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Integrative taxonomy reveals a rare and new cusk-eel species of *Luciobrotula* (Teleostei, Ophidiidae) from the Solomon Sea, West Pacific

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Abstract. With six valid species, *Luciobrotula* is a small genus of the family Ophidiidae, commonly known as cusk-eels. They are benthopelagic fishes occurring at depths ranging from 115–2300 m in the Atlantic, Indian, and Pacific Oceans. Among them, *Luciobrotula bartschi* is the only known species in the West Pacific. Three specimens of *Luciobrotula* were collected from the Philippine Sea, Bismarck Sea, and Solomon Sea in the West Pacific during the AURORA, PAPUA NIUGINI, and MADEEP expeditions under the *Tropical Deep-Sea Benthos* program, and all of them were initially identified as *L. bartschi*. Subsequent examination with integrative taxonomy indicates that they belong to two distinct species, with the specimen collected from the Solomon Sea representing a new species, which is described here. In terms of morphology, *Luciobrotula polylepis* sp. nov. differs from its congeners by having a relatively longer lateral line (end of the lateral line below the 33rd dorsal-fin ray) and fewer vertebrae (abdominal vertebrae 13, total vertebrae 50). In the inferred *COI* gene tree, the two western Pacific species of *Luciobrotula* do not form a monophyletic group. The genetic K2P distance between the two species is 13.8% on average at the *COI* locus.

Key words. Biodiversity exploration, DNA barcoding, Ophidiiformes, species delimitation, tropical deep-sea benthos.

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Introduction

Luciobrotula Smith & Radcliffe, 1913, a rare deep-sea fish genus, is currently classified in the subfamily Neobythitinae (Ophidiidae). Representatives of this genus differ from other ophidiids by having a much depressed head, much less (3–4) developed long gill rakers on the first gill arch, and one median and a pair of basibranchial tooth patches (Nielsen 2009). Currently, six species of *Luciobrotula* are considered valid (Nielsen 2009; Fricke *et al.* 2020): *L. bartschi* Smith & Radcliffe, 1913, *L. brasiliensis* Nielsen, 2009, *L. coheni* Nielsen, 2009, *L. corethromycter* Cohen, 1964, *L. lineata* (Gosline, 1954), and *L. nolfi* Cohen, 1981. All of them are benthic dwellers and can usually be found in the tropical deep waters of continental slopes worldwide (Cohen 1974; Nielsen 2009). They are probably carnivorous, though only a single study mentioned a partially digested caridean shrimp found in the gut of *L. corethromycter* (Cohen 1964). Fishes in this genus are rarely caught and usually from benthic trawling over sand or mud bottoms at depths between 110 and 2300 m (Radcliffe 1913; Cohen 1964; Nielsen *et al*. 1999; Nielsen & Møller 2008; Robertson *et al*. 2017). Among them, *L. bartschi* has the widest distribution in the Indo-West Pacific, ranging from the Gulf of Aden and South Africa, east to the Hawaiian Islands, and north to Japan. It is the only species previously known from the West Pacific (Nielsen 2009).

Luciobrotula bartschi was the first described species of the genus on the basis of a single specimen collected during the *Albatross* Philippines expedition 1907–1910 from the Palawan Passage at a depth of 686 m (Radcliffe 1913). Since its discovery and description, several advanced taxonomic studies have been performed. Cohen (1964) conducted the first taxonomic review of *Luciobrotula*. He synonymized the monotypic ophidiid genus *Volcanus* Gosline, 1954 with *Luciobrotula* after examining the type species, *Volcanus lineatus* Gosline, 1954, concluding that there were no apparent differences between the two genera. In the same study, he also provided the first identification key for three nominal species including a newly described one, *L. corethromycter*, from the Atlantic Ocean. Later, Cohen (1981) described *L. nolfi* from the East Pacific, which was previously often misidentified as *L. bartschi* or *L. corethromycter*. Nielsen *et al.* (1999) conducted a thorough revision on the taxonomy of ophidiiform fishes. In that study, they also revised the identification key for four nominal species within the *Luciobrotula*. Nielsen (2009) carried out the latest review study of *Luciobrotula* by describing two additional species, *L. brasiliensis* from Brazil in the West Atlantic, and *L. coheni* from the Gulf of Panama in the East Pacific as well as providing an updated identification key for all known species of the genus.

Over the last 13 years, a total of 498 ophidiid fish samples were collected during 14 biodiversity expeditions (mainly in the tropical West Pacific) undertaken by the *Tropical Deep-Sea Benthos* (*TDSB*) program (Bouchet *et al*. 2008) and the cooperative project between Taiwan and France entitled “Taiwan-France marine diversity exploration and evolution of deep-sea fauna” (TFDeepEvo, 2013–2016). Among them, only three specimens of *Luciobrotula* were collected from the Philippine Sea, Bismarck Sea, and Solomon Sea. These specimens were tentatively identified as *L. bartschi* as this was the only nominal species known from the West Pacific. However, after re-examining them based on a mitochondrial gene, we found that they are genetically distinct from each other. The purpose of this study is to validate the specimen collected from the Solomon Sea as representing a new species of *Luciobrotula* by using an integrated approach in taxonomy (Dayrat 2005; Hung *et al.* 2017; Lo *et al.* 2017; Lee S.-H. *et al.* 2019). The new species is herein described and an updated identification key for congenic species is provided.

Material and methods

Sample collection

The three examined samples of *Luciobrotula* (sample ID: ASIZP 0913925, PNG1082, and PNG2363) were collected from the Philippine Sea, Bismarck Sea, and Solomon Sea (Fig. 1) during three *TDSB* expeditions, AURORA, PAPUA NIUGINI, and MADEEP, carried out in 2007, 2012, and 2014, respectively. Detailed information about the expeditions can be referenced at https://expeditions.mnhn.fr/. A small piece of muscle was excised from each sample and preserved in 95% ethanol for molecular examination. The specimens were then photographed before fixing with 10%

formalin and later transferred to 70% ethanol for long-term preservation. The specimens were deposited in the ichthyological collections of the NTUM and ASIZP.

Institutional abbreviations

NTUM = National Taiwan University Museums, Taipei
ASIZP = Academia Sinica, Taipei

Morphological examination

The three specimens collected in this study and four voucher specimens of *Luciobrotula bartschi* (ASIZP 0070170, ASIZP 0063749, ASIZP 0066071, and ASIZP 0075076) from the East China Sea and South China Sea (Fig. 1) were morphologically examined (see below and Appendix). Methods for measuring, counting, and general terminology followed Nielsen (2009). Specimens were measured with a dial caliper to the nearest 0.1 mm. Internal osteological characters were examined through radiographs. Color pattern was based on freshly collected specimens and photos, with additional information provided after preservation.

DNA data collection

Total genomic DNA was extracted from each tissue using a commercial DNA extraction kit and a robot (LabTurbo 48 Compact System extractor, Taigene Biosciences Corp., Taipei, Taiwan) following the manufacturer's protocols. The cytochrome c oxidase subunit I (*COI*) gene was chosen as a marker for molecular examination of the specimens. A polymerase chain reaction (PCR) was used to amplify the

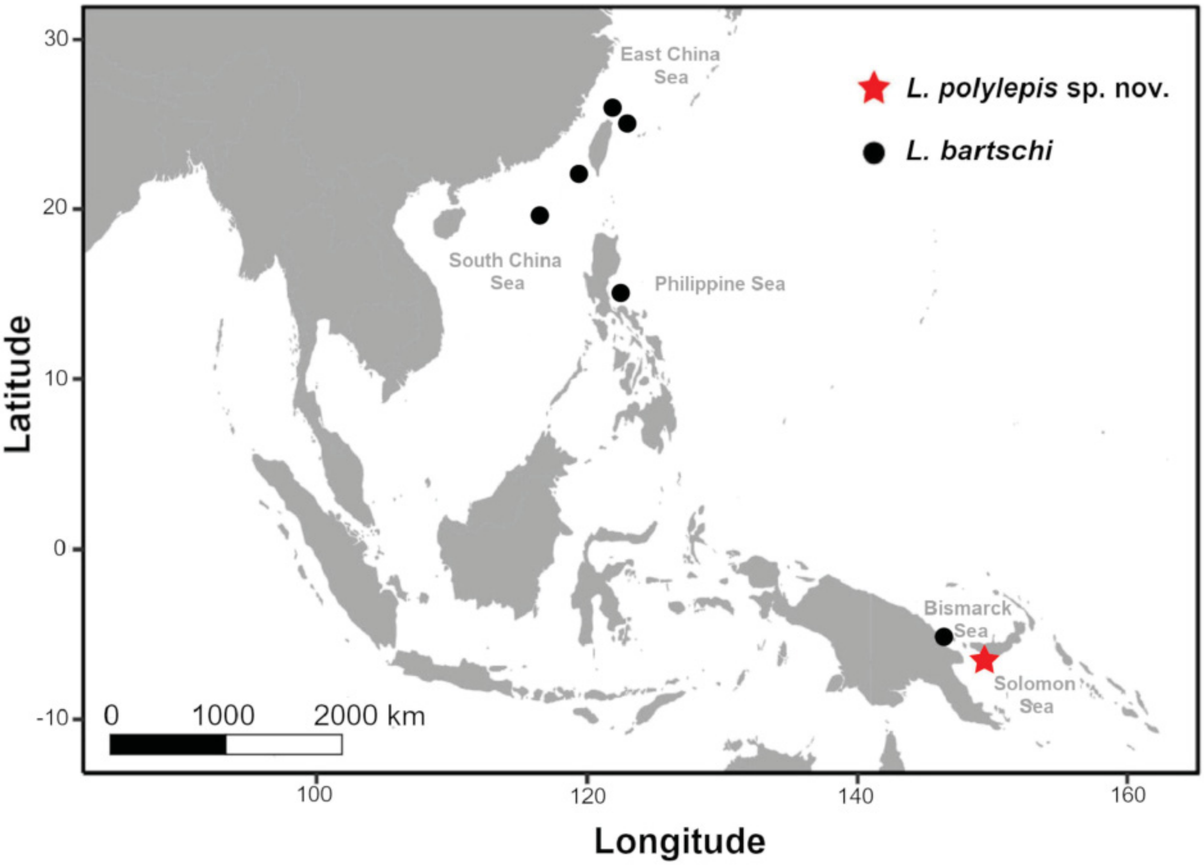

Fig. 1. Distribution of *Luciobrotula polylepis* sp. nov. (red star) and *L. bartschi* Smith & Radcliffe 1913 (solid circles) based on specimens examined in this study.

target gene fragment using the universal fish primers provided in Ward *et al*. (2005). PCR was carried out in a 25 µl volume containing 9 µl sterile distilled water, 0.5 µl of each primer (10 µM), 12.5 µl of EmeraldAmp MAX HS PCR Master Mix (TaKaRa), and 2.5 µl of DNA template (around 10~20 ng). The thermal cycling profile for amplification consisted of an initial denaturation stage (95°C, 60 sec), followed by 35 cycles each with a denaturation step (95°C, 30 sec), an annealing step (51°C, 30 sec), and an elongation step (72°C, 40 sec), before a final extension stage (72°C, 7 min). The successfully amplified products were then purified using the AMPure magnetic bead cleanup protocol (Agencourt Bioscience Corp.) and sequenced by Sanger sequencing at Genomics BioSci and Tech (Taipei). The same primers used for PCR were also used for sequencing; only the forward *COI* primer was used.

Sequence alignment and phylogenetic analysis

The obtained *COI* sequences were viewed and edited using CodonCode Aligner ver. 7.2.1 (CodonCode Corporation, Dedham, MA, USA) and were then aligned with eight other homologous sequences of *Luciobrotula* species retrieved from GenBank (NCBI, Nation Center for Biotechnology Information) (n = 7) and BOLD (The Barcode of Life Data Systems) (n = 1) (Table 1) using the automatic multiple-alignment program MUSCLE (Edgar 2004). MEGA X (Kumar *et al*. 2018) software was further used to manage the compiled dataset and compute pairwise distances of compared sequences with the Kimura-2-Parameter model (K2P) (Kimura 1980). The phylogenetic analysis was conducted based on the compiled *COI* dataset using the maximum likelihood method (ML) with the nucleotide substitution model GTR+G as implemented in the software RAxML ver. 8.0.4 (Stamatakis 2014). Nodal support was assessed with bootstrapping (Felsenstein 1985) under the ML criterion, based on 1000 pseudo-replicates. *Neobythites bimarginatus* Fourmanoir & Rivaton, 1979 and *Neobythites stigmosus* Machida, 1984 were used as outgroups to root the inferred *COI* tree.

Species delimitation analysis

The same *COI* gene dataset was used in three DNA-based species delimitation analyses, Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2012), Bayesian based Poisson Tree Processes (bPTP) (Zhang *et al.* 2013), and Character-Based DNA Barcoding (CBB) (Desalle *et al.* 2005). ABGD is a tool for detecting significant differences between intra- and interspecific variation (barcode gap) by examining pairwise genetic distances. Operational taxonomic units (OTUs) or putative species were redefined through the analytical algorithm. The analysis was performed at the web interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the default value (1.5) for relative gap width (X), and the intraspecific divergence (P) value (0.001 to 0.1) with 20 steps under the K2P distance.

bPTP is a method for delimiting species based on a rooted phylogenetic tree, and mutations are modeled as speciation or branching events. Here, we used the inferred *COI* gene tree (see above) as the input tree. The bPTP settings are: number of MCMC generations = 100 000; thinning = 100; burn-in = 0.1; and seed = 123. The analysis was performed at the web interface available from https://species.h-its.org/.

CBB is a method derived from the standard DNA barcoding approach (Hebert *et al.* 2004; Ward *et al.* 2005). Instead of simple cut-off distance thresholds for delimiting the species presented in the DNA dataset, the putative species are identified through the presence or absence of discrete and unique nucleotide substitutions within the DNA sequences of taxa (Desalle *et al.* 2005; Rach *et al.* 2008; Brower *et al.* 2010; Guimarães *et al.* 2020). Here, MEGA X software and inferred *COI* gene tree were used to determine apomorphic nucleotide sites in *L. polylepis* sp. nov. at the *COI* locus. Numbering of the determined nucleotide sites starts from the first nucleotide of the gene defined through sequence alignment with the complete *COI* sequence retrieved from the whole mitochondrial genome of *Neobythites unimaculatus* Smith & Radcliffe 1913 (AP018428: 5544-7094).

The congruent results from bPTP and ABGD analyses were considered to be primary support of the OTUs (i.e., inferred potential species); other criteria (CBB result, morphological evidence, etc.) were also used for final validation of delimited species.

Table 1. List of samples used for molecular analyses in this study, with sampling location and GenBank/BOLD accession numbers of nucleotide sequences at *COI* locus. Numbers in bold: sequences newly obtained in this study

Taxa	Voucher specimens	Sample ID	Locality[**]	Latitude	Longitude	Depth (m)	GenBank/ BOLD system accession no.
Neobythites bimarginatus[*]	NTUM 17062	NC1657	Coral Sea	23°34′ S	169°37′ E	340	**MW315789**
Neobythites stigmosus[*]	NTUM 14381	WJC7153	Japan (Tosa Bay, Mimase fishing port)	NA	NA	NA	**MW315788**
Luciobrotula bartschi	ASIZP 0075076	ASIZP 0916618	South China Sea	22°12′ N	120°23′ E	68–347	KU943175
Luciobrotula bartschi	ASIZP 0066071	ASIZP 0911588	South China Sea	20°43′ N	117°32′ E	954	KU943157
Luciobrotula bartschi	ASIZP 0068164	ASIZP 0913925	Philippine Sea (CP2729)	15°19′ N	121°37′ E	593–600	**MW315786**
Luciobrotula bartschi	NTUM 16627	PNG1082	Bismarck Sea (CP4033)	4°52′ S	145°53′ E	780	**MW315787**
Luciobrotula bartschi	CSIRO H 6593-01	BW-A4706	Northwest Cape Leveque of Australia	14°36′ S	121°20′ E	705	FOAG829-08
Luciobrotula polylepis sp. nov.	NTUM 11915	PNG2363	Solomon Sea (CP4334)	6°08′ S	149°10′ E	430–620	**MW218670**
Luciobrotula coheni	USNM 422550	MOP110383	Off Costa Rica	9°12′ N	84°29′ W	656–668	MF956762
Luciobrotula coheni	USNM 421491	MOP110705	Gulf of Panama	7°24′ N	78°7′ W	165–183	MF956763
Luciobrotula coheni	USNM 421356	MOP110782	Off Panama	7°0′ N	81°43′ W	716–842	MF956764
Luciobrotula coheni	USNM 421528	MOP110488	Gulf of Panama	7°37′ N	78°41′ W	115–116	MF956765
Luciobrotula coheni	USNM 421217	MOP110814	Off Costa Rica	9°25′ N	85°9′ W	841–920	MF956766

[*] = Outgroups used.
[**] = details on collection information can be referenced at our survey database of the *TDSB* available from https://expeditions.mnhn.fr/.

Results

Class Actinopterygii Klein, 1885
Order Ophidiiformes Berg, 1937
Family Ophidiidae Rafinesque, 1810

Genus ***Luciobrotula*** Smith & Radcliffe, 1913

Molecular phylogeny and species delimitation

The *COI* dataset comprised 13 aligned sequences including three newly obtained sequences from the collected specimens, three additional sequences of *L. bartschi* from the South China Sea and Western Australia, five sequences of *L. coheni* from the Eastern Pacific, plus two outgroup sequences (Table 1). The length of the aligned sequences of the dataset is 618 bp. Figure 2 shows the phylogenetic tree inferred from the ML analysis based on the dataset. The monophyly of the genus *Luciobrotula* is strongly supported (bootstrap value = 98%), and ingroup sequences form three clades or lineages among which two contain sequences from the two known species (Fig. 2). While two of our newly obtained sequences (ASIZP 0913925 and PNG1082) fall into the *L. bartschi* clade, the third one (PNG2363) appears to be a previously unknown lineage. Advanced species delimitation analyses with ABGD and bPTP based on the same *COI* dataset reveal a congruent result with a prediction of three OTUs, corroborating the phylogenetic finding (Fig. 2). The delimited OTUs (or inferred species) are genetically distinct from each other. The unknown lineage is distinct from others by 37 unique nucleotide sites based on CBB analysis. The average genetic distances measured using the K2P model among them are from 0.130 to 0.138 at the *COI* locus. Further morphological examination on the specimens indicates that the features of the sample collected from the Solomon Sea (PNG2363) are unique among all known *Luciobrotula* species (see below), and we validate it herein as a new species.

Fig. 2. Phylogenetic tree of species of *Luciobrotula* Smith & Radcliffe 1913 inferred by the partitioned maximum-likelihood method with GTR+G nucleotide substitution model based on the *COI* gene dataset, and results from species delimitation based on *COI* gene analyses with ABGD, bPTP and CBB. Branch lengths are proportional to inferred nucleotide substitutions. Numbers at nodes represent bootstrap values in percentages. Values < 50% are not shown. Taxa names in bold indicate newly obtained sequences in this study.

Description of new species

Luciobrotula polylepis sp. nov.

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Figs 3–4; Table 2

Diagnosis

Luciobrotula polylepis sp. nov. is morphologically distinct from all congeners by the following combination of characters: lateral line ending below 33rd dorsal-fin ray; dorsal-fin rays 86, anal-fin rays 70, precaudal vertebrae 13, total vertebrae 50; gill rakers 17 (3 long rakers and 14 dentigerous plates); longest gill raker 2.1% SL; height of posterior margin of maxilla 3.2% SL; distance from the snout to end of lateral line 60% SL; one interorbital pore and four occipital pores.

Differential diagnosis

The new species is most similar to *L. brasiliensis* because both share the low number of vertebrae. It differs from *L. brasiliensis* by having a much longer lateral line (ending at the 33rd dorsal-fin ray vs ending at the 2nd dorsal-fin ray), a slightly more posterior position of the anal-fin origin (first anal ray below dorsal ray no. 22 vs first anal ray below dorsal ray no. 17), more pectoral-fin rays (32 vs 26), more gill rakers (17 vs 13–14), longer gill raker on first arch (2.1% SL vs 1.2% SL).

Luciobrotula polylepis sp. nov. differs from *L. bartschi* (Figs 5–7) in having a slightly longer lateral line (ending at the 33rd dorsal-fin ray vs the 18th–26th dorsal-fin ray) and narrower posterior margin of maxilla (3.2% SL vs 3.6–4.7% SL).

It differs from *L. coheni* by having more anal-fin rays (70 vs 59–65), fewer total gill rakers (17 vs 21–26), a more anterior anal-fin origin (anterior anal-fin ray below 17th vertebra vs anterior anal-fin ray below 21st–22nd vertebrae), a narrower interorbital space (3.5% SL vs 3.9–5.6% SL), and a narrower posterior margin of the maxilla (3.2% SL vs 3.9–4.9% SL).

It differs from *L. corethromycter* by having fewer dorsal-fin rays (86 vs 91–96), fewer gill rakers (17 vs 18–21), and anterior position of the anal-fin origin (first anal-fin ray below the 17th vertebra vs first anal-fin ray below the 20th–22nd vertebrae).

It differs from *L. nolfi* by having a slightly longer lateral line (ending at the 33rd dorsal-fin ray vs ending at the 27th–31st dorsal-fin ray), slightly more anterior position of the anal-fin origin (first anal-fin ray below the 17th vertebra vs first anal-fin ray below the 19th–20th vertebrae), smaller head (23.9% SL vs 24.5–28.0% SL), and relatively deeper body (16.3% SL vs 12.5–15.0% SL).

It differs from *L. lineata* by having a much longer lateral line (ending at the 33rd dorsal-fin ray vs ending at the 2nd dorsal-fin ray), fewer dorsal-fin rays (86 vs 92), more pectoral-fin rays (32 vs 26), shorter pelvic-fin rays (10.9% SL vs 15.0% SL) and longer gill raker on the first arch (2.1% SL vs 0.7% SL). A detailed comparison between the new species and other congeners is provided in Table 2.

Along the *COI* gene, the following apomorphic sites are unique nucleotides from the only specimen of *L. polylepis* sp. nov. examined here; these nucleotide sites can be used for the molecular diagnosis of the species to differentiate it from *L. coheni* and *L. bartschi* examined in this study. Nos. 97 (C vs T), 120 (A vs G), 147 (G vs A), 177 (G vs A), 180 (C vs T), 198 (C vs T), 219 (T vs C), 225 (C vs T), 294 (C vs T), 321 (A vs C), 324 (G vs A), 330 (A vs G), 336 (A vs C), 348 (G vs A or C), 363 (G vs T or C), 369 (T vs C), 372 (A vs C or T), 375 (C vs T), 381 (G vs A), 387 (C vs T), 390 (T vs C), 405 (C vs T), 420 (A vs G or C), 426 (T vs C), 465 (A vs C or G), 477 (A vs G), 540 (A vs G), 555 (G vs A), 565 (T vs C), 597 (T vs C), 603 (T vs A), 615 (C vs A), 648 (T vs C), 682 (C vs A or G), 675 (C vs T), 684 (G vs A), 687 (T vs C).

Table 2. Counts and measurements for species of *Luciobrotula* Smith & Radcliffe, 1913.

	L. polylepis* sp. nov.*	***L. bartschi*****	***L. bartschi****	***L. brasiliensis****	***L. coheni****	***L. corethromycter****	***L. lineata****	***L. nolfi****
	Holotype	**n = 6**	**n = 9**	**n = 1**	**n = 9**	**n = 9**	**n = 1**	**n = 8**
Standard length (SL, mm)	168.4	97.9–393.7	85.0–405.0	275.0	164.0–455.0	123.0–534.0	267.0	137.0–590.0
Counts								
Dorsal-fin rays	86	85–92	87–94	84	81–89	91–96	92	86–95
Caudal-fin rays	11	10–11	11–12	12	11	10	12	10–11
Anal-fin rays	70	68–75	69–75	69	59–65	68–74	76	66–70
Pectoral-fin rays	32	25–27	25–27	26	27–31	26–27	26	27–28
Pelvic-fin rays	2	2	2	2	2	2	2	2
Precaudal vertebrae	13	(14#)15–16	15–16	13	15	16	15	15–16
Total vertebrae	50	(49#)52–53	53–55	51	53–54	56–57	56	53–55
Pseudobranchial filaments	NA	NA	2	2	2	2	2–3	2
Ant. dorsal ray above vertebra no.	8	7–9	7–9	8	6–8	7–9	11	6–8
Ant. anal ray below dorsal ray no.	22	19–24	20–24	17	25–29	24–28	20	24–26
Ant. anal-fin ray below vertebra no.	17	17–20	18–20	17	21–22	20–22	20	19–20
End of lateral line below dorsal ray no.	33	18–26	19–26	2	28–37	24–32	2	27–31
Total gill rakers	17	14–16	16–17	13–14	21–26	18–21	16–17	16–19
Long gill rakers	3	3	3	3	3	3	3	3
Measurements in % of SL								
Head	23.9	22.6–24.9	22.5–26.5	22.5	24.5–30.5	21.0–25.0	22.5	24.5–28.0
Depth at origin of anal fin	16.3	13.4–17.6	12.5–16.5	14.5	14.5–17.5	12.0–15.5	15.0	12.5–15.0
Upper jaw	10.6	10.2–12.6	11.0–13.5	12.0	12.0–15.0	11.0–13.0	11.0	11.5–12.5
Dia. of eye window	2.9	2.6–3.2	2.4–3.1	2.5	2.7–3.3	2.4–2.9	3.3	3.0–3.5
Interorbital	3.5	3.4–3.9	3.4–4.5	3.8	3.9–5.6	3.8–4.5	3.3	4.0–5.0
Postorbital	14.3	14.1–15.9	15.0–16.5	15.0	16.5–19.5	13.0–16.5	15.0	15.0–16.5
Height of posterior margin of maxilla	3.2	3.7–4.5	3.6–4.7	4.5	3.9–4.9	3.3–4.9	NA	3.9–5.0
Snout to end of lateral line	59.6	47.5–50.7	48.0–56.0	34.0	60.0–71.0	45.5–57.0	33.0	51.0–55.0
Preanal	48.2	48.9–52.1	48.0–54.0	48.5	52.0–62.0	47.0–59.0	49.5	47.0–58.0
Predorsal	31.1	28.0–32.9	30.0–34.5	32.5	26.0–32.5	26.5–33.0	32.0	28.5–35.5
Base of pelvic to origin of anal fin	33.2	31.7–36.8	30.0–35.0	32.0	36.5–40.5	32.5–41.0	29.0	32.0–33.5
Pectoral fin	9.1	9.0–11.1	9.1–12.0	11.5	9.1–12.0	9.6–12.0	10.5	11.0–12.5
Pelvic-fin longest ray	10.9	10.5–14.2	11.5–16.0	14.5	11.5–15.0	7.9–9.6	15.0	11.0–12.5
Longest gill filament on anterior arch	1.1	1.3–1.8	1.3–2.5	1.1	2.8–3.6	1.3–1.8	1.0	1.4–2.0
Longest gill rakers on anterior arch	2.1	2.0–3.2	2.2–2.7	1.2	1.8–3.1	1.9–2.5	0.7	2.1–2.4

* Data from Nielsen (2009).

** Data from examined specimens of this study

\# Data (in the parentheses) from an abnormal vertebrae development specimen.

Etymology

The name *polylepis* is derived from the Greek '*poly*', meaning 'many' or 'numerous', and '*lepis*', meaning 'scales', in reference to the much longer lateral line and therefore more lateral line scales compared with *L. bartschi*, the only congener distributed in the West Pacific.

Type material

Holotype

SOLOMON SEA • 168.4 mm SL, sample ID: PNG2363; Ainto Bay, SE of New Britain Island, Papua New Guinea, Solomon Sea, West Pacific, stn CP4334; 6°08′ S, 149°10′ E; 430–620 m depth; 6 May 2014; R/V *ALIS*; French beam trawl; MADEEP expedition; GenBank registration: MW218670; NTUM 11915.

Description

Measurements and counts of the holotype given in Table 2. Body elongate with tapering caudal portion, snout and head slightly depressed; eye small and round, horizontal eye diameter about half of snout length. Mouth large, oblique; upper jaw reaching a vertical through the posterior margin of orbit,

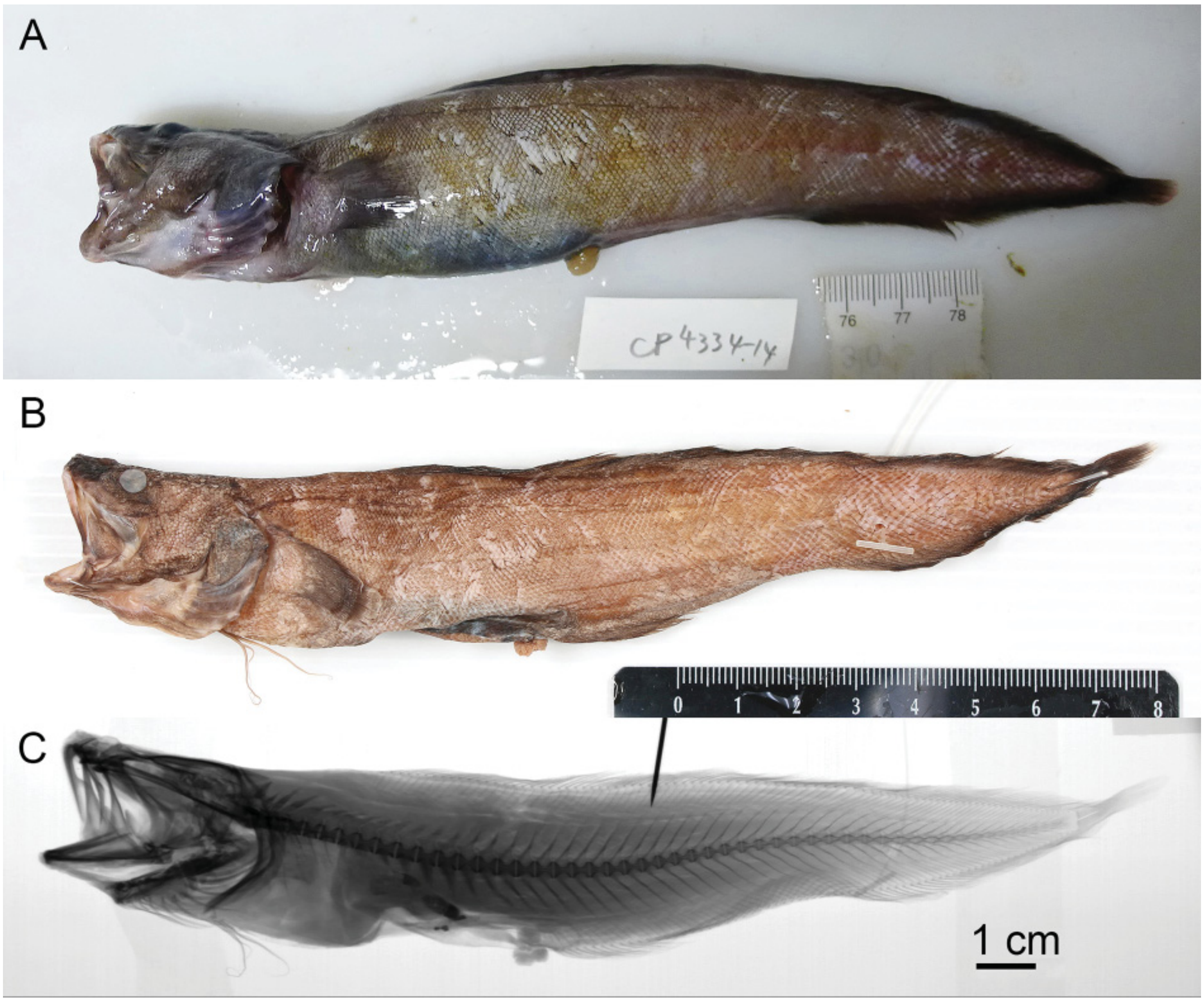

Fig. 3. Holotype of *Luciobrotula polylepis* sp. nov., 168.4 mm SL (NTUM 11915). **A**. Fresh specimen. **B**. Preserved specimen. **C**. Radiograph. Needle points to the lateral line end.

posterior part vertically much extended, slightly protruding beyond lower jaw when mouth closed. Boomerang-formed vomer; palatine, and upper and lower jaw with many small, close-set, rather blunt teeth in several irregular rows; fang-like teeth absent in both jaws. One median and a pair of two large basibranchial tooth patches. Anterior nostril with low rim and placed midway between upper lip and posterior nostril, with small rounded flap rising from anterior rim. Posterior margins of preopercle, interopercle, and subopercle rounded, without spine. First gill arch with four finely dentigerous plates on upper branch, one long raker on the angle, and lower branch with two long rakers interspaced with 10 dentigerous plates (Fig. 4D); gill filaments ca 100, the longest about half as long as longest gill raker; pseudobranchial filament damaged, unavailable count.

Sensory pores are found all over head (Fig. 4A–B). Supraorbital with group of eight pores behind eye, five pores immediately above eye, and five small pores in a row on tip of snout, larger pore between flaps on tip of snout, and above each nostril, one interorbital pore, four occipital pores, six suborbital pores

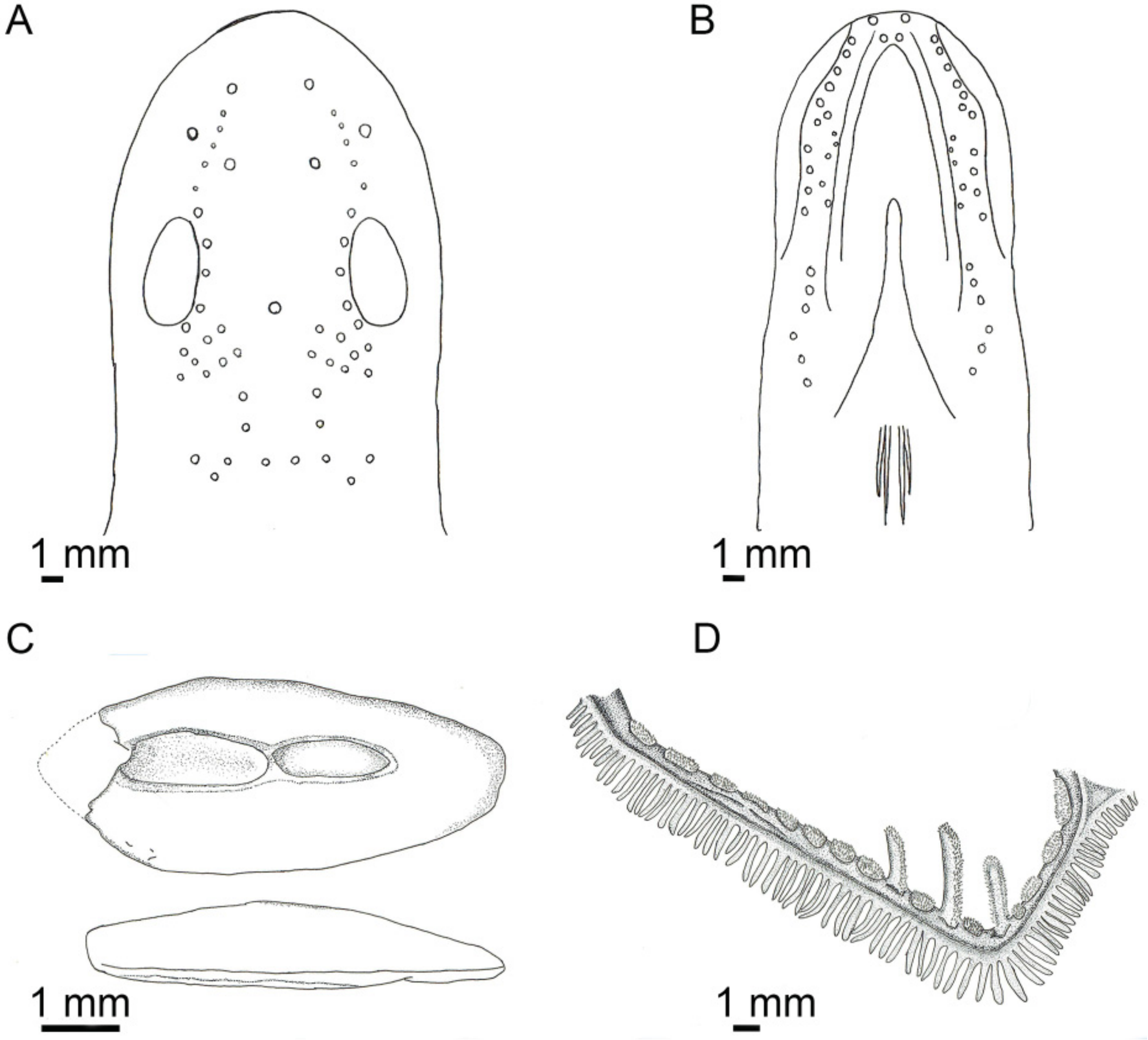

Fig. 4. *Luciobrotula polylepis* sp. nov., holotype (NTUM 11915). **A**. Dorsal view of sensory pores on head. **B**. Ventral view of sensory pores on head. **C**. Median and ventral view of right sagitta. **D**. First gill raker (left side).

and four mandibular pores, 10 small pores close to lower jaw, between this row and mandibular having four small pores, and finally a row of six pores above posterior mandibular, two pores behind posterior end of maxilla, and preopercle with six pores.

Sagittal otolith is elongate and thin, about 2.5 times as long as high. Sulcus divided into ostium and cauda. Cauda is about ⅔ of ostium (Fig. 4C). Due to the damaged anterior rim, the presence of an ostial channel could not be ascertained.

Body, top of head, and opercle covered with small cycloid scales, with ca 72 scales in oblique line from origin of anal fin forwards and ca 111 scales from upper part of gill slit to base of caudal fin; single lateral line originating at upper angle of opercle and extending posteriorly in straight line placed about midway between midline and profile of body, ending below 33rd dorsal-fin ray. Dorsal-fin origin above end of pectoral fin; anal-fin origin at about mid-body of fish, pectoral fin placed medially and pelvic fin reaching one third from base to anal fin.

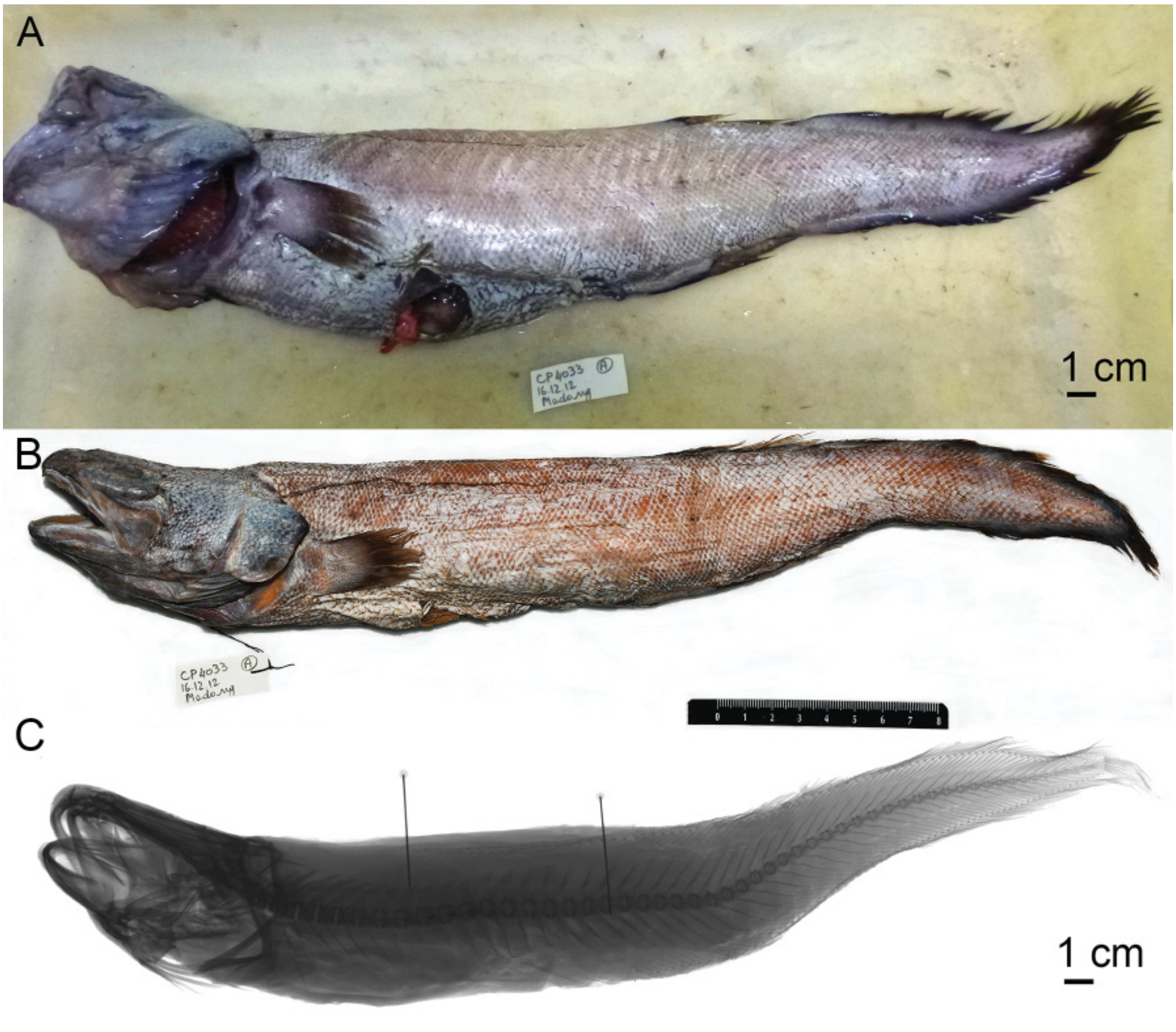

Fig. 5. *Luciobrotula bartschi* Smith & Radcliffe 1913. Voucher specimen, 393.7 mm SL (NTUM 16627). **A**. Fresh specimen. **B**. Preserved specimen. **C**. Radiograph. Second needle points to the end of the lateral line.

Third neural spine pointed, length of first spine half as long as second spine (Fig. 3C), neural spines of posterior 10 pre-caudal vertebrae with blunt tips and broad bases, 4th–11th precaudal vertebrae with broad bases and depressed neural spines, 7th–13th precaudal vertebrae with parapophyses, and pleural ribs on 3rd–6th precaudal vertebrae. Epipleural ribs hard to observe.

Head brown; body brownish-yellow with bluish-brown abdomen (Fig. 3A). Dorsal, pectoral, anal, and caudal fins black. Color of preserved specimen similar to that of fresh specimens, the head and body uniformly brown with dark bluish-brown abdomen (Fig. 3B).

Distribution

Possibly endemic to waters off Papua New Guinea; the only known specimen was collected on the SE continental slope of New Britain Island, Papua New Guinea, at depths of 430–620 m (Fig. 1).

Accompanying fauna

Monomitopus sp. and *Glyptophidium lucidum* Smith & Radcliffe, 1913 were the only two other ophidiids collected along with *L. polylepis* sp. nov., in addition to *Epigonus atherinoides* (Gilbert, 1905) (Epigonidae Poey, 1861) (Okamoto *et al.* 2018). The mud bottom living invertebrates collected from the same site included sea cradles, sea snails, sea stars, deep-sea barnacles, decapods (https://expeditions.mnhn.fr/campaign/madeep/event/cp4334#les_photos), and a recently described deep-sea spider crab, *Tunepugettia corbariae* Lee, Richer de Forges & Ng 2019 (Epialtidae MacLeay, 1838) (Lee B.-Y. *et al.* 2019).

Fig. 6. *Luciobrotula bartschi* Smith & Radcliffe 1913. Voucher specimen, 301.7 mm SL (ASIZP 0068164). **A**. Preserved specimen. **B**. Radiograph. Needle points to the end of the lateral line.

Key to all known species of Luciobrotula Smith & Radcliffe, 1913

(modified from Nielsen 2009)

1. Precaudal vertebrae 13; total vertebrae 50 or 51 2
– Precaudal vertebrae 15 or 16; total vertebrae 52–57 3

2. Lateral line ending at 2nd dorsal-fin ray; total gill rakers 13–14 .... ***L. brasiliensis*** Nielsen, 2009 (off Brazil)
– Lateral line ending at 33rd dorsal-fin ray; total gill rakers 17 .... ***L. polylepis*** sp. nov. (off Papua New Guinea)

3. Lateral line short and distinct, ending at 2nd dorsal-fin ray....***L. lineata*** (Gosline, 1954) (off Hawaii)
– Lateral line long, usually indistinct, ending at 19th–37th dorsal-fin ray.......... 4

4. Dorsal-fin rays 81–89; anal-fin rays 59–65; first gill arch with 3 developed rakers and 18–23 dentigerous plates; longest filaments on first gill arch 2.8–3.6% SL ....***L. coheni*** Nielsen, 2009 (East Pacific)
– Dorsal-fin rays 86–96; anal-fin rays 66–75; first gill arch with 3–4 developed rakers and 12–18 dentigerous plates; longest filaments on fist gill arch 1.3–2.7% SL 5

5. Four occipital pores, one interorbital pore; first anal-fin ray below 18th–24th dorsal-fin rays; dorsal rim of otolith without concavity (large specimens) .... ***L. bartschi*** Smith & Radcliffe, 1913 (Indo-West Pacific)
– Pores absent on occipital and interorbital region; first anal-fin ray below 24th–28th dorsal-fin rays; dorsal rim of otolith with or without concavity 6

6. Head brown; otolith with a distinct concavity in dorsal rim in specimens larger than 300 mm SL; total vertebrae 56–57; longest pelvic-fin ray 7.9–9.6% SL .... ***L. corethromycter*** Cohen, 1964 (Gulf of Mexico, Caribbean Sea)
– Head pale; otolith without a distinct concavity in dorsal rim in specimens larger than 300 mm SL; total vertebrae 53–55; longest pelvic-fin ray 11.0–12.5% SL.......... ***L. nolfi*** Cohen, 1981 (tropical East Atlantic)

Discussion

The number of vertebrae is an important diagnostic character in distinguishing species of *Luciobrotula*. Based on that, the species of this genus can be split into two groups, either possessing 13 precaudal vertebrae or possessing 15 or 16 precaudal vertebrae. Our newly described species, *L. polylepis* sp. nov., is grouped together with *L. brasiliensis* in having a lower precaudal vertebrae count. Another group with a higher precaudal vertebrae count consists of *L. bartschi*, *L. coheni*, *L. corethromycter*, *L. lineata*, and *L. nolfi*. In this study, six specimens of *L. bartschi* were examined (Table 2). We noticed that one of them (ASIZP 0063749) failed to reach the measurement range of the species. In fact, ASIZP 0063749 has fewer precaudal vertebrae (14 instead of 15–16) and fewer total vertebrae (49 instead of 52–55). When we further examined this specimen through radiographs, we observed that the 24th and 25th, and the 28th and 29th vertebrae of ASIZP 0063749 were fused together (Fig. 7) and that it should be regarded as a specimen with abnormal vertebrae. In addition to the number of vertebrae, the lateral line length is another important character for identifying species of *Luciobrotula*. *Luciobrotula brasiliensis* and *L. lineata* possess lateral lines reaching below the second dorsal-fin ray; others, including *L. polylepis* sp. nov., possess lateral lines reaching beyond the 19th dorsal-fin ray.

Among the species of *Luciobrotula*, *L. bartschi* is the only known widespread species. In the West Pacific, its distribution ranges from the Philippines north to Japan and west to Hawaii; it was also recorded from Papua New Guinean waters, first by Nielsen & Møller (2008) (n = 1), and later by Fricke *et al.* (2014) (n = 1). However, the specimen of '*L. bartschi*' (NTUM 10054) examined by Fricke *et al.* (2014) represented a misidentification. Upon our reexamination, we found that it possesses a combination of characters (a copulatory organ and the caudal fin fused with the dorsal and anal fins) that matches fishes from another ophidiiform family, the Bythitidae Gill, 1861 (Møller *et al.* 2016). Nevertheless, the six samples of *L. bartschi* examined in this study were all collected from sites within the reported range of the species (Fig. 1). The new species is possibly endemic to Papua New Guinea, as it is so far known from its type locality only. These two western Pacific species appear to share a similar bathymetric range (430–620 m vs 400–2283 m depth) and habitat (mud bottom), and both are found in Papua New Guinean waters. Certainly, their distribution and ecology require more investigations.

Specimens of *Luciobrotula* seem to be rare. Despite intensive sampling efforts from either local organizations in Taiwan or international expeditions through the *TDSB* for over a decade, only a few specimens were made available for scientific investigations. The difficulty in sampling has limited our understanding of biodiversity, phylogeny, biogeography, and ecology of deep-sea fishes such as those from the rare genus *Luciobrotula* or others (e.g., *Chelidoperca* Boulenger, 1895) (Lee S.-H. *et al.* 2019). In spite of that, in this study we successfully uncover the hidden diversity of the *Luciobrotula* in the West Pacific using an integrated approach in taxonomy and conduct the first phylogenetic study of *Luciobrotula*. From the inferred phylogenetic tree, the two western Pacific species of *Luciobrotula* are shown to be distantly related to each other despite their geographic proximity. Our preliminary phylogenetic result also indicates that the species (*L. bartschi* and *L. coheni*) sharing a similar morphology may not be closely related.

Fig. 7. *Luciobrotula bartschi* Smith & Radcliffe 1913. Voucher specimen, 181.6 mm SL (ASIZP 0063749). **A**. Preserved specimen. **B**. Radiograph. Needle points to the end of the lateral line. Arrows point to fused vertebrae.

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Appendix

Comparative material

Luciobrotula bartschi Smith & Radcliffe, 1913
(6 specimens, 97.9–393.7 mm SL)

BISMARCK SEA • 393.7 mm SL; Cape Croisilles off Papua New Guinea, stn CP4033; 4°52′ S, 145°53′ E; 780 m depth; R/V ALIS, beam trawl, PAPUA NIUGINI expedition; 16 Dec. 2012; NTUM 16627 (tissue sample ID: PNG1082).

EAST CHINA SEA • 181.6 mm SL; stn CD210, 24° 28′ N, 122° 12′ E; 1185 m depth; beam trawl; 30 May 2003; ASIZP0063749.

PHILIPPINE SEA • 301.7 mm SL; stn CP2729, 15°19′ N, 121°37′ E; 593–600 m depth; R/V DA-BFAR, beam trawl, AURORA expedition; 31 May 2007; ASIZP 0068164 (tissue sample ID: ASIZP 0913925).

TAIWAN • 227.4 mm SL; Dashi fishing port; 23 May 2007; ASIZP0070170.

TAIWAN • 97.9 mm SL; South China Sea, NE of Dongsha Island; stn CD321; 20° 43′ N, 117° 32′ E; 954 m depth; beam trawl; 19 Aug. 2005; ASIZP 0066071 (tissue sample ID: ASIZP0911588).

TAIWAN • 202 mm SL; South China Sea, SE of Little Liuqiu Island; 22° 12′ N, 120° 23′ E; 68–347 m depth; beam trawl; 29 Jul. 2014; ASIZP 0075076 (tissue sample ID: ASIZP 0916618).