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## First record of *Larsonella pumilus* (Teleostei: Gobiidae) from Japan, with phylogenetic placement of the genus *Larsonella*

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### Abstract

During a survey of deep-sea fauna, using a Remotely Operated Vehicle, a single specimen (21.6 mm in standard length) of *Larsonella pumilus* (Larson & Hoese, 1980) was collected at a depth of 214 m off the coast of Okinawa Island, Japan. It represents the first record of this species from Japan. The collection site was far deeper than previous reports for this species. This suggests that the main habitat of *L. pumilus* is deeper than previously recognized and it may explain the paucity of records of this species. As the previously available morphological description of *L. pumilus* was based on only a single specimen (holotype), this new specimen is described herein. Its morphology corresponds closely to the original description of the holotype, except that faint melanophores are arranged radially around the eyes and scattered on the trunk and the fins. Mitochondrial genome sequences of *L. pumilus* and 19 related species demonstrate close relationships between *L. pumilus* and the genus *Priolepis*. These data also indicate that the genus *Priolepis* is not monophyletic.

**Key words:** goby, deep sea, mitochondrial genome, Ryukyu Archipelago

### Introduction

The order Gobiiformes (= suborder Gobioidae in Nelson 2006) is a very diverse fish taxon, including more than 2,000 goby species, belonging to more than 300 genera (Nelson *et al.* 2016). Gobies are distributed worldwide and occur in fresh, brackish, and marine waters. In the sea, they occupy nearshore environments, including rocky and sandy bottoms, mudflats, tidepools, coral reefs, and caves (Patzner *et al.* 2011). Although the deep sea may seem to be atypical goby habitat, more than ten genera (*Antilligobius*, *Karsten*, *Lepidogobius*, *Lesueurigobius*, *Obliquogobius*, *Palatogobius*, *Pinnichthys*, *Priolepis*, *Sufflogobius*, *Suruga*, *Thorogobius*, and *Varicus*) have been reported from >200 m depth (Eschmeyer & Herald 1983; Goren 1992; Goren & Baranes 1995; Bianchi *et al.* 1999; Shinohara *et al.* 2001; Greenfield 2002; Murdy 2002; Mytilineou *et al.* 2005; Shibukawa & Aonuma 2007; Tornabene *et al.* 2016; Baldwin *et al.* 2018; Sauberer *et al.* 2018). However, information about deep-sea gobies is extremely limited. Probably due to their small body sizes (usually <10 cm) and their cryptic natures (often dwelling in substrata or abandoned invertebrate exoskeletons), gobies are not easily detected. Most deep-sea gobies recorded were collected by trawling or dredging. We collected a small goby specimen identified as *Larsonella pumilus* from a depth of 214 m off the coast of Okinawa Island, Japan, using a Remotely Operated Vehicle (ROV) during a survey of deep-sea fauna.

*Larsonella pumilus* was originally described by Larson & Hoese (1980) as *Lubricogobius pumilus*. Subsequently, Randall & Senou (2001) reviewed the genus *Lubricogobius* and established a new genus *Larsonella* for this species on the basis of its depressed head, slenderer body, lack of a pelvic frenum, five rows of sensory papillae that radiate perpendicularly from the lower edge of the orbit, a snout longer than the eye, and the presence of scales posteriorly on the body. Currently *L. pumilus* is the only member of the genus *Larsonella*. The phylogenetic placement of *Larsonella* has not been studied, but Hoese & Larson (2010) suggested close relationships among *Larson-*

*ella*, *Lubricogobius*, and *Priolepis* based on their morphological similarity. On the other hand, Thacker (2015) put *Larsonella* and *Lubricogobius* into the “*Gobiodon* lineage” sensu Agorreta *et al.* (2013) (= the “coral gobies” group of Thacker & Roje 2011) with *Gobiodon*, *Eviota*, etc., rather than into the “*Priolepis* lineage” sensu Agorreta *et al.* (2013) (= the “tiny banded gobies” group of Thacker & Roje 2011).

In this paper, we report the first record of *Larsonella pumilus* from Japan. The morphology of this specimen is described because all morphological information about *L. pumilus* (see Larson & Hoese 1980 and Randall & Senou 2001) is based only on a single specimen (holotype) and our material had melanophores which are not described for the holotype. We also discuss the phylogenetic placement of *Larsonella* based on mitochondrial genome sequences of *Larsonella pumilus* and species in related genera.

## Materials and methods

**Sampling.** A single specimen of *Larsonella pumilus* was collected with invertebrate specimens (see details in the “Habitat” section) on a muddy bottom at a depth of 209–220 m off East China Sea coast of Seragaki, Onna Village, Okinawa Island, Japan (26°31'N, 127°52'E), during a survey of deep-sea fauna. Sampling was conducted using a ROV (LEO, KOWA Corporation, Osaka, Japan) on 11 August 2017. The specimen was kept alive in a tank, and fixed in 10% formalin after it died on 22 November 2017, and then preserved in 70% ethanol. A piece of muscle was removed from a damaged part of the left side of the body prior to 10% formalin fixation and was preserved in 99.5% ethanol for mitochondrial DNA analysis.

**Morphological observation.** Measurements and counts were taken from the right side of the fish, because of damage to the left side. Measurements were made point-to-point to the nearest 0.1 mm, using a vernier caliper or a divider under a stereomicroscope, and were expressed as a percentage of standard length (SL). Measurements and counts follow Nakabo (2002), with the following modifications: body depth was measured at the pelvic- and anal-fin origins; length of first dorsal-fin base was measured from the origin of first dorsal fin to the base of the spine of the second dorsal fin. Scales and cephalic sensory organs were observed after staining with cyanine-blue solution. Teeth and osteological features were observed using computed tomography (CT) data. In order to observe nondestructively, the specimen was placed in a plastic pack containing water and scanned with X-ray microcomputed tomography (micro-CT) R\_mCT2 (Rigaku Co., Tokyo, Japan) at an X-ray setting of 90 kV and 200 mA. Micro-CT images were reconstructed from CT-slices (0.02 mm interval) using DICOM editing software AZE VirtualPlace (AZE Ltd., Tokyo, Japan). Teeth were also observed with a stereomicroscope. The dorsal-fin pterygiophore formula follows Birdsong *et al.* (1988). Color in life was described from a photograph taken when it was alive (Fig. 1A). Symbolic codes used to represent collections and institutions follow Sabaj (2016), except OCF (Okinawa Churashima Foundation Research Center).

**Mitochondrial DNA analysis.** Total genomic DNA was extracted from muscle of the *Larsonella pumilus* specimen, and right pectoral fins, right eyes, or muscle piece of 19 related species preserved in 99.5 % ethanol, using Maxwell RSC Blood DNA Kit (Promega, Fitchburg, Wisconsin, USA) or DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Whole-genome shotgun sequencing libraries were prepared using a KAPA HyperPlus Kit, PCR-free (KAPA Biosystems, Wilmington, Massachusetts, USA). Extracted genomic DNA was enzymatically fragmented into pieces of 200–1000 bp. After repairing protruding ends and A-tailing, sequencing adaptors were ligated onto both ends of DNA fragments. Shotgun libraries were then sequenced on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, California, USA) in Rapid Run mode version 2 using a HiSeq Rapid Cluster Kit v2 – Paired-End (Illumina) and a HiSeq Rapid SBS Kit v2 (Illumina) or an Illumina HiSeq 4000 sequencer (Illumina) using HiSeq 3000/4000 PE Cluster Kit (Illumina) following manufacturer’s instructions.

Sequencing data from each library were assembled with the IDBA\_UD assembler version 1.1.1 (Peng *et al.* 2012) with different kmer lengths (60, 80, 100). Identification of complete mitochondrial genomes from assembled contigs was performed by 1) comparing them with the complete *Stiphodon alcedo* mitochondrial genome (accession: AB613000.1) (BLASTN e-value  $\leq 1e-100$ ), and by 2) confirming that 100 bp of both head and tail DNA sequences of a contig were identical, indicating that the sequence was circular. Complete mitochondrial genomes were aligned using MAFFT v7.244 (Katoh & Standley 2013) and all positions with gaps were removed using trimAl (Capella-Gutierrez *et al.* 2009). Phylogenetic model selection for aligned whole mitochondrial genomes was performed using ModelTest-NG version 0.1.5 (Darriba *et al.* 2019). We performed molecular phylogenetic analyses

of aligned mitochondrial genomes using the GTR+I+Gamma model suggested by the above evolutionary model selection. Maximum likelihood (ML) analysis was performed using RAxML version 8.2.3 (Stamatakis 2014) with 100 bootstrap replicates, and Bayesian inference analysis was performed using MrBayes version 3.2.7 (Ronquist *et al.* 2012) with one million generations. All sequenced raw data are available in the DDBJ Sequence Read Archive under BioProject accession number PRJDB5763. Assembled mitochondrial genome sequences with gene annotations are available in the DDBJ under accession numbers: AP019315–AP019347. Accession numbers for each individual are shown in Table 1.

**TABLE 1.** Accession numbers and lengths of mitochondrial genome sequences analyzed in this study.

Species	Catalog number of voucher	Locality	Accession number for assembled mitochondrial genome sequence	Length of mitochondrial genome sequences (bp)
<i>Larsonella pumilus</i>	OCF-P 3808	Okinawa I.	AP019338	16609
<i>Amblygobius phalaena</i>	OCF-P 4014	Okinawa I.	AP019316	16622
<i>Asterropteryx semipunctata</i>	OCF-P 4007	Okinawa I.	AP019328	16456
<i>Asterropteryx semipunctata</i>	OCF-P 4008	Okinawa I.	AP019329	16456
<i>Callogobius okinawae</i>	URM-P 48120	Okinawa I.	AP019317	16503
<i>Callogobius okinawae</i>	URM-P 48121	Okinawa I.	AP019318	16565
<i>Callogobius tanegasimae</i>	URM-P 48462	Okinawa I.	AP019319	16579
<i>Callogobius tanegasimae</i>	URM-P 48463	Okinawa I.	AP019320	16579
<i>Eviota japonica</i>	URM-P 48635	Okinawa I.	AP019334	17484
<i>Eviota ocellifer</i>	OCF-P 4017	Iriomote I.	AP019333	17027
<i>Eviota prasina</i>	OCF-P 4004	Okinawa I.	AP019335	16530
<i>Eviota prasina</i>	OCF-P 4012	Okinawa I.	AP019336	16531
<i>Eviota prasina</i>	OCF-P 4013	Okinawa I.	AP019337	16531
<i>Gobiodon micropus</i>	OCF-P 3248	Okinawa I.	AP019346	16695
<i>Gobiodon erythrospilus</i>	OCF-P 3292	Okinawa I.	AP019347	16702
<i>Parioglossus dotui</i>	OCF-P 4010	Okinawa I.	AP019321	16511
<i>Parioglossus dotui</i>	OCF-P 4011	Okinawa I.	AP019322	16511
<i>Parioglossus dotui</i>	OCF-P 4016	Okinawa I.	AP019332	16511
<i>Parioglossus formosus</i>	URM-P 48487	Okinawa I.	AP019323	16495
<i>Parioglossus formosus</i>	OCF-P 4015	Okinawa I.	AP019331	16494
<i>Parioglossus raoi</i>	URM-P 48490	Okinawa I.	AP019324	16497
<i>Parioglossus raoi</i>	OCF-P 4003	Miyako I.	AP019325	16497
<i>Priolepis cincta</i>	OCF-P 2829	Okinawa I.	AP019342	17059
<i>Priolepis latifascima</i>	OCF-P 2830	Okinawa I.	AP019343	16652
<i>Priolepis semidoliata</i>	URM-P 48119	Okinawa I.	AP019330	16651
<i>Priolepis semidoliata</i>	OCF-P 4018	Okinawa I.	AP019339	16739
<i>Priolepis semidoliata</i>	OCF-P 4019	Okinawa I.	AP019340	16829
<i>Priolepis semidoliata</i>	OCF-P 4020	Okinawa I.	AP019341	16650
<i>Trimma caesiura</i>	OCF-P 2833	Okinawa I.	AP019344	17137
<i>Trimma okinawae</i>	OCF-P 3851	Okinawa I.	AP019345	17969
<i>Valenciennesa longipinnis</i>	OCF-P 4005	Okinawa I.	AP019326	16499
<i>Valenciennesa longipinnis</i>	OCF-P 4006	Okinawa I.	AP019327	16499
<i>Vanderhorstia</i> sp. ‘Komon-yatsushi-haze’	OCF-P 4009	Iriomote I.	AP019315	16548

*Larsonella pumilus* (Larson & Hoese, 1980)

[New Japanese name: Yuuna-haze]

(Figs. 1–4; Table 2)

*Lubricogobius pumilus* Larson & Hoese, 1980: 41 (type locality: Indian Ocean, 3°25'N 47°14.8'E, 37–38 m depth).

*Larsonella pumilus* (Larson & Hoese, 1980): Randall & Senou 2001: 11.

**Material examined.** OCF-P 3808, 21.6 mm SL, East China Sea off Seragaki, Onna Village, Okinawa, Japan, 11 August 2017.

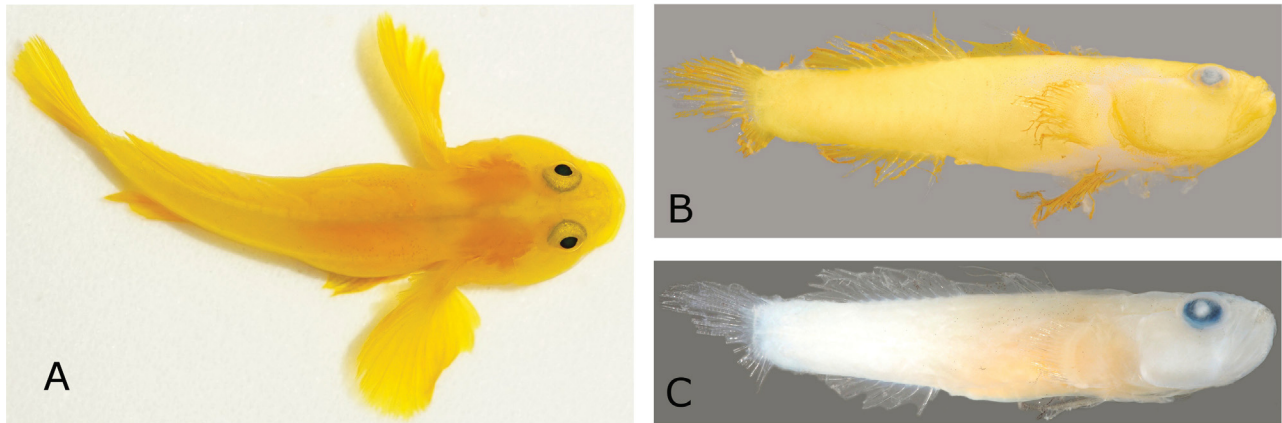


FIGURE 1. *Larsonella pumilus*, OCF-P 3808 (21.6 mm in standard length), in life (A), fresh (B), and after preservation (C).

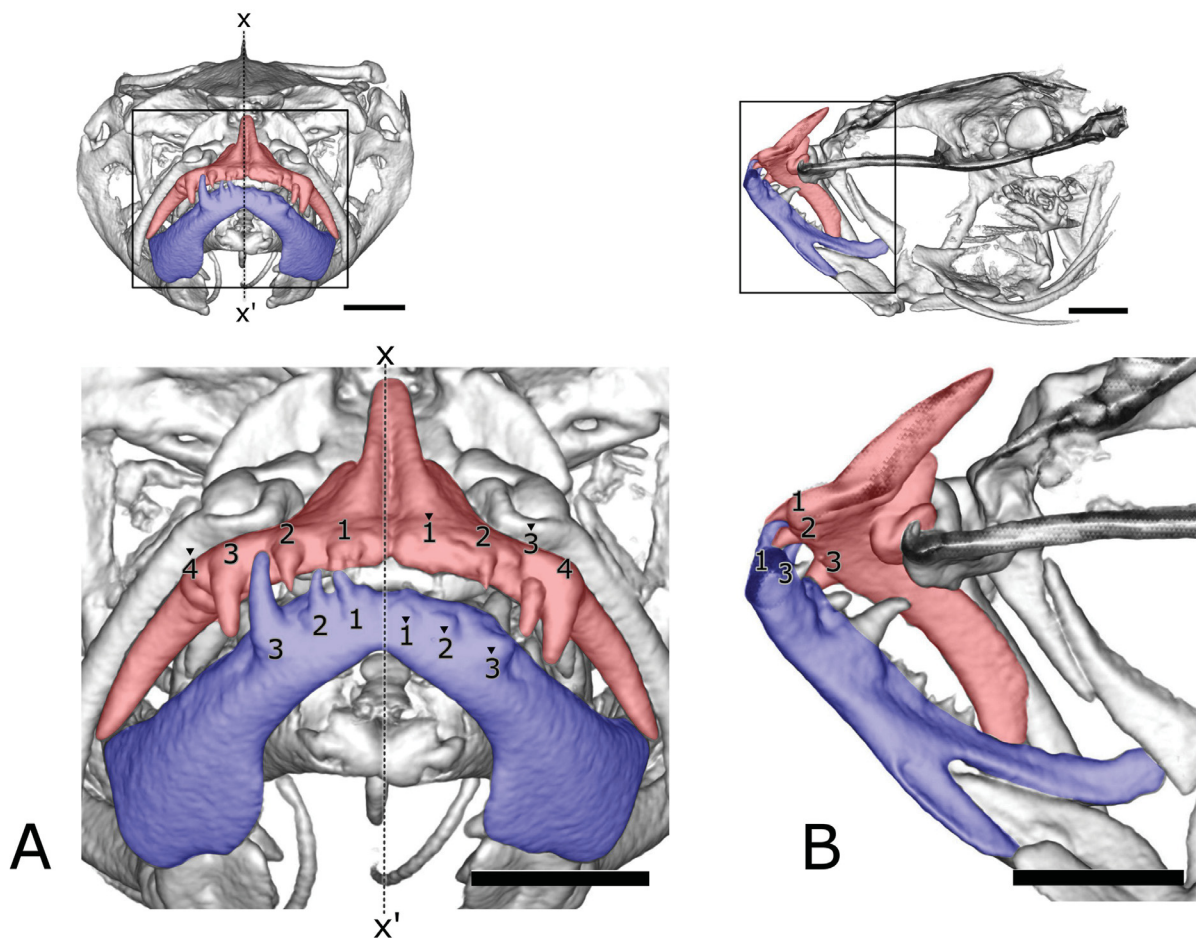
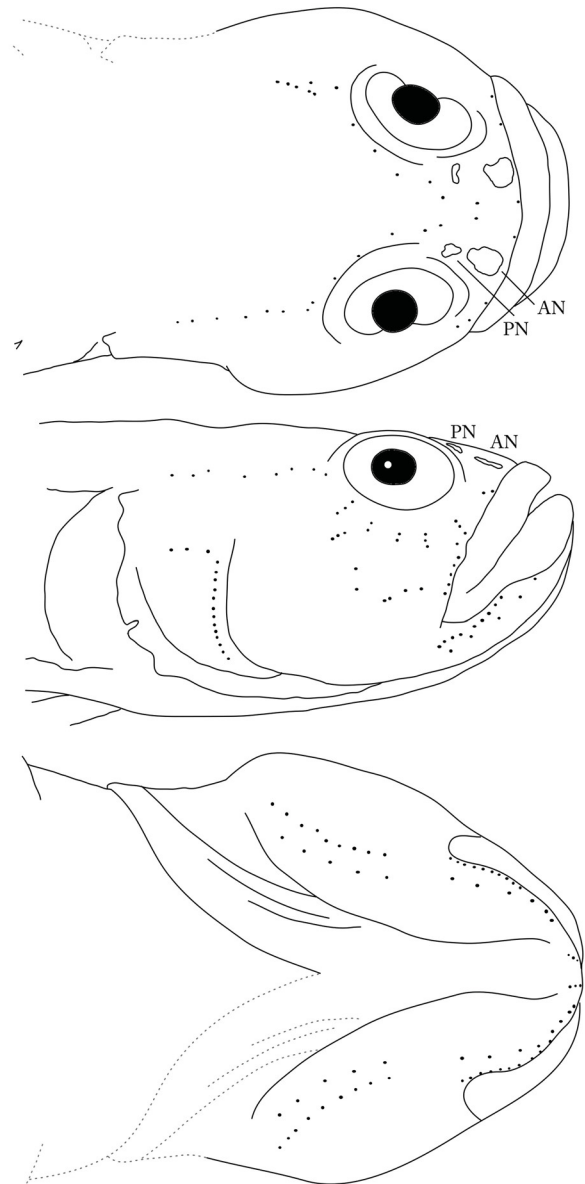


FIGURE 2. Three-dimensional images of the head of *Larsonella pumilus* reconstructed from microcomputed tomography data. A, frontal view, showing the outermost teeth. B, mid-sagittal view of the right side (cut along section x-x' in A), showing inner teeth without a part of ceratohyal and 5th branchiostegal ray to observe teeth easily. Red, premaxilla and outermost teeth on the premaxilla; blue, dentary and outermost teeth on the dentary. Triangles, vestiges of missing teeth. Scale bars, 1 mm.



**FIGURE 3.** Picture of the caudal peduncle, stained with cyanine-blue solution to show scale arrangements (left) and schematic illustrations of cephalic sensory papillae (right) of *Larsonella pumilus* (OCF-P 3808, 21.6 mm SL) in dorsal (top), lateral (middle), and ventral (bottom) views. AN and PN, anterior and posterior nares, respectively. Dotted lines, the probable undamaged states of the damaged parts.

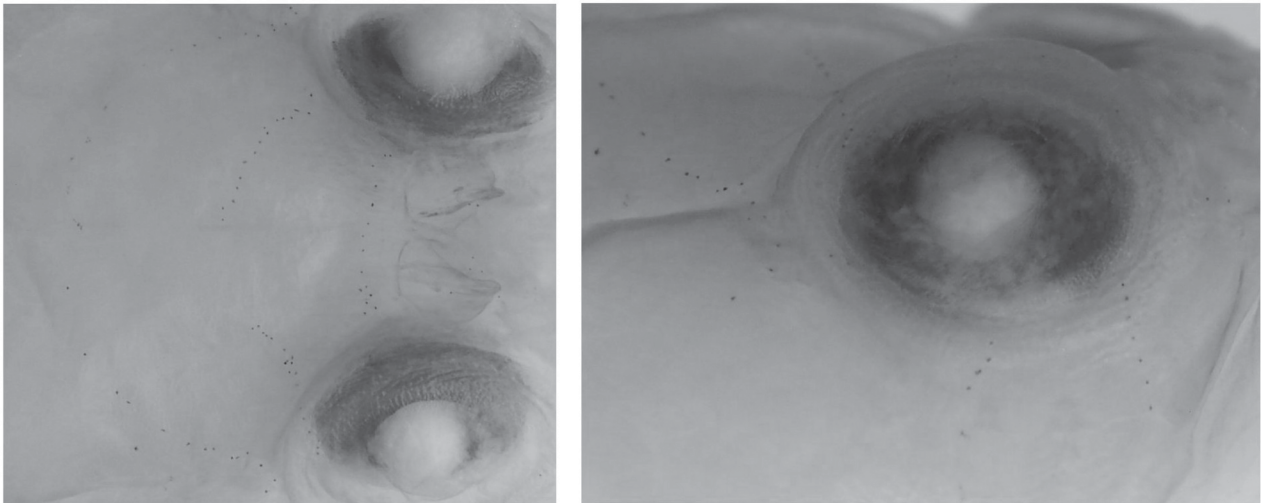
**Description.** Counts and measurements are shown in Table 2. Head depressed, trunk nearly cylindrical, and tail compressed. Eyes located dorsolaterally. Mouth strongly oblique with angle to body axis about 60 degrees. Lower jaw protruding beyond upper jaw. Posterior end of upper jaw reaching below middle point between anterior margin of iris and anterior margin of pupil. Canine-like teeth aligned on edges of anterior halves of premaxilla and dentary; four and three teeth on one side of premaxilla and dentary, respectively; posterior teeth larger (Fig. 2A). An inner row of conical teeth extending from anterior part to more posterior part of dentary than the outer canine-like teeth row (Fig. 2B). Additional small conical teeth observed on inner parts of premaxilla and dentary with a stereomicroscope, but no such small teeth with micro-CT.

First dorsal fin with six spines. Second dorsal fin with one spine and nine soft rays. First and second dorsal fins connected by a low membrane behind last spine of the first dorsal fin. Anal fin with one spine and eight soft rays. Caudal fin rounded, with 17 segmented rays. Pectoral fin with 18 soft-rays. Pelvic fin with one spine and five soft rays. Posterior tips of pectoral fins reaching or exceeding position of anus, according to a photograph taken in life

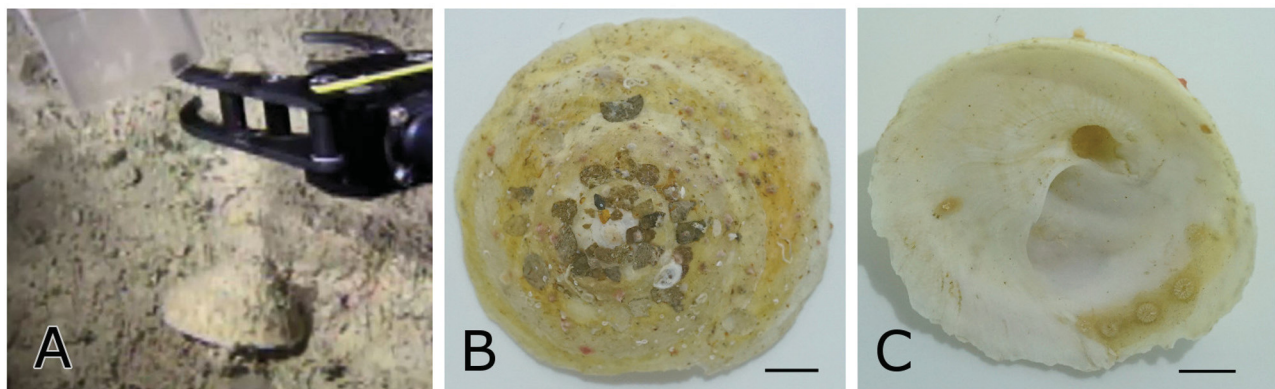
(Fig. 1A), although they are broken in the preserved specimen. Pelvic fins without frenum. Vertebrae  $10 + 16 = 26$ ; dorsal-fin pterygiophore formula 3-22110; epural 1; anal-fin pterygiophores anterior to first haemal spine 2.

Head and body largely naked except for posterior part of caudal peduncle, involving three rows composed of 7–10 ctenoid scales along dorsal midline. A row of three ctenoid scales along lateral midline, and three rows composed of 6–8 ctenoid scales along ventral midline (Fig. 3). No sensory canals or associated pores on head. Cephalic sensory papillae patterns illustrated in Fig. 3. Infraorbital area with five transverse rows of sensory papillae.

Color in preservative (Fig. 1C): Background of head and tail white, trunk yellowish white. All fin membranes transparent. Melanophores scattered on dorsal half of trunk and on membranes of first and second dorsal, anal, and pelvic fins. Pectoral fin also with a few melanophores. Three rows of tiny melanophores arched between right and left eyes (Fig. 4A). Infraorbital area with two transverse rows of tiny melanophores (Fig. 4B) and another row of tiny melanophores behind eyes (Fig. 4B). These melanophore rows arranged radially around eyes.



**FIGURE 4.** Pigmentations around the eyes of *Larsonella pumilus* (OCF-P 3808, 21.6 mm SL) in dorsal (left) and lateral (right) views. Photos were taken after preservation.



**FIGURE 5.** Underwater photograph (A) and a dried shell (B and C) of *Xenophora chinensis*. A, Collecting the shell in which *Larsonella pumilus* may have taken refuge, using a Remotely Operated Vehicle at a depth of 214 m in the East China Sea off Seragaki, Onna Village, Okinawa, Japan. B (dorsal view) and C (ventral view) are likely the same shell as A. Scale bars, 10 mm.

Color in life (Fig. 1A): Body and all fins yellow or yellowish orange. Arrangement of melanophores same as after preservation, described above (Fig. 1A).

**Habitat.** We collected ten specimens of five invertebrate species from the muddy bottom at a depth of 209–220 m using the ROV, on 11 August 2017. These included five live comb jellies, *Lyrocteis imperatoris*, a sea cucumber, *Holothuria dura*, a starfish, *Asterodiscides japonicus*, a heart urchin, *Pericosmus* sp., and an empty shell of *Xenophora chinensis* (Fig. 5). After these animals were put into a tank on the boat, the *L. pumilus* specimen was found





**TABLE 2.** Counts and measurements of *Larsonella pumilus*. Values of the holotype are based on Larson & Hoese (1980). D<sub>1</sub>, first dorsal fin; D<sub>2</sub>, second dorsal fin; A, anal fin; C, caudal fin; P<sub>1</sub>, pectoral fin; P<sub>2</sub>, pelvic fin; PO, preopercular.

	OCF-P 3808	Holotype ZMH 6165
Standard length (mm)	21.6	14.5
Counts		
D <sub>1</sub>	VI	VI
D <sub>2</sub>	I, 9	I, 9
A	I, 8	I, 8
C (segmented rays)	17	17
P <sub>1</sub>	18	17
P <sub>2</sub>	I, 5	-
Measurements as % of standard length		
Head length	31.9	34.5
Head depth at PO margin	20.4	24.1
Head width at PO margin	27.8	28.3
Snout length	7.4	9.7
Eye diameter	8.8	6.9
Upper jaw length	15.3	15.2
Body depth at P <sub>2</sub> origin	21.8	-
Body depth at A origin	18.1	18.6
Caudal peduncle depth	13.9	13.8
Caudal peduncle length	19.0	20.7
Predorsal length	39.8	-
Preanal length	65.7	-
Length of D <sub>1</sub> base	19.9	-
Length of D <sub>2</sub> base	23.6	23.4
Length of A base	19.4	-

## Discussion

**Identification.** The present specimen was identified as *Larsonella pumilus* (Larson & Hoese, 1980) as its morphological characters correspond almost exactly to those of the holotype described by Larson & Hoese (1980), although some characters, including fin morphology and nare shapes could not be confirmed due to their damaged condition. The only major difference found was the presence of melanophores. Larson & Hoese (1980) noted “no traces of dark pigment”, while our specimen has melanophores arranged radially around eyes and scattered on trunk and fins. Because these melanophores were very small and invisible to the naked eye, we suppose that the melanophores were overlooked in the original description. It is also possible that melanophores vary individually or depending on body size and/or sex (we could not determine the sex of our specimen).

**Distribution and habitat.** There have been few records of this species since the holotype of *Lubricogobius pumilus* was collected from the Indian Ocean off Somalia using an Agassiz trawl at a depth of 37–38 m (Larson & Hoese 1980). Other available records from the Indian Ocean are from La Digue in the Seychelles (4°23'S 55°49'E) from dredging at a depth of 30 m (Randall & van Egmond 1994; BPBM 35515 and ROM 66149; as *L. pumilus*) and from the northwest shelf of Australia (16°49'S to 19°52'S) (Hoese & Larson 2006). This species also appeared in a list of marine and brackish water goby species in the western central Pacific (Larson & Murdy 2001), but detailed locality information was not provided. According to the GBIF database (GBIF Secretariat 2017), *L. pumilus* was also collected in New Caledonia and Tonga (69 and 67 m in depth, respectively). The specimen reported in the present study was caught at a depth of 214 m off Okinawa Island. This is the first record of *L. pumilus* from Japan and the only report substantiated with a voucher from the Pacific Ocean. The depth is far greater than other records (214 m vs. 30–69 m). Thus, the main habitat of *L. pumilus* may be deeper sea than previously recognized. This may also

explain the paucity of records since this area has been poorly explored. It also suggests that this species is distributed much more widely in the Indo-Pacific.

**Phylogenetic placement.** Among their hypothesized lineages based on molecular data, morphological characters, and literature review, Thacker (2015) placed the genus *Larsonella* into the “*Gobiodon* lineage (sensu Agorreta *et al.* 2013)” with the genera *Bryaninops*, *Eviota*, *Gobiodon*, etc. However, our results clearly demonstrate that *L. pumilus* belongs to the “*Priolepis* lineage (sensu Agorreta *et al.* 2013)” with *Priolepis* spp. and *Trimma* spp., not to the “*Gobiodon* lineage” (Fig. 6). Our tree also indicates that the genus *Priolepis* is not monophyletic, as *L. pumilus* nested within a clade composed of three species of *Priolepis*. But we could analyze only three of 35 species in *Priolepis* and no *Lubricogobius* species were involved in the present study, although *L. pumilus* is considered to be a close relative of *Lubricogobius* (Hoese & Larson 2010). Hoese & Larson (2010) doubted the validity of *Larsonella* based on the fact that a species of *Lubricogobius* actually has scales on its caudal peduncle, which is one of the major diagnostic characters to distinguish *Larsonella* from *Lubricogobius*. The mitochondrial genome sequence of *L. pumilus* included in the present study will help future research to review phylogenetic relationships among species in the genera *Priolepis*, *Lubricogobius*, and *Larsonella*.

**Remarks.** *Larsonella pumilus* is mentioned as “*Larsonella pumila*” in some online databases (e.g. Fricke *et al.* 2018; Froese & Pauly 2018), although no author spelled it this way. This species was originally described as a new species in *Lubricogobius* and the specific epithet is considered as a noun as the authors stated “Derivation of name: Latin – pumilus = dwarf fish” (Larson & Hoese 1980). Therefore, the original spelling of the specific epithet is to be retained with gender ending unchanged, even if gender of the generic name is changed. Although the specific epithet of this species is spelled “pumilis” in figure 536 of Larson & Hoese (1980) and Randall & van Egmond (1994), this is also a misspelling.

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