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

by

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Abstract. – A new species of *Schismatogobius*, a freshwater goby, is described from Halmahera (Indonesia). It differs from other species belonging to the genus by a high percentage of genetic divergence in partial COI gene (652 bp) and by several characters, including the number of pectoral fin rays, the pattern of the ventral surface of the head, the pectoral fin colour pattern and the jaw length/head length ratio of male and female.

Résumé. – Une nouvelle espèce de *Schismatogobius* (Teleostei : Gobiidae) d'Halmahera (Indonésie).

Une espèce nouvelle de *Schismatogobius*, gobie dulçaquicole, est décrite sur la base de spécimens collectés à Halmahera (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 de la surface ventrale de la tête, la coloration des nageoires pectorales et le ratio longueur de la mâchoire/longueur de la tête du mâle et de la femelle.

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The species of *Schismatogobius* de Beaufort, 1912 are distinctive scaleless freshwater gobies found in the tropical Indo-West Pacific. Recently, Keith *et al*. (2017a, b) reviewed the species found in Indonesia and between Papua New Guinea and Samoa, describing 11 new species and Maeda *et al*. (2018) also described a new species from Japan.

In the Indonesian region, six 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, Dahruddin, Sukmono & Hubert, 2017; and *S. bussoni* Keith, Hubert, Limmon & Dahruddin, 2017. In March 2017, a collaborative work between the Institute for Research and Development (IRD), the Indonesian Institute of Sciences (LIPI), the Universitas Pattimura and the National Museum of Natural History of Paris (MNHN) was conducted in rivers of Halmahera Island (Moluccas) where *Schismatogobius* specimens were collected.

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

MATERIALS AND METHODS

DNA Barcode analysis

Material examined

A total of 79 *Schismatogobius* specimens were used for this analysis. They were those used by Keith *et al*. (2017b) added to the specimens cited below and listed in table I.

Schismatogobius nsp: 5 specimens, Samuda, Air Turjun Sapoli, Halmahera, Indonesia, 23 Mar. 2017; Hubert *et al*. coll.: MZB 24586 (1 spm); BIF 6802. MZB 24587 (2 spms); BIF 6803 & 6805. MNHN 2016-0626 (1 spm); BIF 6801. MNHN 2016-0627 (1 spm); BIF 6804.

Schismatogobius insignus: 3 specimens; MZB (uncata-

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Table I. – Specimens used for the DNA barcode analysis (names, sequences and Barecode Index Numbers).

Species	Sample ID	Sequence ID	BIN
Schismatogobius bruynisi	BIF 4177	BIFZI007-17	BOLD:ACP9882
Schismatogobius bruynisi	BIF 4173	BIFZI004-17	BOLD:ACP9882
Schismatogobius bussoni	BIF 5291	BIFZI026-17	BOLD:ADF3589
Schismatogobius bussoni	BIF 5310	BIFZI051-17	BOLD:ADF3589
Schismatogobius insignus	BIF 4059	BIFZI025-17	BOLD:ADF3590
Schismatogobius insignus	BIF 5087	BIFZI056-17	BOLD:ADF3590
Schismatogobius insignus	BIF 4060	BIFZI057-17	BOLD:ADF3590
Schismatogobius nsp	BIF 6801	BIFZI001-18	BOLD: ADL4589
Schismatogobius nsp	BIF 6802	BIFZI002-18	BOLD: ADL4589
Schismatogobius nsp	BIF 6803	BIFZI003-18	BOLD: ADL4589
Schismatogobius nsp	BIF 6804	BIFZI004-18	BOLD: ADL4589
Schismatogobius nsp	BIF 6805	BIFZI005-18	BOLD: ADL4589
Schismatogobius risdawatiae	BIF 6037	BIFZI010-17	BOLD:ADF3588
Schismatogobius risdawatiae	BIF 6300	BIFZI032-17	BOLD:ADF3588
Schismatogobius saurii	BIF 4175	BIFZI038-17	BOLD:ACP9881
Schismatogobius saurii	BIF 4174	BIFZI042-17	BOLD:ACP9881
Schismatogobius saurii	BIF 4172	BIFZI045-17	BOLD:ACP9881
Schismatogobius saurii	BIF 4171	BIFZI049-17	BOLD:ACP9881

logued): BIF 4059 & 4060, Lombok Utara, 2 Apr. 2015, Hubert *et al*. coll.; BIF 5087, Ambon, 25 Mar. 2016; Hubert *et al*. coll.

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

Schismatogobius saurii: 4 specimens; MZB (uncatalogued): BIF 4171, 4172, 4174 & 4175, Lampung Barat, Wai Ngarip, Sumatra, Indonesia, 22 May 2015, Hubert *et al*. coll.

Schismatogobius bussoni: 2 specimens; MZB (uncatalogued): BIF 5291, Ceram Tengah, Wai Sia, Ceram, Indonesia, 28 Mar. 2016, Hubert *et al*. coll.; BIF 5310, Ceram Tengah, Wai Hetu, Ceram, Indonesia, 28 Mar. 2016, Hubert *et al*. coll.

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

DNA extraction and amplification

Pectoral fin tissue was used to extract total genomic DNA from 18 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. Purification and Sanger sequencing of PCR products were performed by Eurofins (http://www.eurofins.fr) using the same forward and reverse PCR primers. Chromatograms were assembled and edited using Geneious 8.1.5. All the sequences were aligned with MAFFT Alignment (implemented in Geneious). The translation into amino acids was checked for the partial fragment of COI gene, using the vertebrate mitochondrial genetic code. After translation, one or two bases were discarded at the beginning and the end of the sequences and as a result all the sequences in the alignment started and ended with a codon. All the sequences have been deposited in the barcode of life data system (www.boldsystems.org; projects BIFB, BIFFA and BIFFB) 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-Neighbor 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 right side. The size is given in standard length (SL). Abbreviation are as follow: P, pectoral rays; D, dorsal rays; A, anal rays; PDL, predorsal length (% SL); PAL, preanal length (% SL); HL, head length (% SL); JL, jaw length (% SL); CPL, caudal peduncle length (% SL); Pect-L, pectoral fin length (% SL); BDa, body depth at

Table II. – 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; PDL, predorsal length (% SL); PAL, preanal length (% SL); HL, head length (% SL); JL, jaw length (% SL); CPL, caudal peduncle length (% 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. sapoliensis	
	Holotype	Paratypes
P	16	16-17
D	VI 9	VI 8-9
A	I9	I8-9
PDL	44.7	41.5-47.8
PAL	61.5	61.4-62.8
HL male	31.8	29.8
JL male	15.1	15.8
JL/HL male	47.4	52.8
HL female	_	28.2-30.3
JL female	_	9.1-11.1
JL/HL female	_	32-36.7
CPD	7.4	6.7-8.6
Pect L	24.6	23.9-25.9
BDa	15.7	13.1-15.4
SDFL	31.5	27-30.9
AFL	33	30.9-37.8
CFL	26.3	22.2-24.8

anus (% SL); SDFL, second dorsal fin length (% SL); AFL, anal fin length (% SL); CFL, caudal fin length (% SL); SL, standard length (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).

Abbreviations for institutions and collections cited follow the American Society of Ichthyologists and Herpetologists (http://www.asih.org/sites/default/files/documents/resources/symbolic_codes_for_ collections_v5.0_sabajperez_2014.pdf).

Morphomeristic data are summarized in table II.

RESULTS

DNA Barcode analysis

A total of 652 base pairs were amplified for the COI gene. The most likely substitution model selected by jmodeltest was TrN + I. The ML tree (Fig. 1) allowed delimiting nine species, each corresponding to a distinct mitochondrial lineage as evidenced by the RESL algorithm (Tab. I). (BOLD:ACP9881, BOLD:ADF3589, BOLD:ADF3588, BOLD:ACP9882, BOLD:ADF3590, BOLD:ADG5049, BOLD:ADG7314, BOLD:ADB0451, BOLD: ADL4589).

Morphomeristics

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

Schismatogobius sapoliensis, n. sp.

(Figs 1-3; Tabs I-II)

Material examined

Five specimens from Halmahera with a size range of 16.2-18 mm SL.

Holotype. – MZB 24586, male (17.9 mm SL); Samuda, Sapoli, Halmahera, Indonesia, 23 March 2017, Hubert *et al*. coll.; BIF 6802.

Paratypes. – MZB 24587, 2 females (16.2-17.6 mm SL); same data as for holotype; BIF 6803 & 6805. MNHN 2016-0626, 1 female (18 mm SL); same data as for holotype; BIF 6801. MNHN 2016-0627, 1 male (17.8 mm SL); same data as for holotype; BIF 6804.

Diagnosis

Usually 16 pectoral rays; pectoral fins with a large transverse medium black band. 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 with a white mentum. Frenum whitish with a blackish border. Ventral surface of head in female blackish or greyish, except the isthmus, which is whitish, and with a white mentum. Frenum whitish, sometimes with a distal black dot. A single mitochondrial lineage was observed for this species (BOLD: ADL4589).

Description

A small sized *Schismatogobius* (average adult size < 20 mm SL). Body naked, slender, almost circular in cross-section. Head rounded, snout rather pointed. Mouth oblique, lower lip more prominent. Jaw length in male much greater than in female; jaw length 47.4-52.8% of HL in males and 32-36.7% of HL in females. Lower jaw reaching vertical of 1/3 to 1/2 of the eye in female and exceeding a vertical of posterior margin of eye in male. Eyes high on head, close together with interorbital width about equal to 1/3 eye diameter. Anterior nostril short and tube-like.

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 10-11 branched rays, posterior margin straight. Pectoral

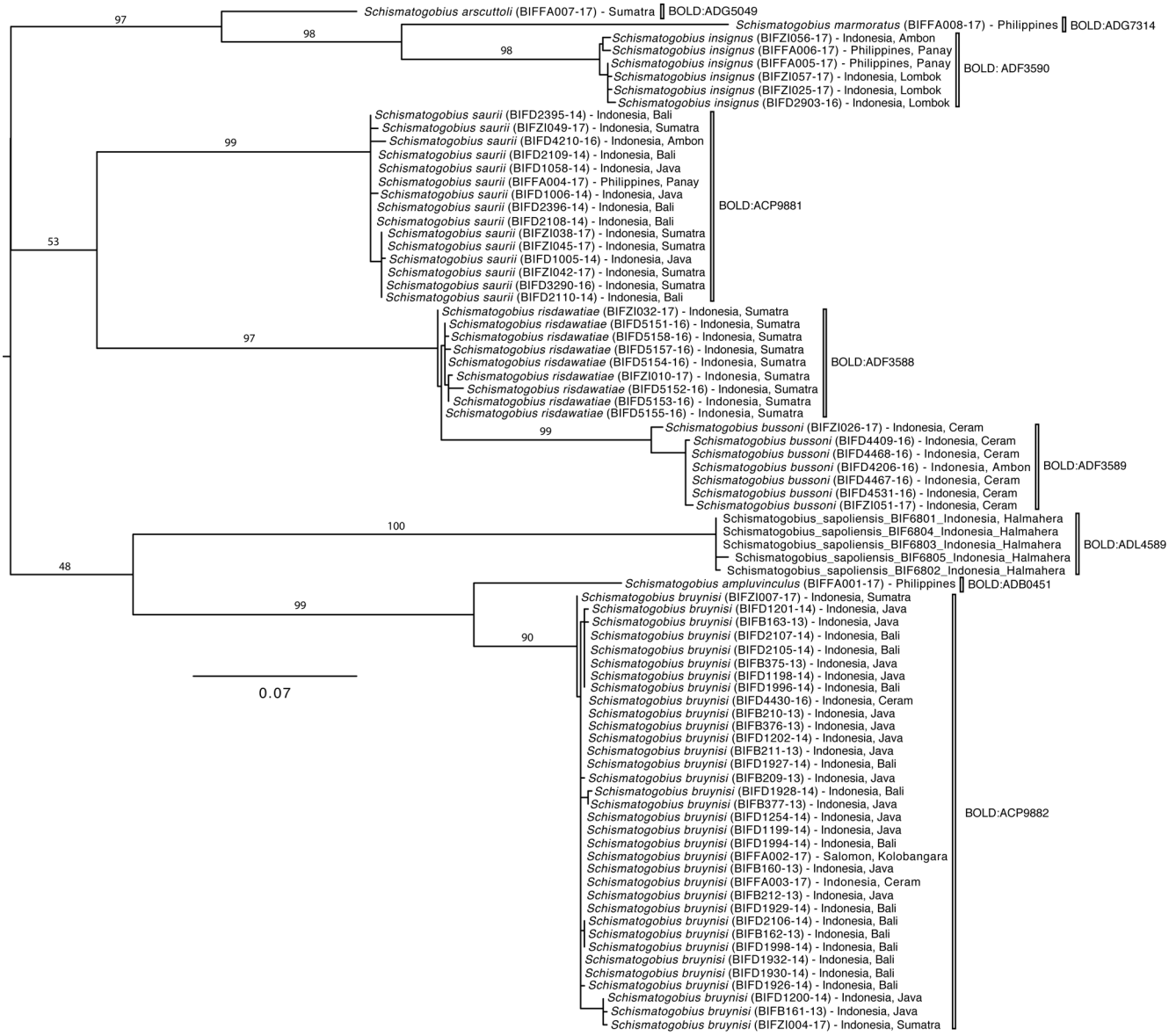

Figure 1. – Most likely ML tree inferred using the TrN+I model (-lnL = 3272.52860.77, I = 0.656, f(A) = 0.23604, f(C) = 0.30606, f(G) = 0.17125, f(T) = 0.28665, AC = AT = CG = GT = 1.0; AG = 14.38; CT = 6.78613). BP are given above each branch.

fins oblong with posterior margin rounded and 16(4)-17(1) rays, ventralmost ray unbranched. Pelvic fins always I,5, with both fins joined together for their entire length between fifth rays to form a strong cup-like disc and a well-developed and lobed frenum between spines; fins not extending beyond anus. Morphomeristic data given in table II.

Tongue bilobed. Teeth in upper jaw (first row 8-10) in two rows, teeth conical and slightly recurved. Teeth in lower jaw (first row 12-13) in 2-3 rows, all teeth conical with outer row teeth only slightly enlarged and somewhat recurved.

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 double terminal pores B slightly posterior to posterior nostril. D pore at rear of interorbital. 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.

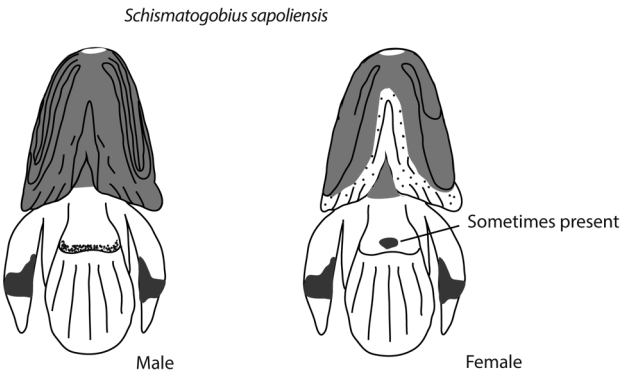

Figure 2. – Schematic drawing of the ventral surface of head of *Schismatogobius sapoliensis*.

Colour in preservation

Body with three or four vertical black bands in dorsal view. When four bands, first band below first dorsal fin, second and third bands below second dorsal fin (sometimes these two bands are overlap) and fourth band at hypural crease. These lateral black body markings alternate with 3 vertical white to grey stripes. Head dusky grey. Ventral surface of head in male blackish or greyish with a white mentum. Frenum whitish with a blackish distal border. Ventral surface of head in female blackish or greyish except the isthmus, which is whitish, and with a white mentum. Frenum whitish, sometimes with a distal black dot (Fig. 2). First dorsal fin with a large horizontal black band. Second dorsal fin mostly cream with rows of black spots on rays. Caudal fin black and white, with a black spot at centre of hypural crease and two white spots posteriorly. Anal fin mostly greyish. Pectoral fins greyish with a large transverse medium black band.

Colour in life (Figs 3, 4)

Three or four vertical black bands in dorsal view. When four bands, first band below first dorsal fin, second and third bands below second dorsal fin and fourth band at hypural crease; the two middle bands are very close to each other. These bands are not uniform but with dorsal or lateral brown to rose spots. Colour of body between each band rose to greyish. Head and cheeks usually mottled with closely spaced black spots and markings, and an overall shade of greyish-brown. Ventral surface of head in male blackish or greyish with a white mentum. Frenum whitish with a blackish border. Ventral surface of head in female blackish or greyish except the isthmus, which is whitish, and with a white mentum. Frenum whitish, sometimes with a distal black dot. Belly whitish to greyish. First dorsal fin with a large horizontal black band. Second dorsal fin with rows of black spots on rays. Caudal fin mainly black with two median white spots dorsally and ventrally. Pectoral fins translucent with a large transverse medium black band.

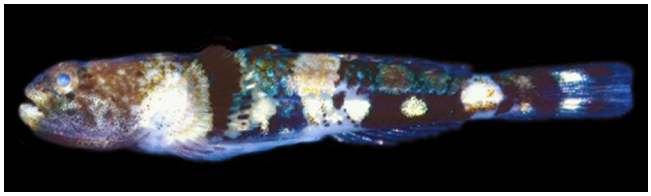

Figure 3. – *Schismatogobius sapoliensis* n. sp., male, holotype MZB 24586, BIF 6802 (17.9 mm) (Photo N. Hubert).

Figure 4. – *Schismatogobius sapoliensis* n. sp., female, *in vivo* (Photo F. Busson).

Habitat

Schismatogobius sapoliensis has been collected in a freshwater stream with moderate to fast flow in shallow areas of rocks and gravel (depth 0.2-0.3 m), just below a waterfall.

Etymology

The new species name is derived from the geographic area, Sapoli, where the specimens were caught in Halmahera.

Affinities

Schismatogobius sapoliensis differs from the other species sequenced and present in the area studied displaying reciprocal monophyly from its closest relatives and high TrN+I genetic distances to its relatives at COI gene. It is also the only known species of the area studied with 16-17 pectorals rays and having a large transverse medium black band 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 banded with rows of dark spots (Keith *et al.,* 2017b).

Distribution

This species is known only from Halmahera (Moluccas).

Comparative material

See Keith *et al*., 2017a, b.

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