

# Evolution of the Light Organ System in Ponyfishes (Teleostei: Leiognathidae)

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**ABSTRACT** Members of the leiognathid subfamily Gazzinae, comprising approximately two-thirds of ponyfish species, are sexually dimorphic with regard to features of the light organ system (LOS). In Gazzinae, the circumesophageal light organ (LO) of males is enlarged and varies in shape compared with similarly sized conspecific females. In association with male species-specific translucent external patches on the head and flank, these sexually dimorphic LO features are hypothesized to be correlated with species-specific luminescence displays. Anatomical differences in LO shape, volume, and orientation, and its association with the gas bladder and other internal structures that function in light emission, are compared to observations of luminescence displays for every major lineage within Leiognathidae. We reconstruct the character evolution of both internal and external morphological features of the LOS to investigate the evolution of LO sexual dimorphism and morphology. Both internal and external sexual dimorphism in the ponyfish LOs were recovered as most likely to have evolved in the common ancestor of Leiognathidae, and likelihood-based correlation analyses indicate that the evolution of internal and external dimorphism in males is statistically correlated. Magnetic resonance imaging technology was applied to examine the unique internal LOs of ponyfishes *in situ*, which provides a new metric (LO index) for comparison of LO structure across lineages. *J. Morphol.* 272:704–721, 2011. © 2011 Wiley-Liss, Inc.

**KEY WORDS:** bioluminescence; character evolution; ichthyology; magnetic resonance imaging; photic display; sexual dimorphism

## INTRODUCTION

Leiognathidae (ponyfishes) are silvery shallow-water fishes with a unique internal light organ system (LOS) that comprises a number of distinct anatomical structures in addition to the circumesophageal light organ (LO) itself (Sparks et al., 2005). The LO houses a high concentration of luminescent bacteria, *Photobacterium leiognathi* (Vibrionaceae), whose light the fish co-opt for external luminescent displays (Hastings and Mitchell, 1971; Dunlap and McFall-Ngai, 1984a). Ponyfishes utilize luminescence as a means of

predator avoidance and, in many species, for photic communication during sexual interactions (Woodland et al., 2002; Sasaki et al., 2003; Herring, 2007). Many species of ponyfishes possess a LO that is sexually dimorphic in both volume and shape, with males having a larger and more complex organ than similarly sized conspecific females. A larger LO permits males to house more bacteria and, consequently, emit more intense light over a larger surface area (Sparks et al., 2005).

The LO abuts the gas bladder, into which bacterially generated luminescence is transmitted via a “window.” The gas bladder in turn is lined internally with silvery, reflective guanine. In most sexually dimorphic lineages, the gas bladder is characterized by species-specific guanine-free translucent regions, which permit light to be emitted in a variety of unique patterns (Fig. 1). In some species, the LO also exhibits paired contralateral “windows,” which emit light into silvery, guanine-lined chambers and internal “light tubes.” The LO, reflective internal chambers, and “light tubes,” and translucent portions of the otherwise reflective gas bladder are in turn closely associated with translucent flank, opercular, gular, or nuchal patches. Together, these internal and external components comprise the ponyfish’s LOS (Sparks et al., 2005).

At the generic level, most ponyfish clades are diagnosable based on the size, shape, and internal

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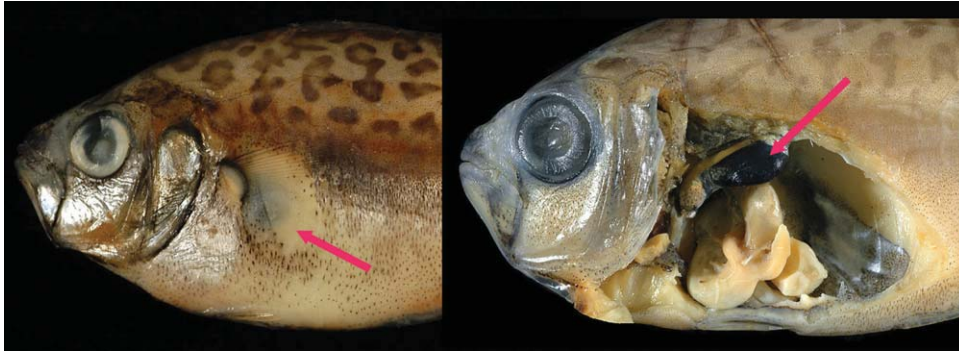


Fig. 1. Image of a ponyfish showing the external translucent skin patch (red arrow on left) and image of a dissection exposing the light organ (red arrow on right), gut, and inner portion of the guanine-lined gas bladder.

orientation of the LO in males (Sparks et al., 2005). Moreover, species within externally sexually dimorphic genera can be distinguished by the male-specific (in terms of size, location, shape, and orientation) translucent patches on the exterior surface of the fish (Dunlap and McFall-Ngai, 1984a,b; Sparks and Chakrabarty, 2007; Chakrabarty et al., 2010). Species-specific translucent windows further enhance and refine the ability of males to present unique luminescent displays to females. Light emission in ponyfishes is hypothesized to be controlled by a combination of muscular shutters and chromatophore-studded “windows” on the LO itself and by rapid dispersion of chromatophores on the translucent external patches (Hastings, 1971; McFall-Ngai and Dunlap, 1984).

Excision of the LO can result in an inaccurate morphological description of the structure because of distortions that result from manipulating the fleshy and easily damaged organ, which is closely associated with and fused to adjacent tissues (e.g., gas bladder and esophagus). An *in situ* methodology is therefore desirable to more accurately explore the comparative morphology of the LOS, given that association of the LO with other structures is critical to understanding overall system function. In this study, we provide a comprehensive review of LO morphology among ponyfish lineages and use magnetic resonance imaging (MRI) reconstructions to visualize and compare the shape, orientation, and relative volumes of the LOs of males *in situ*. MRI provides a noninvasive method of visualizing soft tissues based on water content, allowing internal structures to be visualized and manipulated in three dimensions (3D) without the artifacts associated with traditional dissections. Three-dimensional digital images acquired from these scans permit anatomical structures to be segmented (i.e., the process of digitally partitioning tissues based on specified MR image characteristics) and quantitatively analyzed. To objectively compare the various sizes of the

ponyfish LOs, a novel index is presented that represents the normalized measure of LO size relative to specimen size (i.e., LO index, LOI). This metric is used to explore whether closely related ponyfish lineages exhibit less disparity in LO morphology compared with taxa from other more distantly related clades, with different overall LOS features.

A likelihood approach is used to provide a robust phylogenetic framework for exploring the evolution of sexually dimorphic features of the LOS (e.g., increases in volume and shape variation of the LO in males). Within this phylogenetic framework, we investigate character evolution of the ponyfish LOS to explore and reconstruct the evolution of both internal (LO) and external (external translucent LOS patches/patterns) sexually dimorphic traits. Likelihood-based ancestral character-state reconstructions and character correlation studies are utilized to address the following evolutionary questions, which will allow us to develop a phylogenetic model of the evolution and diversification of the LOS in ponyfishes: 1) Determine whether the evolution of internal and external sexual dimorphism of the LOS occurred independently or concurrently in ponyfishes; 2) identify the lineage(s) in which evolution of internal sexual dimorphism occurred; 3) reconstruct the evolutionary history of shape variation and volume expansion in the LO; and 4) examine the evolution and diversification of the corresponding translucent external LOS patches.

## MATERIALS AND METHODS

### Magnetic Resonance Imaging

The LOSs of males representing eight currently recognized ponyfish genera were visualized using MRI to compare the shape, orientation, and relative volume of the LO and associated structures *in situ*. Adult males representing 10 species of ponyfishes with distinct LOS morphologies were scanned to encompass the range of interspecific LO anatomy and luminescent signaling behaviors known within Leionathidae (Sparks et al., 2005; Table 1). Species were chosen as the best represen-

TABLE 1. Species scanned using MRI, including standard lengths of specimens examined

Clade	Species	SL (mm)	Body vol. (mm <sup>3</sup> )	GB vol. (mm <sup>3</sup> )	LO vol. (mm <sup>3</sup> )	LO index
Equulitini	<i>Equulites rivulatus</i>	73	4,852	274	86	17.7
Eubleekerini	<i>Photopectoralis aureus</i>	84	11,382	819	199	17.4
Eubleekerini	<i>Photopectoralis bindus</i>	72	7,767	230	51	6.6
Gazzini	<i>Secutor insidiator</i>	71	6,086	250	14	2.3
Equulitini	<i>Equulites laterofenestra</i>	122	12,994	770	21	1.6
Gazzini	<i>Gazza achlamys</i>	70	23,656	1,970	34	1.4
Nuचेquulini	<i>Nuचेquula nuchalis</i>	83	12,602	554	11	0.9
Eubleekerini	<i>Eubleekeria splendens</i>	92	19,562	1,810	14	0.7
Equulitini	<i>Equulites moretoniensis</i>	67	6,837	629	4	0.6
Leiognathinae	<i>Leiognathus equulus</i>	85	16,638	1,262	5	0.3

GB, gas bladder; LO, light organ; SL, standard length.

Volumes for the light organ, gas bladder, and whole body, calculated from MRI data segmentation analysis in ITKSNAP, are presented in mm<sup>3</sup>. The light organ (LO) index is calculated by dividing light organ volume by whole body volume and multiplying by 10<sup>3</sup>.

tatives of particular clades based on our previous comparative anatomical studies of the LOS (e.g., Sparks et al., 2005; Chakrabarty and Sparks, 2007, 2008; Sparks and Chakrabarty, 2007), which involved removal of the LO from all ponyfish taxa available for dissection. The 10 species imaged and reconstructed represent all major lineages (i.e., each distinct LO morphotype is analyzed) and include at least one representative from each leiognathid tribe. For tribes known to exhibit interspecific variation in LO morphology (e.g., Equulitini), representative males were imaged for each LO morphotype. All imaged specimens are deposited at the American Museum of Natural History: *Eubleekeria splendens* AMNH 239268; *Equulites moretoniensis* AMNH 231298; *E. rivulatus* AMNH uncat.; *E. laterofenestra* AMNH 241311; *Gazza achlamys* 88067; *Leiognathus equulus* AMNH 244305; *Nuचेquula nuchalis* AMNH 238766; *Photopectoralis aureus* AMNH 241285; *P. bindus* AMNH uncat.; and *Secutor insidiator* AMNH 239285-a. All research was conducted in compliance with AMNH IACUC protocols for the study of vertebrates and adhered to the legal requirements of the United States of America.

All scanned specimens were initially preserved in 10% buffered formalin, post collection, and stored in 75% EtOH. In preparation for MRI analysis, specimens were removed from 75% EtOH and sequentially rehydrated via successive reduction in EtOH concentration (i.e., 50, 25, and 0%, respectively). These solutions were diluted with phosphate-buffered saline (PBS) and 0.01% sodium azide (NaN<sub>3</sub>). To improve scan contrast, specimens were then placed in fresh PBS + NaN<sub>3</sub>, with the addition of a 2.5 mmol l<sup>-1</sup> solution of a gadolinium-based imaging contrast agent, ProHance (Bracco Diagnostics, NJ), for an additional week before imaging.

Imaging was conducted on a 7 T 21-cm bore Bruker Avance II console scanner using a circularly polarized 1H whole-body RF coil (Bruker AXS, Madison, WI). A T1/T2-weighted standard fast low-angle shot (FLASH) GRE acquisition pulse sequence was used to acquire whole-body images at a resolution of 100 μm. Three-dimensional image segmentation and subsequent volume measurements of the LO and associated structures, gas bladder, and whole body were performed using ITK-SNAP v. 1.6 (Insight Segmentation and Registration Toolkit; www.itk-snap.org). LO volumes (mm<sup>3</sup>) were calculated in ITK-SNAP. To provide a normalized measure of LO size relative to the size of the specimen analyzed, a LOI was calculated by dividing LO volume by whole-body volume and multiplying by 10<sup>3</sup>.

## Phylogenetic Analyses

Outgroup taxonomic sampling (Supporting Information Table S1) included groups historically hypothesized to be closely related to ponyfishes (e.g., Gerreidae and Chaetodontidae; Smith and Wheeler, 2004; Springer and Orrell, 2004; Sparks et al., 2005; Thacker, 2009). Sampling within Leiognathidae

(Supporting Information Table S1) is taxonomically comprehensive with multiple representatives from every genus currently described. Nucleotide characters were sampled from seven mitochondrial (16S, COI, ND4, ND5, tRNA-His, tRNA-Ser, and tRNA-Leu) and two nuclear (28S and histone H3) genes for a total of 5,888 base pairs (Supporting Information Table S1). (Note that ND4 and tRNAs are incorporated into the ND5 sequences referred to in Supporting Information Table S1.) Sequences were aligned with ClustalW (Thompson et al., 1994) using default values and concatenated in Geneious 4.6.4 (Drummond et al., 2006). Maximum likelihood analyses were conducted in GARLI v1.0 (Zwickl, 2006) under a GTR+I+Γ model of molecular evolution. The tree with the best likelihood score from 10 independent analyses was used to evaluate evolutionary relationships. A nonparametric bootstrap analysis was conducted for 100 random pseudoreplicates.

## Ancestral Character-State Reconstruction

Ancestral character states were reconstructed in Mesquite 2.7 (Maddison and Maddison, 2009) using likelihood methodology. The Mk1 model (Lewis, 2001) was used to identify the state at each node that maximizes the probability of the states observed in the terminal taxa under a likelihood framework. Character states were reconstructed on the hypothesis of ponyfish intrarelationships presented in this study. In further discussion, the “stem species” refers to the inferred ancestor of a particular clade. Apomorphic features of the LOS, based on the analysis of Sparks et al. (2005), were observed via rendered MRI images and include the following:

- LO sexually dimorphic in volume and shape:
  - 0: No volume or shape dimorphism between male and females.
  - 1: Volume larger and lobes hypertrophied in males.
- External translucent patch(es) in males:
  - 0: Absent.
  - 1: Present and sexually dimorphic with regard to size and/or shape.
- LO shape in males (as seen in Fig. 2):
  - 0: Ventral expansion only.
  - 1: Dorsolateral and ventral expansion.
  - 2: Dorsolateral expansion only.
- Location and orientation of translucent external patch(es) in males:
  - 0: Absent
  - 1: Head region (gular, opercular, and/or buccal).
  - 2: Nuchal saddle.
  - 3: Pectoral-fin axil.
  - 4: Flank stripe (complete or broken and comprising discrete windows).
  - 5: Expansive flank patch (polygonal- or crescent-shaped).



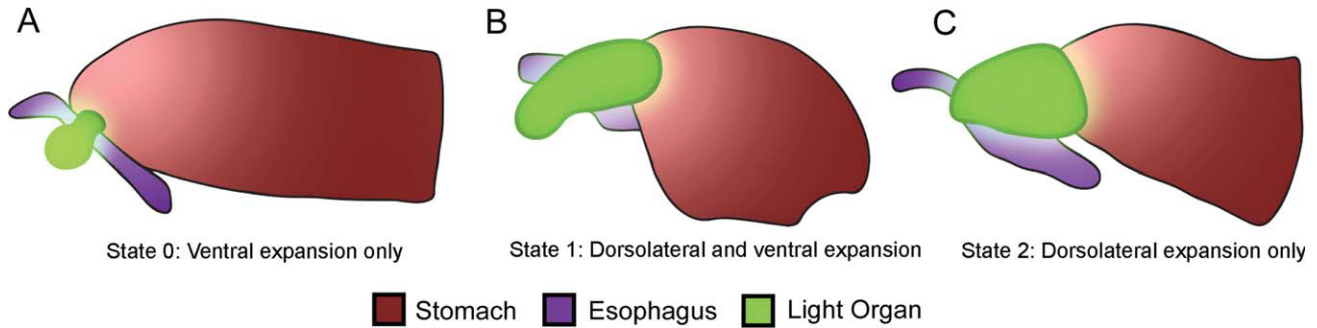


Fig. 2. Illustrations of the light organ and associated structures for (A) *Gazza achlamys*, (B) *Photopectoralis aureus*, and (C) *Equulites rivulatus*, depicting major differences in light organ shape among lineages.

## Correlation Analysis

Using the correl package in Mesquite 2.7 (Maddison and Maddison, 2009), we implemented Pagel's (1994) likelihood method for testing whether the evolution of two discrete binary characters was independent. Pagel's (1994) method compares the likelihoods of models in which the rates of character change are 1) independent from each other and 2) dependent on the other character. Significance of the likelihood difference between the two models was calculated by comparing the observed likelihood difference with that of 1,000 simulations. Using this approach, we tested the null hypothesis that the evolution of internal (Character 1) and external (Character 2) sexual dimorphism of the LOS is independent in ponyfishes.

## RESULTS

### Review of LOS Morphology and Luminescence Displays

A summary of all MRI results is shown in Figure 3, with enlarged images of the LOS morphologies illustrated in Figures 4 and 5. Below, we provide a review of LOS morphology and a description of MRI results among the ponyfish lineages.

**Subfamily Leiognathinae.** Leiognathinae includes members of the genus *Leiognathus* (viz., *L. equulus*, *L. robustus*, and *L. striatus*) and genus *Aurigequula* (viz., *A. fasciata* and *A. longispinna*; Chakrabarty et al., 2009). The subfamily is recovered as the sister group to all other ponyfishes and is the only ponyfish lineage that lacks sexually dimorphic features of the LOS (Sparks and Dunlap, 2004).

Members of Leiognathinae possess a circumesophageal LO and the additional associated features of the LOS that are synapomorphic and diagnostic for the family as a whole (Sparks et al., 2005). Flashing patterns have rarely been observed for members of this subfamily, and in the absence of sexually dimorphic external translucent patches in males or any observable sexual dimorphism of the LOS, we infer that a sexually dimorphic flashing pattern that functions in intraspecific communication is unlikely within this clade.

Hastings (1971) observed ventral luminance in *Leiognathus equulus*, which is consistent with

ventral counterillumination, the emission of light over the ventral surface of the body to match the intensity of downwelling light from the surface. It is hypothesized that ventral counterillumination functions to camouflage the individual from potential predators lurking below (Hastings, 1971; McFall-Ngai and Morin, 1987). Hastings (1971) noted that the emitted light was blue with a maximum wavelength of 490 nm and that it occurred in direct response to distressing situations (i.e., "a stunning blow") or directed light from a flashlight, the latter response being consistent with a camouflaging function. Haneda (1940) also reported flashing in *Leiognathus equulus* and *Aurigequula fasciatus*, describing the emitted light only as "intense" or "mild." Presumably, Haneda (1940) observed a continuous emission of light, not rapid flashes, given that he does not distinguish any unique patterns of flashing from the continuous ventral luminescence he observed and described for another taxon, *Gazza minuta*.

Based on anatomical similarities observed throughout the family, notably the presence of a caudal LO window oriented into the gas bladder and caudoventral clearing of the silvery gas bladder lining, ventral counterillumination is presumed to occur in all leiognathid taxa and in both sexes (McFall-Ngai and Morin, 1991).

**Taxonomic diagnosis.** Leiognathinae comprises all ponyfish species that lack sexually dimorphic anatomical features of the LOS. Members of Leiognathinae possess a circumesophageal LO and associated features of the LOS (e.g., guanine-lined gas bladder with a ventrocaudal clearing) that are synapomorphic and diagnostic for the family (Sparks et al., 2005), but lack any discernable sexual dimorphism of the LOS.

**Light organ indices.** The lowest LOI score overall was recorded for a nonsexually dimorphic species, *Leiognathus equulus* (Figs. 3J and 5E; Table 1). Nonsexually dimorphic species attain the largest adult sizes of any ponyfishes (frequently reaching over 150-mm SL). Despite their large adult body size, based on our previous comparative

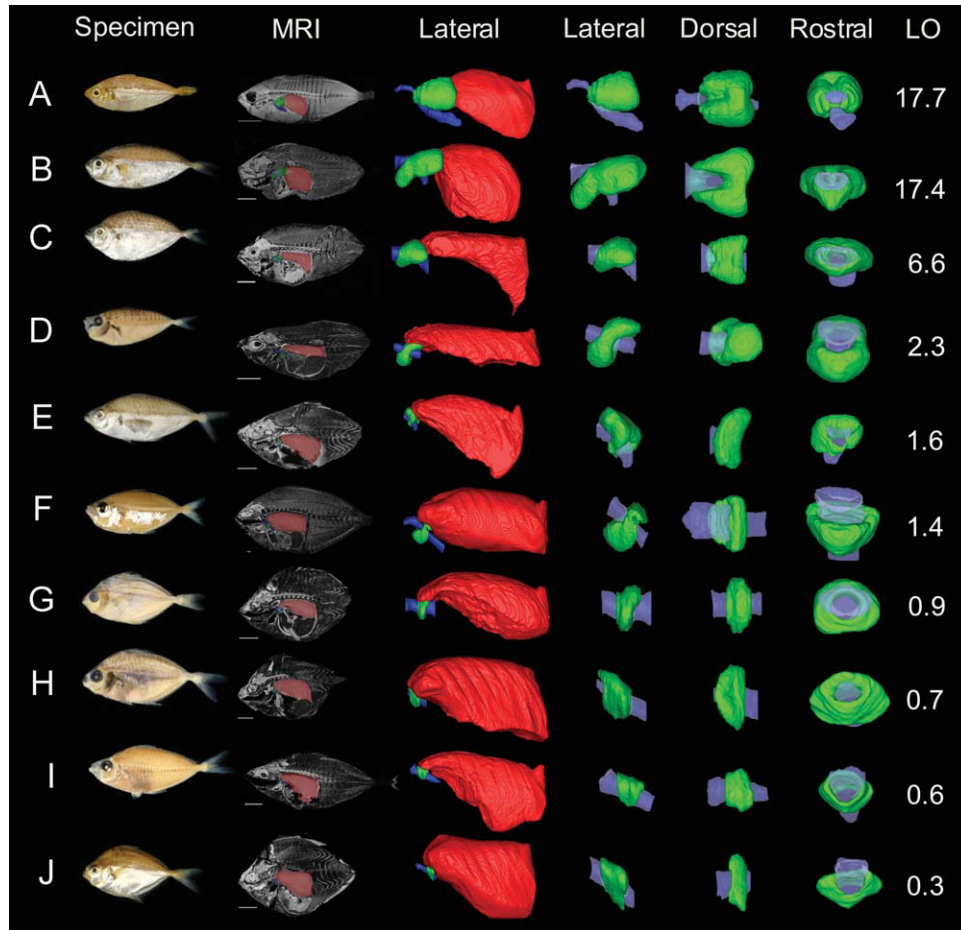


Fig. 3. Summary of MRI 3D reconstructions for all major lineages of ponyfishes. Specimens ranked by LO index score (highest on top). Each row presents a photograph of the specimen and a segmentation image showing the light organ in green and gas bladder in pink. The columns “lateral,” “dorsal,” and “rostral” show the isolated esophagus (purple), light organ (green), and gas bladder (maroon) in those respective orientations. Specimens include: (A) *Equulites rivulatus*, (B) *Photopectoralis aureus*, (C) *Photopectoralis bindus*, (D) *Secutor insidiator*, (E) *Equulites laterofenestra*, (F) *Gazza achlamys*, (G) *Nuclequula nuchalis*, (H) *Eubleekeria splendens*, (I) *Equulites moretoniensis*, and (J) *Leiognathus equulus*.

anatomical studies and quantitatively demonstrated in this analysis, members of Leiognathinae have a disproportionately small LO (Figs. 3J and 5E, Table 1). In contrast, the comparatively large gas bladder size reflects the large body mass of members of this tribe compared with congeners. (Note: Because of size constraints imposed by the MRI coil, only a small adult member of Leiognathinae could be scanned.)

**Subfamily Gazzinae.** This subfamily includes four strongly supported monophyletic tribes, which are discussed and diagnosed below.

**Taxonomic diagnosis.** All members of the subfamily Gazzinae exhibit sexually dimorphic LOS. Males of all included species possess LOs that are enlarged in volume with regard to similarly sized conspecific females. Males of most species within Gazzinae also exhibit shape dimorphism of the LO (e.g., hypertrophied lobes) compared with conspecific females.

**Subfamily Gazzinae: Tribe Gazzini.** The clade Gazzini comprises the sister genera, *Gazza* and *Secutor*. *Gazza* and *Secutor* are easily distinguished from each other and the remaining leiognathid genera on the basis of numerous apomorphic anatomical features (summarized in Sparks et al., 2005). Members of *Gazza* (viz., *G. achlamys*, *G. dentex*, *G. minuta*, *G. rhombea*, and *G. squamiventralis*) are piscivorous as adults, with large caniniform teeth (versus small, weak, conical teeth in all other ponyfishes) and mouths that protract anteriorly (versus dorsally or ventrally as in other ponyfishes). Members of *Secutor* (viz., *S. hanedai*, *S. indicus*, *S. insidiator*, *S. interruptus*, *S. megalolepis*, and *S. ruconius*) are unique among leiognathids in having mouths that protract strongly dorsally. In addition, they have very broad ventral aspects of their cleithrum, and they are strongly laterally compressed. Male members of Gazzini exhibit hypertrophied ventrolateral LO lobes and

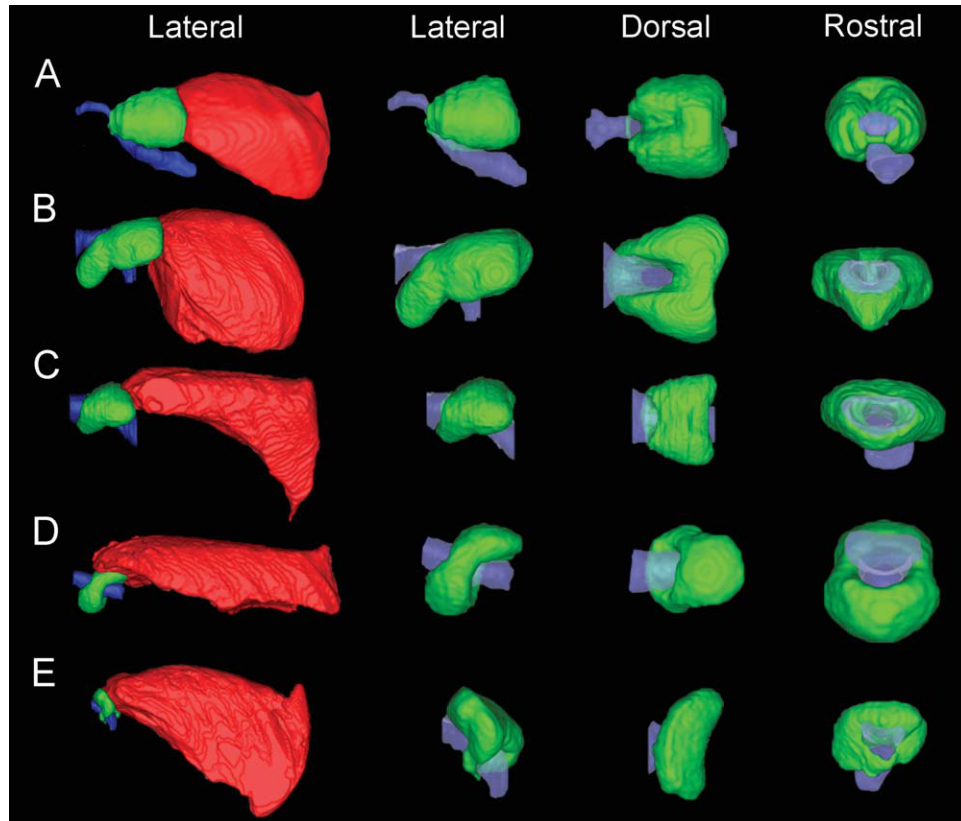


Fig. 4. Magnetic resonance imaging 3D reconstructions of LOS with the isolated esophagus (purple), light organ (green), and gas bladder (maroon). Specimens include: (A) *Equulites rivulatus*, (B) *Photopectoralis aureus*, (C) *Photopectoralis bindus*, (D) *Secutor insidator*, and (E) *Equulites laterofenestra*.

corresponding rostroventrally oriented windows on these contralateral lobes, which are oriented into a silvery, guanine-lined, reflective chamber (see Fig. 3 of Sparks et al., 2005). Unlike other ponyfishes, both male and female members of Gazzini have external translucent opercular (*Gazza*) or gular (*Secutor*) patches that do not emit light via reflection and emission through the gas bladder. Nevertheless, all members of this clade are sexually dimorphic with regard to both LO volume and surface area of the translucent opercular or gular patches; both of which are enlarged in males compared with similarly sized conspecific females. In *Secutor*, unique “light tubes” (i.e., silvery, guanine-lined reflective chambers) facilitate the transmission of light from the contralateral LO windows to the translucent gular patches (see Fig. 8 of Sparks et al., 2005).

In Gazzini, photic displays have been observed in *Gazza minuta*, and five distinct “modes” of luminescence have been described (McFall-Ngai and Dunlap, 1983). Philippine specimens of *Gazza minuta* were observed in aquaria (McFall-Ngai and Dunlap, 1983) and Japanese specimens in the wild (Haneda, 1940). McFall-Ngai and Dunlap (1983) concluded that *Gazza minuta* was capable of ven-

tral body flashing, buccal luminescence, opercular cheek flashing, ventral counterillumination, and discrete projected luminescence. “Discrete projected luminescence” was described as a bright “emanating beam” from distinct spots at the posterior margin of the opercular cavity (i.e., the translucent patches). Displays took place during periods of low light, often beginning as short 1-s flashes that became longer in duration (i.e., up to 2 min on/off periods of display). Ventral body flashing was reported as bright 1-s flashes over the entire ventral two-thirds of the body. Moribund specimens were also observed to exhibit buccal luminescence in which light was projected out of the mouth. Haneda (1940) reported that *G. minuta* “exhibited a bluish white luminescence,” the same color as the ventral portion of the fish. Bacteria in the luminous organ were reported to emit a continuous glow up to several days after host death. Haneda (1940) noted that he observed light of the strongest intensity in the genus *Secutor* (relative to *Aurigequula fasciatus*, *Eubleekeria splendens*, and *Karalla daura*, which he reported as having at most a “mild” intensity). The fact that both sexes possess translucent external patches in Gazzini is suggestive of two-way sexual photic



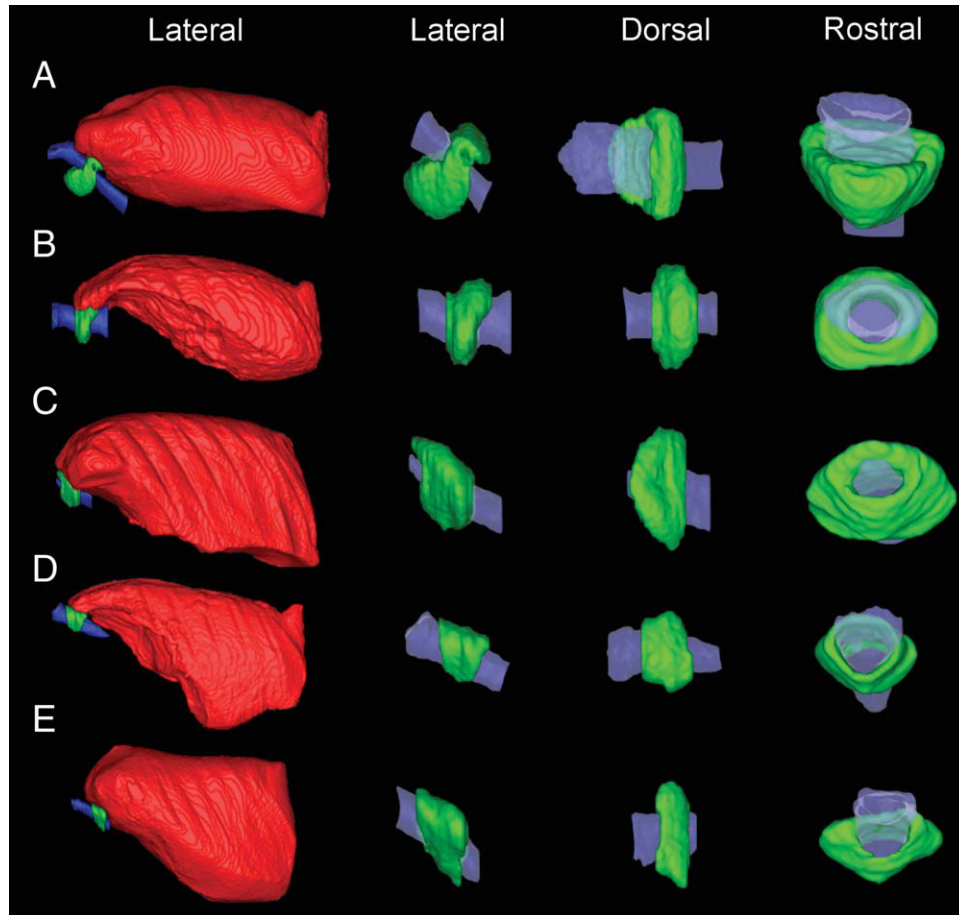


Fig. 5. Magnetic resonance imaging 3D reconstructions of LOS with the isolated esophagus (purple), light organ (green), and gas bladder (maroon). Specimens include: (A) *Gazza achlamys*, (B) *Nuchequula nuchalis*, (C) *Eubleekeria splendens*, (D) *Equulites moretoniensis*, and (E) *Leiognathus equulus*.

communication (see Lloyd, 1966 for an example of a similar system in an invertebrate).

**Taxonomic diagnosis.** Uniquely with Leiognathidae, male members of Gazzini exhibit hypertrophied ventral lobes of the LO with corresponding rostroventrally oriented “windows,” in addition to the posteriorly directed gas bladder window common to all ponyfishes. The “windows” of the contralateral lobes are oriented into a silvery, guanine-lined, reflective chamber that reflects and transmits luminescence from the LO in various ways (see Fig. 3 of Sparks et al., 2005). Uniquely among ponyfishes, both male and female members of Gazzini possess translucent external opercular (*Gazza*) or gular (*Secutor*) patches that are not associated with light reflection and emission via the gas bladder, although the patches are more expansive in males.

**LO indices.** We calculated LO volume indices that ranked fourth and sixth overall for members of Gazzini, *Secutor insidiator* and *Gazza achlamys*, respectively, despite both genera exhibiting at most moderate external sexual dimorphism of the

LOS (Figs. 3D,F, 4D, and 5A; Table 1). Members of Gazzini do, nevertheless, exhibit strong internal sexual dimorphism of the LO itself. The translucent external patches of both genera are relatively inconspicuous; no other leiognathids exhibit translucent patches on the opercular margin or in the gular region, as opposed to the flank. As noted by Sparks et al. (2005), the ventrolateral lobes of the LO in *Gazza* bear “windows” that are located directly medial to the translucent opercular patch. Overall, the LO appears to be anteroventrally displaced in Gazzini relative to its orientation in other leiognathid clades. LO displacement may be associated with the unique mouth protraction mechanisms and rostral placement of the translucent external patches in Gazzini, and/or it may facilitate buccal luminescence as observed by McFall-Ngai and Dunlap (1983). The overall structure and degree of sexual dimorphism of the LO are quite similar between *Gazza* and *Secutor*, comprising a pronounced, paired ventrolateral expansion and contralateral windows on the ventrolateral lobes. Lateral expansion of the ventrolateral

lobes is considerably more pronounced in *Gazza* than in *Secutor*, which is most likely due to placement of the translucent opercular patches. In contrast to placement of the ventrolateral LO windows in close proximity to the corresponding translucent external patches in *Gazza*, *Secutor* relies instead on a “light tube” to facilitate light transmission from the LO to the distally placed translucent gular patches (Sparks et al., 2005: Fig. 8).

**Subfamily Gazzinae: Tribe Eubleekerini.**

Eubleekerini includes all members of *Photopectoralis* and its sister group, *Eubleekeria*. *Photopectoralis* comprises *P. aureus*, *P. bindus*, *P. hataii*, and *P. panayensis*, whereas *Eubleekeria* is represented by *E. jonesi*, *E. kupanensis*, *E. rapsoni*, and *E. splendens* (see Kimura et al., 2005; Chakrabarty and Sparks, 2008). Although members of *Eubleekeria* are sexually dimorphic with respect to LO volume (the LO of males is larger than similarly sized conspecific females), via our comparative studies, we have not been able to identify any sexually dimorphic external features of the LOS in this genus. Members of *Eubleekeria* lack the translucent pectoral-axil patch that is diagnostic for its sister group, *Photopectoralis*, and do not appear to possess translucent flank patches of any kind.

*Eubleekeria* was recently redescribed and diagnosed by the combination of a vermiculate dorsal flank pigmentation pattern, black markings in the membrane of the spinous dorsal fin, and chest scales (Chakrabarty and Sparks, 2008; Kimura et al., 2008). In contrast to *Eubleekeria*, which exhibits only internal sexual dimorphism of the LOS, members of *Photopectoralis* are sexually dimorphic with regard to both internal and external features of its LOS (Sparks et al., 2005). *Photopectoralis* was recently described and diagnosed on the basis of apomorphic features of the LOS (Sparks et al., 2005). Internally, the LO of males is not only considerably larger than conspecific females of similar size but also exhibits greatly enlarged ventrolateral lobes. As in members of Gazzini, the ventrolateral lobes in *Photopectoralis* possess contralateral LO windows that are oriented rostrally, in addition to the LO window abutting the gas bladder, a feature common to all ponyfishes (Sparks et al., 2005). In addition to these internal sexually dimorphic features of the LOS, externally male members of *Photopectoralis* have a translucent patch near the base of their pectoral fin (i.e., the pectoral-fin axil), which varies interspecifically in both surface area and shape.

Within Eubleekerini, photic communication in *Eubleekeria splendens* has been observed in the wild (Woodland et al., 2002). To date, however, none of the externally sexually dimorphic members of this tribe (viz., *Photopectoralis*) have been observed flashing. The LO of members of *Photopectoralis* can be observed through the translucent

pectoral-fin axil patch and the mostly translucent musculature surrounding the ventrolateral lobes, whereas members of *Eubleekeria* are more or less entirely silvery externally and lack translucent flank patches. A large school of *E. splendens* was observed flashing in unison very close to the surface (between 2- and ca. 10-m deep) at night in Ambon, Indonesia by Woodland et al. (2002). The flashes were reported to emanate from the entire lateroventral surface of the fish and consisted of alternating 0.2 s “on” periods and about 0.4 s “off” periods.

**Taxonomic diagnosis.** Members of Eubleekerini are sexually dimorphic with respect to LO volume (i.e., the LO of males is considerably larger than similarly sized conspecific females). Diagnostic features of *Eubleekeria* include a vermiculate dorsal flank pigmentation pattern, black markings in the membrane of the spinous dorsal fin, and chest scales (Chakrabarty and Sparks, 2008; Kimura et al., 2008). In contrast to *Eubleekeria*, which exhibits only internal sexual dimorphism of the LOS, members of *Photopectoralis* are strongly sexually dimorphic with regard to both internal and external features of its LOS (Sparks et al., 2005). Internally, the LO of males is not only considerably larger in volume than conspecific females of similar size but also exhibits greatly enlarged dorsal lobes that are laterally expanded and abut corresponding species-specific translucent patches located in the pectoral-fin axil. Translucent external pectoral-axil patches are lacking in females.

**LO indices.** The second and third highest LOI scores were recovered for members of *Photopectoralis*. *Photopectoralis aureus* and *P. bindus* are similar morphologically, with the exception that *P. bindus* is deep bodied (>50% BD) and nearly disk shaped, whereas *P. aureus* is elongate and more or less oval in body shape. These species exhibit very similar LO morphologies; however, the LOI of *P. aureus* is nearly three times that of *P. bindus* (Figs. 3B,C and 4B,C, Table 1). Interestingly, *P. aureus* is also more strongly sexually dimorphic externally than *P. bindus*, with males possessing an expansive translucent pectoral-axil patch, whereas in similarly sized *P. bindus* males this patch is considerably smaller. Both species exhibit large dorsal and ventrolateral expansions of the LO, such that the dorsal lobes nearly abut the pectoral-fin axil just medial to the translucent external patches (Figs. 3B,C and 4B,C). The distance between the external surface of the body and the LO is far less in this genus than in other leiognathid taxa. As noted by Sparks et al. (2005), “males of *Photopectoralis* have a translucent patch in the pectoral-fin axil, with greatly enlarged dorsolateral lobes of the LO that abut this patch.” Close association of the LO and translucent external windows no doubt facilitates greater transmission of light in this genus.



The species of *Eubleekeria* scanned and measured had the third lowest LOI score: a score that was 10 to 25-fold smaller than those recorded for members of its sister genus, *Photopectoralis* (Table 1). Despite the relatively smaller size of the LO in *Eubleekeria* compared with *Photopectoralis*, its overall shape is similar. Members of both genera exhibit pronounced lateral, ventral, and dorsal expansion of the LO (Figs 3–5).

**Subfamily Gazzinae: Tribe Equulitini.** Equulitini comprises all members of genus *Equulites*, which are strongly sexually dimorphic both internally (e.g., LO volume enlarged in males) and externally (i.e., males possess expansive translucent external patches, whereas females have none) with regard to features of the LOS. Equulitini comprises two distinct morphological lineages. One clade comprises species with a translucent lateral flank stripe, which we informally refer to here as the “lateral-stripe” group (viz., *E. antongil*, *E. moretoniensis*, and *E. stercorarius*). Its sister clade, the subgenus *Photoplagios*, comprises all species of Equulitini with an expansive and species-specific translucent triangular, polygonal-, or crescent-shaped flank patch (viz., *E. absconditus*, *E. elongatus*, *E. laterofenestra*, *E. leuciscus*, *E. klunzingeri*, and *E. rivulatus*; Sparks and Chakrabarty, 2007; Chakrabarty et al., 2010).

Observations of photic communication in Equulitini have been reported for *Equulites rivulatus* in aquaria (Wada et al., 2005b) and for *E. elongatus* in the wild (Sasaki et al., 2003). Males of both species possess a large translucent flank patch, whereas females do not (Sparks and Chakrabarty, 2007). Sasaki et al. (2003) documented nocturnal flashing behavior in male *E. elongatus* from Japanese waters and reported that light was emitted from “the lateroventral portion of the body, as bright rectangular-shaped luminescence patches turned on and off periodically.” Females were non-luminous and were pursued by the males within a shoal, which may be indicative of lekking behavior. Luminescence in *E. elongatus* was reported to be “bright greenish” with periodic on–off sequences that lasted from one to a few seconds. Males were also reported to school and produce synchronous on–off flashing patterns. In a closely related species with quite similar LOS morphology, *E. rivulatus*, Wada et al. (2005b) found that males and females harbored different strains (from each other) of the luminescent bacterium *Photobacterium leiognathi* in June, but in no other month. *Equulites rivulatus* was not observed flashing in these studies, so whether temporal change in the bacterial fauna corresponded with the breeding season is not known. To date, there are no published accounts of flashing behavior in species of *Equulites* that are characterized by a translucent lateral stripe or horizontal series of translucent lateral spots or patches (i.e., *E. antongil*, *E. more-*

*toniensis*, and *E. stercorarius*). Our comparative studies demonstrate that the length and structure of the translucent stripe as well as the number, size, orientation, and shape of the translucent spots or patches are species specific for members of this clade (Sparks, 2006; Sparks and Chakrabarty, 2007; Chakrabarty et al., 2009). It is possible that these unique species-specific morphologies correspond to distinct luminescent signals that play a role in both species and mate recognition.

**Taxonomic diagnosis.** Although Equulitini comprises two quite distinct morphological lineages, the clade as a whole is characterized by males that possess either an expansive translucent flank patch or stripe, dorsal lobes of the LO that are hypertrophied, laterally expanded, and extend posteriorly into the gas bladder, and lateral clearing of the gas bladder lining corresponding directly to the translucent external patches or stripe (Sparks et al., 2005). *Equulites* comprises species with a translucent lateral flank stripe, that is either continuous or comprised of discrete, horizontally arrayed translucent windows (e.g., *E. stercorarius*), and dorsal lobes of the LO that extend posteriorly only slightly into the gas bladder. In contrast, the subgenus *Photoplagios* comprises all members of Equulitini in which males possess an expansive and species-specific translucent flank patch, which may be triangular, polygonal, or crescent shaped (Sparks and Chakrabarty, 2007; Chakrabarty et al., 2010). The dorsolateral LO lobes of males in this clade are markedly enlarged compared with similarly sized conspecific females and extend posteriorly well into the gas bladder (Sparks et al., 2005; Fig. 4). Members of *Photoplagios* exhibit the highest degree of sexual dimorphism of the LOS of any lineage within Leiognathidae.

**LO indices.** MRI analyses indicate that LO volume and morphology are radically different among members of Equulitini. Sparks et al. (2005) diagnosed this clade partially on the basis of having “hypertrophied dorsolateral lobes of the LO”; however, a more accurate and quantitative in situ description is available through MRI 3D reconstructions. *Equulites (Photoplagios) rivulatus* had the highest overall LOI score and exhibited both extensive lateral and dorsal expansion of the LO lobes in males (Figs. 3A and 4A). In contrast, *Equulites moretoniensis* exhibited primarily ventral expansion of the LO with little lateral or dorsal enlargement in males, which translated to one of the lowest LOI scores (Figs. 3I and 5D; Table 1).

Externally, the LOSs of these two members of Equulitini are also markedly different: *E. moretoniensis* has a translucent lateral stripe comprising numerous discrete windows, whereas *E. P. rivulatus* and other members of the subgenus *Photoplagios* are characterized by an expansive translucent flank patch (triangular and bullet shaped in

*E. P. rivulatus*). For example, *E. P. laterofenestra* also has an expansive translucent lateral flank patch, which is trapezoidal or crescent shaped. Although its LO is dorsolaterally expanded and similar in structure to *E. P. rivulatus*, enlargement of the dorsolateral lobes is only moderate, not extreme as in *E. P. rivulatus*. The LOI score for *E. P. laterofenestra* is fifth among ponyfishes that were measured and intermediate between the two other members of Equulitini that were examined (Table 1).

**Subfamily Gazzinae: Tribe Nuchequulini.** Nuchequulini comprises members of the genera *Nuchequula* (viz., *N. blochii*, *N. flavaxilla*, *N. gerreoides*, *N. glenysae*, *N. longicornis*, *N. mannusella*, *N. decora*, and *N. nuchalis*) and *Karalla* (viz., *K. dussumieri* and *K. daura*). *Nuchequula* was recently elevated from subgeneric to generic rank by Chakrabarty and Sparks (2007) to encompass all leiognathid species possessing a darkly pigmented, saddle-shaped, nuchal marking and a pigment-free, more or less mitten-shaped region posteroventral to the pectoral-fin base. *Karalla* was recently described by Chakrabarty and Sparks (2008) to encompass leiognathid species with a deep golden coloration on the flank and a retrognathous lateral snout outline.

Members of *Karalla* and *Nuchequula* are sexually dimorphic with regard to LO volume, with males exhibiting enlarged LOs compared with conspecific females of similar size. All members of *Nuchequula* are characterized by a large, darkly pigmented, nuchal marking (saddle) centered along the dorsal midline in the predorsal region. In males, a translucent region is present within this marking. Given that the underlying musculature is generally translucent in this region, it is possible that light may be emitted dorsally via the translucent section within the nuchal marking, although this mode of luminescence has yet to be confirmed via direct observation (Chakrabarty and Sparks, 2007).

Within Nuchequulini, photic communication in aquaria has been observed in *Nuchequula nuchalis* (Azuma et al., 2005; Wada et al., 2005a). Light emission in *N. nuchalis* was confined almost entirely to a known breeding season (mid-July) and primarily around midnight spawning periods. Few flashing events were recorded outside of this narrow time period. According to Azuma et al. (2005), "the light of this species is very weak, because we could not detect the light by eye or using CCD camera." Azuma et al. (2005) presented photometer measurements, in which no more than 35 light signaling events were observed in any 30-min period from a total of five individuals. Unfortunately, photometer readings provide no information regarding the duration, intensity, or pattern of flashing. Interestingly, results of the Azuma et al. (2005) study raise the question of

how (or if) the fish are able to detect such a weak flashing pattern if the investigators could not.

*Karalla daura* and *K. dussumieri* do not appear to be sexually dimorphic for external features of the LOS, although they exhibit LO volume dimorphism that is common to all members of Nuchequulini. There are no published observations regarding the flashing behavior for members of this genus, although Haneda (1940) reported that he observed light emission in *K. daura*. Given that Haneda (1940) did not describe the light emission in *K. daura* as distinctive in any way, we presume he observed the same steady ventral emission of light (ventral counterillumination) that he reported for other leiognathid species.

**Taxonomic diagnosis.** Members of Nuchequulini are sexually dimorphic with regard to LO volume, with males exhibiting an enlarged LO compared with conspecific females of similar size. All members of *Nuchequula* are characterized by a large, darkly pigmented, more or less translucent, nuchal marking (saddle) centered along the dorsal midline as described by Chakrabarty and Sparks (2007). Although members of *Karalla* do not appear to exhibit any externally sexually dimorphic features of the LOS, they are distinguished from other leiognathid species by a deep golden coloration on the flank and a retrognathous lateral snout outline (Chakrabarty and Sparks, 2008).

**LO indices.** Within Nuchequulini, an individual of *Nuchequula nuchalis* was scanned, and its LOI was calculated to be the fourth lowest of all the ponyfishes measured (Table 1). Ventrolateral expansion of the LO in this species was considerable; however, little dorsal or dorsolateral expansion was evident (Figs. 3G and 5B).

### Phylogenetic Relationships of Leiognathidae

All 10 independent likelihood analyses recovered identical topologies, with the best likelihood score being  $\ln -50115.92$ . This reconstruction of leiognathid evolutionary relationships is shown in Figure 6. The interrelationships of the family are consistent with findings from previous studies (e.g., Sparks et al., 2005; Chakrabarty et al., manuscript under review), with the described and diagnosed subfamilies and tribes recovered as monophyletic (Fig. 6).

### Character Evolution

**Subfamily Leiognathinae.** The LO of male members of Leiognathinae is not sexually dimorphic with respect to either volume or shape (Character 1: State 0); however, the LO is expanded ventrally (Character 3: State 0). Based on ancestral character reconstructions, the inferred common ancestor of Leiognathinae unambiguously possessed

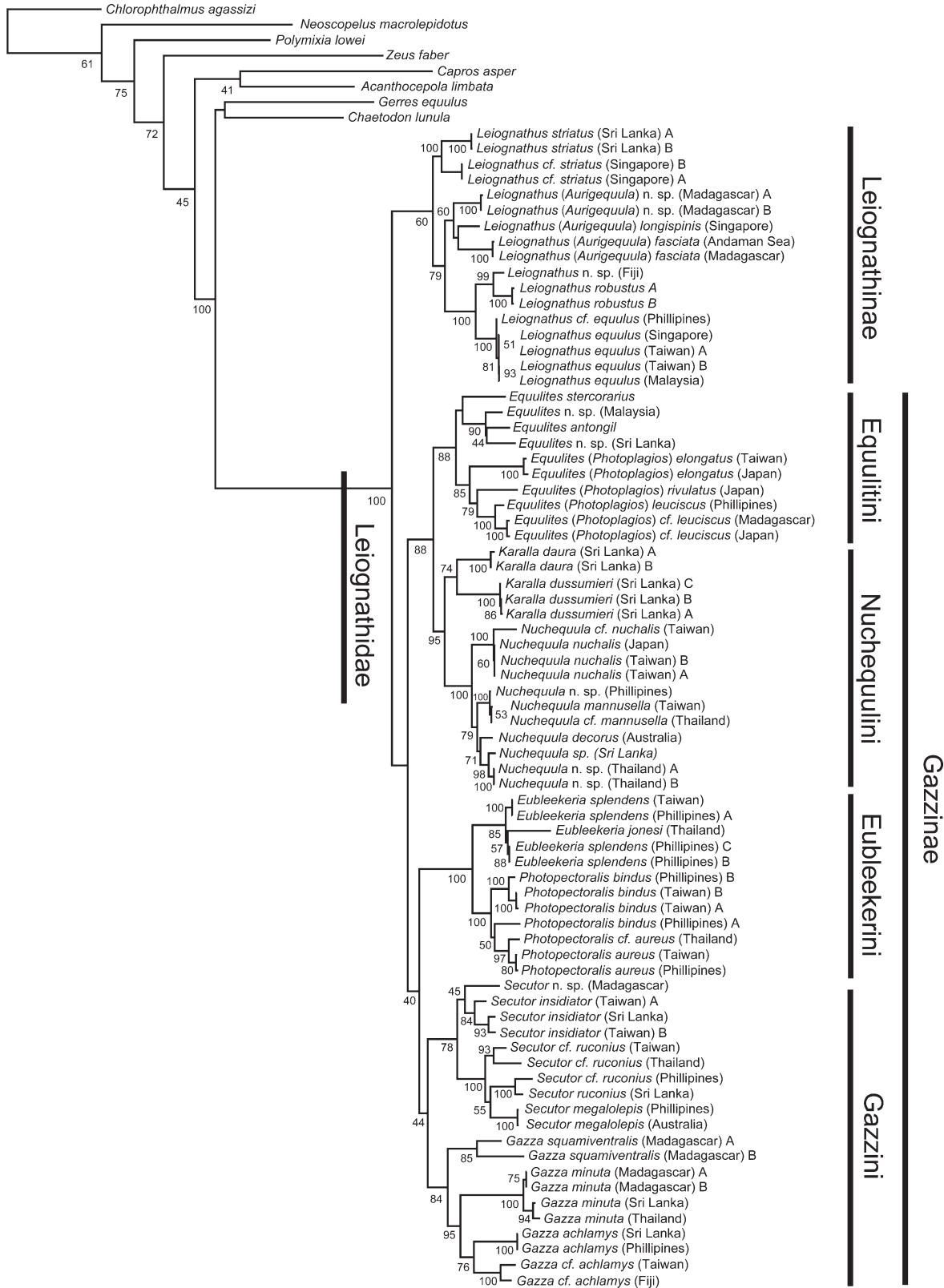
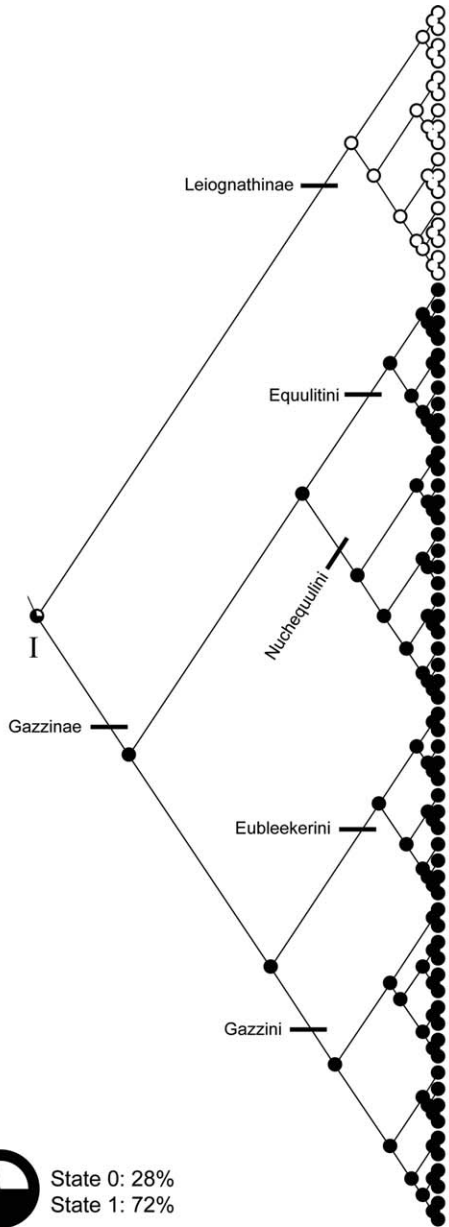


Fig. 6. Maximum likelihood reconstruction of leignathid relationships. Bootstrap support values greater than 40 are shown at each node. (High-resolution image can be viewed in Supporting Information online).



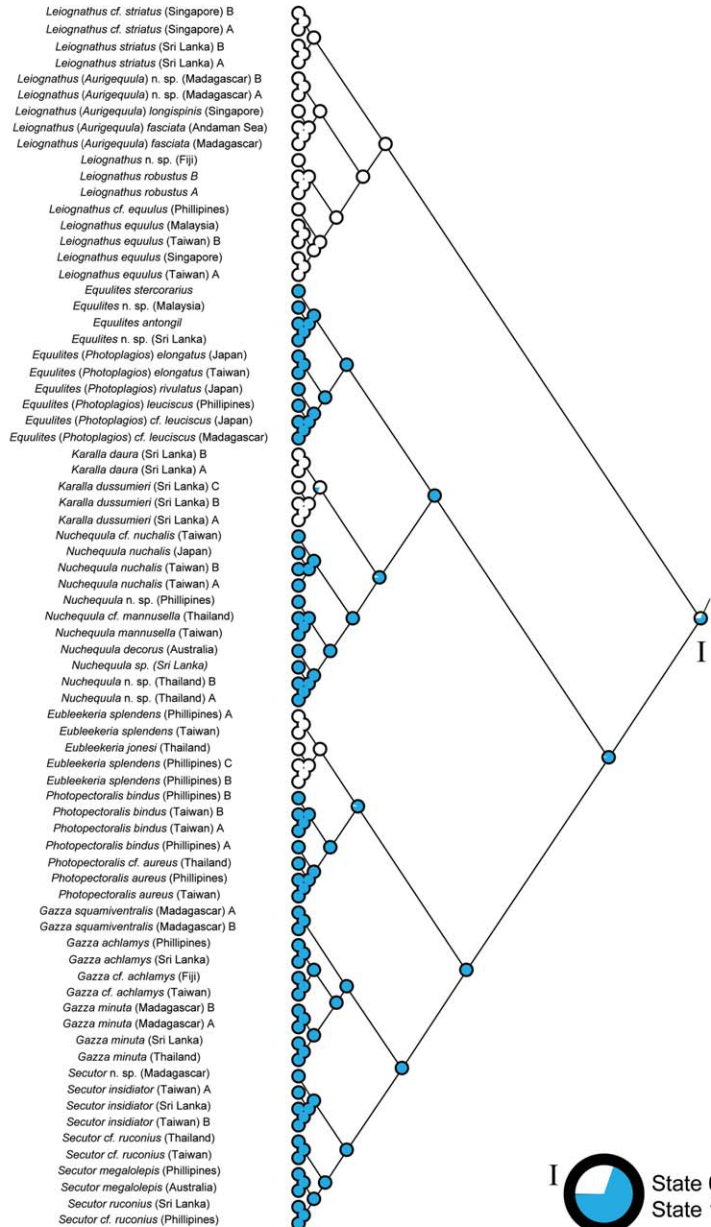
A



Character 1

- State 0: No light organ volume or shape dimorphism between male and female
- State 1: Light organ volume larger and shape different (lobes hypertrophied) in males

B



Character 2

- State 0: LOS patch dimorphism absent
- State 1: LOS patch sexually dimorphic in size or shape

Fig. 7. Likelihood character evolution of LOS sexual dimorphism. Pie charts represent probabilities of character-state likelihoods. (A) Character 1: Light organ sexually dimorphic in males. (B) Character 2: External translucent patch sexually dimorphic in males. (High-resolution image can be viewed in Supporting Information online).

these characteristics (Figs. 7A and 8A). Male members of this clade also lack any sexually dimorphic traits in associated LOS structures (Character 2: State 0; Fig. 7B), including translucent external patches (Character 4: State 0; Fig. 8B).

**Subfamily Gazzinae: Tribe Gazzini.** The LO of members of Gazzini is strongly sexually dimorphic and characterized by an increase in volume in males (Character 1: State 1). The LO is also markedly expanded ventrally in male members of

Gazzini (Character 3: State 0). Based on ancestral state reconstructions, these two LO features are inferred as having been present in the common ancestor of *Gazza* and *Secutor* (Figs. 7A and 8A). Members of Gazzini also exhibit external sexual dimorphism in males with regard to the size (surface area) of the rostrally placed translucent LOS patch(es), with males possessing moderately larger opercular (*Gazza*) or gular (*Secutor*) patches than similarly sized conspecific females (Character 2: State 1; Fig. 7B). Our character reconstructions suggest that sexually dimorphic rostrally placed translucent external windows (Character 4: State 1) most likely originated in the common ancestor of Gazzini (Fig. 8B).

**Subfamily Gazzinae: Tribe Eubleekerini.**

Members of Eubleekerini exhibit sexually dimorphic LOs, characterized by an increase in volume in males (Character 1: State 1; Fig. 7A). In members of this tribe, the dorsolateral and ventral LO lobes are markedly expanded (Character 3: State 1; Fig. 8A). Ancestral character state reconstructions indicate that these unique attributes of the LO were present in the common ancestor of Eubleekerini (Figs. 7A and 8A). Although members of *Eubleekeria* are not externally sexually dimorphic with regard to features of the LOS (Character 2: State 0; Fig. 7B), *Photopectoralis* males possess characteristic translucent pectoral-axil patches (Character 2: State 1). Character-state reconstruction indicates that the inferred common ancestor of Eubleekerini most likely was externally sexually dimorphic (94%), and that external LOS dimorphism was subsequently lost in *Eubleekeria* (Fig. 7B). Externally, the specialized translucent pectoral-fin axil in *Photopectoralis* (Character 4: State 3) most likely evolved a single time in the inferred ancestor of *Photopectoralis* (Fig. 8B).

**Subfamily Gazzinae: Tribe Equulitini.**

All members of Equulitini are internally sexually dimorphic and characterized by LOs in males that are enlarged in volume (Character 1: State 1). In members of *Equulites* characterized by a lateral flank stripe or horizontal series of discrete windows, the LO is moderately expanded ventrally (Character 3: State 0), whereas members of the subgenus *E. Photoplagios*, characterized by expansive translucent flank patches, have LOs that are greatly expanded dorsolaterally (Character 3: State 2), corresponding to the highest degree of LO sexual dimorphism within Leiognathidae.

Character-state reconstructions indicate that the inferred common ancestor of Equulitini exhibited ventral expansion of the LO and was sexually dimorphic (Fig. 7A,B). Members of Equulitini are externally sexually dimorphic with males possessing highly variable and species-specific translucent LOS patches, stripes, or distinct patterns of serially arrayed patches on the flank (Character 2: State 1), a trait that is also inferred to have been

present in the common ancestor of Equulitini (Fig. 7B). There are two unique, sexually dimorphic, external specializations of the LOS in Equulitini. The first is the presence of a translucent flank stripe in the “lateral-stripe” group of *Equulites* (Character 4: State 4), which is recovered as the most likely state of the inferred ancestor of that clade (Fig. 8B). The second is the presence of expansive, translucent lateral flank patches (e.g., polygonal, bullet, or crescent shaped) in the subgenus *Photoplagios* (Character 4: State 5), which also is recovered as the most likely state in the inferred common ancestor of *Photoplagios* (Fig. 8B). The inferred state of the common ancestor of Equulitini with regard to morphology of the translucent external patch(es) is ambiguous, with three potential reconstructed states and probabilities (State 0: 17%, State 4: 55%, and State 5: 28 %).

**Subfamily Gazzinae: Tribe Nuchequulini.**

All members of Nuchequulini are internally sexually dimorphic with regard to features of the LOS. Males are characterized by LOs that are both larger in volume (Character 1: State 1) and that exhibit considerable ventral expansion (Character 3: State 0) compared with conspecific females of similar size. Ancestral character-state reconstructions indicate that both of these sexually dimorphic LO features were present in the inferred common ancestor of Nuchequulini (Figs. 7A and 8A). Members of *Karalla* are not sexually dimorphic with regard to external features of the LOS (Character 2: State 0), whereas *Nuchequula* males are externally sexually dimorphic and possess a translucent central region within the nuchal patch (Character 2: State 1). The common ancestor of Nuchequulini is hypothesized to have exhibited external sexual dimorphism of the LOS, suggesting that external dimorphism was subsequently lost in *Karalla* (Fig. 7B). A novel LOS specialization is present in Nuchequulini, a translucent central region within the nuchal patch in *Nuchequula* males (Character 4: State 2), which is inferred to have evolved in the common ancestor of *Nuchequula* (Fig. 8B).

**Character Correlation**

**Internal and external sexual dimorphism of the LOS.** The null hypothesis that sexually dimorphic internal (Character 1) and external (Character 2) features of the LOS have evolved independently of each other is statistically rejected with a *P* value of 0.02. A log likelihood difference of 4.55 was recovered between the likelihoods of the independent model (log likelihood, 14.57) and the dependent model (log likelihood, 10.02).

**DISCUSSION**

**Evolution of LOS Sexual Dimorphism**

Internal sexual dimorphism, characterized by the presence of a circumesophageal LO that is

A

B

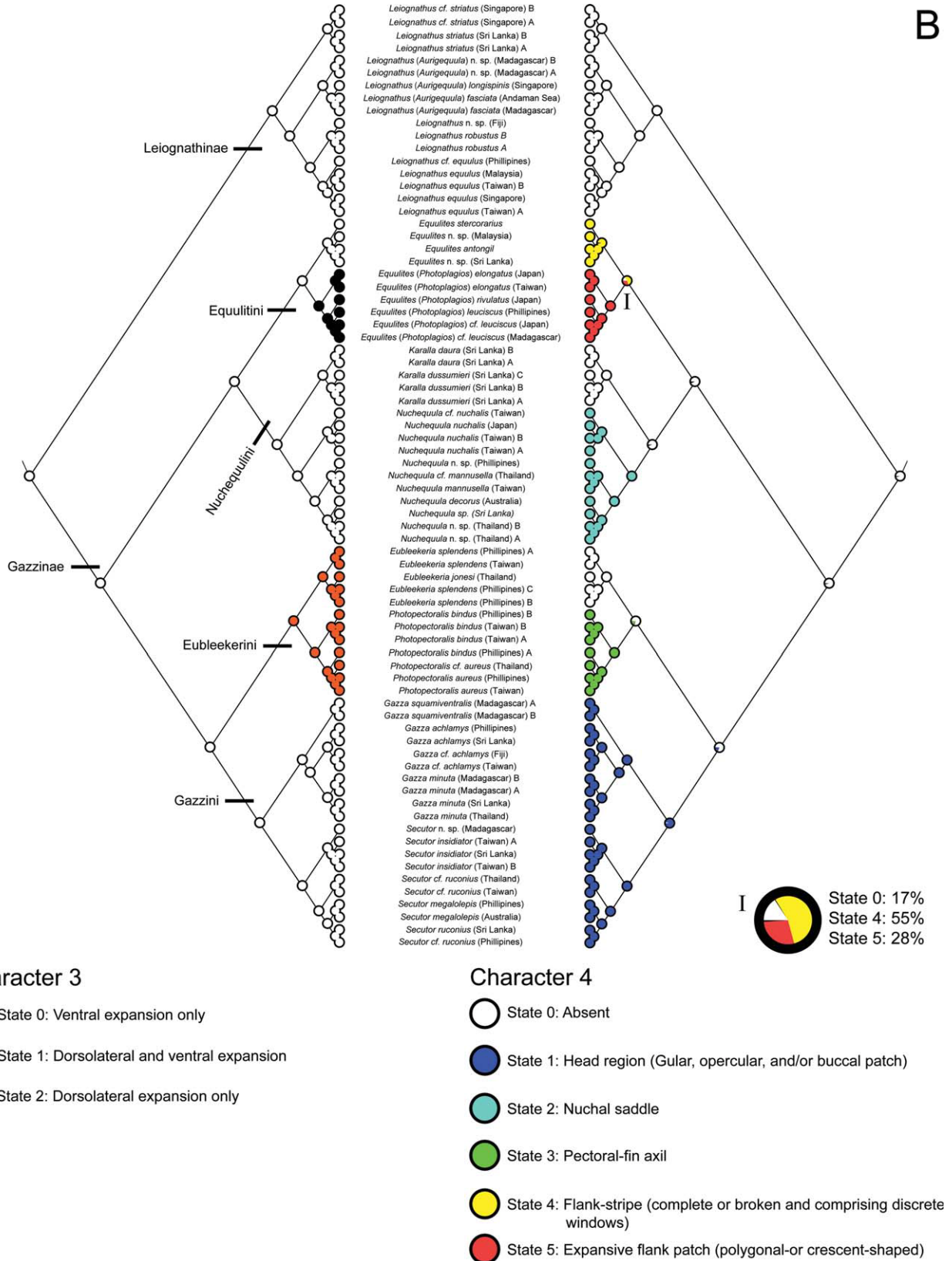


Fig. 8. Likelihood character evolution of LOS morphology. Pie charts represent probabilities of character-state likelihoods. (A) Character 3: Light organ shape. (B) Character 4: LOS translucent patch specialization and orientation. (High-resolution image can be viewed in Supporting Information online).



enlarged with regard to volume in males, appears to have evolved a single time in the stem species of the family Leiognathidae. Two potential scenarios can be invoked to explain this evolutionary transformation. The first is that internal sexual dimorphism of the LO evolved in the common ancestor of the ponyfish lineage and was subsequently lost in the subfamily Leiognathinae. Our reconstructions of character evolution indicate that this is the most likely scenario (Fig. 7A), with a probability of 72%. The alternative, and less likely scenario according to our analyses, is that the common ancestor of ponyfishes possessed a nonsexually dimorphic LO (28% probability), and that this trait was retained in Leiognathinae. In this scenario, the evolution of internal sexual dimorphism of the LO would have occurred a single time in the stem species of the subfamily Gazzinae (Fig. 7A).

The evolution of external sexual dimorphism of the LOS follows a similar pattern, with two distinct scenarios that mirror those described for internal sexual dimorphism. Based on our analyses of character evolution, the most likely scenario posits that the evolution of external sexually dimorphic translucent LOS patches evolved in the common ancestor of ponyfishes (70%), with three independent losses in the inferred common ancestors of Leiognathinae, *Eubleekeria*, and *Karalla* (Fig. 7B). The less likely scenario is that the common ancestor of ponyfishes lacked externally sexually dimorphic translucent patches (30%), which would suggest that sexually dimorphic external LOS patches evolved a single time in the common ancestor of the clade comprising Gazzini, *Eubleekerini*, *Equulitini*, and *Nuchequulini*, with two independent losses in the common ancestors of *Eubleekeria* and *Karalla* (Fig. 7B).

Although it is possible that the evolution of sexually dimorphic internal and external features of the leiognathid LOS was temporally decoupled, results of our analyses of character evolution indicate that this particular scenario is statistically unlikely. Our results utilizing Pagel's (1994) correlation test reject the null hypothesis that internal and external sexual dimorphism of the LOS are evolving independently from one another ( $P = 0.02$ ), suggesting that the evolution of sexually dimorphic internal and external traits of the LOS is linked in some fashion. On the basis of these analyses, we hypothesize that the most likely scenario for the evolution of sexual dimorphism of the luminescent system in ponyfishes is that internal and external sexually dimorphic features evolved in the stem species of Leiognathidae. Subsequently, sexually dimorphic internal and external features of the LOS were lost in the subfamily Leiognathinae. Finally, two additional losses of externally sexually dimorphic LOS traits have occurred in the genera *Eubleekeria* and *Karalla*; however, in both cases, internal sexual dimor-

phism of the LO has been retained. Non-independent evolution of internal and external LOS features is not particularly surprising, given that external sexually dimorphic features of the LOS are functionally dependent upon internal LO features.

### Evolution of the Light Organ System

The unique circumesophageal LO of ponyfishes is formed early on in development as an outpocketing of esophageal tissue. In its basic, nonsexually dimorphic form, the LO is more or less donut shaped. Among the sexually dimorphic leiognathid lineages, a remarkable array of shape and size variation is observed (Sparks et al., 2005). A similar luminescent system is not known to occur in any other acanthomorph taxa. Most ponyfishes exhibit some ventral expansion of the LO, which is presumably associated with a ventral counterillumination function. Comparative anatomical studies show quite clearly that further expansion (hypertrophy) of particular LO lobes in males is highly correlated with the location and orientation of the species-specific translucent external LOS patch(es) observed among various leiognathid lineages (Sparks et al., 2005). Ventral expansion of the LO is recovered in the inferred common ancestor of Leiognathidae, and this feature is retained in a majority of ponyfish lineages, including Leiognathinae, Gazzini, *Equulitini*, and *Nuchequulini* (Fig. 8A). Pronounced dorsolateral and ventrolateral expansion of the LO is characteristic of members of *Eubleekerini* (Figs. 3H and 5C) and most likely evolved a single time in the common ancestor of this lineage (Fig. 8A). Pronounced dorsal and dorsolateral expansion of the LO is unique to members of the subgenus *Photoplagios* (subclade within *Equulitini*) and is recovered as most likely having evolved in the common ancestor of that clade (Fig. 8A).

Although the results from ancestral character-state reconstructions and the correlation analysis indicate that the common ancestor of Leiognathidae most likely possessed external dimorphism of the LOS in males (Fig. 7B), the location and orientation of these sexually dimorphic features on the body are unclear in the common ancestor of the family (Fig. 8B). Placement on the body and shape of the translucent external patches is extremely variable across ponyfish lineages, with distinct patch shapes, orientations, and locations evolving in the common ancestors of various tribes and genera (Fig. 8B). This includes the evolution of translucent buccal, gular, and/or opercular patches in the common ancestor of Gazzini, a translucent patch in the pectoral-fin axil in the common ancestor of *Photopectoralis*, a translucent lateral flank stripe in either the common ancestor of *Equulitini* or *Equulites*, and expansive translucent flank

patches in either the common ancestor of Equulitini or the *E. Photoplagios* lineage (Fig. 8B). Because of substantial variability, it is difficult to reconstruct the potential patch shapes and locations on the body in the stem species of deeper ponyfish nodes (Fig. 8B), although all of these nodes were reconstructed to have most likely possessed some kind of external dimorphism of the LOS (Fig. 7B).

### Morphology of the Light Organ From MRI

A comparison of LO indices from the taxa analyzed in this study reveals that closely related species within the tribe Equulitini possess widely divergent LO volumes in relation to body size (Table 1). *Equulites rivulatus* had the highest LOI of all ponyfishes measured. LO morphology and associated features of the LOS in *E. rivulatus* are very similar to its sister species, *E. elongatus* (Sparks et al., 2005), which also exhibits a greatly enlarged LO in males characterized by hypertrophied dorso-lateral lobes (Sparks et al., 2005). *Equulites moretoniensis* had the second lowest LO score, whereas *E. laterofenestra* was intermediate among the taxa analyzed. *Equulites rivulatus* is a small-bodied (<100-mm SL) species, whereas *E. laterofenestra* is medium sized, with both species possessing an expansive translucent flank patch. *Equulites moretoniensis* is a medium-sized ponyfish and is recovered within a subclade of *Equulites* that is characterized by a translucent lateral stripe in contrast to an expansive polygonal, or bullet-shaped flank patch as is diagnostic for members of *E. Photoplagios*. The wide ranging LOI scores within this genus suggest that LO volume is independent (in some cases) with regard to the size of the individual, translucent external patch morphology, or phylogenetic affinity.

In contrast to the pattern recovered for Equulitini, in which the LO exhibits both significant shape and volume disparity, members of Eubleekerini (*Eubleekeria* + *Photopectoralis*) exhibit remarkably similarly shaped LOs despite large differences in volume. For example, the second and third highest LO indices are found in members of *Photopectoralis*. Males of this genus have a translucent flank patch located in the pectoral-fin axil that is, in general, much smaller in terms of surface area than the translucent flank patches that are present in members of *Equulites*, except those taxa characterized by a translucent lateral stripe (e.g., *E. moretoniensis*). Surprisingly, although *E. laterofenestra* possesses a very expansive translucent external flank patch, much larger than any pectoral-axil patch observed in *Photopectoralis*, it is nevertheless ranked fifth in its LOI score. Although *Eubleekeria*, the closest relative of *Photopectoralis*, has a much smaller LO than its sister genus, the LOs of these two genera are quite simi-

lar in overall morphology. These data again suggest that there is not a clear correlation between external patch size and relative LO volume. Other factors certainly also play a role in the luminescent display capabilities of these fishes, including: position of the LO relative to the translucent flank patch(es) or stripes, presence of reflective guanine-lined chambers, guanine-lined light tubes, and translucent regions of the otherwise reflective guanine-lined gas bladder.

Both *Nuclequula* and *Eubleekeria* had low LO indices. Although internal sexual dimorphism of the LOS is well documented in these two genera (Sparks et al., 2005), *Eubleekeria* appears to lack any externally sexually dimorphic features of the LOS [i.e., no external patch(es)], whereas male members of *Nuclequula* are characterized by a translucent region within the dark nuchal marking characteristic of the genus. Individuals of *Eubleekeria* have been observed flashing in unison within a shoal (Woodland et al., 2002); thus, it is possible that flashing pattern of the LO itself could be sexually dimorphic and that sexual communication might be feasible in the absence of external sexual dimorphism. *Photopectoralis*, the sister group to *Eubleekeria*, in contrast had a high LOI, providing further evidence that phylogenetic relationship is not necessarily a good predictor of relative LO volume.

In all of the taxa analyzed in this study, ventral expansion of the LO was present to some degree. Although *Equulites (Photoplagios) rivulatus* and *E. P. laterofenestra* exhibited the least amount of ventral expansion, the former species had the highest LOI score and exhibited extreme dorsal and dorsolateral expansion of the LO. Expansion of the lateral LO lobes is pronounced in five of the ponyfish species for which we calculated LO indices (Fig. 3; Table 1). Ventral expansion of the LO is most pronounced in those taxa with generally low LOI scores. However, a certain degree of ventral expansion of the LO is present in all ponyfishes, and ventral expansion of the LO was recovered as occurring the common ancestor of the family (Fig. 8A). Dorsal and dorsolateral expansion of the LO lobes is most pronounced in the ponyfish species with the top three LOI scores, *Equulites rivulatus*, *Photopectoralis aureus*, and *Photopectoralis bindus*. This correlation may be due to an emphasis on sexual luminescence displays centered on the flank in males of these two genera versus ventral counterillumination; however, further study regarding the flashing behavior of ponyfishes and the function of various components of the LOS is required before we are in a position to test this hypothesis.

Although this study presents an analysis of the evolution of the luminescent system in ponyfishes from the perspective of LOS functional anatomy, a greater knowledge of sexual photic communication

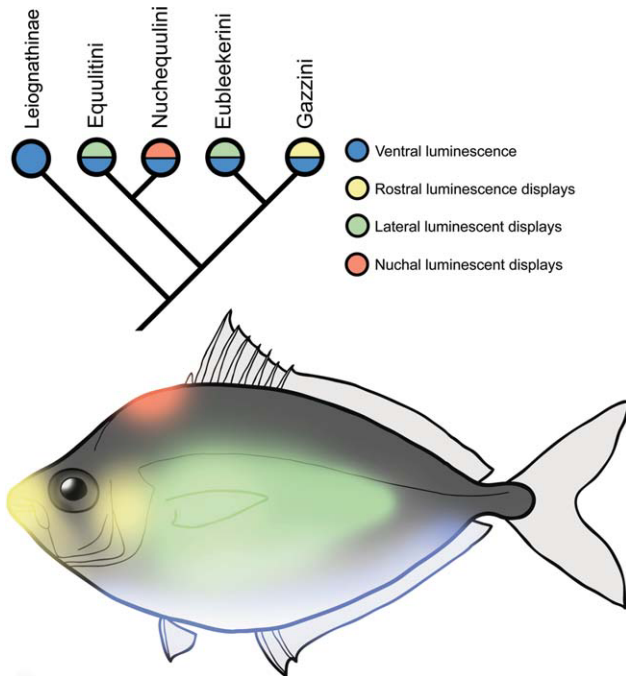


Fig. 9. Summary of empirically observed luminescent displays within ponyfishes.

patterns within and among clades is needed. Although observations of photic displays in leiognathids are rare, they nevertheless do exist for members of each of the major clades (Haneda, 1940; Hastings, 1971; Sasaki et al., 2003; Wada et al., 2005a) as summarized in Figure 9. Representatives from all ponyfish tribes have been observed to display ventral counterillumination, whereas additional modes of luminescent display have been observed in members of the tribes Gazzini, Equulitini, and Eubleekerini. Unfortunately, these observations offer little insight regarding the potential for intraspecific sexual communication or species recognition in the wild, such as mate choice, dominance displays, or schooling behavior. Only Sasaki et al. (2003) and Woodland et al. (2002) documented sexual luminescent displays of males and females.

Although rather inconspicuous and morphologically conservative externally, ponyfishes are unique in possessing a LOS that is remarkably anatomically variable across the various lineages that comprise the family, including pronounced internal and external sexual dimorphism of LOS features in more than two-thirds of all leiognathid taxa. A similar system is unknown in any other extant taxa, and the relative roles of sexual selection and natural selection in maintaining sexual dimorphism of the leiognathid LOS remain unclear. Significant additional observational data documenting luminescent displays and mating behavior in ponyfishes are needed to further explore the biological significance of the ponyfish LOS.

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