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タイトル	Systematics of the circum-global scorpionfish family Setarchidae
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キーワード (taxonomy) (re-description) (*Lythrichthys*)

※ Nomenclatural acts on this manuscript are invalid.

Introduction

The circumglobal deepwater scorpionfish family Setarchidae Matsubara 1943 (Scorpaenoidei) is one of the most widely distributed scorpionfish family, ranging from tropical to temperate waters of the Indo-Pacific and Atlantic deepseas about 150–2400 m depth (Eschmeyer and Collette 1966; Poss 1999; Nakabo and Kai 2013). The Setarchidae previously treated as a subfamily in the family Scorpaenidae Risso 1827 (Eschmeyer and Collette 1966; Eschmeyer 1998; Nakabo and Kai 2013), is characterized by having a highly modified lateral line, reduced ossification, and reduction in the head supination (Eschmeyer and Collette 1966, as subfamily; Motomura and Struthers 2015). This family currently includes eight nominal genera, three of which, *Setarches* Johnson 1862, *Lioscorpius* Günther 1880, and *Ectreposebastes* Garman 1899, are regarded as valid, and 14 nominal species, of which six within these three genera are treated as valid species (Eschmeyer and Collette 1966; Eschmeyer 1998; Fricke et al. 2020).

As a consequence of a generic re-definition of the family Setarchidae, based on an examination of 1,322 specimens, a total of 10 species belonging to four genera are currently recognized. *Lythrichthys* Jordan and Starks 1904, previously regarded as a junior synonym of *Setarches*, is recognized as valid in the present study. Consequently, *Lioscorpius longiceps* var. *longimanus* Alcock 1894, *Lythrichthys eulabes* Jordan and Starks 1904, and *Scorpaenella cypho* Fowler 1938, previously regarded as synonyms of *Setarches longimanus* (Alcock 1894) by Eschmeyer and Collette (1966), are re-described as valid species in the genus *Lythrichthys*, based on holotypes of each nominal species with 84, 501, and 392 non-type specimens, respectively. In addition, 15 and 24 unidentified specimens within the genus, collected from deepsea regions of the western Pacific Ocean, are described herein as constituting two new species, respectively.

Lioscorpius and *Ectreposebastes*, along with four currently valid species in each of these genera are confirmed as valid based on original descriptions and all existing holotypes of each nominal species, and re-described in the present study based on newly established diagnoses.

Materials and methods

Counts and measurements followed Wada et al. (2020). Standard and head lengths are abbreviated as SL and HL, respectively. Osteological characters were observed from soft X-ray photographs. The last two soft rays of the dorsal and anal fins are counted as single rays, each pair being associated with a single pterygiophore. Full counts and measurements of each species of Setarchidae used for diagnosis

and description are given by the present specimens in good condition.

For a phylogenetic analysis of the family Setarchidae, total DNA was extracted from muscle tissue preserved in 99.5% ethanol, using the Wizard Genomic DNA Purification Kit (Promega Inc.), according to the manufacturer's protocols. The partial Cytochrome c Oxidase subunit I (COI) and Cytochrome *b* (Cyt *b*) genes were amplified using the primers designed by Ward et al. (2005) and Martin and Palumbi (1993), respectively. The PCR proceeded for 30 cycles, with denaturation at 94°C for 15 sec, annealing for 15 sec (54°C for COI, and 45°C for Cyt *b*), and extension at 72°C for 30 sec, using the KAPA2G Robust PCR Kit (KAPA Biosystems). In addition, a nuclear DNA locus, ryanodine receptor 3 (RYR3), was determined following the method of Li et al. (2007). The PCR products were purified with ExoSAP-IT (USB Corporation) enzyme. Automated sequencing was performed for both directions using the BigDye terminator sequencing kit (Applied Biosystems), and analyzed on a model 310 Sequencer (Applied Biosystems). The sequences determined here were aligned using Clustal X (Thompson et al. 1997) with previously determined sequences of species of Setarchidae, deposited at INSDC (International Nucleotide Sequence Database Collaboration) and BOLD (Barcode of Life Data System). For comparison, the COI, Cyt *b*, and RYR3 sequences from specimens of Sebastidae and Neosebastidae were also determined using the above protocols. All sequences determined here have been deposited in INSDC. Separate alignments of the Cyt *b*, COI, and RYR3 sequences were straightforward, as they did not require indels, the final alignment combining the three genes comprising 2,067 nucleotide positions. From the aligned sequences, the best evolutionary model was found by MEGA X software (Kumar et al. 2018). A maximum likelihood (ML) tree was reconstructed using MEGA X, and branch support measured using nonparametric bootstrapping with 1,000 replications, based on the same algorithm (Felsenstein 1981). Neosebastidae was set as an out-group in the phylogenetic reconstruction. Evolutionary connections in *S. guentheri* were estimated with an unrooted parsimony-based network of COI gene haplotypes using Network 4.6 (Fluxus Technology Ltd.). The data were first processed through the MJ (median-joining) algorithm (Bandelt et al. 1999), followed by the maximum parsimony (MP) option (Polzin and Daneschmand 2003).

Results and Discussion

Setarchidae Matsubara 1943: Members of the family Setarchidae can be readily distinguished from those of all other families, including those of Scorpaenoidei, based on the following diagnostic characters: cranium thin, cavernous, bones weakly ossified; suborbital stay uniformly broad or gradually becoming wider posteriorly, without spines; no fleshy appendages on head or body; small slit present behind fourth gill arch; lateral line continuous, covered by thin membranous scales; cycloid scales on head and body; total vertebrae 24; and pyloric caecum 4 or 5. Setarchinae (= Setarchidae) was established by Matsubara (1943) as a subfamily of Scorpaenidae and includes the genera *Setarches* and *Macroscorpius* Fowler 1938. This family was reviewed by Eschmeyer and Collette (1966) and is still treated as one of the subfamilies of Scorpaenidae. Eschmeyer and Collette (1966) also considered the genera *Setarches*, *Lioscorpius*, and *Ectreposebastes* to be valid members of the subfamily Setarchinae, and synonymized *Macroscorpius* under *Lioscorpius*. On the basis of osteological and myological evidence, Ishida (1994) treated Setarchinae as a distinct family, due to sufficient

differences from other families within Scorpaenoidei. However, although Ishida (1994) also treated Setarchidae as a newly established family, this designation is considered invalid, given that Setarchinae was originally established by Matsubara (1943), and a name established for a taxon of any rank within a family group is deemed to have been simultaneously established for nominal taxa at all other ranks in the family group (Article 36.1, ICZN 1999). Setarchidae has subsequently been confirmed as a distinct family, as a consequence of morphological-based phylogenetic classification by Imamura (2004). This designation has been generally recognized by other authors, including Motomura and Struthers (2015) and Fricke et al. (2020). The genus *Lythrichthys*, previously regarded as a junior synonym of *Setarches*, is found to be valid, and the validities of all currently included genera of Setarchidae, namely, *Setarches*, *Lioscorpius*, and *Ectreposebastes*, have also been confirmed in the present study (see results and discussion for each genus). All three genera constituting this family are readily distinguishable based on stable morphological characters. Although some of the species are missing in our phylogenetic analysis, all genera of Setarchidae are considered to be monophyletic (bootstrap values above 89%) on the ML tree, based on the COI, Cyt *b*, and RYR3 gene sequences (2,067 bp in total).

***Ectreposebastes* Garman 1899:** A genus of Setarchidae with the following characters: anal-fin rays III, 6; predorsal scales 8–17; body depth at pelvic-fin origin and interorbital width at vertical midline of eye being 33.1–44.5% and 10.0–15.0% of standard length, respectively; snout, maxilla, and dorsal and ventral surface of head scaled; lateral surface of maxilla with distinct single median ridge; lip on lateral surface of premaxilla rudimentary; first lacrimal spine minute or rudimentary; 5 preopercular spines, third spine longest, and remaining spines short and similar in length; scales of thorax and abdomen exposed; swimbladder rudimentary or absent; intestine and pyloric caeca pale; abdominal vertebrae 10, caudal vertebrae 14; tip of second anal proximal-pterygiophore located between tenth abdominal and first caudal vertebrae. The genus contains two valid species, namely, *E. imus* Garman 1899, which has a circumglobal distribution, and *E. niger* (Fourmanoir 1971), which occurs in Indo-West Pacific Ocean.

***Lioscorpius* Günther 1880:** A genus of Setarchidae with the following characters: Anal-fin rays II, 6 or III, 5; predorsal scales 7–12; body depth at pelvic-fin origin and interorbital width at vertical midline of eye being 20.6–28.1% and 5.3–6.7% of standard length, respectively; snout, maxilla, and dorsal and ventral surface of head without scales; lateral surface of maxilla with distinct single median ridge; lip on lateral surface of premaxilla rudimentary; first lacrimal rudimentary; 5 preopercular spines, all short and almost same in length; scales of thorax and abdomen exposed; swimbladder well developed; intestine and pyloric caeca black or grey; abdominal vertebrae 10, caudal vertebrae 14; tip of second anal proximal-pterygiophore located between tenth abdominal and first caudal vertebrae. The genus contains *Li. longiceps* Günther 1880 and *Li. trifasciatus* Last, Yearsley and Motomura 2005, which co-occur in the western Pacific Ocean, as valid species.

***Lythrichthys* Jordan and Starks 1904:** A genus of Setarchidae with the following characters: Anal-fin rays usually III, 5 (rarely III, 4 or III, 6); predorsal scales 7–12; body depth at pelvic-fin origin and interorbital width at vertical midline of eye 29.1–38.6% and 8.1–12.9% of SL, respectively; snout, dorsal and ventral surface of head without scales; at least three-quarters of lateral surface of

premaxilla covered by well-developed lip; first lacrimal spine well developed, about same length as second and third spines; 5 preopercular spines, second rudimentary or much shorter than first and third, first and third spine much longer than lower 2, fourth and fifth subequal in length; tip of first lacrimal spine just reaching or extending over upper lip; thoracic and abdominal scales well embedded in skin; swimbladder well developed; intestine and pyloric caeca black or grey; single supraneural; abdominal vertebrae 9, caudal vertebrae 15; tip of second anal proximal-pterygiophore located between ninth abdominal and first caudal vertebrae. The genus contains three co-occurring species, *Ly. longimanus* (Alcock 1894), *Ly. eulabes* Jordan and Starks 1904, and *Ly. cypho* (Fowler 1938), distributed in Indo-West Pacific Ocean, and two new species, namely, *Ly. dentatus* sp. nov. from the Sulu and Timor seas, and Australia, and *Ly. grahami* sp. nov., which occurs in the southwestern Pacific Ocean. *Lythrichthys grahami* can be distinguished from all other congeners by the following combination of characters: 8–11 (usually 9) scale rows between 6th dorsal-fin spine base and lateral line; more than 10–12 (11) scale rows between last dorsal-fin spine base and lateral line; lower opercular spine length 30.6–33.5% (31.9%) of head length, its tip beyond posterior margin of opercle (sometimes slightly short of margin in specimens up to ca 80 mm SL); caudal-peduncle depth 8.8–10.9% (9.9%) of SL; dorsal margin of interorbital space slightly raised; body base color blackish-red when fresh; and oral surface uniformly gray in preserved specimens of ca. 100 mm SL.

Setarches Johnson 1862: A genus of Setarchidae with the following characters: anal-fin rays usually III, 5 (rarely III, 4 or III, 6); pectoral-fin rays 20–25; predorsal scales 14–17; body depth at pelvic-fin origin and interorbital width at vertical midline of eye being 29.8–42.3% and 7.4–10.1% of standard length, respectively snout, maxilla, and dorsal and ventral surface of head without scales; lateral surface of maxilla with several indistinct ridge or smooth; lip on lateral surface of premaxilla rudimentary; first lacrimal spine well developed about same length as second and third spines; 5 preopercular spines, second preopercular spine, almost same as first and third spines in length, much longer than lower 2; tip of first lacrimal spine not reaching to premaxilla (just reaching or extending past in juveniles); scales of thorax and abdomen exposed; swimbladder well developed; intestine and pyloric caeca black or grey; abdominal vertebrae 10, caudal vertebrae 14; tip of second anal proximal-pterygiophore located between tenth abdominal and first caudal vertebrae. This monotypic genus contains only the circum-globally distributed *S. guentheri* Johnson 1862 as valid species.

Within the amplified regions of mtDNA, 591 bp of the COI gene sequences from 43 specimens were successfully aligned. The *p*-distance within *S. guentheri* (0–1.7) was found to be considerably smaller than that between *S. guentheri* and *E. imus* (8.1–9.3) and notably less than the uppermost divergence levels considered indicative of intraspecific variation in fishes, including those in the closely related family Scorpaenidae. In addition, undefined geographical isolation in the haplotype network is only considered indicative of intraspecific variation.

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