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Lacydoniidae (Annelida) Off the Coast of North-eastern Japan: A Description of *Lacydonia japonica* sp. nov.

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Lacydonia japonica sp. nov. (Annelida, Lacydoniidae) is described based on material found in sediments collected off the Pacific coast of northern Honshu, Japan, at depths of 262 m and 407 m. The sediments were obtained by a remotely operated vehicle equipped with a suction sampler during a Tohoku Ecosystem-Associated Marine Sciences (TEAMS) project in 2019. *Lacydonia japonica* sp. nov. belongs to the eyeless group of lacydoniids and is discriminated from the morphologically most similar congener, *Lacydonia papillata* Uschakov, 1958 by its reddish pigments on both the dorsal and ventral parapodial cirri and four pigment spots on the pygidium. To assess the phylogenetic position of the new species among other lacydoniids for which sequence data are available in public databases, analyses were performed using the mitochondrial cytochrome *c* oxidase subunit I (*COI*) and 16S rRNA, as well as the nuclear 18S rRNA and 28S rRNA genes. We additionally obtained some lacydoniids by sledging off western Japan, but these were severely fragmented and broken during collection. Using the paucity of morphological data, they were left unidentified as *Lacydonia* sp. but included in the molecular analyses. Genetic distances between *Lacydonia eliasoni* Hartmann-Schröder, 1996, *Lacydonia japonica*, and *Lacydonia* sp. off western Japan were 10.4–17.1% uncorrected *p*-distance (11.3–18.6% K2P) in terms of 658-bp *COI* sequences.

Key words: Benthos, Deep sea, Marine invertebrates, Polychaetes, ROV.

BACKGROUND

The annelid family Lacydoniidae Bergström, 1914 is currently monotypic with the sole genus *Lacydonia* Marion & Bobretzky, 1875 harboring 13 valid species, which inhabit sandy and muddy unconsolidated substrata mixed with shell debris and gravel (Rizzo et al. 2016) and rock bottoms (Marion and Bobretzky 1875; Rouse and Pleijel 2001). Since the first representative *Lacydonia miranda* Marion & Bobretzky, 1875 was discovered from southern France, other members have been reported from the Pacific, Atlantic, Arctic, and Antarctic (Rizzo et al. 2016; Mazurkiewicz et al. 2017). Their bathymetric distribution ranges from the shallow subtidal to 5,700-m deep bottom (Uschakov 1958; Rouse and Pleijel 2001). Lacydoniids are small in body size, mostly less than 1 cm in length, with up to 50 chaetigers (Hartman 1967). They are rarely collected in a large number at one time (Rouse and Pleijel 2001) and usually described with one or a few specimens (Magalhães et al. 2012) partially due to its easily overlooked small body and difficulty in efficient extraction from sediment samples (Rouse and Pleijel 2001). Based on the nature of the eyes, the

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congeners can be divided into three groups: i) species with a couple of large eyes, *i.e.*, *L. miranda* Marion & Bobretzky, 1875, *L. oculata* (Hartman, 1967), and *L. jacki* Rizzo, Magalhães & Santos, 2016; ii) species with two or more small eyes, *i.e.*, *L. mikrops* Ehlers, 1913, *L. quadrioculata* Magalhães, Bailey-Brock & Rizzo, 2012, and *L. brasiliensis* Rizzo, Magalhães & Santos, 2016; and iii) species lacking eyes, *i.e.*, *L. papillata*, Uschakov, 1958, *L. cirrata* (Hartman & Fauchald, 1971), *L. laureci* Laubier, 1975, *L. gordia* Hartmann-Schröder, 1993, *L. hampsoni* Blake, 1994, *L. eliasoni* Hartmann-Schröder, 1996, and *L. anapaulae* Rizzo, Magalhães & Santos, 2016 (Table 1). From Japanese waters, *Lacydonia papillata* (Uschakov 1972) and an unidentified *Lacydonia* sp. (Rouse and Pleijel 2001) have been recorded. The former does not possess eyespots, while the latter has a pair of reddish eyes.

In the present study, we describe a new species of *Lacydonia* from the Pacific coast of northern Honshu, Japan. We also report another unidentified *Lacydonia* sp. off western Japan. Using DNA sequence data available in public databases as well as newly determined ones, we reconstruct the phylogeny of the genus based on partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) and 16S rRNA (16S), as well as the nuclear 18S rRNA (18S) and 28S rRNA (28S) genes. Furthermore, we compare genetic distances between selected congeners in terms of the *COI* sequences.

MATERIALS AND METHODS

Specimens were collected by the remotely operated vehicle (ROV) *Crambon* dive #35 during the KM19-05C cruise of the R/V *Kaimei* in Tohoku Ecosystem-Associated Marine Sciences (TEAMS) project on 16–17 July 2019 at depths of 262 m (39°06.79'N, 142°09.87'E) and 407 m (39°06.79'N, 142°09.87'E) off Ofunato, Iwate, Japan. Specimens were also collected by sledging during a cruise of the oceanographic research-training vessel *Toyohata-maru* on 24 July 2019 at a depth of 100 m (33°18.45'N, 133°35.82'E), Tosa Bay, Kochi, off western Japan.

Table 1. List of morphological characters (eyes and body pigmentation) of *Lacydonia* species, largely based on Magalhães et al. (2012)

Species	Eyes	Body pigmentation
Lacydonia miranda Marion & Bobretzky, 1875	a couple of large eyes	not reported
L. oculata (Hartman, 1967)	a couple of large eyes	not reported
L. jacki Rizzo, Magalhães & Santos, 2016	a couple of large eyes	pigmented spots on prostomium, dorsal side of parapodia, and dorsal cirri
L. mikrops Ehlers, 1913	two or more small eyes	two spots on pygidium
L. quadrioculata Magalhães, Bailey-Brock & Rizzo, 2012	two or more small eyes	dark brown pigments on prostomium, anterior margin of achaetous segment; dark brown pigment spots present in all chaetigers (rarely on achaetous segments), one spot per parapodium, near notopodial base; one pair of pigment spots present on pygidium; sometimes more than one spot present per parapodium
L. brasiliensis Rizzo, Magalhães & Santos, 2016	two or more small eyes	pale dorsal pigmentation with reddish-brown punctiform pigments mainly on anterior region of prostomium, parapodial lobes, pre-anal segments, and pygidium
L. papillata Uschakov, 1958	absent	four large papilliform dark spots behind chaetiger 1
L. cirrata (Hartman & Fauchald, 1971)	absent	not reported
L. laureci Laubier, 1975	absent	absent in Laubier (1975); red-brown pigmented spots on prostomium and first segment in Böggemann (2009)
L. gordia Hartmann-Schröder, 1993	absent	not reported
L. hampsoni Blake, 1994	absent	dark brown pigment spots on prostomium and first 3 chaetigers; pigment spots on borders of parapodia and cirri
L. eliasoni Hartmann-Schröder, 1996	absent	a single pair of pigments on achaetous segment
L. anapaulae Rizzo, Magalhães & Santos, 2016	absent	small red-brown pigmented spots; prostomium slightly pigmented
L. japonica sp. nov.	absent	body with yellowish to brownish mottled pattern; without dorsal dark spots around chaetiger 2; red pigment spots in both dorsal and ventral parapodial cirri, not base of parapodia; pygidium with four red to brown pigments

Sediment samples were agitated in seawater to extract animals. The suspended water was filtered by a 250-µm hand mesh net, and the residue was subsequently transferred into seawater. Animals were picked up under a dissecting microscope Olympus SZ40 (Olympus, Tokyo, Japan) and photographed with a digital still camera OM-D E-M1 Mark II (Olympus, Tokyo, Japan). For each specimen, width and length of the prostomium were measured under a light microscope (LM) Olympus BX51 (Olympus, Tokyo, Japan) after fixation. Specimens for molecular studies were preserved in 99% ethanol. For morphological observation by scanning electron microscopy (SEM), specimens were anaesthetized with a $MgCl_2$ solution isotonic to seawater, fixed in 10% seawater-buffered formalin or 70% ethanol, dehydrated in an ethanol series, critical-point dried in a Hitachi HCP-1 (Hitachi, Tokyo, Japan) mounted on an aluminum stub, coated with gold in a JEOL JFC-1100 ion spatter (JEOL, Tokyo, Japan), and then examined with a Hitachi S-3000N scanning electron microscope (Hitachi, Tokyo, Japan), at 15–30 kV accelerating voltage. Type and voucher specimens were deposited into the National Museum of Science and Technology, Tsukuba (NSMT), Japan.

Total DNA was extracted from a piece of the ethanol-fixed posterior tip of the body with a DNeasy Tissue Kit (Qiagen, German). PCR amplification was performed with the primer pairs LCO1490/HCO2198 (Folmer et al. 1994) for the partial sequence of *COI*, 16Sar-L/16br-H (Palumbi et al. 1991) for 16S, 1F/9R (Giribet et al. 1996) for 18S, and LSU5/rd5b (Littlewood 1994; Schwendinger and Giribet 2005) for 28S, with an Applied Systems 2720 thermal cycler. 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. Nucleotide sequencing was performed using internal primers in addition to the same primer pairs with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and an ABI 3100 Avant Genetic Analyzer (Applied Biosystems, US). Internal primers used in this study were as follows: 3F/5R (Giribet et al. 1996) and 18Sbi/S2.0 (Whiting et al. 1997) for 18S; and LSU3/D2F (Littlewood 1994), 28Z (Hillis and Dixon 1991), Sa (Whiting et al. 1997) for 28S. Newly obtained sequences in this study were deposited into the DNA Data Bank of Japan (DDBJ) under accession numbers listed in table 2.

Sequences listed in table 1 were combined by using MEGA7 (Kumar et al. 2016); *Eulalia viridis* (Linnaeus, 1767), *Paranaitis wahlbergi* (Malmgren, 1865), and *Phyllodoce longipes* Kinberg, 1866 were selected as outgroups. Alignment was performed with MAFFT ver. 7 with the G-INS-i strategy (Katoh and Standley 2013). Ambiguous sites were removed using Gblocks ver. 0.91b (Castresana 2000), which resulted in a 3698 bp (505 bp for 16S; 626 bp for *COI*; 1755 bp for 18S; 812 bp for 28S) as a final dataset. To assess the phylogeny of the genus, maximum-likelihood (ML) and Bayesian inference (BI) analyses were carried out using the concatenated sequences. The best-fit partition scheme for ML was the GTR + G model for the concatenated sequences according to PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) employing the greedy algorithm. ML analysis was performed with RAxML ver. 8.0.0 (Stamatakis 2014). Nodal values were derived from 1000 bootstrap pseudoreplicates. BI was performed using MrBayes ver. 3.2.3 (Ronquist et al. 2012) launching two independent Metropolis-coupled analyses with four Markov chains for 10^7 generations, sampling every 100 generations from the chain, based on the GTR + I model selected by PartitionFinder ver. 2.1.1. Run convergence was assessed by Tracer ver. 1.7 (Rambaut et al. 2018); for all parameters, effective sample sizes were above 200.

Uncorrected pairwise genetic distances and Kimura (1980) two-parameter (K2P) genetic distances were calculated based on 658 bp of *COI* by MEGA ver. 7 (Kumar et al. 2016).

Table 2. List of species included in the phylogenetic analyses with GenBank accession numbers

Species	*COI*	16S	18S	28S	References
Lacydonia eliasoni	AY996120	AY996061	-	AY996102	Eklöf et al. (2007)
Lacydonia laureci	-	-	GQ426579	-	Böggemann (2009)
Lacydonia japonica sp. nov.	LC520110	-	LC520118	LC520119	present study
Lacydonia sp. MB-2010	-	GQ426617	GQ426580	-	Böggemann (2009)
Lacydonia sp. Tosa	LC520109	LC520111	LC520117	LC520120	present study
Outgroups					
Eulalia viridis	AY996122	AY996064	AY996085	AY996104	Eklöf et al. (2007)
Paranaitis wahlbergi	AY996115	AY996058	AY996077	AY996098	Eklöf et al. (2007)
Phyllodoce longipes	AY996113	AY996056	AY996075	AY996096	Eklöf et al. (2007)

RESULTS

SYSTEMATICS
Family Lacydoniidae Bergström, 1914
Lacydonia Marion & Bobretzky, 1875

Lacydonia japonica sp. nov.
[Japanese name: yamato-rakidonia]

(Figs 1–4)

urn:lsid:zoobank.org:act: 9326307E-F7BC-47FF-A026-D26B6051B8AB

Material examined: Three specimens. Holotype, NSMT-Pol-H-810, preserved in 10% formalin, collected at a depth of 262 m, 39°06.7054'N, 142°06.4487'E, off Ofunato, Iwate, northern Honshu, Japan, by the ROV *Crambon* dive #35 during the KM19-05C cruise of the R/V *Kaimei* on 16 July 2019; posterior tip used for DNA extraction. Two paratypes, both collected at a depth of 407 m, 39°06.7979'N, 142°09.8719'E, off Ofunato, Iwate, Japan, by the ROV *Crambon* dive #36 during the same cruise as the holotype, on 17 July 2019: NSMT-Pol-P-811, Au-coated and mounted on a SEM stub; NSMT NSMT-Pol-P-812, Au-coated and mounted on a SEM stub.

Etymology: The specific name is a Latin adjective (*japonicus*, *-a*, *-um*), referring to the occurrence of the new species in Japan. The first component of the Japanese name "yamato-" is taken after an ancient name for Japan.

Description of holotype: Body 2.0 mm in length, 0.3 mm in width, 20 chaetigers (complete specimen), generally transparent; white spots present on dorsal surface; pale yellow intestine visible through integument in life (Fig. 1). Peristomium followed by 1 achaetiger segment, 3 uniramous chaetigers, 16 biramous chaetigers, and pygidium (Fig. 2).

Prostomium anteriorly rounded, 1.4 times wider than long, without slit on median anterior edge. Cilia developed near nuchal organs and around median antenna (Fig. 3b). Single pair of lateral antennae and single median antenna present, short, conical (Figs. 2b, c, 3b); lateral antennae (25 µm in length) slightly longer than median antenna (15 µm in length). Eyespots absent (Fig. 2a). Single pair of palps present on ventral side of prostomium, short, conical. Nuchal organs forming incomplete ring, opening dorsally (Fig. 3b). Peristomium not well demarcated.

Tentacular segment (segment 1) achaetigerous bearing single pair of short ovoid cirri (Fig. 3c). Uniramous chaetigers 1–3 (segments 2–4) possessing weakly developed neuropodial lobes with dorsal and ventral cirri (Fig. 3c). Chaetiger 4 and following biramous. Notopodia each possessing conical lobe with ovoid dorsal cirrus (Figs. 2d, 3d, 4). Notopodial cirri 30–40 µm in length (Figs. 2d, 3d, 4). Notochaetae (Fig. 4b) up to 15 simple capillaries (Fig. 3f). Neuropodia each possessing conical lobe, being longer than notopodial lobe (Figs. 2e, 3e, 4). Neuropodial lobe with ovoid ventral cirrus (Figs. 2e, 3e, 4a). Neuropodial cirri 40–50 µm in length (Figs. 2e, 3e, 4a). Neurochaetae (Fig. 4c) up to 17 compound spinigers with weakly serrated blades (Fig. 3g). Pygidium with a ventral papilla between pair of lateral cirri (Fig. 2h).

Body surface of preserved specimens with yellowish to brownish mottled pattern (Fig. 2g), without dorsal dark spots around chaetiger 2 (segment 3). One red pigment spot observed in both dorsal and ventral parapodial cirri, not base of parapodia (Figs. 2f, 4). Pygidium with four red to brown pigments (Fig. 2h). Body pigmentations observed regardless of body size of individuals.

Description of paratype (used for SEM observation): Body 1.2–5.0 mm in length, 0.25–0.3 mm in width, 17–25 chaetigers. Peristomium followed by 1 achaetiger, 3 uniramous chaetigers, biramous 14–22 chaetigers, and pygidium (Fig. 3a). Other characters are same as holotype.

Distribution and habitat: The species is only known from the type locality, off Ofunato, Iwate, Japan; at depths of 262 and 407 m; muddy to sandy sediments.

Lacydonia sp. Tosa

Material examined: Four specimens, collected by sledging during a cruise of the oceanographic research-training vessel *Toyohata-maru* at a depth of 100 m

Fig. 1. *Lacydonia japonica* sp. nov., holotype (NSMT-Pol-H-810), living specimen, dorsal view. Scale bar = 250 µm.

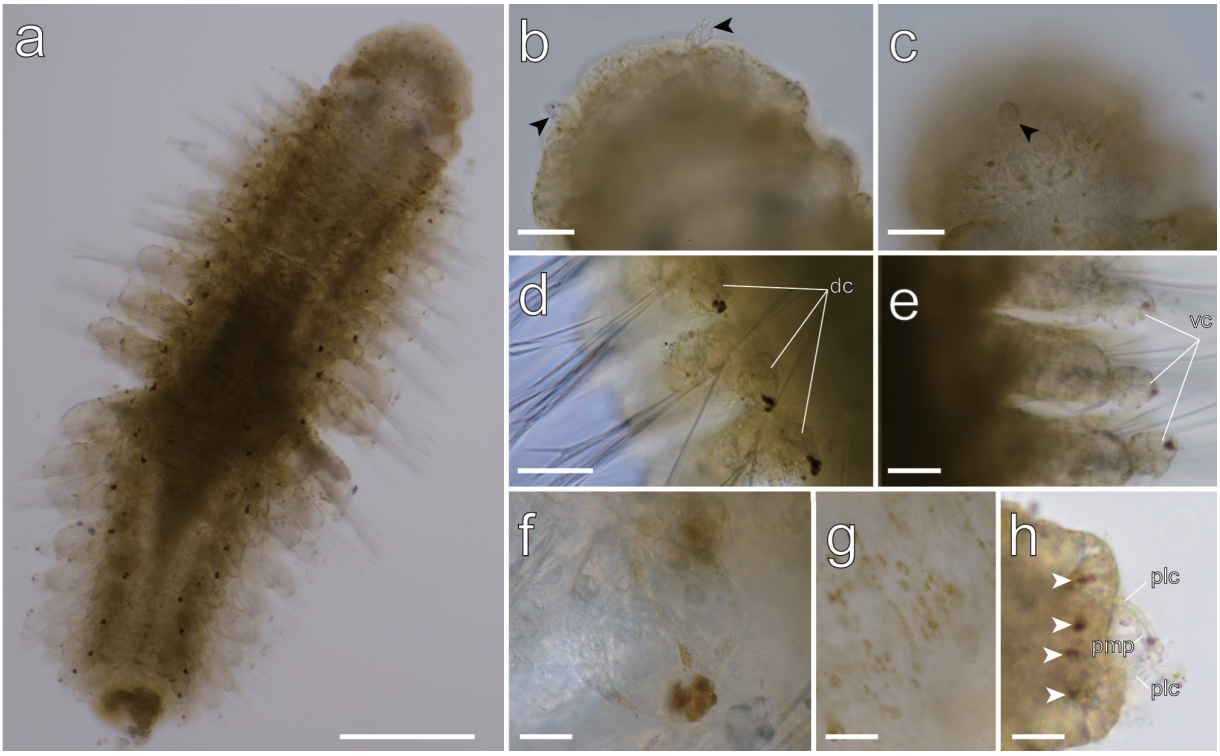

Fig. 2. *Lacydonia japonica* sp. nov., holotype (NSMT-Pol-H-810), LM. a, Whole body, dorsal view; b, Magnification of prostomium focusing on lateral antennae (black arrowheads); c, Magnification of prostomium focusing on median antenna (black arrowhead); d, Parapodial dorsal cirri of mid-body; e, Parapodial ventral cirri of mid-body; f, Magnification of parapodial dorsal cirrus showing a reddish pigment spot; g, Body surface; h, Pygidium, white arrowheads pointing to pigment spots. Abbreviations: dc, parapodial dorsal cirri; plc, pygidial lateral cirrus; pmp, pygidial median papilla; vc, parapodial ventral cirri. Scale bars: a = 200 µm; b–e, h = 50 µm; f, g = 20 µm.

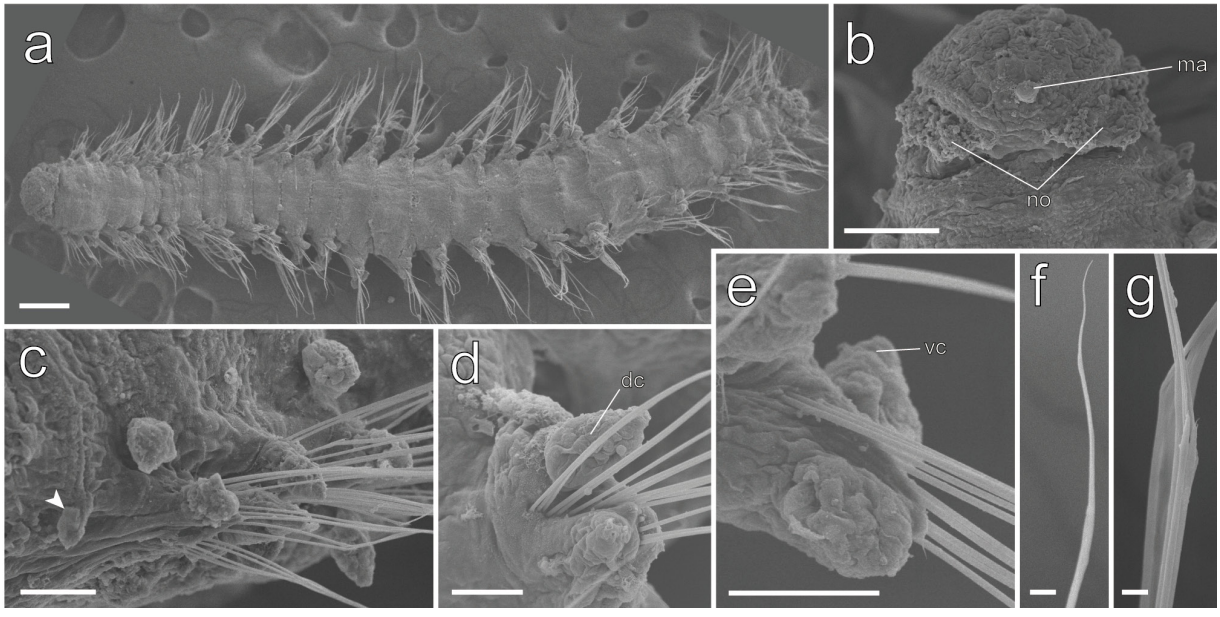

Fig. 3. *Lacydonia japonica* sp. nov., paratype (NSMT-Pol-P-811), SEM. a, Whole body, dorsal view; b, Magnification of prostomium; c, Achaetigerous segment (segment 1, pointed with a white arrowhead) and uniramous segments (segments 2–4), dorsal cirri lost in segment 3; d, Notopodium of mid-body segment, anterior view; e, Neuropodium of mid-body segment, anterior view; f, Notochaeta of mid-body; g, Neurochaeta of mid-body. Abbreviations: dc, parapodial dorsal cirrus; ma, median antenna; no, nuchal organ; vc, parapodial ventral cirrus. Scale bars: a = 200 µm; b, c, e = 50 µm; d= 30 µm; f =5 µm; g = 1 µm.

about 33°18.452'N, 133°35.829'E, Tosa Bay, Kochi, off western Japan, on 24 July 2019: one specimen, preserved in formalin; another specimen, Au-coated and mounted on a SEM stub; the other two specimens used for DNA extraction.

Remarks: All the specimens were represented by anterior or posterior fragments and heavily damaged during sledging on the bottom and/or onboard sieving while extraction from bottom sediments. Detailed morphological data were not available from these specimens.

Genetic distances and phylogenetic analyses

The interspecific genetic distance based on *COI* was 10.4–11.3% in *p*-distance and 11.3–12.3% in K2P between *Lacydonia japonica* sp. nov. and the unidentified *Lacydonia* sp. Tosa (Table 3). Our phylogenetic tree of *Lacydonia* shows that the two Japanese species form a clade, while the relationships among *Lacydonia eliasoni*, *L. laureci*, and another unidentified *Lacydonia* sp. MB-2010 were not well resolved with the sequences currently available in GenBank (Fig. 5).

DISCUSSION

Lacydonia japonica sp. nov. morphologically resembles *L. eliasoni*, *L. papillata*, *L. papillata sensu* Böggemann (2009), and *L.* cf. *papillata sensu* Rizzo et al. (2016) in the absence of the eyes, the absence of two lateral lobes on the posterior margin of the prostomium, and the approximately 1:1 width-to-length ratio of the prostomium. However, the new species differs from them by lacking pigmentation in chaetiger 2 (segment 3), and having pigmentation in its dorsal/ventral parapodial cirri, as well as in the pygidium. Furthermore, the genetic distance (Table 3) and phylogenetic relationship (Fig. 5) between *L. eliasoni* and *L. japonica* sp. nov. were in concordance with the morphological difference at the species level (Table 1), although no molecular data were available for *L. papillata*, *L. papillata sensu* Böggemann (2009), or *L.* cf. *papillata sensu* Rizzo et al. (2016). The present species differs from *L. laureci* in its body pigmentation (Table 1), the absence of two lateral lobes on the posterior margin of the prostomium,

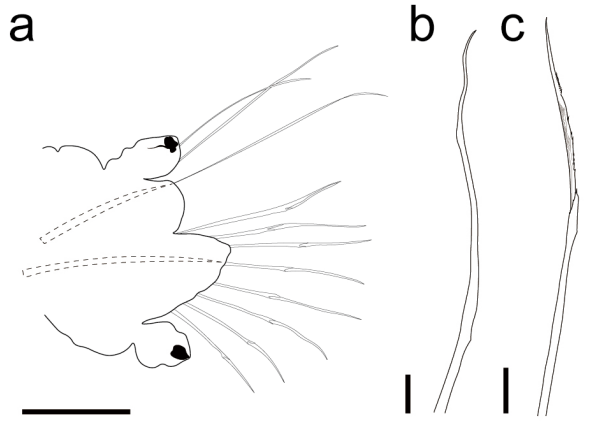

Fig. 4. *Lacydonia japonica* sp. nov., paratype (NSMT-Pol-P-811). a, Mid-body parapodium; b, Notochaetae; c, Neurochaetae. Scale bars: a = 50 μm; b, c = 10 μm.

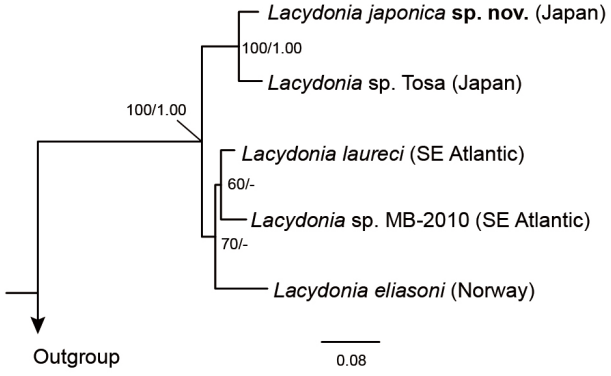

Fig. 5. A preliminary tree of the genus *Lacydonia* reconstructed with a maximum likelihood analysis based on concatenated partial sequences of *COI*, 16S rRNA, 18S rRNA, and 28S rRNA genes. Numbers near nodes indicate bootstrap support values (BS) generated by maximum likelihood analysis with 1,000 replicates and posterior probability (PP) of a separate partitioned Bayesian analysis (BS/PP). *Eulalia viridis*, *Paranaitis wahlbergi*, and *Phyllodoce longipes* were used as outgroup taxa.

Table 3. Range of intra- and interspecific genetic distances (%) among species of the genus *Lacydonia*, represented by uncorrected *p*-distance (values below diagonal) and K2P (values above diagonal). The intraspecific value for *Lacydonia* sp. Tosa was the same in terms of uncorrected *p*-distance and K2P

Species (Number of individuals)	*Lacydonia eliasoni*	*Lacydonia japonica* sp. nov.	*Lacydonia* sp. Tosa
Lacydonia eliasoni (1)	-	17.1	18.4–18.6
Lacydonia japonica sp. nov. (1)	17.1	-	11.3–12.3
Lacydonia sp. Tosa (2)	16.4–16.5	10.4–11.3	1.3

and the approximately 1:1 width-to-length ratio of prostomium, which is in concordance with the result of our phylogenetic analyses (Fig. 5). The genetic distance based on *COI* between *L. japonica* and *Lacydonia* sp. Tosa was comparable to the interspecific *COI* divergences among phyllodocids (Nygren and Pleijel 2011), suggesting that the specimens from the two distinct localities can be regarded as different species.

Lacydonia japonica is morphologically most similar to *L. papillata*; the only morphological difference between them is their body pigmentations. *Lacydonia papillata* was originally described from Kuril-Kamchatka Trench. Since then, specimens tentatively identified as *L. papillata* have been reported from almost all over the world: Kuril-Kamchatka Trench (Uschakov 1958; Alalykina 2015), the Canadian Arctic and Beaufort Sea (Paul and Menzies 1974; Carey 1977), and Angola and Guinea Basin (Böggemann 2009), although *L. papillata sensu* Böggemann (2009) should have been identified as *L. elongata* rather than *L. papillata* in terms of morphology, as indicated by Rizzo et al. (2016). To assess whether such morphological differences between them are useful for species delimitation, morphological examination accompanied with molecular data is necessary. Over the past two decades, many molecular studies on polychaetes have revealed the existence of cryptic species (*e.g.*, Manchenko and Radashevsky 2002; Maltagliati et al. 2004; Barroso et al. 2010; Nygren and Pleijel 2011; Nygren 2014; Tosuji et al. 2019). *Lacydonia papillata* might include several undescribed cryptic species inferred by Magalhães et al. (2012) with respect to the common large-eyed species *L. miranda*. To recognize presence of cryptic or pseudocryptic species, a molecular approach using proper gene makers (*e.g.*, *COI*) is useful; however, published sequence data on *Lacydonia* are currently scarce. Future taxonomic studies on *Lacydonia* with DNA barcoding might explore the hidden diversity of the genus.

CONCLUSIONS

In the present study, we described a new species in the genus *Lacydonia* off the coast of north-eastern Japan. The new species is distinguishable from *L. eliasoni*, *L. papillata*, *L. papillata sensu* Böggemann (2009), and *L.* cf. *papillata sensu* Rizzo et al. (2016) in that it lacks pigmentation in chaetiger 2 (segment 3), and having pigmentation in dorsal/ventral parapodial cirri, as well as in the pygidium. Our preliminary results from the phylogenetic analyses suggested the species-level morphological difference between *L. eliasoni* and *L. japonica* sp. nov. Detailed morphological examinations including type materials and molecular approach are necessary to understand the systematics of the poorly known polychaetes *Lacydonia*.

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Authors' contributions: NH and NJ designed the present study. NH, NJ and HK drafted the manuscript. ST and YF organized KM19-05C cruise of the R/V *Kaimei*. YF provided the photos of living material. NH and NJ conducted the morphological observation. NH conducted the phylogenetic analyses. 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: The manuscript is incorporated in ZooBank. Materials are deposited in the collections of NSMT.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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