

A new species of freshwater pipefish (Teleostei: Syngnathidae: *Coelonotus*) from Papua New Guinea

by

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Abstract. – A new species of *Coelonotus*, a freshwater pipefish, is described on the basis of six specimens from the Gavuvu River of West New Britain Island (Papua New Guinea). It differs from other *Coelonotus* species by a combination of morphomeristic values including: number of dorsal fin rays (45-47), number of subdorsal (4-5) and tail rings (35-37). The Folmer DNA barcode fragment of the *COI* mitochondrial gene shows that this new species is indeed a different genetic lineage from other *Coelonotus* species of this area.

Résumé. – Une nouvelle espèce de *Coelonotus* (Teleostei : Syngnathidae) d'eau douce de Papouasie-Nouvelle-Guinée.

Une nouvelle espèce de *Coelonotus*, un syngnathe dulçaquicole, est décrite sur la base de six spécimens de la rivière Gavuvu de l'île de Nouvelle-Bretagne occidentale (Papouasie-Nouvelle-Guinée). Elle diffère des autres *Coelonotus* par une combinaison de plusieurs valeurs morpho-méristiques comprenant : le nombre de rayons de la nageoire dorsale (45-47), le nombre d'anneaux subdorsaux (4-5) et le nombre d'anneaux de la queue (35-37). Le fragment *Barcode* du gène mitochondrial *COI*, montre que cette nouvelle espèce correspond bien à une lignée génétique différente des autres espèces de *Coelonotus* de cette région.

Key words

Coelonotus kaipuae
New species
Syngnathidae
Taxonomy
Cytochrome oxidase 1

INTRODUCTION

Papua New Guinea (PNG) is often considered as a biodiversity sanctuary (Novotný *et al.*, 2006; Marshall and Beehler, 2007). Located north of the Australian mainland, PNG is an archipelago composed of a large main continental island and several volcanic islands (Heming, 1974), known as the Bismarck Archipelago. Several main islands are part of it: New Britain, New Ireland and the Admiralty Islands. The ruggedness and the inaccessibility make these areas poorly known (Amick and Toko, 2021). However, these islands harbour numerous species and many of them are endemic to PNG. Freshwater fish biodiversity is no exception and many species are restricted to relatively small isolated catchments (Allen *et al.*, 2008; Keith *et al.*, 2019; Keith and Mennesson, 2020; Lord *et al.*, 2020) like *Sicyopus beremeensis* Keith, Amick, Toko & Lord, 2019 or *Sicyopterus elomionearum* Lord, Keith, Causse & Amick, 2020. Several species of Syngnathidae (pipefish) inhabit these tropical rivers.

The Syngnathidae family includes about 50 genera and 300 species and is represented by seahorses, pipefishes and sea dragons and the vast majority is distributed in tropical and temperate marine coastal waters (Hamilton *et al.*, 2017). However, about thirty of them are part of the fish communities in tropical island rivers of the Indo-Pacific (Nelson *et al.*, 2016). Syngnathidae have a quite particular reproductive behaviour. The male carries the eggs in a ventral pouch and takes care of them until they hatch. The position of the brood pouch, under the trunk or the tail, led to the subdivision into two subfamilies: Syngnathinae (tail-brooders) and Nerophinae (trunk-brooders) (Herald, 1959). Freshwater pipefishes, which are mainly part of the Nerophinae (one genus of Syngnathinae is known from freshwater, *Hippichthys* Bleeker, 1849), have not been studied for over 35 years and the taxonomy and nomenclature are solely based on morphological characters (Dawson, 1985; Kottelat, 2013; Miesen *et al.*, 2016). The specific diversity of this group is therefore probably largely underestimated. According to

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Kottelat (2013), there are six genera of Nerophinae freshwater pipefish: *Micropis* Kaup, 1853; *Coelonotus* Peters, 1855; *Doryichthys* Kaup, 1856; *Belonichthys* Peters, 1868; *Oostethus* Hubbs, 1929; and *Lophocampus* Dawson, 1984.

In 2018, a survey has been carried out by the Muséum national d'Histoire naturelle in West New Britain rivers (Papua New Guinea) with many specimens of freshwater pipefish collected. Several specimens, closely related to *Coelonotus argulus* (Peters, 1855) and *Coelonotus biocellatus* Günther, 1870 but which differ in some counting, were caught. Three species are known under the genus *Coelonotus*: *Coelonotus leiaspis* (Bleeker, 1854) distributed in Japan, Indonesia, New Caledonia, Solomon and PNG; *Coelonotus argulus* (Peters, 1855) distributed from Comoro to Marquesas and *Coelonotus biocellatus* Günther, 1870 distributed in Indonesia. Nowadays, the study and the description of species is no longer only based on morphomeristic data; taxonomists increasingly use integrative taxonomy which very often combines morphological and molecular data as well as environmental, geographical or behavioural data to improve species delimitation (e.g. Padial *et al.*, 2010). The purpose of this paper is to describe a new species of *Coelonotus* from New Britain, Papua New Guinea (*Coelonotus kaipuae*), comparing them to all known *Coelonotus* species, based on morphomeristic and on a mitochondrial fragment of *Cytochrome oxidase subunit I* gene.

MATERIALS AND METHODS

Abbreviations used

COI, *Cytochrome oxidase subunit I*; MNHN, Muséum national d'Histoire naturelle, Paris, France; BMNH, British Museum of Natural History, London, UK; ZMB, Zoologisches Museum, Museum für Naturkunde der Humboldt-Universität, Berlin, Germany; RMNH, Rijksmuseum van Natuurlijke Historie, Leiden, the Netherlands; SL, standard length.

Sample collection

The fish included in the study (Tab. I) were sampled using a DEKA 3000 electrofishing system (Gerätebau, Marsberg, Germany) (except for type specimens). Following the annex IV of the directive 2010/63/EU, fish were euthanized using an overdose of clove essential oil. Entire fish were stored and preserved in 95% ethanol.

Comparative material

Coelonotus argulus (Peters, 1855). ZMB 6232, Syntypes, 2 females, 119.2 and 110.3 mm SL, Streams of St. Johanna, Anjouan Island, Comoro Islands. MNHN-IC-2006-0616, 1 female, 97.46 mm SL, Anjouan Island, Comoro Islands, 30 Oct. 2005, Keith & Marquet coll. MNHN-IC-2021-0308,

1 male, 106.12 mm SL, 1 female, 98.57 mm SL, Ranongga Island, Solomon Islands, 26 Oct. 2016, Keith coll. MNHN-IC-2021-0309, 2 females, 86.45 and 101.12 mm SL, Ranongga Island, Solomon Islands, 28 Oct. 2016, Keith coll. MNHN-IC-2021-0310, 1 male, 108.78 mm SL, Kolombogara Island, Solomon Islands, 13 Nov. 2015, Keith coll. MNHN-IC-2004-1651, 1 female, 93.6 mm SL, Tahuata Island, Marquesas, 16 Feb. 2000, Keith coll.

Coelonotus biocellatus Günther, 1870. BMNH 1868.11.17.37, Holotype, 1 female, 117.9 mm SL, Java, Indonesia.

Coelonotus leiaspis (Bleeker, 1854). RMNH 7252, Syntype, 1 female, Java, Indonesia (specimen highly damaged). MNHN-IC-2021-0311, 1 male, 120.46 mm SL, Ambon, Indonesia, 04 Sep. 1916, unknown coll. MNHN-2021-0312, 1 female, 98.57 mm SL, West New Britain, Papua New Guinea, 30 Oct. 2018, Keith coll. MNHN-IC-2021-0313, 1 female, 136.22 mm SL, Kolombogara Island, Solomon Islands, 11 Nov. 2015, Keith coll. MNHN-IC-2021-0314, 1 female, 107.64 mm SL, Ceram, Indonesia, 29 Mar. 1916, unknown coll.

Hippichthys heptagonus Bleeker, 1849. MNHN-IC-2021-0315, 1 female, 72.07 mm SL, Isabel Island, Solomon Islands, 25 Oct. 2019, Keith *et al.* coll. MNHN-IC-2021-0316, 1 female, 59.28 mm SL, Isabel Island, Solomon Islands, 26 Oct. 2019, Keith coll.

Morphomeristic

Methods follow Dawson (1977) with several additional measurements. All counts and measurements were taken from the right side of specimens. Measurements were taken with a dial calliper to the nearest tenth of a millimetre and expressed to the nearest whole percent of standard length (% SL). The size is given as standard length (SL). Length measurements are reported as: HL, head length; SnL, snout length; SnD, snout depth; BD, body depth. Ring and fin ray counts are reported as: P, pectoral fin rays; D, dorsal fin rays; C, caudal fin rays; TrR, trunk rings, dermal rings counted from operculum to urogenital papilla; TaR, tail rings, dermal rings counted from urogenital papilla to the last ring before the caudal fin; STr, subdorsal trunk rings, dermal rings counted from anterior dorsal fin insertion to urogenital papilla; STa, subdorsal tail rings, dermal rings counted from urogenital papilla to posterior dorsal fin insertion; STt, subdorsal rings, total dermal rings counted under dorsal fin. In addition to counts and measurements, the observation of ridges on certain body parts (patterns of trunk and tail ridges, opercular ridges) were done. Sex identification was made for each specimen by observation of the urogenital papilla.

DNA extraction and amplification

DNA sampling was performed from caudal fin clips for seventeen specimens of four species (see Tab. I) and from

Table 1. – Samples for morpho-meristic and molecular analyses. Type specimens: (●) Holotype; (■) Syntype. MNHN: Muséum national d'Histoire naturelle; ZMB: Zoologisches Museum; BMNH: British Museum of Natural History; RMNH: Rijksmuseum van Natuurlijke Historie. M: male; F: female. PNG: Papua New Guinea.

Collection numbers	Tag nbs	Species	Standard length (mm)	Sex	Archipelagos	Islands	Rivers	Date	Collectors	GenBank number	COI	Morpho-meristic
MNHN-IC-2021-0306	19161	<i>Coelonotus kaupuae</i>	86.22	M	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465467	X	X
MNHN-IC-2021-0307	19163	<i>Coelonotus kaupuae</i>	70.88	M	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465469	X	X
MNHN-IC-2021-0307	19164	<i>Coelonotus kaupuae</i>	77.25	F	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465470	X	X
MNHN-IC-2021-0307	19166	<i>Coelonotus kaupuae</i>	82.76	M	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465471	X	X
MNHN-IC-2021-0307	19167	<i>Coelonotus kaupuae</i>	83.87	F	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465472	X	X
MNHN-IC-2021-0307	19168	<i>Coelonotus kaupuae</i>	84.83	M	PNG	New Britain	Gavuvu	28/10/18	Keith et al.	OK465468	X	X
ZMB 6232 ■	1	<i>Coelonotus argulus</i>	119.2	F	Comoros	Anjouan	St. Johanna	1855	Peters	–	–	X
ZMB 6232 ■	2	<i>Coelonotus argulus</i>	110.3	F	Comoros	Anjouan	St. Johanna	1855	Peters	–	–	X
MNHN-IC-2006-0616	–	<i>Coelonotus argulus</i>	97.46	M	Comoros	Moheli	–	30/10/05	Keith & Marquet	OK465461	X	X
MNHN-IC-2021-0308	14958	<i>Coelonotus argulus</i>	106.12	M	Solomon	Ranongga	Mondo	26/10/16	Keith et al.	OK465463	X	X
MNHN-IC-2021-0308	14959	<i>Coelonotus argulus</i>	98.57	F	Solomon	Ranongga	Mondo	26/10/16	Keith et al.	OK465464	X	X
MNHN-IC-2021-0309	14965	<i>Coelonotus argulus</i>	86.45	F	Solomon	Ranongga	Qiloe Paro	28/10/16	Keith et al.	OK465462	X	X
MNHN-IC-2021-0309	14966	<i>Coelonotus argulus</i>	101.12	F	Solomon	Ranongga	Qiloe Paro	28/10/16	Keith et al.	OK465465	X	X
MNHN-IC-2021-0310	19189	<i>Coelonotus argulus</i>	108.78	M	Solomon	Kolombogara	Poitete	13/11/15	Keith et al.	OK465466	X	X
MNHN-IC-2004-1651	–	<i>Coelonotus argulus</i>	93.6	F	Marquesas	Tahuata	–	16/02/00	Keith et al.	–	–	X
BMNH 1868-11-17-37 ●	–	<i>Coelonotus biocellatus</i>	117.93	F	Indonesia	Java	–	1852	Günther	–	–	X
RMNH 7252 ■	–	<i>Coelonotus leiaspis</i>	–	F	Indonesia	Java	–	1854	Bleeker	–	–	X
MNHN-IC-2021-0311	14970	<i>Coelonotus leiaspis</i>	120.46	M	Indonesia	Ambon	–	04/09/16	unknown	OK465473	X	X
MNHN-IC-2021-0312	19061	<i>Coelonotus leiaspis</i>	98.57	F	PNG	New Britain	Walindi	30/10/18	Keith et al.	OK465474	X	X
MNHN-IC-2021-0313	19192	<i>Coelonotus leiaspis</i>	136.22	F	Solomon	Kolombogara	Kiva	11/11/15	Keith et al.	OK465475	X	X
MNHN-IC-2021-0314	19200	<i>Coelonotus leiaspis</i>	107.64	F	Indonesia	Ceram	Wahai	09/09/16	unknown	OK465476	X	X
–	–	<i>Oostethus brachyurus</i>	–	–	China	–	–	–	Lin & Qin	KT355100	X	–
–	–	<i>Oostethus brachyurus</i>	–	–	China	–	–	–	Lin & Qin	KT355102	X	–
–	–	<i>Oostethus brachyurus</i>	–	–	China	–	–	–	Lin & Qin	KT355103	X	–
MNHN-IC-2021-0315	18257	<i>Hippichthys heptagonus</i>	72.07	F	Solomon	Isabel	Kolopakissa	25/10/19	Keith et al.	OK465477	X	X
MNHN-IC-2021-0316	18273	<i>Hippichthys heptagonus</i>	59.28	F	Solomon	Isabel	Kolopakissa	26/10/19	Keith et al.	OK465478	X	X

the internal tissue for one specimen (*C. argulus* MNHN-IC-2006-0616) following non-destructive sampling protocol described by Haï *et al.* (2020). DNA was extracted using the Macherey and Nagel NucleoSpin® Tissue kit following the manufacturer’s instructions on an Eppendorf EpMotion 5075. A mitochondrial fragment of the *COI* gene (650 pb) was amplified using the tailed fish specific primers VF2-t1 5’ TGTAACGACGGCCAGTCAACCAACCACAGACATTGGCAC3’; FishF2-t1 5’TGTAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC3’; Fishr2-t1 5’CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGA3’ (Ward *et al.*, 2005); Fr1d-t15’CAGGAAACAGCTATGACACCACAGGGTGTCCGARRAAYCARAA3’ (Ivanova *et al.*, 2007). DNA was amplified by PCR in a final 20 µL volume containing 1 µL DMSO, 1 µL BSA 1 µL of dNTP 6.6 µM, 0.15 µL of Qiagen Taq DNA polymerase, using 2 µL of the buffer provided by the manufacturer and 0.4 µL of each of the four primers at 10 pM; 2 µL of DNA extract was added. After a 2 min denaturation at 94°C, the PCR was run for 60 cycles (30 s, 94°C; 45 s, 54°C; 1 min, 72°C), with a 2-minute terminal elongation on a Bio-rad t100™ thermal Cycler. Successful PCRs were selected on ethidium-bromide stained agarose gels. Sanger sequencing was performed in both directions by

a commercial company (Eurofins) (<http://www.eurofins.fr>) using M13 tail primers M13F (–21) 5’TGTAACGACGGCCAGT3’; M13R (–27) 5’CAGGAAACAGCTATGAC3’ (Messing, 1983). Sequences were quality checked and aligned with MAFFT alignment (Kato *et al.*, 2002) in Geneious Prime 2020.2.4 (<http://www.geneious.com>).

Eighteen sequences were obtained for the phylogenetic reconstruction and three sequences of *Oostethus brachyurus* were added from GenBank (KT355100, KT355102 and KT355103) to compare *Coelonotus* species with another genus of freshwater Nerophinae. The percentage of divergence between sequences was calculated in Geneious Prime 2020.2.4. PartitionFinder v.2.1.1 (Lanfear *et al.*, 2012) was used to estimate the best evolution model. Three models depending to the three-codon position were selected under the Bayesian Information Criterion (1st position TRNEF + G; 2nd position F81 + G; 3rd position HKY + G). A phylogenetic tree was constructed using Bayesian inference (MrBayes v.3.2.6; Ronquist *et al.*, 2012). Bayesian inference was run for 10 million generations, sampling every 200 generations with two independent runs. Run convergence was checked using TRACER v. 1.6.0 (Rambaut *et al.*, 2018). The tree obtained is a majority consensus with 25% of the trees discarded as burn-in and visualized using FigTree v.1.4.0 (Ram-

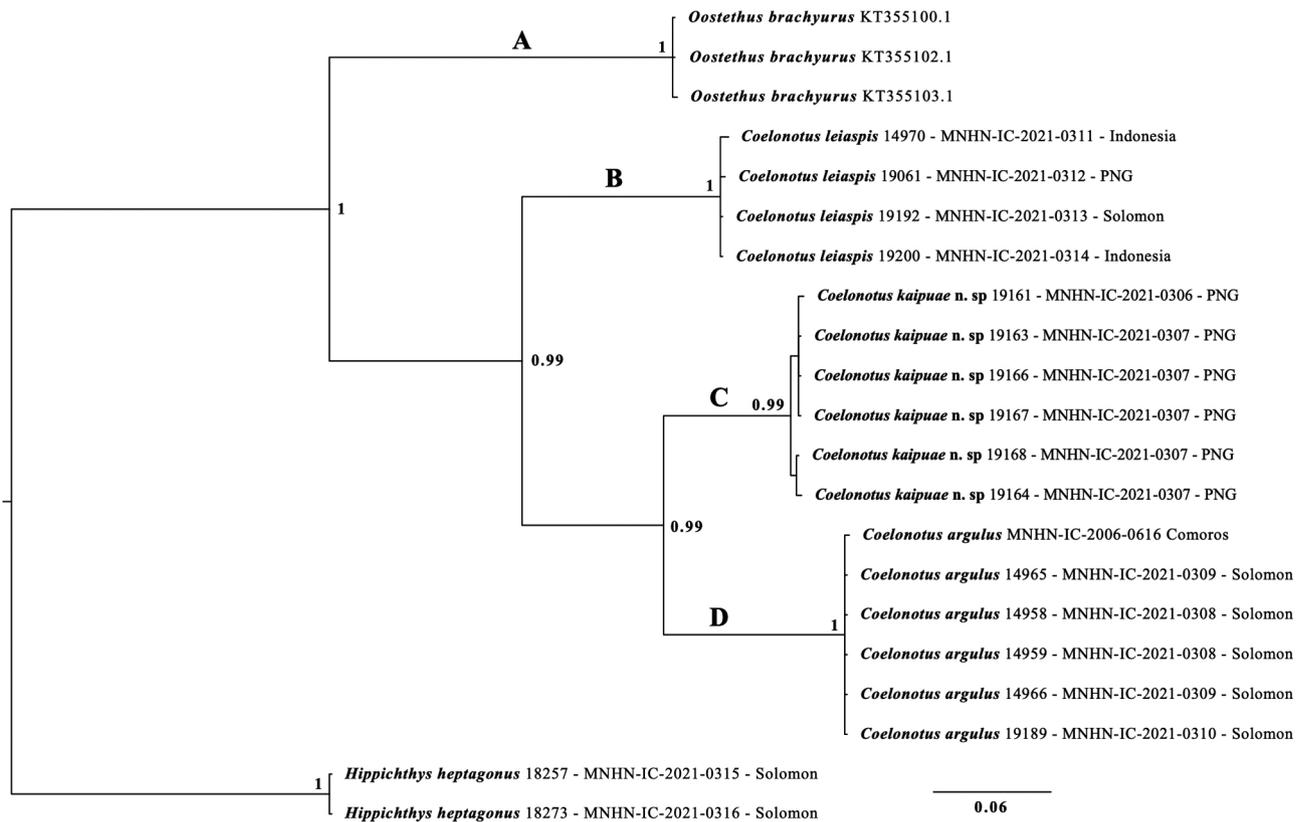


Figure 1. – Bayesian tree of the cytochrome c oxidase subunit (*COI* – 564 bp) for sequenced specimens of *Coelonotus*. Numbers at each node represent posterior probabilities. Outgroups are represented by *Hippichthys heptagonus*. PNG: Papua New Guinea.

baut, 2012). Two sequences of Syngnathinae (tail-brooders) freshwater pipefish, *Hippichthys heptagonus*, were included as outgroups.

RESULTS

DNA analysis

A total of 21 partial *COI* sequences were used for the phylogenetic reconstruction based on an alignment of 564 base pairs (bp) (Fig. 1). The phylogenetic tree based on partial *COI* shows four clades: clade A (*Oostethus brachyurus*), clade B (*Coelonotus leiaspis*), clade C (*Coelonotus kaupuae* n. sp.) and clade D (*Coelonotus argulus*). No specimen of *C. biocellatus* was available for DNA sequencing. This tree allowed the delimitation of one *Oostethus* species and three *Coelonotus* species with the presence of a new *Coelonotus* species. All clades are strongly supported by posterior probability values (pp = 1). The percentage of divergence between the new species from New Britain and the other species is between 12.1 and 18.7%. The most closely related species to the new species is *Coelonotus argulus* with 12.1% of divergence. There is 15.6% of divergence between the new *Coelonotus* species and *Coelonotus leiaspis*. The most distant species to the new *Coelonotus* is *Oostethus brachyurus* with 18.7% of divergence.

Coelonotus kaupuae n. sp.

(Figs 2-3; Tab. II)

Diagnosis

Coelonotus kaupuae is distinguished from other *Coelonotus* species with 45-47 dorsal fin rays, with 4-5 subdorsal trunk rings, with 12-13 subdorsal rings and 35-37 tail rings (Tab. II).

It therefore differs from *C. argulus* in having 4-5 subdorsal trunk rings (vs 2.5-3 in *C. argulus*) and 12-13 subdorsal rings (vs 10.5-11.5 in *C. argulus*).

It differs from *C. leiaspis* in having 35-37 tail rings (vs 30-33 in *C. leiaspis*) and 45-47 dorsal fin rays (vs 53-60 in *C. leiaspis*).

It differs from *C. biocellatus* in having 45-47 dorsal fin rays (vs 52 in *C. biocellatus*), 35-37 tail rings (vs 39 *C. biocellatus*) and 4-5 subdorsal trunk rings (vs 3.5 *C. biocellatus*).

Material examined

Holotype. MNHN-IC-2021-0306 (tag 19161), male, 86.22 mm SL. Gavuvu River, New Britain (Papua New Guinea), 28 Oct. 2018, Keith coll.

Paratypes. MNHN-IC-2021-0307, 3 males (tag 19163, 19166, 19168), 2 females (tag 19164, 19167), size range

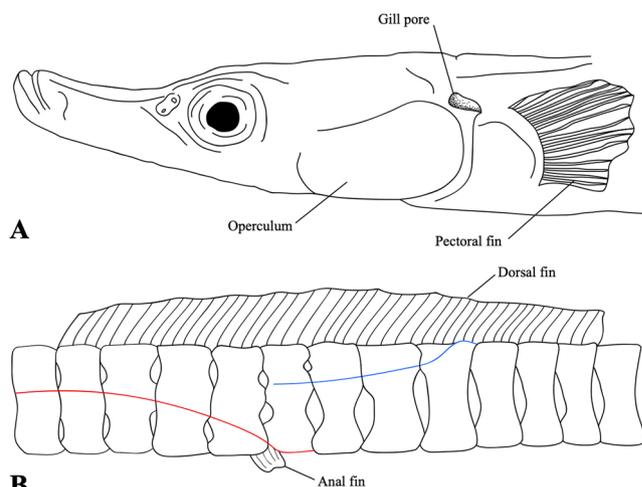


Figure 2. – A: Diagram of the head of *Coelonotus kaupuae* n. sp. B: Diagram of the lateral trunk (in red) and tail (in blue) ridges of *Coelonotus kaupuae* n. sp.



Figure 3. – *Coelonotus kaupuae* n. sp. Up: Holotype, MNHN IC-2021-0306 (tag 19161), male, 86.22 mm SL. Gavuvu river, New Britain (Papua New Guinea), 28 Oct. 2018, Keith coll. Down: Paratype, in MNHN IC-2021-0307 (tag 19167), female, 83.87 mm SL. Gavuvu river, New Britain (Papua New Guinea), 28 Oct. 2018, Keith coll.

70.88-84.83 mm SL. Gavuvu River, New Britain (Papua New Guinea), 28 Oct. 2018, Keith coll.

Description

Morphometric data is given in table II. Holotype counts are given first, followed in brackets, by paratypes' counts.

Body is smooth, tapered and covered in dermal plates forming a series of rings. Dorsal fin rays 37 (35-37). Caudal fin rays 9, like other *Coelonotus* species. Pectoral fin rays 16 (16-18). Trunks rings 16 (16-17), tail rings 37 (35-37) and total rings 53 (51-53). Subdorsal trunk rings 4.5 (4.5-5), subdorsal tail rings 8 (7.5-8.5) and total subdorsal rings 12.5 (12-13). Anal fin is atrophied and located near the urogenital papilla. The pelvic fins are absent. The head length (10% of SL) has a short (inferior or equal to 5% of SL) and thin (inferior or equal to 1-2% of SL) snout. The body depth at the middle of the trunk is 4-5 (% SL). Size up to 86.22 mm SL.

Operculum without ridge and pectoral-fin base without distinct ridge (Fig. 2A). Scutella are smooth. Pattern of lateral trunk and tail ridges, superior trunk and tail ridges are discontinuous with lateral trunk ridge confluent with the infe-

Table II. – Morphomeristics values for *Coelonotus kaupuae* n. sp. and related species. (*) values for type specimens.

		Dorsal fin rays																		
		42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
<i>C. leiaspis</i>	Male																1			
	Female												1	–	–	1	–	–	–	1
<i>C. argulus</i>	Male					3														
	Female	1*	1*	–	–	–	3	1												
<i>C. biocellatus</i>	Male																			
	Female										1*									
<i>C. nsp</i>	Male				1	2	1													
	Female				1	1														

		Trunk rings (TrR)			
		15	16	17	18
<i>C. leiaspis</i>	Male				1
	Female			3*	1
<i>C. argulus</i>	Male				
	Female		9**		
<i>C. biocellatus</i>	Male				
	Female		1*		
<i>C. nsp</i>	Male		4		
	Female		1	1	

		Tail rings (TaR)										
		30	31	32	33	34	35	36	37	38	39	
<i>C. leiaspis</i>	Male			1								
	Female	1	–	–	2							
<i>C. argulus</i>	Male									2	1	
	Female								3*	3*		
<i>C. biocellatus</i>	Male											
	Female										1*	
<i>C. nsp</i>	Male						1	1	2			
	Female						2					

		Tail rings (TaR)									
		30	31	32	33	34	35	36	37	38	39
<i>C. leiaspis</i>	Male			1							
	Female	1	–	–	2						
<i>C. argulus</i>	Male								2	1	
	Female							3*	3*		
<i>C. biocellatus</i>	Male										
	Female										1*
<i>C. nsp</i>	Male						1	1	2		
	Female						2				

		Subdorsal trunk rings (STr)					
		2.5	3	3.5	4	4.5	5
<i>C. leiaspis</i>	Male			1			
	Female			1	3*		
<i>C. argulus</i>	Male	2	1				
	Female	3**	3				
<i>C. biocellatus</i>	Male						
	Female			1*			
<i>C. nsp</i>	Male				2	2	
	Female				1	–	1

		Subdorsal tail rings (STa)			
		7.5	8	8.5	9
<i>C. leiaspis</i>	Male			1	
	Female		2*	1	1
<i>C. argulus</i>	Male		2	1	
	Female		5**	1	
<i>C. biocellatus</i>	Male				
	Female			1*	
<i>C. nsp</i>	Male	1	2	1	
	Female	1	1		

		Subdorsal rings (STt)					
		10.5	11	11.5	12	12.5	13
<i>C. leiaspis</i>	Male				1		
	Female			1	1*	1	1
<i>C. argulus</i>	Male	1	2				
	Female	2*	3*	1			
<i>C. biocellatus</i>	Male						
	Female				1*		
<i>C. nsp</i>	Male				1	2	1
	Female				1	1	

		Snout length (%SL)	
		4	5
<i>C. leiaspis</i>	Male	1	
	Female	1	2
<i>C. argulus</i>	Male	3	
	Female	6**	
<i>C. biocellatus</i>	Male		
	Female	1*	
<i>C. nsp</i>	Male	4	
	Female	1	1

rior tail ridge under the dorsal fin (Fig. 2B). The section of trunk has a V shape while section of tail is square-like. There is a sexual dimorphism, with a brood pouch for males. Brood pouch is located under trunk with pouch plates slightly con-

vergent and pouch folds absent; begins on the first trunk ring and ends at the urogenital papilla. Eggs are small, deposited in a single layer of three to four rows with about a hundred eggs per pouch.

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