trawls, the specific host(s) of *F. cf. abyssicola* could not be established.

Discussion

Identification and taxonomy

Because we did not obtain adult specimens, we are unable to fully confirm whether the present species is identical to *Fecampia abyssicola*. However, our juveniles lacked eyes, whereas eyes are present in juveniles of *Fecampia xanthocephala* Caullery & Mesnil, 1902 and most likely also in those of *Fecampia balanicola* Christensen & Hurley, 1977 (Christen and Hurley, 1977; Christensen, 1981a). One morphological difference between our specimens and *F. abyssicola* concerns the coloration of the cocoons. For *F. abyssicola*, coloration of the cocoons was reported as dirty white (Sluys and van Ginkel, 1989), whereas our cocoons were pale pink (Fig. 2A, C), with empty cocoons being pale to

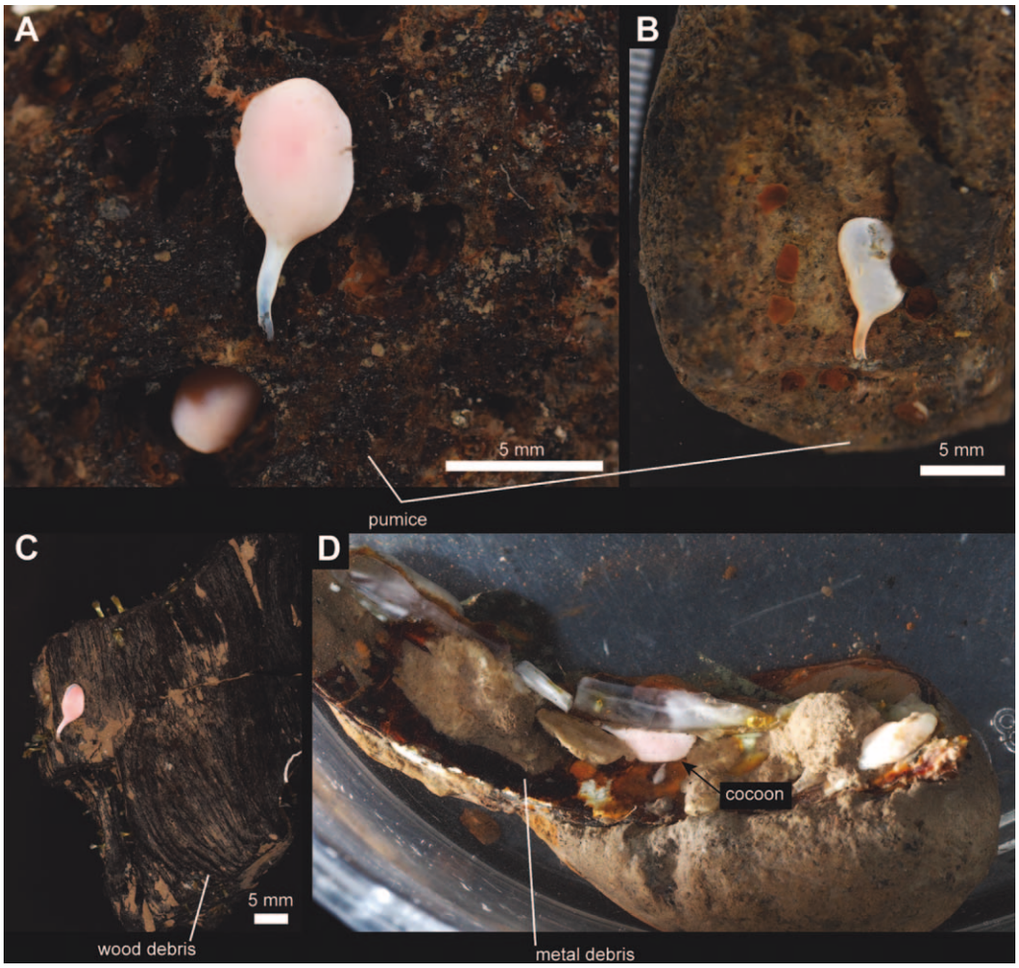

Figure 2. Cocoons of *Fecampia cf. abyssicola* (photographs taken on board). Cocoons attached to pumice (A, B), wood debris (C), and metal debris (D).

translucent (Fig. 2B). With our current data, we cannot conclude whether this is due to intraspecific variation, whether it resulted from environmental factors, or whether it signals a different species. Despite the difference in coloration, the shape of our cocoons, including their long and slightly curved necks, agrees with that previously reported for *F. abyssicola* (Christensen, 1981b; Sluys and van Ginkel, 1989). The size of our cocoons (3.5–4.0 mm in length without the neck and 2.3–4.1 mm in width) also falls within the range of size variation previously reported for *F. abyssicola* (*i.e.*, 2.8–8.6 mm in length without the neck and 2.0–5.0 mm in width) (Christensen, 1981b; Sluys and van Ginkel, 1989). Furthermore, for the juveniles of *F. abyssicola*, Christensen (1981a) mentioned a length of 155–165 μm and a width of 30–40 μm; the length largely agrees with that of our specimens, 150 μm. The fact that our juveniles were broader (75 μm) than those of Christensen (1981a) may be due to the fact that we examined anesthetized live specimens, while flatworms usually shrink during the fixation process. Christensen (1981a) mentioned that in his juvenile larval specimens, a mouth and pharynx appeared to be present, although this may have been illusory. The information that we could glean from our specimens indeed suggested the presence of a mouth and a tubular pharynx (Fig. 3). In view of the above, we herein follow a conservative approach to taxonomy and refer to our specimens as *F. cf. abyssicola*.

The body wall musculature of *F. cf. abyssicola* consisted of longitudinal, circular, and diagonal muscle fibers (Fig. 4B). However, diagonal muscles were not reported for *F. abyssicola*, with the caveat that the internal morphology was studied only on the basis of HE staining (Christensen, 1981b). But diagonal muscles fibers have been reported for other members of the Fecampiida on the basis of immunohistochemical studies, namely, *Notentera ivanovi* and *Urastoma cyprinae* (Hooge and Tyler, 1999; Raikova *et al.*, 2017).

Adiaphanida, a platyhelminth assemblage comprising the three orders Tricladida, Prolecithophora, and Fecampiida, is a well-supported phylogenetic clade (Norén and Jondelius, 1999, 2002; Laumer and Giribet, 2014, 2017; Laumer *et al.*, 2015), although the internal relationships remain unresolved (Laumer and Giribet, 2017). Our molecular analysis squarely placed *F. cf. abyssicola* within the Fecampiida (Fig. 5).

Although *F. cf. abyssicola* shares similarities with other adiaphanidans in the arrangement of the body wall musculature, which comprises longitudinal, circular, and diagonal

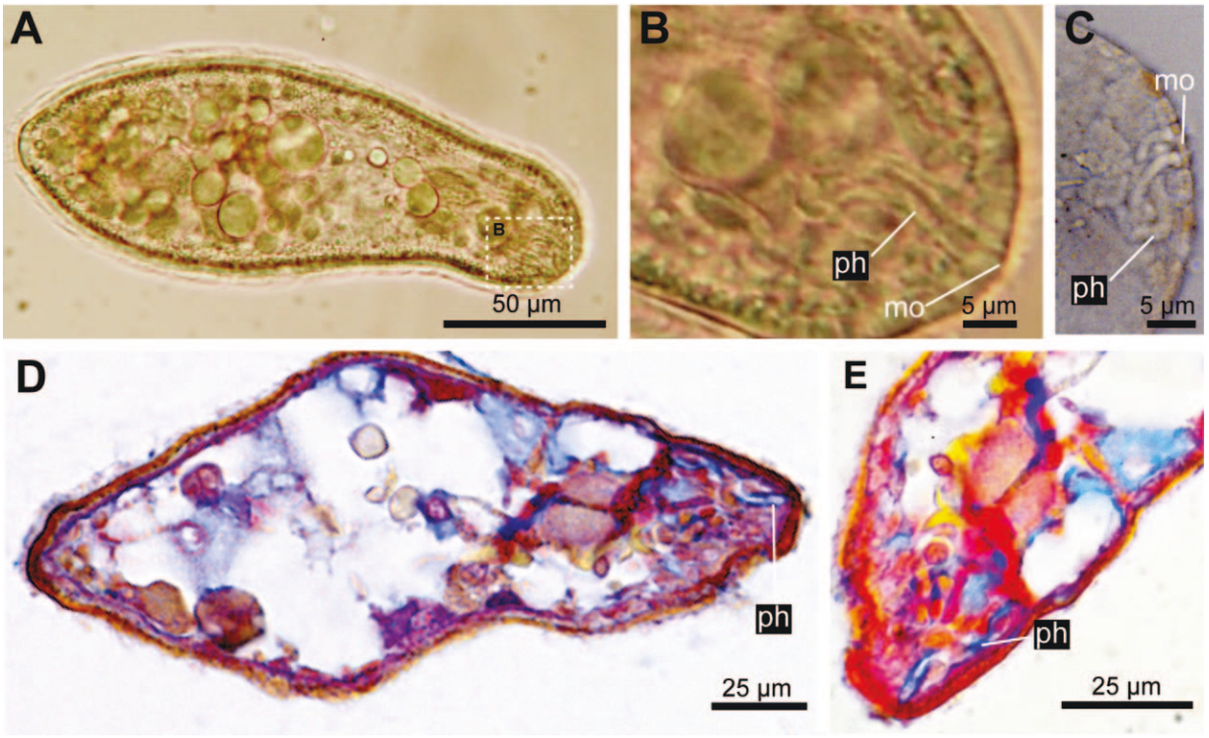

Figure 3. *Fecampia cf. abyssicola* anatomy. (A–C) Live specimens. (D, E) Histological preparations. (A) Photograph of *Fecampia cf. abyssicola* in swimming stage; inset enlarged in (B). (B) Magnification of duct presumed to be the pharynx. (C) Mouth opening and pharynx. (D) Horizontal section of entire animal. (E) Horizontal section of the anterior end of the body. mo, mouth opening; ph, pharynx.

muscle fibers (Fig. 4B), the presumed close relationship between Fecampiida and Prolecithophora in terms of nervous and muscular morphology (Grosbusch *et al.*, 2019, 2021) appears not to be supported by our phylogenetic analysis (Fig. 5).

Nervous system

A previous study comparing the nervous systems of several taxa of the Adiaphanida suggested that prolecithophorans are more similar to fecampiids than to triclads in having three pairs of longitudinal nerve cords (dorsal, lateral, and ventral) (Grosbusch *et al.*, 2021). In the present study, we observed three pairs of longitudinal nerve cords in juvenile *F. cf. abyssicola* specimens, thus corroborating the conclusion of Grosbusch *et al.* (2021).

The present study provides also some detailed insight into the nervous system of *Fecampia*, as we found it to be more complex than previously reported. The nervous system of our juvenile specimens comprised dorsal, lateral, and ventral pairs of longitudinal nerve cords that were transversely connected through both the anterior neuropile and the posterior transverse commissures (Fig. 4F). Earlier studies, in contrast, described a simpler morphology of the nervous system of *F. abyssicola*, with a brain-like neuropile situated in the anterior mesenchyme and paired lateral nerve cords (Christensen, 1981a). A similar nervous system was reported in *Fecampia erythrocephala* Giard, 1886, in which a bilobed cerebral ganglion in the anterior region is connected with paired lateral nerve cords, albeit the cords could not be followed far behind the brain, as they appeared to divide to form a fine plexus (Caullery and Mesnil, 1903; Bellon-Humbert, 1983). However, it may also be the case that previous studies were unable to discern the minor longitudinal nerve cords because of challenges involved in the examination of these structures in such small worms on the basis of traditional histological methods, whereas we used immunocytochemical techniques.

Musculature

Prolecithophorans are mainly free-living predators or scavengers (Noren and Jondelius, 2002) that have a ventral petal-like structure formed by strong longitudinal muscle fibers that radiate from the pharynx (Grosbusch *et al.*, 2019). This structure is considered important for anchoring the pharynx to the gut (Grosbusch *et al.*, 2019). In the facultative parasitic fecampiid *U. cyprinae*, which has both free-living and parasitic phases in its life cycle, a few radial muscle fibers extend from the middle of the pharynx (Hooge and Tyler, 1999); it has been hypothesized that these fibers are reminiscent of prolecithophoran anchoring muscles (Grosbusch *et al.*, 2019). In the present study, we did not detect with phalloidin staining any of such anchoring muscles of the pharynx in *F. cf. abyssicola*. *Fecampia* is an obligate parasite that absorbs nutrients through its epidermis from host tissues (Christensen, 1981b) and presumably therefore completely lost any anchoring muscles as an adaptation to its parasitic lifestyle.

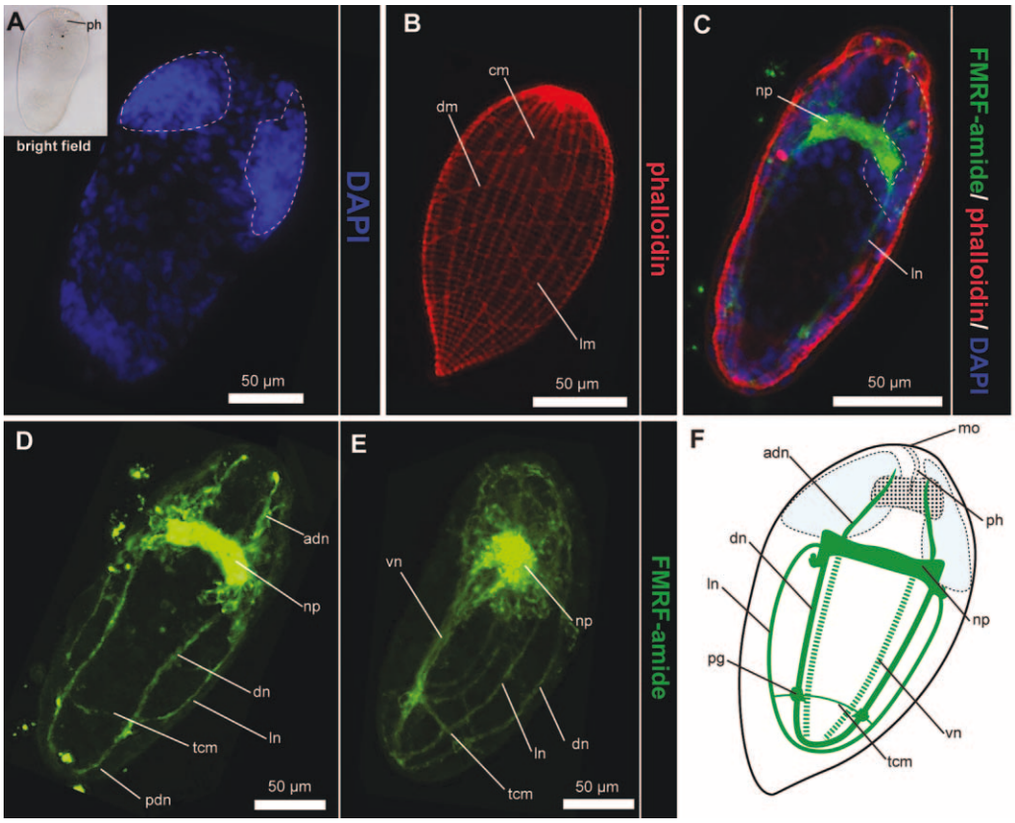

Figure 4. *Fecampia cf. abyssicola*, immunohistochemistry. (A) Dorsal view of the entire body, showing DAPI-staining cells anteriorly densely distributed (blue), with the same specimen in bright field shown in the inset. (B) Dorsal view of the entire body, showing the musculature by phalloidin staining (red). (C) Dorsal view of the entire body, showing anterior and posterior pairs of ganglia immunoreactive to FMRF-amide (green), musculature labeled with phalloidin (red), and nuclei labeled with DAPI (blue). (D) Dorsal view of the entire body, showing anterior neuropiles and longitudinal nerve cords immunoreactive to FMRF-amide (green). (E) Lateral view of the entire body, showing anterior neuropiles and longitudinal nerve cords immunoreactive to FMRF-amide (green). (F) Schematic diagram of the nervous and reproductive system of *F. cf. abyssicola*. In all panels the anterior tip of the body is in the top right-hand corner and the posterior end in the lower left-hand corner. adn, anterior dorsal longitudinal nerve cord; cm, circular muscle; dm, diagonal muscle; ln, lateral longitudinal nerve cord; mo, mouth opening; np, neuropile; pg, posterior ganglia; ph, pharynx; tcm, transverse commissure; vn, ventral longitudinal nerve cord.

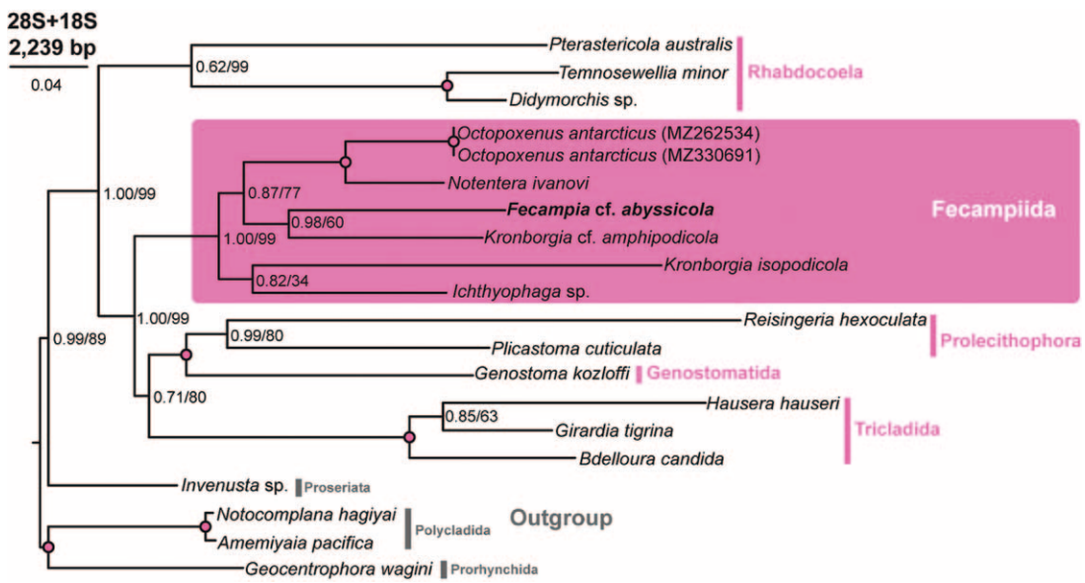

Figure 5. Maximum likelihood (ML) tree of selected species in Adiaphanida. Numbers at the nodes indicate bootstrap values and posterior probabilities (bs/pp). Solid circles indicate full support of bs values (ML) and pp (Bayesian inference).

Ecology

The fact that we found fecampiid cocoons on pumice, wood debris, and metal debris (Fig. 2), as well as on plastic debris and dead *Hyalonema* sponge stalks (NH, pers. obs.) suggests that the worms are not very particular about the kind of substrate for settling and subsequently attaching their cocoons, as long as it concerns a hard substrate. This notion is corroborated by the fact that in the South China Sea cocoons of *F. abyssicola* appeared to also be attached to plastic debris (Song *et al.*, 2021).

Although we have been unable to determine the actual host species of our specimens of *F. cf. abyssicola*, it has been suggested that isopods and amphipods most likely form the host or hosts of *F. abyssicola* (Christensen, 1981b), which agrees with the fact that our trawling samples in the northwestern Pacific brought deep-water amphipods and shrimps to the surface.

The collection depths (1861–4438 m) of our *F. cf. abyssicola* specimens were consistent with previous records of widely distributed *F. abyssicola* (Fig. 1), which has been found at depths ranging from about 600 to 5000 m (Christensen, 1981a, b; Sluys and Ginkel, 1989).

Geographic distribution

Fecampia abyssicola was already known to have a wide geographic distribution, with sampling localities in the Atlantic Ocean, Indian Ocean, and eastern Pacific Ocean (Sluys and van Ginkel, 1989, fig. 3). Recently, the species was also reported from the South China Sea, where it was found attached to plastic debris (Song *et al.*, 2021). Our records of *F. cf. abyssicola* from the northwestern Pacific Ocean are comparatively close to the records of Song *et al.* (2021; Fig. 1), although we excluded the *Fecampia* sequences from our analyses, as they do not overlap with our sequences or with those of other fecampiids. Although lack of molecular data from topotype specimens or other specimens of *F. abyssicola* precludes definitive species identification of our *F. cf. abyssicola* specimens, the present study provides, at least, what seem to be the first records of abyssal *Fecampia* in Japanese waters.

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Data Accessibility

The datasets generated and/or analyzed during the current study are available in the GenBank repository (https://www.ncbi.nlm.nih.gov/nuccore/OR677932).

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