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A Clade of Non-Sexually Dimorphic Ponyfishes (Teleostei: Perciformes: Leiognathidae): Phylogeny, Taxonomy, and Description of a New Species

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ABSTRACT

A phylogeny was generated for Leiognathidae, commonly known as ponyfishes, using nucleotide characters from two mitochondrial genes. Results indicate that Leiognathidae comprises two major clades, one consisting of species that exhibit internally sexually dimorphic light-organ systems (LOS), and the *Leiognathus equulus* species complex, whose members exhibit neither internal nor external sexual dimorphism of the LOS. Species with internally sexually dimorphic LOS generally also exhibit associated male-specific external modifications in the form of transparent patches on the margin of the opercle, the midlateral flank, or behind the pectoral fin axil. The *L. equulus* species complex is the sister group to all other leiognathids, and a new species, *L. robustus*, recovered within this clade is described herein. Results demonstrate that *Leiognathus* is paraphyletic, whereas *Gazza* and *Secutor* are each monophyletic and are nested within the sexually dimorphic clade. The morphology of the LOS of non-sexually dimorphic leiognathids is compared to the more common sexually dimorphic state, and differences in these systems are discussed and illustrated. In the context of a family-level phylogeny, we can trace the evolution of the leiognathid LOS from a ''simple'' non-sexually dimorphic circumesophageal light organ to a complex and species-specific luminescence system involving not only major structural modifications of the light organ itself but also numerous associated tissues.

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INTRODUCTION

Leiognathids, commonly known as ponyfishes or slipmouths, are bioluminescent, schooling fishes common in near-shore and estuarine Indo-Pacific waters from the east coast of Africa to islands of the west Pacific and from Japan to Australia. As locally abundant fishes in turbid coastal waters, they often are captured in mixed assemblages of a few to several species (McFall-Ngai and Dunlap, 1984; Woodland et al., 2001; P.V. Dunlap, personal obs.). Approximately 40 species in three genera, *Gazza*, *Leiognathus*, and *Secutor*, are currently recognized (Eschmeyer, 1998; Froese and Pauly, 2003; Woodland et al., 2001).

Luminescence in leiognathids is produced from an internal light organ, a circumesophageal ring of tissue in which are harbored large numbers of the symbiotic luminous bacterium, *Photobacterium leiognathi* (Boisvert et al., 1967; Hastings and Mitchell, 1971; Bassot, 1975; Reichelt et al., 1977; Dunlap, 1984). Together with the light organ, the light-organ system (LOS) of leiognathids is composed of reflectors and chromatophore-embedded light-organ shutters, transparent and reflective tissues of the gasbladder, and transparent bone, musculature, and skin (fig. 1). These accessory tissues function to control, direct, and diffuse the intense blue-green bacterial light over the ventral surface of the fish (Harms, 1928; Ahrens, 1965; Bassot, 1975; McFall-Ngai, 1983; McFall-Ngai and Dunlap, 1983; Dunlap and McFall-Ngai, 1987). Hypothesized functions of the bacterial light include camouflage illumination against bottom-dwelling piscivorous fishes and other forms of predator avoidance, prey attraction, schooling, and sex-specific signaling (Hastings, 1971; Herring and Morin, 1978; McFall-Ngai, 1983; McFall-Ngai and Dunlap, 1983, 1984; Dunlap and McFall-Ngai, 1987; McFall-Ngai and Morin, 1991; Woodland et al., 2002).

In the present study, to gain a more detailed understanding regarding evolution of the LOS in ponyfishes, we generated a phylogeny for the family based on nucleotide characters from two mitochondrial genes. In addition, we examined numerous museum lots of specimens belonging to both the nondimorphic and sexually dimorphic clades that were recovered in this analysis. Together with a morphological analysis of the light organ and associated tissues of the LOS in the nondimorphic clade, we describe a new species of leiognathid bearing a non-sexually dimorphic light organ. In the context of the recovered phylogeny, we discuss the insights this nondimorphic clade provides into the evolution and diversification of the leiognathid LOS. Further, taxonomic and nomenclatural implications (i.e., paraphyly of *Leiognathus*) are discussed in light of the recovered phylogenetic pattern.

MATERIALS AND METHODS

Morphology

Osteological characters of the new species and related taxa were examined using radiographs, specimens cleared and stained for bone and cartilage, and dry skeletal preparations. Materials examined are listed in appendix 1. When sufficient material was available, multiple males and females of each species were dissected and examined for LOS features. Light organs were isolated from each taxon to permit detailed comparison. Specimens were cleared and stained for bone and cartilage using a modified protocol based on Taylor and Van Dyke (1985). Morphometric measurements were recorded to the nearest 0.1 mm using dial calipers. Standard length (SL) is used throughout. Body depth *A* was measured at a vertical from the origin of the anal fin, and body depth *B* at a vertical from the origin of the dorsal fin. Vertebral counts exclude the ural centrum (= last half-centrum). Vertebral and fin spine/ray counts were obtained from radiographs. The terminal dorsal-fin and anal-fin rays, which are branched to the base of the fin, are counted as a single element. Pored scales of the lateral line are counted in series from the dorsal margin of the gill opening to the caudal flexure. Scale counts should be interpreted as approximations, due to high intra- and interspecific variability, irregular arrangement, and because small scale size and the degree to which scales are embedded make accurate counts problematic. Institutional abbreviations follow Leviton et al. (1985).

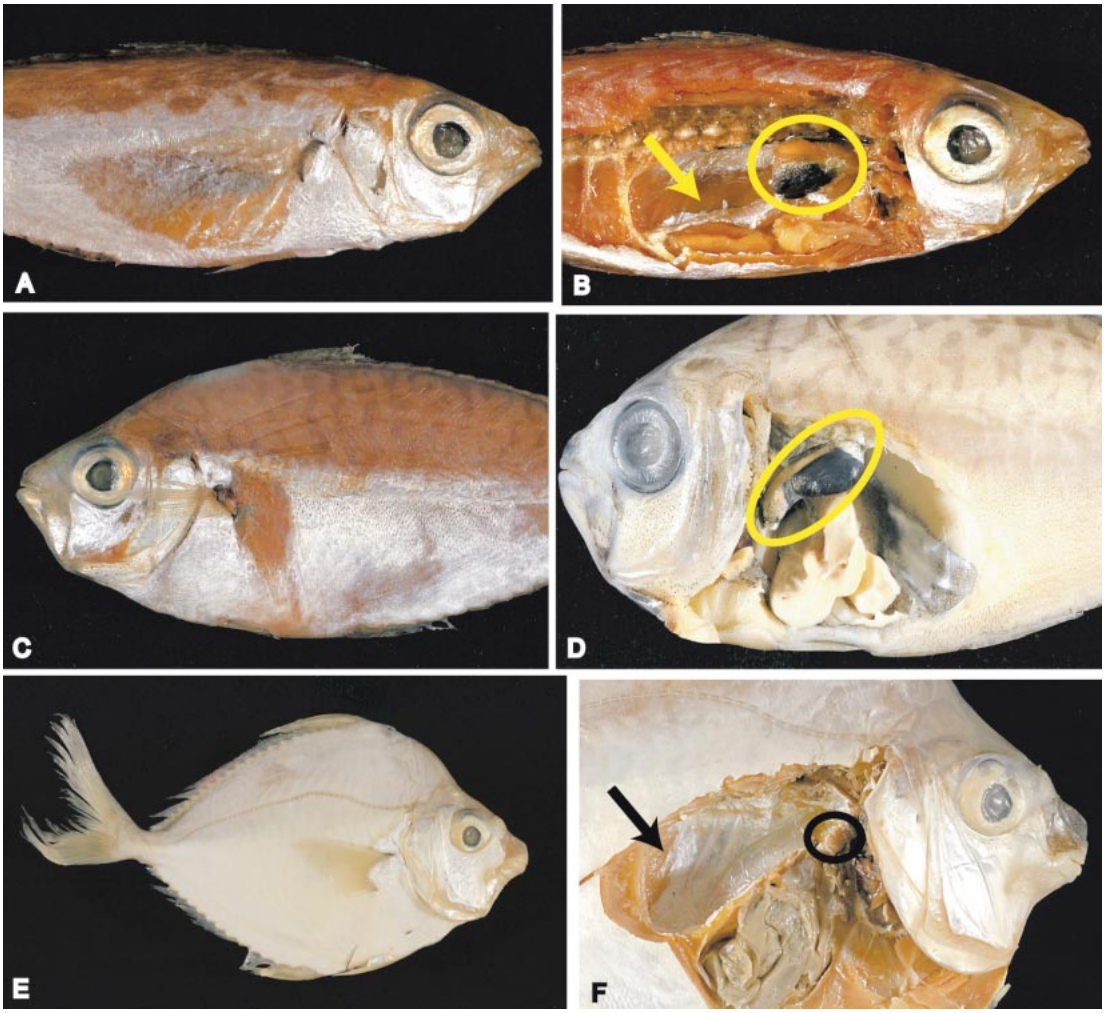

Fig. 1. Comparison of light organs (circled) and associated features of the LOS in male leiognathids exhibiting both sexually dimorphic and nondimorphic states. *Leiognathus elongatus*, extreme sexual dimorphism: (**A**) external anatomy illustrating expansive transparent lateral flank patch characteristic of males, which is located just external to the clear gasbladder wall and enlarged dorsal lobes of the male light organ; (**B**) internal anatomy illustrating lateral clearing of the silvery gasbladder lining (arrow) and hypertrophied dorsal lobes of the light organ, which lie internal to the gasbladder lining (removed). *Leiognathus aureus*, moderate to extreme sexual dimorphism: (**C**) external anatomy illustrating transparent pectoral-axil patch characteristic of males, which lies just exterior to the hypertrophied dorsolateral light-organ lobes; (**D**) internal anatomy illustrating enlarged dorsolateral light-organ lobes that abut lateral clearing of the integument just internal to the pectoral-fin axil. *Leiognathus equulus*, nondimorphic: (**E**) external anatomy; (**F**) internal anatomy. In members of the *L. equulus* species complex the light organ is not enlarged in males and there is no corresponding lateral clearing of the silvery gasbladder lining (arrow indicates posterior clear region common to all leiognathids) or integument proximal to the light organ.

TABLE 1
Taxa Used in Molecular Phylogenetic Analysis, Including Collection Locality, Tissue Voucher Code, and GenBank Accession Numbers

Taxon	Collection locality	Tissue voucher code	GenBank accession number	
			16S	COI
Leiognathidae				
Gazza achlamys	Philippines	GA-1	AY541648	AY541623
Gazza minuta	Philippines	GM-1	AY541649	AY541624
Leiognathus aureus	Philippines	LA-1P	AY541650	AY541625
Leiognathus bindus	Philippines	LB-1P	AY541651	AY541626
Leiognathus elongatus	Japan	LE-1J	AY541652	AY541627
Leiognathus equulus (Philippines)	Philippines	LEQ-1P	AY541653	AY541628
Leiognathus equulus (Singapore)	Singapore	LEQ-2S	AY541654	AY541629
Leiognathus fasciatus	Philippines	LF-2P	AY541655	AY541630
Leiognathus jonesi	Philippines	LJ-1P	AY541656	AY541631
Leiognathus leuciscus	Philippines	LL-1P	AY541657	AY541632
Leiognathus nuchalis	Japan	LN-1J	AY541658	AY541633
Leiognathus panayensis	Philippines	LH-1P	AY541659	AY541634
Leiognathus philippinus	Philippines	LP-1P	AY541660	AY541635
Leiognathus rivulatus	Japan	LR-1J	AY541661	AY541636
Leiognathus robustus, n.sp.	Singapore	LEQ-1S	AY541664	AY541639
Leiognathus splendens	Philippines	LS-2P	AY541662	AY541637
Leiognathus stercorarius	Philippines	LST-1P	AY541663	AY541638
Secutor indicius	Philippines	SI-1P	AY541665	AY541640
Secutor megalolepis	Philippines	SM-1P	AY541666	AY541641
Gerreidae				
Gerres abbreviatus	Philippines	GAB-1	AY541667	AY541642
Gerres equulus	Japan	GE-1	AY541668	AY541643
Gerres filamentosus	Philippines	GF-1	AY541669	AY541644
Carangidae				
Carangoides equula	Japan	KE-1	AY541670	AY541645
Carangoides malabaricus	Philippines	CM-1	AY541671	AY541646
Selar crumenophthalmus	Japan	SC-1	AY541672	AY541647

Institutional Abbreviations

AMNH	American Museum of Natural History, New York
AMS	Australian Museum, Sydney
BMNH	Natural History Museum, London
CAS	California Academy of Sciences, San Francisco
LACM	Los Angeles County Museum of Natural History
SIO	Scripps Institution of Oceanography, Marine Vertebrates Collection, La Jolla
UMMZ	University of Michigan, Museum of Zoology, Ann Arbor
USNM	National Museum of Natural History, Smithsonian Institution, Washington, D.C.
ZMB	Universitat Humboldt, Museum fur Naturkunde, Berlin
ZMUC	Kobenhavns Universitet, Zoologisk Museum, Copenhagen

DNA Sequencing and Sequence Analysis

A total of 1251 nucleotide characters from two mitochondrial genes (the large ribosomal subunit [16S] and cytochrome *c* oxidase subunit I [COI]) were used in the phylogenetic analysis. Taxon sampling was designed to include a diverse assemblage of leiognathid species representative of overall familial diversity (table 1 and appendix 1). In addition to all leiognathid species included in the molecular analysis, a number of species for which tissue samples suitable for molecular studies could not be obtained were examined for LOS features. Outgroup taxa were selected from perciform families hypothesized to be closely related to leiognathids, including members of Gerreidae (mojarras) and Carangidae (jacks) (Günther 1862; Weber

and de Beaufort 1931; James 1975; Jones 1985; unpubl. data).

Fish tissues were either preserved in 95% ethanol or stored frozen at −75°C prior to extraction of DNA. Total genomic DNA was extracted from muscle 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 a segment (~600 bp) of DNA from the mitochondrial large ribosomal subunit (16S) and a segment (~750 bp) of cytochrome *c* oxidase subunit I (COI). Double-stranded amplifications were performed in either 25 or 50μl volumes containing 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2–0.5 μM of each primer, 10–1000 ng of genomic DNA (1–2 μl), and 1 unit of Taq polymerase. To amplify and sequence the 16S fragment, the primers 16S ar-L 5′-CGCCTGTTTATCAAAAACAT-3′ and 16S br-H 5′-CCGGTCTGAACTCAGATCACGT-3′ (Kocher et al., 1989; Palumbi, 1996) were used. To amplify and sequence the COI fragment, the Folmer et al. (1994) primers LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ were used. Amplifications for 16S were carried out in 30 cycles according to the following temperature profile: denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C, with an additional terminal extension at 72°C for 10 min. For COI, 35 cycles were run according to the following temperature profile: denaturation for 30 sec at 94°C, annealing for 1 min at 45°C, and extension for 2 min at 72°C, with an additional terminal extension at 72°C for 10 min. The double-stranded amplification products were 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. The sequencing reactions were electrophoresced on ABI 377 or ABI 3700 automated DNA sequencers.

DNA sequences were aligned and compiled using Sequence Navigator 1.0.1 (Applied Biosystems), Sequencher v.4.1 (Gene Codes), CLUSTAL X (Thompson et al., 1994, 1997; available at http://ncbi.nlm.nih.gov), and Sequence Monkey 2.9.1 (Graf, 2000; available at http://www.monkeysoftwerks.com). The initial 16S alignment was folded into stem and loop regions using available secondary-structure models (Guttel and Fox, 1988; De Rijk et al., 1994; Orti et al., 1996). Base-pair complementarity was verified for all stem regions. The protein-coding fragment (COI) was unambiguously aligned using CLUSTAL X and required no further adjustment.

Phylogeny Reconstruction

All 1251 nucleotide characters were analyzed simultaneously under the optimality criterion of parsimony. Gaps were treated as a fifth character state. Parsimony analyses were conducted using PAUP* 4.0b3 (Swofford, 1998). No weighting schemes of any kind were applied. Heuristic searches were performed with 1000 replications and random stepwise addition of taxa. Consistency indices (CI), retention indices (RI), and rescaled consistency indices (RC) (Kluge and Farris, 1969; Farris, 1989) were computed in PAUP*. To estimate the robustness of the phylogenetic hypothesis, Bremer support (Bremer, 1988, 1995) was calculated for all recovered clades using TreeRot v.2 (Sorenson, 1999; available at http://mightyduck.bu.edu/TreeRot), and Jackknife resampling analyses (10,000 replications, heuristic searches, 10 random stepwise additions per replication, emulate Jac option selected) were performed using PAUP*. Patterns of character evolution were examined using both PAUP*, PAUP v.3.1.1 (D.L. Swofford, unpubl.), and MacClade (Maddison and Maddison, 1997). Ingroup relationships were unaffected regardless of whether carangids or gerreids were used to root the topology. Sequences are deposited at GenBank under the accession numbers listed in table 1.

RESULTS

Molecular Analysis

A single optimal tree was recovered by analysis of the combined, equally weighted nucleotides from the mitochondrial 16S and COI genes (1251 characters; 432 parsimony-informative sites; 1976 steps including only

parsimony-informative sites; CI = 0.402; RI = 0.555; RC = 0.223), representing a diverse assemblage of leiognathid species (fig. 2). Monophyly of Leiognathidae (clade A) is very strongly supported by nucleotide characters (Bremer support [BS] = 47; Jackknife [JK] = 100%). Within Leiognathidae, two major clades were recovered and are also robustly supported: clade B (BS = 22; JK = 100%), comprising *L. equulus* and a morphologically similar species described herein; and clade C (BS = 8; JK = 94), containing all other members of *Leiognathus* together with all members of *Gazza* and *Secutor*. Both *Gazza* and *Secutor* are monophyletic, as is an assemblage comprising *Gazza* + *Secutor*. These relationships render the genus *Leiognathus* paraphyletic. Within clade C, a number of less inclusive clades are recovered and most receive strong support.

SYSTEMATIC ACCOUNT

Leiognathus robustus, new species

Figures 3–6

HOLOTYPE: UMMZ 242144, 183.4 mm SL, adult male; Singapore: fish market; H.H. Ng, 16 July 2001.

PARATYPES: AMNH 233607, 1 ex., 167.9 mm SL, male; Singapore: fish market; H.H. Ng, July 2002; UMMZ 240362, 1 ex., 165.5 mm SL, female; Singapore: fish market; H.H. Ng, July 2002.

DIAGNOSIS: *Leiognathus robustus* is distinguished from the only other species of leiognathid known to possess a non-sexually dimorphic LOS, *L. equulus*, by the absence of an occipital hump (vs. pronounced hump), the presence of a mildly sloping predorsal profile (vs. strongly curved, creating the image of an arched back), frontal and lateral ethmoid ossifications that project anterodorsally and extend well anterior of the orbit to form a distinct preorbital protuberance (vs. slight bulge above orbit), and a nuchal spine that is not exposed in lateral view (vs. exposed and projecting, particularly distally).

DESCRIPTION: Selected proportional measurements and meristic data are presented in table 2. A deep-bodied and robust *Leiognathus*, which grows to a large size (>180 mm SL) (figs. 3, 4). Body laterally compressed. No pronounced supraoccipital (= predorsal) hump (fig. 5A). Lateral snout outline mildly concave. Strong preorbital protuberance due to hypertrophy and protrusion of frontal and lateral ethmoid ossifications (fig. 6A). Predorsal head profile mostly straight to mildly curved; back not strongly arched. Nuchal spine not protruding, and distal tip not exposed (figs. 3A, 4, 5A). Nuchal spine with distinct median keel. Two short and stout postnasal spines present on lateral ethmoid, located posterior to nasal foramina and just rostrodorsal of orbit. Postnasal spines followed posteriorly by well-developed supraorbital ridges; ridges converge posteriorly. Dorsal and ventral profiles about evenly curved. Dorsal-fin origin about midway between pelvic-fin and anal-fin origins. Anal-fin origin at about level of vertical through seventh dorsal-fin spine. Eye large. Caudal peduncle slender and shallow. Mouth small and terminal in position, directed slightly downward when protruded. Caudal margin of maxilla exposed, reaching to level of vertical through anterior margin of orbit. Anterior nasal pore round, posterior foramen crescent-shaped, partially encircling anterior pore. Lower preopercular margin weakly serrate. Vertebral count: 9 precaudal + 14 caudal = 23. Neural and hemal spines of vertebral centrum PU4 expanded and bladelike (fig. 6A). Fifteen or 16 stout and triangular outer ceratobranchial gill rakers arrayed along lower limb (= ceratobranchial one) of first gill arch.

Fins: Dorsal fin with VIII spines and 16 branched rays. First dorsal-fin spine greatly reduced in length, yet relatively robust. Second through fourth dorsal-fin spines elongate and robust, second spine longest. Third and fourth dorsal-fin spines serrate along anterior margin, ''lock'' into groove on preceding spine when erect. Dorsal-fin spines five through eight feeble, shorter than second through fourth spines. Anal fin with III spines and 14 branched rays. First anal-fin spine very short. Second and third anal-fin spines robust and elongate, second spine longest. Third anal-fin spine serrate on anterior margin, ''locks'' into groove on posterior margin of second spine when erect. Spinous dorsal and anal fins with asquamate basal sheath, creating furrow into which fins may retract. Pelvic fins short, not reaching first

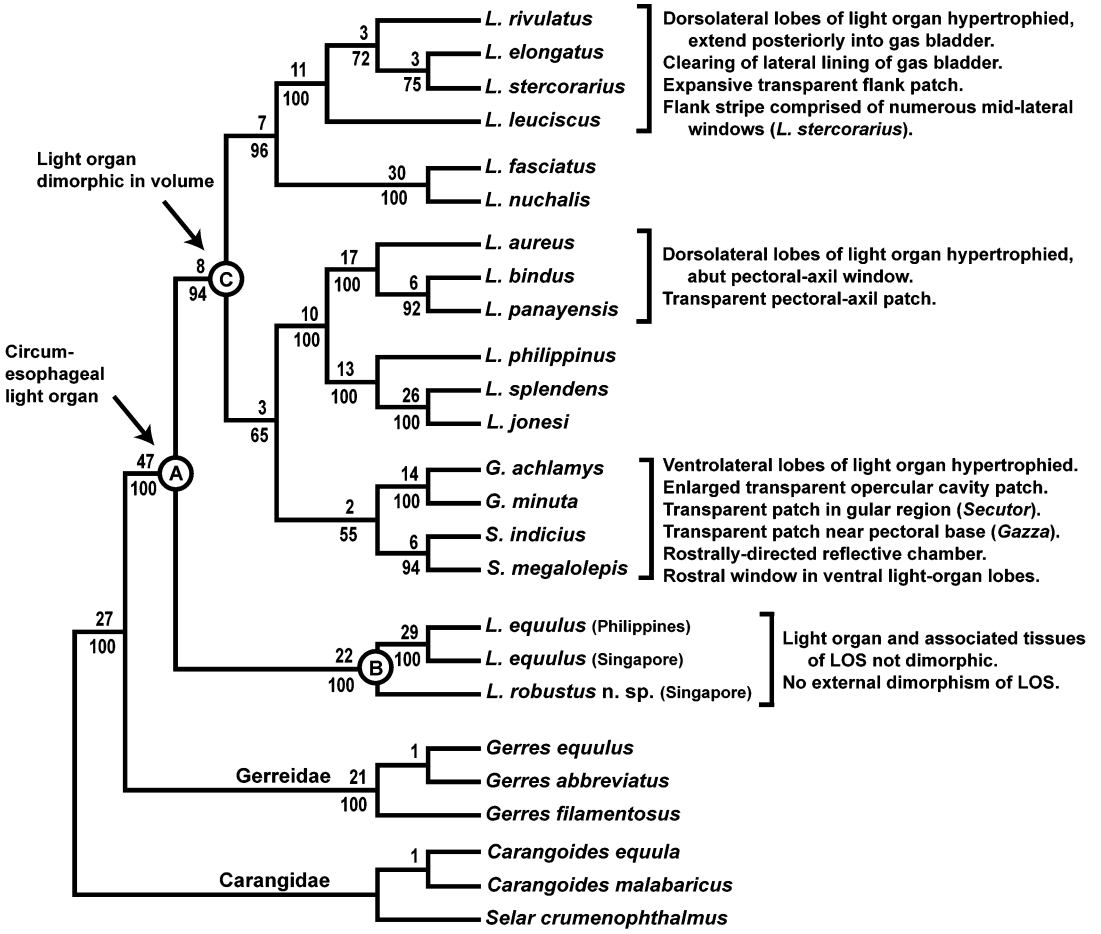

Fig. 2. Single optimal tree of leiognathid relationships recovered by combined analysis of mitochondrial (16S and COI) nucleotide characters. *L.* = *Leiognathus*; *G.* = *Gazza*; *S.* = *Secutor*. Numbers above branches represent Bremer support and numbers below branches represent Jackknife resampling percentages (>50%). Letters at nodes correspond to clades discussed in text: clade A = Leiognathidae; clade B = leiognathids with non-sexually dimorphic LOS; clade C = leiognathids bearing sexually dimorphic LOS. LOS features characteristic of members of recovered clades (Sparks and Dunlap, in review) are indicated on the topology.

anal spine when adducted. Eight upper and seven lower branched caudal-fin rays. Nineteen total pectoral-fin rays.

Dentition: Multiple rows of small, closely set, elongate and moderately recurved, villiform teeth present in both upper and lower jaws. Four to five closely set rows anteriorly and one to two rows posteriorly in upper jaw. Three to four rows anteriorly and one to two rows posteriorly in lower jaw. Lips fleshy and rugose.

Squamation: Body scales small and cycloid. Head and opercular region naked. Breast naked, asquamate region extending to pectoral-fin base. Lateral line arched and complete. Pored scales in lateral line number 60–65. Pores well developed. Pelvic axillary scale well developed and elongate. All fins asquamate.

PIGMENTATION IN PRESERVATION: Body ground coloration gray to grayish-blue dorsal of midline, and pale yellow, white, or golden ventral of midline. Iridescent golden patches present to varying degree along lateral midline. Cheek and opercular region iridescent silvery to golden. Head above orbit and nape

TABLE 2

Morphometric and Meristic Data of *Leiognathus robustus*, new species, and *L. equulus*

Measurements (mm) are percentage standard length (SL) or percentage head length (HL), unless noted otherwise. H indicates count corresponding to holotype.

	L. robustus					*L. equulus*				
Character	*N*	Holotype	Range	Mean	SD	*N*	Holotype	Range	Mean	SD
Standard length (mm)	3	183.4	165.5–183.4	172.3		23	131.0	69.0–177.8	110.6	
Head length % SL	3	29.9	28.8–30.1	29.6	0.58	22	n.a.	28.6–33.1	30.3	1.09
Body depth (origin AF) % SL (A)	3	52.3	52.3–53.5	52.8	0.56	22	n.a.	51.2–60.5	56.2	2.50
Body depth (origin DF) % SL (B)	3	52.2	52.2–58.4	54.3	2.87	22	n.a.	49.5–58.3	55.1	2.60
Predorsal length % SL	3	50.0	49.5–50.2	49.9	0.30	22	n.a.	48.4–53.3	50.8	1.40
Preanal length % SL	3	54.6	54.1–56.1	55.0	0.83	22	n.a.	50.2–58.5	54.1	2.08
Prepelvic length % SL	3	37.3	35.6–37.9	36.9	0.97	22	n.a.	35.1–40.2	37.3	1.29
Dorsal-fin base length % SL	3	52.3	52.3–54.1	53.4	0.80	22	n.a.	53.2–59.1	56.2	1.50
Anal-fin base length % SL	3	42.9	42.9–44.3	43.7	0.58	22	n.a.	42.3–48.8	45.6	1.59
Caudal peduncle length % SL	3	12.3	10.6–13.1	12.0	1.03	22	n.a.	9.4–13.7	11.1	1.19
Caudal peduncle width % SL	3	4.9	4.7–5.1	4.9	0.19	22	n.a.	3.3–4.7	4.0	0.34
Caudal peduncle depth % SL	3	6.6	6.6–7.1	6.8	0.23	22	n.a.	5.9–7.4	6.7	0.39
Pectoral fin length % SL	3	23.5	22.9–24.0	23.5	0.48	22	n.a.	21.2–26.2	23.9	1.27
Pelvic fin length % SL	3	15.3	14.8–15.4	15.1	0.25	22	n.a.	11.4–16.8	15.5	1.14
Snout length % HL	3	35.9	34.4–37.1	35.8	1.11	22	n.a.	33.7–39.6	37.0	1.73
Head width % HL	3	53.9	52.6–54.5	53.7	0.80	22	n.a.	47.9–54.4	50.9	1.98
Upper jaw length % HL	3	22.8	22.7–24.1	23.2	0.64	22	n.a.	23.1–27.6	25.4	1.02
Lower jaw length % HL	3	50.3	50.3–55.9	53.4	2.36	22	n.a.	48.0–57.4	52.0	2.36
Interorbital width % HL	3	35.5	35.0–35.5	35.2	0.21	22	n.a.	31.7–36.6	34.3	1.32
Orbit diameter % HL	3	37.2	37.1–38.7	37.7	0.76	22	n.a.	33.3–41.7	37.3	2.46
Preorbital depth % HL	3	20.4	19.8–22.2	20.8	1.04	22	n.a.	19.6–25.9	22.7	1.60
Caudal peduncle length/width	3	2.5	2.1–2.8	2.5	0.30	21	n.a.	2.1–3.7	2.8	0.35
Caudal peduncle length/depth	3	1.9	1.5–2.0	1.8	0.21	21	n.a.	1.3–2.3	1.7	0.23
Pored scales in lateral line	3	60–65, 61 (H)				12	56–65			
Dorsal fin	3	VIII 16				22	VII 17 (1), VIII 16 (20) (H), VIII 17 (1)			
Anal fin	3	III 14				22	III 14 (H)			
Vertebrae (precaudal + caudal)	3	9 + 14 = 23				21	9 + 14 = 23			

grayish to grayish-brown. Snout dusky to blackish; appears spotty due to concentrated melanophores. Nasal region and lips pale yellow. Gular region with iridescent silvery or golden patches. Chest and belly white or pale gray. Caudal peduncle and base of caudal fin iridescent silvery or golden. Dorsal, anal, pectoral, and pelvic fins whitish to yellow. Dorsal fin with black pigment distally. Pectoral-fin axil blackish due to concentration of melanophores, surrounded by large iridescent silver patch. Pectoral-fin base silvery. Caudal fin yellowish to light brown, dorsal and ventral rays with black pigment, especially proximal to base. Caudal fin with prominent black terminal band. Pelvic axillary scale and body along anal-fin base silvery or golden. Pores of lateral line scales edged dorsally and ventrally with melanophores.

LIGHT-ORGAN SYSTEM (LOS) (fig. 3): Sexual dimorphism of the light organ and associated structures is not detected. The light organ of males is not enlarged compared to the similarly sized conspecific female and does not exhibit any apparent shape dimorphism. The light organ is a comparatively simple, dorsoventrally compressed, doughnut-shaped structure surrounding the esophagus (fig. 3B). Neither the dorsal nor ventral lobes of males are hypertrophied compared to the conspecific female examined. Likewise, associated structures of the LOS (e.g., clearing of the gasbladder lining, modifications of the integument) do not exhibit any sexually dimorphic attributes. As in all other leiognathids, the posteroventral margin of the gasbladder chamber and the small, thin anteroventral patch separating the light organ from the gasbladder are transparent. This 'window' is sparsely 'peppered' with iridescent silvery and bluish chromatophores. No lateral clearing of the silvery gasbladder lining is evident (fig. 3B). Externally sexually dimorphic features of the LOS (i.e., male specific transparent patches or stripes in the opercular region or on the flanks) are absent (figs. 3A, 4).

DISTRIBUTION: Known at this time only from market specimens purchased in Singapore. Given that the Singapore fleet fishes throughout much of the Indo-Pacific basin, we are unable to report the collection locality of the type series. The overall similarity of *L. robustus* to *L. equulus*, a common and widespread species, or to other large leiognathid species such as *L. dussumieri* or *L. fasciatus*, suggest that *L. robustus* may be easily misidentified, and therefore may traditionally have been overlooked in collections.

ETYMOLOGY: Named in reference to the robust nature and large size of the species compared to all congeners except *L. equulus*, its sister taxon, and *L. fasciatus*. The specific epithet, *robustus*, is used as an adjective.

DISCUSSION

COMPARISONS: This study describes *Leiognathus robustus*, a new species and the second member of the non-sexually dimorphic clade of leiognathid fishes recovered by analysis of nucleotide characters (fig. 2, clade B). Like *L. equulus*, the other member of this clade, the light organ and associated tissues of the LOS are not sexually dimorphic. The clade formed by these two species is quite distinct from all other leiognathids based on both morphology of the LOS (Sparks and Dunlap, in review) and the analysis of nucleotide characters.

Leiognathus robustus is easily misidentified as *L. equulus* (fig. 7) and is also similar in overall morphology to *L. fasciatus* and *L. longispinis*. These species exhibit no obvious external sexual dimorphism, grow to large size for leiognathids (>150 mm SL) (Woodland et al., 2001; Froese and Pauly, 2003; personal obs.), and exhibit similar overall body shape, coloration, and pigmentation. *Leiognathus fasciatus* males, however, bear moderately enlarged light organs compared to similarly sized conspecific females, though like *L. robustus* they lack any external sexually dimorphic features of the LOS (McFall-Ngai and Dunlap, 1984; Sparks and Dunlap, in review). It is not known at this time whether the LOS of *L. longispinis* is sexually dimorphic; suitable material currently is lacking.

Leiognathus robustus is distinguished from *L. equulus* (Forsskål, 1775) by the features listed above under the differential diagnosis (viz. the absence of an occipital hump [vs. prominent hump], the presence of

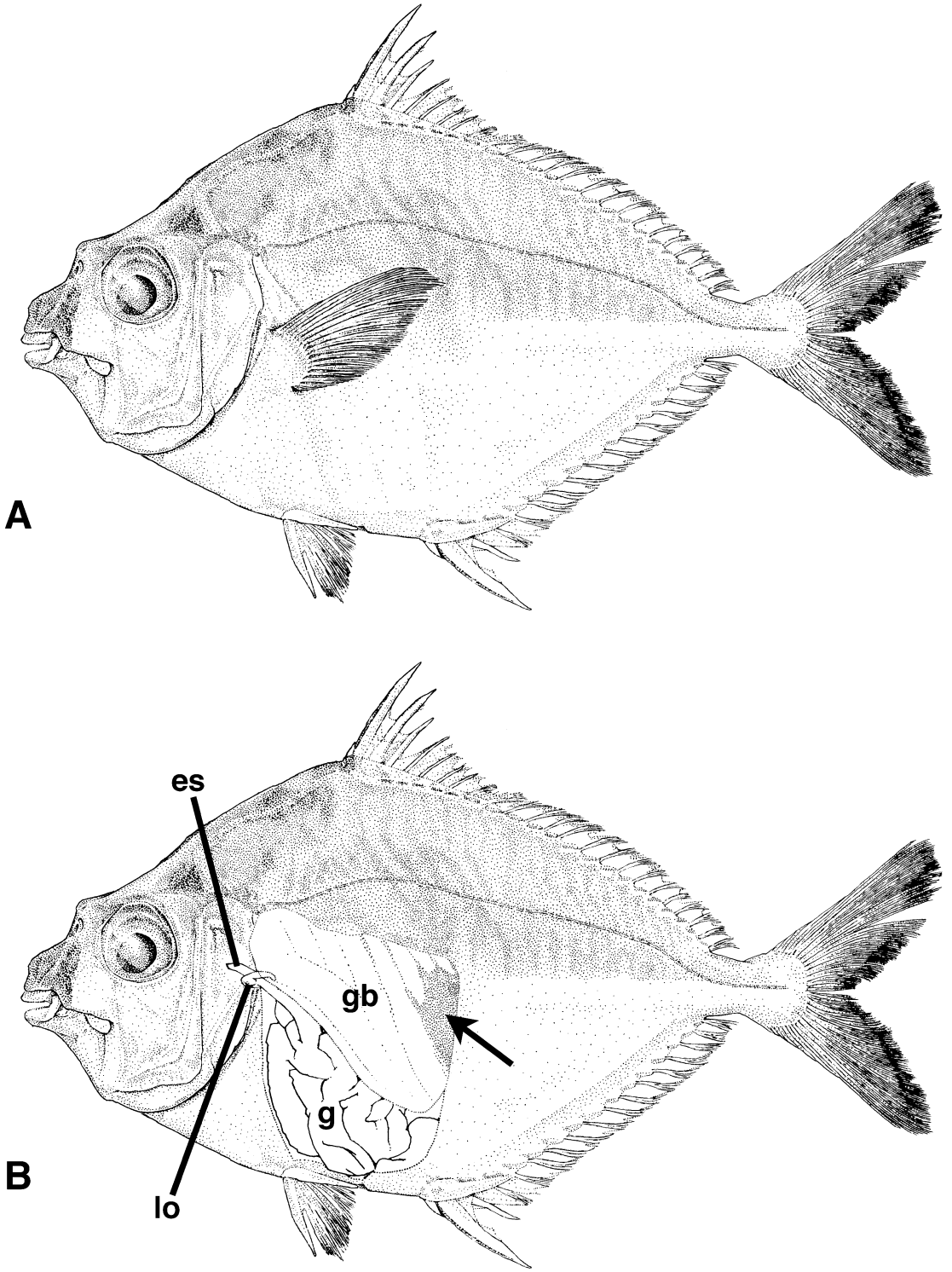

Fig. 3. *Leiognathus robustus*, holotype, UMMZ 242144, 183.4 mm SL, adult male; Singapore. **A**. External anatomy, illustrating general pigmentation pattern, and absence of transparent flank or opercular

Fig. 4. *Leiognathus robustus*, holotype, UMMZ 242144, adult male, 183.4 mm SL, Singapore.

a gently sloping predorsal profile [vs. strongly curved, arched back], a distinct preorbital protuberance [vs. small bump above orbit], a nuchal spine that is not visible in lateral view [vs. exposed and projecting distally]; figs 3–7), and by the analysis of mitochondrial nucleotide characters (fig. 2). In lateral view, the nuchal spine is clearly exposed in both the lectotype and paralectotype of *L. equulus* (ZMUC P48219 and ZMUC P48220, respectively) (fig. 8A). Width of the caudal peduncle (4.7–5.1% in *L. robustus* vs. 3.3–4.7% SL in *L. equulus*) and body depth at origin of the anal fin in adults (52.3–53.4% in *L. robustus* vs. 55.7–56.7% SL in *L. equulus*) are also generally useful features for distinguishing between the new species and *L. equulus*.

The new species is distinguished from *L. dussumieri* (Valenciennes, 1835) by the absence of scales on the breast (vs. conspicuous in *L. dussumieri*; Woodland et al., 2001), the absence of a yellow patch between the pectoral and anal fins (although this patch could be faded in preservation and not discernable in our material), the absence of distinctive and easily visualized, thin, dark vertical bars on the flanks that extend to just below midline, by short pelvic fins that do not extend to the first anal spine (fig. 3A), and a larger maximum adult size (>150 mm SL and >200 mm TL vs. 140 mm max. TL).

Leiognathus robustus is distinguished from *L. fasciatus* (Lacepède, 1803) by a much shorter second dorsal-fin spine (in *L. fasciatus* this spine is distinctly elongate), by the presence of a non-sexually dimorphic light organ and LOS (vs. light organ moderately enlarged in *L. fasciatus* males), and by the absence of prominent dark vertical bars on the flanks and above the lateral midline.

←

patches. **B**. Schematic of internal LOS morphology. Light organ of males not enlarged and clearing of silvery gasbladder lining restricted to posterior of chamber, a condition common to all leiognathids. No lateral clearing of integument present in region of light organ. Abbreviations: es, esophagus; g, gut; gb, gasbladder; lo, light organ. Shaded region (designated by arrow) indicates clearing of silvery gasbladder lining. Drawings by Ian Hart.

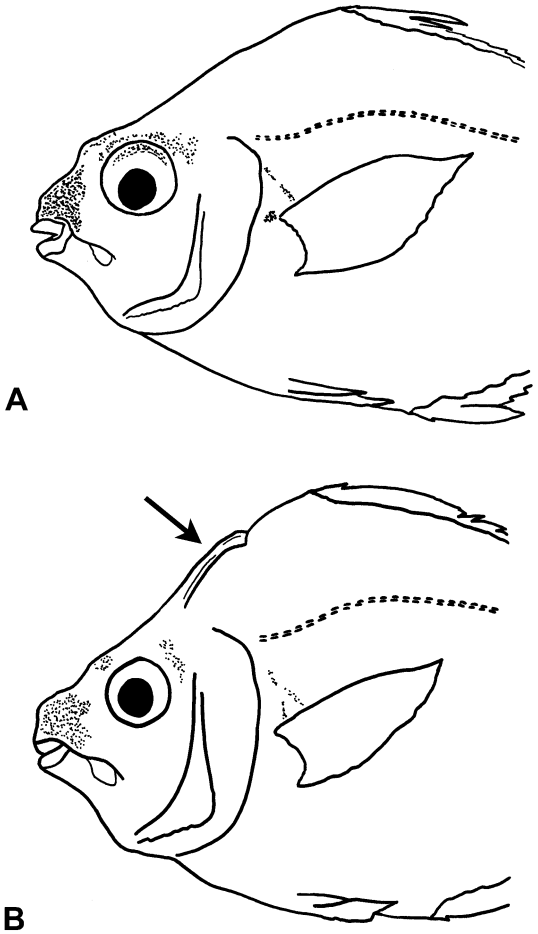

Fig. 5. Left lateral view of head of: (**A**) *Leiognathus robustus*, holotype, UMMZ 242144, adult male, 183.4 mm SL; (**B**) *Leiognathus equulus*, UMMZ 238805, adult male, 173.4 mm SL. Arrow indicates nuchal spine.

The new species is distinguished from *L. longispinis* (Valenciennes, 1835) by markedly shorter second dorsal- and anal-fin spines (vs. spines exceedingly elongate and occasionally reaching to origin of the caudal fin, Woodland et al., 2001). In addition, *L. robustus* lacks the faint and unevenly spaced, dorsal-flank blotches characteristic of *L. longispinis*. As mentioned, it is not currently known whether the LOS of *L. longispinis* exhibits any sexually dimorphic attributes, although we are working to obtain suitable study material.

TAXONOMY: Prior to our molecular phylogenetic analysis of leiognathid intrafamilial relationships, *Gazza*, *Leiognathus*, and *Secutor* were each generally assumed to be monophyletic. Our analysis of nucleotide characters revealed however that while *Gazza* and *Secutor* are indeed monophyletic, the genus *Leiognathus* comprises a paraphyletic assemblage (see also Sparks and Dunlap, in review) (fig. 2). Consistent with this finding, apomorphic features supporting monophyly of *Gazza* and *Secutor* have been identified (e.g., Mochizuki and Hayashi, 1989; Kimura et al., 2000; Woodland et al., 2001; Sparks and Dunlap, in review), whereas none has been advanced to unite members of *Leiognathus*. Based on features of the LOS, members of *Leiognathus* comprise three morphologically distinct groups (fig. 2): a clade whose members possess non-sexually dimorphic light organs and that exhibit no internal or external sexually dimorphic attributes of the LOS (clade B; fig. 1E, F), and two clades nested within clade C whose members possess sexually dimorphic light organs, most of which also possess associated internal and external sexually dimorphic features of the LOS (fig. 1A–D). One of these latter sexually dimorphic clades is the sister taxon to a clade comprising all members of *Gazza* and *Secutor* (fig. 2). Thus, the generally accepted but erroneous classification scheme of leiognathids based on overall external similarity is now corrected in favor of a scheme based on derived features of the LOS (Sparks and Dunlap, in review).

Given that *Leiognathus* must now be recognized as a paraphyletic assemblage, provenance of the generic name is problematic. Forsskål (1775) described the first fish we currently recognize as a leiognathid, *Scomber equula*, from two dry skins collected in the Red Sea off of Yemen (fig. 8A). Klausewitz and Nielsen (1965: 23) later designated a lectotype and paralectotype from these specimens. We have examined photographs and radiographs of both type specimens (ZMUC P48219, lectotype, 131 mm SL; ZMUC P48220, paralectotype, 120 mm SL). The diagnostic arched back and prominent nuchal spine of specimens subsequently attributed to *L. equulus* are clearly visible in both the radiographs and photographs of the type specimens (fig. 8A). Although the lec-

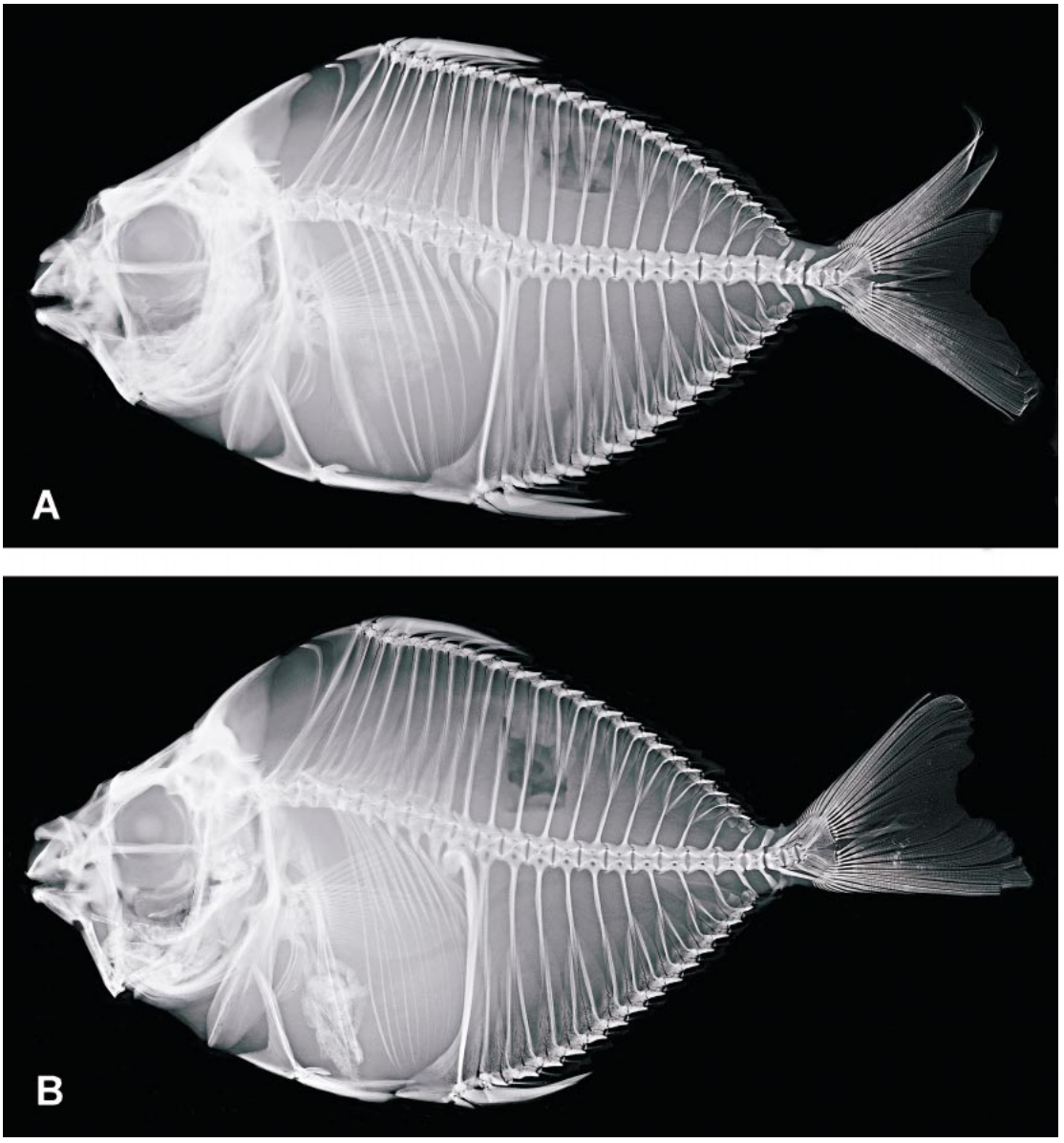

Fig. 6. Comparative radiographs of similarly sized (**A**) *Leiognathus robustus*, holotype, UMMZ 242144, adult male, 183.4 mm SL, and (**B**) *Leiognathus equulus*, UMMZ 238805, adult male, 173.4 mm SL.

totype and paralectotype of *Scomber equula* could not be examined directly due to their delicate nature, all relevant anatomical features, aside from those of the LOS, could easily be visualized via the examination of detailed photographs and radiographs.

Twenty years subsequent to Forsskål's description of *Scomber equula*, Bloch (1795) described *Scomber edentulus*, apparently from a single specimen, ZMB 8756 (dry left skin), for which he provided no collection locality (fig. 8B). Thereafter, Lacepède (1802) described the genus *Leiognathus* from the same specimen (ZMB 8756), which he

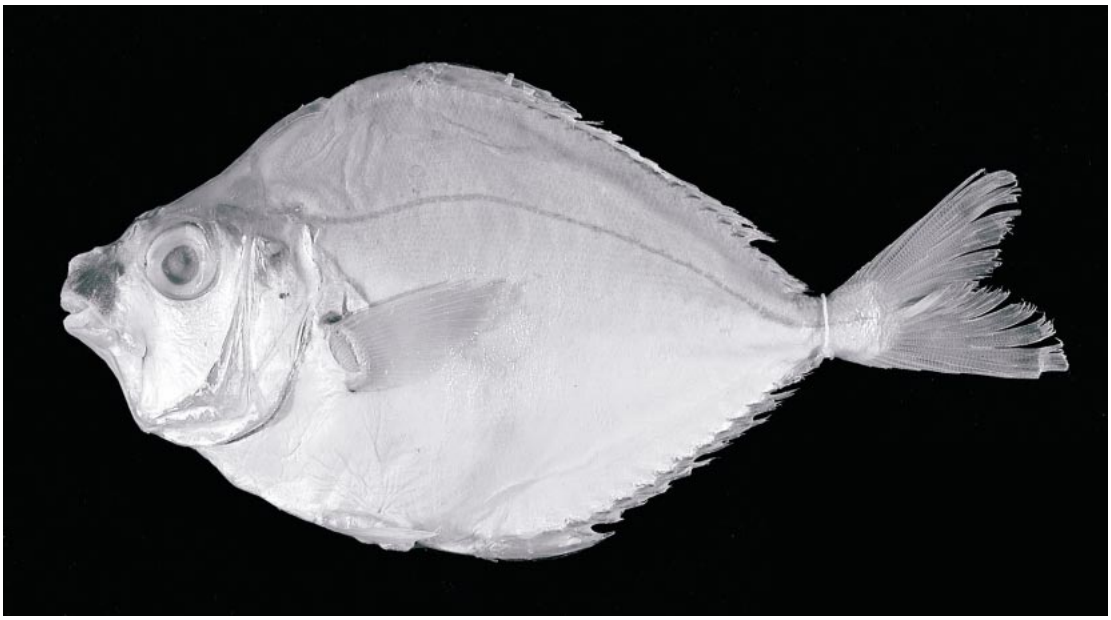

Fig. 7. *Leiognathus equulus*, UMMZ 238805, adult male, 173.4 mm SL, Singapore.

listed as having been collected in Tranquebar, India, and designated *L. argenteus* as the type species of the genus. Subsequent to this, Cuvier (1829: 212) described *Equula ensifera* once again from the same specimen (ZMB 8756). As Eschmeyer (1998) pointed out, *Equula ensifera* is an available name from the footnote in Cuvier (1829: 212), ''*Eq. ensifera*, Nob., ou *Scomber edentulus*, Bl., 428, ou *Leyognathe argenté*, Lacep.'' This taxon was described again by Valenciennes in Cuvier and Valenciennes (1835: 66). Cuvier (1815: 463) named *Equula* (''je nommerai *equula*''), with *Scomber equula* (Forsskål, 1775) (= *Centrogaster equula* of Gmelin, 1788) becoming the type species of the genus by absolute tautonymy (Eschmeyer, 1998). The subsequent synonymy of *Leiognathus argenteus* Lacepède, 1802 (= *Scomber edentulus* Bloch) with *Scomber equula* (= *Equula equula*) required *Equula* therefore to become a junior synonym of *Leiognathus* Lacepède, 1802 (James, 1975; Dor, 1984; Fricke, 1999). Eschmeyer (1998) was correct in his assertion that *L. argenteus* was an unneeded new name for *Scomber edentulus* Bloch, 1795. Likewise, *Equula ensifera* was also an unneeded new name for *Scomber edentulus* Bloch, 1795.

Clearly, *Scomber edentulus*, *Leiognathus argenteus*, and *Equula ensifera* are conspecific, having all been described from the same specimen (ZMB 8756), with the name *Scomber edentulus* having priority. However, in the absence of detailed comparative analyses we are hesitant to accept the synonymy of *Scomber edentulus* with *Scomber equula* as James (1975: 145), Dor (1984: 135), and Fricke (1999: 260) have proposed. It is apparent that James (1975), Dor (1984), and Fricke (1999) did not examine and compare type material of the relevant taxa to reach this conclusion. For example, Fricke (1999: 260) stated under ''Remarks'' that the synonymies he listed for *Leiognathus equulus* are a ''Taxonomic decision of James (1975: 145–147).'' Likewise, in the study of James that Fricke refers to, no type specimens of the relevant taxa were examined, and in fact only a single lot of (presumably) *L. equulus* from Indian waters, Palk Bay and the Gulf of Mannar, was listed under ''Material'' by James (1975: 146). James (1975: 147) mentioned that the chest region in the specimens he examined is covered by diaphanous scales; however, we have examined material spanning the putative geographic range of *L. equulus*, including the type series, and con-

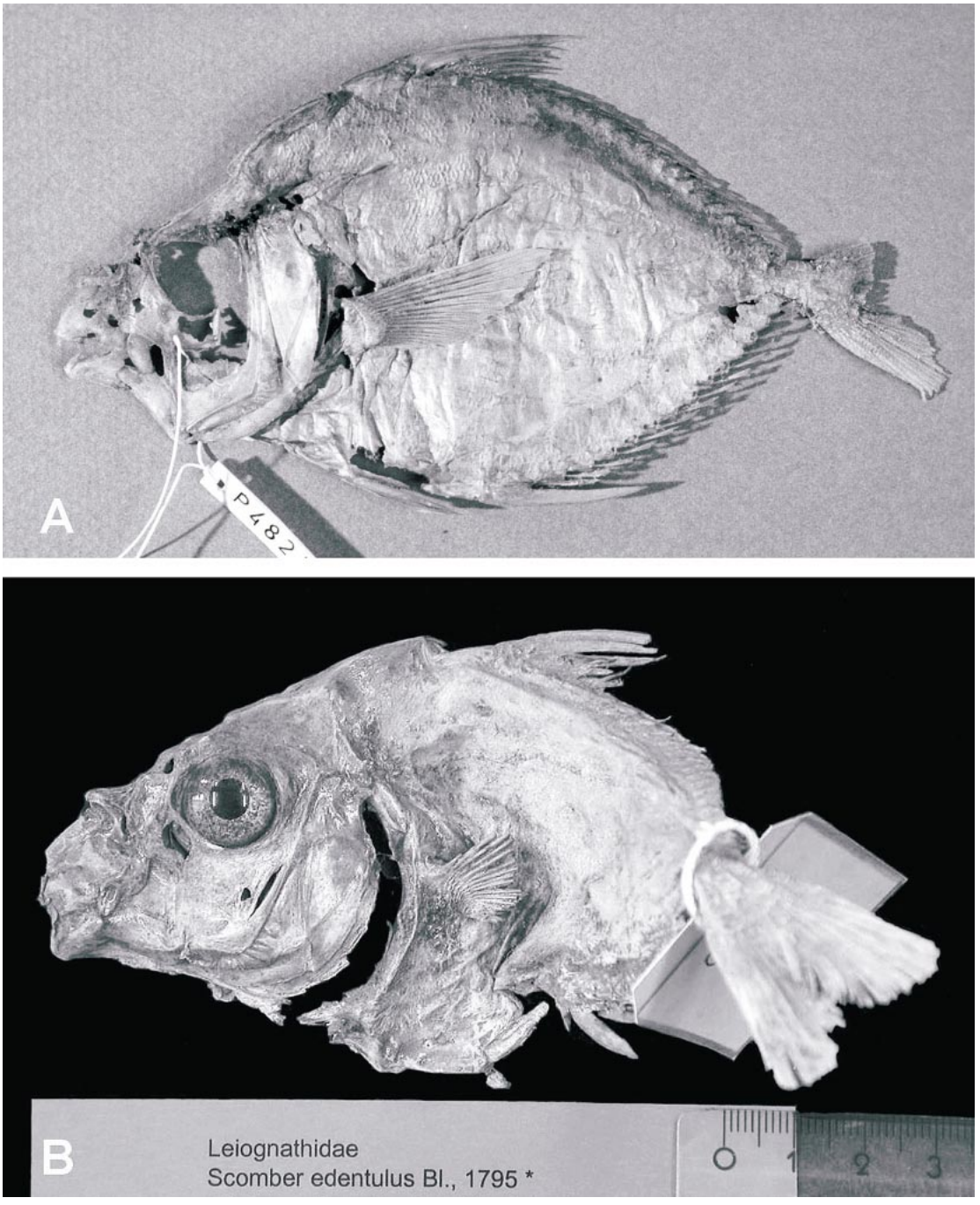

Fig. 8. **A**. *Scomber equula* Forsskål, lectotype, ZMUC P48219, dry skin, 131 mm SL, Yemen: Red Sea: Luhaiya. **B**. *Scomber edentulus* Bloch, holotype, ZMB 8756, dry left skin, India: Tranquebar.

cur with Woodland et al. (2001) that this region is indeed naked in *L. equulus*. Squamation extends anteriorly in this region at most to about the level of a vertical through pectoral-fin insertion. Without the direct comparison of type material for these morphologically very similar taxa, James (1975) certainly had no justifiable means for proposing the synonymies he lists for *L. equulus*. Dor (1984: viii) likewise stated that ''My work is mostly based on the literature'' and nowhere in the text does he present a list of comparative material. Thus, in our opinion the synonymies proposed in these three works cannot be justified at the present time.

The type specimens of both *Scomber equula* and *Scomber edentulus* are dry partial skins, and detailed comparative analyses are problematic (fig. 8). Certainly the illustration of *Scomber edentulus* (Bloch, 1795: pl. 428) does not match well the overall gross external morphology of *L. equulus*. The back (= dorsal profile) does not appear strongly arched as in *L. equulus* and there is no prominent occipital hump. Examination of a photograph of the holotype of *Scomber edentulous*, however, appears to reveal both an arched back and prominent nuchal spine, despite the specimen's poor state of preservation (fig. 8B). Moreover, *L. equulus* lacks the distinctive broad vertical bars present on the flanks of *Scomber edentulus*, and the number of dorsal fin spines in *L. equulus* is seven to eight, never five as illustrated by Bloch (1795: pl. 428). Interestingly, as the species name implies, Bloch (1795) reported that ''the small mouth [is] toothless.'' Contrary to this claim, small teeth are indeed visible in the desiccated lip tissue of the type specimen of *S. edentulus* (P. Bartsch [ZMB], personal comm.] and must have been overlooked by Bloch. These discrepancies could simply be due to errors or to exaggerated features in Bloch's illustration and description, or they could result from damage to the holotype prior to description; however, we think that they are significant enough that we are hesitant to accept the proposed synonymy of *Scomber edentulus* with *Scomber equula*. Even if one could obtain permission to directly examine the types of *Scomber equula* and *Scomber edentulus*, all dry partial skins in poor condition, it likely would not be possible to formulate a formal conclusion regarding the identity of the two synonymized species. Given the poor condition and delicate nature of type material for these taxa, and that features of LOS morphology cannot be studied, we may never be able to reach a firm conclusion regarding the identity and status of *L. argenteus*, the type species of *Leiognathus*. Without direct examination of the LOS we cannot determine the intrafamilial placement of *Leiognathus argenteus* (= *Scomber edentulus*) with certainty. There is little doubt that *Scomber equula* and *Leiognathus argenteus* (= *Scomber edentulus*) are closely related; whether they are conspecific remains to be determined. In light of these limitations, and until a more comprehensive phylogenetic revision of the family is completed, we consider it prudent (and least disruptive) to treat *Scomber equula* as a member of *Leiognathus*. This taxon is the most common and widespread member of the family, and we think that any change in generic assignment at this time, without certainty regarding the placement and status of *L. argenteus* (= *Scomber edentulus*), would be counterproductive and lead to more confusion.

LIGHT ORGAN SYSTEM: To examine evolution of the leiognathid LOS within a phylogenetic context we conducted a parsimony analysis of extant forms based on DNA sequence data from two mitochondrial genes (COI and 16S). Two distinct clades were recovered in this analysis, which revealed a major phylogenetic divergence within Leiognathidae. Members of one clade, which contains most leiognathid species, exhibit sexual dimorphism of the light organ (fig. 2, clade C). Most members of this clade also exhibit sexual dimorphism of the associated tissues of the LOS. Members of the other clade, restricted to *Leiognathus equulus* and *L. robustus*, n. sp., bear light organs that are not sexually dimorphic, and they exhibit no internal or external sexual dimorphism of associated LOS tissues (fig. 2, clade B). Our phylogenetic results indicate that the nondimorphic state is plesiomorphic. Given that presumably all leiognathids are capable of emitting light over their ventral surface (McFall-Ngai and Dunlap, 1983, 1984; McFall-Ngai and Morin, 1991), whereas only some sexually dimorphic species possess the

modifications necessary for lateral luminescence (fig. 2) (McFall-Ngai and Dunlap, 1984; Sparks and Dunlap, in review), we hypothesize that the LOS originally evolved for ventral counterillumination, possibly as a means of avoiding bottom-dwelling predators (Sparks and Dunlap, in review).

The LOS of leiognathid fishes exhibits species-specific differences in the size and shape of the light organs (fig. 1). In addition, most leiognathid species exhibit a sexually dimorphic LOS, with the light organs of males being moderately to highly enlarged compared to that of similarly sized conspecific females (Haneda and Tsuji, 1976; Dunlap and McFall-Ngai, 1984; McFall-Ngai and Dunlap, 1984; Jayabalan and Ramamoorthi, 1985; Jayabalan, 1989; Kimura et al., 2003; Sparks and Dunlap, in review) (fig. 1B, D). For example, the light organ of a male *Leiognathus elongatus* typically is 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) (fig. 1B). In most cases, leiognathids bearing sexually dimorphic light organs also exhibit male-specific transparency of the internal reflective lateral lining of the gasbladder (certain *Leiognathus* species) (figs. 1B, 2), male-specific transparent patches (i.e., windows) on the external lateral flank or behind the pectoral-fin axil (certain *Leiognathus* species) (figs. 1A, C, 2), or male-enhanced transparent patches on the margin of the opercular cavity (*Gazza* and *Secutor*) (fig. 2). The presence of these modifications correlates with hypertrophy of dorsolateral or ventrolateral lobes of the light organ in males, enabling males to emit light laterally (Haneda and Tsuji, 1976; McFall-Ngai and Dunlap, 1984; Kimura et al., 2003). Like 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). A major function of the leiognathid LOS therefore may be mate-specific recognition (Andersson, 1994; Paterson, 1985), with luminescence signaling by males operating 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 and ostracodes (Lloyd, 1966; Morin, 1986; Morin and Cohen, 1991; Branham and Greenfield, 1996). Sexual selection for species-specific luminescence signaling presumably plays a key role in generating and maintaining species diversity within Leiognathidae (Sparks and Dunlap, in review).

Features of the LOS are reliable markers of phylogeny and their use is critical in comparative studies of ponyfishes (fig. 2), the members of which otherwise exhibit a high degree of morphological conservatism (Dunlap and McFall-Ngai, 1984; Sparks and Dunlap, in review). Many taxonomic questions concerning this group of fishes can only be addressed through examination and comparison of LOS features, and even when other morphological characters clearly distinguish species, features of the LOS can serve to corroborate and support the membership of individual species in species assemblages (Kimura et al., 2003). The very substantial diversification of leiognathids bearing sexually dimorphic LOS, evolving apparently under selection pressure for species-specific male luminescence signaling, from a relatively simple, non-sexually dimorphic state that is retained by very few extant species (fig. 2, clade B), accounts for the large number of phylogenetically-informative characters of the LOS (Sparks and Dunlap, in review).

The sexually dimorphic nature of the leiognathid LOS suggests that it functions in mate choice, possibly as a reproductive isolating mechanism similar to that documented for fireflies (Lloyd, 1966) and ostracodes (Morin, 1986; Morin and Cohen, 1991). Although direct observation of unique flashing patterns for most species of leiognathid are currently lacking (McFall-Ngai and Dunlap, 1983; Sasaki et al., 2003), we think that the morphological variation and modifications reported for the LOS in male ponyfishes (Sparks and Dunlap, in review), in light of the recovered phylogeny, present compelling evidence for a system of sexual selection based on species-specific male flashing pattern. We hypothesize that variation of the LOS has permitted a number of morpholog-

ically similar forms to coexist and maintain species fidelity, frequently in habitats with extremely poor visibility. This system, which apparently represents a unique mechanism of sexual selection in fishes, demonstrates that bioluminescent symbiosis has been important not only in determining the patterns of species diversification in ponyfishes, but in maintaining that diversity as well (Sparks and Dunlap, in review). The recovery of a clade of non-sexually dimorphic leiognathids, containing at this time just two member species, *L. equulus* and *L. robustus*, serves as an evolutionarily and ecologically intriguing counterpoint to the much more numerous and phylogenetically diverse, sexually dimorphic ponyfishes.

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APPENDIX 1

MATERIAL EXAMINED

Nucleotide sequences for taxa included in the molecular phylogenetic analysis are deposited in GenBank under accession numbers AY541648–AY541672 for 16S, and AY541623–AY541647 for COI (table 1). The notation ''(in part)'' following some catalog numbers indicates that the alcoholic lot examined was found to contain more than a single species.

LEIOGNATHIDAE

Gazza achlamys: UMMZ 240128; UMMZ 240132; UMMZ 240139.

Gazza minuta: AMNH 220748; AMNH uncat.; UMMZ 191542; UMMZ 240126; UMMZ 240140; UMMZ 240141.

Leiognathus aureus: UMMZ 240129; UMMZ uncat.

Leiognathus bindus: CAS 51097; UMMZ 240131; UMMZ 240142; UMMZ uncat.

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

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

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

Leiognathus elongatus: BMNH 1872.4.6, holotype; CAS 52602; LACM 42993-1; LACM 43584-1; SIO 83-55; USNM 55613; UMMZ 226771; UMMZ 240145; UMMZ uncat.

Leiognathus equulus: ZMUC P48219, lectotype (dry skin; photographs and radiographs examined); ZMUC P48220, paralectotype (dry skin, photograph and radiograph examined); AMNH 59535; AMNH 88039; 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 240360; UMMZ uncat.

Leiognathus fasciatus: AMNH 15520; CAS 1872; UMMZ 240504; UMMZ 240361; UMMZ uncat.

Leiognathus hataii: UMMZ uncat.

Leiognathus cf. *hataii*: AMNH 89922.

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

Leiognathus leuciscus: UMMZ 240125; UMMZ uncat.

Leiognathus longispinis (= *L. smithursti*): AMNH 219296; AMS I.20907036; AMS I.22974001; AMS 22981001; AMS 23044001.

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

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

Leiognathus panayensis: UMMZ 240137; UMMZ uncat.

Leiognathus philippinus: UMMZ 240130.

Leiognathus rivulatus: AMNH 34850; UMMZ 240144; UMMZ uncat.

Leiognathus splendens: CAS 1485; CAS 38789; CAS 56438; CAS 56441; UMMZ 191202; UMMZ uncat.

Leiognathus stercorarius: USNM 55906, holotype; USNM 126395, cotype; CAS 42171, paratype; CAS 17678; CAS-SU 20004, paratype; UMMZ 240138; UMMZ uncat.

Secutor indicius: UMMZ 240127; UMMZ uncat.

Secutor insidiator: CAS 29894; UMMZ uncat.

Secutor megalolepis: UMMZ 240135.

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

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