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Morphological Variations in the Scleral Ossicles of 172 Families of Actinopterygian Fishes with Notes on their Phylogenetic Implications

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Hin-kui Mok and Shu-Hui Liu (2012) Morphological variations in the scleral ossicles of 172 families of actinopterygian fishes with notes on their phylogenetic implications. *Zoological Studies* **51**(8): 1490-1506. This study reports on (1) variations in the number and position of scleral ossicles in 283 actinopterygian species representing 172 families, (2) the distribution of the morphological variants of these bony elements, (3) the phylogenetic significance of these variations, and (4) a phylogenetic hypothesis relevant to the position of the Callionymoidei, Dactylopteridae, and Syngnathoidei based on these osteological variations. The results suggest that the Callionymoidei (not including the Gobiesocidae), Dactylopteridae, and Syngnathoidei are closely related. This conclusion was based on the apomorphic character state of having only the anterior scleral ossicle. Having only the anterior scleral ossicle should have evolved independently in the Syngnathioidei + Dactylopteridae + Callionymoidei, Gobioidei + Apogonidae, and Pleuronectiformes among the actinopterygians studied in this paper. http://zoolstud.sinica.edu.tw/Journals/51.8/1490.pdf

Key words: Scleral ossicle, Actinopterygii, Phylogeny.

Scleral ossicles of the teleostome fish eye comprise a ring of cartilage supporting the eye internally (i.e., the sclerotic ring; Moy-Thomas and Miles 1971). Springer (1983) pointed out that perciform fishes commonly have 2 scleral ossicles in each eye. Miller (1973) indicated the presence of a single scleral ossicle in each eye of *Rhyacichthys*. A single scleral ossicle also occurs in some eleotrids (see Springer 1983, *Terateleotris*, Shibukawa et al. 2001) and callionymids. But other gobioids, including some eleotrids may have only circular scleral cartilage (Springer 1983). Springer (1983: 83) commented that “the value of…. the presence of only one scleral ossicle in each eye for basing gobioid intra- and/or interrelationships, is, perhaps, worthy of further investigation.” Fink and Fink (1981) found that scleral ossicles were absent from their Siluriformes (including the Gymnotoidei and Siluroidei). Besides these reports, teleost scleral ossicles and scleral cartilage have received little attention. It was not until a recent paper by Franz-Odendaal and Hall (2006) that the homology of skeletal elements within teleost eyes and other vertebrates was considered; notably, the teleost scleral skeletal elements might not be homologous to those in reptiles. The origin and evolution of the cartilaginous and bony components in the vertebrate ocular skeleton were studied by Franz-Odendaal (2012) from an evolutionary developmental approach. Franz-Odendaal and Hall (2006) concluded that from an ancestral condition of 4 scleral ossicles, there have been both gains and losses in the number of scleral ossicles within actinopterygians. Although scleral ossicles are variable in size in the 375 species they examined and mentioned in their article, no further information about these skeletal elements was provided. More-recent extensive work by Franz-

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Odendaal (2008) on scleral ossicles of teleosts included a total of 547 species from 36 orders and 163 families. According to her study, (1) the scleral ossicle number was found to decrease from the basal condition of 4 elements per eye to no ossicles in eyes of derived teleosts; (2) the scleral ossicle number was not found to be correlated with the phylogenetic distribution at the ordinal level, but some trends were identified with the activity and vertical distribution of fishes; and (3) the character state of having a single scleral ossicle was only seen in derived Percomorphs, namely the Tetraodontiformes, Pleuronectiformes, Perciformes, and Scorpaeniformes. However, species with this character state were not provided in that paper.

On the basis of the above results and suggestions, we made an extensive survey of scleral and other skeletal elements over a wide spectrum of actinopterygians. Our preliminary observations made on these structures revealed other informative variations of scleral ossicles in the examined actinopterygians. The objectives of this study were to (1) describe the variation in the number and position of scleral ossicles in 283 actinopterygian species representing 172 families, (2) elucidate the possible phylogenetic trend between the morphological variants of these bony elements, and (3) apply the morphological variations of these ossicles to resolve phylogenetic relationships of some relevant actinopterygian groups.

MATERIALS AND METHODS

The entire specimen of a studied species or one of its eyes was cleared and stained following the method of Dingerkus and Uhler (1977). The scleral cartilage and ossicles were observed under a dissecting microscope. The number, size (estimated by the length relative to the circumference of the scleral cartilage), shape of the scleral ossicles, and their position relative to the eye axes and to each other were recorded. On the basis of the concept of cladistics (Hennig 1966, Nelson and Platnick 1981), morphological characters were reviewed in a variety of actinopterygian taxa in order to make hypotheses of character homology, synapomorphy, and monophyly among relevant taxa.

In total, 283 actinopterygian species were examined (Table 1). The vast majority of specimens examined were of un-catalogued materials in our personal possession, while some were specimens from museums: AMNH, American Museum of Natural History (New York, USA); AMS, Australian Museum (Sydney, Australia); BSKU, Laboratory of Marine Biology, and the Department of Environmental Science, Kochi Univ. (Kochi, Japan); and NMMB, National Museum of Marine Biology and Aquarium (Checheng, Pingtung, Taiwan).

RESULTS

The number, size, and position of scleral ossicles observed in 39 actinopterygian orders are listed in table 1. Scleral ossicles appeared as early as in elopiforms among the actinopterygians we examined. Numbers of ossicles in osteichthyans varied from 0 to 4 with a mode of 2. Within the 39 orders examined, 24 orders (62%) had no ossicles as a condition within the order; and 7 orders had 1 scleral ossicle, 19 orders (49%) had 2 scleral ossicles as an option, and only 2 orders had 3 or 4 scleral ossicles. In the majority of these fishes, the centers of the 2 scleral ossicles were on a horizontal axis with the eye; there were only a few exceptions in which the center of the ossicle did not lie on this axis (see below), and the 2 ossicles were only symmetrical with the vertical axis of the eye. The size and shape of the 2 scleral ossicles were similar in most species, with some exceptions in which these parameters differed between the 2 ossicles in the same eyeball (e.g., *Esox*, *Oreochromis*, embiotocids, and labrids). No detailed information on size differences between the 2 ossicles is given in this paper. Unless stated otherwise, species examined in this study had 2 symmetric scleral ossicles located at 0° and 180° on the horizontal axis of the eye.

Within-species variability of the size, shape, and location of the 2 scleral ossicles in most actinopterygians was considered to be low based on data from 12 serranids of the genus *Epinephelus* and 12 specimens of shrimpfish *Aeoliscus strigatus* (Günther). The former was selected as a representative of basal percoids, whereas the latter was selected to decipher variations in the position of the scleral ossicle (see below).

1. Three to 4 scleral ossicles

Two to 3 stripes of ribbon-like ossicles (*Elops machnata* (Forsskål), 3 ossicles; *Albula*

Table 1. Taxa included in this study and their classification following Nelson (2006)

Subclass / Division / Subdivision	Order / Suborder	Family	Species	Ossicle type[a]	Specimens[b]
Cladistia					
	Polypteriformes	Polypteridae	*Polypterus* sp.	0	1 (110 mm)
Chrondorstei					
	Acipenseriformes	Acipsensieridae	*Acipenser* sp.	0	1 (220 mm)
Neopterygii					
	Lepisosteiformes	Lepisosteidae	*Lepisosteus* sp.	0	1 (172 mm)
	Amiiformes	Amiidae	*Amia calva* Linnaeus	0	1 (195 mm)
	Teleostei				
	Osteoglossomorpha				
	Osteoglossiformes	Notopteridae	*Notopterus notopterus* (Pallas)	0	1 (195 mm)
		Osteoglossidae	*Osteoglossum bicirrhosum* (Cuvier)	0	1 (165 mm)
		Mormyridae	*Brienomyrus niger* (Günther)	0	1 (90 mm)
			Gnathonemus sp.	0	1 (75 mm)
	Elopomorpha				
	Elopiformes	Elopidae	*Elops machnata* (Forsskål)	4	1 (125 mm)
		Megalopidae	*Megalops cyprinoides* (Broussonet)	3	2 (260, 380 mm)
	Albuliformes	Albulidae	*Albula glossodonta* (Forsskål)	3	1 (320 mm)
	Auguilliformes	Synaphobranchidae	*Dysomma anguillare* Barnard	0	1 (605 mm)
			Simenchelys parasitica Gill	0	1 (285 mm)
		Congridae	*Ariosoma anago* (Temminck & Schlegel)	0	1 (350 mm)
			Rhynchoconger ectenurus (Jordan & Richardson)	0	1 (660 mm)
		Muraenidae	*Gymnothorax fimbriatus* (Bennett)	0	1 (275 mm)
	Notacathiformes	Notacanthidae	*Notacanthus* sp.	0	1 (250 mm)
	Ostarioclupeomorph				
	Clupeiformes	Pristigasteridae	*Ilisha elongata* (Anonymous [Bennett])	2A	1 (170 mm)
			Spratelloides gracilis (Temminck & Schlegel)	2B	1 (54 mm)
		Engraulidae	*Engraulis japonicus* Temminck & Schlegel	2B	1 (120 mm)
			Setipinna taty (Valenciennes)	2B	1 (136 mm)
			Lycengraulis poeyi (Kner, 1863) (AMNH4211)	2B	1 (75 mm)
			Stolephorus sp.	2C	1 (60 mm)
		Chirocentridae	*Chirocentrus dorab* (Forsskål)	2A	1 (37.5 mm)
	Gonorynchiformes	Chanidae	*Chanos chanos* (Forsskål)	2D	1 (305 mm)
		Gonorynchidae	*Gonorynchus abbreviates* Temminck & Schlegel	1pD	2 (152, 180 mm)
	Cypriniformes	Cyprinidae	*Cyprinus carpio* Linnaeus	2C	1 (135 mm)
			Hemiculter leucisculus (Basilewsky)	2B	1 (132 mm)
		Balitoridae	*Crossostoma lacustre* Steindachner	2C	1 (42 mm)
			Hemimyzon formosanus (Boulenger)	2D	1 (40 mm)
		Cobitidae	*Misgurnus anguillicaudatus* (Cantor)	0	1 (84 mm)
	Siluriformes	Ariidae	*Arius maculatus* (Thunberg)	0	1 (130 mm)
		Plotosidae	*Plotosus lineatus* (Thunberg)	0	1 (145 mm)
		Loricariidae	*Hypostomus* sp.	0	1 (55 mm)
	Characiformes	Characidae	*Moenkhausia* sp.	2A	1 (30 mm)
		Gasteropelecidae	*Carnegiella* sp.	1r	1 (28 mm)
	Euteleostei				
	Esociformes	Esocidae	*Esox americanus* Gmelin	2C	1 (176 mm)
	Argentiniformes	Argentinidae	*Argentina kagoshimae* Jordan & Snyder	2C	1 (165 mm)
		Alepocephalidae	*Alepocephalus bicolor* Alcock	0	2 (170, 194 mm)
	Osmeriformes	Galaxiidae	*Galaxias maculatus* (Jenyns)	0	1 (70 mm)
	Stomiiformes	Stomiidae	*Astronesthes lucifer* Gilbert	0	2 (80, 120 mm)
	Aulopiformes	Chlorophthalmidae	*Chlorophthalmus nigromarginatus* Kamohara	2C	2 (110,160 mm)
		Synodontidae	*Saurida undosquamis* (Richardson)	2D	1 (95 mm)
			Trachinocephalus myops Bloch & Schneider	2C	1 (70 mm)
		Paralepididae	*Lestrolepis japonica* (Tanaka)	0	1 (190 mm)
	Myctophiformes	Myctophidae	*Benthosema pterotum* (Alcock)	0	1 (38 mm)

Table 1. (Continued)

Subclass / Division / Subdivision / Order / Suborder	Family	Species	Ossicle type[a]	Specimens[b]
		Ceratoscopelus warmingii (Lütken)	0	1 (26 mm)
		Diaphus sagamiensis Gilbert	0	1 (82 mm)
		Lampanyctus alatus Goode & Bean	0	1 (28 mm)
		Myctophum obtusirostre Tåning	0	1 (60 mm)
	Neoscopelidae	*Neoscopelus microchir* Matsubara	0	1 (150 mm)
	Neoscopelidae	*Neoscopelus microchir* Matsubara	0	1 (150 mm)
Percopsiformes	Percopsidae	*Percopsis omiscomaycus* (Walbaum)	0	1 (50 mm)
Gadiformes	Bregmacerotidae	*Bregmaceros* sp.	0	1 (84 mm)
	Macrouridae	*Hymenocephalus striatissimus* Jordan & Gilbert	0	1 (104 mm)
Ophidiiformes	Ophidiidae	*Neobythites stigmosus* Machida	0	1 (135 mm)
	Bythitidae	*Diancistrus fuscus* (Fowler)	0	1 (42 mm)
		Porocephalichthys dasyrhynchus (Cohea & Hutchins)	0	1 (72 mm)
Batrachoidiformes	Batrachoididae	*Allenbatrachus grunniens* (Linnaeus)	0	1 (100 mm)
Lophiiformes	Lophiidae	*Lophiomus setigerus* (Vahl)	0	1 (135 mm)
	Himantolophidae	*Himantolophus* sp.	0	1 (74 mm)
Lampriformes	Veliferidae	*Velifer hypselopterus* Bleeker	2C	1 (140 mm)
Polymixiiformes	Polymixiidae	*Polymixia japonica* Günther	2D	1 (65 mm)
Mugiliformes	Mugilidae	*Mugil cephalus* Linnaeus	2C	1 (110 mm)
Atheriniformes	Atherinidae	*Atherinomorus lacunosus* (Forster)	2B	1 (90 mm)
	Melanotaeniidae	*Melanotaenia* sp.	2C	1 (60 mm)
Beloniformes	Exocoetidae	*Exocoetus monocirrhus* Richardson	2C	1 (150 mm)
	Scomberesocidae	*Cololabis saira* (Brevoort)	2A	1 (260 mm)
Cyprinodontiformes	Fundulidae	*Fundulus heteroclitus* (Linnaeus)	0	1 (70 mm)
	Poeciliidae	*Gambusia affinis* (Baird & Girard)	2B	1 (38 mm)
Beryciformes	Anomalopidae	*Anomalops katoptron* (Bleeker)	0	1 (65 mm)
	Berycidae	*Centroberyx rubricaudus* Liu & Shen	2C	1 (160 mm)
	Holocentridae	*Sargocentron rubrum* (Forsskål)	2C	1 (46 mm)
	Trachichthyidae	*Hoplostethus crassispinus* Kotlyar	0	1 (58 mm)
Zeiformes	Parazenidae	*Parazen pacificus* Kamohara	0	1 (100 mm)
		Cyttopsis rosea (Lowe)	0	1 (42 mm)
Gasterosteiformes	Gasterosteidae	*Culaea inconstans* (Kirtland)	0	1 (44 mm)
	Pegasidae	*Pegasus laternarius* Cuvier	0	5 (78, 80, 83, 83, 84 mm)
	Aulostomidae	*Aulostomus chinensis* (Linnaeus)	2B	1 (425 mm)
	Fistulariidae	*Fistularia petimba* Lacepède	0	3 (131, 284, 334 mm)
	Macroramphosidae	*Macroramphosus scolopax* (Linnaeus)	1adC	2 (80, 150 mm)
	Centriscidae	*Aeoliscus strigatus* (Günther)	1adC	12 (82, 113, 115, 118, 120, 120, 122, 122, 122, 125, 125, 140 mm)
	Syngnathidae	*Trachyrhamphus serratus* (Temminck & Schlegel)	0	2 (240, 245 mm)
		Hippocampus kuda Bleeker	0	1 (150 mm)
Scorpaeniformes	Dactylopteridae	*Dactyloptena orientalis* (Cuvier)	1aD	4 (86, 110, 144, 257 mm)
		Dactyloptena gilberti Snyder	1aC	1 (140 mm)
	Scorpaenidae	*Parapterois heterurus* (Bleeker)	2D	1 (75 mm)
		Pontinus tentacularis Fowler	0	1 (40 mm)
		Scorpaenodes guamensis (Quoy & Gaimard)	2C	1 (72 mm)
	Hexagrammidae	*Hexagrammos otakii* Jordan & Starks	2B	1 (121 mm)
	Triglidae	*Pterygotrigla hemistica* (Temminck & Schlegel)	2C	1 (210 mm)
	Platycephalidae	*Inegocia japonica* (Tilesius)	2D	1 (79 mm)
		Onigocia spinosa (Temminck & Schlegel)	2D	1 (30 mm)

Table 1. (Continued)

Subclass Division Subdivision Order Suborder	Family	Species	Ossicle type[a]	Specimens[b]
		Sorsogona tuberculata (Cuvier)	2C	1 (110 mm)
	Hoplichthyidae	*Hoplichthys ogilbyi* McCulloch	0	1 (130 mm, AMSI. 25800-018)[b]
		Hoplichthys regani Jordan	0	1 (85 mm)
Perciformes				
Percoidei	Acropomatidae	*Acropoma japonicum* Günther	2C	1 (66 mm)
		Doederleinia berycoides (Hilgendorf)	2C	1 (140 mm)
		Malakichthys wakiyae Jordan & Hubbs	2C	1 (70 mm)
		Synagrops serratospinosus Smith & Radcliffe	2C	1 (65 mm)
	Ambassidae	*Ambassis urotaenia* Bleeker	2B	1 (40 mm)
	Serranidae	*Cephalopholis argus* Bloch & Schneider	2B	1 (95 mm)
		Cephalopholis miniata (Forsskål)	2B	1 (164 mm)
		Chromileptes altivelis (Valenciennes)	2B	2 (58, 78 mm)
		Epinephelus sp.	2B	5 (45, 48,48, 50, 50 mm)
		Plectranthias sp.	2C	1 (62 mm)
	Grammistidae	*Grammistes sexlineatus* (Thunberg)	2B	1 (72 mm)
		Diploprion bifasciatum Cuvier	2B	1 (100 mm)
	Plesiopidae	*Calloplesiops altivelis* (Steindachner)	2C	1 (130 mm)
	Glaucosomatidae	*Glaucosoma buergeri* Richardson	2C	1 (130 mm)
	Terapontidae	*Terapon jarbua* (Forsskål)	2B	1 (65 mm)
	Banjosidae	*Banjos banjos* (Richardson)	2D	1 (70 mm)
	Kuhliidae	*Kuhlia mugil* (Forster)	2C	1 (71 mm)
	Centrarchidae	*Lepomis* sp.	2C	1 (52 mm)
	Percidae	*Perca* sp.	2B	1 (50 mm)
	Priacanthidae	*Heteropriacanthus cruentatus* (Lacepède)	2C	2 (178, 250 mm)
		Cookeolus boops (Bloch & Schneider)	2C	1 (258 mm)
		Priacanthus blochii Bleeker	2C	1 (80 mm)
		Priacanthus macracanthus Cuveir	2C	2 (60, 130 mm)
		Priacanthus tayenus Richardson	2C	1 (252 mm)
		Pristigenys niphonia (Cuvier)	2C	1 (130 mm)
	Apogonidae	*Apogon aureus* (Lacepède)	2C	1 (105 mm)
		Apogon endekataenia Bleeker	2C	1 (45 mm)
		Apogon novemfasciatus Cuvier	2C	1 (54 mm)
		Archamia lineolata (Cuvier)	2C	1 (50 mm)
	Sillaginidae	*Sillago maculata* Quoy & Gaimard	2B	1 (130 mm)
	Malacanthidae	*Branchiostegus japonicus* (Houttuyn)	2B	1 (130 mm)
		Malacanthus brevirostris Guichenot	2B	2 (173, 176 mm)
	Scombropidae	*Scombrops boops* (Houttuyn)	2C	1 (320 mm)
	Pomatomidae	*Pomatomus saltatrix* (Linnaeus)	2C	2 (80, 140 mm)
	Coryphaenidae	*Coryphaena hippurus* Linnaeus	2A	1 (450 mm)
	Menidae	*Mene maculata* (Bloch & Schneider)	2A	2 (60, 130 mm)
	Leiognathidae	*Eubleekeria splendens* (Cuvier)	2A	2 (65, 85 mm)
		Gazza minuta (Bloch)	2A	1 (65 mm)
		Secutor insidiator (Bloch)	2A	1 (88 mm)
	Bramidae	*Brama japonica* Hilgendorf	2D	1 (230 mm)
		Taractichthys steindachneri (Döderlein)	0	1 (98 mm)
	Lutjanidae	*Lutjanus monostigma* (Cuvier)	2C	2 (70, 80 mm)
		Paracaesio caeruleus (Katayama)	2A	1 (202 mm)
	Lobotidae	*Lobotes surinamensis* (Bloch)	2C	1 (254 mm)
	Gerreidae	*Eucinostomus gula* (Quoy & Gaimard)	2C	1 (66 mm)
		Gerres filamentosus Cuvier	2C	1 (75 mm)
	Haemulidae	*Plectorhinchus pictus* (Tortonese)	2C	1 (108 mm)
	Sparidae	*Acanthopagrus schlegelii* (Bleeker)	2B	1 (178 mm)

Table 1. (Continued)

Subclass Division Subdivision Order Suborder	Family	Species	Ossicle type[a]	Specimens[b]
	Lethrinidae	*Lethrinus* sp.	2B	1 (73 mm)
	Nemipteridae	*Nemipterus japonicus* (Bloch)	2B	1 (135 mm)
		Paracaesio coeruleus (Katayama)	2A	1 (200 mm)
		Scolopsis vosmeri (Bloch)	2B	1 (120 mm)
	Caesionidae	*Pterocaesio diagramma* (Bleeker)	2A	2 (174, 178 mm)
	Sciaenidae	*Johnius dussumieri* (Cuvier)	2C	1 (143 mm)
		Johnius distinctus (Tang)	2C	1 (148 mm)
		Larimichthys crocea (Richardson)	2B	1 (95 mm)
		Otolithes ruber (Bloch & Schneider)	2B	1 (114 mm)
		Pennahia pawak (Lin)	2B	1 (100 mm)
	Mullidae	*Mulloidichthys flavolineatus* (Lacepède)	2B	1 (100 mm)
		M. vanicolensis (Valenciennes)	2B	1 (84 mm)
		Mullus auratus Jordan & Gilbert	2B	1 (114 mm) (UF11667)
		Parupeneus chrysopleuron (Temminck & Schlegel)	2B	1 (127 mm)
		P. cyclostomus (Lacepède)	2B	1 (70 mm)
		P. multifasciatus (Quoy & Gaimard)	2B	1 (150 mm)
		P. pleurostigma (Bennett)	2B	1 (190 mm)
		P. spilurus (Bleeker)	2B	1 (115 mm)
		Pseudupeneus grandisquamis (Gill)	2B	1 (145 mm, SIO68-72-39)
		Upeneus japonicus (Houttuyn)	2B	2 (40, 62 mm)
		U. tragula Richardson	2B	1 (77 mm)
		U. moluccensis (Bleeker)	2B	1 (89 mm)
		Upeneichthys lineatus (Bloch & Schneider)	2B	1 (210 mm, (NMNZ P.21610)
	Monodactylidae	*Monodactylus argenteus* (Linnaeus)	2B	1 (58 mm)
	Pempheridae	*Pempheris oualensis* Cuvier	2C	1 (110 mm)
	Kyphosidae	*Girella punctata* Gray	2D	1 (50 mm)
		Kyphosus vaigiensis (Quoy & Gaimard)	2C	1 (130 mm)
	Toxotidae	*Toxotes* sp.	2B	1 (90 mm)
	Drepaneidae	*Drepane punctata* (Linnaeus)	2A	1 (142 mm)
	Chaetodontidae	*Chaetodon* sp.	2C	1 (86 mm)
		Forcipiger longirostris (Broussonet)	2C	1 (90 mm)
		Heniochus varius (Cuvier)	2C	1 (45 mm)
	Pomacanthidae	*Pomacanthus imperator* (Bloch)	2B	1 (142 mm)
	Histiperidae	*Pentaceros capensis* Cuvier	2C	1 (128 mm)
	Nandidae	*Nandus nandus* (Hamilton)	0	1 (70 mm)
	Oplegnathidae	*Oplegnathus punctatus* (Temminck & Schlegel)	2B	1 (312 mm)
	Cichlidae	*Oreochromis mossambicus* (Peters)	2B	2 (65, 130 mm)
		Pseudtropheus sp.	2C	1 (62 mm)
	Embiotocidae	*Hysterocarpus traskii* Gibbons	2C	1 (150 mm)
	Pomacentridae	*Abudefduf sexfasciatus* (Lacepède)	2A	1 (52 mm)
		Amphiprion clarkii (Bennett)	2A	1 (98 mm)
		A. frenatus Brevoort	2A	1 (72 mm)
		Chrysiptera brownriggii (Bennett)	2A	1 (54 mm)
		Pomacentrus nigromarginatus Allen	2A	1 (48 mm)
	Cirrhitidae	*Cirrhitus pinnulatus* (Forster)	2B	1 (135 mm)
		Oxycirrhites typus Bleeker	2B	1 (68 mm)
	Cheilodactylidae	*Goniistius zonatus* Cuvier	2C	1 (125 mm)
	Cepolidae	*Acanthocepola indica* (Day)	2avD	2 (195, 228 mm)
		Acanthocepola krusensternii (Temminck & Schlegel)	2avD	1 (201 mm)
		Owstonia tosaensis Kamohara	2avD	2 (80, 194 mm)

Table 1. (Continued)

Subclass / Division / Subdivision / Order / Suborder	Family	Species	Ossicle type[a]	Specimens[b]
	Polynemidae	*Polydactylus microstomus* (Bleeker)	2A	1 (120 mm)
	Opistognathidae	*Opistognathus aurifrons* (Jordan & Thompson)	0	1 (56 mm)
		O. hongkongiensis Chan	1pD	1 (110 mm)
		O. randalli Smith-Vaniz	1pD	1 (85 mm)
		O. darwiniensis Macleay	0	1 (120 mm)
Carangoidei	Rachycentridae	*Rachycentron canadum* (Linnaeus)	2B	1 (132 mm)
	Echeneidae	*Echeneis naucrates* Linnaeus	2C	1 (120 mm)
	Carangidae	*Alectis indicus* (Rüppell)	2B	1 (65 mm)
		Elagatis bipinnulata (Quoy & Gaimard)	2B	1 (82 mm)
		Carangoides armatus (Rüppell)	2B	1 (82 mm)
		Seriola nigrofasciata (Rüppell)	2B	1 (232 mm)
		Trachinotus baillonii (Lacepède)	2B	1 (152 mm)
Kurtoidei	Kurtidae	*Kurtus gulliveri* Castelnau	2C	1 (210 mm; TMB 01-20-9)
Acanthuroidei	Ephippidae	*Ephippus orbis* (Bloch)	2B	2 (51, 120 mm)
		Platax orbicularis (Forsskål)	2C	1 (140 mm)
	Scatophagidae	*Scatophagus argus* (Linnaeus)	2C	1 (70 mm)
	Acanthuridae	*Acanthurus dussumieri* Valenciennes	2B	1 (270 mm)
	Siganidae	*Siganus* sp.	2C	1 (90 mm)
Labroidei	Labridae	*Bodianus leucostictus* (Bennett)	2B	1 (132 mm)
		Oxycheilinus unifasciatus (Streets)	2C	1 (190 mm)
		Choerodon azurio (Jordan & Snyder)	2B	1 (165 mm)
		Novaculichthys taeniourus (Lacepède)	2B	1 (73 mm)
		Pseudocheilinus hexataenia (Bleeker)	2A	1 (35 mm)
		Xyrichtys dea Temminck & Schlegel	2B	1 (115 mm)
	Scaridae	*Scarus ghobban* Forsskål	2C	1 (142 mm)
Zoarcoidei	Pholidae	*Rhodymenichthys dolichogaster* (Pallas)	0	1 (105 mm)
Notothenioidei	Channichthyidae	*Channichthys* sp.	2C	1 (252 mm)
Trachinoidei	Champsodontidae	*Champsodon guentheri* Regan	0	1 (56 mm)
	Uranoscopidae	*Uranoscopus japonicus* Houttuyn	2C	1 (110 mm)
	Uranoscopidae	*Uranoscopus japonicus* Houttuyn	2C	1 (110 mm)
	Trichonotidae	*Trichonotus setiger* Bloch & Schneider	2C	1 (172 mm)
	Pinguipedidae	*Parapercis mimaseana* (Kamohara)	2B	1 (128 mm)
		Parapercis muronis (Tanaka)	2B	1 (118 mm)
	Ammodytidae	*Bleekeria mitsukurii* Jordan & Evermann	2C	1 (100 mm)
Blennioidei	Tripterygiidae	*Enneapterygius* sp.	2B	1 (21 mm)
	Blenniidae	*Istiblennius edentulus* (Forster & Schneider)	2B	1 (85 mm)
Gobiesocoidei	Gobiesocidae	*Conidens laticephalus* (Tanaka)	0	1 (21 mm)
		Lepadichthys frenatus Waite	0	1 (42 mm)
Callionymoidei	Callionymidae	*Bathycallionymus kaianus* (Günther)	1aC	1 (115 mm)
		Callionymus curvicornis Valenciennes	1aC	3 (27, 28, 34 mm)
		Callionymus doryssus (Jordan & Fowler)	1aC	1 (185 mm)
		Callionymus formosanus Fricke	1aC	2 (16, 112 mm)
		Callionymus japonicus Houttuyn	1aC	4 (48, 52, 76, 104 mm)
		Foetorepus masudai Nakabo	0	1 (143 mm)
		Repomucenus planus Ochiai	1aC	2 (18, 56 mm)
		Repomucenus virgis (Jordan & Fowler)	1aC	2 (64, 84 mm)
		Synchiropus altivelis (Temminck & Schlegel)	1aC	1 (120 mm)
		Synchiropus splendididus (Herre)	1aC	1 (108 mm)
	Draconettidae	*Draconetta xenica* Jordan & Fowler	1aC	2 (90 mm, BSKU54010; 28 mm, BSKU 77527)

Table 1. (Continued)

Subclass Division Subdivision Order Suborder	Family	Species	Ossicle type[a]	Specimens[b]
Gobioidei	Rhyacichthyidae	*Rhyacichthys aspro* (Valenciennes)	1aC	2 (56, 58 mm)
	Gobiidae	*Gobiodon citrinus* (Rüppell)	0	1 (45 mm)
		Yongeichthys nebulosus (Forsskål)	0	1 (48 mm)
		Acentrogobius caninus (Valenciennes)	0	2 (56, 58 mm)
		Acentrogobius caninus (Valenciennes)	0	2 (56, 58 mm)
	Eleotridae	*Eleotris fusca* Forster	0	1 (100 mm)
Scombroidei	Sphyraenidae	*Sphyraena japonica* Bloch & Schneider	2A	1 (145 mm)
	Gempylidae	*Promethichthys prometheus* (Cuvier)	2D	1 (235 mm)
		Rexea prometheoides (Bleeker)	2D	1 (108 mm)
	Trichiuridae	*Trichiurus lepturus* Linnaeus	0	2 (380, 405 mm)
	Scombridae	*Rastrelliger kanagurta* (Cuvier)	2A	1 (165 mm)
		Scomber japonicus Houttuyn	2A	1 (190 mm)
		Thunnus albacares (Bonnaterre)	2A	1 (615 mm)
	Istiophoridae	*Istiophorus platypterus* (Shaw)	2B	1 (198 mm)
Stromateoidei	Centrolophidae	*Psenopsis anomala* (Temminck & Schlegel)	2B	1 (138 mm)
	Stromateidae	*Cubiceps whiteleggii* (Waite)	2B	1 (162 mm)
		Pampus argenteus (Euphrasen)	2C	1 (95 mm)
Anabantoidei	Osphronemidae	*Betta splendens* Regan	0	1 (47 mm)
Channoidei	Channidae	*Channa micropeltes* Cuvier	2C	1 (80 mm)
Caproidei	Caproidae	*Antigonia rubescens* (Günther)	2B	1 (34 mm)
Synbranchiformes	Mastacembelidae	*Mastacembelus* sp.	2B	1 (260 mm)
Pleuronectiformes	Psettodidae	*Psettodes erumei* (Bloch & Schneider)	2C	1 (182 mm)
	Bothidae	*Arnoglossus tenuis* Günther	2C	1 (64 mm)
		Chascanopsetta lugubris Alcock	0	1 (132 mm)
		Engyprosopon multisquama Amaoka	2C	2 (105, 120 mm)
	Samaridae	*Samariscus latus* Matsubara & Takamuki	2D	1 (76 mm)
	Pleuronectidae	*Pleuronichthys cornutus* (Temminck & Schlegel)	1aC	1 (125 mm)
		Poecilopsetta natalensis Norman	0	1 (95 mm, NMMB5429)
		Poecilopsetta praelonga Alcock	0	1 (127 mm) (NMMB 4420)
	Soleidae	*Aesopia cornuta* Kaup	2C	1 (118 mm)
		Zebrias crossolepis Zheng & Chang	0	1 (108 mm)
Tetraodontiformes	Triacanthidae	*Triacanthus biaculeatus* (Bloch)	2B	1 (115 mm)
	Triacanthodidae	*Triacanthodes anomalus* (Temminck & Schlegel)	1pC	1 (74 mm)
	Monacanthidae	*Stephanolepis cirrhifer* (Temminck & Schlegel)	0	1 (60 mm)
	Balistidae	*Sufflamen chrysopterum* (Bloch & Schneider)	0	1 (80 mm)
		Rhinecanthus sp.	0	1 (60 mm)
	Ostraciidae	*Ostracion cubicus* Linnaeus	0	1 (32 mm)
	Tetraodontidae	*Arothron nigropunctatus* (Bloch & Schneider)	0	1 (100 mm)
		Takifugu poecilonotus (Temminck & Schlegel)	0	1 (145 mm)
		Canthigaster solandri (Richardson)	0	1 (40 mm)
		Canthigaster compressa (arion de Procé)	0	1 (70 mm)
	Diodontidae	*Diodon holocanthus* Linnaeus	0	1 (105 mm)
	Molidae	*Mola mola* (Linnaeus)	0	1 (1638 mm)

[a]Numbers and lowercase and uppercase letters represent the number, position, and size of scleral ossicles, respectively; a, anterior; ad, anterodorsal to the horizontal axis of the eye; av, anterior and posterior scleral ossicles located on and below the horizontal axis of the eye, respectively; p, posterior; r, a complete ring; length of scleral ossicle relative to circumference of the eye: A, 1/2 ≥ X > 3/8; B, 3/8 ≥ X > 1/4; C, 1/4 ≥ X ≥ 1/8; D, X < 1/8. [b]Numbers in parentheses: standard length in millimeters and catalogue numbers are in parentheses.

glossodonta (Forsskål), 2 ossicles; *Megalops cyprinoides* (Broussonet), 2 ossicles) surrounding almost the entire circumference of the eyeballs of the examined elopiforms and albuliforms. In a 380-mm *M. cyprinoides* specimen, an additional small shield-like ossicle was attached to the posterior scleral ossicle with a suture between them (Fig. 1A). However, this small ossicle did not contact the posterior scleral ossicle in a smaller *M. cyprinoides* specimen (260 mm SL). A similar bone was also found in the *E. machnata* and *A. glossodonta* (Fig. 1B, C). Complete fusion among these 2 bones was found in *A. glossodonta* and some *M. cyprinoids* specimens.

2. Two scleral ossicles

Sizes of the scleral ossicles were variable in fishes with 2 scleral ossicles. They were divided into 4 categories. In category A, the length of scleral ossicle was > 3/8 but < 1/2 of the circumference. In category B, the length of scleral ossicle was ≤ 3/8 but longer than 1/4. In category C, the length of scleral ossicle was ≤ 1/4 but > 1/8. In category D, the length of scleral ossicle was < 1/8.

2.1 Category 2A

There were 2 arrangement patterns in this category. One was of 2 long ribbon-like scleral ossicles surrounding the dorsal and ventral sides of the eye leaving only a narrow cartilage strip located near the anterior and posterior midpoints of the eye circumference (Fig. 2A). This category occurred in *Ilisha elongata* (Anonymous [Bennett]) (Pristigasteridae). The other one was of 2 long ossicles on the anterior and posterior halves of the eyeball occupying almost the entire circumference (the length of each ossicle was ca. 1/2 of the circumference of the eyeball; Fig. 2B-D): *Moenkhausia* (Characidae), *Cololabis* (Exocoetoidei), *Mene* (Menidae), *Secutor* and *Gazza* (Leiognathidae), *Caesio* (Caesioidae), *Drepane* (Drepaneidae), *Amphiprion*, *Chrysiptera* (Pomacentridae), *Polydactylus* (Polynemidae), *Sphyraena* (Sphyraenidae), *Scomber*, *Thunnus* (Scombridae), etc. All of these fishes are teleosts.

2.2 Category 2B (Fig. 2E)

This category occurred in *Lycengraulis*, *Spratelloides* (the anterior ossicle larger than the posterior one; Clupeoidei), *Hexagrammos* (Scorpaeniformes), *Trachinotus*, *Cephalopholis*, *Sillago*, *Lethrinus*, *Parupeneus*, *Nemipterus*, *Cirrhitus*, *Acanthurus* (Percoidei), *Novaculichthys* (Labroidei), *Enneapterygius* (Blennioidei), *Istiophorus* (Scombroidei), *Antigonia* (Caproidei), *Triacanthus* (Balistoidei), etc.

2.3 Category 2C (Fig. 2F)

This category occurred in *Stolephorus*, *Esox* (Esociformes), *Chlorophthalmus* (Synodontoidei), *Velifer* (Lampriformes), *Melanotaenia* (Atheriniformes), *Exocoetus* (Beloniformes), *Sorsogona* (Platycephaloidei), *Synagrops*, *Malakichthys*, *Glaucosoma*, *Kuhlia*, *Priacanthus*, *Pristigenys*, *Apogon*, *Archamia*, *Scombrops*, *Pomatomus*, *Lutjanus*, *Lobotes*, *Chaetodon*, *Forcipiger*, *Heniochus* (Chaetodontidae),

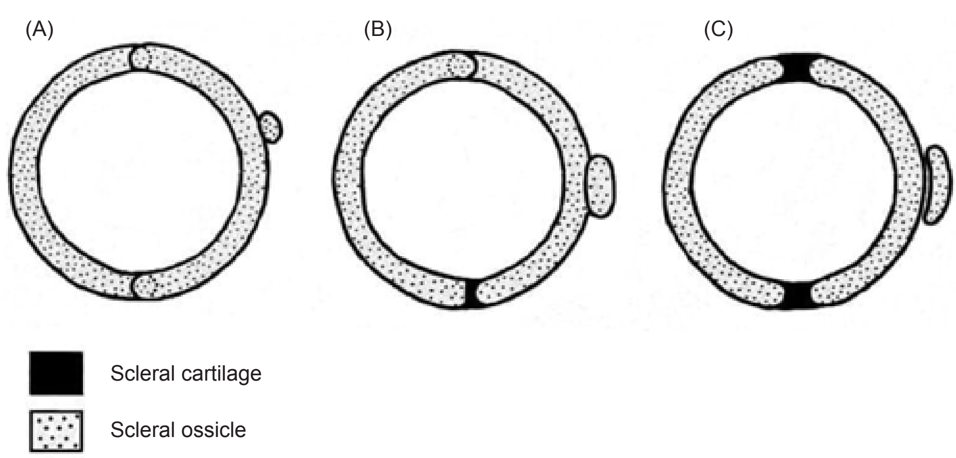

Fig. 1. Diagrammatic drawings showing a side view of the left eye (anterior to the left) and the scleral ossicles. (A) *Megalops cyprinoides*; (B) *Elops machnata*; (C) *Albula glossodonta*.

Haemulidae, Sciaenidae (Percoidei), *Pentaceros*, *Goniistius*, *Platax* (Acanthuroidei), *Siganus* (Acanthuroidei), *Scarus* (Labroidei), *Bleekeria* (Trachinoidei), *Channa* (Channoidei), *Psettodes* (Psettodoidei), *Arnoglossus*, *Aesopia* (Pleuronectoidei), etc.

2.4 Category 2D (Fig. 2G, H)

This category occurred in *Chanos* (Chanoidei), *Saurida* (Aulopiformes, Alepisauroidei), *Polymixia* (Polymixiiformes), *Onigocia*, *Banjos*, *Brama*, *Girella*, *Acanthocepola* (Percoidei), and *Promethichthys* (Scombroidei).

2.5 Symmetry of the 2 scleral ossicles in position, size, and shape

The 2 scleral ossicles of *Cololabis* (Scomberesocoidei), *Sphyraena*, some carangids (e.g., *Alectis* and *Trachinotus*) (Percoidei), and *Psenopsis* (Stromatoidei) were only symmetrical with the vertical axis of the eye (Fig. 2C, D). The upper and lower halves of these ossicles were not symmetrical; the upper ends of the 2 scleral ossicles were closer to each other than that of the ventral ends, which was an unusual condition seen only in these species. The axis that the 2 ossicles of *Trachiotus*, *Harengula*, and *Lycengraulis* were symmetrical to was a 45° angle of inclination (Fig. 2E).

In cepolids (including Cepolinae and Owstoniinae; Nelson 2006) the anterior and posterior scleral ossicles were not symmetrical to the horizontal axis of the eye; the anterior one was at the axis, whereas the posterior one was below the axis (i.e., at about 225°; Fig. 2H). This position of the posterior ossicle is unique to fishes and is treated as an autapomorphic character of the Cepolidae.

The size and shape of the anterior and posterior scleral ossicles might or might not have been the same. When they were not the same (e.g., *Calloplesiops*, *Oxycirrhites*, *Apogon*, and *Oxycheilinus*), the anterior one tended to be larger (Fig. 3A, C). The shape of these scleral ossicles may also have differed. Curvatures of the posterior edges of the ossicles of *Calloplesiops* (Plesiopidae), *Abudefduf*, *Amphiprion*, *Chrysiptera*, *Pomacentrus* (Pomacentridae), *Bodianus*, *Oxycheilinus*, *Choerodon*, *Novaculichthys*, and *Xyrichthys* (Labridae) differed (Fig. 3B, D).

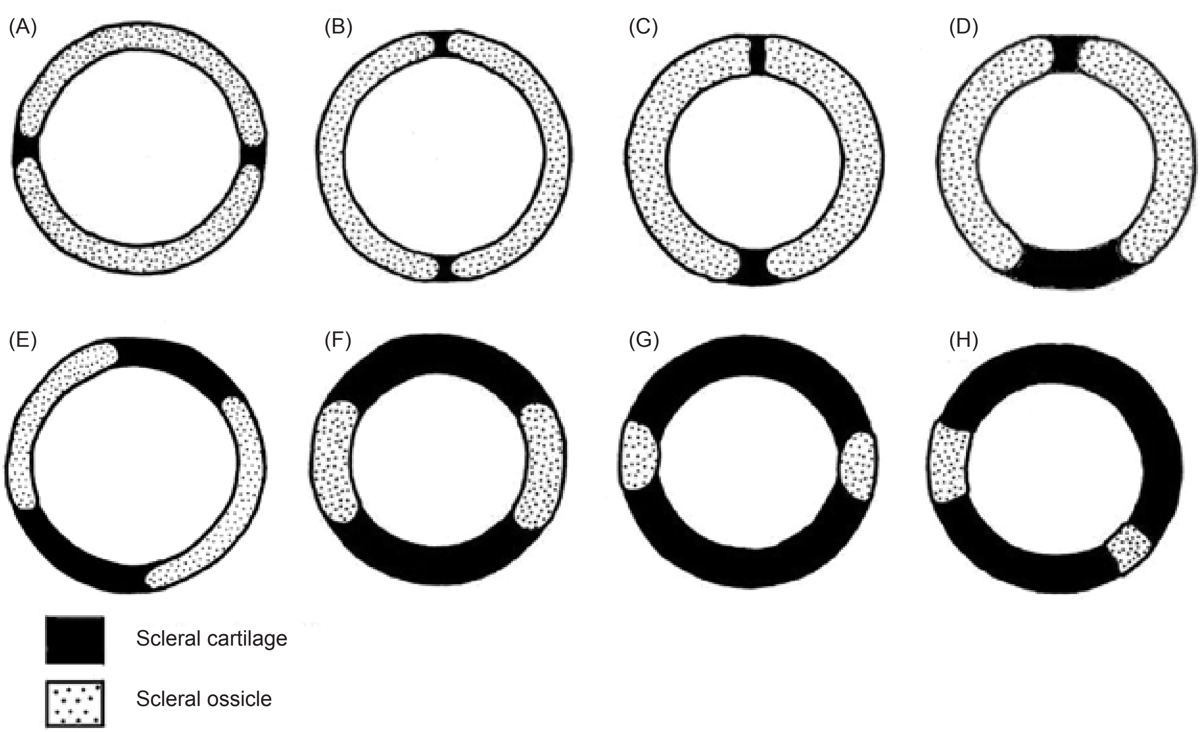

Fig. 2. Diagrammatic drawings showing an external side view of the left eyes (anterior to the left) of *Ilisha elongata* (A), *Secutor insidiator* (B), *Cololabis saira* (C), *Sphyraena japonica* (D), *Trachinotus baillonii* (E), *Bleekeria mitsukurii* (F), *Promethichthys prometheus* (G), and *Acanthocepola kruseusterni* (H).

3. A single ossicle

The position of the scleral ossicle varied among groups with only 1 scleral ossicle. In the 1st group, the only ossicle was located at the midpoint of the anterior 1/2 of the eyeball circumference (at 0° to the horizontal axis of the eye; Fig. 4A-D). This character state of lacking the posterior scleral ossicle and having the anterior scleral ossicle at 0° occurred among the examined species only in *Dactyloptena orientalis* (Cuvier), *Dactyloptena gilberti* Snyder (Dactylopteridae), *Draconetta xenica* Jordan & Fowler (Draconettidae), *Callionymus curvicornis* Valenciennes, *Callionymus doryssus* (Jordan & Fowler), *Callionymus formosanus* Fricke,

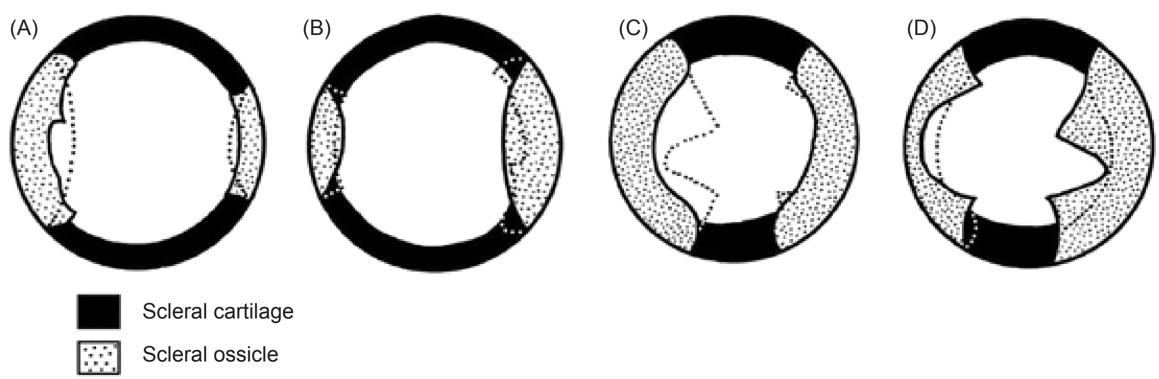

Fig. 3. Diagrammatic drawings showing an external side view (left) and internal side view (right) of the left eye of *Calloplesiops altivelis* (top insets; diameter of the eye = 7.5 mm) and *Oxycheilinus unifasciatus* (bottom insets, diameter of the eye = 11.0 mm).

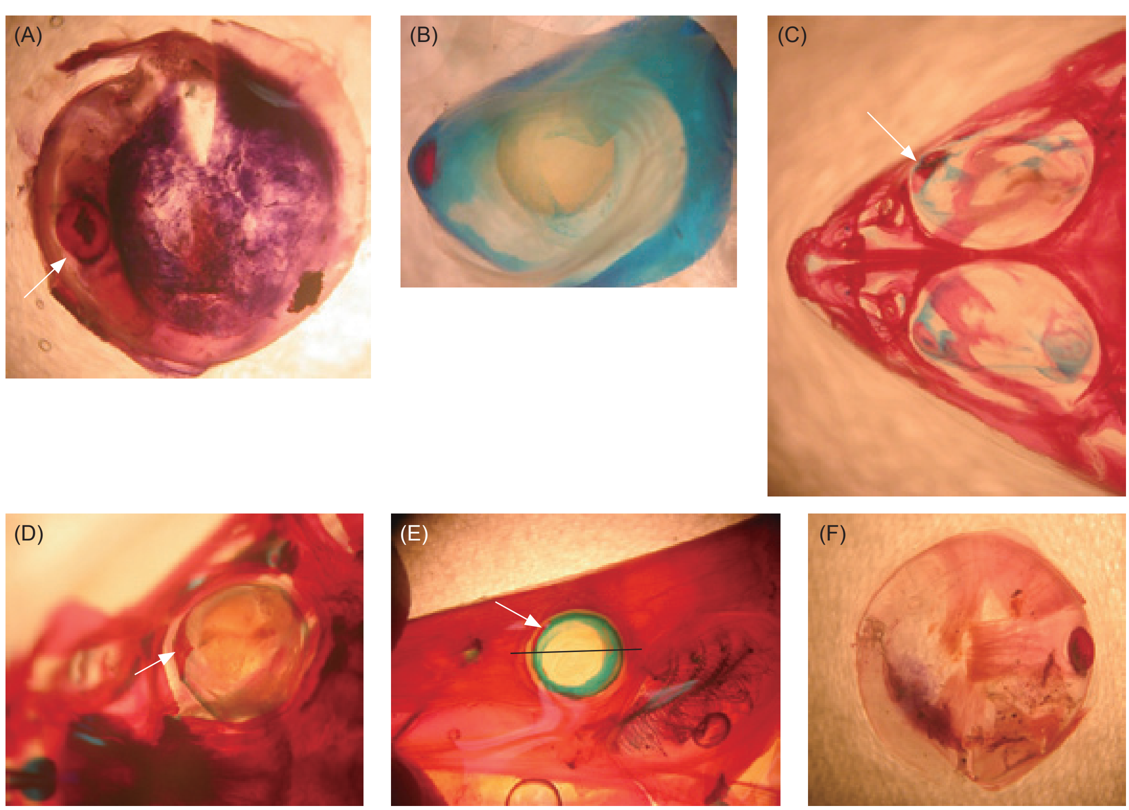

Fig. 4. External side view of the left eye of *Dactyloptena orientalis* (A), *Draconetta xenica* (B), *Callionymus doryssus* (C), *Rhyacichthys aspro* (D), *Aeoliscus strigatus* (E), and *Gonorynchus abbreviatus* (F).

Callionymus japonicus Houttuyn, *Repomucenus virgis* (Jordan & Fowler), *Bathycallionymus kaianus* (Günther), *Synchiropus altivelis* (Temminck & Schlegel), *Synchiropus splendidus* (Herre) (Callionymidae), *Rhyacichthys aspro* (Valenciennes) (Gobioidei), and *Pleuronichthys cornutus* Temminck & Schlegel (Pleuronectidae). Other examined adult callionymoids (i.e., *Foetorepus masudai* Nakabo), small callionymid specimens (about 15-mm *Callionymus planus* Ochiai and 15-mm *Callionymus formosanus* Fricke), and gobioids had no scleral ossicles.

In the 2nd group, the only ossicle was located at approximately 30° above the horizontal axis of the eyeball at the anterior 1/2 of the eye circumference. This character state of lacking the posterior scleral ossicle and having the anterior scleral ossicle located at the particular position occurred only in aulostomoids, centriscids, and macroramphosids among the species examined and was considered a synapomorphic character state (Fig. 4E). These 2 families were therefore hypothesized to be sister groups. Other aulostomoids (including aulostomids and fistulariids) had no scleral ossicles.

In the 3rd group, the only ossicle was located at the midpoint of the posterior 1/2 of the eyeball circumference (i.e., at 180° to the horizontal axis of eye; Fig. 4F). This character state in lacking the anterior scleral ossicle and having the posterior scleral ossicle at 180° only occurred in *Gonorynchus* (Ostariophysii), *Opistognathus hongkongiensis* (Opistognathidae), and *Triacanthodes* (Tetraodontiformes).

4. Absence (or loss) of scleral ossicles

The absence of scleral ossicles occurred in many remotely related actinopterygian groups in various taxonomic categories. This character state occurred in *Polypterus* (Polypteriformes), *Acipenser* (Acipenseriformes), *Lepisosteus* (Lepisosteiformes), *Amia* (Amiiformes), *Gnathonemus*, *Osteoglossum*, *Notopterus* (Osteoglossiformes), *Notacanthus* (Notacanthiformes), some anguilliforms (e.g., *Simenchelys* and *Ariosoma*), some cypriniforms (e.g., *Misgurnus*), some siluriforms (e.g., *Arius* and *Plotosus*), some salmoniforms (e.g., *Alepocephalus* and *Galaxias*), *Astronesthes* (Stomiiformes), some aulopiforms (e.g., *Lestrolepis*), *Benthosema*, *Diaphus*, *Lampanyctus*, *Myctophum*, *Neoscopelus* (Myctophiformes), *Percopsis* (Percopsiformes), *Bregmaceros*, *Hymenocephalus* (Gadiformes), *Neobythites* (Ophidiiformes), *Allenbatrachus* (Batrachoidiformes), *Diancistrus*, *Himantolophus* (Lophiiformes), *Fundulus* (Fundulidae), *Conidens* (Gobiesociformes), some beryciforms (e.g., *Hoplostethus*), *Culaea* (Gasterosteiformes), *Pegasus* (Pegasiformes), some syngnathids (e.g., *Fistularia*, *Hippocampus*, and *Trachyrhamphus*), some scorpaeniforms (e.g., *Pontinus* and *Hoplichthys*), some perciforms (e.g., *Opistognathus aurifrons*, *Opistognathus darwiniensis*, *Trichiurus*, *Taractichthys*, *Nandus*, *Champsodon*, *Foetorepus masudai*, callionymids of < 18 mm, Betta, some pleuronectiforms (e.g., *Zebrias*, *Chascanopsetta*, *Poecilopsetta praelonga*, and *P. natalensis*), and the majority of tetraodontiforms (i.e., *Sufflamen*, *Rhinecanthus*, *Canthigaster*, *Arothron*, *Takifugu*, *Ostracion*, *Diodon*, and *Mola*).

DISCUSSION

Results of our observations are similar to that of Franz-Odendaal (2008): having no scleral ossicles per eye is more common at the order level (91% of orders) and scleral ossicle numbers are stable at the family level (96% of families are identical). But we found a greater variety in these orders: Elopiformes (0, 2, 3, 4), Albuliformes (0, 3), Gonorynchiformes (0, 1, 2), Cypriniformes (0, 2), Characiformes (0, 1, 2), Percopsiformes (0, 2), Gasterosteiformes (0, 1, 2), and Synbranchiformes (0, 2).

Because the observed families in Franz-Odendaal's study (2008) and this study were not the same (76 families in Franz-Odendaal's paper were not included in the present study, and 78 families in this study were not present in Franz-Odendall's paper), and numbers of scleral ossicles of none of the families were reported in her paper, we cannot combine these 2 datasets for a more comprehensive analysis.

Franz-Odendaal (2008) presented the most parsimonious evolutionary scenario of scleral ossicle numbers in teleost orders. Most orders with 2 ossicles were gained from 0, and families in the Tetraodontiformes, Pleuronectiformes, Perciformes, and Scorpaeniformes, with 1 ossicle, were the most advanced groups. We reexamined this hypothesis by attaching scleral ossicle numbers to lineages of the molecular phylogenetic tree. In the phylogenetic tree of the Tetraodontiformes inferred from RAG1 and mitochondrial 12S and 16S rRNA genes (Alfaro et al. 2007; Fig. 5A), the evolutionary scenario should be a gain from 0 to 2 ossicles, and a loss from 2 to 1 ossicle, and finally another loss to 0 ossicles (0 to 2 to 1 to 0).

In the Scorpaeniformes, we found no families with only 1 scleral ossicle, and we do not know which taxa reported by Franz-Odendaal (2008) had only 1 scleral ossicle. Referring to the phylogenetic tree of Smith and Wheeler (2004) (Fig. 5B), the evolutionary process in this order should be a gain from 0 to 2 ossicles, and a loss from 2 to 0 ossicles (0 to 2 to 0). Cypriniformes showed the same trend based on the phylogenetic tree of Lavoué et al. (2005) (Fig. 5C).

In the Pleuronectiformes, there may be 2 evolutionary processes in this order: one is 2 to 1 to 0 and the other is 2 to 0. This result was derived from the phylogenetic trees of Azevedo et al. (2008), Pardo et al. (2005), and Berendzen and Dimmick (2002).

We also found the 2 patterns (2 and 0 scleral ossicles) in the Beryciformes. As reports of Colgan et al. (2000) and Miya et al. (2003) both showed that the Beryciformes is not a monophyletic group, we did not infer the phylogenetic trend of scleral ossicle number in this order.

Phylogenetic significance of the scleral ossicles

The Elopiformes is an unusual group in having the eye surrounded by 2 or 3 scleral ossicles with partial overlapping or complete fusion between adjacent ones. Only *Elops* has 3 ossicles in this bony frame. In these fishes, the small ossicle at the rear edge of the posterior ossicle is unique in teleostomes. The homology status between this small posterior ossicle and the only scleral ossicle in the eyes of the *Gonorhynchus* located at the posterior part of the eye (see below) is uncertain. Franz-Odendaal and Hall (2006) proposed that 4 scleral ossicles is a primitive state in teleosts, and following this point, the Elopiformes should be the most primitive group among teleosts.

The anterior scleral ossicle above the horizontal axis of the eye (i.e., located at about ≥ 30° above the horizontal axis) is unique to the Centriscidae and Mascroramphosidae, supporting the monophyly of these 2 families, which are placed in the superfamily Macroramphosoidea (which only includes these 2 families) in the infraorder Macroramphosa (Syngnathoidei, Gasterosteiformes; Pietsch 1978) or in the Centriscoidea of the Aulostomoida, Syngnathoidei, Gasterosteiformes (Nelson 2006). This super-

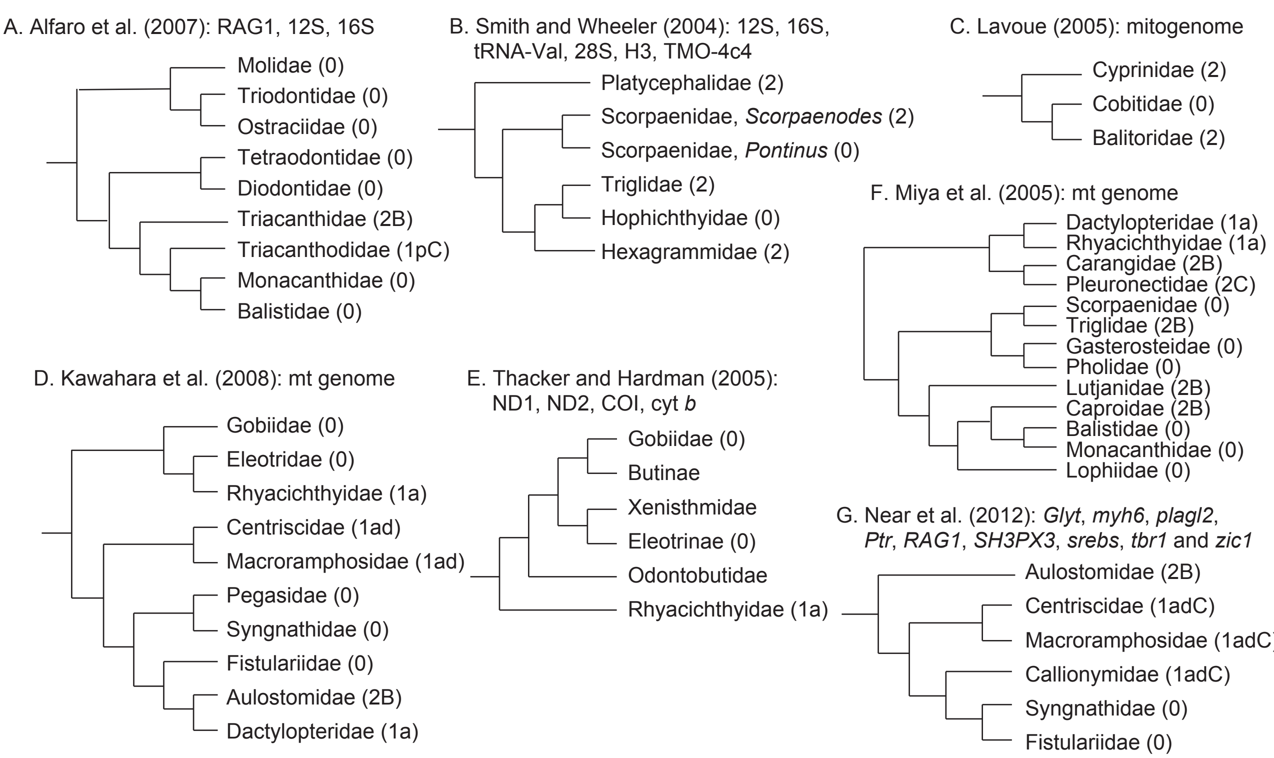

Fig. 5. Alternative phylogenetic hypotheses for acanthomorph fishes. Molecular phylogenetic hypotheses based on partial mitochondrial and nuclear genes (A, Alfaro et al. 2007; B, Smith and Wheeler 2004; E, Thacker and Hardman 2005; Near et al. 2012) and the mitochondrial genome (C, Lavoue 2005; D, Kawahara et al. 2008; F, Miya et al. 2005).

family is characterized by the anterior 5 or 6 vertebrae being elongated (Nelson 2006). Consequently, evidence of the scleral ossicle position points to a conclusion which agrees with that derived from the character state of the anterior vertebrae. This agreement suggests the reliability of the information related to the position of the anterior scleral ossicle in reflecting a monophyletic relationship. This relationship was also demonstrated by Kawahara et al. (2008) as inferred from the complete mitochondrial genome and Near et al. (2012) from 9 nuclear genes (Fig. 5D, G). An additional piece of evidence supporting the notion that the spatial relation of the scleral ossicles is informative in falsifying phylogenetic hypotheses was demonstrated by the Cepolidae (see above).

Having only a single scleral ossicle located at the anterior part of the eye circumference (or losing the posterior ossicle) is very unusual, being seen only in centriscid, macroramphosid, dactylopterid, draconettid, callionymid, rhyacichthyid (a basal plesiomorphic goboid), and one of 3 examined pleuronectid species (the other 2 congeners lack scleral ossicles). The similarity among these groups in sharing this unusual character may either be a case of homology or convergence.

In the Pleuronectiformes, the Psettodidae, a plesiomorphic group, had 2 scleral ossicles. Among pleuronectoid species, *Pleuronichthys cornutus* was the only species with just the anterior scleral ossicle, while the other examined species had 2 or no scleral ossicles. The common ancestor of pleuronectiforms would very likely have had 2 scleral ossicles; this inference fits the parsimonious criterion because possible sister groups to pleuronectiforms (i.e., carangoids; Miya et al. 2003, Azevedo et al. 2007) also possess 2 scleral ossicles.

Within the Callionymoidei, the Draconettidae is treated as a plesiomorphic sister group of the Callionymidae, as the gill opening of draconettids is comparatively broader than that of callionymids; a broader opening was hypothesized to be a more-general character state. The presence of a single scleral ossicle placed in the anterior part of the eye in (1) the primitive callionymoid family (i.e., the Draconettidae) and in only some callionymids, and (2) the primitive gobioid family (i.e., the Rhyacichthyidae) represents the 1st step (losing the posterior ossicle first) towards the complete loss of both ossicles (i.e., further loss of the anterior ossicle in other callionymids and gobioids) (Fig. 5E). As such, the presence of only the anterior ossicle represents an ancestral condition of these 2 suborders. The similarity among the Macroramphosoidea (or Centriscoidea), Dacterylopteridae, Callionymoidei, and Gobioidei in sharing this unusual character may either be a case of homology or convergence (see above); the former would imply that these 4 taxa are closely related.

Molecular evidence suggests a possible close relationship between (1) the Gobioidei and Dactylopteridae (Miya et al. 2003 2005) (Fig. 5F), (2) the Gobioidei and Dacterylopteridae + Syngnathoidei (Kawahara et al. 2008) (Fig. 5D), (3) the Gobioidei and Apogonidae + Pempheridae (Thacker 2009), and (4) the Gobioidei and Apogonidae (Smith and Wheeler 2006, Li et al. 2009, Wainwright et al. 2012). Miya et al. (2005) used 102 whole mitochondrial genome sequences to infer the phylogenetic position of the order Batrachoidiformes and recovered a poorly supported clade formed by the *Dactyloptena* and gobioids (*Rhyacichthys* + *Eleotris*). Those authors, however, did not mention a clade of dactylopterids and gobioids in the text. Kawahara et al. (2008) studied the interrelationships of the 11 gasterosteiform families and reached several conclusions based on entire mitogenome sequences from 75 teleosts, which resulted in: (1) the Syngnathoidei (together with Dactylopteridae) forming a monophyletic group; and (2) within the Syngnathoidei, the Centriscidae and Macroramphosidae (or Centriscoidea) forming a clade which is the sister group to a clade including the ((Pegasidae + Syngnathoidea), (*Fistularia* (*Aulostomus*, Dactylopteridae))). Another interesting result from their study was shown in the maximum-likelihood tree (Kawahara et al. 2008, Fig. 5D); it indicated that the Gobioidei is a sister group to the Syngnathoidei (including the Dactylopteroidei). However, this node was supported by a low bootstrap value and was not recovered in another analysis. Consequently, they did not recognize a clade composed of the Gobioidei and Syngnathoidei. In addition, as no apogonids were run in their study, the results are hard to compare with other published works relevant to gobioid relationships. Works that hypothesized a possible close relationship between the Gobioidei and Apogonidae include Smith and Wheeler (2006) based on mitochondrial genes (12S and 16S) and nuclear genes (28S and H3), Thacker (2009) based on 4 mitochondrial genes (ND1, ND2, COI, and cyt *b*), Li et al. (2009) based on 3 nuclear genes (rhodopsin, MLL, and

IRBP), and Wainwright et al. (2012) based on 10 nuclear genes (*ENC1*, *Glyt*, *myh6*, *plagl2*, *Ptr*, *RAG1*, *SH3PX3*, *srebs*, *tbr1*, and *zic1*), and these nodes were supported by high bootstrap values or posterior probabilities. As such, the presence of 2 ossicles represents the ancestral condition of this Gobioidei and Apogonidae clade; loss of the posterior ossicle and loss of the 2 ossicles are thus an intermediate and the most derived character states in this clade, respectively.

In the results of Kawahara (2008), *Aeoliscus* and *Macroramphosus* form a plesiomorphic sister group to other syngnathoids, whereas aulostomids and *Dactyloptena* are the most derived group in the Syngnathoidei. In the results of Near et al. (2012), the Syngnathidae and Fistulariidae are the most derived groups in the Syngnathoidei, but *Aulostomus* is a plesiomorphic sister group to other syngnathoids. In Kawahara et al.'s (2008) hypothesis, *Aeoliscus*, *Macroramphosus*, and *Dactyloptena* possess the ancestral condition in having only the anterior scleral ossicle; other advanced syngnathoids (i.e., pegasids, syngnathids, and fistulariids) express the apomorphic condition in having lost both scleral ossicles. Interestingly, aulostomids with 2 scleral ossicles are the sister group of *Dactyloptena*; the presence of 2 scleral ossicles is a derived character state in this scenario. They may have lost the posterior ossicle first, and then gained 2 ossicles, finally losing the posterior one again. On the other hand, according to Near et al.'s (2012) hypothesis, the presence of 2 ossicles in the most plesiomorphic group, the aulostomids, is a plesiomorphic condition in this group. Considering the transforming series of sclera ossicles (i.e., 2 to 1 to 0), the relationship hypothesized by Near et al. (2012) is more reasonable than that of Kawahara et al. (2008).

Except for the small order Gonorhynchiformes, whenever there are members in a particular larger taxonomic group of fishes with only a single scleral ossicle, there are also other species within the same group with no scleral ossicle at all. The absence of 1 scleral ossicle seems to be an intermediate step towards the complete absence of these ossicles. Interestingly, whether the anterior or posterior scleral ossicle is the 1st one to be lost is a matter of phylogenetic significance. The distribution of scleral ossicle variants within the Tetraodontiformes is interesting. The primitive groups (i.e., triacanthids and triacanthodids; Santini and Tyler 2003) have 2 (triacanthid) and 1 (triacanthodid) scleral ossicles, while the more-advanced groups (i.e., monacanthids, balistids, tetraodontids, canthigasterids, diodontids, ostraciids, and molids) lack scleral ossicles. Unlike other fishes that have only 1 scleral ossicle, the single scleral ossicle of the triacanthodids is placed on the posterior part of the eyeball. A trend seems to exist only in the Tetraodontiformes among other actinopterygians in which the anterior scleral ossicle was the 1st scleral ossicle to be lost during the evolution of the Tetraodontiformes. Although the single scleral ossicle in gonorhynchiforms is also found at the posterior part of the eye, it might not be a homologous bone to that in the triacanthodid. On the other hand, it is likely a structure homologous to the small bone next to the posterior scleral ossicle in elopiforms (see above). Similarity between some opistognathids and triacanthodid in having the only scleral ossicle on the posterior 1/2 of the eye is possibly a result of convergence. Because *Opistognathus aurifrons* and *O. darwinensis* lack scleral ossicles, the presence of a single scleral ossicle at the posterior midpoint in *O. hongkongiensis* indicates that loss of both scleral ossicles in this family has taken a different path (i.e., the anterior scleral ossicle was first lost in the family) compared to the aulostomoids, gobioids, and callionymids mentioned above.

Within the Scombroidei, pelagic scombroids have 2 very long scleral ossicles; some other scombroids with a similar habit exhibit a relatively similar character state of the scleral ossicles being well developed. The deep-water snake mackerels, such as the *Promethichthys* and *Rexea*, have much-reduced scleral ossicles, whereas cutlassfishes (Trichiuridae), which exhibit a vertical diel migration, lack scleral ossicles. Other more-remote scombroid relatives, such as sphyraenids and pomatomids (Johnson 1986), also have well-developed scleral ossicles.

The character states of scleral ossicle counts in an actinopterygian taxon may vary; a taxon can be fit into one of the following categories in terms of the states it includes: (1) a single circular ossicle occupying the complete circumference of the eye (characiforms); (2) 2 or 3 ossicles occupying more or less the entire circumference of the eye (elopiforms and albuliforms); (3) 2 separate (anterior and posterior ones) ossicles (most actinopterygian taxa); (4) 2 separate ossicles, 1 anterior ossicle, and no scleral ossicle (Pleuronectiformes); (5) 2 separate ossicles, 1 posterior ossicle, no ossicle (tetraodontiforms); (6) 2 separate ossicles (anterior and posterior), no ossicle (Scombroidei); (7) 1 anterior ossicle (Centrisoidea); (8) 1 posterior ossicle (Gonorynchidae and Triacanthodidae); (9) 1 anterior ossicle, no ossicle (Callionymoidei, Gobioidei, and Pleuronectidae); and (10) no ossicle (e.g., Zeidae, Trichiuridae, and Gobiidae). It is obvious that (1) the loss of all scleral ossicles has independently occurred several times during the evolution of actinopterygians. In groups showing multiple character states in ossicle counts (i.e., groups 4 and 5), basal taxa carry the primitive state in having 2 ossicles (e.g., tetraodontiforms and pleuronectiforms). When there are 2 states in a taxon, the basal group also carries the primitive state, e.g., Gobioidei: *Rhyacichthys* with 1 ossicle while other gobioids have 1 (*Terateleotris* (Shibukawa et al. 2001) or no ossicle; Scombroidei: all families with 2 ossicles except for the derived family Trichiuridae with no ossicle). During the process of losing all ossicles, either the anterior or posterior ossicle would be the first to be lost; the former condition occurred in the Tetraodontiformes, and the latter condition occurred in the Syngnathoidei, Gobioidei, Callionymoidei, and Pleuronectiformes. It was interesting to note that no gobioids retained the plesiomorphic character state of having 2 scleral ossicles. Despite the loss of a structure possibly not being a strong indication for a sister-group relationship, there may be a mechanism that controls which scleral ossicle (the anterior or posterior) is the 1st to be lost. Under this assumption, such a mechanism may be informative, and it was treated as an indication favoring a close relationship among groups (including the Gobioidei, Callionymoidei, Dactylopteridae, and Syngnathoidei) retaining the anterior but losing the posterior ossicle. This assumption, however, leads to conflicting results when it is applied to inferring the Pleuronectiformes as an order that has never been hypothesized to have a close relationship with these 4 taxa. This conflict suggests the likelihood of convergence. It remains possible that the Dactylopteridae and Macroramphosoidea are sister groups to other syngnathoids with no scleral ossicles.

The asymmetrical dorsal and ventral portions of the scleral ossicles observed in *Cololabis*, some carangids (e.g., *Alectis* and *Trachinotus*), *Sphyraena*, and *Psenopsis* do not broadly appear in other families. Information from more species in these groups may provide insights into this character.

Franz-Odendaal et al. (2007) reported that eye growth in the alewife, *Alosa pseudoharengus*, exhibits positive allometry with body length. Different-sized priacanthid specimens (20%-72% of maximum length) and apogonids specimens (32%-72% of maximum length) are located in the same size category (i.e., 2C) implying that the relative size of the scleral ossicles remains stable with growth in groups with shorter scleral ossicles (Table 1). According to the present dataset, sizes of scleral ossicles in some clupeiforms were long (i.e., categories 2A and 2B), whereas others had short scleral ossicles (i.e., 2C; Table 1). The above growth pattern can only be applied to groups with long scleral ossicles.

Intra-family variations in the size of the scleral ossicles were observed in some families. In the Priacanthidae, ossicles of the *Pristigenys* and *Cookeolus* were smaller (i.e., about 3/16) than those of *Heteropriacanthus* and *Priacanthus* (i.e., about 4/16). If a reduction in size is considered to be an apomorphic character, a closer phylogenetic relationship between *Pristigenys* and *Cookeolus* can be inferred. This phylogenetic hypothesis, however, disagrees with that based on other morphological characters (*Pristigenys* (*Cookeolus* (*Heteropriacanthus*, *Priacanthus*))) (Starnes 1988). If an increase in ossicle size is hypothesized to be the synapomorphic character state, then Starnes's phylogenetic hypothesis cannot be falsified by the scleral ossicle transformation series (i.e., *Heteropriacanthus* and *Priacanthus* being sister groups). However, sizes of scleral ossicles of possible sister groups to the Priacanthidae (e.g., the Letheridae; Sanciangco et al. 2011) are not small, making the 1st inference more plausible.

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