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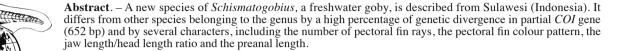
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Regular paper

A new species of Schismatogobius (Teleostei: Gobiidae) from Sulawesi (Indonesia)

by

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Résumé. – Une nouvelle espèce de Schismatogobius (Teleostei : Gobiidae) de Sulawesi (Indonésie).

Une espèce nouvelle de Schismatogobius, gobie dulçaquicole, est décrite sur la base de spécimens collectés à Sulawesi (Indonésie). Elle diffère des autres espèces du genre par un fort pourcentage de divergence au niveau du gène COI partiel (652 pb) et par plusieurs caractères incluant principalement le nombre de rayons aux nageoires pectorales, la coloration des nageoires pectorales, le ratio longueur de la mâchoire/longueur de la tête et la longueur préanale.

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Key words

Schismatogobius New species Gobiidae Sulawesi Indonesia

Schismatogobius de Beaufort, 1912 species are distinctive scaleless freshwater gobies found in the tropical Indo-West Pacific. Keith et al. (2017a, b; 2018) reviewed the species found in Indonesia

and between Papua New Guinea and Samoa, describing 11 new species.

In the Indonesian region, seven species are known: Schismatogobius insignus (Herre, 1927), S. bruynisi de Beaufort, 1912, S. arscuttoli Keith, Lord & Hubert, 2017, S. saurii Keith, Lord, Hadiaty & Hubert, 2017, S. risdawatiae Keith, Darhuddin, Sukmono & Hubert, 2017, S. bussoni Keith, Hubert, Limmon & Darhuddin, 2017 and S. sapoliensis Keith, Darhuddin, Limmon & Hubert, 2018. In 2019, a collaborative work between the Institute for Research and Development (IRD), the Indonesian Institute of Sciences (LIPI), the Universitas Pattimura, the Department of Fisheries, Faculty of Marine Science and Fisheries of Hasanuddin University in Sulawesi and the National Museum of Natural History of Paris (MNHN) was conducted in rivers of Sulawesi where Schismatogobius specimens of seven species were collected (all the species above except S. risdawatiae, plus a new species).

The purpose of this paper is to describe a new Schismatogobius species found in Sulawesi, using genetic and morphometric approaches.

MATERIAL AND METHODS

DNA Barcode analysis

Material examined

A total of 139 Schismatogobius specimens were used for this analysis. They include those used by Keith et al. (2017b; 2018) and the specimens cited below. All specimens are listed in BOLD (dx.doi.org/10.5883/DS-SCHISMA).

Schismatogobius n. sp.: in MZB.25492 (1 specimen: BIF 10058), Wera Waterfall Nature Park Palu, Sulawesi Ten-

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gah, Sulawesi, Indonesia, 12 Jul. 2019, Hubert *et al.* coll.; MNHN-IC 2020-0176 (2 spms: BIF 10054, BIF 10055), same data as MZB.25492.

Schismatogobius sapoliensis: MZB.24586 (1 spm: BIF 6802), Samuda, Air Turjun Sapoli, Halmahera, Indonesia, 23 Mar. 2017, Hubert *et al.* coll.; MZB 24587 (2 spms: BIF 6803, BIF 6805), same data as MZB.24586; MNHN-IC 2016-0626 (1 spm: BIF 6801), same data as MZB.24586; MNHN-IC 2016-0627 (1 spm: BIF 6804), same data as MZB.24586.

Schismatogobius insignus: MZB (uncatalogued) (2 spms: BIF 4059, BIF 4060), Lombok Utara, 2 Apr. 2015, Hubert *et al.* coll.; MZB (uncatalogued) (1 spm: BIF 5087), Ambon, 25 Mar. 2016, Hubert *et al.* coll.

Schismatogobius risdawatiae: in MZB.23801 (1 spm: BIF 6037), Padang, Air Terjun Lubuk Hitam, West Sumatra, Indonesia, 1st May 2016, Hubert *et al.* coll.; MZB (uncatalogued) (1 spm: BIF 6300), Padang, Sunga Lunda, West Sumatra, Indonesia, 2nd May 2016, Hubert *et al.* coll.

Schismatogobius saurii: in MZB.23797 (1 spm: BIF 4172), Lampung Barat, Wai Ngarip, Sumatra, Indonesia, 22 May 2015, Hubert *et al.* coll.; MZB (uncatalogued) (3 spms: BIF 4171, BIF 4174, BIF 4175), same data as MZB.23797.

Schismatogobius bussoni: MZB (uncatalogued) (1 spm: BIF 5291), Ceram Tengah, Wai Sia, Ceram, Indonesia, 28 Mar. 2016, Hubert *et al.* coll.; MZB (uncatalogued) (1 spm: BIF 5310), Ceram Tengah, Wai Hetu, Ceram, Indonesia, 28 Mar. 2016, Hubert *et al.* coll.

Schismatogobius bruynisi: MZB (uncatalogued) (2 spms: BIF 4173, BIF 4177), Lampung Barat, Wai Ngarip, Sumatra, Indonesia, 22 May 2015, Hubert *et al.* coll.

NB: *Schismatogobius ninja* Maeda, Saeki & Satoh, 2017 is a junior synonym of *Schismatogobius saurii* Keith, Lord, Hadiaty & Hubert 2017.

DNA extraction and amplification

Pectoral fin tissue was used to extract total genomic DNA from 143 individuals using the Macherey & Nagel NucleoSpin® Tissue kits following the manufacturer's instructions on an Eppendorf EpMotion 5075.

The DNA barcode fragment of the *cytochrome oxydase* I (*COI*) mitochondrial gene was amplified using primers FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward *et al.*, 2005). All PCRs were performed on Biometra thermocyclers in a 25 µl volume of 5% of DMSO, 5 µg of bovine serum albumin, 300 µM of each dNTP, 0.3 µM of Taq DNA polymerase from Qiagen, 2.5 µl of the corresponding buffer, and 1.7 pM of each of the two primers. After a 2-minute denaturation at 94°C, the PCR ran 50 cycles of 25 seconds at 94°C, 25 seconds at 52°C and 1 minute at 72°C, with a 3-minute terminal elongation. Amplification, purification and Sanger sequencing were performed by the Centre for Biodiversity Genomics (https://biodiversitygenomics.net/). Chromatograms were assembled and edited using Geneious 8.1.5. All the sequences were aligned using BOLD amino-acid alignment procedure. The translation into amino acids was checked for the partial fragment of *COI* gene, using the vertebrate mitochondrial genetic code. All the sequences have been deposited in the barcode of life data system (www.boldsystems.org; dx.doi.org/10.5883/DS-SCHISMA) as well as GenBank (accession numbers accessible through BOLD).

Phylogenetic relationships were inferred using the Maximum Likelihood (ML) algorithm as implemented in phyml 3.0.1 (Guindon and Gascuel, 2003). The optimization of the ML tree topology was conducted using the BEST tree rearrangement option combining both Nearest-Neighbour Interchange (NNI) and Subtree Pruning and Regrafting (SPR). The best-fit ML substitution model was selected among 88 models according to the Bayesian Information Criterion (BIC) as implemented in jmodeltest 2.1.7 (Darriba et al., 2012). The statistical support of the tree topology was estimated through 2000 replicates of nonparametric bootstrapping (BP) as implemented in phyml 3.0.1. Delineation of mitochondrial lineages with independent evolutionary dynamics was performed using the Refined Single Linkage (RESL) algorithm as implemented in BOLD and each cluster of sequence was assigned to a Barcode Index Number (BIN) in BOLD (Ratnasingham and Hebert, 2013).

Morphomeristics

Methods follow Keith *et al.* (2017a). Measurements were taken with a dial calliper to the nearest tenth of a millimetre. All counts were taken from the left side. The size is given in standard length (SL). Abbreviation are as follow: P, Pectoral rays; D, Dorsal rays; A, Anal rays; C, Caudal branched rays; PDL, Predorsal length (% SL); PAL, Preanal length (% SL); HL, Head length (% SL); JL, Upper jaw length (% SL); CPD, Caudal peduncle depth (% SL); Pect-L, Pectoral fin length (% SL); BDa, Body depth at anus (% SL); SDFL, Second dorsal fin length (% SL); AFL, Anal fin length (% SL); CFL, Caudal fin length (% SL); SL, Standard length (SL) (mm).

Teeth were always counted to the right of the symphysis, from the tooth closest to the symphysis to the posteriormost dentary or premaxillary tooth; outer row of teeth were counted in the upper jaw and inner row counted in the lower jaw.

Abbreviations used to represent cephalic sensory pores follow Akihito (1986) and sensory papilla rows as in Sanzo (1911).

Morphomeristic data is summarized in table I.

RESULTS

DNA Barcode analysis

A total of 652 base pairs of the *COI* gene were amplified for 143 specimens. The most likely substitution model selected by jmodeltest was TIM + I. The ML tree (Fig. 1) allowed delimiting ten species, each corresponding to a distinct mitochondrial lineage as evidenced by the RESL algorithm (BOLD: dx.doi.org/10.5883/DS-SCHISMA).

Morphomeristics

Specimen examination led to the discovery of a new species to science and its description is given below.

Schismatogobius limmoni, n. sp. Keith & Hubert (Figs 1-4; Tab. I)

Material examined

Seven specimens from Sulawesi with a size range of 21.7-26.3 mm SL.

Holotype. – MZB.25491, male (22.2 mm SL); Sulawesi, Sulawesi Tengah, Palu, Wera Waterfall Nature Park, Indonesia, 12 Jul. 2019, Hubert *et al*. coll. (field number BIF 10059).

Paratypes. – MZB.25492, 4 females (21.7-26.3 mm SL); same data as for holotype (field numbers BIF 10050, 10058, 10060, 10061). MNHN-IC 2020-0176, 2 females (24.7-24.8 mm SL); same data as for holotype (field numbers BIF 10054, 10055).

Diagnosis

A small *Schismatogobius*. 16-17 pectoral rays (mostly 17); pectoral fins with a medium black triangular wedge dorsally usually with 2 vertical rows of dark spots below it. First dorsal fin membrane posterior to spine 6 not connected to base of spine of second dorsal fin. Ventral surface of head in male blackish or greyish in the medium part. Frenum whitish. Ventral surface of head in female mainly whitish. Frenum whitish. A single mitochondrial lineage was observed for this species (BOLD: AEA7944).

Description

A small-sized *Schismatogobius* (average adult size < 27 mm SL). Body naked, slender, almost circular in cross-section. Head (27.8-29.8 %SL) rounded, snout rather pointed. Mouth oblique, lower lip more prominent. Upper jaw length in male (13.5 %SL) much greater than in female (10.5-11.7 %SL); jaw length 45.5 % of HL in male (holo-type) and 36.5-39% of HL in females. Lower jaw reaching vertical of 1/2 to 2/3 of the eye in female and > 2/3 in male. Eyes high on head, close together with interorbital width

Table I. – Morphomeristics of the new *Schismatogobius* species. Morphometrics are given as percentages of standard length, except JL/HL. P, Pectoral rays; D, Dorsal rays; A, Anal rays; C, Caudal branched rays; PDL, Predorsal length (% SL); PAL, Preanal length (% SL); HL, Head length (% SL); JL, jaw length (% SL); CPD, Caudal peduncle depth (% SL); Pect-L, Pectoral fin length (% SL); BDa, Body depth at anus (% SL); SDFL, Second dorsal fin length (% SL); AFL, Anal fin length (% SL); CFL, Caudal fin length (% SL).

	S. limmoni	
	Holotype	Paratypes
Р	17	16-17
D	VI,9	VI,8-9
А	I,9	I,8-9
С	11	10-12
PDL	45.6	41.1-45.3
PAL	67.7	62.9-68.4
HL male	29.8	_
JL male	13.5	_
JL/HL male	45.5	-
HL female	_	27.8-29.8
JL female	_	10.5-11.7
JL/HL female	—	36.5-39
CPD	7.7	6.9-8.2
Pect L	28.4	23.8-28.3
BDa	14.4	14.1-15.2
SDFL	33.8	31.2-35.4
AFL	24.4	23.9-27.4
CFL	27.1	21.8-26.2

about equal to 2/3 eye diameter. Anterior nostril short and tube-like. Morphomeristic data given in table I.

Dorsal fins VI-I,8-9, membrane in first dorsal fin posterior to spine 6 not connected to base of spine of second dorsal fin. D1 with all spines about equal in length. Anal fin I,8-9, origin directly opposite to second dorsal fin origin. Caudal fin with 10-12 branched rays, posterior margin straight. Pectoral fins oblong with posterior margin rounded and 16(2)-17(5) rays, ventralmost ray unbranched. Pelvic fins always I,5, with both fins joined together on their entire length between fifth rays to form a strong cup-like disc and a well developed and lobed fraenum between spines; fins not extending beyond anus. Body depth at anus 14.1-15.2%SL, predorsal length 41.1-45.6 %SL and preanal length 62.9-68.4 %SL.

Tongue bilobed. Teeth in upper jaw in two rows (first row 12-17), conical and slightly recurved, mostly caniniform in frontal position in male. Teeth in lower jaw in 2-3 rows (first row 12-17), all teeth conical; only outer row with slightly enlarged and somewhat recurved teeth.

Cephalic sensory pore system always with pores B, D, F, K, L, N and O, pore D singular with all other pores paired; oculoscapular canal absent between pores F and K. Anterior interorbital extension of anterior oculoscapular canal with

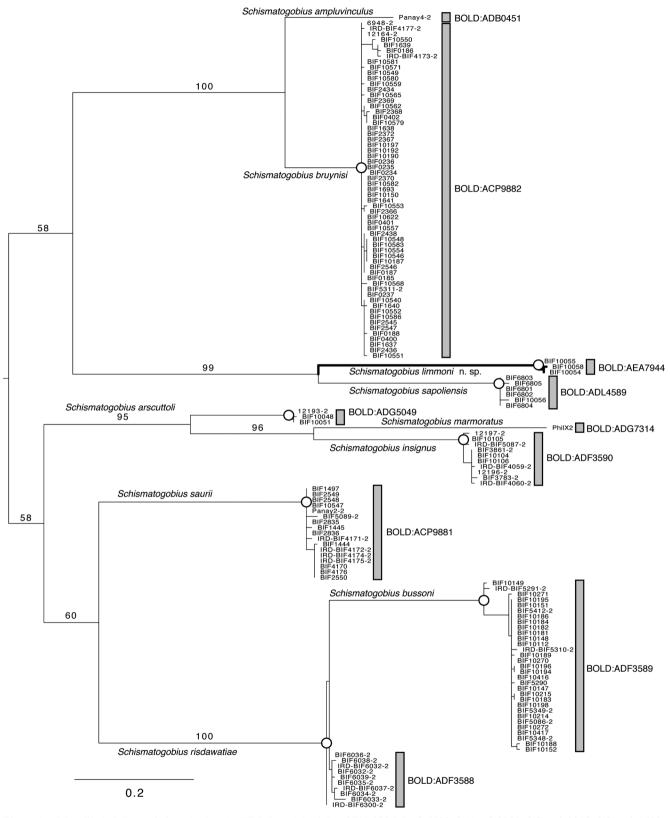


Figure 1. – Most likely ML tree inferred using the TIM+I model (-lnL = 3711.2906, I = 0.6520, f(A) = 0.2382, f(C) = 0.3018, f(G) = 0.1692, f(T) = 0.2909, AC = GT = 1.0, AT = CG = 0.6842, AG = 12.0806, CT = 6.0413). BP are given above each branch. *Schismatogobius limmoni* branch is highlighted in bold.

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double terminal pores B slightly posterior to posterior nostril. D pore at rear of intertobital. Posterior extension of anterior oculoscapular canal terminating laterally on each side of head at pore F, just behind posterior edge of eye. Posterior oculoscapular canal with 2 terminal pores, K and L; preopercular canal with 2 pores, N and O. Cutaneous sensory papillae not well developed.

Sexual dimorphism fairly well developed with male having jaws longer than female and a different colour pattern on ventral surface of head. Urogenital papilla oval in female and slightly pointed in male.

Colour in preservation

Five vertical black bands in dorsal view. The first one on the nape, the second one below the first dorsal fin, the third and fourth below the second dorsal fin and the fifth band at hypural crease. These bands are not uniform but with dorsal or lateral brown to greyish spots. Colour of body between each band whitish to grevish. Head and cheeks usually mottled with closely spaced black or grey spots and markings. Inferior half of lateral part of head brownish in male. Ventral surface of head in male blackish or greyish in the medium part. Fraenum whitish (Fig. 2). Ventral surface of head in female whitish, lightly spotted of brown. Fraenum whitish (Fig. 2). Belly whitish to greyish. First dorsal and second dorsal fins with rows of black spots on rays. Caudal fin mainly translucent with two black spots posteriorly. A black band at hypural base. Pectoral fins translucent with a medium black triangular wedge dorsally usually with 2 vertical rows of dark spots below it.

Colour in life (Figs 3-4)

Five vertical blackish bands in dorsal view. The first one on the nape, the second one below the first dorsal fin, the third and fourth below the second dorsal fin and the fifth band at hypural crease. These bands are not uniformly black but with dorsal or lateral greyish spots. Colour of body, on the

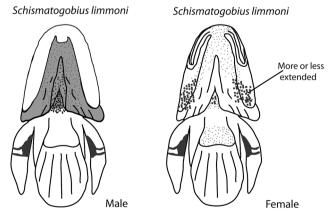


Figure 2. – Schematic drawing of the ventral surface of head of *Schismatogobius limmoni*.



Figure 3. – *Schismatogobius limmoni* n. sp., male, holotype MZB.25491 (SL 22.2 mm; BIF 10059) (Photo N. Hubert).



Figure 4. – *Schismatogobius limmoni* n. sp., female, paratype *in* MNHN-IC 2020-0176 (SL 24.7 mm; BIF 10054) (Photo N. Hubert).

upper part and between each band greyish with some pinkish to orange areas, particularly between the second and the third bands. On the inferior longitudinal part, the body is mottled black and grey with three aligned horizontal ovoid whitish to silvery spots on the posterior part. Head and cheeks mottled with closely spaced black or pink-orange spots and markings, and an overall shade of greyish-yellow. Sub-inferior part of head blackish. Ventral surface of head in male blackish or greyish in the medium part. Fraenum whitish. Ventral surface of head in female whitish. Fraenum whitish. Belly whitish to greyish. First and second dorsal fins with rows of black spots on rays. Caudal fin mainly translucent with two median white spots dorsally and ventrally, two black spots posteriorly and a large white spot at hypural base. Pectoral fins translucent with a medium black triangular wedge dorsally usually with 2 vertical rows of dark spots below it.

Habitat

Schismatogobius limmoni has been collected in a freshwater stream with moderate to fast flow in shallow areas of boulders, gravel and sand.

Etymology

The new species is named *limmoni* in dedication to Gino Limmon, from Universitas Pattimura, for all his work to improve our knowledge on Indonesian fishes and its application for the conservation of the Indonesian aquatic fauna.

Affinities

Schismatogobius limmoni differs from the other species sequenced and present in the area studied by displaying distinct mitochondrial lineages from its closest relatives and high TIM+I genetic distances to its relatives at *COI* gene (0.6749 to its closest relative *S. sapoliensis*). It is also the second only known species of the area studied (with *S. sapoliensis*) with 16-17 pectorals rays and having a broad black triangular wedge dorsally on pectoral fins. Indeed, *S. saurii, S. bussoni* and *S. bruynisi* have 14-15 pectorals rays, *S. risdawatiae* has 15-16 pectorals rays, and *S. insignus* and *S. arscuttoli* have 16-17 pectorals rays but the pectoral fins are striped with rows of dark spots (Keith *et al.* 2017b, 2018). It differs from *S. sapoliensis* in having a greater jaw length/head length ratio in female (36.5-39% vs 32-36.7%), a greater jaw length in female (10.5-11.7% vs 9.1-11% SL), pectoral fins (usually 17 rays vs usually 16) with a medium black triangular wedge dorsally usually with 2 vertical rows of dark spots below it *versus* pectoral fins with a large transverse medium black band crossing usually all the fin, and a greater preanal length (62.9-68.4 vs 61.4-62.8) (Tab. I).

Distribution

This species is known only from Sulawesi, Indonesia

Comparative material

See Keith et al. 2017a, b and 2018.

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