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Evolution and diversification of a sexually dimorphic luminescent system in ponyfishes (Teleostei: Leiognathidae), including diagnoses for two new genera

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Abstract

A phylogeny was generated for Leiognathidae, an assemblage of bioluminescent, Indo-Pacific schooling fishes, using 6175 characters derived from seven mitochondrial genes (*16S*, *COI*, *ND4*, *ND5*, *tRNA-His*, *tRNA-Ser*, *tRNA-Leu*), two nuclear genes (*28S*, *histone H3*), and 15 morphological transformations corresponding to features of the fishes' sexually dimorphic light-organ system (LOS; e.g., circumesophageal light organ, lateral lining of the gas bladder, transparent flank and opercular patches). Leiognathidae comprises three genera, *Gazza*, *Leiognathus*, and *Secutor*. Our results demonstrate that Leiognathidae, *Gazza*, and *Secutor* are monophyletic, whereas *Leiognathus* is not. The recovered pattern of relationships reveals that a structurally complex, strongly sexually dimorphic and highly variable species-specific light organ is derived from a comparatively simple non-dimorphic structure, and that evolution of other sexually dimorphic internal and external features of the male LOS are closely linked with these light-organ modifications. Our results demonstrate the utility of LOS features, both for recovering phylogeny and resolving taxonomic issues in a clade whose members otherwise exhibit little morphological variation. We diagnose two new leiognathid genera, *Photopectoralis* and *Photoplagios*, on the basis of these apomorphic LOS features and also present derived features of the LOS to diagnose several additional leiognathid clades, including *Gazza* and *Secutor*. Furthermore, we show that five distinct and highly specialized morphologies for male-specific lateral luminescence signaling, which exhibit species-specific variation in structure, have evolved in these otherwise outwardly conservative fishes. Leiognathids inhabit turbid coastal waters with poor visibility and are often captured in mixed assemblages of several species. We hypothesize that the species-specific, sexually dimorphic internal and external modifications of the leiognathid LOS provide compelling evidence for an assortative mating scheme in which males use species-specific patterns of lateral luminescence signaling to attract mates, and that this system functions to maintain reproductive isolation in these turbid coastal environments.

Leiognathids, commonly known as ponyfishes or slipmouths, are bioluminescent, schooling fishes common in the near-shore and estuarine waters of the Indo-West Pacific. They are locally abundant and are often captured in mixed assemblages of a few to several species in these turbid coastal waters of poor visibility (McFall-Ngai and Dunlap, 1984; Woodland et al., 2001; P.V. Dunlap, pers. obs.). Approximately 40 species in three genera, *Gazza*, *Leiognathus* and *Secutor*, are currently recognized as valid (Eschmeyer, 2005; Froese and Pauly, 2005).

Luminescence in leiognathids is produced from an internal, circumesophageal ring of tissue, the light organ, in which are harbored large numbers of the symbiotic luminous bacterium, *Photobacterium leiognathi*, the source of the animal's light (Boisvert et al., 1967;

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Hastings and Mitchell, 1971; Bassot, 1975; Reichelt et al., 1977; Dunlap, 1984; Dunlap et al., 2004). The light organ is composed of epithelial cells forming many individual tubules, with bacteria housed within the lumen of each tubule (Harms, 1928; Haneda, 1940, 1950; Ahrens, 1965; Bassot, 1975; McFall-Ngai, 1983). Circumesophageal light organs are unknown in other fish groups, including those traditionally hypothesized to be closely related to leiognathids, gerreids and carangoids (including menids) (Bleeker, 1845, 1859; Günther, 1862; Regan, 1913; Weber and de Beaufort, 1931; James, 1975; Jones, 1985).

Leiognathids use reflective layers and chromatophore-embedded shutters of the light organ, together with reflective and transparent tissues of the gas bladder and transparent bone and hypaxial musculature to control, direct and diffuse the bacterial light over the animal's ventral surface (Harms, 1928; Haneda, 1940, 1950; Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1983; McFall-Ngai, 1983). Ventral luminescence in leiognathids is hypothesized to provide camouflage, through disruptive illumination, against bottom-dwelling piscivorous fishes (Hastings, 1971; Herring and Morin, 1978; McFall-Ngai and Dunlap, 1983; McFall-Ngai, 1983; McFall-Ngai and Morin, 1991). In addition, flashing from the opercular, buccal, anteroventral, and lateral flank areas by individuals, and synchronized flashing in schools, has been observed and interpreted as functioning in avoiding predators, attracting prey, spacing of individuals in a school, and sex-specific signaling (Haneda, 1940; Haneda and Tsuji, 1976; Herring and Morin, 1978; McFall-Ngai and Dunlap, 1983; Woodland et al., 2002; Sparks and Dunlap, 2004).

Most species of leiognathids exhibit sexual dimorphism of the light organ, which is moderately to highly enlarged in males compared to similarly sized conspecific females (Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1984; Jayabalan and Ramamoorthi, 1985; Jayabalan, 1989; Kimura et al., 2003; Sparks and Dunlap, 2004; this study). For example, the light organ of a male *Leiognathus elongatus* is typically 20 times larger in volume than conspecific females of similar standard length, and may be up to 100 times larger (Dunlap and McFall-Ngai, 1984; McFall-Ngai and Dunlap, 1984). In a majority of cases, leiognathids bearing sexually dimorphic light organs also exhibit male-specific transparency of the internal reflective lateral lining of the gas bladder (certain *Leiognathus* species), male-specific external transparent patches (i.e., windows) on the lateral flank or behind the pectoral fin axil (certain *Leiognathus* species), or male-enhanced transparent patches on the margin of the opercular cavity (*Gazza* and *Secutor*). The presence of these modifications correlates with hypertrophy of dorsolateral or ventrolateral lobes of the light organ in males, such that males can emit light laterally (Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1984; Kimura et al., 2003; Sparks and Dunlap, 2004). Like the emission of light from the light organ, which is under control of the fish via retraction and relaxation of the light-organ shutters, light emission from the transparent external windows also is under the fish's control (McFall-Ngai and Dunlap, 1983, 1984; Sasaki et al., 2003).

These sexually dimorphic attributes, together with the species-specific size and shape of the light organ (Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1984; this study), suggest that a major function of the leiognathid LOS is mate-specific recognition (Paterson, 1985; Andersson, 1994). Sexual selection by female choice plays an important role in maintaining species identity through reproductive isolation in many animals. Examples include assortative mating based on male coloration in rift lake cichlid fishes and luminescence courtship signaling in male fireflies. Luminescence signaling by male leiognathids may operate to attract females, induce spawning, or segregate species spatially or temporally for reproduction (McFall-Ngai and Dunlap, 1984; Herring and Morin, 1978), in a manner analogous to the species-specific male courtship flashing utilized by fireflies (Lloyd, 1966; Branham and Greenfield, 1996). Thus, sexual selection for species-specific luminescence signaling could play a key role in generating and maintaining species diversity within Leiognathidae.

To date, the evolution of a sexually dimorphic bioluminescent system in vertebrates has not been examined in detail in the context of a robust phylogeny. The only cladistic studies to investigate relationships within Leiognathidae included less than half of the nominal species (Ikejima et al., 2004; Sparks and Dunlap, 2004); all other family level studies to date have been nonexplicit, descriptive anatomical reviews (James, 1975, 1985a,b; Jones, 1985; Woodland et al., 2001). James (1985a,b) presented comparative osteological features for leiognathids, but he did not analyze these data using cladistic methodology. In the absence of a formal analysis of these data, James (1985b, p. 395) proposed a set of conflicting (i.e., incompatible) relationships for the three leiognathid genera. A robust phylogenetic hypothesis for the assemblage, however, is a necessary precursor to understanding and interpreting major trends in the evolution of the LOS.

Reconstructing leiognathid phylogeny using "traditional" hard anatomical features has been problematic. Not only are leiognathids morphologically very similar in external appearance, less LOS features (external variation in the LOS is frequently cryptic in preservation and generally overlooked), they are also meristically quite constant (Jones, 1985). The fossil record also does not provide insight into these issues. The few fossil leiognathids known, the earliest mid-Oligocene in age,

are morphologically similar to extant forms (Hess and Weiler, 1955; Danil'chenko, 1967; Yabumoto and Uyeno, 1994), and soft tissues of the LOS have not been preserved. Alternatively, nucleotide characters provide an "independent" means for testing historical hypotheses of evolutionary processes and are especially useful for reconstructing phylogeny in groups that exhibit a high degree of morphological conservatism, such as ponyfishes. The study of Sparks and Dunlap (2004) demonstrated the utility of both nucleotide characters and soft anatomical features of the fishes' LOS for reconstructing phylogeny in Leiognathidae.

Although about 40 species of ponyfishes are currently recognized as valid (Eschmeyer, 2005; Froese and Pauly, 2005), the actual number of diagnosable species may be considerably higher. Ambiguity regarding leiognathid species diversity exists for a number of reasons. First, the descriptions of many species are rudimentary, frequently lacking adequate diagnoses, and were written with a limited taxonomic comparison to existing species of the time. Second, type specimens are either missing or were never deposited for many of these insufficiently diagnosed species, which has precluded reliable identifications. Third, no explicit morphology-based phylogenetic study of the family has been attempted to date, and appropriate taxonomic comparisons and placement below the family level have been problematic; only recently (e.g., Ikejima et al., 2004; Sparks and Dunlap, 2004) have preliminary molecular phylogenetic hypotheses for the family become available. If features of the LOS are not taken into account, leiognathids are difficult to diagnose and identify because they are otherwise morphologically conservative, both internally and externally, which has resulted in several putatively widespread "wastebasket" species (e.g., *L. equulus* and *L. fasciatus*). We hypothesize that many of these widespread species, in fact, represent species complexes, and that these species will be diagnosable both on the basis of nucleotide characters and soft anatomical features of the LOS.

To examine the evolution and diversification of the leiognathid LOS within a phylogenetic context, we conducted a parsimony analysis of extant forms based on DNA sequence data from nine genes, representing both mitochondrial and nuclear loci (Table 1), and 15 morphological transformations corresponding to features of the LOS (Table 2). Results based on the simultaneous analysis of nucleotide characters and morphological features of the LOS were used to interpret the evolution of the sexually dimorphic anatomical modifications that form this functional, luminescent signaling system. On the basis of derived features of the LOS, we diagnose two new genera of sexually dimorphic species. Diagnostic features of the LOS are also presented for several additional leiognathid clades, including *Gazza* and *Secutor*.

Materials and methods

DNA sequencing and sequence analysis

A total of 6160 aligned nucleotide characters (based on the implied alignment; Wheeler, 2003b) from seven mitochondrial (*16S*, *COI*, *ND4*, *ND5*, *tRNA-His*, *tRNA-Ser*, *tRNA-Leu*) and two nuclear genes (*28S*, *histone H3*), as well as 15 morphological transformations, were used in the phylogenetic analyses. All *ND4*, *tRNA-His*, *tRNA-Ser*, *tRNA-Leu*, and some *ND5* sequences used in this study were obtained from GenBank. Taxon sampling was designed to include a diverse assemblage of leiognathid species representative of overall familial diversity (Table 1, Appendix 1). In addition to all leiognathid species included in the simultaneous analysis of nucleotide and morphological characters, a number of species for which tissue samples suitable for molecular studies could not be obtained were included in the comparative morphological analysis and examined for internal and external features of the LOS (see below) to further clarify leiognathid generic and clade boundaries. Outgroup sampling was comprehensive and designed to provide a robust test of leiognathid monophyly. Outgroup taxa were selected from perciform families traditionally hypothesized to be closely related to leiognathids, including members of Gerreidae (mojarras), Carangidae (jacks), Menidae (moonfishes), and other carangoid lineages (e.g., Günther, 1862; Weber and de Beaufort, 1931; James, 1975; Jones, 1985; Springer and Johnson, 2004). In addition, a broad range of both perciform and non-perciform lineages were included to address the interrelationships of Leiognathidae, following preliminary work of one of the authors (Smith and Wheeler, unpubl. data) and to test a recent hypothesis (based on two morphological features, both of which were highly homoplasious) that placed leiognathids within a clade comprised of both lampridiform and perciform lineages (Springer and Orrell, 2004).

Fish tissues were preserved in 70–95% ethanol, stored frozen at −75 °C, or used fresh for DNA extraction. Total genomic DNA was extracted from muscle, liver, or fin clips via use of a Qiagen Tissue Extraction Kit (QIAamp or QIAquick Tissue Kit) following the manufacturer's protocol. PCR was used to amplify the target segments from each gene sequenced. Double-stranded amplifications were performed in either 25 or 50 μL volumes containing 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2–0.5 μL of each primer, 10–1000 ng of genomic DNA (1–2 μL), and 1 μL of Taq polymerase, or a 25 μL volume containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.25 μL of each primer, and 2–5 μL of genomic DNA. Amplification profiles for all genes can be found in Smith and Wheeler (2004), Sparks (2004), and Sparks and Smith

Table 1
Classification of taxa included in molecular phylogenetic analysis and corresponding GenBank accession numbers. Tissue voucher numbers or original specimen citations are presented for Leiognathidae

Taxon	Voucher no./Source	16S	COI	ND5+	H3	28S
Aulopiformes						
Evermannellidae	*Coccorella atlantica* (ROOT)	DQ027905	DQ027974	DQ028044	DQ028077	DQ028165
Chlorophthalmidae	*Chlorophthalmus agassizi*	DQ027906	DQ027975	NC003160*	DQ028078	DQ028166
Lampridiformes						
Veliferidae	*Velifer hypselopterus*	DQ027907	DQ027976	DQ028045	DQ028079	Unavailable
Lampridae	*Lampris guttatus*	DQ027908	DQ027977	NC003165*	DQ028080	DQ028167
Trachipteridae	*Trachipterus trachypterus*	DQ027909	DQ027978	NC003166*	DQ028081	DQ028168
Polymixiiformes						
Polymixiidae	*Polymixia lowei*	AY538966	AY662744	NC003181*	AY539175	AY539071
Percopsiformes						
Aphredoderidae	*Aphredoderus sayanus*	DQ027910	DQ027979	NC004372*	DQ028082	DQ028169
Gadiformes						
Lotidae	*Lota lota*	DQ027911	DQ027980	NC004379*	DQ028083	DQ028170
Ophidiiformes						
Ophidiidae	*Chilara taylori*	AY538967	DQ027981	Unavailable	AY539176	AY539072
	Lepophidium profundorum	DQ027912	Unavailable	Unavailable	DQ028084	DQ028171
Bythitidae	*Brotulina fusca*	DQ027913	DQ027982	Unavailable	Unavailable	DQ028172
	Brosmophycis marginata	DQ027914	DQ027983	DQ028046	Unavailable	DQ028173
Lophiiformes						
Antennariidae	*Antennarius avalonis*	DQ027915	DQ027984	DQ028047	DQ028085	DQ028174
Zeiformes						
Zeidae	*Zeus faber*	DQ027916	DQ027985	NC003190*	DQ028086	DQ028175
Stephanoberyciformes						
Rondeletiidae	*Rondeletia loricata*	DQ027917	DQ027986	NC003186*	DQ028087	DQ028176
Beryciformes						
Berycidae	*Beryx splendens*	DQ027918	DQ027987	NC003188*	DQ028088	DQ028177
Gasterosteiformes						
Gasterosteidae	*Gasterosteus aculeatus*	DQ027919	DQ027988	NC003174*	DQ028089	DQ028178
Scorpaeniformes						
Scorpaenidae	*Scorpaena guttata*	AY538984	DQ027989	DQ028048	AY539193	AY539089
Perciformes						
Bramidae	*Brama japonica*	DQ027920	DQ027990	DQ028049	DQ028090	DQ028179
Carangidae	*Trachinotus ovatus*	DQ027921	DQ027991	DQ028050	DQ028091	DQ028180
	Carangoides malabaricus	AY541671	AY541646	DQ028051	DQ028092	DQ028181
	Scomberoides lysan	DQ027922	DQ027992	DQ028052	DQ028093	DQ028182
Cepolidae	*Cepola macrophthalma*	DQ027923	DQ027993	Unavailable	DQ028094	DQ028183
	Cepola pauciradiata	DQ027924	DQ027994	Unavailable	DQ028095	DQ028184
Coryphaenidae	*Coryphaena hippurus*	DQ027925	DQ027994	Unavailable	DQ028096	DQ028185
Gerreidae	*Diapterus auratus*	DQ027926	DQ027996	Unavailable	DQ028097	DQ028186
	Gerres equulus	AY541668	AY541643	DQ028053	DQ028098	DQ028187
Haemulidae	*Haemulon plumierii*	AY539057	AY662752	DQ028054	AY539266	AY539161
Labridae	*Tautoga onitis*	AY662710	AY662761	DQ028055	AY662886	DQ028188
Menidae	*Mene maculatus*	DQ027927	DQ027997	DQ028056	DQ028099	DQ028189
Moronidae	*Morone saxatilis*	AY538941	AY662754	DQ028057	AY539255	AY539150
Nematistiidae	*Nematistius pectoralis*	DQ027928	DQ027998	DQ028058	DQ028100	DQ028190
Scombridae	*Scomber scombrus*	DQ027929	DQ027999	DQ028059	DQ028101	DQ028191
Serranidae	*Diplectrum formosum*	AY539048	AY662750	DQ028060	AY539257	AY539152

Sparidae	*Calamus penna*		AY662700	AY662747	DQ028061	AY662876	DQ028192
Leiognathidae	*Gazza* sp. "Fiji"	KU 4444	DQ027930	DQ028000	Unavailable	DQ028102	DQ028193
	Gazza sp. "Madagascar L22"	AMNH 120340	DQ027931	DQ028001	DQ028062	DQ028103	DQ028194
	Gazza sp. "Madagascar"	WLSMadT240	DQ027932	DQ028002	DQ028063	DQ028104	DQ028195
	Gazza sp. "Madagascar WLS54"	AMNH 120364	DQ027933	DQ028003	DQ028064	DQ028105	DQ028196
	Gazza sp. "Madagascar WLS56"	AMNH 119978	DQ027934	DQ028004	DQ028065	DQ028106	DQ028197
	Gazza achlamys "Philippines"	Sparks & Dunlap 2004	AY541648	AY541623	AB100025*	DQ028107	DQ028198
	Gazza achlamys "Sri Lanka"	LEI 1 SL	DQ027935	DQ028005	AB100025*	DQ028108	DQ028199
	Gazza dentex	Ikejima et al. 2004	Unavailable	Unavailable	AB100026*	Unavailable	Unavailable
	Gazza minuta "Philippines GM1"	Sparks & Dunlap 2004	AY541649	AY541624	AB100027*	DQ028109	DQ028200
	Gazza minuta "Sri Lanka L4"	LEI 6 SL	DQ027936	DQ028006	DQ028066	DQ028110	DQ028201
	Gazza minuta "Sri Lanka L29"	LEI 9 SL	DQ027937	DQ028007	AB100027*	DQ028111	DQ028202
	Gazza squamiventralis "Madagascar WLS51"	AMNH 120341	DQ027938	DQ028008	Unavailable	DQ028112	DQ028203
	Gazza squamiventralis "Madagascar WLS52"	AMNH 120342	DQ027939	DQ028009	Unavailable	DQ028113	DQ028204
	Leiognathus sp. "Fiji"	KU 4440	DQ027940	DQ028010	DQ028067	DQ028114	DQ028205
	Leiognathus sp. "Madagascar L25"	AMNH 119979	DQ027941	DQ028011	DQ028068	DQ028115	DQ028206
	Leiognathus sp. "Madagascar L39"	AMNH 120338	DQ027942	DQ028012	DQ028069	DQ028116	DQ028207
	Leiognathus sp. "Madagascar L41"	AMNH 120356	DQ027943	DQ028013	DQ028070	DQ028117	DQ028208
	Leiognathus sp. "Singapore"	UMMZ 240361	DQ027944	DQ028014	Unavailable	DQ028118	DQ028209
	Leiognathus sp. "Sri Lanka L2"	LEI 2 SL	DQ027945	DQ028015	Unavailable	DQ028119	DQ028210
	Leiognathus sp. "Sri Lanka L9"	LEI 17 SL	DQ027946	DQ028016	Unavailable	DQ028120	DQ028211
	Leiognathus equulus "Okinawa"	OKI-LE-1	DQ027947	DQ028017	AB100017*	DQ028121	DQ028212
	Leiognathus equulus "Philippines"	AMNH 122185	AY541653	AY541628	AB100017*	DQ028122	DQ028213
	Leiognathus equulus "Singapore"	UMMZ 238805	AY541654	AY541629	AB100017*	DQ028123	DQ028214
	Leiognathus equulus "Taiwan"	WLS 14 Tai	DQ027948	DQ028018	AB100017*	DQ028124	DQ028215
	Leiognathus fasciatus "Fiji L16"	KU 4649	DQ027949	DQ028019	Unavailable	DQ028125	DQ028216
	Leiognathus fasciatus "Fiji L36"	KU 4645	DQ027950	DQ028020	Unavailable	DQ028126	DQ028217
	Leiognathus fasciatus "Madagascar"	JSS 28-2003/WLS 55	DQ027951	DQ028021	Unavailable	DQ028127	DQ028218
	Leiognathus fasciatus "Okinawa"	FRLM 28999/OKI-LF-1	DQ027952	DQ028022	Unavailable	DQ028128	DQ028219
	Leiognathus robustus "L35"	UMMZ 240360	DQ027953	DQ028023	Unavailable	DQ028129	DQ028220
	Leiognathus robustus "LEQ1S"	UMMZ 242144	AY541664	AY541639	Unavailable	DQ028130	DQ028221
	"*Leiognathus*" sp. "Philippines"	AMNH 122171	AY541655	AY541630	DQ028071	DQ028131	DQ028222
	"*Leiognathus*" *daura* "Sri Lanka L1"	LEI 1 SL	DQ027954	DQ028024	Unavailable	DQ028132	DQ028223
	"*Leiognathus*" *daura* "Sri Lanka L28"	LEI 8 SL	DQ027955	DQ028025	Unavailable	DQ028133	DQ028224
	"*Leiognathus*" *decorus* "Australia"	WI-02–04	DQ027956	DQ028026	AB100015*	DQ028134	DQ028225
	"*Leiognathus*" *decorus* "Sri Lanka"	AMNH 234765	DQ027957	DQ028027	DQ028072	DQ028135	DQ028226
	"*Leiognathus*" *dussumieri* "Sri Lanka L5"	LEI 10 SL	DQ027958	DQ028028	DQ028073	DQ028136	DQ028227
	"*Leiognathus*" *dussumieri* "Sri Lanka L15"	AMNH 234763	DQ027959	DQ028029	DQ028074	DQ028137	DQ028228
	"*Leiognathus*" *dussumieri* "Sri Lanka L30"	LEI 18 SL	DQ027960	DQ028030	DQ028075	DQ028138	DQ028229
	"*Leiognathus*" *nuchalis*	Sparks & Dunlap 2004	AY541658	AY541633	AB100028*	DQ028139	DQ028230
	"Leiognathus" pan	Ikejima et al. 2004	Unavailable	Unavailable	AB100018*	Unavailable	Unavailable
	"*Leiognathus*" *philippinus*	AMNH 122187	AY541660	AY541635	Unavailable	DQ028140	DQ028231
	"*Leiognathus*" *splendens* "Philippines LS1"	Sparks & Dunlap 2004	AY541656	AY541631	AB100020*	DQ028141	DQ028232

Table 1
Continued

Taxon	Voucher no./Source		16S	COI	ND5+	H3	28S
Leiognathidae	"*Leiognathus*" *splendens* "Philippines LS2"	Sparks & Dunlap 2004	AY541662	AY541637	AB100020*	DQ028142	DQ028233
	Photopectoralis sp. "Okinawa"	OKI-LB-1	DQ027961	DQ028031	Unavailable	DQ028143	DQ028234
	Photopectoralis sp. "Taiwan"	WLS 31 Tai	DQ027962	DQ028032	Unavailable	DQ028144	DQ028235
	Photopectoralis aureus	AMNH 122186	AY541650	AY541625	Unavailable	DQ028145	DQ028236
	Photopectoralis bindus	Sparks & Dunlap 2004	AY541651	AY541626	Unavailable	DQ028146	DQ028237
	Photopectoralis panayensis	AMNH 122174	AY541659	AY541634	Unavailable	DQ028147	DQ028238
	Photoplagios sp. "Madagascar"	AMNH 119981	DQ027963	DQ028033	Unavailable	DQ028148	DQ028239
	Photoplagios elongatus	AMNH 122175	AY541652	AY541627	AB100016*	DQ028149	DQ028240
	Photoplagios leuciscus "Madagascar"	AMNH 120336	DQ027964	DQ028034	Unavailable	DQ028150	DQ028241
	Photoplagios leuciscus "Okinawa"	OKI-LL-1	DQ027965	DQ028035	Unavailable	DQ028151	DQ028242
	Photoplagios leuciscus "Philippines"	AMNH 122173	AY541657	AY541632	Unavailable	DQ028152	DQ028243
	Photoplagios lineolatus	LEI 16 SL	DQ027966	DQ028036	Unavailable	DQ028153	DQ028244
	Photoplagios rivulatus	Sparks & Dunlap 2004	AY541661	AY541636	AB100019*	DQ028154	DQ028245
	Photoplagios stercorarius	AMNH 122172	AY541663	AY541638	AB100021*	DQ028155	DQ028246
	Secutor sp. "Madagascar L26"	AMNH 119980	DQ027967	DQ028037	Unavailable	DQ028156	DQ028247
	Secutor sp. "Madagascar L37"	AMNH 120194	DQ027968	DQ028038	Unavailable	DQ028157	DQ028248
	Secutor sp. "Philippines"	WLS 757 Phil	DQ027969	DQ028039	Unavailable	DQ028158	DQ028249
	Secutor hanedai	Ikejima et al. 2004	Unavailable	Unavailable	AB100022*	Unavailable	Unavailable
	Secutor indicius "Philippines"	Sparks & Dunlap 2004	AY541665	AY541640	AB100023*	DQ028159	DQ028250
	Secutor indicius "Sri Lanka"	LEI 12 SL	DQ027970	DQ028040	AB100023*	DQ028160	DQ028251
	Secutor cf. *insidiator*	WLS 32 Tai	DQ027971	DQ028041	Unavailable	DQ028161	DQ028252
	Secutor megalolepis "Australia"	WI-02-11	DQ027972	DQ028042	DQ028076	DQ028162	DQ028253
	Secutor megalolepis "Philippines"	Sparks & Dunlap 2004	AY541666	AY541641	AB100024*	DQ028163	DQ028254
	Secutor cf. *ruconius*	LEI 14 SL	DQ027973	DQ028043	Unavailable	DQ028164	DQ028255

*Denotes taxa that include ND4 and tRNAs $^{His,\ Ser,\ and\ Leu}$ with ND5 fragment.

Table 2
Morphological character matrix of internal and external features of the leiognathid light-organ system (LOS). Inapplicable characters are designated by (–)

	Characters														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Outgroups	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Clade I: *Leiognathus fasciatus* complex	1	0	0	0	0	0	0	0	–	–	0	0	0	0	0
Clade J: *Leiognathus equulus* complex	1	0	0	0	0	0	0	0	–	–	0	0	0	0	0
Clade K: *Leiognathus* sp. "Sri Lanka"	1	?	0	0	0	0	0	0	–	–	0	0	0	0	0
Clade L: *Photoplagios*	1	1	1	0	0	1	0	1	0	–	0	0	0	0	0
Clade M: *Photoplagios stercorarius*	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0
Clade M: *Photoplagios lineolatus* & *P.* sp. "Madagascar"	1	1	1	0	0	1	0	1	1	0	0	0	0	0	0
Clade E: *Photopectoralis*	1	1	0	1	1	0	1	0	–	–	0	0	1	1	0
Clade D: *Secutor*	1	1	0	0	1	0	0	0	–	–	1	0	1	1	1
Clade F: *Gazza*	1	1	0	0	1	0	0	0	–	–	0	1	1	1	0
Clade G: "*Leiognathus*"	1	1	0	0	0	0	0	0	–	–	0	0	0	0	0
Clade H: "*Leiognathus*"	1	1	0	0	0	0	0	0	–	–	0	0	0	0	0

(2004a,b). To amplify and sequence the *16S* fragment, the primers 16S ar-L 5′-CGCCTGTTTATCAAAAAC-AT-3′ and 16S br-H 5′-CCGGTCTGAACTCAGATC-ACGT-3′ (Kocher et al., 1989; Palumbi, 1996) were used. To amplify and sequence the *ND5* fragment, the primers ND5PercA – L 5′-GGYTGATGATACGGNC-GAGCAGA-3′, ND5PercB – H 5′-AGGGCTCAGGC-GTTNAGGTG-3′, ND5AthA – L 5′-CTCCACCCTT-GACTACCTTCC-3′, and ND5AthB – H 5′-GGTGA-GATGTGTTDAGTGCTTCA-3′ (Sparks and Smith, 2004a) were used. To amplify and sequence the *cytochrome c oxidase subunit I* (*COI*) fragment, the primers LCO1490 5′-GGTCAACAAATCATAAAGATATTG-G-3′ and HCO2198 5′-TAAACTTCAGGGTGACCA-AAAAATCA-3′ (Folmer et al., 1994) or Pros1Fwd 5′- TTCTCGACTAATCACAAAGACATYGG-3′ and Pros2Rev 5′-TCAAARAAGGTTGTGTTAGGTTYC-3′ (P. Chakrabarty, pers. comm.) were used. To amplify and sequence the *histone H3* fragment, the primers H3-L 5′-ATGGCTCGTACCAAGCAGACVGC-3′ and H3-H 5′-ATATCCTTRGGCATRATRGTGAC-3′ (Colgan et al., 1998) were used. To amplify and sequence the *28S* fragment, the primers 28SV 5′-AAGGTAGCCAAAT-GCCTCGTCATC-3′ and 28SJJ 5′-AGGTTAGTTTT-ACCCTACT-3′ (Hillis and Dixon, 1991) were used.

The double-stranded amplification products were either desalted and concentrated using Qiagen QuickSpin PCR Purification Columns, using AMPure (Agencourt Biosciences Corporation), or isolated on 1% agarose gels, excised under UV light, and extracted using a Qiagen Gel Extraction Kit. Both strands of the purified PCR fragments were used as templates and directly cycle-sequenced using the original amplification primers and an ABI Prism Dye Terminator Reaction Kit (version 1.1). The sequencing reactions were cleaned and desalted using standard isopropanol-ethanol precipitation or using cleanSEQ (Agencourt Biosciences Corporation). The sequencing reactions were electrophoresed on ABI 377, ABI 3700, or ABI 3730xl automated DNA sequencers. Contigs were built in Sequencher version 4.1 (Gene Codes) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Sequencher and Bioedit (Hall, 1999). All novel sequences are deposited in GenBank under accession numbers DQ027905–DQ028255 (Table 1).

Morphological analysis

Morphological features include both internal and external features of the leiognathid LOS (Table 2, Appendix 2) that were examined for all taxa for which nucleotide sequence data were collected. Whenever possible, multiple males and females of each included species were dissected and examined for all of the included LOS features. A number of additional leiognathid species, for which tissue samples could not be obtained for inclusion in the simultaneous analysis, were also examined for these LOS features (Appendix 1). The placement of these additional taxa is discussed in the text. Numbering of characters (Appendix 2) corresponds to that presented in the morphological character matrix (Table 2). A parsimony analysis of the 15 features of the LOS that were coded was conducted using NONA (Goloboff, 1998) and PAUP* (Swofford, 2002). Consistency indices (CI, Kluge and Farris, 1969) follow the individual character descriptions, and indicate the fit of the character on both the cladogram generated using only DNA sequence data, and that based on the simultaneous analysis of morphological and nucleotide characters. Patterns of character evolution were examined using NONA in conjunction with WinClada. Unambiguous morphological transformations common to all most-parsimonious dichotomized trees were used to diagnose clades (Goloboff, 1995).

Specimens used in comparative morphological analyses are deposited at the following institutions: American Museum of Natural History, New York (AMNH); Australian Museum, Sydney (AMS); Natural History

Museum, London (BMNH); California Academy of Sciences, San Francisco (CAS); Faculty of Fisheries, Fisheries Research Laboratory, Mie University, Japan (FRLM); Los Angeles County Museum of Natural History (LACM); Museum National d'Histoire Naturelle, Paris (MNHN); Scripps Institution of Oceanography, Marine Vertebrates Collection, La Jolla (SIO); University of Michigan, Museum of Zoology, Ann Arbor (UMMZ); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). Institutional abbreviations follow Leviton et al. (1985).

Phylogeny reconstruction

For the phylogenetic analysis, 6160 nucleotide characters [based on the implied alignment (Wheeler, 2003b)] from the nine gene fragments and 15 morphological transformations, were simultaneously analyzed under the optimality criterion of parsimony with all transformations given equal weight. Because we were not able to obtain tissue samples for *Leiognathus pan*, *Secutor hanedai* and *Gazza dentex,* we were unable to amplify the *16S*, *COI*, *28S* and *histone H3* genes for these three taxa, although they are included based on GenBank sequences for *ND4*, *ND5*, *tRNA-His*, *tRNA-Ser* and *tRNA-Leu*. Additionally, we were unable to successfully amplify or sequence particular gene fragments for some of the included taxa. Missing gene fragments are designated as "unavailable" in Table 1. Base positions corresponding to missing gene fragments are treated as missing data in the parsimony analysis.

The parsimony analysis was conducted using direct optimization (Wheeler, 1996) as implemented in the program POY (Wheeler et al., 2003), and run on the American Museum of Natural History Parallel Computing Cluster with default settings unless noted otherwise below. The method of direct optimization was used to avoid the potential biases inherent in standard sequence alignment procedures (e.g., manual alignment), which may not necessarily result in the most-parsimonious topology due to a potentially suboptimal static input alignment (Slowinski, 1998; Wheeler, 2001). Unlike standard multiple sequence alignment, which is divorced from the search for optimal tree topologies, direct optimization combines alignment and tree-search into a single procedure (i.e., nucleotide homology is dynamic) to produce globally most-parsimonious trees.

The analysis began by generating 10 random addition sequences (RAS), which were improved by TBR branch swapping, tree fusing (Goloboff, 1999; specifying fuselimit 2000 and fusemingroup 3), and 20 rounds of ratcheting (Nixon, 1999; specifying ratchettbr, ratchetseverity 4, and ratchetpercent 35). This procedure was repeated 40 times for a total of 400 RAS with extensive tree searching. All of the unique optimal trees resulting from these 40 replicates were submitted as starting points to POY for an additional round of TBR branch swapping, tree fusing (specifying fusemingroup 3), and 50 rounds of ratcheting (specifying ratchettbr, ratchetseverity 4, and ratchetpercent 35). This suite of analyses resulted in 12 equally most-parsimonious trees with lengths of 20 148 steps. These 12 trees were submitted to POY for further tree searching [specifying iterative pass (Wheeler, 2003a) and exact (Wheeler et al., 2003), which reduce the heuristics in nucleotide optimization], including TBR branch swapping, tree fusing (specifying fuselimit 1000 and fusemingroup 3), and ratcheting (specifying ratchettbr).

The length of the resulting implied alignment (Wheeler, 2003b) was verified in NONA (Goloboff, 1998) and PAUP* (Swofford, 2002). To estimate the "robustness" of the recovered phylogenetic hypotheses, Bremer supports (Bremer, 1988, 1995) were calculated using Tree Rot (Sorenson, 1999) in conjunction with PAUP*, and jackknife resampling analyses were performed using NONA (500 replications, heuristic searches, 10 random additions per replication) via the WinClada interface (Nixon, 2000). Patterns of character evolution on the recovered topology were examined using NONA in conjunction with WinClada (see *Morphological analysis*).

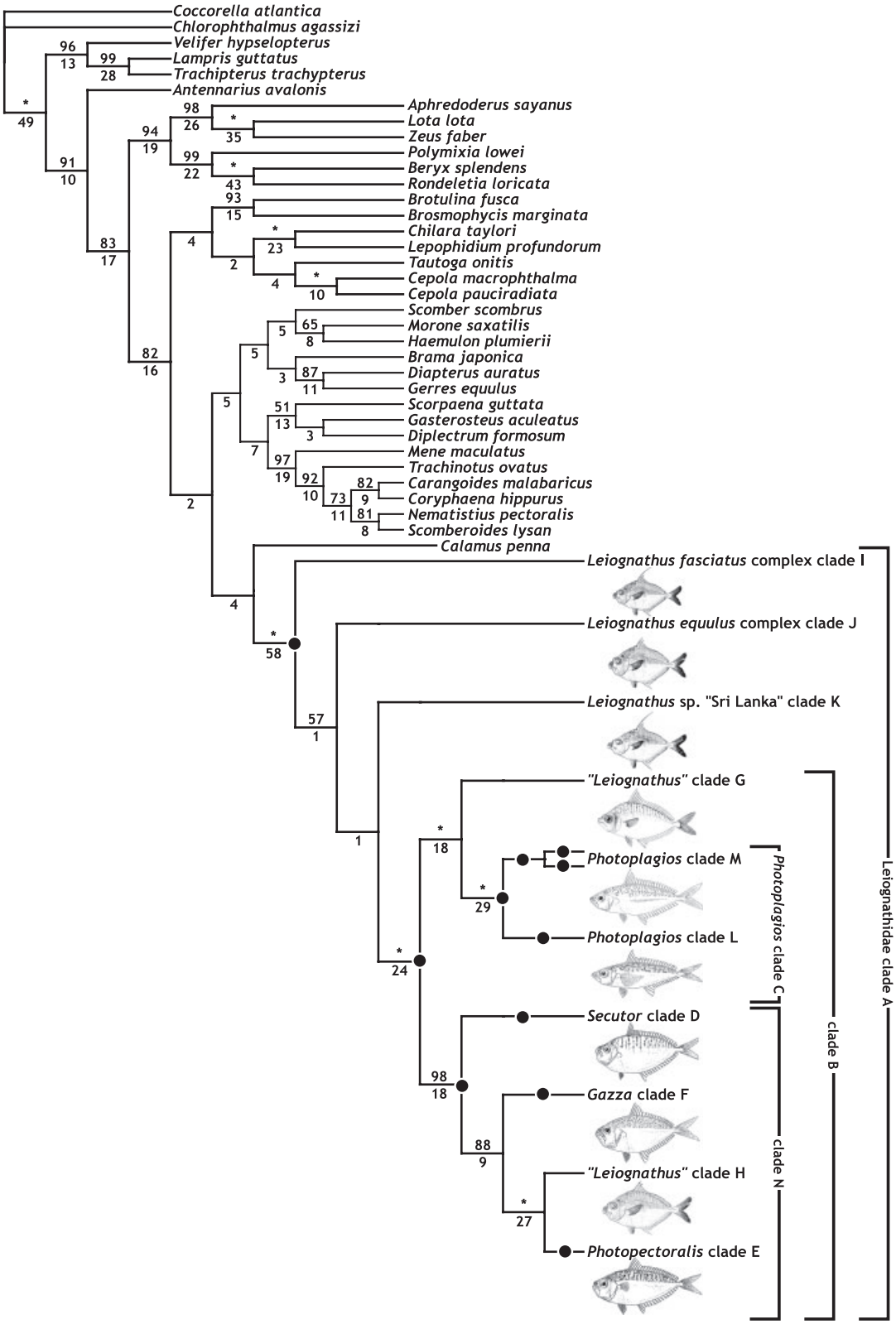
Results

Phylogenetic analysis

Simultaneous analysis of the nucleotide and morphological transformations resulted in nine equally most-parsimonious trees with lengths of 20 078 steps [CI: 0.30 and retention indices (RI, Farris, 1989): 0.55 (when uninformative characters are retained)]. A strict consensus topology of these nine optimal trees, collapsed to the level of major leiognathid clades for clarity, is presented in Fig. 1. Identical relationships are hypothesized using

Fig. 1. Strict consensus of nine equally most-parsimonious trees (length 20 078, CI = 0.30, RI = 0.55) recovered, based on the simultaneous analysis of 6160 mitochondrial and nuclear nucleotide characters and 15 morphological transformations, depicting the relationships of the major leiognathid clades. Species-level relationships for Leiognathidae are presented in Fig. 2. Solid black circles designate nodes that are supported by the following unique LOS features (character numbers correspond to the morphological transformations listed in Table 2 and Appendix 2; character number is followed by state in parentheses): Clade A: 1(1); Clade B: 2(1); Clade C: 3(1), 6(1), 8(1); Clade D: 11(1), 15(1); Clade E: 4(1), 7(1); Clade F: 12(1); Clade L: 9(0); Clade M: 9(1); *Photoplagios lineolatus* + *P.* sp. "Madagascar": 10(0); Clade N: 5(1), 13(1), 14(1). Numbers above branches represent Bremer support and numbers below branches jackknife resampling percentages (> 50%). Nodes with jackknife support of 100% are indicated by an asterisk (*).

the molecular data alone, albeit with a cost of 20 060 steps. Results of the simultaneous analysis are presented at the species level for Leiognathidae in Fig. 2. In this reconstruction, Leiognathidae (clade A) is monophyletic with strong support. Within Leiognathidae several major clades are recovered and strongly supported:



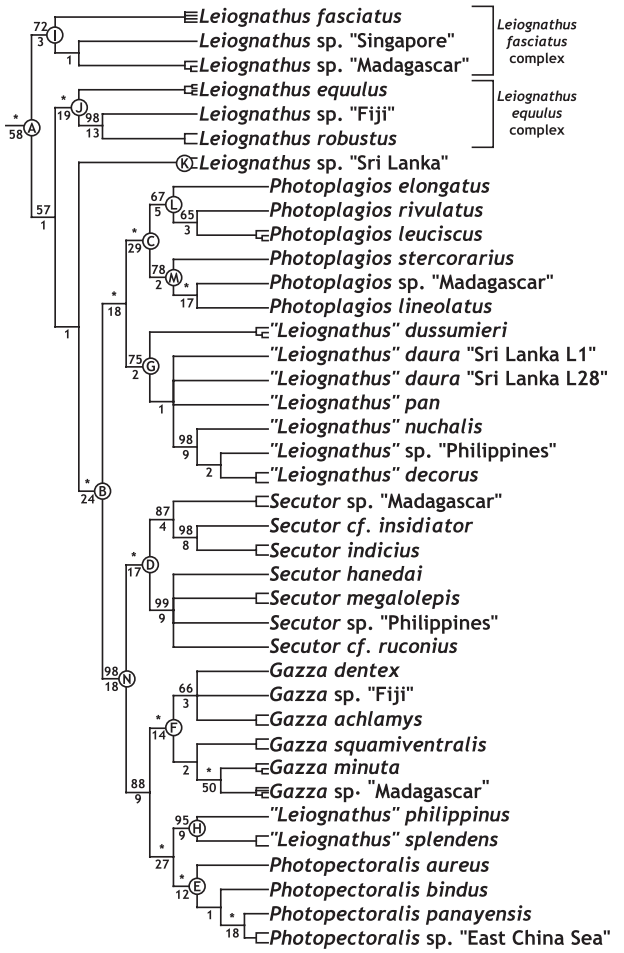


Fig. 2. Species-level cladogram depicting relationships within Leiognathidae (phylogeny expanded from Fig. 1). Letters at nodes correspond to clades discussed in the text and presented in Fig. 1. Branching within terminals indicates that multiple intraspecific populations were sampled for particular ponyfish species. Numbers above branches represent Bremer support and numbers below branches denote jackknife resampling percentages (> 50%). Nodes with jackknife support of 100% are indicated by an asterisk (*).

clade B comprises all members of *Leiognathus* together with all members of *Gazza* and *Secutor* that exhibit internal sexual dimorphism, in terms of volume and/or shape, of the circumesophageal light organ. *Gazza* (clade F) and *Secutor* (clade D) each are monophyletic, although they are not sister taxa. These relationships render the genus *Leiognathus* paraphyletic, with the generic name currently applied to three ''basal'' lineages (clades I, J and K), the former two which do not appear to be internally or externally sexually dimorphic with respect to features of the LOS (insufficient material is available for clade K; see below), as well as two clades (G and H) nested within the sexually dimorphic clade (clade B), which exhibit only internal sexual dimorphism of the LOS.

In this reconstruction, sparids (porgies) were recovered as the sister group to leiognathids, however, Bremer support for this clade is not strong. In taxonomically more comprehensive studies of acanthomorph relationships, cepolids (bandfishes) are recovered as the sister group to leiognathids (Smith and Wheeler, unpubl. data). In the current study, the sister group to the leiognathid-sparid clade is a large assemblage comprising groups [i.e., carangoids (jacks and allies), gerreids (mojarras), and menids (moonfishes)] traditionally hypothesized to be close relatives of ponyfishes, as well as a number of other percomorph lineages that have not previously been hypothesized as closely related to leiognathids.

The hypothesis of relationships based on internal and external features of the leiognathid LOS is less resolved than the phylogeny generated using only nucleotide characters or by simultaneous analysis of both data sets, due to the large number of morphological matrix entries for which it was necessary to code as inapplicable. However, the resulting morphological tree is entirely congruent/consistent with that generated using nucleotide characters (or a combination of nucleotide characters and these 15 LOS features) (Fig. 1). Furthermore, 12 of the 15 morphological characters optimized on the simultaneous analysis topology are recovered as uniquely derived with no homoplasy; the remaining three characters are hypothesized to have a single reversal (characters 5, 13 and 14).

The evolution of internal and external features of the LOS was examined by optimizing the 15 morphological transformations on the strict consensus topology (Fig. 1). Solid black circles in Fig. 1 designate clades supported by apomorphic or unique features (i.e., character states for which polarity cannot be established) of the LOS. These LOS features are listed (by number) for each clade in the figure legend and are discussed below. Figure 3 is a schematic illustrating the internal anatomy of a generalized leiognathid, and the various derived light-organ morphologies characteristic of males belonging to the clades recovered in Figs 1 and 2. The morphological character matrix is presented in Table 2; morphological character descriptions and the corresponding distributions of plesiomorphic and derived states are presented in Appendix 2. Figures 4–8 illustrate derived internal and external features of the leiognathid LOS in males corresponding to the major lateral luminescence morphologies identified, and in particular the relationship of sexually dimorphic internal LOS structures to the external male species-specific transparent opercular patches, flank patches, or midlateral stripes.

Systematic accounts

Photoplagios, new genus

Diagnosis: Males of *Photoplagios* are distinguished from all other members of Leiognathidae by the

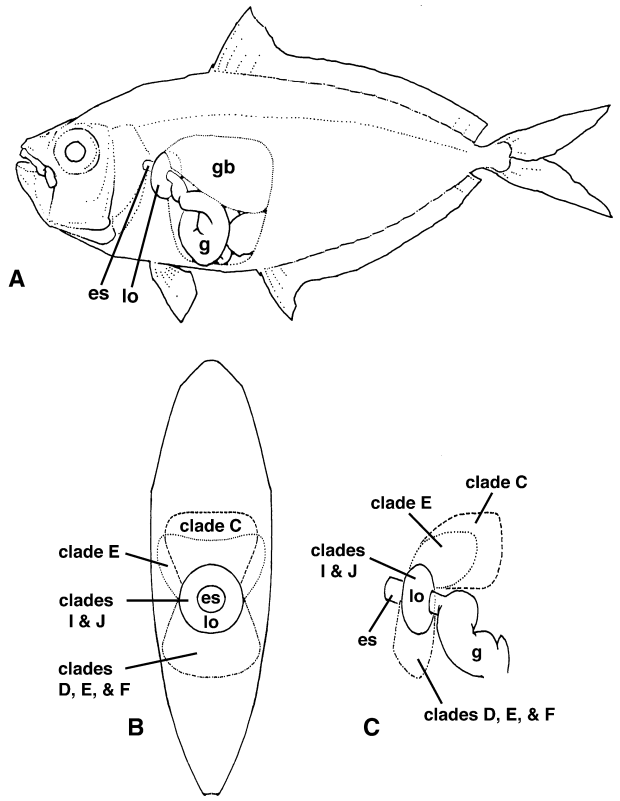


Fig. 3. (A) Schematic illustrating the relationship of the light organ, gastrointestinal tract, and the gas bladder in a representative adult leiognathid. (B) Cross-sectional view at the level of the light organ, and (C) lateral view comparing the shape and development of the non-dimorphic (solid outline) light organ characteristic of males of clades I and J, versus range of morphological variation exhibited by sexually dimorphic light organs (stippled outlines) characteristic of males belonging to clades C, D, E, and F. See Appendix 2 for descriptions of character states. Abbreviations: es = esophagus; g = gastrointestinal tract; gb = gas bladder; lo = circumesophageal light organ.

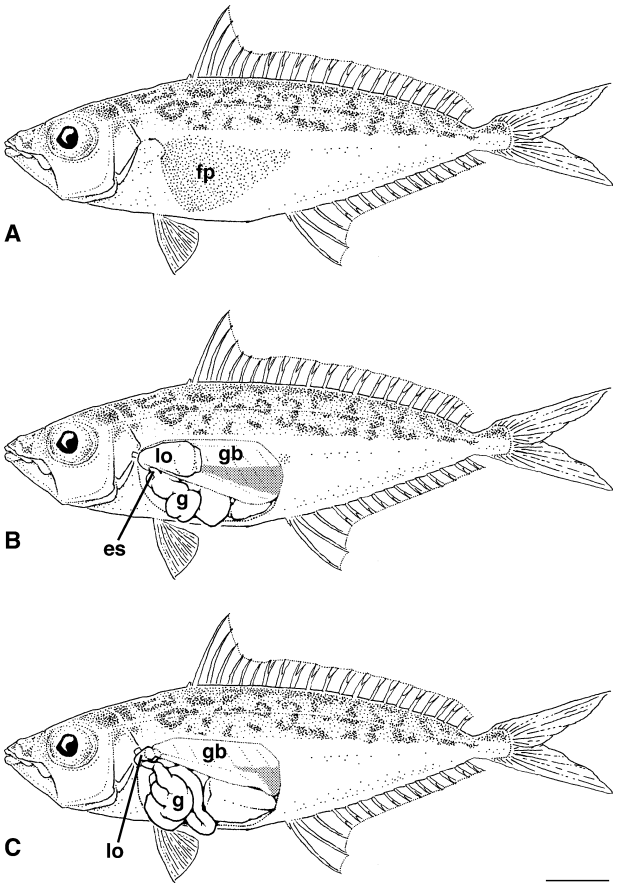


Fig. 4. *Photoplagios elongatus*: (A) external and (B) internal LOS anatomy of an adult male illustrating the relationship of the light organ, which bears hypertrophied dorsolateral lobes that extend posteriorly well into the gas bladder, clear gas bladder lining in this region, and associated transparent external flank patch characteristic of males belonging to clade C; (C) adult female for comparison. Abbreviations (Figs 4–8): aw = anterior light-organ window; es = esophagus; fp = transparent flank patch; g = gastrointestinal tract; gb = gas bladder; gp = transparent gular patch; lo = circumesophageal light organ; mls = transparent mid-lateral stripe; ocp = transparent opercular cavity patch; pap = transparent pectoral-axil patch; sc = silvery (guanine-lined) anteroventrally directed chamber. Stippling scheme (Figs 4–8): internal anatomy—finely stippled regions within the gas bladder indicate clearing of the silvery reflective lining; external anatomy—labeled stippled regions indicate transparent lateral flank patches, opercular cavity patches, or mid-lateral stripes. Pectoral fins omitted to simplify visualization of internal and external structures. Scale bar = 10 mm.

presence of an expansive, translucent lateral flank patch or stripe, dorsolateral lobes of the light organ that are hypertrophied and extend posteriorly into the gas bladder (extensively in members of clade L, less *P. leuciscus*, and only slightly in members of clade M), and lateral clearing of the silvery lining of the gas bladder.

Type species: Photoplagios elongatus (Günther, 1874).

Included species: Photoplagios lineolatus (Valenciennes, in Cuvier and Valenciennes, 1835), *P. leuciscus* (Günther, 1860), *P. moretoniensis* (Ogilby, 1912), *P. rivulatus* (Temminck & Schlegel, 1845), and *P. stercorarius* (Evermann & Seale, 1907), plus an undescribed species from Madagascar, *P.* sp. ''Madagascar''.

Additional remarks: The dorsal light-organ lobes of males of clade M are somewhat enlarged and extend slightly (at least in some specimens of *P. stercorarius* and *P.* sp. ''Madagascar'') into the gas bladder (interior of the lining). The condition is about the same as we observe in *P. leuciscus*. Males of the remaining members of *Photoplagios* (viz., *P. elongatus* and *P. rivulatus*) have enormous dorsal light-organ lobes that extend posteriorly well into the gas bladder (Fig. 4B).

A tissue sample suitable for molecular studies was lacking for *Leiognathus klunzingeri*; therefore this species could not be included in the phylogenetic analyses. Based on external morphology and detailed light organ comparisons, however, we tentatively also place *L. klunzingeri* in this new genus. The light organ of *Photoplagios* (*Leiognathus*) *klunzingeri* is nearly indistinguishable

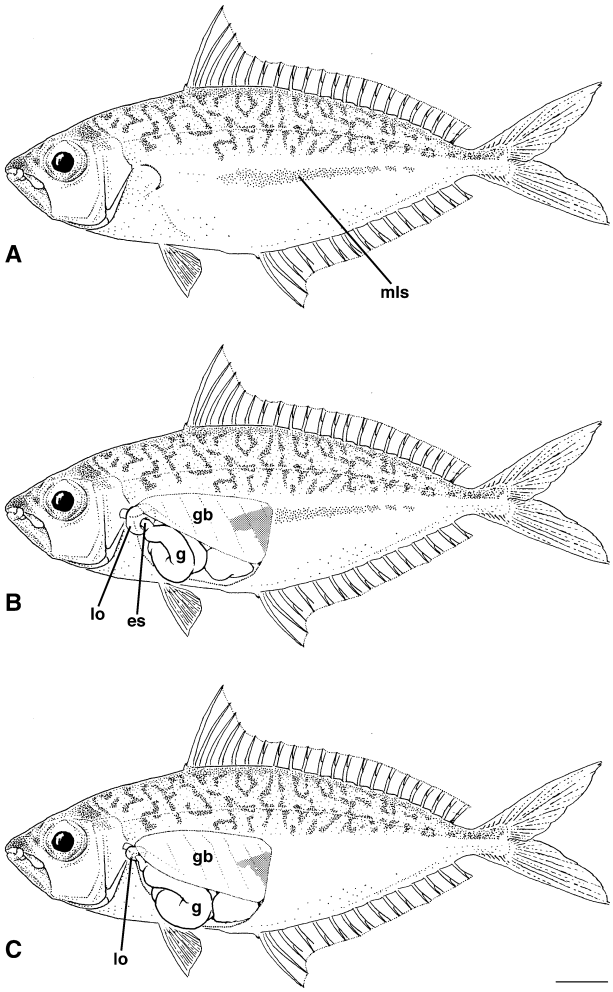


Fig. 5. *Photoplagios stercorarius*: (A) external and (B) internal LOS anatomy of an adult male illustrating the enlarged light organ and lateral clearing of the silvery gas bladder lining just internal to the horizontal series of transparent, overlapping mid-lateral "windows" (i.e., mid-lateral stripe) characteristic of males belonging to clade M; (C) adult female for comparison. Scale bar = 10 mm.

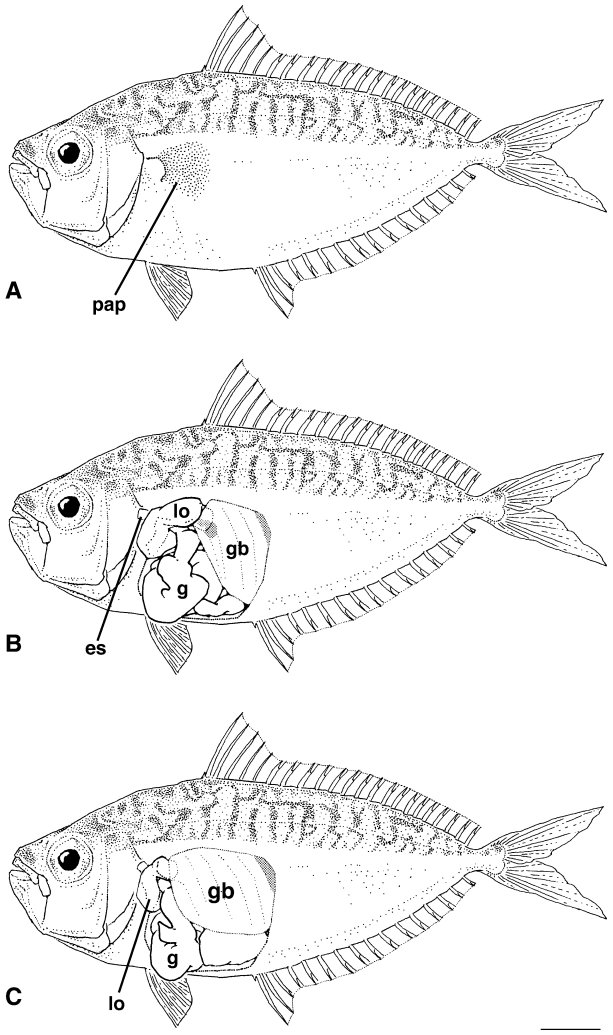


Fig. 6. *Photopectoralis aureus*: (A) external and (B) internal LOS anatomy of an adult male illustrating the hypertrophied and laterally expanded dorsolateral light-organ lobes, which abut the transparent pectoral-axil patch, characteristic of males of clade E; (C) adult female for comparison. Scale bar = 10 mm.

from that of *P. leuciscus* in terms of size, shape, sexual dimorphism, and pigmentation pattern (i.e., highly speckled). Externally, *P. klunzingeri* also closely resembles *P. leuciscus*. Unfortunately, the silvery, guanine layer is lost in the *P. klunzingeri* material available to us and we are unable to determine whether a translucent flank patch (or stripe) is present in males and if there is any lateral clearing of the silvery, reflective gas bladder lining.

Etymology: The generic name refers to the lateral flank luminescence produced by males of this clade [*photos* (Greek) = light and *plagios* (Greek) = flank or side]. Gender masculine.

Photopectoralis, new genus

Diagnosis: Males of *Photopectoralis* are distinguished from all other members of Leiognathidae by the presence of a translucent patch located in the pectoral-fin axil, and greatly enlarged dorsolateral lobes of the light organ that abut this pectoral-axil window.

Type species: Photopectoralis aureus (Abe and Haneda, 1972).

Included species: Photopectoralis bindus (Valenciennes, in Cuvier and Valenciennes, 1835), *P. panayensis* (Kimura and Dunlap, in Kimura et al., 2003), *P. hataii* (Abe and Haneda, 1972), and an undescribed species from the East China Sea, *P.* sp. "Okinawa/Taiwan".

Etymology: The generic name refers to the pectoral-axil luminescence produced by males of this clade (Greek, *photos* = light and *pectoralis* = pectoral region or chest). Gender masculine.

The diagnoses of these two new genera (clades C and E), which are sexually dimorphic for both internal and

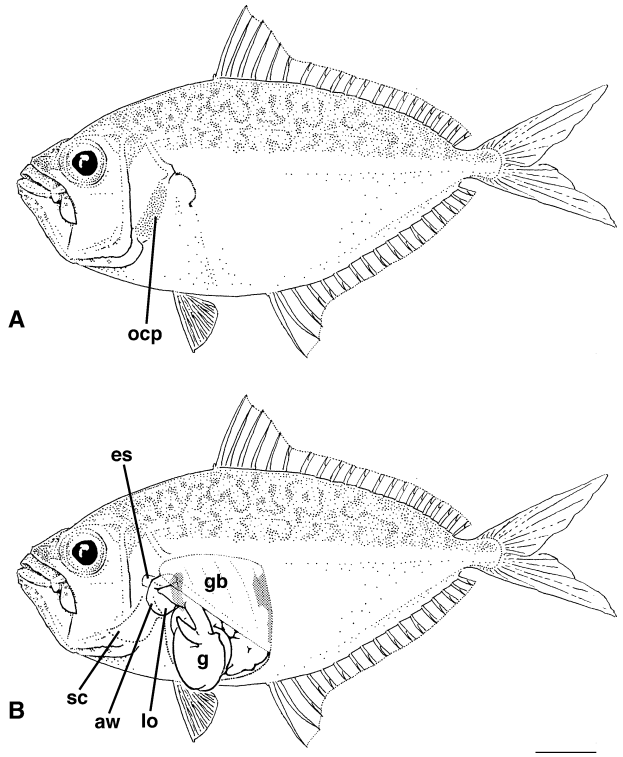


Fig. 7. *Gazza minuta*: (A) external and (B) internal LOS anatomy of an adult male belonging to clade F illustrating the closely apposed ventrolateral light-organ lobes, which bear large anterolaterally directed windows, and transparent opercular cavity patches. Males of clades D (*Gazza*), F (*Secutor*), and E (*Photopectoralis*) also exhibit hypertrophied ventrolateral light-organ lobes and associated silvery, guanine-lined reflective chambers (sc) that facilitate transmission of bacterial luminescence to enlarged transparent opercular cavity patches and/or the buccal cavity. Scale bar = 10 mm.

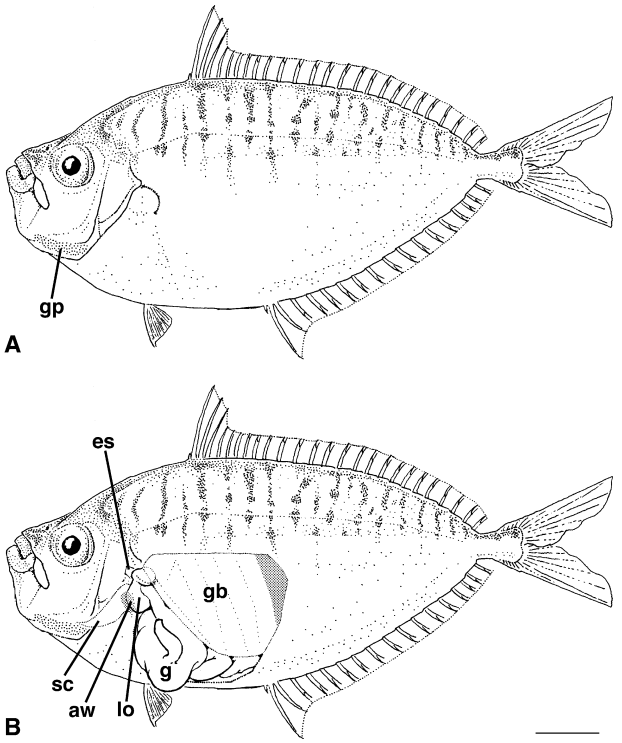


Fig. 8. *Secutor indicius*: (A) external and (B) internal LOS anatomy of an adult male belonging to clade D illustrating the relationship of the hypertrophied ventrolateral lobes of the light organ, anterior light-organ window, associated guanine-lined reflective chamber, and distally situated transparent gular patch. Scale bar = 10 mm.

external features of the LOS, do not render the genus *Leiognathus* monophyletic. *Photoplagios* and *Photopectoralis* are, nevertheless, easily distinguished on the basis of apomorphic internal and external LOS features, which justifies their erection. Both the transparent lateral flank patches or stripes of *Photoplagios* (Figs 4 and 5), and the pectoral-axil patches of *Photopectoralis* (Fig. 6) are species-specific in terms of size, shape, and orientation. In addition, the light organs in both new genera exhibit species-specific modifications. Unfortunately, apomorphic morphological features, including those of the LOS, could not be identified for the sister clades to both of the new genera (clades G and H), which are currently not diagnosable and retain the generic name *Leiognathus*. As discussed by Sparks and Dunlap (2004), the generic name *Leiognathus* applies to a member of the *L. equulus* complex. The sexually dimorphic species remaining in *Leiognathus* are placed in double quotes in Figs 1 and 2 to signify the need for naming additional genera. We anticipate that with additional study, particularly finer-scale analyses focusing on the LOS and associated structures, we will be able to clarify the taxonomy of Leiognathidae and diagnose these remaining sexually dimorphic clades.

Non-sexually dimorphic ponyfishes (clades I, J, and K(?))

The light organ of members of clades I and J, regardless of sex, and K (see below), is a relatively simple, dorsoventrally compressed, doughnut-shaped structure surrounding the esophagus (Fig. 3). Clades I and J comprise members of *L. equulus*, *L. fasciatus* and *L. robustus*, as well as several potentially novel species, none of which appear to be sexually dimorphic with respect to features of the LOS. If features of the LOS are sexually dimorphic in these species, the degree of dimorphism is subtle; we are not able to characterize any variation if it exists. To date, we do not have sufficient specimens of *Leiognathus* n. sp. "Sri Lanka" (clade K), the sister group to clade B, to determine whether or not their LOS is sexually dimorphic (i.e., all three known specimens are immature females). Nevertheless, the light organ and associated LOS features in these three females comprising clade K are anatomically indistinguishable from those of clades I and J.

In most respects, members of clades I, J and K, all of which are large and deep bodied, are indistinguishable in terms of both internal and external morphology. These lineages are particularly important taxonomically given that provenance of the generic name *Leiognathus* remains uncertain (see Discussion in Sparks and Dunlap, 2004). Regrettably, apart from *L. robustus*, which was recently described from material obtained in Singapore (Sparks and Dunlap, 2004), type material for these non-dimorphic species has either been lost or was not preserved (e.g., *L. fasciatus*), or is in extremely poor condition [e.g., *L.* (*Scomber*) *equulus* and *L.* (*Scomber*) *edentulus* dried partial specimens], which precludes comparative studies of their LOS (Sparks and Dunlap, 2004).

The specimens referred to as *L. fasciatus* by Sparks and Dunlap (2004: Fig. 2), recovered as a member of clade G in that study and reported to be internally sexually dimorphic, were mistakenly identified. That terminal, recovered as a member of clade H in the current study, is now determined to represent an undescribed species, "*L.*" sp. "Philippines" (Fig. 1). Our re-identification is based on the examination of, and comparison with, much additional material, including several large, mature adults, that we believe more closely match the original (albeit rudimentary) description of *L. fasciatus* (Lacepède, 1803). Based on these observations, we conclude that *L. fasciatus* is not sexually dimorphic with respect to internal or external features of the LOS. We note however, that no type specimens are known for *L. fasciatus*, the description of which Lacepède apparently based on a manuscript and illustration by Commerson describing material collected in Mauritius (Eschmeyer, 2005). Necessarily, our identifications are based on the comparison of preserved specimens to the original description of *L. fasciatus* (Lacepède, 1803), and rely heavily on the presence of an elongate dorsal (but not corresponding anal) spine and spotting below the lateral midline. Clearly, the absence of type material is problematic; nevertheless, our Malagasy specimen, which of the material available to us was collected closest to the putative type locality (Mauritius) of *L. fasciatus*, matches Lacepède's (1803) original description of this species well.

We hypothesize that *Leiognathus longispinis* (= *L. smithursti*) is the sister taxon to clade I (= "*L. fasciatus* complex") based on morphological comparisons to our putative *L. fasciatus* material and the other members of the "*L. fasciatus* complex". These two lineages, *L. longispinis* and the "*L. fasciatus* complex", are deep bodied, they exhibit similar lateral pigmentation patterns, and both possess an elongate second dorsal-fin spine (*L. longispinis* uniquely possesses a markedly elongate second anal-fin spine).

Our phylogenetic results indicate that a great deal of ponyfish diversity has gone unnoticed, particularly among the non-sexually dimorphic lineages (clades I and J), and that the status of putatively widespread species, including *Leiognathus equulus* and *L. fasciatus*, needs to be re-examined in this context. Although we have examined the LOS of all lineages comprising these three clades for evidence of sexual dimorphism, we note that specimen availability was limited for some taxa. Therefore, the possibility exists that subtle sex-specific differences (e.g., light organ volume) in these systems may have been missed. Clearly, none of these lineages exhibit the striking degree of sexual dimorphism observed in members of clade B; however, we believe further study is needed to rule out the possibility of slight sexual dissimilarities.

Sexually dimorphic ponyfishes (clade B)

A distinctive grouping of sexually dimorphic species within clade B based only on morphology of the LOS was observed; this grouping matched the pattern of relationships recovered in the simultaneous analysis of nucleotide characters and morphological features (Figs 1 and 2). In all species of clade B, the light organ is sexually dimorphic and greater in volume in males than conspecific females (character 2).

Photoplagios (clade C): In males of clade C, the dorsolateral lobes of the light organ are hypertrophied and extend posteriorly into the gas bladder, interior of the gas bladder lining (character 3; Fig. 3 [clade C] and 4B, C) (McFall-Ngai and Dunlap, 1984; this study). In *P. elongatus* and *P. rivulatus*, the dorsolateral light-organ lobes of males are greatly enlarged and extend well into the gas bladder (Fig. 4B). *Photoplagios leuciscus*, *P. stercorarius*, and *P.* sp. "Madagascar" males exhibit moderately enlarged dorsolateral lobes that extend only slightly into the gas bladder (Fig. 5B). We currently lack sufficient comparative material that can be reliably identified as *P. lineolatus* to make a conclusive statement regarding its LOS morphology. Based on the phylogenetic placement of *P. lineolatus* and the configuration of the LOS in its sister taxon, *P.* sp. "Madagascar", we hypothesize that *P. lineolatus* also shares the derived LOS features that characterize members of clade C.

Extensive lateral clearing of the silvery gas bladder lining (character 6) occurs only in males belonging to clade C. Externally, males of clade C also exhibit an associated transparent flank patch or stripe (character 8; Figs 4A, B and 5A, B), a modification that has been shown to permit lateral luminescence (Sasaki et al., 2003). Males of clade L are characterized by large, transparent triangular flank patches (character 9; Fig. 4A), whereas males of clade M exhibit a range of mid-lateral stripe morphologies (character 9; Fig. 5A). The dark-blue flank stripe diagnostic of male *P. stercorarius*, for example, is not only composed of numerous closely spaced or overlapping oval windows

(character 10), but is transparent (Fig. 5A). Posterior clearing of the reflective lining of the gas bladder, characteristic of leiognathids, extends more anterolaterally in males than in females of *P. stercorarius* (Fig. 5B, C), though to a lesser extent than in other members of clade C, where lateral clearing in males may extend the length of the gas bladder (e.g., *P. elongatus*; Fig. 4B).

Although *Photoplagios moretoniensis* was not included in the simultaneous analysis due to the lack of a suitable tissue sample, we have been able to verify that the LOS of this elongate leiognathid is quite similar to that of *P. stercorarius*. Like *P. stercorarius*, males appear to possess a well-developed, transparent mid-lateral stripe composed of numerous, closely spaced windows (character 10). This stripe, which frequently appears dark in preservation due to a concentration of melanophores, extends the length of the flank in *P. moretoniensis*, whereas in *P. stercorarius* it is restricted posterior to a vertical through the dorsal-fin origin (Fig. 5A). Internally, the LOS of *P. moretoniensis* is also comparable in structure to *P. stercorarius*. The light organ of males is moderately enlarged, although, like *P. stercorarius*, extends at most slightly into the gas bladder. Moreover, lateral clearing of the silvery gas bladder lining in males is enhanced compared to females, but does not extend the length of the chamber as in some members of clade C. Based on these shared LOS morphologies and external features, including body shape and pigmentation pattern, we hypothesize that *P. moretoniensis* and *P. stercorarius* are sister taxa, justifying the placement of the former species in *Photoplagios*.

Members of the sister clade to *Photoplagios stercorarius* (+ *P. moretoniensis*), comprising *P. lineolatus* and *P.* sp. ''Madagascar'', also lack a large translucent flank patch, and instead possess a wide and presumably transparent mid-lateral flank stripe. We note, however, that we have limited material of both *P. lineolatus* and *P.* sp. ''Madagascar'' to examine, and that these specimens are not ideally preserved for detecting translucent external patches (i.e., the silvery, guanine layer is mostly to completely lost in preservation).

Based on the examination of type material, we also note that *P. leuciscus* Günther, 1860 is closely related to and possibly conspecific with *Leiognathus parviceps* Valenciennes, in Cuvier & Valenciennes, 1835. The syntype of *Photoplagios* (*Leiognathus*) *parviceps* (MNHN A-0580) we have to examine and the holotype of *Photoplagios leuciscus* (BMNH 1858.4.21.243) presumably are both females, and we can neither compare the size and shape of the translucent lateral flank patch (assuming one exists in males of *L. parviceps*), nor dissect the specimens for internal LOS comparisons or sex determination.

Photopectoralis (clade E): Members of clade E, *Photopectoralis aureus*, *P. bindus*, a recently described species, *P. panayensis* (Kimura et al., 2003), and an undescribed species, *P.* sp. ''East China Sea'', all exhibit volume and shape dimorphism of the light organ, with hypertrophy of the dorsolateral lobes in males (character 4), such that the lobes extend laterally, exterior of the gas bladder lining and abut a lateral clearing of the internal skin integument just posterior to the pectoral-fin axils and just internal to the male-specific external transparent pectoral-axil patches (character 7; Figs 3 [clade E] and 6A–C). The LOS of a morphologically similar fish, *P. hataii* (Kimura et al., 2003), fits that of the species comprising clade E, but tissue of this rare species was not available for sequencing. Compared to conspecific females, the ventral light-organ lobes are also enlarged and somewhat laterally expanded in males of clade E (see Discussion below; Fig. 6B, C).

Gazza (clade F) and Secutor (clade D): Males of *Secutor* and *Gazza* exhibit light-organ volume and shape dimorphism through hypertrophy of the ventrolateral lobes, as well as more expansive transparent patches on the margin of the opercular cavity than conspecific females (McFall-Ngai and Dunlap, 1983, 1984; this study) (Figs 3 [clades D and F], 7 and 8). These transparent opercular margin patches are located posteriorly proximal to the pectoral-fin base in *Gazza* (character 12; Fig. 7A) and anteriorly in the gular region in *Secutor* (character 11; Fig. 8A). An additional species of *Gazza*, *G. rhombea*, that could not be sequenced due to the lack of a suitable tissue sample, was examined and conforms well externally for these LOS features to other members of the genus that we included in both our morphological and molecular analyses. We were only able to examine specimens of the type series of *G. rhombea*, which could not be dissected to examine the LOS internally.

In male *Gazza*, *Secutor*, and *Photopectoralis* (clade N), the hypertrophied ventrolateral light-organ lobes are associated with several additional LOS modifications. Specifically, rostroventrally oriented windows in the enlarged contralateral ventral light-organ lobes are directed into a silvery, guanine-lined reflective chamber (characters 5, 13 and 14; Figs 6B, 7B and 8B), presumably allowing for light transmission and reflection to the enlarged opercular margin patches and buccal cavity in *Gazza* and *Secutor* (Fig. 7A and 8B) or to the buccal cavity in *Photopectoralis*, which lacks opercular patches (Fig. 6B). In *Gazza*, the enlarged ventrolateral lobes of males abut the transparent opercular cavity patches (Fig. 7A, B), whereas in *Secutor* the light organ is not directly associated with the considerably more rostrally placed transparent gular patches characteristic of this taxon (Fig. 8A, B). Although the light organ and transparent gular patch are not in close proximity in *Secutor*, the silvery reflective chamber described above extends rostrally along the opercular margin in this taxon, presumably functioning as a light tube to facilitate transmission and reflection of bacterial luminescence

directly from the light organ to the clear gular patch (character 15; Fig. 8B).

"Leiognathus" (clades G and H): In contrast to males of clades C, D, E and F, males of clade G, "*Leiognathus*" *daura*, "*L.*" *decorus*, "*L.*" *dussumieri*, "*L.*" *nuchalis*, "*L.*" *pan*, and an undescribed species, "*L.*" sp. "Philippines", and clade H, "*L.*" *philippinus* and "*L.*" *splendens*, apparently exhibit only volume dimorphism of the light organ; no discernable internal shape dimorphism or external dimorphism in the form of transparent opercular or flank patches was noted in these taxa (McFall-Ngai and Dunlap, 1984; Sparks and Dunlap, 2004; this study). We note, however, that volume dimorphism of the light organ can be significant in some members of these clades (e.g., "*L.*" *splendens*).

Discussion

Sexually dimorphic light organs, not simply the sex-specific arrangement of photophores on the body, are documented in a number of fishes other than leiognathids. For example, in stomiiforms and myctophids the light organs of males are often considerably enlarged compared to conspecific females (Nafpaktitis, 1966; Gibbs, 1969; Goodyear and Gibbs, 1969; Marshall, 1979; Herring and Widder, 2001), or light organs are present only in one of the sexes, as in most ceratioid anglerfishes, where the females possess photophores on very elaborate escae that presumably function as lures to attract both prey and males (Herring and Widder, 2001; Bertelsen and Pietsch, 2002).

Although the evolution of a sexually dimorphic bioluminescent system based on male species-specific signaling is well documented in fireflies (Lloyd, 1966), in vertebrates these systems, restricted to marine fishes, remain poorly understood and their function(s) the subject of much conjecture (see Buck, 1978; Herring, 1990, 2002 for reviews). This is in large part due to the difficulty inherent in studying and interpreting the behavior of marine fishes. Nevertheless, in addition to numerous observations that leiognathids possess the ability to emit light in rapid flashes from the opercular region, buccal cavity, and flanks, as well as the ventrum (Haneda, 1940; Hastings, 1971; Herring and Morin, 1978; McFall-Ngai and Dunlap, 1983; McFall-Ngai, 1991; Woodland et al., 2002; Sasaki et al., 2003), the degree of species-specific morphological specialization and strong sexual dimorphism of the light organ and associated structures of the LOS observed throughout the family, suggest a system of mate recognition based on male species-specific luminescent signaling.

A comparison of leiognathid clade B with the "basal" lineages (i.e., clades I and J, which encompass *L. equulus*, *L. fasciatus*, *L. robustus*, and potentially a number of undescribed species), revealed a distinct morphological dichotomy (Fig. 1). Members of clades I and J (and presumably also members of clade K; see Results) bear non-dimorphic light organs and exhibit no obvious dimorphism in associated tissues of the LOS, whereas all members of clade B exhibit sexual dimorphism of the light organ in terms of volume (i.e., male light organs are enlarged), and most members also exhibit dimorphism of the associated internal and external tissues of the LOS (e.g., clearing of the lateral silvery lining of the gas bladder, transparent flank and opercular patches, guanine-lined reflective structures) (Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1984; Kimura et al., 2003; Sparks and Dunlap, 2004; this study) (Figs 3–8). In the context of this phylogeny, we examine and discuss the evolution and diversification of the LOS in ponyfishes.

Patterns of LOS evolution

Given that the family contains both non-dimorphic and sexually dimorphic species, in which males exhibit highly variable and species-specific LOS morphologies, leiognathids provide an ideal system in which to examine the development and differentiation of a structurally complex and sexually dimorphic luminescent system in vertebrates. In the context of the recovered phylogeny, we can trace the evolution of the leiognathid LOS from a comparatively simple ring-like structure surrounding the esophagus (clades I and J), to a complex, highly modified, sexually dimorphic system (clade B), involving not only the light organ itself, but numerous associated structures that allow for the emission of light from the lateral surfaces of these fishes, either in the opercular region (Figs 7 and 8) or from the flanks (Figs 4–6), as well as the buccal cavity (Figs 7 and 8).

The pattern of relationships recovered in the simultaneous analysis of nucleotide and morphological characters indicates that *Gazza* and *Secutor* are each monophyletic, whereas *Leiognathus* is not (Fig. 1). These results are congruent with those reported in other recent, but less taxonomically comprehensive, phylogenetic studies of ponyfishes (Ikejima et al., 2004; Sparks and Dunlap, 2004). The optimization of LOS features on this topology reveals that the major patterns of LOS evolution are wholly congruent with the recovered phylogenetic pattern, and demonstrates the utility of LOS features for phylogeny reconstruction (and taxonomy) in a clade that otherwise exhibits little morphological variation (Fig. 1). The recovered phylogenetic pattern also reveals that a sexually dimorphic light organ evolved once in leiognathids from the non-dimorphic, plesiomorphic condition.

From the cladogram it can be seen that five distinct modes of lateral luminescence involving sexually dimorphic tissues associated with the LOS, and which exhibit

species-specific variation in structure, have evolved within Leiognathidae: (1) Via an expansive, yet single, transparent flank patch (clade L, Fig. 4A). In males exhibiting this derived external morphology, lateral luminescence is facilitated by dorsolateral lobes of the light organ that are hypertrophied and extend posteriorly into the gas bladder, such that they lie just internal to the lateral flank patch, and by clearing of the lateral silvery lining of the gas bladder in this region (Fig. 4A–C). (2) Via a series of closely spaced to overlapping "windows" arrayed along the lateral midline (clade M, viz., *Photoplagios stercorarius* and *P. moretoniensis*, Fig. 5A) or a mid-lateral stripe that is presumably transparent (clade M, viz., *P. lineolatus* and *P.* sp. "Madagascar"). In comparison to females, males of this clade exhibit more extensive lateral clearing of the gas bladder lining just internal to the transparent mid-lateral "windows" or stripe, as well as an enlarged light organ that may extend slightly into the gas bladder (Fig. 5A–C). (3) Via a transparent pectoral-axil patch (clade E, Fig. 6A). Lateral luminescence in males of clade E is facilitated by greatly enlarged dorsolateral lobes of the light organ that lie just internal to and abut the clear pectoral-axil patch (Fig. 6A–C). (4) Via transparent patches located on the margin of the opercular cavity anteriorly in the gular region (*Secutor*, clade D, Fig. 8A). (5) Via transparent patches located on the margin of the opercular cavity posteriorly proximal to the pectoral-fin base (*Gazza*, clade F, Fig. 7A). Within each clade of externally sexually dimorphic leiognathids, the size, shape, location, or orientation of the transparent external patches varies interspecifically.

Excluding LOS variation, leiognathids are extremely conservative anatomically, and reconstructing their interrelationships based on osteology, external morphology and meristics has been problematic (Jones, 1985; Woodland et al., 2001). Data collected to date demonstrate the utility of LOS features for recovering phylogeny, as well as for resolving taxonomic problems in leiognathids (Dunlap and McFall-Ngai, 1984; Sparks and Dunlap, 2004). The use of additional techniques such as electron microscopy, histology and high-resolution computed microtomography, has the potential to reveal additional phylogenetically informative features of the LOS. The pigmentation pattern of the light organ appears to be consistent intraspecifically, but varies interpsecifically, and may also provide a rich source of characters (unpubl. data).

Luminescent signaling and ponyfish diversification

All leiognathids possess the ability to emit light over the ventrum, presumably as a means of camouflage, through disruptive illumination, against bottom-dwelling piscivorous fishes; however, only some leiognathids (i.e., members of clades C, D, E and F) possess the structural modifications necessary for lateral luminescence. The species-richness of clade B relative to clades I, J and K, and the high proportion of species within clade B that also exhibit species-specific sexual dimorphism of associated tissues of the LOS in addition to the light organ itself, suggest strong sexual selection for species-specific lateral luminescence signaling in males (Figs 2 and 4–8).

The habitat of leiognathids: frequently murky, turbid bays and estuaries characterized by poor visibility, may also be correlated with LOS variability and specialization. It is common to find several species of leiognathids co-occurring within a relatively small area (P.V. Dunlap, pers. obs.). The morphological specializations documented for the LOS of male leiognathids suggest that species-specific variation in male flashing or signaling pattern, as well as the location (or possibly even the wavelength) of emitted light on males, may at least partly explain why a number of morphologically similar species are able to co-occur and maintain species fidelity in habitats with limited visibility.

Our observations of the external transparent patches in members of clade B suggest that emitted light is filtered in some species of ponyfishes. For example, in *Photoplagios stercorarius* the transparent mid-lateral stripe, composed of numerous closely spaced or overlapping rectangular or oval windows (Fig. 5A, mls), is frequently dark blue. It seems likely that this dark-blue pigment, which would absorb the blue-green luminescence from the light organ, acts to prevent the lateral emission of light at inappropriate times, and that the fish can decrease the absorptive quality of this pigment at times when lateral light emission would be appropriate.

Further evidence for a signaling function for the leiognathid LOS comes from field studies and observations made under controlled conditions. Numerous researchers have reported distinct discrete rapid flashes in a number of leiognathid species (Haneda, 1940; Hastings, 1971; Haneda and Tsuji, 1976; Herring and Morin, 1978; McFall-Ngai and Dunlap, 1983; McFall-Ngai, 1991; Sasaki et al., 2003; P.V. Dunlap, pers. obs.), even synchronized rhythmic flashing in schools (Woodland et al., 2002), a behavior that is inconsistent with a mechanism of predator avoidance via ventral counter-illumination against bottom-dwelling piscivorous fishes. As Hastings (1971) postulated, if the function of bioluminescent light is for camouflage to match background light intensity, it would be emitted as a continuous, diffuse glow over the ventrum ("not in flashes"), and would occur during daylight hours and crepuscular periods. However, in addition to a diffuse glow emitted over the entire ventrum, characterized by a slow onset and decay, discrete rapid flashes are reported from the anterolateral, lateroventral, opercular, and ventral regions of leiognathids, and are also reported to occur at night (Haneda and Tsuji, 1976; Herring and

Morin, 1978; McFall-Ngai and Dunlap, 1983; Woodland et al., 2002; Sasaki et al., 2003). In fact, McFall-Ngai and Dunlap (1983) documented no less than three (possibly four) modes of light emission by flashing (= rapid onset and decay of emitted light) for *Gazza minuta* alone. In light of this exceptional versatility in luminescent display exhibited by a single species, McFall-Ngai and Dunlap (1983) posited that the diversity of luminescent behaviors exhibited by leiognathids might be greater than those of any other organism studied to date. More recently, Sasaki et al. (2003) provided direct (field) evidence for luminescence signaling between male and female *Photoplagios elongatus*, with observed light emitted only from the clear flank patch of males.

Based on these results, and the extent and degree of taxon-specific sexual dimorphism observed throughout the family, we consider it unlikely that the leiognathid LOS functions principally for avoiding predators or attracting prey (Hastings, 1971; McFall-Ngai and Dunlap, 1983; McFall-Ngai and Morin, 1991). The male-specific modifications described here would appear to make the individuals possessing them far more conspicuous targets to predators (Andersson, 1994), and if this system had evolved entirely under selection pressure to avoid predators or facilitate prey capture, both sexes would be expected to exhibit similar LOS morphologies. In the absence of sexual selection, it is difficult to envision a plausible mechanism under which such pronounced and extensive sexual dimorphism could have evolved, or once evolved be maintained.

Female choice plays a critical role in ensuring species fidelity through reproductive isolation in numerous animal groups (Lloyd, 1966; Andersson, 1994; Seehausen et al., 1997). Species-specific signals frequently function to create prezygotic reproductive barriers among closely related, sympatric species. Our results establish a phylogenetic basis for reproductive isolation in leiognathid fishes based on LOS morphologies that are uniquely modified to facilitate male species-specific luminescence signaling from the flank, opercular region, or buccal cavity. Although a similar function has been proposed for other bioluminescent fishes (e.g., Morin et al., 1975; Buck, 1978; Herring and Morin, 1978; Nicol, 1978), here we adopt an explicitly phylogenetic approach to examine and document the evolution of a highly variable, sexually dimorphic bioluminescent system in a well circumscribed assemblage of nearshore marine fishes. We hypothesize that male species-specific luminescence signaling permits morphologically similar leiognathid species to coexist and maintain species-fidelity in habitats with markedly reduced visibility, and that reproductive isolation by luminescence signaling has therefore likely been instrumental in the diversification of this clade.

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Appendix 1

Material examined

Type specimens are listed first, followed alphabetically by museum abbreviation. The notation "(in part)" following some catalog numbers indicates that the alcoholic lot examined was found to contain more than a single species.

Leiognathidae:

Clade C: *Photoplagios*

Photoplagios elongatus: BMNH 1872.4.6.105, holotype; CAS 52602; LACM 42993-1; LACM 43584-1; SIO 83-55; USNM 55613; UMMZ 226771; UMMZ 240145; UMMZ uncat. (PVD 82-06/19a).

Photoplagios klunzingeri: AMNH 44488; AMNH 44491; AMNH 44493.

Photoplagios leuciscus: BMNH 1858.4.21.243, holotype; AMNH uncat. (Mad 25-2003, WLS 51, Leo 38); AMNH uncat. (Mad 25-2003); AMNH uncat. (Mad 25-2003, WLS 52); AMS I.22967001; AMS 22978004; AMS I.34365015; UMMZ 240125; UMMZ uncat. (PVD 02-01/30a); UMMZ uncat. (PVD 00-10/18 61); USNM 76609; USNM 191991; USNM 373280.

Photoplagios lineolatus: MNHN 1988-0327, paralectotype, 1 ex.

Photoplagios n. sp. "Madagascar": AMNH uncat. (Mad 29-2003, WLS 57, Leo 27).

Photoplagios moretoniensis: AMS I.21700001; AMS I.22983001.

Photoplagios (*Leiognathus*) *parviceps*: MNHN A-0580, syntype, 1 ex.

Photoplagios rivulatus: AMNH 34850; UMMZ 240144; UMMZ uncat. (PVD 82-06/19a).

Photoplagios stercorarius: USNM 55906, holotype; USNM 126395, cotype; CAS 42171, paratype; CAS 17678; CAS-SU 20004, paratype; UMMZ 240138; UMMZ uncat. (PVD 99-11/30a); UMMZ uncat. (PVD 02-03/11a); UMMZ 02-03/19 (29); UMMZ uncat. (PVD 03-04/07a); USNM 191996.

Clade D: *Secutor*

Secutor indicius: UMMZ 240127; UMMZ uncat. (PVD 02-03/11a).

Secutor insidiator: CAS 29894; UMMZ uncat.

Secutor megalolepis: UMMZ 240135.

Secutor ruconius: CAS-SU 29895; UMMZ 225240; UMMZ uncat.

Secutor n. sp. "Madagascar": AMNH 232550; AMNH uncat. (Mad 8-2003); AMNH uncat. (Mad 25-2003, WLS 52); AMNH uncat. (Mad 25-2003); AMNH uncat. (Mad 29-2003, WLS 57).

Clade E: *Photopectoralis*

Photopectoralis aureus: UMMZ 240129; UMMZ 240309; UMMZ uncat.; USNM 373277.

Photopectoralis bindus: AMS I.34367021; CAS 51097; UMMZ 240131; UMMZ 240142; UMMZ uncat. (PVD 00-01/18a); UMMZ uncat. (PVD 99-11/24 75); UMMZ uncat. (PVD 02-03/19a); USNM 373284.

Photopectoralis cf. *bindus*: AMNH uncat. (Taiwan, Leo 31).

Photopectoralis hataii: UMMZ uncat.

Photopectoralis cf. *hataii*: AMNH 89922.

Photopectoralis panayensis: UMMZ 240300, holotype; UMMZ 240301, paratypes, 4 ex.; UMMZ 240302, paratypes, 5 ex.; UMMZ 240303, paratypes, 8 ex.; UMMZ 240304, paratypes, 16 ex.; UMMZ 240137; UMMZ uncat. (PVD 02-03/06a).

Photopectoralis sp. "East China Sea": AMNH uncat.

Clade F: *Gazza*

Gazza achlamys: SU-21652, paratype, 1 ex.; SU-22853, paratype, 1 ex.; UMMZ 240128; UMMZ 240132; UMMZ 240139.

Gazza dentex: MNHN A-578, lectotype.

Gazza minuta: AMNH 220748; AMNH uncat.; UMMZ 191542; UMMZ 240126; UMMZ 240140; UMMZ 240141; UMMZ uncat. (PVD 01-02/07a).

Gazza rhombea: USNM 332347, paratype, 1 ex.; USNM 350467, paratype, 1 ex.

Gazza squamiventralis: USNM 345525, holotype; USNM 345526, paratype, 1 ex.; AMNH uncat. (Mad 25-2003, WLS 52); AMNH uncat. (Mad 25-2003, WLS 51).

Gazza n. sp. "Madagascar": AMNH uncat. (Mad 25-2003, WLS 51).

Clade G:

"Leiognathus" daura: USNM 100291; USNM 373281.

"Leiognathus" decorus: AMNH 231297; AMNH 234765; AMNH uncat. (Aust. WI-02-14); AMNH uncat. (Aust. WI-02-04); AMNH uncat. (Aust. WI-02-11); AMNH uncat.; AMS I.22990002; AMS I.26927001.

"Leiognathus" dussumieri: MNHN A-6721, syntype, 1 ex.; AMNH 234763.

"Leiognathus" nuchalis: AMNH 26819; CAS-SU 4757; UMMZ 240143.

"Leiognathus" pan: USNM 276536, paratype, 1 ex.

"Leiognathus" blochii: MNHN A-6757, syntype, 1 ex.; MNHN A-6759, syntype, 1 ex.

Clade H:

"Leiognathus" jonesi: UMMZ 240134; UMMZ 240505; UMMZ uncat.

"Leiognathus" philippinus: UMMZ 240130.

"Leiognathus" splendens: CAS 1485; CAS 38789; CAS 56438; CAS 56441; MNHN A-6724; UMMZ 191202; UMMZ uncat.; USNM 190258; USNM 190263.

Clade I: *Leiognathus fasciatus* complex

Leiognathus fasciatus: AMNH 15520; AMNH uncat. (Mad 28-2003, WLS 55, Leo 21); CAS 1872; UMMZ 240504; UMMZ uncat.; USNM 191962; USNM 191966.

Leiognathus n. sp. "Madagascar": AMNH uncat (Mad 29- 2003, WLS 57, Leo 25); AMNH uncat. (Mad 25-2003, WLS 51, Leo 39); AMNH uncat. (Mad 25- 2003, WLS 52); AMNH uncat. (Mad 26-2003, WLS 53, Leo 41).

Leiognathus n. sp. "Singapore": UMMZ 240361.

Leiognathus longispinis (= *L. smithursti*): MNHN A-0579, holotype; AMNH 219296; AMS I.20907036; AMS I.22974001; AMS 22981001; AMS 23044001; USNM 324651.

Clade J: *Leiognathus equulus* complex

Leiognathus edentulus: ZMB 8756, holotype (dry skin; photograph and radiographs examined).

Leiognathus edwardsi: USNM 55904, holotype.

Leiognathus equulus: ZMUC P48219, lectotype (dry skin; photographs and radiographs examined); ZMUC P48220, paralectotype (dry skin, photograph and radiograph examined); AMNH 59535; AMNH 88039; AMNH uncat. (Taiwan, Leo 14); CAS 57306; CAS-SU 35627; CAS-SU 38781; MNHN A-6723; UMMZ 191520; UMMZ 235029; UMMZ 238805 (in part); UMMZ 240133; UMMZ 240502; UMMZ 240503; UMMZ uncat.

Leiognathus robustus: UMMZ 242144, holotype; AMNH 233607, 1 ex., paratype; UMMZ 240362, 1 ex, paratype.; UMMZ 240360.

Clade K:
Leiognathus n. sp. "Sri Lanka": FRLM uncat.

Outgroups

Lampridiformes:
Veliferidae: *Velifer hypselopterus*: AMNH 49575; AMNH 90147.

Ophidiiformes:
Ophidiidae: *Brotula multibarbata*: AMNH 212126; *Chilara taylori*: AMNH 38157; *Lepophidium brevibarbe*: AMNH 83648.

Perciformes:
Bramidae: *Eumegistus illustris*: AMNH 29742.
Carangidae: *Carangoides equula*: UMMZ uncat.; *Carangoides malabaricus*: UMMZ uncat.; *Carangoides ferdau*: AMNH 51991; *Scomberoides* sp. AMNH 218737; *Selar crumenophthalmus*: AMNH 222013; UMMZ uncat.; *Trachinotus carolinus*: AMNH 74161.
Cepolidae: *Cepola macrophthalma*: AMNH 49646.
Coryphaenidae: *Coryphaena hippurus*: AMNH 222751.
Gerreidae: *Diapterus rhombeus*: AMNH 224760; *Gerres abbreviatus*: UMMZ uncat.; *Gerres equulus*: UMMZ uncat.; *Gerres filamentosus*: AMNH 232145; UMMZ uncat.
Haemulidae: *Haemulon plumierii*: AMNH 225401.
Menidae: *Mene maculatus*: USNM 200297; AMNH uncat. (Mad 2003).
Moronidae: *Morone saxatilis*: AMNH 51028.
Nematistiidae: *Nematistius pectoralis*: AMNH 237.
Scombridae: *Scomber japonicus*: AMNH 76919; AMNH 76992.
Sparidae: *Calamus leucosteus*: AMNH 86356; *Calamus penna*: AMNH 84471.

Appendix 2

Character descriptions

Fifteen morphological features were coded, corresponding to both internal and external features of the leiognathid LOS (Table 2), for all species for which molecular sequence data were collected (Table 1). Multiple specimens of each species were dissected and examined. The numbering of characters corresponds to that presented in the data matrix. Consistency indices follow the individual character descriptions and indicate the fit of the character on the cladogram generated using DNA sequence data alone or using nucleotide characters in combination with the morphological transformations. The characters are:

1. *Circumesophageal light organ containing symbiotic bioluminescent bacteria* (Photobacterium leiognathi) (Fig. 3). 0: Absent. 1: Present. State one is restricted to Leiognathidae. A similar structure is unknown in any other group of fishes. (1.00)

2. *Light organ dimorphic in volume*. 0: Absent. 1: Present. In male leiognathids exhibiting state one, the light organ is enlarged in volume compared to conspecific females (Fig. 4B). An enlarged light organ is present in males of all leiognathid species in clade B. (1.00)

3. *Dorsolateral lobes of light organ hypertrophied in males, lobes confined to interior of gas bladder lining and extend posteriorly into gas bladder*. 0: Absent. 1: Present. State one is restricted to members of clade C, and is pronounced in members of clade L, in which the greatly enlarged dorsal light-organ lobes of males extend well into the gas bladder (e.g., *Photoplagios elongatus* and *P. rivulatus*; Fig. 4B), less *P. leuciscus*. In members of clade M, the dorsal light-organ lobes of males are moderately enlarged and extend only slightly into the gas bladder (Fig. 5B). (1.00)

4. *Dorsolateral lobes of light organ hypertrophied in males, lobes extend laterally, exterior of gas bladder lining and abut pectoral-axil window*. 0: Absent. 1: Present. State one is restricted to clade E, comprising *Photopectoralis aureus*, *P. bindus*, *P. hataii*, *P. panayensis* and *P.* sp. "East China Sea" (Fig. 6B). (1.00)

5. *Ventrolateral lobes of light organ hypertrophied in males*. 0: Absent. 1: Present. State one is restricted to clade N (Fig. 1). State one is present in clades F (*Gazza*), D (*Secutor*) and E (*Photopectoralis*) (Figs 7B and 8B). The ventrolateral lobes are not enlarged in members of clade H ("*Leiognathus*"). Only members of *Photopectoralis* exhibit both enlarged dorsolateral and ventrolateral light-organ lobes. (0.50)

6. *Clearing of lateral silvery lining of gas bladder in males*. 0: Absent. 1: Present. Clearing of the lateral gas bladder lining is present only in members of clade C, comprising *Photoplagios elongatus*, *P. leuciscus*, *P. moretoniensis*, *P. rivulatus*, *P. stercorarius* and *P.* sp. "Madagascar" (i.e., all leiognathids which exhibit a corresponding transparent lateral flank patch or stripe) (Fig. 4B). Suitably preserved material (i.e., with silvery guanine layer intact) of *P. lineolatus* was unavailable for comparison. (1.00)

7. *Lateral luminescence via transparent pectoral-axil patch in males*. 0: Absent. 1: Present. State one is restricted to members of clade E, comprising *Photopectoralis aureus*, *P. bindus*, *P. hataii*, *P. panayensis* and *P.* sp. "East China Sea" (Fig. 6A). (1.00)

8. *Lateral luminescence via transparent flank patches in males*. 0: Absent. 1: Present. State one is restricted to

members of clade C, comprising *Photoplagios elongatus*, *P. leuciscus*, *P. lineolatus*, *P. moretoniensis*, *P. rivulatus*, *P. stercorarius* and *P.* sp. "Madagascar" (Figs 4A and 5A). Although ideally preserved material (i.e., with silvery guanine layer intact) of *P. lineolatus* and *P.* sp. "Madagascar" was lacking, a wide mid-lateral stripe is present on the posterior flanks, which appears to be (at least partially) transparent. (1.00)

9. *Morphology of transparent flank patch(es) in males*. 0: Expansive triangular patch. 1: Mid-lateral stripe. State zero is restricted to members of clade L (*Photoplagios elongatus*, *P. rivulatus* and *P. leuciscus*) (Fig. 4A). State one is restricted to members of clade M (*Photoplagios stercorarius*, *P. moretoniensis*, *P. lineolatus* and *P.* sp. "Madagascar" (Fig. 5A). (1.00)

10. *Transparent flank stripe in males*. 0: Continuous. 1: Comprised of numerous, serially arranged, mid-lateral windows, which may be discrete or overlapping. State one is present only in *Photoplagios stercorarius* and *P. moretoniensis* (Fig. 5A). Although our comparative material is limited and not ideally preserved (i.e., silvery, guanine layer is faded), state zero, a continuous mid-lateral stripe that appears to be at least partially transparent, is present in *P. lineolatus* and *P.* sp. "Madagascar". (1.00)

11. *Lateral luminescence via enlarged transparent opercular patch in males, located anteriorly in opercular cavity and occluded by interopercle*. 0: Absent. 1: Present. State one is restricted to *Secutor* (clade D) (Fig. 8A). (1.00)

12. *Lateral luminescence via enlarged transparent opercular patch in males, posteriorly positioned in opercular cavity and occluded by subopercle*. 0: Absent. 1: Present. State one is restricted to *Gazza* (clade F) (Fig. 7A). (1.00)

13. *Silvery, guanine-lined reflective chamber surrounding and extending rostrally and ventrally from contralateral ventral light-organ lobes*. 0: Absent. 1: Present. State one is restricted to clade N (Fig. 1) and is present in *Gazza (*clade F), *Secutor* (clade D) and *Photopectoralis* (clade E) (Figs 7B and 8B), but absent in members of clade H ("*Leiognathus*"). (0.50)

14. *Large anteroventrally directed windows present on ventral light-organ lobes, which are oriented into silvery reflective chamber*. 0: Absent. 1: Present. State one is restricted to clade N (Fig. 1) and is present in *Gazza* (clade F), *Secutor* (clade D), and *Photopectoralis* (clade E) (Figs 7B and 8B), but absent in members of clade H ("*Leiognathus*"). (0.50)

15. *Silvery, guanine-lined reflective chamber extends rostrally along opercular cavity margin to gular region*. 0: Absent. 1: Present. State one is restricted to *Secutor* (clade D) and presumably links the light organ and transparent gular patches (Fig. 8B). (1.00)