FULL PAPER

Cusk-eel confusion: revisions of larval *Luciobrotula* and *Pycnocraspedum* and re-descriptions of two bythitid larvae (Ophidiiformes)

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Abstract

Since 2006, an ophidiiform larva with an ovoid body, elongate anterior dorsal-fin ray, and long trailing fleshy filament has been identified as *Pycnocraspedum squamipinne*. Similarly, the larvae of the ophidiid genus *Luciobrotula* have been tentatively identified since 1988, with posteriorly displaced dorsal fins and bulging or exterilium guts. However, neither of these larval forms morphologically agree with their adult counterparts. Recently, blackwater divers captured and photographed specimens of larval *Luciobrotula* and *Pycnocraspedum* off the coast of Hawai'i and Florida, making them available for both morphological and molecular sampling. After examining these larvae and analyzing DNA barcode sequences, as well as a newly captured and sequenced adult of *Pycnocraspedum phyllosoma*, we revise the previously identified "*Pycnocraspedum*" larvae to species of *Luciobrotula*. We describe the larvae of *Luciobrotula bartschi* and *Luciobrotula corethromycter* for the first time, highlighting an extraordinary loss of multiple anterior dorsal-fin elements in their ontogeny. We also generate the first DNA sequences for *L. corethromycter* and *P. phyllosoma*, adding to the depauperate number of sequences available for ophidiiforms. For the previously identified "*Luciobrotula*" larvae, neither morphological nor molecular characters provide definitive identification other than recovering them among the Bythitidae. We provide new morphological observations, revised descriptions, and generate a phylogeny of ophidiiform fishes based on COI to place these larvae in a phylogenetic context, prompting further investigation into the relationships of the Ophidiiformes using additional genetic markers. Our study emphasizes the importance of blackwater diving to improving our understanding of marine larval fishes and the need for additional molecular sampling of the diverse order of brotulas, cusk-eels, pearlfishes, and their allies.

Keywords *Benthocometes* · Blackwater · COI · Integrative taxonomy · Ophidiidae

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"Ophidiiform fishes, in general, are too poorly known anatomically to resolve questions of phylogenetic relationships (Cohen & Nielsen 1978), and details of ontogeny, including developmental osteology, have been described for too few genera to contribute to a resolution of these questions" (Fahay and Hare in Richards 2005).

Introduction

In 2006, Evseenko and Okiyama described a remarkable ophidiid larva from the New Guinean "Dana" larval flatfish collection. The 22.5 mm standard length (SL) specimen possesses many bothid-like features, including an ovoid body, elongate anterior dorsal-fin ray, long and thin cartilaginous ventral process of the coracoid, and a protruding, but not exterilium, gut (see Fraser and Smith 1974). The larva also

has a long fleshy appendage trailing away from the body, attaching near the anus (Evseenko and Okiyama 2006: fig. 1; Fig. 1). Based on a suite of characters, the authors placed the larva among the Ophidiiformes and within Group 1 of Howes (1992) classification, which includes the genera *Brosmophyciops*, *Brotula*, *Cherublemma*, *Dicrolene*, *Genypterus*, *Glyptophidium*, *Hoplobrotula*, *Hypopleuron*, *Lamprogrammus*, *Lepophidium*, *Monomitopus*, *Neobythites*, *Ogilbia*, *Ophidion*, *Parophidion*, *Petrotyx*, *Pycnocraspedum*, and *Sirembo*. Highlighting the anterior position of the dorsal fin (compare Figs. 1 with 2a–b), the number of elongate gill rakers on the first arch (4), and the first rib attaching to the vertebral centrum, Evseenko and Okiyama (2006) identified the larva as "*Pycnocraspedum squamipinne*" (hereafter, taxonomic names in quotes refer to larval identifications that are revised in this study). However, the authors noted a "lack of agreement" in the number of precaudal vertebrae and dorsal-fin rays between their larva and adult *Pycnocraspedum*, with the larva having a greater number for both (14 or 15 larval vs. 12–13 adult precaudal vertebrae; 102 larval vs. 88–92 adult dorsal-fin rays; see Evseenko and Okiyama 2006: 195). Counts of fin rays, myomeres, and vertebrae are critical to the recognition and diagnosis of larval fishes (e.g., Moser et al. 1984; Okiyama 1988, 2014; Richards 2005; Fahay

2007), and such discrepancies in counts could indicate the larva belongs to another genus or group of ophidiids.

In discussing Group 1 of his classification, Howes (1992: 117) noted the genus *Luciobrotula* (Figs. 2c–d) "possibly also belongs with this group" based on expanded second and third ribs and connections between these ribs and the gas bladder. The larvae of *Luciobrotula* were tentatively identified by Okiyama (1988, 2014; translated by an author) based on specimens 10.1–23.0 mm SL from the waters of Japan and the Philippines. These described "*Luciobrotula*" larvae have compressed bodies, various levels of bulging or exterilium guts, posteriorly displaced dorsal fins, and 13 caudal-fin rays (see Okiyama 2014: 434–435; Fig. 3). In 2014, Okiyama separated these larvae into four types (types 1–4) largely based on differences in dorsal- and anal-fin-ray counts, pigmentation, and exterilium-gut length relative to SL. Okiyama (2014) also noted that the amount of variation seen in these four types of "*Luciobrotula*" larvae exceeds the known number of species that occur in Japan (only *Luciobrotula bartschi*) and listed these larvae as "*Luciobrotula*?" to indicate their tentative identification.

Blackwater diving, nighttime open water drift dives (see Nonaka et al. 2021 for more information), and photography have opened new opportunities to learn about marine larval

Fig. 1 Larvae of *Luciobrotula* from the Hawaiian Islands, morphologically similar to larval "*Pycnocraspedum*" described by Evseenko and Okiyama (2006). **a** Blackwater photo *L. bartschi*, USNM 454562, captured by A. and N. Deloach, offshore of Kona, Hawai'i, 11 November 2021; **b** preserved USNM 454562; **c** preserved *L.* cf. *bartschi*, USNM 454451. Scale bars = 1 cm
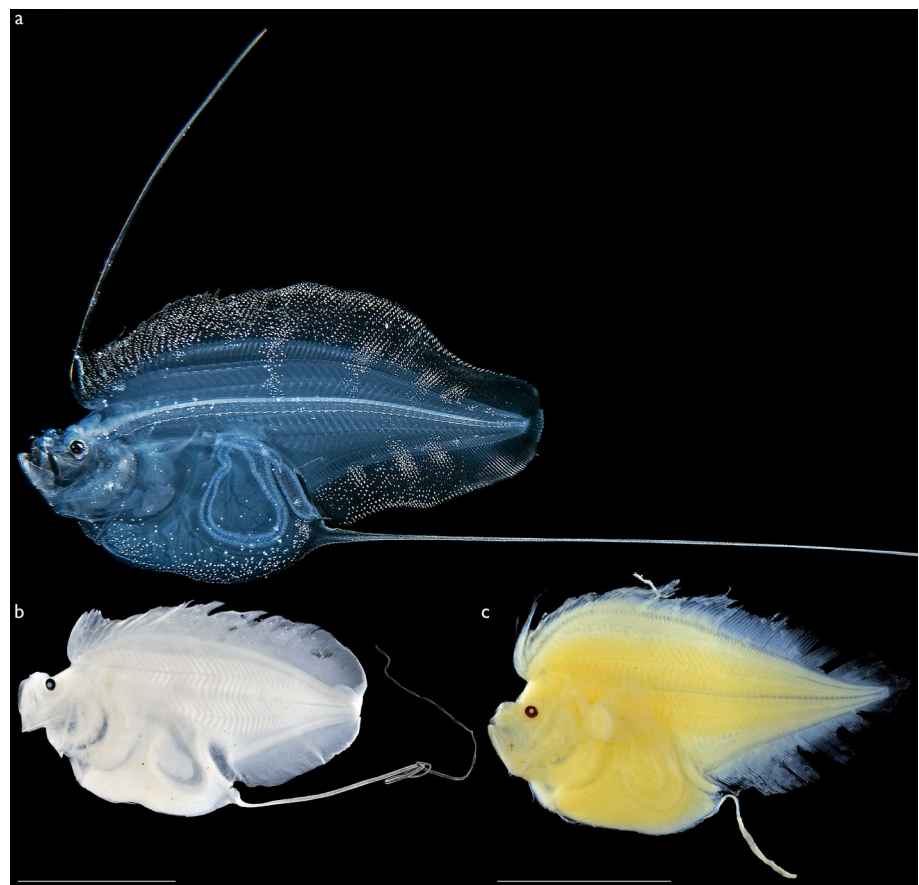
Fig. 2 Adult **a** *Pycnocraspedum armatum* (USNM 162717 holotype) μCT scan, **b** *P. phyllosoma* (USNM 227388), **c** *Luciobrotula bartschi* (USNM 74151 holotype) μCT scan, **d** *L. corethromycter* (USNM 188549 paratype). Arrows indicate origin of dorsal and anal fins. Scale bars = 1 cm

fishes. Photographs of these larvae have allowed new morphological characters to be observed and behaviors to be described (e.g., Nonaka et al. 2021; Pastana et al. 2022). In 2017, a larva similar to Okiyama's (2014) "*Luciobrotula*" types 2 and 3 was photographed and captured by blackwater divers off Hawai'i (Figs. 3d–e). The Hawaiian specimen was barcoded for cytochrome c oxidase subunit I (COI) and included in the study by Nonaka et al. (2021) but the sequence was only able to be verified to "Actinopterygii" when compared to the Barcode of Life Database (BOLD; see their table 1). Recently, blackwater divers and photographers encountered a larva matching Okiyama's (2014) "*Luciobrotula*" type 4 off Florida (Figs. 3a–c) and larvae similar to Evseenko and Okiyama's (2006) "*Pycnocraspedum*" off Hawai'i and Florida. After generating and comparing COI sequences from these newly captured larvae and an adult specimen of *Pycnocraspedum phyllosoma* from the Gulf of Mexico to publicly available sequences on BOLD and GenBank, we find these larvae have been misidentified by previous authors. In this study, we revise the identity of larval "*Pycnocraspedum*" to the genus *Luciobrotula* and describe the larvae of *L. bartschi* and *Luciobrotula corethromycter*. With the larvae of these two species of *Luciobrotula* identified, we then examine larvae that morphologically match Okiyama's

(2014) "*Luciobrotula*" types. While neither morphological nor molecular characters could provide definitive identifications of these larvae, our phylogenetic analysis places these larvae among the Bythitidae. We provide revised descriptions of these larvae, reducing the number of larval types originally described by Okiyama (2014) from four to two based on new morphological observations, and highlight a connection between the coracoid and intestine.

Materials and methods

Classification. We follow the classifications of the Ophidiiformes by Møller et al. (2016) and Fricke et al. (2022).

Morphological identification, examination, and laboratory imaging of larvae and adults. Seven larval specimens, four of "*Pycnocraspedum*" and three of "*Luciobrotula*", were examined, along with type and non-type adult specimens of *Luciobrotula* and *Pycnocraspedum*. All specimens used, their lengths, preparations, and museum catalog numbers are listed in the Specimens Examined section. Museum codes follow Sabaj (2020) except for NMNH referring to non-Fishes Division personnel and resources at the National Museum of Natural History, Smithsonian Institution.
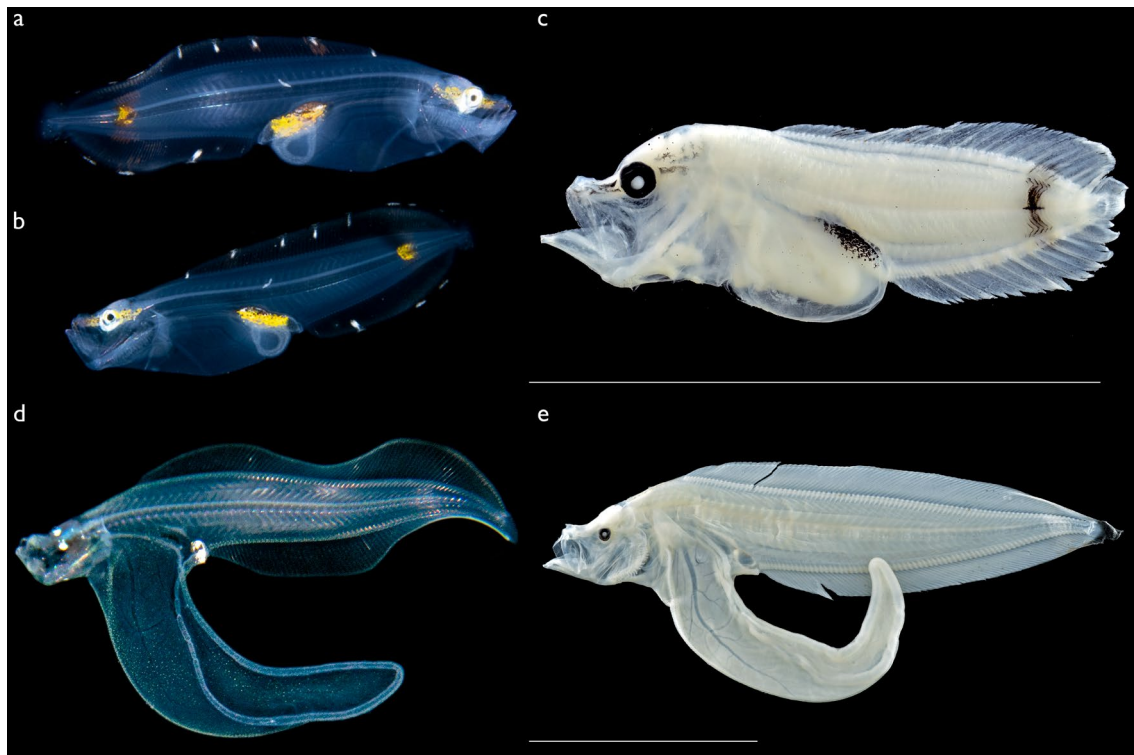
Fig. 3 Larval Bythitidae types 1 and 2. **a, b** Blackwater photos of type 1 larva, USNM 465411, captured offshore of West Palm Beach, Florida, 18 February 2022, photos © D. Devers; **c** preserved USNM 465411; **d** blackwater photo of type 2 larva, USNM 447052, captured by J. Milisen, offshore of Kona, Hawai'i, 13 May 2017, figure from Nonaka et al. (2021: fig. 9C) published in *Ichthyology* & *Herpetology* 109 by the American Society of Ichthyologists and Herpetologists (https://www.asih.org) and is licensed under CC BY 4.0; **e** preserved USNM 447052. Scale bars = 1 cm

Measurements of larval specimens were taken with a digital caliper to the nearest 0.1 mm. Measurements of adult specimens were taken with a measuring tape to the nearest 1 mm. Larval specimens were cleared and stained following Potthoff (1984) with the modifications listed in Girard et al. (2020). Morphological features were documented using equipment listed in Girard et al. (2020). Type and large specimens were either x-rayed or scanned using microcomputed tomography (μCT) to view internal osteology. Specimens were x-rayed using a Thermo Scientific PXS5-927 MicroFocus 90kV X-Ray Source and a duraSCAN 1417-NDI Digital Flat Panel X-Ray Detector at NMNH. Specimens were μCT scanned using either a GE Phoenix v|tome| x M 240/180kV Dual Tube μCT at NMNH or a Nikon Metrology XT H 225 ST at the Chemical and Biophysical Instrumentation Center, Yale University. Specimens were scanned using 110–120 kV, 90–100 μA, a 250–333 ms exposure time, and a 62.9–98.9 μm voxel size. Resulting scans were then visualized and segmented using the protocol described in Girard et al. (2022a). MorphoSource identifiers for the μCT scans can be found in the Specimens Examined section.

DNA extraction and amplification. Protocols for tissue sampling, DNA extraction, PCR, and sequencing COI follow the methods described in Nonaka et al. (2021) and Weigt et al. (2012a) using primers from Baldwin et al. (2009). Sequence contigs were built, edited, and assembled into FASTA files using Geneious, vers. 11.1.5 (Kearse et al. 2012). Sequences were deposited on both Gen-Bank (OQ359786–OQ359790) and BOLD [LUPY001-23–LUPY005-23; see Electronic Supplementary Material (ESM) S1].

Taxon identification and analyses of molecular data. To identity larvae using molecular characters, we downloaded all publicly available COI sequences of *Luciobrotula* (11) and *Pycnocraspedum* (6) from BOLD and GenBank, as well as an additional 131 sequences representing of all four recognized families, 61 genera (of 121, ~50%), and 131 species (of 562, ~23%) of ophidiiforms (see ESM S1). Sequences came from a series of published and unpublished works, including Miya et al. (2003), Ward and Holmes (2007), Steinke et al. (2009), Lara et al. (2010), Cawthorn et al. (2011), Mabragana et al. (2011), Hubert et al. (2012), Weigt et al. (2012b), McCusker et al. (2013), Chen et al. (2014), Landi et al. (2014), Parmentier et al. (2016), Campbell et al. (2017), Chang et al. (2017), Robertson et al. (2017 [but see Lea et al. 2023]), Delrieu-Trottin et al. (2019), Bañón et al.

(2020), Nonaka et al. (2021), Jayakumar et al. (2021), Wong et al. (2021), Marín et al. (2022), and Pham et al. (2022). Downloaded sequences were collated into a single file with newly sequenced larvae and adults, aligned with MAFFT vers. 7 (Katoh and Standley 2013) within Geneious and exported as a PHYLIP-format file for phylogenetic analysis. The aligned matrix, which contained 154 terminals, 655 base pairs (bps) in length (~98% complete at the level of individual bps), and 297 parsimony-informative sites, was broken into three partitions, one for each of the three codon positions in the protein-coding locus. These three partitions were input for ModelFinder function within IQ-Tree vers. 2.2.0 (i.e., MFP + MERGE, Chernomor et al. 2016; Kalyaanamoorthy et al. 2017; Minh et al. 2020) that selected a partitioning scheme and models based on Bayesian information criterion. Phylogenetic analysis was performed by 10 independent analyses within IQ-Tree with the number of unsuccessful iterations to stop (-nstop) set to 1,000 and perturbation strength (-pers) set to 0.1. The 10 resulting trees were then used as starting trees for a second set of 10 independent analyses within IQ-Tree with the -nstop set to 2,000 and more-thorough nearest-neighbor interchange search (-allnni). Support for the best-fitting topology of the dataset was generated using 5,000 Ultrafast bootstrap replicates (-bb, -wbtl) and reconciled with the most likely phylogeny using IQ-Tree (-con). Analyses were rooted on species of *Polymixia* (see ESM S1–2).

Results

Placement of newly sequenced larvae. The hypothesis of relationships recovered from our analysis of COI is shown in Fig. 4 and ESM S2 and has a ln L = -27836.101. Out of 147 nodes, 96 (~65%) were supported by a bootstrap value ≥95% and 133 (~90%) nodes had a bootstrap value ≥70% (see ESM S2). The resulting topology recovers the specimens sequenced in this study in the following positions: all three larval "*Pycnocraspedum*" are recovered in a clade containing all specimens of adult *Luciobrotula* sampled. Of these "*Pycnocraspedum*" larvae, the larva from Hawai'i (USNM 454562) is recovered in a clade with all adult samples of *Luciobrotula bartschi*; the larvae from Florida (USNM 465301, USNM 465380) are recovered in a clade sister to all samples of adult *Luciobrotula coheni*. The adult specimen *P. phyllosoma* is recovered sister to samples of *P. squamipinne*. This clade of *Pycnocraspedum* is recovered sister to *Neobythites*, not monophyletic with any "*Pycnocraspedum*" larvae sequenced. Larval "*Luciobrotula*" from Hawai'i (USNM 447052) is recovered in a clade of bythitid genera *Barathronus*, *Cataetyx*, *Diplacanthopoma*, *Paraphyonus*, and *Sciadonus*. Larval "*Luciobrotula*" from Florida (USNM

465411) is also recovered in a clade of bythitids, among the genera *Brosmophyciops*, *Grammonus*, and *Saccogaster*.

Relationships among ophidiiforms. While testing the monophyly and interrelationships among the Ophidiiformes are beyond the focus of this study, our phylogeny reflects previously published multi-locus phylogenies (e.g.,Møller et al. 2016; Ghezelayagh et al. 2022) with respect to members of the Carapidae nested within the Ophidiidae and a monophyletic Dinematichthyidae. However, there are several differences between our topology and those from multi-locus datasets. These include the non-monophyly of Bythitidae, where Dinematichthyidae is nested within the family, and members of the former Aphyonidae in a clade separate from Bythitidae (Fig. 4; ESM S2). We also recover the following four ophidiiform genera as non-monophyletic: *Cataetyx*, *Encheliophis*, *Lepophidium*, and *Ophidion*. These results may be because our analysis is limited to a single locus. We do not modify the classification of the Ophidiiformes based on our results because our recovered relationships should be tested using additional data.

Revised identification of "*Luciobrotula*" and "*Pycnocraspedum*" larvae. Based on our analysis of COI, distributions of ophidiiform species in the localities where the larvae were captured, and counts of precaudal vertebrae, total vertebrae, elongate gill rakers, anal-fin rays, pectoral-fin rays, and caudal-fin rays, we revise the identification of the following "*Pycnocraspedum*" larvae: larva from Hawai'i (USNM 454562; Fig. 1) is revised to *Luciobrotula bartschi*; larva described and illustrated from New Guinea by Evseenko and Okiyama (2006) and larva from Hawai'i (USNM 454451; Fig. 1) are revised to *L.* cf. *bartschi*; larvae from Florida (USNM 465301, USNM 465380; Fig. 5) are revised to *L. corethromycter*. Dorsal-fin-ray counts disagree with these revised identifications and discrepancies are discussed below.

For previously identified "*Luciobrotula*" larvae, we combine two of the four originally described types by Okiyama (2014) based on overlapping morphological counts and characters (see Discussion section) and revise the identifications of the following: larvae a and b described and illustrated for "*Luciobrotula*" type 1 in Okiyama (2014: 434), larva described and illustrated for "*Luciobrotula*" type 4 in Okiyama (2014: 435), and larva from Florida (USNM 465411) are revised to Bythitidae type 1; larvae described and illustrated for "*Luciobrotula*" types 2 and 3 in Okiyama (2014: 434–435), larva from Hawai'i (USNM 447052), and larva from Japan (USNM 465770) are revised to Bythitidae type 2. Hereafter, we refer to these larvae by their revised names.

General morphology of larval *Luciobrotula bartschi*. USNM 454451 and USNM 454562 (Figs. 1, 6a–b), postflexion. Note: USNM 454451 is identified as *L.* cf. *bartschi* but is included in this section (see Discussion). Counts (Table 1): dorsal-fin rays 102–109; anal-fin rays 68–75;

Table 1 Counts for larval and adult specimens

Species	Developmental stage	Museum catalog number	Capture locality	Precaudal vertebrae	Total vertebrae	Dorsal-fin rays	Anal-fin rays	Pectoral-fin rays	Pelvic-fin rays	Caudal-fin rays	Elongate gill rakers
Luciobrotula bartschi	Adult	See 2, 5, 9	Pacific	14–15 (15)	50–55 (53)	85–97 (89)	66–75 (68)	25–28 (26)	2 (2)	11–12 (11)	3–4 (3)
Luciobrotula bartschi	Larva	USNM 454562	Pacific, Hawai'i	15	53	102	72	28	2	11	3
Luciobrotula cf. *bartschi*	Larva	See 4	Pacific, New Guinea	14 or 15	54	102	68	26	2	10?	3
Luciobrotula cf. *bartschi*	Larva	USNM 454451	Pacific, Hawai'i	15	57	109	75	28	2	11	3
Luciobrotula lineata	Adult	See 1, 3, 9	Pacific	15 (15)	51–56 (56)	90–92 (92)	67–76 (76)	26 (26)	2 (2)	12 (12)	3 (3)
Luciobrotula polylepis	Adult	See 8	Pacific, New Guinea	(13)	(50)	(86)	(70)	(32)	(2)	(11)	(3)
Pycnocraspedum armatum	Adult	See 1, 9	Pacific, Hawai'i	12 (12)	47–55 (55)	90–99 (97)	61–72 (71)	26 (26)	2 (2)	10 (10)	4–6 (4)
Pycnocraspedum squamipinne	Adult	See 7	Indian	12	47–49	63–92	54–79	24	2	10	4
Luciobrotula corethromycter	Adult	See 2, 5, 9	Atlantic	15–16 (16)	53–57 (56)	91–103 (93)	68–77 (70)	26–30 (28)	2 (2)	10–12 (11)	3 (3)
Luciobrotula corethromycter	Larva	USNM 465301	Atlantic, Florida	16	56	108	77	29	2	11	3
Luciobrotula corethromycter	Larva	USNM 465380	Atlantic, Florida	16	56	105	69	31	2	10	3
Pycnocraspedum phylosoma	Adult	See 9	Atlantic	12 (12)	51–54	96–101 (97)	59–72 (71)	24–26 (26)	2 (2)	10	4
Bythitidae type 1	Larva	USNM 465411	Atlantic, Florida	12	53	~76	~58	U	U	U	U
"*Luciobrotula*" type 1 and 4	Larva	See 6	Pacific, Japan	?	51–54	77–80	61–62	25	1 or 2	13	?
Bythitidae type 2	Larva	USNM 447052	Pacific, Hawai'i	~11	~55	87	70	U	1	13	3
Bythitidae type 2	Larva	USNM 465770	Pacific, Japan	12	55	88	68	U	1	13	3

Table 1 (continued)

Species	Developmental stage	Museum catalog number	Capture locality	Precaudal vertebrae	Total vertebrae	Dorsal-fin rays	Anal-fin rays	Pectoral-fin rays	Pelvic-fin rays	Caudal-fin rays	Elongate gill rakers
"*Luciobrotula*" type 2 and 3	Larva	See 6	Pacific, Japan, and Philippines	?	57	90–92	68–72	25	1 or 2	13	?

Values listed in paratheses indicate count from holotype

U fin rays undifferentiated, *?* value unknown

In-table citations: 1, Gosline 1954; 2, Cohen 1964; 3, Prokofiev 2005; 4, Evseenko and Okiyama 2006; 5, Nielsen 2009; 6 Okiyama 2014; 7, Jayakumar et al. 2021; 8, Wong et al. 2021; 9, see specimens examined section

pectoral-fin rays 26–28; pelvic-fin rays 2; caudal-fin rays 10–11; precaudal vertebrae 14–15, total vertebrae 54–57. Following description based on *in-situ* images as well as ethanol and cleared-and-stained specimens. Head large, nearly as deep as long, body broadly ovoid, tapering posteriorly to a narrow hypural plate. Maxilla and premaxilla short, at oblique upturned angle. Premaxilla and dentary with small, distantly spaced teeth. Distal end of maxilla dorsoventrally expanded, posterior margin convex. Posterior tip of premaxilla nearly reaching posterior margin of maxilla. Supramaxilla indistinguishable. Large rostral cartilage attached to ascending process of premaxilla. Spine associated with symphysis of dentary. Eight branchiostegals (full complement). Three elongate gill rakers present on first arch. Other tooth plates of branchial arches not developed. Body and head scaleless. Dorsal fin origin over supraoccipital. Distinct notch between dorsal margin of head and first dorsal-fin pterygiophore. Anterior three proximal-middle radials undifferentiated, cartilaginous, and anteriorly directed, with ventral margin following contour of neurocranium. First dorsal-fin ray elongate and robust, approaching or exceeding SL of specimen. *In-situ* images of fixed specimen show the elongate rays were damaged and truncated during capture and subsequent fixation (compare Figs. 1a with 1b–c, 6a). Accordingly, total length of elongate ray not reported. Remaining dorsal- and anal-fin rays approximately subequal in length. Pectoral fin large, fan-like, with broad base. Four pectoral radials present, cartilaginous. Coracoid with elongate cartilaginous ventral process, extending to and associated with ascending loop of gut. Gut massive, protruding ventrally below the body musculature, lacking exterilium morphology of some larval ophidiids (e.g., *Brotulotaenia*, *Lamprogrammus*, *Leptobrotula*; Fraser and Smith 1974; Fahay and Nielsen 2003; Okiyama and Yamaguchi 2004). Gut with single, broad intestinal loop in posterior half of tract, near the 12th-to-13th vertebra. Tissue surrounding gut vascularized, possibly liver. End of gut loop and anus extends posteroventrally from gut loop, surrounded by bolus of tissue, widely separated from body contour. Anus slightly anterior to anal fin. Long fleshy filament present anterior to anus, extending from body wall. No ossification present in filament. *In-situ* images of fixed specimens show the elongate filament was damaged during capture and subsequent fixation (compare Figs. 1a with 1b–c, 6a). Accordingly, total length of filament not reported but approaches or exceeds SL of specimen. Pelvic fin minute, girdle reduced and associated with cleithral symphysis. Caudal fin small, without procurrent rays, middle rays longest. Lower two hypurals differentiated, upper hypurals as single element.

Melanophores few on upper part of premaxilla and elongate dorsal-fin ray. Melanophores on body minute, almost entirely restricted to areas near looped gut. Several melanophores near anus. Fleshy filament near anus with dense

Fig. 4 Hypothesis of ophidii-
form relationships based on an
analysis of COI. Terminals col-
lapsed to genus level. Voucher
information can be found in
ESM S1. Phylogeny with all
terminals available in ESM S2.
Black, gray, patterned, or white
bars between phylogeny and ter-
minal names note family-level
classification (see Møller et al.
2016; Fricke et al. 2022). We
do not modify the classification
of the Ophidiiformes based on
our results as they are based on
a single locus. Terminals with
blue text highlight larval and
adult taxa targeted in this study.
"*" indicates newly sequenced
adult specimen (see Specimens
Examined). Illustrations associ-
ated with terminals USNM
447052 and USNM 465411 ©
M. Okiyama (2014) reproduced
with permission of Tokai Uni-
versity Press. Illustration associ-
ated with clade of *Luciobrotula*
based on USNM 454451
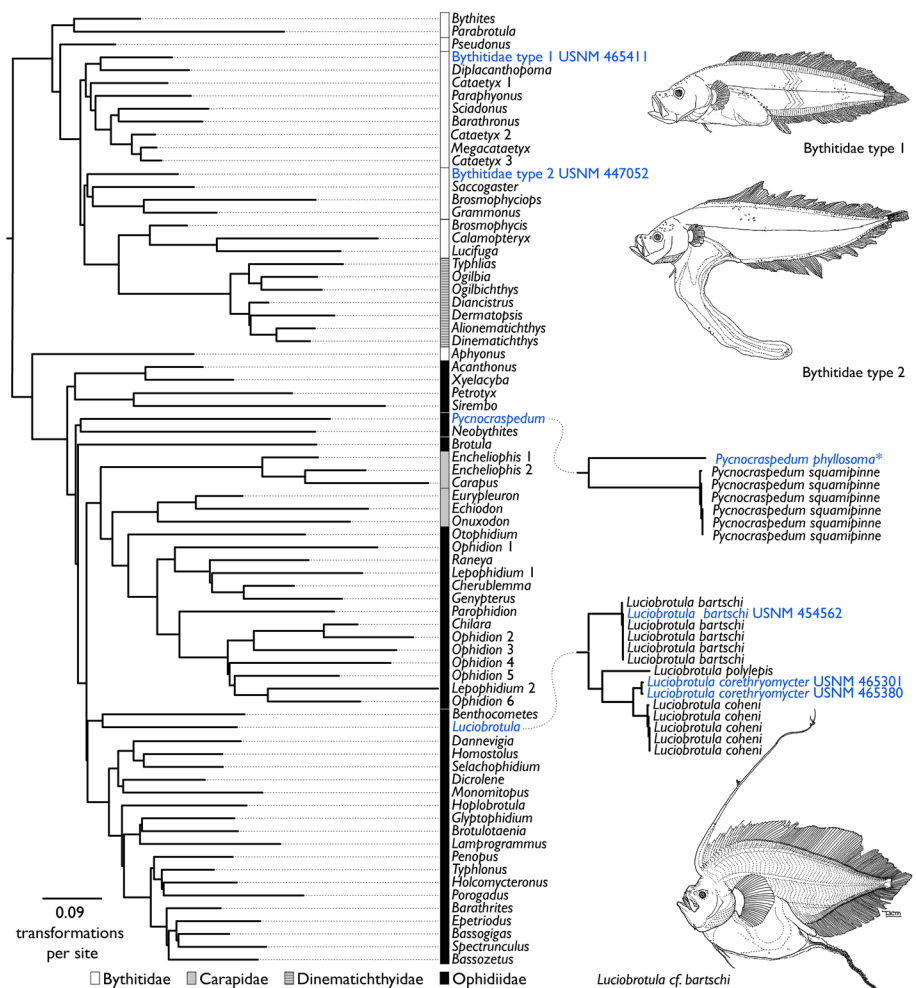
melanistic pigmentation, increasing in density distally to
almost completely black tip. First dorsal-fin ray directed
anteriorly when fully flexed. Fleshy filament near anus
trails in straight line behind, appearing rigid. Body almost
completely transparent. Broad blotches of reflectance or
iridescence covering ventral margin of gut, dorsal fin, and
anal fin, with these areas extending onto the body in discrete
bands (white blotches in Fig. 1a). At least four broad bands
of reflectance or iridescence present on upper flank, three
on lower flank. These areas not discernable in preserved
specimens.

**General morphology of larval *Luciobrotula corethro-
mycter*.** USNM 465301, flexion (Figs. 5a–b, 6c–d), and
USNM 465380, postflexion (Figs. 5c–d). Counts (Table 1):
dorsal-fin rays 105–108; anal-fin rays 69–78; pectoral-fin
rays 29–31; pelvic-fin rays 2; caudal-fin rays 10–11; precau-
dal vertebrae 16, total vertebrae 56–57.

General morphology same as larval *L. bartschi* (see
above). Differences include: melanophores present on
elongate first dorsal-fin ray, increasing in density distally
to almost completely black tip (may also be present in *L.*

bartschi but specimens examined missing distal part of fin
ray); fewer areas of reflectance or iridescence along the body
(compare Figs. 1a with 5a–c). It is possible that this differ-
ence in reflectance or iridescence is an artifact of different
angles and/or intensities of camera strobes used. However,
given the multiple angles at which the specimens were pho-
tographed, we doubt that camera strobes are the sole reason
for these differences.

General morphology of Bythitidae type 1 larva.
USNM 465411, flexion, (Figs. 3a–c; ESM S3), and Okiy-
ama (2014: 434, type 1 figs. a–b; 435 type 4, figs. a–c).
Counts (Table 1): dorsal-fin rays 76–80; anal-fin rays 58–62;
pectoral-fin rays 25; pelvic-fin rays 1 or 2; caudal-fin rays
13; precaudal vertebrae 12, total vertebrae 51–54. Follow-
ing description based on *in-situ* images of USNM 465411
(Figs. 3a–b), a cleared-and-stained specimen (ESM S3),
and specimens described and illustrated by Okiyama (2014,
see above). Head large, nearly as deep as long, body com-
pressed, tapering posteriorly to a narrow hypural plate.
Premaxilla and dentary with small, distantly spaced teeth.
Distal end of maxilla dorsoventrally expanded with concave

Fig. 5 Larvae of *Luciobrotula corethromycter* from Florida. **a** Blackwater photo USNM 465301 captured by R. Collins, 24 June 2021; **b** preserved USNM 465301; **c** blackwater photo USNM 465380 captured by N. Deloach, 4 August 2021; **d** preserved USNM 465380. Scale bars = 1 cm
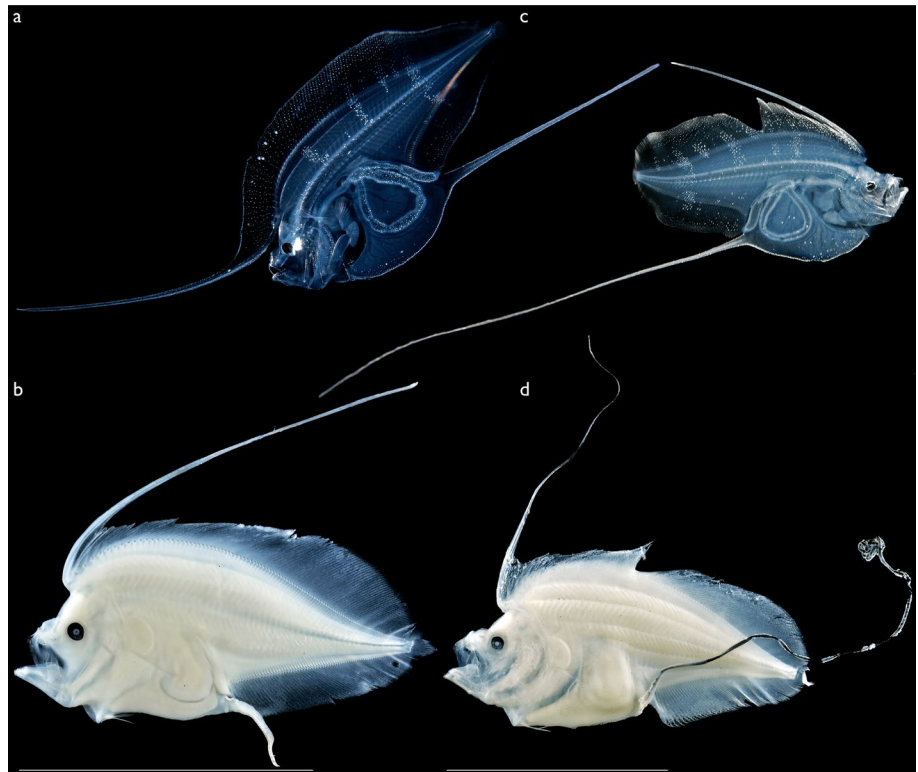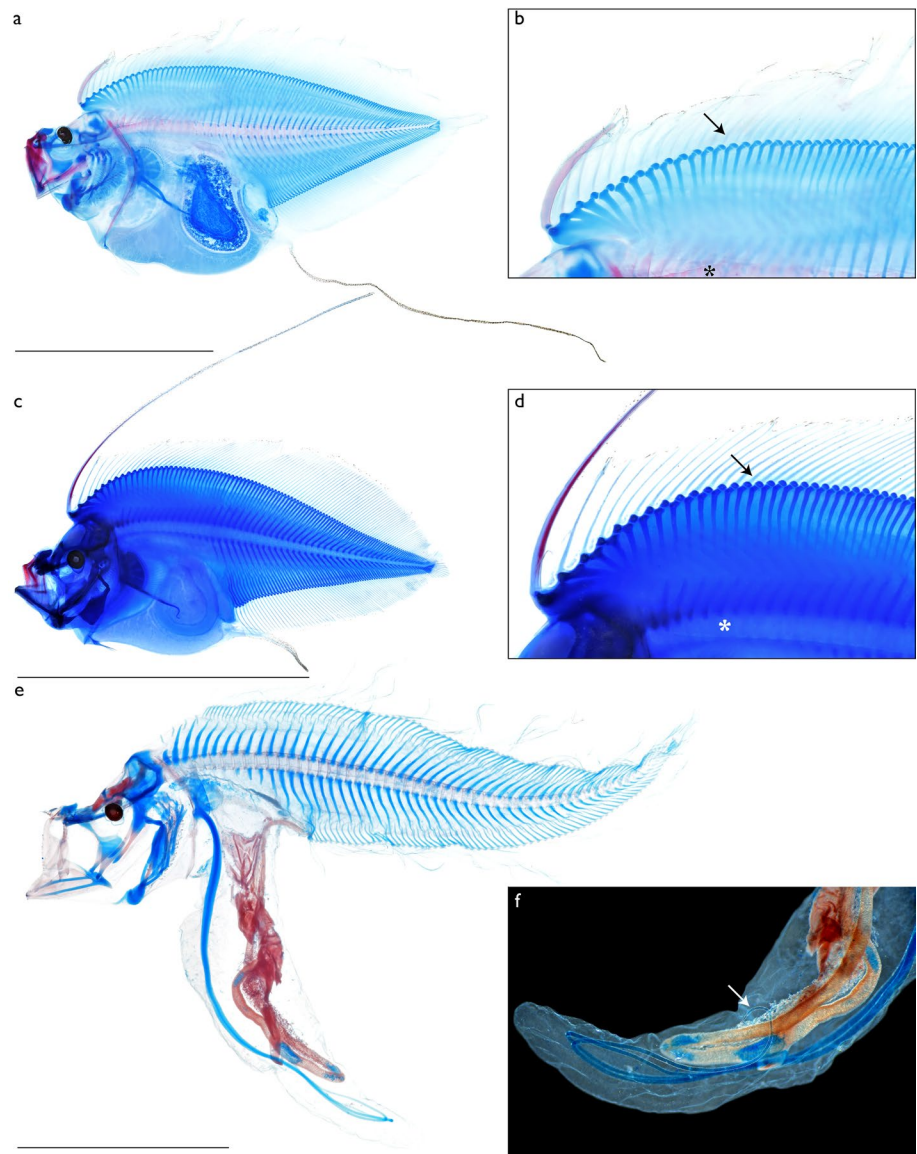

posterior margin. Posterior tip of premaxilla reaching posterior margin of maxilla. Supramaxilla indistinguishable. Large rostral cartilage attached to ascending process of premaxilla. Articular with acute spine-like process ventrally. Seven branchiostegals. Gill rakers not developed on first arch. Other tooth plates of branchial arches not developed. Body and head scaleless. At least three supraneurals anterior to first dorsal-fin pterygiophore, one in each interneural space between first, second, and third vertebrae. Dorsal fin origin over fifth vertebra. Dorsal- and anal-fin rays approximately subequal in length. Pectoral fin fan-like, with broad base, rays undifferentiated. Coracoid with elongate cartilaginous process, distally wavy, extending to and forming symphysis at ventral margin of body. Gut bulging ventrally to slightly below the body musculature, without exterilium morphology. Single intestinal loop in the posterior part of the tract, near anus. Gut tissue vascularized, possibly liver. End of intestinal tract and anus large, slightly anterior to anal fin, extending posteroventrally from body wall, widely separated from body contour. Pelvic fin minute, single ray present, girdle reduced and associated with cleithral symphysis. Caudal fin small, fan like.

Melanophores on head concentrated in longitudinal band from tip of premaxilla to posterior margin of neurocranium. Few large melanophores above brain. Melanophores on body concentrated above anus and in vertical band near the caudal peduncle. Melanophores in vertical band extend onto both dorsal and anal fin. Few small melanophores speckle body.

Head with strong, horizontal strip of yellow, overlapping areas of melanophores in fixed specimen (see above). Body almost completely transparent, with three discrete areas of color, one white diagonal blotch on flank above anus, one yellow blotch near caudal peduncle, anus with strong horizontal strip of yellow. Yellow coloration overlapping areas of dense melanophores in fixed specimen (see above). Dorsal fin with eight discrete blotches of white, anterior five vertically oriented, posterior three horizontally oriented. Anal fin with three discrete blotches of white coloration, anterior most vertically oriented, posterior two horizontally oriented. Slight dots of reflectance or iridescence covering exterilium gut, dorsal fin, and anal fin, most dense on exterilium. Anus highly reflective anterior to anal fin.

General morphology of Bythitidae type 2 larvae. USNM 447052 and USNM 465770, postflexion (Figs. 3d–e, 6e–f), and Okiyama (2014: 434, type 2 figs. a–b; 435 type 3, fig. a). Counts (Table 1): dorsal-fin rays 87–92; anal-fin rays 68–72; pectoral-fin rays 25; pelvic-fin rays 1 or 2; caudal-fin rays 13; precaudal vertebrae 11–12, total vertebrae 55–57. Following description based on *in-situ* images of USNM 447052 (Fig. 3d), ethanol and cleared-and-stained specimens, and specimens described and illustrated by Okiyama (2014, see above). Head large, nearly as deep as long, body compressed, tapering posteriorly to a narrow hypural plate. Two nostrils, anterior nostril low on snout, slightly above upper lip, characteristic of the Bythitidae (Nielsen et al. 1999). Premaxilla with small, distantly spaced teeth.

Fig. 6 Cleared-and-stained larval specimens. **a** *Luciobrotula bartschi* USNM 454562. **b** Focused view of anterior dorsal-fin rays in larval *L. bartschi*; "*" highlights sixth precaudal vertebra; arrow points to 13th dorsal-fin ray. **c** *Luciobrotula corethromycter* USNM 465301. **d** Focused view of anterior dorsal-fin rays in larval *L. bartschi*; "*" highlights sixth precaudal vertebra; arrow points to 14th dorsal-fin ray. **e** Bythitidae type 2 USNM 465770, note elongate coracoid cartilage within exterilium. **f** Focused view of looping coracoid cartilage within Bythitidae type 2 USNM 465770; arrows highlight distal end of right cartilage attaching to intestinal loop. Scale bars = 1 cm

Distal end of maxilla dorsoventrally expanded with concave posterior margin. Posterior tip of premaxilla nearly reaching posterior margin of maxilla. Supramaxilla present. Large rostral cartilage attached to ascending process of premaxilla. Articular with acute spine-like process ventrally. Eight branchiostegals. Three elongate gill rakers present on first arch. Other tooth plates of branchial arches not developed. Body and head scaleless. Four supraneurals anterior to first dorsal-fin pterygiophore. Dorsal fin origin over fourth or fifth vertebra. Dorsal- and anal-fin rays approximately subequal in length. Pectoral fin large, fan-like, with broad base, rays undifferentiated. Four pectoral radials present. Coracoid with highly elongate cartilaginous posterior process, extending along the entire anterior margin of exterilium gut. Distal tips of processes loop near ventral margin of gut, attaching separately to ascending portion of intestine, do not form discrete symphysis. Gut with single, broad intestinal loop in middle of tract, near base of exterilium. Intestine muscular, extending throughout exterilium in USNM 447052, contracted in USNM 465770, not extending to exterilium ventral margin (compare Figs. 3d–e with 6e–f). Tissue surrounding gut loop vascularized, possibly liver. Anus above exterilium, slightly anterior to anal fin, extending posteroventral from body wall, widely separated from body contour. Pelvic fin minute, girdle reduced and associated with cleithral symphysis. Caudal fin small, without procurrent rays, middle rays longest. Upper two hypurals differentiated completely, lower two hypurals separated at proximal end.

Melanophores on body and fins few, minute, almost entirely restricted to medial fins and upper flank (Fig. 3e). Body almost completely transparent. Dorsal fin with three discrete blotches of pigment. Slight dots of reflectance or

iridescence covering exterilium gut, dorsal fin, and anal fin, most dense on exterilium (Fig. 3d). Anus highly reflective anterior to anal fin.

Discussion

Larvae of *Luciobrotula*. Despite the bothid-like appearance of their specimen, Evseenko and Okiyama (2006) correctly identified their larva from New Guinea as an ophidiiform. However, counts of precaudal vertebrae and dorsal-fin rays disagree with their identification of "*Pycnocraspedum squamipinne*" (see above, Table 1). Our morphological examination and molecular sequencing of newly captured "*Pycnocraspedum*" larvae from Hawai'i and Florida identifies them as species of *Luciobrotula*. Phenotypically, counts of precaudal vertebrae, total vertebrae, elongate gill rakers, anal-fin rays, pectoral-fin rays, and caudal-fin rays overlap between these larvae and adults of *Luciobrotula* (see Table 1; Cohen 1964; Nielsen 2009). Genotypically, previously generated COI sequences of adult specimens of *L. bartschi* are identical to those generated from our Hawaiian larva (USNM 454562), and all sequences of this species analyzed were recovered as monophyletic in our phylogeny (Fig. 4, ESM S2). One Hawaiian larva was unable to be sequenced (USNM 454451) and we identify it as *L.* cf. *bartschi*, recognizing that a second species in the genus, *Luciobrotula lineata*, also occurs in Hawai'i (Gosline 1954). As these two Hawaiian species are primary differentiated by the length of the lateral line (see Nielsen 2009), which is not developed in the larva, it is difficult to confidently assign USNM 454451 to one of these species. Ultimately, we identified this larva as *L.* cf. *bartschi* until additional specimens of *L. lineata* can be captured and examined. We also tentatively revise the identity of the larva described by Evseenko and Okiyama (2006) to *L.* cf. *bartschi* despite two species in the genus occurring near or in New Guinea. Aside from its type locality of Hawai'i, *L. lineata* is also known from Kyushu-Palau Ridge (Prokofiev 2005) and may have a wider range that includes New Guinea. This species has more caudal-fin rays (12; Prokofiev 2005) than the larva described by Evseenko and Okiyama (2006). Additionally, *Luciobrotula polylepis* from the Solomon Sea near New Guinea has fewer precaudal vertebrae (13) and more pectoral fin rays (32, Wong et al. 2021) than the larva described by Evseenko and Okiyama (2006, see Table 1). Although our sequences from the Floridian larvae were identical to each other, they did not match any previously sequenced ophidiiform taxon and were recovered within the larger clade of *Luciobrotula* in our phylogeny. Only one species of *Luciobrotula*, *L. corethromycter*, is known from the waters of Florida (Cohen 1964; Nielsen 2009), however, a COI sequence has yet to be publicly released for this taxon. Counts from the two larval

specimens are within the ranges of precaudal vertebrae, total vertebrae, anal-fin rays, pectoral-fin rays, and caudal-fin rays of adult *L. corethromycter* (see Table 1; Cohen 1964; Nielsen 2009). Therefore, we identify these larvae as *L. corethromycter*, with the genetic resources generated in this study being the first available for this taxon.

While we have identified the larvae of *L. bartschi* and *L. corethromycter* using both genotypic and phenotypic characters, characteristics of the larval dorsal fin disagree with these identifications in three key areas: the number of dorsal-fin rays are greater in the larvae than the adult (*L. bartschi*: 102–109 vs. 85–97; *L. corethromycter*: 105–108 vs. 91–103; see Table 1; Cohen 1964; Nielsen 2009); the dorsal fin originates above the neurocranium in the larvae compared to the middle of the abdomen in adults; the elongate first dorsal-fin ray is present in the larvae but absent in adults. For the counts and dorsal-fin morphology to agree between developmental stages, the larvae of *Luciobrotula* would lose ~2–24 anterior dorsal-fin elements through ontogeny. While reductions of dorsal-fin-ray lengths between larvae and adults are common among marine fishes broadly (e.g., Bathysauridae, Bothidae, Carangidae, Macrouridae; Fahay 2007; Okiyama 2014), including members of ophidiiforms (e.g., *Brotulotaenia*, *Lamprogrammus*, see Fahay and Nielsen 2003), losses of entire elements and associated posterior shifts of fins are limited to a few groups. One example of such a loss occurs the ophidiiform family Carapidae, that lose the vexillum anterior to the adult first dorsal-fin ray ontogenetically (Olney and Markle 1979; Markle and Olney 1990). Nielsen and Evseenko (1989) highlighted an unusual loss of dorsal-fin elements and consequent posterior shift of fin origin in the ontogeny of the ophidid *Benthocometes robustus*. Approximately 10 pterygiophores and elongate anterior dorsal-fin rays that insert above the neurocranium in larval *B. robustus* become lost or reduced, represented by rudimentary ossifications between the posterior margin of the neurocranium and anterior margin of the dorsal fin in the adult (compare Nielsen and Evseenko 1989: figs. 4 and 6). The larval dorsal fin of *B. robustus* is similar to the anterior dorsal-fin morphology of larval *Luciobrotula* described here, but with more elongate anterior rays and fin elements well ossified (compare Nielsen and Evseenko 1989: figs. 3, 4 with Figs. 6a–d). Further, our analysis of COI recovers *Benthocometes* as the sister genus to *Luciobrotula* (Fig. 4). Based on the counts and insertion of the dorsal-fin elements between larvae and adults and a putative close ally, *Benthocometes*, having ontogenetic reductions in dorsal-fin elements, we hypothesize that an extraordinary number of dorsal-fin elements are lost in the ontogeny of *Luciobrotula*. While we did not find cartilaginous or ossified elements that resembled pterygiophores between the posterior margin of the neurocranium and anterior-most dorsal-fin pterygiophore in adult specimens of *Luciobrotula*, we found the dorsal fin inserts

between the fifth and eighth vertebrae in the adult specimens (Fig. 2). Our cleared-and-stained larvae have 13–14 dorsal-fin elements anterior to the sixth vertebra (Figs. 6a–d), 17–18 elements anterior to the eighth vertebra, and these counts are within the range needed to be lost through ontogeny to conform with adult dorsal-fin-ray counts (i.e., 2–24; see Table 1). While specimens of transitioning *Luciobrotula* will need to be captured and examined to fully understand the ontogenetic changes occurring in the dorsal fins of these fishes, this is only the second example of 10+ fin elements being lost through ontogeny, along with a putative close ophidiid relative *Benthocometes* (Nielsen and Evseenko 1989). Our findings call into question the ubiquitous utility of fin-ray counts when identifying larval ophidiids and we encourage researchers to use multiple data types to identify larvae going forward.

Unknown larvae of *Pycnocraspedum*. Considering the revisions made above, the larvae of *Pycnocraspedum* are now unknown. We recover our newly sequenced *P. phyllosoma* and previously generated sequences of *P. squamipinne* in a distantly related clade from *Luciobrotula*, sister to species of *Neobythites* (Fig. 4). The larvae of *Neobythites* are similar in overall physiognomy to the adult form (Fahay 2007; Okiyama 2014) and the unknown larvae of *Pycnocraspedum* may also be more similar in overall appearance to their adult counterparts. We encourage subsequent sampling to use both genotypic and phenotypic characters to correctly identify the larvae of these fishes.

Larval bythitid types 1 and 2. Okiyama (2014) tentatively identified the larvae of "*Luciobrotula*", separating them into four types (types 1–4) largely based on differences in exterilium-gut length and dorsal- and anal-fin-ray counts. However, the substantial overlap in counts (see Table 1) and descriptions for these larvae by Okiyama (2014) suggest they are more similar than different. For example, three of the four larval descriptions direct the reader to the description of type 1, with the type 4 identification section noting "See type 1… type 4 shares main characters/counts including caudal fin counts (13)" (Okiyama 2014: 435, translated by an author). The length of the exterilium appears to be the most important character in differentiating the four types described by Okiyama (2014). "*Luciobrotula*" types 1 and 4 (Okiyama 2014) have the shortest guts among the four types, with only slight bulging or exterilium guts illustrated, and are listed as having 77–80 dorsal-fin rays, 61–62 anal-fin rays, 25 pectoral-fin rays, 13 caudal-fin rays, and 51–54 total vertebrae (see Table 1). These ranges are all within expected variation for an ophidiiform species (see Table 1 and references therein). Furthermore, the specimens are listed as having two pelvic-fin rays, but the illustrations show highly reduced pelvic girdles and a single ray (see Okiyama 2014). Although the newly captured larva (USNM 465411) is from the Atlantic Ocean—Okiyama's (2014) larvae were

from the Pacific—the larva has fin-ray and vertebral counts within the ranges of the types 1 and 4 larvae (see Table 1), a reduced pelvic girdle, and is highly similar in overall physiognomy and pigment. Despite being from different oceans, we interpret the newly captured larva from Florida, as well as those in types 1 and 4 described by Okiyama (2014 see above) to be closely related, possibly of the same genus (i.e., Bythitidae type 1). For the remaining specimens we examined representing larval "*Luciobrotula*", we interpret the larvae captured from Japan (USNM 465770) and Hawai'i (USNM 447052), as well as those in types 2 and 3 described by Okiyama (2014), to be closely related (i.e., Bythitidae type 2) given their overlapping counts and overall physiognomy (see Table 1; Figs. 3d–e, 4, 6e–f).

Although recovered among a larger clade of the Bythitidae, species-level identifications of larval types 1 and 2 are not possible based on molecular characters and the current number of ophidiiform barcodes available on public repositories. Only 66 genera (~54%), and 135 species (~24%) of ophidiiforms currently have barcodes available on BOLD or GenBank, with the most-recent targeted molecular study on ophidiiforms (Møller et al. 2016) not including COI sequences despite sampling other mitochondrial markers (i.e., 16s, ND4). Without greatly increasing the number of taxa sequenced, we cannot confidently identify these and other ophidiiform larvae using standard barcoding methods. As for morphological characters, the dorsal- and anal-fin ray counts of both larval types are not exclusive to any bythitid in these localities. However, caudal-fin-ray counts in these larvae are an unusual 13. A few species in the bythitid genus *Tuamotuichthys* have 13 caudal-fin rays (Nielsen and Møller 2008). However, species of *Tuamotuichthys* are currently known to only occur in the western and southern Pacific Ocean. While the genus *Parasaccogaster* occurs in both Atlantic and Pacific Oceans, only one species, *Parasaccogaster normae* of the southeast Pacific, is known to have 13 caudal-fin rays (Nielsen et al. 2012). It is worth highlighting that the species in both *Parasaccogaster* and *Tuamotuichthys* are represented by one to a few specimens and the fin-ray counts may be broader in range than currently known. The 3–4 supraneurals present in these larvae may also be helpful for identification. Patterson and Rosen (1989) noted that a single ossified supraneural anterior to the second neural spine is the primitive condition for the Ophidiiformes. Bythitids have a variety of conditions, from no supraneurals (e.g., *Dermatopsis* and *Ogilbia*) to as many as six cartilaginous elements (e.g., *Brotulina* [currently *Dinematichthys*], *Calamopteryx*, *Grammonus*, *Lucifuga*, and *Ogilbia*; Patterson and Rosen 1989; Carnevale and Johnson 2015). Although three of these genera are now classified in the Dinematichthyidae (i.e., *Dinematichthys*, *Dermatopsis*, and *Ogilbia* [see Møller et al. 2016]), our phylogeny includes representatives from these genera, none of which

are recovered closely related to larval Bythitidae types 1 and 2. Without a detailed survey of supraneural and pterygiophore patterns across the Bythitidae, the utility of these larval supraneurals are limited. There is also the possibility that these larvae represent an undescribed lineage of ophidiiform, as sampling these fishes from their often deep, rugged, and rocky habitats is difficult. Although our understanding of ophidiiform fauna continues to increase, additional work is needed to fully understand the biodiversity of brotulas, cusk-eels, pearlfishes, and their larvae.

Fahay and Nielsen (2003) suggested that an early-forming, elongate coracoid process descending along the exterilium gut is a character that supports a sister-group relationship between *Brotulotaenia* and *Lamprogrammus* and diagnostic to an expanded Brotulotaeniinae. This character has also been described in the larva of *Leptobrotula* (Okiyama and Yamaguchi 2004), which was also included in the expanded Brotulotaeniinae. Both types of bythitid larvae examined in this study are similar to these brotulotaeniins, with the cartilaginous coracoid having a ventral process with a distal tip that extends near the ventral margin of the gut. In Bythitidae type 2, the cartilaginous coracoid processes form a loop within the exterilium gut (Figs. 3d–e, 6e–f) and the intestine is differentially expanded or contracted (compare Figs. 3d–e with 6e–f). These coracoids and differences in intestine length have not been previously described. The tissue surrounding the expanded intestine in USNM 447052 is taut when compared to the loose tissue surrounding the contracted intestine in USNM 465770 (compare Figs. 3d–e with 6e–f). While we did not find any muscular attachments to the coracoid processes, they are attached to the otherwise muscular intestine and surrounded by vascular tissue (Fig. 6f) that may represent the liver. Given the differences in appearance of the exterilium tissue relative to the length of the muscular intestine, we suspect that the larvae can manipulate the exterilium and intestine length through expansion or contraction. The intestines are stippled in the illustrations by Okiyama (2014: 434–435), highlighting that those with the most-elongate gut have an intestinal loop that reaches the ventral margin of the exterilium, similar to the larva in Figs. 3d–e. Such expansions or contractions would explain the overall variation in exterilium length observed in these larvae. Given this, we question the utility of exterilium gut length as a diagnostic feature among these bythitid type 2 larvae. Further, our phylogeny (Fig. 4) shows the larval elongate coracoid character occurring in multiple separate lineages of ophidiiforms. As this larval character was diagnostic for the Brotulotaeniinae, but phylogenetic hypotheses (e.g., Møller et al. 2016; Ghezelayagh et al. 2022) have found non-monophyly of ophidiiform subfamilies recognized by Nielsen et al. (1999), Fahay and Nielsen (2003), and others, we emphasize that the classification of

the order should be re-evaluated using both morphological and molecular characters.

Conclusion

In-situ photos and specimens captured by blackwater divers and photographers allowed for the examination and sequencing of newly captured ophidiiform larvae, revision of larval *Luciobrotula* morphology, and redescription of two larval bythitids. With newly revealed morphological features in these larvae, such as the elongate coracoid processes and the exceptional losses of anterior dorsal-fin elements, and larvae of many species unknown, now including the larvae of *Pycnocraspedum*, we hope that this study will encourage blackwater divers to continue to capture additional specimens, images, and video footage of ophidiiform larvae to further understand their morphology and diversity. The photographs and specimens captured by the divers continue to increase our understanding of the biology and natural history of marine fish larvae at an accelerated rate (see Nonaka et al. 2021; Pastana et al. 2022). However, our ability to identify these exceptional larvae using molecular characters is directly related to the accuracy and completeness of barcode reference libraries (Pentinsaari et al. 2020; Girard et al. 2022b; Mulcahy et al. 2022; Philips et al. 2022; Lea et al. 2023). Given that less than one quarter of ophidiiform species diversity has been barcoded and even fewer additional mitochondrial and nuclear loci have been made publicly available to date, this study highlights the continual need for generating sequences from vouchered museum specimens of brotulas, cusk-eels, pearlfishes, and allies. The sequences of *L. corethromycter* and *P. phyllosoma* are the first to be generated for these taxa and data from many other species are needed. Such efforts will greatly enhance our understanding of ophidiiform species diversity, their evolutionary history, and the under-explored morphological diversity of their larvae.

Specimens Examined Specimens are adults unless otherwise denoted as "Larva-" preceding specimen preparation type. Specimens examined as cleared and stained are denoted "CS"; specimens examined as whole ethanol specimens are denoted "ET" with a "*" indicating the specimen was also x-rayed or scanned using a μCT. Image stacks of μCT scans have been uploaded to MorphoSource, with associated accession numbers listed in brackets following the preparation type. All measurements listed are SL.

Benthocometes robustus: NHMD P77784, 1 Larva-CS, 20 mm, 27 September 1921, Mediterranean Sea; NHMD

P77785, 1 Larva-CS, 39 mm, 3 October 1921, Alboran Sea; NHMD P77786, 1 CS, 96 mm, 2 March 1968, Brazil.

"Bythitidae type 1": USNM 465411, 1 Larva-CS, 9.1 mm, captured and photographed by D. Devers, 18 February 2022, West Palm Beach, Florida.

"Bythitidae type 2": USNM 465770, 16.6 mm, 1 Larva-CS, 9 May 1987, Japan; USNM 447052, 24.0 mm, 1 Larva-ET, captured and photographed by J. Milisen, 13 May 2017, Kona, Hawai'i.

Luciobrotula bartschi: USNM 74151 holotype, 1 ET* [491478], 260 mm, 27 December 1908, Palawan, Philippines; USNM 179900, 1 ET, 116 mm, 3 June 1909, Samar, Philippines; USNM 454562, Larva-1 CS, 22.0 mm, photographed and captured by A. Deloach, N. Deloach, and S. Kovacs, 11 November 2021, Kona, Hawai'i.

Luciobrotula cf. *bartschi*: USNM 454451, Larva-1 ET, 22.9 mm, captured by S. Yano, 28–29 September 1988, Kona, Hawai'i.

Luciobrotula coheni: USNM 421217, 1 ET, 128 mm, 25 November 2010, Costa Rica; USNM 421356, 1 ET, 208 mm, 19 November 2010, Panama; USNM 421491, 1 ET, 206 mm, 12 November 2010, Panama; USNM 421528, 1 ET, 167 mm, 12 November 2010, Panama; USNM 422550, 1 ET, 99 mm, 24 November 2010, Costa Rica.

Luciobrotula corethromycter: USNM 188547 holotype, 1 ET*, 534 mm, 25 May 1962, Panama; USNM 188548 paratype, 1 ET, 295 mm, 26 July 1962, Alabama; USNM 188549 paratype, 1 ET* [491484], 310 mm, 14 December 1962, Florida; USNM 188550 paratype, 1 ET, 390 mm, 31 May 1962, Panama; USNM 188551 paratype, 1 ET*, 500 mm, 22 March 1963, French Guiana; USNM 334068, 1 CS, 58 mm, 10 June 1985; USNM 395816, 1 CS, 158 mm, 2 June 1964, Columbia; USNM 465301, Larva-1 CS, 15.0 mm, captured and photographed by R. Collins, 24 June 2021, West Palm Beach, Florida; USNM 465380, Larva-1 ET, 15.4 mm, captured and photographed by A. Deloach, N. Deloach, and S. Kovacs, 5 August 2021, West Palm Beach, Florida.

Luciobrotula lineata: USNM 162716 holotype, 1 ET*, 267 mm, 3 June 1950, Hawai'i.

Pycnocraspedum armatum: USNM 162717 holotype, 1 ET* [491490], 302 mm, 2 June 1950, Hawai'i; USNM 227411, 1 ET, 124 mm, 20 November 1968, Hawai'i; USNM 227412, 2 ET*, 248–250 mm, 24 September 1972, Hawai'i; USNM 395796, 1 CS, 123 mm, 1 November 1967, Hawai'i; USNM 395797, 1 CS, 117 mm, 19 November 1968, Hawai'i.

Pycnocraspedum phyllosoma: UF 233512, 1 ET, 94 mm, 23 July 1969, Anegada, British Virgin Islands; USNM 227388, 1 ET, 124 mm, 19 November 1968, Nicaragua; USNM 227413, 1 ET*, 233 mm, 27 May 1965, Turks and Caicos Islands; USNM 421586, 1 ET, 352 mm, 2013, Curaçao; USNM 421587, 1 ET, 301 mm, 2013, Curaçao; DEEPEND PC12-B0923-2790-MTSW6-SN-325A1-PS3661, tissue G176, 90 mm, 23 September 2011,

Gulf of Mexico; YPM ICH 2902 holotype, 1 ET * [491493], 100 mm, 4 April 1927, Turks and Caicos Islands.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethics approval Species of fishes in this study are not listed as threatened or endangered by the IUCN Red List or CITES. All methods of capture and preservation conform to the Guidelines for the Use of Fishes in Research established by the American Fisheries Society, American Institute of Fishery Research Biologists, and American Society of Ichthyologists and Herpetologists. Larvae collected off West Palm Beach, Florida, were acquired under Florida permit SAL-21-2155A-SR. Permit not required for larval collections off Kona, Hawai'i.

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