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Anatomy and evolution of bioluminescent organs in the slimeheads (Teleostei: Trachichthyidae)

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Abstract

Bacterial bioluminescent organs in fishes have a diverse range of tissues of origin, patterns of compartmentalization, and associated light-conducting structures. The morphology of the perianal, bacterial bioluminescent organ of *Aulotrachichthys prosthemius* was described previously, but the light organ in other species of slimeheads, family Trachichthyidae, is poorly known. Here, we describe the anatomy of the bioluminescent organs in trachichthyids and places the evolution of this light-producing system in the context of a new phylogeny of the Trachichthyoidei to test the hypothesis that bioluminescence evolved twice in the suborder and that the light-producing component derives from the perianal ectoderm. We use gross and histological examination to provide the first description of the bioluminescent organ of *Paratrachichthys* and four additional species of *Aulotrachichthys*. Observations also strongly suggest the presence of a perianal bioluminescent organ in *Sorosichthys ananasa*. The updated phylogeny of the Trachichthyoidei is the first to combine morphological and DNA-sequence (11-gene fragments) evidence, and supports a monophyletic Trachichthyidae with component subfamilies Hoplostethinae and Trachichthyinae, supporting continued recognition of the family Anoplogastridae. All bioluminescent trachichthyoids share a similar bioluminescent-organ structure with elongate chambers filled with bacteria and connected to collecting ducts that, in turn, connect to superficial ducts that lead to and have lining epithelia continuous with the epidermis. In the context of the phylogeny, the bioluminescent organ of trachichthyids is inferred to have evolved as an elaboration of the proctodeum in the ancestor of *Aulotrachichthys*, *Paratrachichthys*, and *Sorosichthys* independently from the structurally similar cephalic bioluminescent organs in Anomalopidae and Monocentridae.

KEYWORDS
Aulotrachichthys, bacteria, histology, *Paratrachichthys*, *Sorosichthys*

1 | INTRODUCTION

Bacterial bioluminescent organs in fishes have evolved many times from a diversity of anatomical structures. Because bacterial bioluminescent organs require colonization by an environmental bacterium, they necessarily involve the formation of bacteria-containing compartments by epithelial surfaces of the skin or the digestive tract. Despite this requirement, these organs still have a diverse range of tissues of origin, patterns of compartmentalization, and associated light-conducting structures (Chakrabarty et al., 2011; Ghedotti et al., 2018;

Nishiguchi et al., 2004). Although bacterial bioluminescence is inferred to have evolved less frequently than intrinsic bioluminescence among invertebrate animals (Haddock et al., 2010; Lindgren et al., 2012; Pankey et al., 2014), the converse is true among teleost fishes with bacterial bioluminescence comprising at least 17 of 28 inferred origins of bioluminescence (Davis et al., 2016; Haddock et al., 2010).

Bacterial bioluminescent organs in fishes serve a variety of functions, most commonly to provide camouflage via ventral counter-illumination against a background of downwelling light, although bacterial bioluminescence also is used for prey luring, prey illumination, and mate recognition (Haddock et al., 2010; Paitio et al., 2016). Bacterial bioluminescent organs have bacteria-containing compartments, tubules, or crypts that develop from the endodermal epithelium of the digestive tract or the ectodermal epithelium of the head, dorsal fin, ventrolateral surface of the body, or perianal region (Chakrabarty et al., 2011; Ghedotti et al., 2018; Haneda, 1961; Johnson & Rosenblatt, 1988; Munk, 1999; Thacker & Roje, 2009). In some cases, closely related taxa with bacterial bioluminescent organs may form them in different anatomical regions (Ghedotti et al., 2018; Paitio et al., 2016).

The bioluminescent members of the suborder Trachichthyoidei all exhibit bacterial bioluminescent organs derived from either the cephalic or the perianal integument (Davis et al., 2016). In the Anomalopidae and the Moncentridae, families in which all species are bioluminescent, the light organs are composed of dead-end tubules or crypts derived from the subocular or submandibular integument, respectively (Bassot, 1968; Haneda & Tsuji, 1971; Tebo et al., 1979). The bioluminescent organs of *Anomalops katoptron*, *Photoblepharon palpebratus* (another anomalopid), and *Monocentris japonica* house bacterial symbionts of the species *Photodesmus katoptron*, *Photodesmus blepharus*, and *Allivibrio fischeri*, respectively (Dunlap et al., 2007; Hellinger et al., 2017; Hendry & Dunlap, 2014). The members of the families Anoplogastridae and Diretmidae lack bioluminescent organs, whereas the Trachichthyidae has both bioluminescent and non-bioluminescent species (Davis et al., 2016). Within the Trachichthyidae, a perianal light organ and associated structures for transmitting light called "striated areas" have been noted for the species of *Aulotrachichthys* (Gomon & Kuiter, 1987; Gon, 1983). The bioluminescent organ of *Aulotrachichthys prosthemius* house bacterial symbionts of the species *Photobacterium kishitani*, a common bioluminescent symbiont with fishes in at least seven families (Dunlap et al., 2007; Haneda, 1957; Hendry & Dunlap, 2014). *Aulotrachichthys* is diagnosed by the presence of the light-conducting striated areas, and their presence was the basis for the recognition of the genus with eight species as distinct from *Paratrachichthys* with three species (Fowler, 1938; Gomon & Kuiter, 1987; Paxton et al., 1989). *Paratrachichthys mackleayi*, was noted as having a perianal bioluminescent organ by Jordan and Bruce (1993). However, other authors indicated that the genus *Paratrachichthys* is not bioluminescent (Konishi & Okiyama, 1997; Paitio et al., 2016; Paulin, 1979). The Little Pineapple Fish, *Sorosichthys ananassa* also has striated areas like species of *Aulotrachichthys* and has a far anterior anus associated with the pelvic fins as in both *Aulotrachichthys* and *Paratrachichthys* but has not been identified as having a bioluminescent organ (Gomon & Kuiter, 1987; Grant, 1987; Kotlyar, 1992; Kuiter, 1993).

The bioluminescent organ of *A. prosthemius* was generally described in the 1950s and has not been studied anatomically since then. Kuwabara (1955) identified a perianal organ composed of bacteria-containing crypts with ducts to the epidermis, surrounded by a chromatophore-lined collagenous capsule, underlain by a chromatophore-containing connective-tissue lens, and associated with long slips of possibly light-conducting muscle that he called unknown structures. Haneda (1957) confirmed Kuwabara's (1955) observations, cultured luminous bacteria from the organ, renamed Kuwabara's (1955) unknown structures as the filiform bodies, and observed bioluminescence in live individuals of *A. prosthemius*. Haneda (1957) also documented the role of chromatophores in obscuring or revealing light diffusing from the bacterial crypts through the transparent muscle in the isthmus, the pectoral region, and along the filiform bodies.

Knowing the phylogenetic relationships within the suborder Trachichthyoidei is critical for understanding the evolution of the bioluminescent organs and associated structures in this order. Based on a phylogenetic analysis of 24 adult morphological characters, Moore (1993) recognized two major clades. One clade was composed of the Diretmidae and Anoplogastridae in which all species are non-bioluminescent. The other clade contained the families Anomalopidae, Monocentridae, and Trachichthyidae, families in which some or all species are bioluminescent, and Monocentridae and Trachichthyidae were sister taxa. These relationships were consistent with a single origin of bacterial bioluminescence, possibly with a subsequent loss in a group or groups within the Trachichthyidae. Konishi and Okiyama (1997) conducted a phylogenetic analysis using 26 adult and larval morphological characters which resulted in relationships similar to those found by Moore (1993), except that the Trachichthyidae was paraphyletic with respect to a clade composed of the Monocentridae and Anomalopidae. The relationships depicted by Moore (1993) suggested that there was a single origin of bioluminescence in the order. Recent large-scale phylogenetic studies using DNA-sequence data but with limited trachichthyiform taxon sampling commonly have recovered Diretmidae sister to all other trachichthyoids, and a bioluminescent clade containing the Anomalopidae and Monocentridae sister to a clade composed of Trachichthyidae and *Anoplogaster* (Betancur et al., 2017; Davis et al., 2016; Dornburg et al., 2017). However, one analysis found *Anoplogaster* sister to all other trachichthyoids (Near et al., 2013). The relationships recovered using DNA-sequence data are consistent with at least two origins of bioluminescence.

In this study, we test the hypothesis that bioluminescence evolved twice in the Trachichthyoidei, that the bioluminescent organ in the Trachichthyidae evolved once, and that the light-producing component is derived from the perianal ectoderm which is derived from the developmental proctodeum in all bioluminescent trachichthyids. To this end, we provide the first description of the histological and anatomical structure of the bioluminescent organ of *Paratrachichthys* and four additional species of *Aulotrachichthys*. We

also provide an updated phylogeny of the Trachichthyoidei, the first to combine morphological and DNA-sequence evidence.

2 | MATERIALS AND METHODS

2.1 | Morphological examination

The number of specimens available to be dissected and/or sampled histologically was limited by the relatively few specimens in museum collections. We examined cataloged, preserved museum specimens and did not work with live animals. We externally examined specimens cleared and double stained for bone and cartilage (Dingerkus & Uhler, 1977; Potthoff, 1984), prepared dry skeletons, and/or dissected previously formaldehyde-fixed and ethanol-preserved or museum-cataloged specimens (institutional codes follow Sabaj, 2020) that were from the Academy of Natural Sciences of Drexel University (ANSP), Philadelphia, PA, the American Museum of Natural History (AMNH), New York, NY, the Australian Museum (AMS), Sydney, NSW, Australia, the California Academy of Sciences (CAS), San Francisco, CA, the Field Museum of Natural History (FMNH), Chicago, IL, the Natural History Museum of Los Angeles County (LACM), CA, the Scripps Institution of Oceanography (SIO), La Jolla, CA, and the University of Minnesota Bell Museum of Natural History (JFBM), St. Paul, MN. Specimens examined are listed in supplementary online material Table S1.

We examined the bioluminescent organ and associated structures embedded in the body wall by removing the overlying skin and then separating muscle masses using fine forceps to reveal the bioluminescent organ or associated structures. In cleared and stained specimens, we removed the branchial skeleton by using microdissection scissors to cut cleared tissues around the branchial skeleton and cut the ligamentous connection of the interhyal to the suspensorium. We used a Leica MZ 12.5 stereomicroscope (Leica Microsystems, RRID:SCR_008960) with an attached Q Imaging MicroPublisher 5.0 RTV photodocumentation system (Q Capture Software, RRID:SCR_014432) to examine and photograph specimens. We conducted gross dissection and examination of ethanol-preserved museum specimens at Regis University, Denver, CO.

We examined external photographs of specimens in lateral and ventral view that were unavailable for loan due to rarity or distance from the AMS; Australian National Fish Collection (CSIRO), Hobart, TAS, Australia (CSIRO), and the United States National Museum of Natural History (USNM), Washington, DC. We particularly noted the condition and position of the anus and any light-conducting "striated areas."

2.2 | Histological analysis

We removed histological samples from formaldehyde-fixed and ethanol-preserved museum-cataloged specimens from AMS, FMNH, JFBM, and SIO via dissection of approximately 0.5–1 cm^3 of tissue from the ventral body. Samples were from the area around the anus, immediately anterior to the anal fin, and immediately posterior to the anal fin from the specimens indicated in the specimens examined supplement (supplementary online material, Table S1). We decalcified samples in a citrate buffered 25% formic acid solution for 1–5 h, followed by dehydration in an ethanol series to 100% and clearing in xylene. We infiltrated samples with paraffin, embedded them in paraffin blocks, sectioned blocks on a rotary microtome every 10 μm, and mounted sections on slides using a water bath at 37°C (Humason, 1979). We stained sections using standard Harris's hematoxylin and aqueous eosin with alcian blue (HE + A) to identify acidic polysaccharides such as those found in cartilage and some mucus cells (Charman & Reid, 1972; Ghedotti et al., 2019) and the Masson's trichrome (MT) staining protocol (Bancroft & Stevens, 1982; Sheehan & Hrapchak, 1980) to differentiate collagen and muscle. We conducted all histological procedures at Regis University, Denver, CO where we examined and photographed slide-mounted sections using a Leica DM 2500 compound microscope (Leica Microsystems) with an attached Q Imaging MicroPublisher 5.0 RTV photodocumentation system (Q Capture Software, RRID:SCR_014432). We prepared photos for figures by increasing brightness and contrast evenly across the entire image and eliminating fragments or discoloration in the mounting medium outside the external margin of the organism using image software (Adobe Photoshop, Photoshop CC 2017, RRID: SCR_014199).

2.3 | Taxon sampling for phylogenetic analysis

Taxonomic sampling for the total evidence phylogenetic analyses focused on including representatives of major lineages within the Beryciformes with 40 taxa representing 14 families including 30 species and all families of the bioluminescent focal group, suborder Trachichthyoidei (Anomalopidae, Anoplogastridae, Diretmidae, Monocentridae, and Trachichthyidae). Additional outgroups were stem acanthomorph taxa from the Polymixiiformes (*Polymixia berndti*, root for analysis) and Lampriformes (*Lampris guttatus*) based on the phylogenetic relationships presented by Betancur et al., 2017, Davis et al., 2016, Hughes et al. (2018), Near et al. (2012), Near et al. (2013), and Smith et al. (2016).

2.4 | Acquisition of morphological characters for phylogenetic analysis

We obtained 60 morphological characters within the trachichthyoids from those published in Konishi and Okiyama (1997), Kotlyar (1992), and Moore (1993) that were potentially phylogenetically informative for the taxa included in this study. For taxa outside the Trachichthyoidei, species within a genus were coded at the genus-level unless data were available for multiple species within the genus. We checked morphological characters for all available species examined as cleared and stained specimens. For larval characters or when a specimen was not available for morphological examination of the specific characteristic, we checked character codings against descriptions

in the primary literature (Baldwin & Johnson, 1995; Gonzalez-Rodriguez et al., 2013; Johnson, 1984; Jordan & Bruce, 1993; Kotlyar, 1992; Oka & Higashiji, 2012; Post & Quero, 1981; Richards et al., 2003; Shimizu, 1977; Starks, 1904; Zehren, 1979).

2.5 | Acquisition of nucleotide sequences and phylogenetic analysis

The data matrix included 11 gene fragments [COI, 1557 base pairs (bp); ENC1, 811 bp; GlyT, 870 bp; MYH6, 761 bp; PLAGL2, 780 bp; Ptr, 706 bp; RAG1, 1504 bp; SH3PX3, 727 bp; SREB2, 988 bp; TBR, 690 bp; ZIC1, 865 bp] with previously published sequences available on GenBank and the Barcode of Life Database (supplementary online material, Table S2) and 60 morphological characters (supplementary online material, Table S3). We used multiple gene fragments to best infer relationships among both trachichthyid and related taxa. We aligned sequence data with MAFFT (Katoh et al., 2002, Katoh & Standley, 2013; MAFFT, RRID:SCR_011811) using default values. We used the program PartitionFinder v2.1.1 (Lanfear et al., 2017) to select models of molecular evolution for sequence data using the AICc and rclusterf search method for each codon position within a gene fragment for a total of 33 partitions. PartitionFinder identified 20 subsets (Subset 1: COI-1; Subset 2: COI-2; Subset 3: COI-3; Subset 4: ENC1-1; Subset 5: ENC1-2 and Ptr-1; Subset 6: ENC1-3 and TBR-1; Subset 7: GlyT-1, MYH6-1, and RAG1-2; Subset 8: GlyT-2 and MYH6-2; Subset 9: GlyT-3 and RAG1-3; Subset 10: MYH6-3 and Ptr-2; Subset 11: PLAGL2-1, SH3PX3–1, and ZIC1-1; Subset 12: PLAGL2-2, SH3PX3–2, and ZIC1-2; Subset 13: PLAGL2-3 and SH3PX3–3; Subset 14: Ptr-3 and SREB2-1; Subset 15: RAG1-3; Subset 16: SREB2-2; Subset 17: SREB2-3; Subset 18: TBR-2; Subset 19: TBR-2; Subset 20: ZIC1-3) with associated models for each subset including GTR (Subset 19), GTR + G (Subsets 2, 3, 4, 6, 8, 9, 10, 12, 13, 15, 17, 18, and 20), and GTR + I + G (Subsets 1, 5, 7, 11, 14, and 16). We used a single partition for the morphological data with the MK (Markov) model for morphological data that also included a modification to accommodate ascertainment bias as the morphological alignment did not include constant sites (Lewis & Puterman, 2001). We analyzed the total evidence dataset with IQ-Tree v1.6.12 (Nguyen et al., 2015; IQ-Tree, RRID:SCR_017254) to infer the optimal tree topology with a maximum-likelihood approach. Tree searching was performed 10 independent times with default settings and the tree with the maximum-likelihood score of the repeated analyses is presented herein. Bootstrap replicates were conducted 100 independent times, with bootstrap numbers presented on the most likely tree.

3 | RESULTS

3.1 | Bioluminescent organ structure and histology

The light-producing component in the bioluminescent organ of *Paratrachichthys fernandezianus* is a ring-shaped region around the terminal intestine composed of bacterial crypts with associated tissues that are immediately dorsal to an externally visible, melanistic, perianal ring (Figure 1(a–c)). The bacterial crypts are dorsoventrally oriented, oval to cylindrical chambers filled with bacteria, lined by a simple cuboidal epithelium that is continuous with the epithelium of the ducts. The epithelium becomes stratified by the time it merges with the stratified cuboidal epithelium of the epidermis of the perianal ring. The ducts from each crypt widen into open collecting ducts and that connect to several crypts (Figure 1(c,e,f,h)). The crypts and ducts are surrounded by a vascular connective tissue that supports the raised perianal ring and contains many dark chromatophores in the ventral region between the many ducts (Figure 1(e)). A thick, more superficial, connective tissue layer dorsal and dorsolateral to the crypts contains many dark chromatophores near its ventral margin and connects laterally to the medial surface of anterior and posterior processes of the pelvic girdle along most of the length of the organ (Figure 1(b,f,g)). A deep connective tissue layer is dorsal to the light-producing bacterial crypts except for a continuation of this connective tissue present between the anterior and middle infracarinal muscles near the main body of the bioluminescent organ (Figure 1(d)).

The light-producing component in the bioluminescent organ of the species of *Aulotrachichthys* examined (*A. argyrophanus*, *A. heptalepis*, *A. novaezelandicus*, *A. prosthemius*, and *A. sajademalensis*) is generally similar to the structure in *P. fernandezianus* with the following exceptions (Figure 2). The externally visible, melanistic, perianal ring in the examined specimens of *A. novaezelandicus* is similar to the perianal ring in *Paratrachichthys*, but in the specimens of the other species of *Aulotrachichthys* examined, the perianal ring is narrower than in *Paratrachichthys* and surrounded by an area of asquamous skin (Figure 2(b)). In contrast to the condition observed in *Paratrachichthys*, the dorsal connective tissue and bacterial crypts anterior to the anus are deeper within the body wall and extend further anterior with substantial body-wall musculature lateral to these structures (Figure 2(d,e)). The anteriormost part of the perianal component of the bioluminescent organ has a cartilage cap that is continuous dorsal to the bacterial crypts and the associated connective tissue both anterior and posterior to the anus (Figure 2(c–e)). The light-producing bacterial crypts are dorsoventrally oriented as in *P. fernandezianus* near the center of the organ, but are increasingly inclined obliquely to horizontally around the periphery of the organ (Figure 2(c,e)). The ducts from each crypt widen into collecting ducts as in *P. fernandezianus*, but each collecting duct connects to more crypts (Figure 2(e,f)). Most duct openings in *A. argyrophanus*, *A. heptalepis*, *A. prosthemius*, and *A. sajademalensis* are on the asquamous areas surrounding the narrow perianal swelling that is less extensive than in *Paratrachichthys* (Figure 3(e,f)). However, these ducts open only on the wide perianal swelling in the 18 mm sample from *A. novaezelandicus* (Figure 3(a)), as they do in *P. fernandezianus*.

The light-transmitting component of the bioluminescent organ of the species of *Aulotrachichthys* examined, also called the striated areas (Fowler, 1938; Gomon & Kuiter, 1987; Paxton et al., 1989) or filiform bodies (Haneda, 1957), are areas of transparent muscle in contact with the light-producing component of the bioluminescent organ. We confirmed prior observations that these areas of transparent muscle are

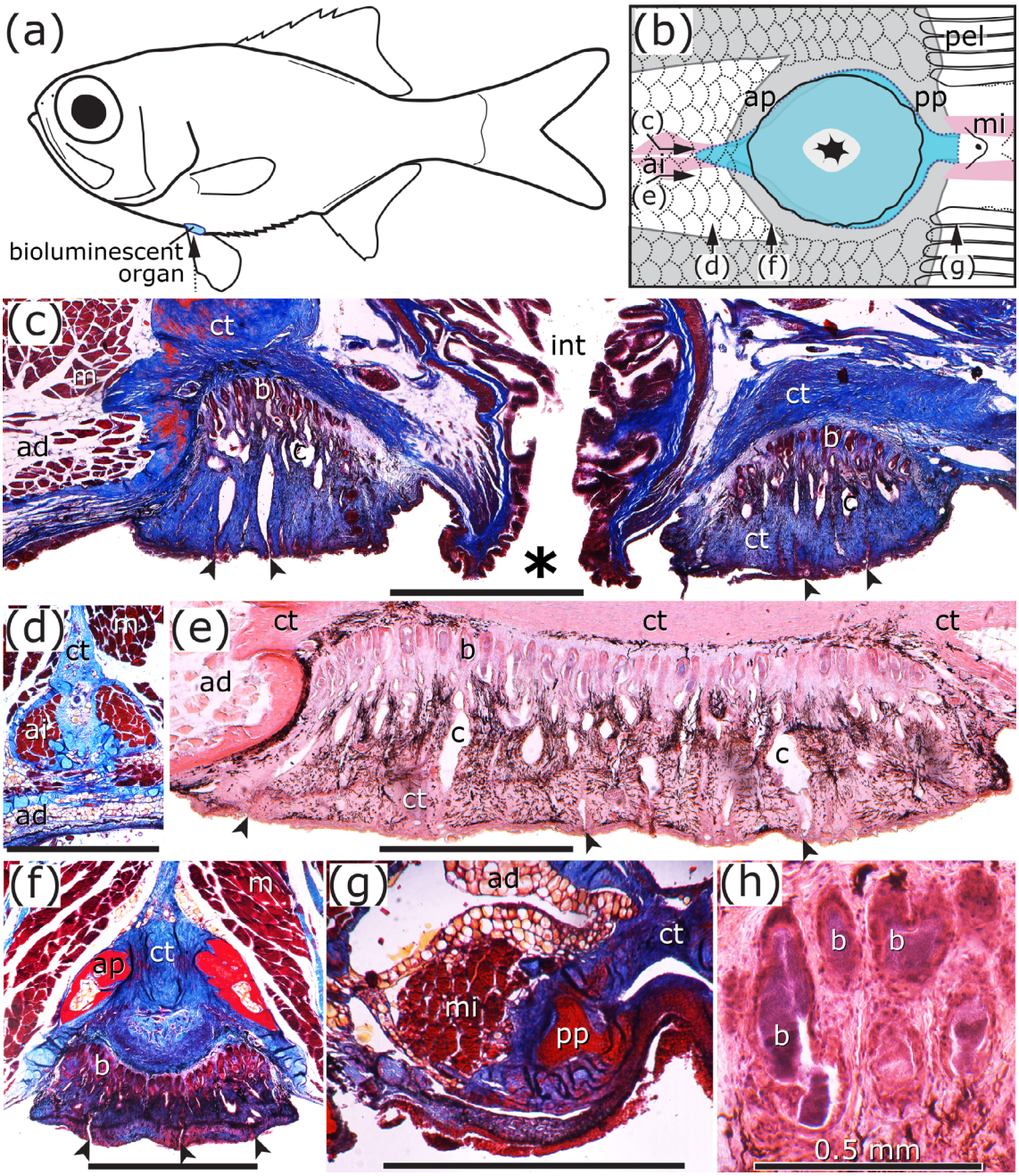

FIGURE 1 The ventral bioluminescent organ in *Paratrachichthys fernandezianus* (FMNH 107298 and SIO 65-637). Sections indicated as FMNH and SIO are from 78 mm SL and 101 mm SL individuals, respectively. (a) Diagrammatic illustration of the bioluminescent organ in left-lateral view of *P. fernandezianus*. Dotted arrow indicates anus position. (b) Diagrammatic illustration of a ventral view of the perianal bioluminescent organ. Gray indicates the pelvic girdle. Letters indicate location of sections depicted in (c–g). (c) Median longitudinal section of bioluminescent organ. SIO. Masson's trichrome (MT). (d) Cross section anterior to anus. FMNH. MT. (e) Parasagital longitudinal section of bioluminescent organ. SIO. Harris's hematoxylin and aqueous eosin with alcian blue (HE + A). (f) Cross section anterior to anus. FMNH. MT. (g) Cross section posterior to anus, left of midline. FMNH. MT. (h) Cross section of crypts from light organ anterior to anus. FMNH. HE + A. Arrow head, external opening of ducts; asterisk (*), anus; ad, adipose tissue; ai, anterior infracarinalis muscle; ap, anterior process of pelvic girdle; b, bacterial crypts; c, collecting duct; ct, collagen-rich connective tissue; int, intestine; m, muscle; mi, middle infracarinalis muscle; pel, pelvic fin; pp, posterior process of pelvic girdle. All scale bars are 1 mm unless otherwise indicated

superficially overlain by oblique rows of dark chromatophores that give the structure a "striated" appearance and are underlain by a deep connective-tissue layer (Figure 3(b)), that is reflective in fresh specimens (Haneda, 1957; Woods, 1961). The muscles anterior to the anus, ventral to the pectoral fin and extending anterior to the isthmus are the anterior component of the light-transmitting tissues. The posterior muscles in the light-transmitting component of the bioluminescent organ extend as bilaterally paired structures attached to the posterior processes of

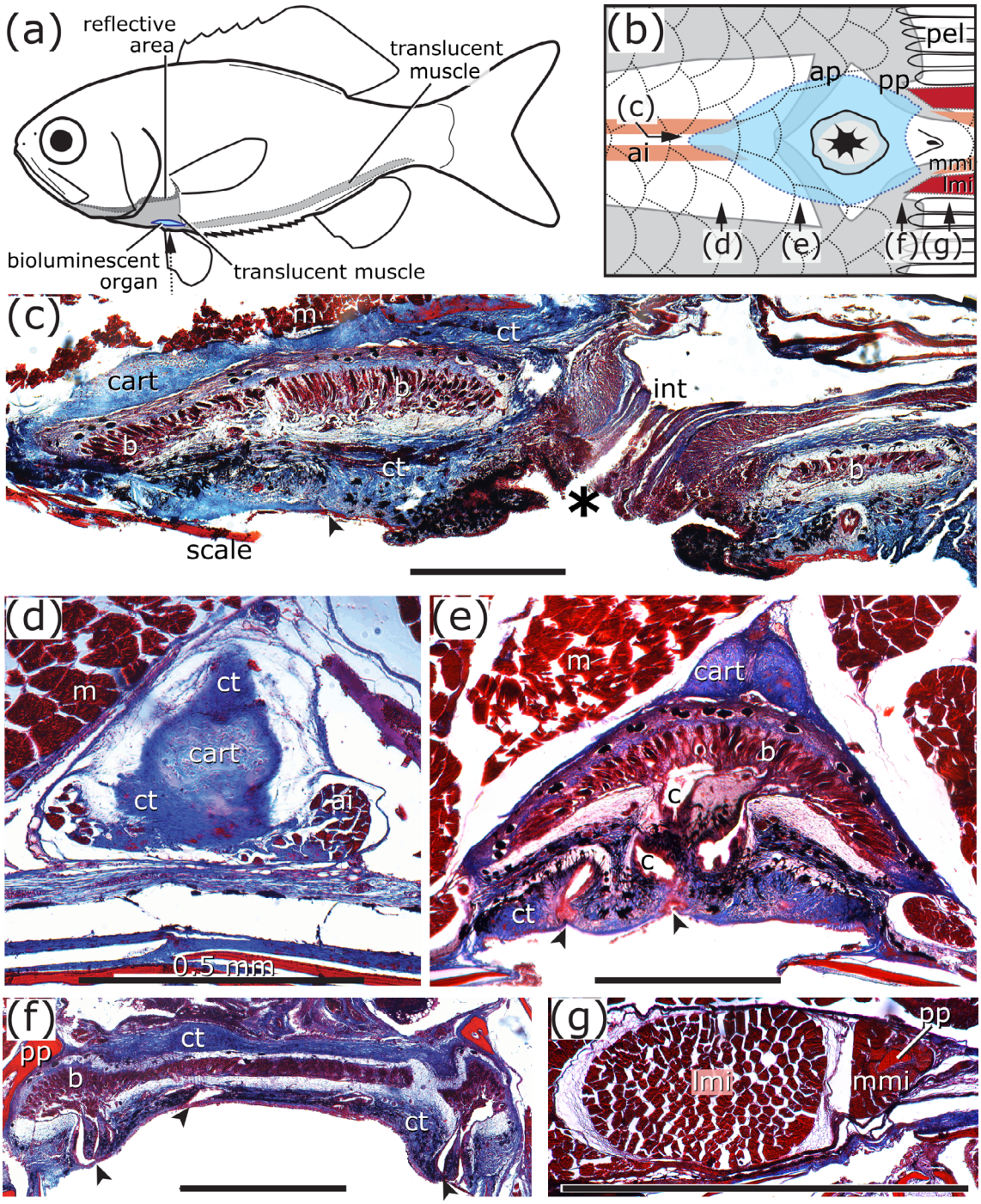

FIGURE 2 The ventral bioluminescent organ in *Aulotrachichthys prosthemius* (JFBM 48685, 71 mm SL, 72 mm SL). All sections Masson's trichrome stained. (a) Diagrammatic illustration of the bioluminescent organ in left-lateral view of *A. prosthemius*. Dotted arrow indicates anus position. (b) Diagrammatic illustration of a ventral view of the perianal light-producing component of the bioluminescent organ. Gray indicates the pelvic girdle. Letters indicate location of sections depicted in (c–g). (c) Median longitudinal section of bioluminescent organ. (d,e) Cross sections anterior to anus. (f) Cross section posterior to anus. (g) Cross section posterior to anus, left of midline. Arrow head, external opening of ducts to crypts; asterisk (*), anus; ai, anterior infracarinalis muscle; ap, anterior process of pelvic girdle; b, bacterial crypts; cart, cartilage; c, collecting duct; ct, collagen-rich connective tissue; int, intestine; lmi, lateral division of middle infracarinalis muscle; m, muscle; mmi, medial division of middle infracarinalis muscle; pel, pelvic fin; pp, posterior process of pelvic girdle. All scale bars are 1 mm unless otherwise indicated

the pelvic girdle and extend posteriorly on both sides of the midline to the caudal peduncle just posterior to the posterior base of the anal fin. These muscles run lateral and parallel to the more medial middle infracarinalis muscles that attach on the posterior processes of the pelvic girdle more posteriorly and medially than the posterior muscles in the light-transmitting component of the bioluminescent organ (Figure 2 (g)). These light-transmitting muscles extend most posteriorly, beyond a vertical at the posterior margin of the anal fin, in *A. prosthemius* and *A.*

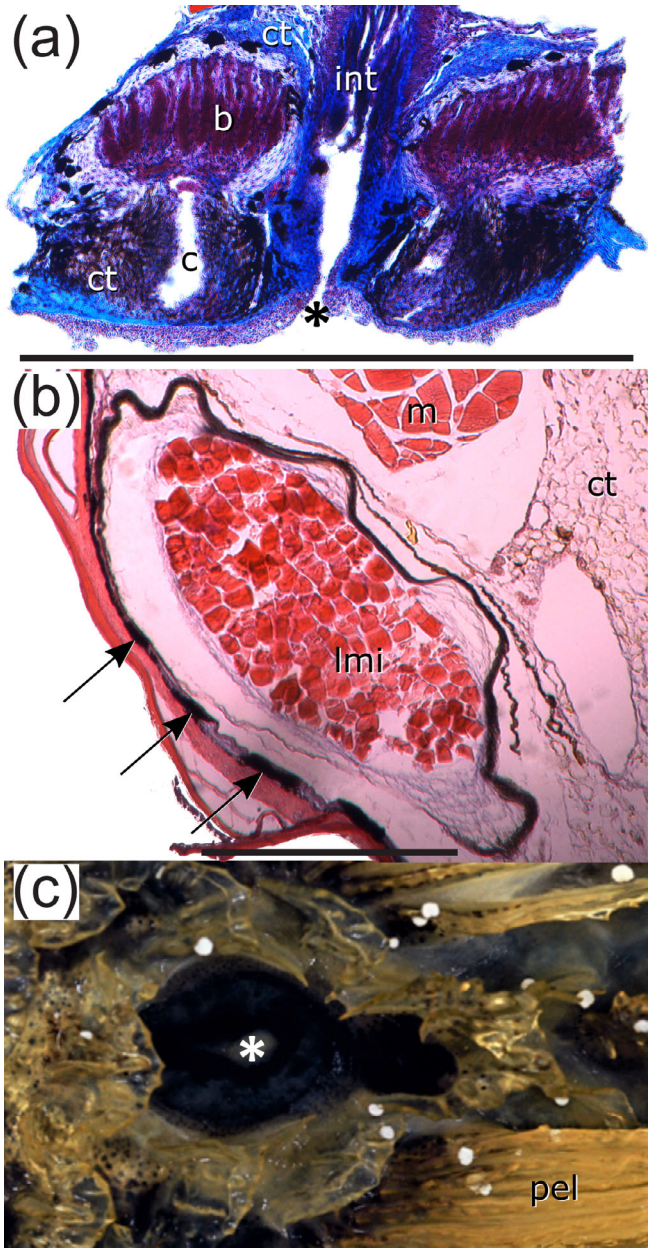

FIGURE 3 (a) Cross section of the light-producing component of the bioluminescent organ at the anus in *Aulotrachichthys novaezelandicus* (AMS I.20313-009, 18 mm SL). MT. (b) Cross section of left posterior light-transmitting structure, likely a lateral division of the middle infracarinalis muscle, in *A. argyrophanus* (FMNH 65600, 53 mm SL) in the caudal peduncle, left of midline. The overlying dark chromatophores and the underlying reflective surface are visible. HE + A. (c) Ventral view of anus in *Sorosichthys ananassa* (CSIRO H 6942.02, 58 mm SL). Anterior at left. Photo obtained from (a). Graham. Arrows, pigment forming light-masking striations; asterisk (*), anus; b, bacterial crypts; c, collecting duct; ct, collagen-rich connective tissue; lmi, lateral division of middle infracarinalis muscle; m, muscle; pel, pelvic fin. All scale bars are 1 mm

pulsator (Gomon & Kuiter, 1987). The middle infracarinalis muscles in *P. fernandezianus* attach to the posterior process of the pelvic girdle as a single pair of bilateral muscles associated with adipose tissue (Figure 1(g)).

S. ananassa has a large raised perianal ring without a larger area of asquamous surrounding skin that is similar to the one observed in *Paratrachichthys* and the observed juvenile specimens of *Aulotrachichthys novaezelandicus* (Figure 3(c)). Striated areas that externally appear similar to the light-transmitting component of the bioluminescent organ of species of *Aulotrachichthys* are visible externally in *S. ananassa* and have the same posterior extent as those observed in *A. prosthemius* and *A. pulsator*. We were unable to histologically sample specimens of *S. ananassa* due to the rarity of specimens in collections. We were limited to external examination via photographs of museum specimens.

We histologically sectioned the light organs of *A. katoptron* and *Monocentris japonica* to confirm prior observations of bioluminescent organ structure. We confirmed the observations of Bassot (1968) that the suborbital bioluminescent organ of *A. katoptron* houses densely packed bacteria in narrow tubules lined by a simple squamous or simple cuboidal epithelium that are superficial to a dark chromatophore-containing and reflective layer. The tubules are continuous with more superficial collecting ducts that lead separately to multiple external pores on the surface of the bioluminescent organ. We also confirmed later observations that the mental bioluminescent organ of *M. japonica* houses densely packed bacteria in oval elongate crypts lined by a simple cuboidal epithelium that are superficial to a dark chromatophore-containing layer (Duchatelet et al., 2019; Tebo et al., 1979). The crypts connect via individual ducts to more superficial collecting ducts that also often contain bacteria in the examined preserved specimens. The collecting ducts connect to smaller emissary ducts that lead to external pores at the dorsal margin of the bioluminescent organ. All trachichthyoid bioluminescent organs share a common morphology with multiple elongate chambers containing bacteria that connect to more superficial collecting ducts that variably may contain bacteria and that connect to ducts leading to pores in the epidermis regardless of position on the body or taxonomic group.

Histological sectioning of the anus and perianal tissues of *Anoplogaster cornuta*, *Diretmoides pauciradiatus*, *Hoplostethus crassipinnus*, *H. occidentalis*, and *Optivus agastos* definitively demonstrated the absence of a perianal bioluminescent organ in these taxa. As also observed externally, none of these species had distinct perianal melanism or swellings as were observed in *Aulotrachichthys* and *Paratrachichthys*. We also observed unmodified and undivided posterior middle infracarinalis muscles in these samples.

3.2 | Morphological characters

We compiled morphological characters from the literature including adult and larval characteristics (Konishi & Okiyama, 1997; Kotlyar, 1992; Moore, 1993) and checked characters using the specimens listed in the specimens examined. The character states reported by Kotlyar (1992) and Moore (1993) all were consistent when we examined specimens of the same species as these authors. Character state distribution among taxa is presented in supplementary online

material, Table S3 and character descriptions are presented in supplementary online material, Table S4.

3.3 | Phylogenetic analysis

The inferred phylogenetic relationships from our total-evidence analysis supports a monophyletic Beryciformes (bootstrap 79) with two broad clades, the Trachichthyoidei (bootstrap 89) and a clade composed of the Holocentridae, Melamphaidae, Berycidae, and the stephanoberycoids with weaker bootstrap support (Figure 4). Overall boostrap support was strong for each genus but was weaker for relationships within a genus, which is likely the result of some taxa within a genus having larger amounts of missing genetic data (supplementary online material, Table S1). The relationships within the non-trachichthyoid clade are consistent with multiple DNA-sequence analyses (Davis et al., 2016; Dornburg et al., 2017; Near et al., 2013). The inferred relationships of the Trachichthyoidei (Figure 4) are consistent with the findings from Dornburg et al. (2017) that included five trachichthyoid species (*A. katoptron*, *Anoplogaster cornuta*, *Aulotrachichthys sajademalensis*,

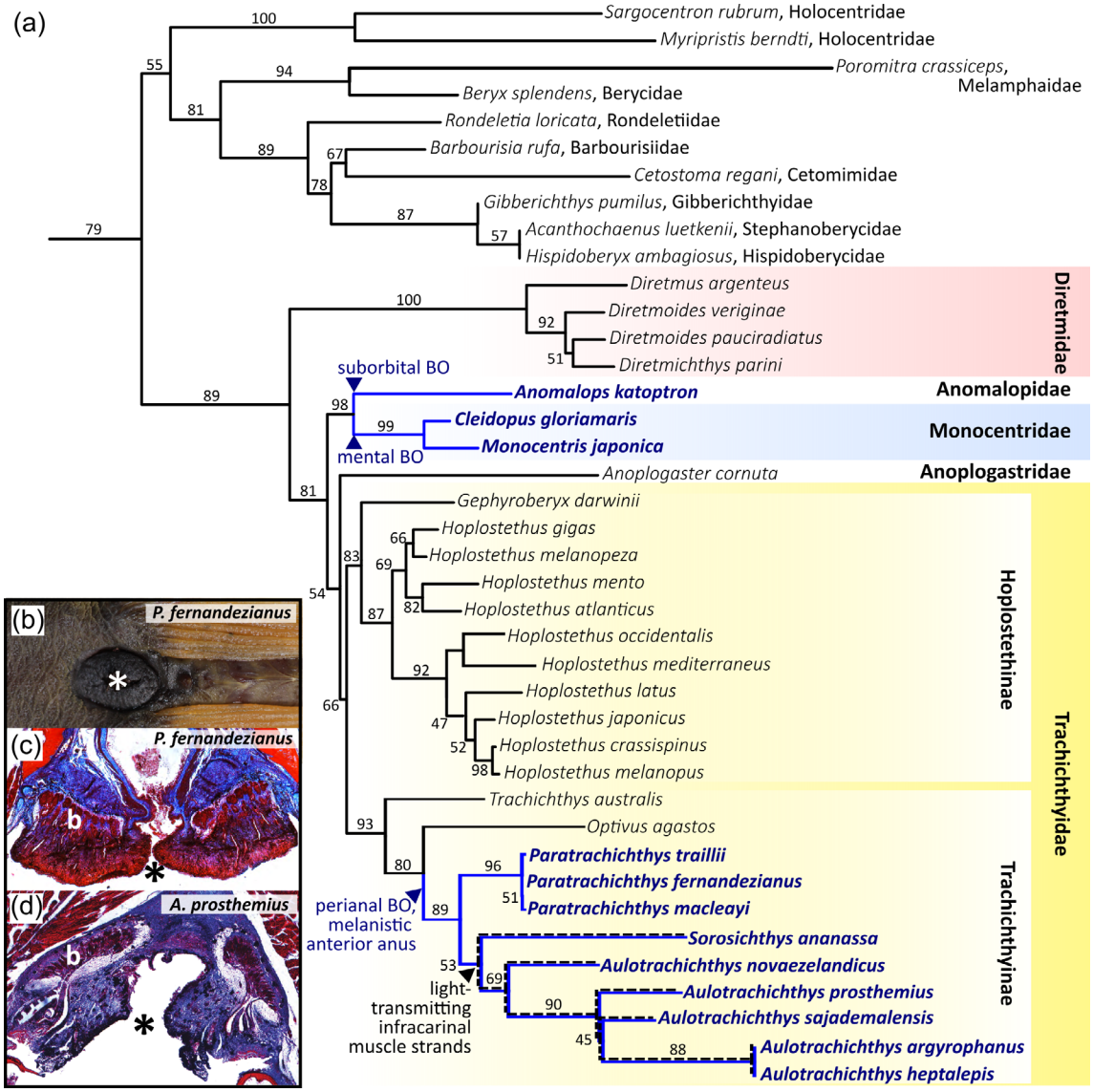

FIGURE 4 (a) Evolutionary relationships among representative trachichthyoids and related taxa based on a maximum likelihood analysis of 11 gene fragments (10 nuclear and 1 mitochondrial) using evolutionary models GTR (one subset), GTR + G (13 subsets) and GTR + I + G (six subsets) for genetic data. See Section 2.5 for specifics. Outgroups not shown. Branch lengths indicate the relative number of character substitutions per site for the mixed nucleotide and morphological dataset. Numbers associated with nodes are percent presence in 100 bootstrap replicates. Blue lines indicate bioluminescent taxa. Dashed lines indicate taxa with specialized light-transmitting infracarinal muscles. Trachichthyoid family and subfamily names indicated at right. (b) Ventral view of anus in *Paratrachichthys fernandezianus* (SIO 65626, 96 mm SL). Anterior at left. (c) Cross section of ventral bioluminescent organ in *P. fernandezianus* (FMNH 107298) at anus. MT. (d) Cross section of ventral bioluminescent organ in *Aulotrachichthys prosthemius* (JFBM 48685) at anus. MT. The cross sections demonstrate the difference in position of the bacterial crypts. Asterisk (*), anus; b, bacterial crypts; BO, bioluminescent organ

Diretmoides pauciradiatus, and *Monocentris reidii*) based on 132 genetic loci, but our findings differ from some prior DNA-sequence analyses in recovering a monophyletic Trachichthyidae exclusive of *Anoplogaster* (Betancur et al., 2017; Davis et al., 2016). Within the Trachichthyidae, our results support recognition of a subfamily Trachichthyinae Bleeker, 1856 composed of *Aulotrachichthys*, *Optivus*, *Paratrachichthys*, *Sorosichthys*, and *Trachichthys* that is morphologically diagnosed by the presence of enlarged tympanic plates derived from intermuscular bones (Kotlyar, 1992; Shimizu, 1977) and a subfamily Hoplostethinae Kaup, 1873 composed of *Gephyroberyx*, *Hoplostethus*, and, presumably, *Parinoberyx* for which we did not have data (Kotlyar, 1984, 1986).

All trachichthyid genera were recovered as monophyletic. The composition of the subgenera of *Hoplostethus* proposed by Kotlyar (1986) are not supported by the phylogeny (Figure 4). The monophyletic clade of the genera *Aulotrachichthys*, *Paratrachichthys*, and *Sorosichthys* is composed of species that have a melanistic anus that is immediately behind or between the pelvic fins and a perianal bioluminescent organ. Within this group, the clade composed of *Aulotrachichthys* and *Sorosichthys* all have modified light-conducting middle infracarinalis and pectoral-region muscles. *Aulotrachichthys novaezelandicus*, an endemic species from the waters around New Zealand, is sister to a clade composed of all other species of *Aulotrachichthys* (Figure 4).

4 | DISCUSSION

Based on our phylogenetic reconstruction (Figure 4) using available DNA sequences and morphological observations we support our hypothesis that bacterial bioluminescent organs with multiple bacterial crypts and multiple external openings likely evolved at least twice within the suborder Trachichthyoidei. Light organs evolved once perianally from the ectodermal proctodeum in the ancestor of *Aulotrachichthys*, *Paratrachichthys*, and *Sorosichthys* within Trachichthyidae and at least once cephalically in the ancestor of Monocentridae and Anomalopidae from the cephalic ectodermal epidermis.

The taxonomic conclusions based on our genetic and morphological data include recognition of the family Anoplogasteridae, the fang tooths, because Trachichthyidae is monophlyletic exclusive of *Anoplogaster*. This is consistent with analysis of genomic data by Dornberg et al. (2017) but contrary to the paraphyly of the trachichthyids with respect to *Anoplogaster* proposed by other studies using DNA-sequence data (Betancur et al., 2017; Davis et al., 2016). Our results also support recognition of a monophyletic subfamily Hoplostethinae composed of *Gephyroberyx*, *Hoplostethus*, and *Parinoberyx* and a subfamily Trachichthyinae composed of *Aulotrachichthys*, *Optivus*, *Paratrachichthys*, *Sorosichthys*, and *Trachichthys* (Figure 4, Kotlyar, 1992; Shimizu, 1977). The Trachichthyinae is clearly diagnosed by the presence of enlarged tympanic plates derived from intermuscular bones.

Using histological sectioning we definitively demonstrate that *P. fernandezianus* has a perianal bacterial organ (*contra* Konishi & Okiyama, 1997; Paitio et al., 2016; Paulin, 1979) and that it is very likely bioluminescent because of the substantial similarity of structure to the bioluminescent organ in species of *Aulotrachichthys* (Figures 1 and 2). The other two species of *Paratrachichthys*, *P. macleayi* and *P. traillii*, likely are similarly bioluminescent because they have the same anterior anus with distinct perianal swelling. Histological examination of the bioluminescent organs in five species of *Aulotrachichthys* (*A. argyrophanus*, *A. heptalepis*, *A. novaezelandicus*, *A. prosthemius*, and *A. sajademalensis*) confirms and significantly expands upon the observations of Kuwabara (1955) and Haneda (1957) in *A. prosthemius*. All species of *Aulotrachichthys* species examined have a perianal light-producing component of the bioluminescent organ associated with ventral light-conducting muscles, referred to as "striated areas" in prior literature (Fowler, 1938; Gomon & Kuiter, 1987; Gon, 1983; Kotlyar, 1992). The posterior light-conducting muscles attach to the posterior processes of the pelvic girdle, and, as inferred based on the position and attachment, these specialized muscle tracts likely are lateral divisions of left and right middle infracarinalis muscles (Figure 2 (g)). The specimen of *A. novaezelandicus* we observed histologically differed from the other four species of *Aulotrachichthys* in having the emissary ducts leading from the bacterial crypts opening on the surface of an enlarged perianal swelling (Figure 3(a)) as occurs in *Paratrachichthys*. The COI-gene data in the phylogenetic analysis resulted in *A. novaezelandicus* being the sister taxon to all other *Aulotrachichthys* species, suggesting that the pronounced perianal swelling is as ancestral characteristic retained in this species. However, this characteristic may represent an early ontogenetic stage in bioluminescent organ development in *Aulotrachichthys* because the only *A. novaezelandicus* specimens available for examination were juveniles under 15 mm SL. We did not have similarly small specimens of other *Aulotrachichthys* species to examine.

The extent of the light-conducting muscles along the ventral surface and Haneda's (1957) observations of light emission in live specimens strongly suggest that bioluminescence in *Aulotrachichthys* functions in camouflage via counterillumination. This function is similar to *Acropoma* (Ghedotti et al., 2019), *Leiognathus* (Chakrabarty et al., 2011), bioluminescent lestidiids (Ghedotti et al., 2015), and opisthoproctids (Poulsen et al., 2016) in which a localized light-producing structure produces light that is diffused by transparent ventral musculature to produce counter illumination. The function of the bioluminescent organ in *Paratrachichthys* is less clear because of the lack of obviously specialized, ventral, light-conducting structures. The perianal position of the bioluminescent organ makes prey illumination and prey luring unlikely functions. Additionally, the allopatric distribution and low number of species of *Paratrachichthys* are not consistent with sexual selection and species recognition that can lead to bioluminescence-facilitated speciation (Davis et al., 2014; Poulsen et al., 2016). Somiya (1977) suggested that the bacterial perianal light organ of *Chlorophthalmus* was used for intraspecific communication because of its small size. However, the bioluminescent organ in *Paratrachichthys* is proportionally much larger than in *Chlorophthalmus*, similar in size to the organ in counter illuminating *Aulotrachichthys*, and parsimoniously is inferred to have evolved from the same

ancestral structure as the organ in *Aulotrachichthys* (Figure 4). Although the muscle around the bioluminescent organ in *Paratrachichthys* is not obviously specialized for conducting light, the adjacent tissues do exhibit more adipose tissue in the perianal region than other trachichthyid species, the bacterial light-producing structure is surrounded with dark chromatophores similar in distribution to those in the counterilluminating *Aulotrachichthys*, and less obviously specialized adjacent tissues could diffuse the light produced by the bacterial crypts. Presumably, less extensive ventral bioluminescence than in the species of *Aulotrachichthys* still would disrupt and reduce the silhouette produced by downwelling light. This makes counterillumination the most likely function of this structure in species of *Paratrachichthys*.

We were not able to directly sample *S. ananassa* due to its rarity in natural history collections, but we examined external photos, used Kotlyar's (1992) extensive osteological survey, and obtained COI sequence from the Barcode of Life Database to include it in the phylogenetic analysis. The phylogenetic analysis supports a sister-group relationship between *Sorosichthys* and *Aulotrachichthys* as previously suggested by Gomon and Kuiter (1987) and Kotlyar (1992). *Sorosichthys ananassa* has striated areas like the species in *Aulotrachichthys* and a large, melanistic perianal swelling like both *Paratrachichthys* and the juvenile specimens of *A. novaezelandicus* examined (Figure 3(c)). Because *S. ananassa* is within a clade with all species of *Aulotrachichthys* and *Paratrachichthys* (Figure 4), has striated areas that are used to transmit light from photogenic bacteria along the ventral surface in *Aulotrachichthys*, and has an external perianal anatomy similar to the bioluminescent *Paratrachichthys* species and *A. novaezelandicus* (Figure 3(c)), this strongly suggests that *S. ananassa* also is bioluminescent.

Morphologically similar bioluminescent organs with different positions and functions evolved at least twice independently within the trachichthyoids (Figure 4). The bioluminescent organ, when present in members of the family Trachichthyidae, surrounds the anus, houses symbionts in the genus *Photodesmus*, and likely is used for counterillumination camouflage (Dunlap et al., 2007; Haneda, 1957; Hendry & Dunlap, 2014). In contrast to trachichthyids, the bioluminescent organs in members of the families Anomalopidae and Monocentridae are cephalic, house symbionts in the genera *Allivibrio* or *Vibrio*, and are used for prey illumination and/or coordination of schooling (Dunlap et al., 2007; Gruber et al., 2019; Hellinger et al., 2017; Hendry & Dunlap, 2014). In addition, the differing locations of the bioluminescent organs in anomalopids and monocentrids, with subocular and mandibular locations, respectively, also suggest a more complicated pattern of evolution with either separate origins or an evolutionary shift in locations within this clade. Despite evolving in different locations on the body, housing different types of bacteria, and having different functions, the anomalopid, monocentrid, and trachichthyid bioluminescent organs have a similar morphology with elongate chambers filled with bacteria and connected to collecting ducts that connect to superficial emissary ducts that lead to and have lining epithelia that are continuous with the epidermis. If this similarity were a constraint on the morphology of ectodermal bioluminescent organs, it would be common in many bioluminescent taxa, which it is not. However, *Acropoma* and *Chlorophthalmus* also have perianal bioluminescent organs derived from the embryonic ectodermal proctodeum like the trachichthyids (Baranowska Körberg et al., 2015; Pyati et al., 2006) but have morphologies that differ substantially from the bioluminescent organs in trachichthyoids (Ghedotti et al., 2018; Somiya, 1977). Therefore, the general similarity of structure within the trachichthyoids may be either a notable incidence of chance parallelism; may suggest non-parsimonious multiple losses of an organ that evolved in the ancestor of the clade containing the trachichthyids, anoplogastrids, anomalopids, and moncentrids; or may reflect different regional expression of some common underlying genetic structure or module derived from a common ancestor within the suborder.

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AUTHOR CONTRIBUTIONS

Michael J. Ghedotti: Conceptualized the work for this manuscript, wrote the first draft of the paper, constructed the figures, completed the gross anatomical work, compiled the morphological phylogenetic data with W. L. S., completed the majority of the histological sectioning, and coordinated the activities of all authors. **Hannah M. DeKay**: Completed a significant amount of the histological sectioning, assisted with the gross anatomical work, assisted MJG with imaging, and consulted with the other authors in the writing of the manuscript. **Alex J. Maile**: Compiled DNA-sequence data with M. P. D. and W. L. S. for the phylogenetic analyses, worked with W. L. S. and M. P. D. on data curation, and consulted with the other authors in the writing of the manuscript. **W. Leo Smith**: Compiled DNA-sequence data with M. P. D. and A. J. M., compiled the morphological phylogenetic data with MJG, worked with A. J. M. and M. P. D. on data curation, and helped write and edit the paper. **Matthew P. Davis**: Compiled the DNA-sequence data with A. J. M. and W. L. S. for the phylogenetic analyses, completed the phylogenetic analyses with W. L. S., worked with A. J. M. and W. L. S. on data curation, and helped write and edit the paper.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmor.21349.

DATA AVAILABILITY STATEMENT

The DNA-sequence data that support the findings of this study are openly available in GenBank and the Barcode of Life Database at https://www.ncbi.nlm.nih.gov/genbank and https://www.ncbi.nlm.nih.gov/genbank/barcode/, reference numbers in Supplementary Table S2. The morphological, phylogenetic data that support the findings of this study are available in supplementary online material, Table S3 of this article.

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Additional supporting information may be found online in the Supporting Information section at the end of this article.

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