

Supplemental re-description of a deep-sea ascidian, *Fimbrora calsubia* (Ascidiacea, Enterogona), with an inference of its phylogenetic position

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Abstract

Fimbrora Monniot & Monniot, 1991, a macrophagous ascidian genus within the family Ascidiidae Adams & Adams, 1858, is currently monotypic, represented by *F. calsubia* Monniot & Monniot, 1991, a species previously recorded from the bottom of the South Pacific at depths of 1000–1860 m. The taxonomic status of *Fimbrora* has remained ambiguous because characteristics in its branchial papillae and neural-gland opening are incompletely known in previous studies, while these traits are essential for distinguishing other ascidiid genera. So far, no nucleotide sequence representing *F. calsubia* is available. In this study, we collected a single specimen of *F. calsubia* at a depth of 2027 m, about 400 km off the Pacific coast of Honshu, Japan. This is the deepest record, as well as the first report from the North Pacific, for the species. Our examination indicates that *Fimbrora* is morphologically similar to another ascidiid genus, *Psammascidia* Monniot, 1962, by having only secondary branchial papillae in the pharynx. Our phylogenetic analysis, based on the 18S ribosomal RNA and cytochrome *c* oxidase subunit I genes, along with those of 27 ascidian species available in public databases, showed that *F. calsubia* was more closely related to *Ascidia zara* Oka, 1935, *Phallusia fumigata* (Grube, 1864) and *Phallusia mammilata* (Cuvier, 1815) than to *Ascidia ceratodes* (Huntsman, 1912), *Ascidella aspersa* (Müller, 1776) and *Ascidella scabra* (Müller, 1776). Our results also indicated that acquisitions of macrophagous feeding by deep-sea members happened independently at least three times in the evolutionary history of the entire Ascidiacea.

Key Words

bathyal zone, biogeography, Chordata, phylogeny, taxonomy, Tunicata, Urochordata

Introduction

The ascidiid genus *Fimbrora* Monniot & Monniot, 1991a is currently monotypic, consisting of the deep-sea ascidian *Fimbrora calsubia* Monniot & Monniot, 1991a. The taxonomic identity of *Fimbrora* is not fully established because states of some characters used for distinguishing other ascidiid genera are not known for this taxon. Apart from *Fimbrora*, the family Ascidiidae Adams & Adams,

1858 also contains four genera: *Ascidia* Linnaeus, 1767; *Ascidella* Roule, 1884; *Phallusia* Savigny, 1816; and *Psammascidia* Monniot, 1962. *Fimbrora* is supposed to be distinguished from the other ascidiid genera by having a combination of three characteristics: *i*) the large, cup-shaped oral siphon with thin, uniformly long, and soft lobes, *ii*) two large blood vessels running on the oral-siphon wall and *iii*) macrophagous feeding behaviour (cf. Monniot and Monniot (1991a)). The remaining four

genera are distinguished from each other, based on: *i*) whether primary and/or secondary branchial papillae in the pharynx are present and *ii*) whether accessory openings of the neural gland are present (e.g. Kott (1985); Monniot et al. (1991); Rocha et al. (2012)). However, while the branchial papillae have been reported to be present in *Fimbrora* (Monniot & Monniot, 1991a), whether they are primary and/or secondary was not mentioned in any of the previous literature (Monniot and Monniot 1991a; Monniot 1993; Monniot and López-Legentil 2017); also, the nature of the neural-gland opening (or, whether accessory openings are present) has not been stated in any of these works.

While ascidians are generally suspension feeders that filter food particles, such as phytoplankton, from the surrounding seawater (Millar 1971), 40 species have hitherto been identified as macrophagous, based on their large oral siphons and unciliated pharynges; direct confirmation of this feeding behaviour was made in 10 species by the presence of small crustaceans in their gut contents (Table 1). These macrophagous ascidians exclusively inhabit deep waters below 200 m with one exception, *Oligotrema psammites* Bourne, 1903, which is also distributed up to 90 m (Table 1). In addition to *Fimbrora*, another three ascidian taxa—the family Octacnemidae, Herdman 1888 (with 26 species in 10 genera), as well as the two molgulid genera *Asajirus* Kott, 1989 (with eight species) and *Oligotrema* Bourne, 1903 (with five species)—are known to consist of macrophagous members (Table 1). Previously, certain morphological data suggested that macrophagous feeding amongst ascidians evolved convergently, probably due to difficulty in filter-feeding in the deep sea (Millar 1959). This view was confirmed by the phylogenetic study of Tatián et al. (2011), including two macrophagous taxa, the octacnemid *Megalodicopia* Oka, 1918 and the molgulid *Oligotrema*, but *F. calsubia* has not been represented with any molecular sequence data.

The taxonomy of macrophagous molgulids has experienced twists and turns. Historically, *Asajirus* and *Oligotrema* were once considered by Kott (1989) to comprise the now-abandoned family Hexacrobylidae Seeliger, 1906, for which the monofamilial order Aspiraculata and the mono-order class Sorberacea had been established by Seeliger (1906) and Monniot et al. (1975), respectively. These suprafamilial higher taxa were rejected by Kott (1989), who also noted morphological similarities between Hexacrobylidae and Molgulidae Lacaze-Duthiers, 1877. At another time, Hexacrobylidae was regarded by Monniot and Monniot (1990) to consist of the four genera *Hexacrobylus* Sluiter, 1905a, *Gasterascidia* Monniot & Monniot, 1968, *Sorbera* Monniot & Monniot, 1974 and *Hexadactylus* Monniot & Monniot, 1990, the first three of which were synonymised with *Oligotrema* by Kott (1989) and the last was synonymised with *Asajirus* by Kott (1992). Later, Hexacrobylidae was demonstrated to be a junior synonym of Molgulidae by a molecular phylogenetic analysis supporting the inclusion of *O. lyra* (Monniot & Monniot, 1973) in the latter family (Tatián

et al. 2011). Until then, Hexacrobylidae/Aspiraculata/Sorberacea had been occasionally considered valid in certain publications (e.g. Monniot (2001)).

So far, *F. calsubia* has been known from the South Pacific bathyal zone in three publications, based on a total of 13 specimens: three specimens at a depth of 1865 m in New Caledonian waters (Monniot and Monniot 1991a), two specimens at about 1000 m depth in Indonesia (Monniot 1993) and eight specimens at 1000–1200 m depth in Papua New Guinea (Monniot and López-Legentil 2017). Meanwhile, during a biodiversity survey in an off-shore submarine nature conservation area around Nishi-Shichito Ridge in the western North Pacific, a 14th individual of *F. calsubia* was obtained. Here, we provide a morphological re-description and an inference of its molecular phylogenetic position within the class Ascidiacea.

Materials and methods

A single specimen of *F. calsubia* was collected near the south of Hiei Seamount, about 400 km off the Pacific coast of Honshu, Japan (Fig. 1), with a manipulator of the human-occupied vehicle *Shinkai 6500* (Dive No. 1651) during the cruise YK22-17C of the R/V *Yokosuka* (Suppl. material 1, 2). The live animal was photographed with an OM-D E-M1X digital still camera (Olympus, Tokyo, Japan) attached to an M.Zuiko Digital ED 30 mm F3.5 Macro lens (Olympus). Two of the thread-like lobes of the specimen were dissected from the oral siphon; one was preserved in 99% ethanol for DNA extraction, the other in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) for future analysis; the remaining body was fixed in 10% formalin seawater for morphological observation. For detailed examination, the pharynx was stained with haematoxylin. The voucher specimens have been deposited in the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, with the catalogue number JAMSTEC No. 111618 for the formalin-fixed specimen, JAMSTEC No. 111619 for the lobe in 99% ethanol and JAMSTEC No. 111620 for the lobe in RNAlater.

Total DNA was extracted using a DNeasy Tissue Kit (Qiagen, Hilden, Germany). For amplification, KOD One PCR Master Mix (TOYOBO, Osaka, Japan) was used. Partial sequences of the 18S rRNA (18S) gene and the mitochondrial cytochrome *c* oxidase subunit I (COI) gene were PCR amplified from the total DNA; the primer pairs 1F/9R (Giribet et al. 1996) and dinF/Nux1R (Brunetti et al. 2017) were used for 18S and COI, respectively. PCRs were performed under the following conditions. For 18S: 94 °C for 2 min; 35 cycles of 94 °C for 45 sec, 52 °C for 50 sec and 72 °C for 90 sec; then 72 °C for 5 min. For COI: 94 °C for 2 min; 35 cycles of 94 °C for 40 sec, 50 °C for 60 sec and 72 °C for 60 sec; then 72 °C for 7 min. Purification of PCR products was conducted by enzymatic reaction with ExoSAP-IT (Applied Biosystem, Waltham, MA, USA). The purified products were

Table 1. List of macrophagous species in Ascidiacea with information about family, species, depth, evidence for macrophagous feeding and references.

Family	Species	Depth (m)	Evidence for macrophagous feeding*	References
Asciidiidae	<i>Fimbrora calsubia</i> Monniot & Monniot, 1991	1000–2027	m/c	Monniot and Monniot (1991a), Monniot (1993), Monniot and López-Legentil (2017), present study
Octacnemidae	<i>Benthascidia michaelsoni</i> Ritter, 1907	399	m	Ritter (1907), Monniot (1998)
	<i>Cibacapsa gulosa</i> Monniot & Monniot, 1983	567	m/c	Monniot and Monniot (1983)
	<i>Cryptia planum</i> Monniot & Monniot, 1985	4930	m/c	Monniot and Monniot (1985a)
	<i>Dicopia antirrhinum</i> Monniot, 1972	600–4300	m/c	Monniot (1972), Monniot and Monniot (1974, 1985a), Sanamyan (2014)
	<i>Dicopia fimbriata</i> Sluiter, 1905	1210	m	Sluiter (1905a), Monniot and Monniot (1991b), Monniot and López-Legentil (2017), Sanamyan and Sanamyan (1999)
	<i>Dicopia japonica</i> Oka, 1913	4526–4609	m	Oka (1913), Millar (1988)
	<i>Kaikoja globosa</i> Monniot, 1998	1978	m	Monniot (1998)
	<i>Kaikoja multitentaculata</i> (Vinogradova, 1975)	4485–4520	m	Vinogradova (1975), Sanamyan and Sanamyan (2002)
	<i>Megalodicopia hians</i> Oka, 1918	200–5325	m/c	Oka (1918), Tokioka (1953), Kott (1969), Nishikawa (1991), Sanamyan (1998), Okuyama et al. (2002), Havenhand et al. (2006)
	<i>Megalodicopia rineharti</i> (Monniot & Monniot, 1989)	695–3970	m	Monniot and Monniot (1989), Sanamyan and Sanamyan (2002)
	<i>Myopegma melanesium</i> Monniot & Monniot, 2003	445–472	m/c	Monniot and Monniot (2003)
	<i>Myopegma midatlantica</i> Monniot, 2011	2087	m	Monniot (2011)
	<i>Octacnemus alatus</i> Monniot & Monniot, 1985	3344	m	Monniot and Monniot (1985b)
	<i>Octacnemus bythius</i> Moseley, 1876	1957–4087	m/c	Moseley (1876), Ritter (1906), Ihle (1935), Millar (1959), Monniot and López-Legentil (2017)
	<i>Octacnemus ingolfi</i> Madsen, 1947	640–4655	m	Madsen (1947), Monniot and Monniot (1973, 1976, 1985a, 1985b, 1985c, 1991b, 2003), Sanamyan (2014)
	<i>Octacnemus kottae</i> Sanamyan & Sanamyan, 2002	3700–3910	m	Sanamyan and Sanamyan (2002)
	<i>Octacnemus vinogradovae</i> Sanamyan & Sanamyan, 1999	5400	m	Sanamyan and Sanamyan (1999)
	<i>Octacnemus zarcoi</i> Monniot & Monniot, 1984	4260–4270	m/c	Monniot and Monniot (1984a), Sanamyan (2014)
	<i>Polyoctacnemus patagoniensis</i> (Metcalf, 1893)	1920	m	Metcalf (1893), Ihle (1935)
	<i>Situla cuculli</i> Monniot & Monniot, 1991	2040	m	Monniot and Monniot (1991b)
	<i>Situla galeata</i> Monniot & Monniot, 1991	1395–4891	m	Monniot and Monniot (1991b), Sanamyan and Sanamyan (1998)
	<i>Situla lanosa</i> Monniot & Monniot, 1973	1800–4990	m	Monniot and Monniot (1973, 1974, 1985a), Sanamyan (2014)
	<i>Situla macdonaldi</i> Monniot & Monniot, 1977	790	m	Monniot and Monniot (1977)
	<i>Situla pelliculosa</i> Vinogradova, 1969	5035–8400	m	Vinogradova (1969)
	<i>Situla rebaini</i> Vinogradova, 1975	3700–5651	m	Vinogradova (1975), Sanamyan and Sanamyan (2002)
	<i>Situla rineharti</i> Monniot & Monniot, 1989	695–3680	m	Monniot and Monniot (1989, 1991b)
Molgulidae	<i>Asajirus arcticus</i> (Hartmeyer, 1923)	905–1283	m	Hartmeyer (1923)
	<i>Asajirus dichotomus</i> (Monniot & Monniot, 1984)	3550	m	Monniot and Monniot (1984a, 1985a), Kott (1989)
	<i>Asajirus eunuchus</i> (Monniot & Monniot, 1976)	2000–5000	m	Monniot and Monniot (1976)
	<i>Asajirus gulosus</i> (Monniot & Monniot, 1984)	1800–2500	m	Monniot and Monniot (1984a), Kott (1989)
	<i>Asajirus hemisphericus</i> (Monniot & Monniot, 1990)	3680–3740	m	Monniot and Monniot (1990)
	<i>Asajirus indicus</i> (Oka, 1913)	800–5000	m/c	Oka (1913), Hartmeyer (1923), Van Name (1945), Millar (1959, 1970), Kott (1957, 1969, 1989), Monniot (1969, 1971), Monniot and Monniot (1968, 1970, 1973, 1974, 1976, 1982, 1984a, 1984b, 1985a, 1985b, 1990), Sanamyan and Sanamyan (2006), Maggioni et al. (2018, 2022)
	<i>Asajirus ledanoisi</i> (Monniot & Monniot, 1990)	720–4829	m	Monniot and Monniot 1973; 1974; 1977; 1985b; 1990; Sanamyan 2014
	<i>Asajirus ovirarus</i> (Monniot & Monniot, 1990)	820–1900	m	Monniot and Monniot 1990; 2003
	<i>Oligotrema lyra</i> (Monniot & Monniot, 1973)	3360–4680	m/c	Monniot C. and Monniot F. (1973, 1974, 1984b, 1985a, 1990), Kott (1989), Sanamyan and Sanamyan (1999), Sanamyan (2014)
	<i>Oligotrema psammotodes</i> (Sluiter, 1905)	1158	m	Millar (1969), Sluiter (1905a, 1905b), Monniot and Monniot (1990)
	<i>Oligotrema psammites</i> Bourne, 1903	90–4000	m	Bourne (1903), Monniot and Monniot (1990), Monniot (2022), Kott (1992, 2009)
	<i>Oligotrema sandersi</i> (Monniot & Monniot, 1968)	2200–5020	m	Monniot and Monniot (1968, 1970, 1974, 1985a, 1990), Millar (1970), Kott (1989), Sanamyan (2014)
	<i>Oligotrema unigonas</i> (Monniot Monniot, 1974)	2300–5500	m	Monniot and Monniot (1974, 1984b, 1985a, 1985b, 1990), Kott (1989), Sanamyan (2014)

*‘m’ indicates that the species was judged to be macrophagous, based on morphological characteristics; ‘m/c’ indicates that gut contents were also observed in addition to morphological features.

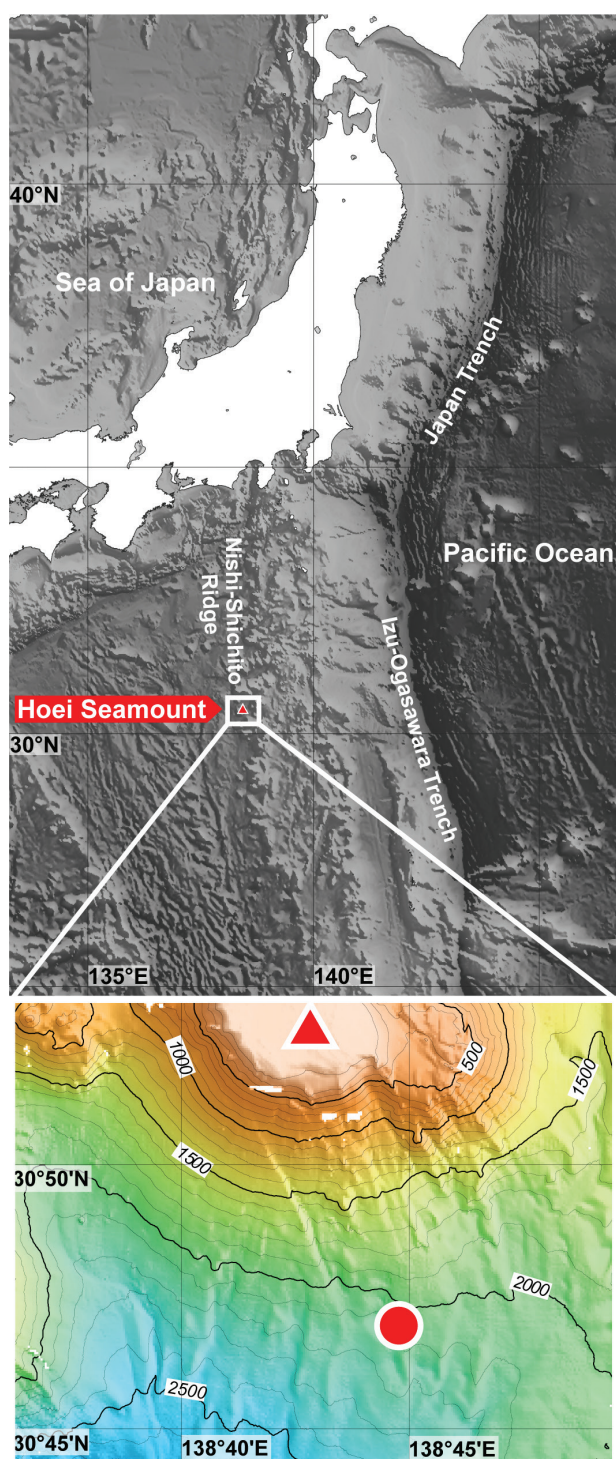


Figure 1. Maps showing the sampling site (red circle), south of Hiei Seamount (of which the top is indicated with a red triangle). The images were generated by using GMT 6 (Wessel et al. 2019), based on grid data provided by the General Bathymetric Chart of the Oceans.

sequenced with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and an ABI 3100 Avant Genetic Analyzer (Applied Biosystem), using the same primer pairs for amplification; for 18S, the internal primers 3F and 5R (Giribet et al. 1996), as well as a2.0 and bi (Whiting et al. 1997), were also used.

For phylogenetic analysis, 18S and COI sequences of 27 ascidian species and those of the lancelet *Branchiostoma floridae* Hubbs, 1922 were downloaded from GenBank (Table 2). The dataset of 18S was aligned using MAFFT ver. 7.310 with *E-INS-I* strategy (Katoh and Standley 2013); the aligned 18S dataset was trimmed by using trimAl ver. 1.4. rev15 with gappyout command (Capella-Gutiérrez et al. 2009). An alignment of COI was obtained by using MEGA X (Kumar et al. 2018) following Hasegawa and Kajihara (2019). Then, the 18S and COI sequences were concatenated on MEGA X (Kumar et al. 2018).

Table 2. The GenBank accession numbers of 18S and COI sequences of *Fimbrora calsubia* Monniot & Monniot, 1991a, as well as 27 ascidian species and the lancelet *Branchiostoma floridae* Hubbs, 1922, used for phylogenetic analysis in this study.

Species	18S	COI
<i>Ascidia ceratodes</i>	L12378	MW872268
<i>Ascidia zara</i>	LC547325	KY235397
<i>Ascidia aspersa</i>	LC547321	KF886702
<i>Ascidia scabra</i>	AB811928	MN064599
<i>Botrylloides violaceus</i>	LC432326	LC432331
<i>Chelyosoma siboga</i>	AF165821	AB104867
<i>Ciona robusta</i>	AB013017	MF479417
<i>Ciona savignyi</i>	LC547329	MK512499
<i>Clavelina lepadiformis</i>	JN573225	AY603104
<i>Clavelina meridionalis</i>	FM244840	AM706470
<i>Corella eumyota</i>	FM244846	KU299765
<i>Ecteinascidia herdmanni</i>	FM244847	AY600968
<i>Ecteinascidia turbinata</i>	FM244848	MT873564
<i>Fimbrora calsubia</i>	LC777587	LC777585
<i>Halocynthia roretzi</i>	AB013016	HM151268
<i>Herdmania momus</i>	AF165827	KM411616
<i>Megalodicopia hians</i>	AB075543	AB104866
<i>Molgula manhattensis</i>	L12426	MT873565
<i>Oligotrema lyra</i>	JN565043	–
<i>Perophora japonica</i>	AB499607	MN064600
<i>Perophora viridis</i>	FM244849	OM912740
<i>Phallusia fumigata</i>	FM244844	KF309548
<i>Phallusia mammillata</i>	AF236803	MN064634
<i>Pycnoclavella diminuta</i>	KJ632948	KC017435
<i>Pyura mirabilis</i>	LC432327	LC432332
<i>Styela clava</i>	LC432329	LC432334
<i>Sympyga reptans</i>	AF165826	LS992553
<i>Syncarpa composita</i>	LC432325	LC432330
<i>Branchiostoma floridae</i>	M97571	AB478593

For constructing phylogenetic trees, Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed; MrBayes ver. 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) for BI and the ultrafast bootstrap method (Hoang et al. 2018) implemented in IQtree (Nguyen et al. 2015) for ML. PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) was used for selecting the best-fit substitution models, which suggested GTR + I + G for 18S and COI first codon position and GTR + G for COI second and third codon positions. For BI, Markov chains were

started from a random tree and run for 10^7 generations; trees were picked up every 100 generations from the chain. Burn-in was set at 25%. The “sumt” command was used for calculating a consensus of trees; the posterior probability (PP) for each node was collected to assess the certainty of the inference. Run convergence was assumed, based on the following values of variables: average standard deviation of split frequencies = 0.002002; average estimated sample size of all parameters > 200; and potential scale reduction factor for all parameters ≤ 1.008 . For ML analysis, branch support was calculated with 1000 ultrafast bootstraps (Minh et al. 2013).

Results

Taxonomy and morphology

Order Enterogona

Suborder Phlebobranchia

Family Ascidiidae Adams & Adams, 1858

Fimbrora calsubia Monniot & Monniot, 1991a

Figs 2–4

Fimbrora calsubia Monniot & Monniot, 1991a, p. 384, figs 1–6; Monniot (1993), p. 356; Monniot and López-Legentil (2017), p. 531, figs 1, 2.

New Japanese name. *Yorifusa-boya*, from *yorifusa*, an ornament for kimonos and Japanese accessories and *boya*, a phonological variant of *hoya*, meaning a sea squirt.

Material examined. One individual, JAMSTEC No. 111618, collected by N. Hookabe on 26 September 2022, about 400 km off the Pacific coast of middle Honshu, Japan, 30°47.05'N, 138°44.72'E, at a depth of 2027 m (Fig. 1).

Description. Individual ca. 20 cm in length including oral siphon (Fig. 2A, B). Tunic opaque and gelatinous; blood vessels running on surface of tunic (Fig. 2B); fine warts, each about 0.5 mm in diameter, scattered evenly over entire tunic. Body attached to substrate with its posterior end (Fig. 2A, B). Oral siphon enlarged, ca. 10 cm in diameter; single annular muscle strand running on outer edge of oral siphon; thread-like lobes, 52 in number, tightly arranged to each other on oral-siphon edge; single groove radially arranged on edge of oral siphon between base of each lobe; muscle strand associated to each lobe, running on inner wall of oral-siphon edge from lobe base for ca. 1 cm; beneath inner surface of oral siphon, neural cords radially running from neural ganglion (Fig. 2C). Oral aperture situated 2.5 cm anterior to neural ganglion. Atrial siphon 1.5 cm in diameter; 37 blood vessels longitudinally running on surface of atrial siphon (Fig. 2D).

Body wall attached to tunic on oral siphon, heart and renal vesicles; irregular cavity existing between tunic and body wall; inner surface of tunic covered with epithelial

tissue. Neural ganglion situated between oral siphon and atrial siphon. On base of oral siphon, 105 oral tentacles present, each being ca. 8 mm in length. Peripharyngeal band made of single lamina running in a short distance posterior to oral tentacles, forming V-shape posterior to neural gland aperture (Fig. 3A); latter being single in number, almost straight in shape (Fig. 3A) and opening at dorsal tubercle. Pharynx connected by mesenteries to peripharyngeal epithelium; mesenteries 0.5–3.0 mm in diameter (Fig. 3B). Smooth dorsal lamina running along mid-line on ventral side of pharynx (Fig. 3A, B). Longitudinal and transverse vessels running on inner surface of pharynx (Fig. 3C); 6–10 stigmata without lateral cilia per mesh (Fig. 3C). Secondary branchial papillae present on intersections of longitudinal and transverse vessels (Fig. 3C).

Digestive tract positioned on left side of body (Fig. 4A). Oesophagus opening to left side of dorso-posterior part of pharynx. Stomach about 1.5 cm in length, having 10 folds, surrounded with renal vesicles (Fig. 4A); multiple crustaceans (probably copepods) found in stomach lumen (Fig. 4B). Intestinal loop S-shaped, having primary loop and secondary loop; intestine ca. 7 cm in length, ca. 5 mm in diameter (Fig. 4A). Anus smoothly edged, opening close to atrial siphon (Fig. 4A).

Gonad situated proximally on intestinal loop (Fig. 4C). Ovaries surrounded with male testis (Fig. 4C, D). Oviduct and spermiduct running along secondary loop, opening close to anus (Fig. 4A). Eggs contained in ovaries and oviduct, up to 0.2 mm in diameter (Fig. 4D).

Habitat. The animal attached itself to a dead sponge in an area with accumulated sand and mud at a depth of 2027 m, where the water temperature was 1.93 °C (Fig. 2A; Suppl. material 1). It opens the oral aperture in the direction facing the water current (Suppl. material 2). An euplectellid sponge was also found attached to the same substrate. Macrobenthos found around this area included other sponges, octocorals, sandy creeplets, sea anemones and sea lilies.

Molecular phylogeny

The clade consisting of four genera in the family Ascidiidae, i.e. *Ascidia*, *Ascidiella*, *Fimbrora* and *Phallusia*, received high support values (97% bootstrap; 1.00 posterior probability) (Fig. 5). In this clade, *F. calsubia* was most closely related to *Ascidia zara* Oka, 1935, but with less-supported values (53% bootstrap; 0.68 posterior probability). The clade of *Ascidia* + *Fimbrora* + *Phallusia* was sister to the genus *Ascidiella*. The genus *Ascidia* was recovered as a non-monophyletic group.

The three macrophagous ascidians included in this analysis—*F. calsubia*, *Megalodicopia hians* Oka, 1918 and *Oligotrema lyra*—were each positioned differently in the phylogenetic tree. As in previous analyses (Kurabayashi et al. 2003; Tatián et al. 2011), *M. hians* was sister to *Corella eumyota* Traustedt, 1882; *O. lyra* was sister to *Molgula manhattensis* (De Kay, 1843).

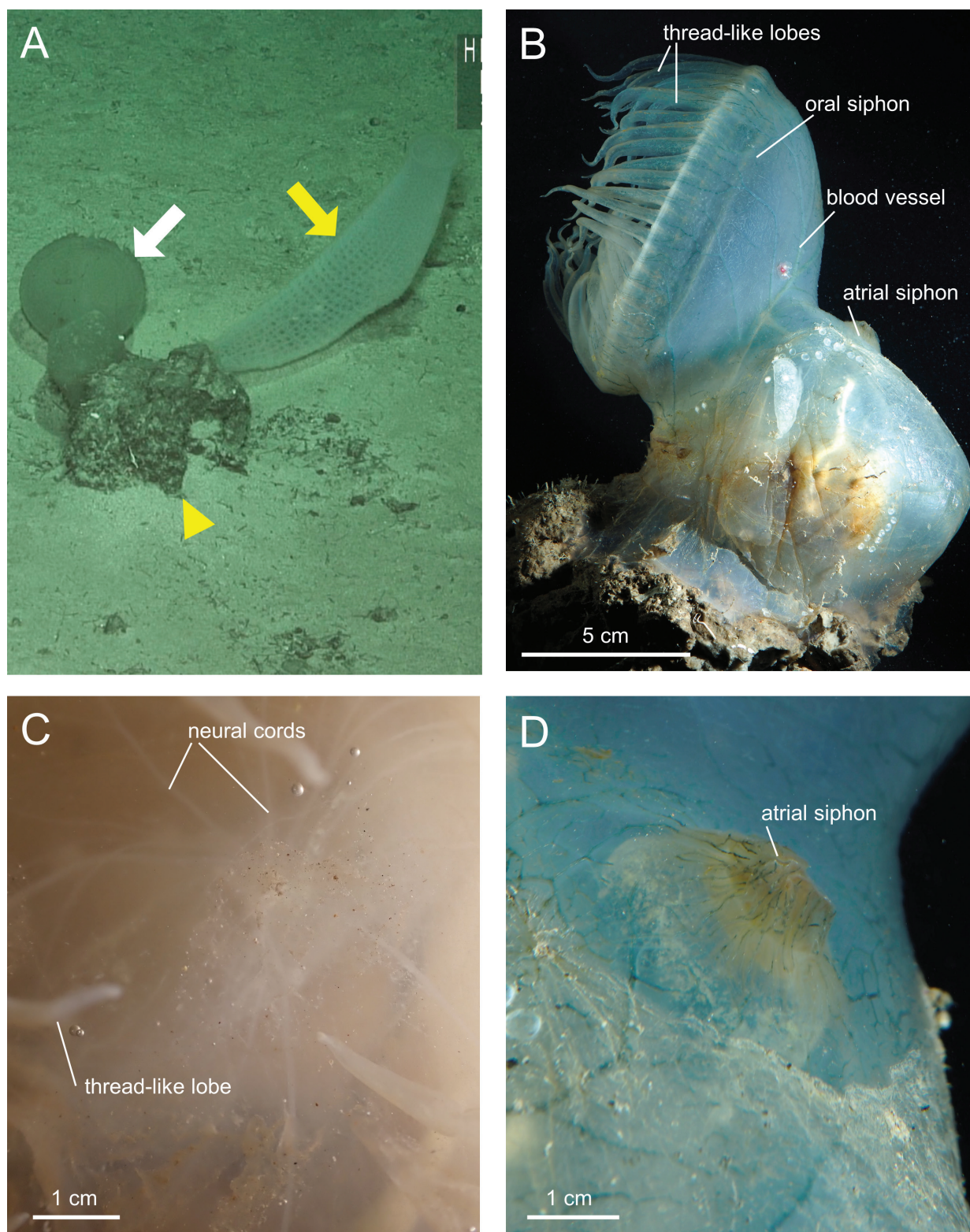


Figure 2. *Fimbrora calsubia* Monniot & Monniot, 1991a, photographs showing external appearance of JAMSTEC No. 111618. **A.** The individual *in situ* (white arrow), attaching to a dead sponge (yellow arrow) along with a euplectellid glass sponge (yellow arrowhead); **B.** Left view in life; **C.** Inner surface of the oral siphon in fixed state; **D.** Enlarged view of atrial siphon in life.

Discussion

Previous studies posited that *Fimbrora* would belong to Ascidiidae (Monniot and Monniot 1991a; Monniot and López-Legentil 2017) and our phylogenetic analysis sup-

ported this view. The morphological characteristics that suggested *Fimbrora*'s familial affiliation were the longitudinal vessels having papillae and straight stigmata in the pharynx (Monniot and Monniot 1991a; Monniot

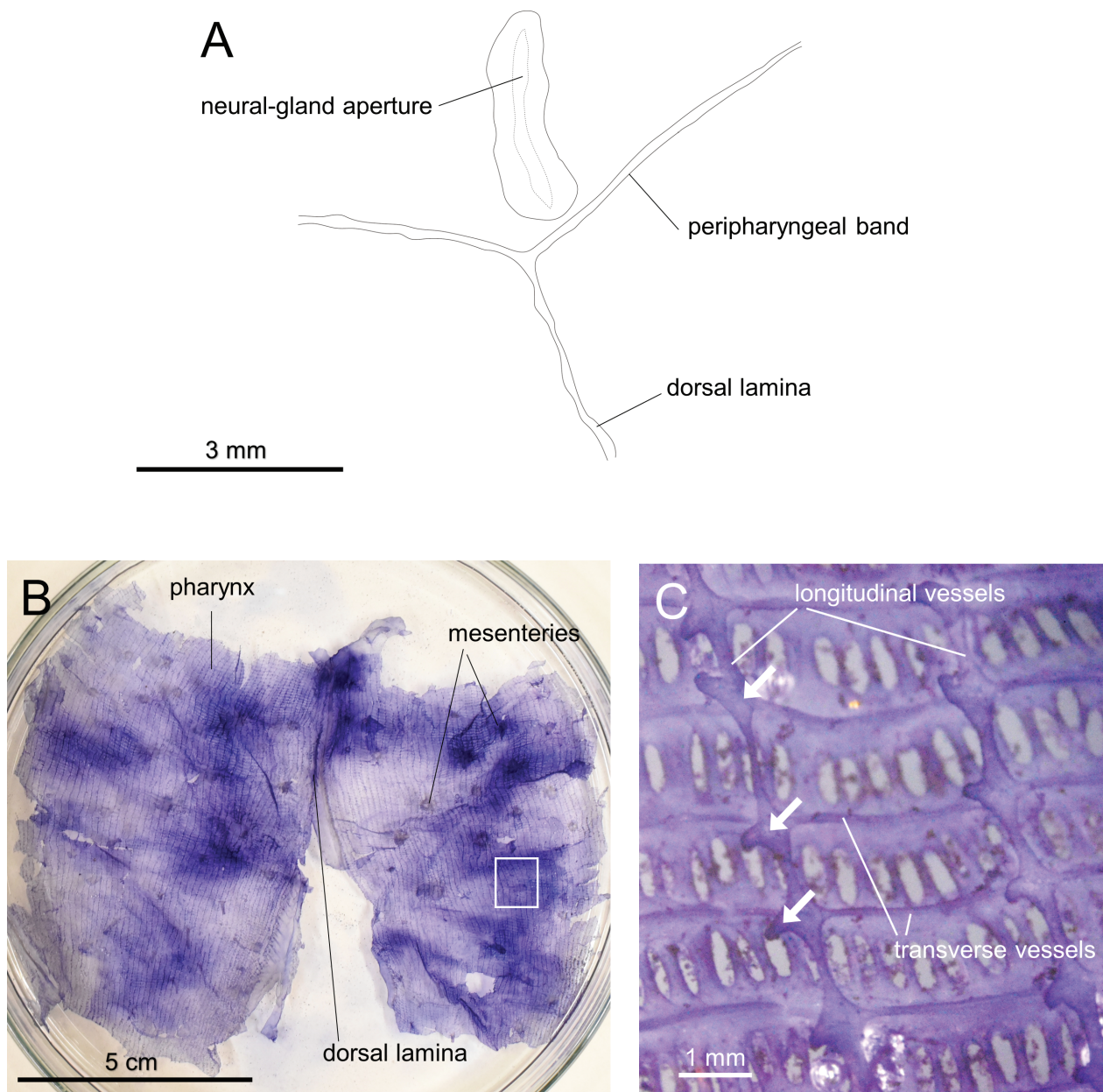


Figure 3. *Fimbrora calsubia* Monniot & Monniot, 1991a (JAMSTEC No. 111618). **A.** Drawing of dissected specimen, showing the shape of neural-gland aperture, peripharyngeal band and dorsal lamina; **B.** Photograph of dissected pharynx cut open from ventral side; **C.** Magnification of the rectangle on B, showing the arrangement of longitudinal vessels, transverse vessels, stigmata and secondary branchial papillae (indicated with arrows).

and López-Legentil 2017), while Monniot and Monniot (1991a) noted the superficial resemblance of the genus with the family Octacnemidae in having an enlarged oral siphon. The phylogenetic position of *Fimbrora* within Ascidiidae was unresolved in our tree (Fig. 5). The more precise phylogenetic position of *Fimbrora* in the family would require the inclusion of additional ascidiid taxa in molecular analyses. One such to-be-included taxa is *Psammascidia*, which shares two characteristics with *Fimbrora*—having secondary branchial papillae on the longitudinal vessels and lacking primary and intermediate branchial papillae (Monniot and Monniot 1973), features that are not found in other ascidiid genera (cf.

Kott (1985); Brunetti and Mastrototaro (2017)). Future molecular analyses may reveal the phylogenetic relationship amongst ascidiid species including *Fimbrora*.

Monniot and Monniot (1991a) suggested that *F. calsubia* has a partly carnivorous diet, based on the finding of copepods in its gut contents mixed with unidentified particles, as well as the shape of the oral siphon. The presence of small crustaceans, likely copepods, in the stomach of our specimen supports this assertion. The reports of *F. calsubia* from Indonesia (Monniot 1993) and Papua New Guinea (Monniot and López-Legentil 2017), however, did not provide any information on gut contents in their specimens. In addition to this, the observed

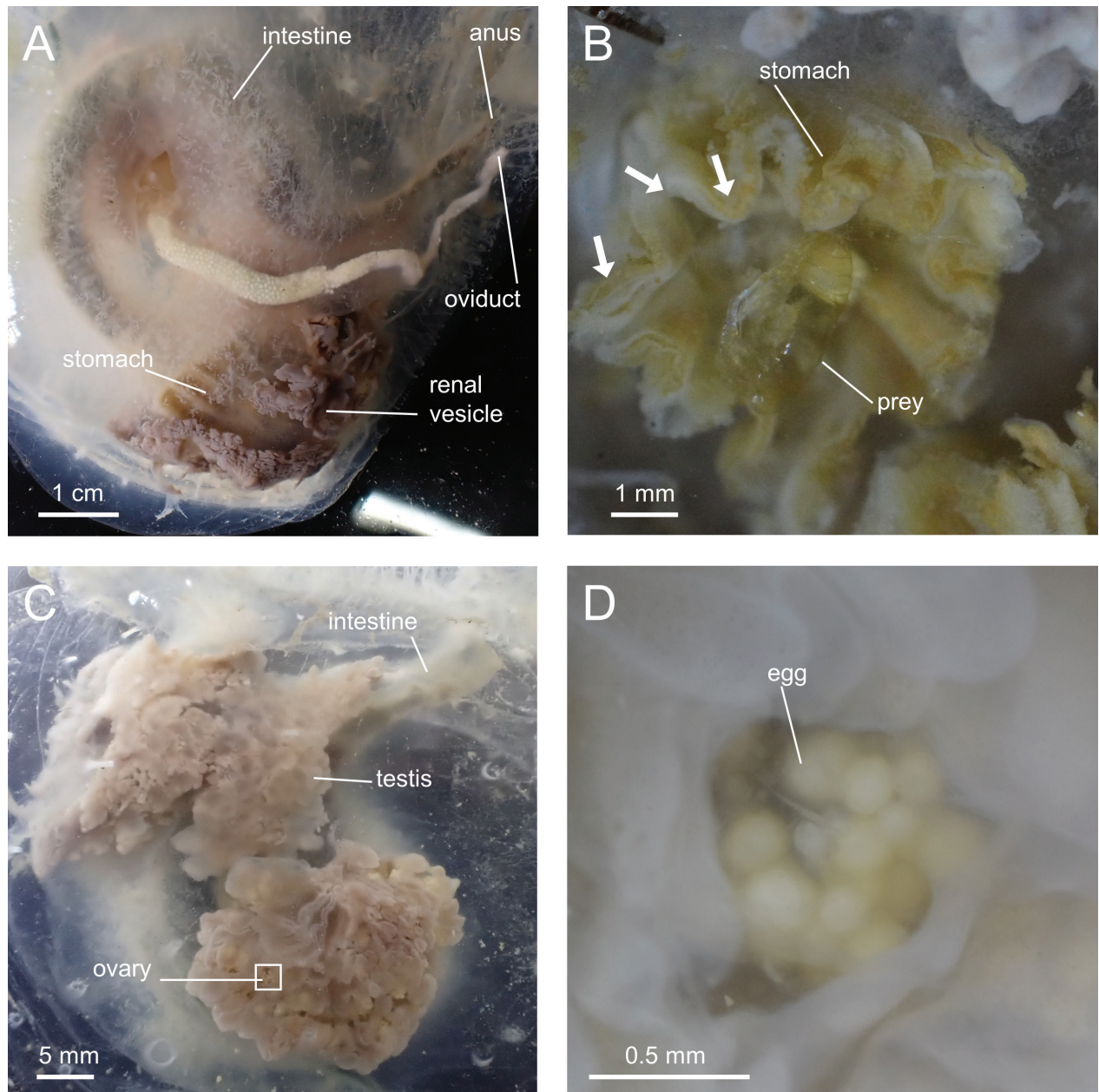


Figure 4. *Fimbrora calsubia* Monniot & Monniot, 1991a (JAMSTEC No. 111618), photographs of fixed specimen. **A.** Sinistaro-posterior portion of body, viewed from outside, showing alimentary canal and reproductive system; **B.** Cross section of stomach, showing the prey crustacean (probably a copepod); arrows indicating stomach folds; **C.** Gonads; **D.** Magnification of the rectangle on **C**, showing an ovary containing multiple eggs.

behaviour of *F. calsubia*, where the individual orientates its oral siphon towards the water flow (Suppl. material 2), is similar to the behaviour found in *M. hians* as described by Okuyama et al. (2002). This suggests that *F. calsubia* also utilises water currents for feeding.

While the convergent evolution of macrophagous feeding in *Megalodicopia* and *Oligotrema* has already been revealed by Tatián et al. (2011), our phylogenetic tree clearly shows that *Fimbrora* is also the case: this trait was acquired at least three times independently within the class Ascidiacea (Fig. 5).

The present study expanded the species' known distribution range for about 4000 km northwards, representing

the first record of the species from the North Pacific. Our material also represents the deepest record for the species with the known vertical distribution range being about 1000–2000 m (Monniot and Monniot 1991a; Monniot 1993; Monniot and López-Legentil 2017; present study).

Conclusions

We present the first report of *F. calsubia* from the North Pacific. Our molecular phylogenetic analysis suggested that macrophagous feeding was convergently acquired at

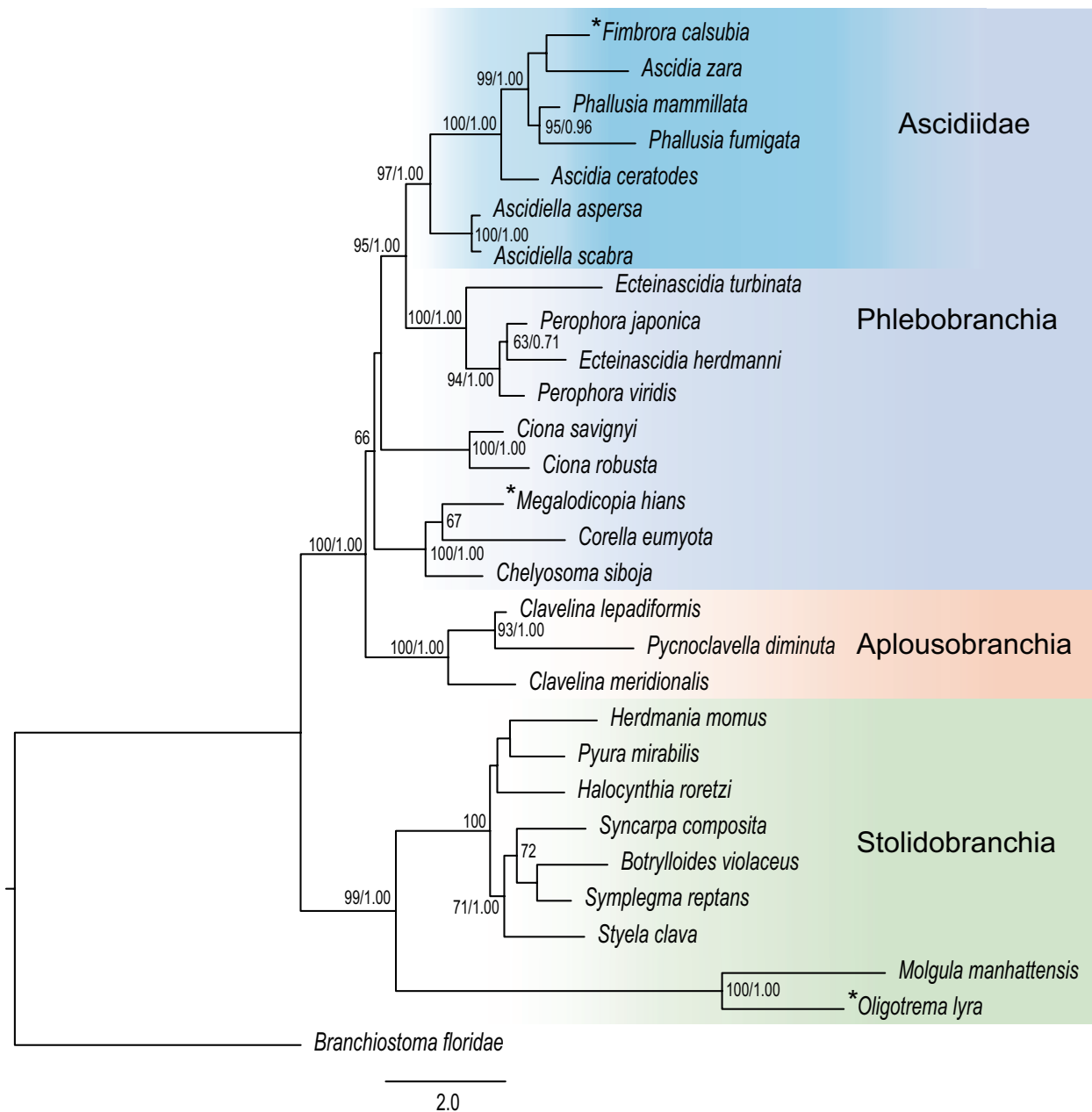


Figure 5. Phylogenetic relationship of 28 ascidian species; a Maximum-Likelihood tree, based on a concatenated dataset consisting of 18S rRNA (1676 bp) and COI (1136 bp) genes. Bootstrap values and posterior probabilities are indicated if they are higher than 60% and 0.70, respectively. Macrophagous species are indicated with an asterisk (*).

least three times independently in Ascidiacea. Our morphological observation indicated a similarity of *Fimbrora* to *Psammascidia* in having secondary papillae and lacking primary and intermediate branchial papillae.

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Supplementary material 1

Video 1. A close encounter with the deep-sea ascidian

Authors: Naohiro Hasegawa, Natsumi Hookabe, Yoshihiro Fujiwara, Naoto Jimi, Hiroshi Kajihara

Data type: mov

Explanation note: Video of the moment the specimen was discovered at a depth of 2027 m.

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Link: <https://doi.org/10.3897/zse.100.113132.suppl1>

Supplementary material 2

Video 2. Grabbing the ascidian with the manipulator of *Shinkai 6500*

Authors: Naohiro Hasegawa, Natsumi Hookabe, Yoshihiro Fujiwara, Naoto Jimi, Hiroshi Kajihara

Data type: mov

Explanation note: Video of the moment the specimen used in this study was collected by *Shinkai 6500*.

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Link: <https://doi.org/10.3897/zse.100.113132.suppl2>