

## *Nemacheilus cacao*, a new species of loach (Teleostei: Nemacheilidae) from the middle Mekong basin in Laos

Jörg Bohlen<sup>1\*</sup>, Maurice Kottelat<sup>2,3</sup> & Vendula Šlechtová<sup>1</sup>

**Abstract.** *Nemacheilus cacao*, new species, is described from the middle Mekong basin in Laos. It differs from all other species of *Nemacheilus* by having an incomplete lateral line; and adult males having a plain grey-brown body and head (except ventral side), and with extensive tuberculation on the flank, on the dorsal side of the pectoral fin and on the head and nape. The phylogenetic position of *N. cacao* in the Selangoricus clade of *Nemacheilus*, as a sister species to *N. platiceps*, is supported by the presence of bars on the body in juveniles and females as well as by genetic data.

**Key words.** Cobitoidea, taxonomy, Khammouan, Nam Thorn, Xe Bangfai

### INTRODUCTION

The diversity within the freshwater fish genus *Nemacheilidae* is still far from being fully documented, and numerous new species and genera are still described every year (e.g., Kottelat, 2017a, b, c, 2018, Hosoya et al., 2018, Shangningam et al., 2019). Species of the genus *Nemacheilus* are distributed throughout most of Southeast Asia (Kottelat, 1984, 1990, 2012). They are small (usually less than 100 mm SL), elongated benthic fishes and inhabit streams and rivers of various size, usually observed in loose groups swimming close to the bottom. For a long time, the name *Nemacheilus* has been a catch-all genus for the majority of nemacheilid species and nearly 400 nominal species and subspecies have, at some time, been placed in this genus (also incorrectly spelled *Nemachilus* and *Noemacheilus*) (Kottelat, 2012). Kottelat (1984, 1990, 2012) restricted *Nemacheilus* to a group of species sharing morphological similarities. At present, *Nemacheilus* includes around 30 species, but the generic position of several of the species placed in the genus remains unclear (Kottelat, 2012, 2013, 2021; Šlechtová et al., 2021). The present study aims to describe a new species of *Nemacheilus* from the middle Mekong drainage in Laos.

### MATERIAL AND METHODS

The holotype and one paratype (ZRC 62554) were collected as juveniles and reared in aquaria. All other type specimens were fixed shortly after capture, either in formalin and later transferred to 70% ethanol for permanent storage or fixed and stored in 96% ethanol. Measurements and counts follow Kottelat (1990) and Kottelat & Freyhof (2007). The last two branched dorsal and anal-fin rays that articulate on a single pterygiophore are counted as '1½'. Measurements were taken point-to-point using a digital calliper and recorded to the nearest 0.1 mm. Abbreviations used: CMK, collection of M. Kottelat; and ZRC, Lee Kong Chian Natural History Museum, National University of Singapore, Singapore.

Sequencing of mitochondrial cytochrome *b* gene and nuclear RAG1 gene of the holotype and one paratype of the new species was carried out as described in Šlechtová et al. (2021). The dataset from Šlechtová et al. (2021) was used as comparative material to assess the phylogenetic position of the new species. The material of *N. platiceps* includes eight specimens that originate from the Mekong drainage in Thailand, including seven specimens from the Tonle Sap basin in Chantaburi province and one specimen from the Mun catchment in Ubon Ratchathani province. *Nemacheilid* sequences from Sember et al. (2015) served as outgroups. The phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) approaches. The best-fit substitution models were estimated using Partition Finder 2 (Lanfear et al., 2016) (implemented in PhyloSuite 1.2.2; Zhang et al., 2020) based on the corrected Akaike Information Criterion (AICc). The partitioned ML analysis was performed using IQ-TREE (Nguyen et al., 2015) implemented in PhyloSuite using the estimated loci and codon position specific models and the node support values were obtained with 1000 ultrafast bootstrap replicates (UFBoot) (Hoang et al., 2018). BI was

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<sup>1</sup>Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Science, Liběchov, Czech Republic; Email: joerg\_bohlen@yahoo.de (\*corresponding author)

<sup>2</sup>Rue des Rauragues 6, 2800 Delémont, Switzerland (permanent address)

<sup>3</sup>Lee Kong Chian Natural History Museum, 2 Conservatory Drive, National University of Singapore, Singapore 117377.

Table 1. Morphometric data of holotype and eight paratypes (ZRC 62553 to 62558) of *Nemacheilus cacao*, new species. Range and mean include holotype data.

	Holotype	Range	Mean
Standard length (mm)	56.9	27.7–61.5	
Total length (mm)	73.5	35.7–78.3	
<b>In percent of standard length</b>			
Total length	129.2	124.6–130.6	127.8
Head length (dorsal)	18.5	17.2–20.2	18.4
Head length (lateral)	20.7	20.4–23.5	21.9
Predorsal length	49.4	46.1–50.6	48.3
Prepelvic length	50.1	49.8–56.3	52.1
Pre-anus length	68.4	67.6–73.5	69.7
Pre-anal length	76.1	75.1–80.2	77.7
Head depth at nape	12.3	12.3–14.6	13.3
Body depth at dorsal-fin origin	20.4	13.9–20.7	17.3
Depth of caudal peduncle	12.7	10.2–12.7	11.5
Length of caudal peduncle	16.9	14.4–17.1	15.8
Maximum head width	14.4	13.1–15.8	14.7
Body width at dorsal-fin origin	11.4	9.4–17.9	13.9
Length of dorsal fin	16.0	14.8–20.3	17.3
Length of upper caudal-fin lobe	28.1	25.7–29.2	27.2
Length of median caudal-fin rays	19.2	18.7–22.4	20.9
Length of lower caudal-fin lobe	–	25.5–29.0	27.1
Length of anal fin	15.1	13.7–22.4	16.9
Length of pelvic fin	16.0	12.9–18.8	15.9
Length of pectoral fin	20.4	15.5–23.1	18.7
<b>In percent of lateral head length</b>			
Snout length	32.2	30.2–38.5	33.4
Eye diameter	22.0	22.0–31.3	27.1
Interorbital width	37.3	30.0–37.3	33.4

conducted in MrBayes 3.2.7 (Ronquist & Huelsenbeck, 2003) via CIPRES Science Gateway (Miller et al., 2010). The analyses were performed in two independent runs of 10 million generations, partitions were unlinked and relative burnin of 25% was applied. The remaining trees were used to build 50% majority rule consensus tree. The topologies of trees yielded by ML and BI were congruent.

In the present study we use the name *N. cf. pallidus* for the species from the Central Mekong, which was originally considered conspecific with the material from the Chao Phraya basin in Thailand (Kottelat, 1990, 1998, 2001) but which apparently is a distinct, unnamed species (Kottelat, 2021).

## TAXONOMY

### *Nemacheilus cacao*, new species (Figs. 1–5, Table 1)

**Holotype.** ZRC 62553, 56.9 mm SL; Laos: Khammouan province: Thakhek district: small tributary of Nam Thorn at cave Tham Nang Eng, 17°26'39"N 104°56'55"E; collected 09 June 2013, reared until October 2013 in aquaria.

**Paratypes.** Laos: Khammouan province: ZRC 62554, 1, 61.5 mm SL, collected and reared together with holotype; ZRC 62555–62558, 7, 27.7–50.4 mm SL; same locality as holotype, March 2018. — CMK 19403, 1, 32.2 mm SL; Southwest of Gnommalat, Tham Pong cave, near Phou Phathoung quarry, inside cave, 17°32'30"N 105°09'58"E; M. Kottelat et al., 7 May 2006. — CMK 23170, 1, 36.9 mm SL; Xe Bangfai drainage: Houay Kalo, a small tributary of Nam Oula near Ban Phonexay, 17°20'56"N 105°20'07"E; M. Kottelat et al., 22 May 2012.

**Diagnosis.** *Nemacheilus cacao* is distinguished from all other species of the genus except *N. platiceps* in having an incomplete lateral line reaching between verticals of pelvic-fin origin and of anus, with 33–49 pores (vs. complete). *Nemacheilus cacao* is most easily distinguished from *N. platiceps* by its colour pattern, with a uniform dark brown body in adult males. In contrast, *N. platiceps* has 12–16 narrow bars on the flank, clearly distinct at all sizes and both sexes. In addition, *N. cacao* is distinguished from *N. platiceps* by males having: a conspicuous suborbital flap (vs. poorly developed); dorsal surface of first 3–7 pectoral-fin rays covered by densely-set small tubercles; flank with a patch of scales each with a small tubercle (vs. absence of tubercles on pectoral fin and flanks).



Fig. 1. Live specimen of *Nemacheilus cacao*, new species, ZRC 62554, paratype, male, 61.5 mm SL; Laos: Khamouane province: Thakhet district: small tributary of Nam Thorn at cave Tham Nang Eng (Photo: J. Kühne).

**Description.** See Figs. 1 and 2 for general appearance and Table 1 for morphometric data of holotype and eight paratypes. A moderately elongated nemacheilid loach with body depth about 5–7 times in SL. Body depth gradually increasing until dorsal-fin origin, then decreasing until posterior extremity of anal-fin base, uniform from there to caudal-fin base. Dorsal outline between nape and dorsal-fin origin slightly convex. Head slightly depressed; body moderately compressed behind head, increasingly compressed until caudal-fin base. Interorbital area flat or nearly flat. In lateral view, upper margin of eye flush with dorsal profile of head. Cheeks not swollen; snout rounded. Depth of caudal peduncle 1.2–1.5 times in its length. Very short and low ventral crest on posterior end of caudal peduncle, absent or nearly absent dorsal crest. Largest recorded size 61.5 mm SL.

Dorsal fin with 4 unbranched and  $9\frac{1}{2}$  branched rays, distal margin straight to slightly convex; second branched ray longest. Anal fin with 3 unbranched and  $5\frac{1}{2}$  branched rays, distal margin straight, second branched ray longest. Pectoral fin with 1 unbranched and 11–12 branched rays, reaching halfway to pelvic-fin base; no axillary lobe. Pelvic fin with 1 unbranched and 7 (6 in 1 specimen) branched rays, reaching half-way to anal-fin base, not or just reaching anus, which is located  $1\frac{1}{2}$ –2 eye diameter in front of anal-fin origin: axillary lobe small and on whole length attached to body. Caudal fin with 9+8 (8+8 in 1 specimen) branched rays, slightly forked, lobes rounded, both lobes same length or upper lobe up to 1.1 times length of lower lobe.

Body entirely scaled. Lateral line incomplete, ending usually below or shortly behind dorsal-fin base, occasionally reaching level of anal-fin origin; visible pores (33–49) only until vertical of anus. Cephalic lateral line system with 5 supraorbital, 4+10–11 infraorbital, 9–10 preoperculo-mandibular and 3 supratemporal pores.

Anterior nare pierced at tip of an obliquely truncate tube. Posterior nare adjacent to anterior one. Mouth moderately arched, gape about twice wider than long (Fig. 3). Upper lip moderately thick, without median notch, with numerous wrinkles. Processus dentiformis present. Lower lip with

narrow median interruption; median part with very weakly marked sulci; thinner towards rictus. Barbels long; inner rostral barbel reaching beyond vertical through middle of eye, outer one and mandibular barbels reaching beyond eye.

**Sexual dimorphism.** Adult males with suborbital flap (Fig. 4) and with uniform brown flank (vs. females and juveniles with narrow brown bars). Adult males (= males with suborbital flap; 48–62 mm SL) with patch of tubercles on flank under dorsal-fin base, on upper surface of pectoral-fin rays, and scattered tubercles on postorbital area of head and nape (Fig. 5).

Extent of patches of tubercles on flank variable, reaching longitudinally from tip of adpressed pectoral fin to end of anal-fin base, single tubercles even until caudal-fin base. Shape of patch irregular, usually elongated oval (Fig. 5a). In dorsoventral direction, patch stretching across 5 rows of scales above lateral line and up to 10 rows below, highest under dorsal fin and shortly behind. In this patch, each scale with a single large, white and triangular tubercle (Fig. 5b, c). Tubercles on pectoral fin much smaller than those on body, white, conical, in 1–5 rows on dorsal side of rays and on unciliferous pad behind; stretching to tip of ray on unbranched and first branched ray, with decreasing length on following branched rays until only on base of third-last ray (Fig. 5d, e). Last two branched rays without tubercles. Tubercles on sides of head small, white, conical, only tip pointing out of skin. Tubercles on body, pectoral fin, and head either all present or all absent.

**Colouration.** *Nemacheilus cacao* undergoes a remarkable ontogenetic and sexual change of colouration. A juvenile of 27.7 mm SL had 22 dark brown bars on body meeting their contralaterals on back, their width at least twice width of interspaces, some vertically split. In females, most bars split and fade with increasing size, resulting in a pattern of numerous narrow brown bars along body and broader dorsal saddles. In adult males, bars and saddles absent and whole fish with uniform chocolate brown body and head, only ventral side of head and body white. A faint dark stripe from snout to eye in most specimens, no black bar on caudal-fin base.



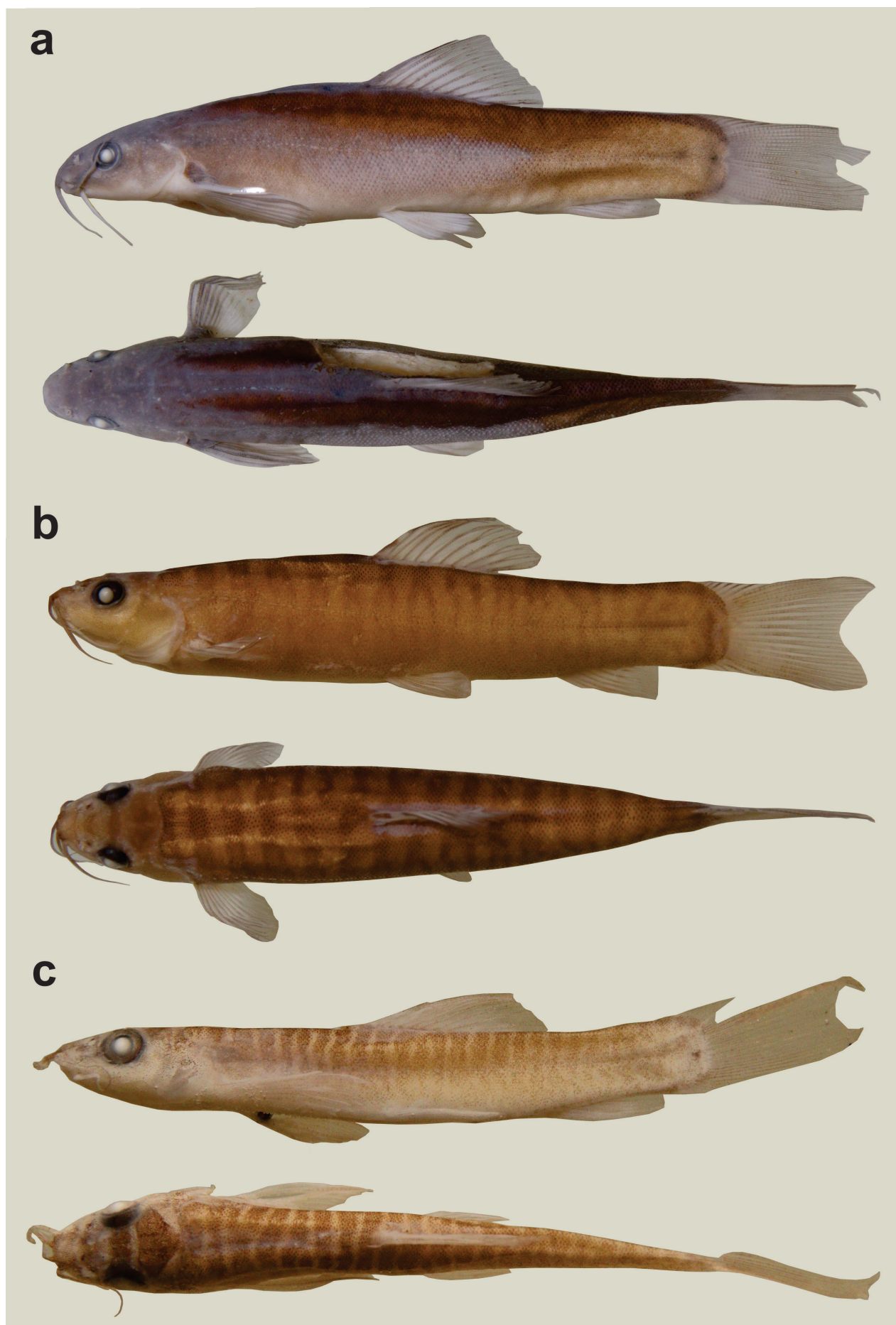


Fig. 2. *Nemacheilus cacao*, new species, Laos: Khammouane prov: Thaket district: small tributary of Nam Thorn at cave Tham Nang Eng. a, ZRC 62553, holotype, 56.9 mm SL, adult male; b, ZRC 62558, paratype, 50.4 mm SL, adult female; c, ZRC 62556, 27.7 mm SL, juvenile.

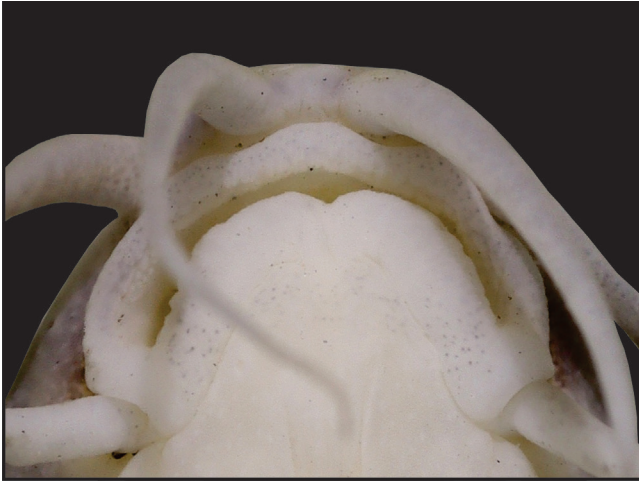


Fig. 3. *Nemacheilus cacao*, new species, ZRC 62553, holotype, 56.9 mm SL; mouth in ventral view.

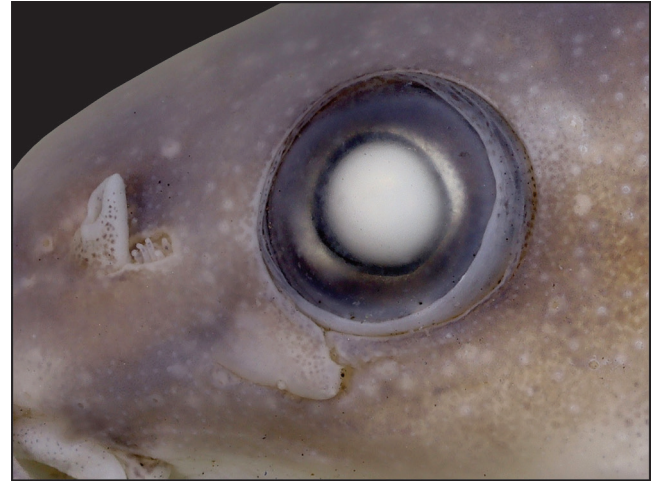


Fig. 4. *Nemacheilus cacao*, new species, ZRC 62553, holotype, 56.9 mm SL; left suborbital flap in lateral view.

**Phylogenetic position.** The topologies of the single gene trees were congruent; therefore, the data have been combined. The analysis of the concatenated dataset estimated the phylogenetic position of *N. cacao* as a member of the genus *Nemacheilus*, member of the Selangoricus clade and sister species to *N. platiceps* (Fig. 6). Genbank accession numbers for the sequences of the holotype (ZRC 62553) and paratype (ZRC 62554) are: ON720270 and ON720269 for cytochrome *b*, ON720272 and ON720271 for RAG-1.

**Etymology.** From *Theobroma cacao*, the cacao tree whose seeds are used to produce chocolate; itself derived from kakawa in some ancient Mesoamerican language (Kaufman & Justeson, 2007). A reference to the chocolate brown colour of large males. A noun in apposition, indeclinable.

## DISCUSSION

Kottelat (1990) identified three species groups within *Nemacheilus* based on their pigmentation pattern and the presence of enlarged scales along the lateral line, and the same groups were recovered in a recent molecular phylogeny (Šlechtová et al., 2021): the Masyae clade, characterised by a series of saddles along the dorsal midline and a series of blackish blotches along the lateral line; the Ornatus clade with a black stripe along the lateral line and a black stripe or blotches along the dorsal midline, and the Selangoricus clade featuring brown to black bars on the body and acuminate scales on the caudal peduncle in some species. *Nemacheilus platiceps*, a species from the lower Mekong drainage, was not placed in one of the three groups by Kottelat (1990); Šlechtová et al. (2021) found it to be a sister species to the whole Selangoricus clade. The present genetic analysis found *N. cacao* and *N. platiceps* to be sister species, basal to the Selangoricus clade, with which they share the presence of

brown to black bars. Compared to other members of the clade, the bars are narrow and irregularly shaped and set, becoming more irregular and more vertically split with increasing size, and fainter or (in the case of males of *N. cacao*) disappearing entirely in the dark brown background colour. While all other species of *Nemacheilus* have a complete lateral line, *N. cacao* and *N. platiceps* share the trait of an incomplete lateral line.

The plain brown body in males distinguishes *N. cacao* from all other species of *Nemacheilus*. Large males of *N. cacao* are also distinguished from those of many of its congeners by the presence of conspicuous tubercles on the flank and pectoral fins. Such tubercles are also present in *N. selangoricus*, *N. spiniferus*, *N. tebo*, *N. tuberigum*, *N. masyae*, *N. fasciatus*, *N. chrysolaimos* and *N. cf. pallidus* (Kottelat, 1984, 1990, Hadiaty & Siebert, 2001, Hadiaty & Kottelat, 2009, and unpublished observations). In *N. selangoricus* and *N. spiniferus*, tubercles are present in both sexes, but on a single row of scales above and below the lateral line on the anterior half of the body; these two species also have acuminate scales on the caudal peduncle (elongated scales with a posterior projection or process, with a tubercle at the tip; Kottelat, 1984, 1990). In *N. tuberigum*, tubercles are present in the mid-body region and on the anterior part of the caudal peduncle in both sexes but they are more numerous and larger in males than in females. In *N. fasciatus*, *N. chrysolaimos*, *N. masyae*, and *N. cf. pallidus* the pattern of tubercles on the flanks is similar to that of *N. cacao* and is only present in males (Kottelat, 1990 and unpublished observations). Tubercles are also present on the pectoral-fin rays of *N. binotatus* (well developed) and *N. ornatus* (very few) but there are no tubercles on the flank scales. For all the aforementioned species, it is not clear whether tubercles are permanent or seasonal.



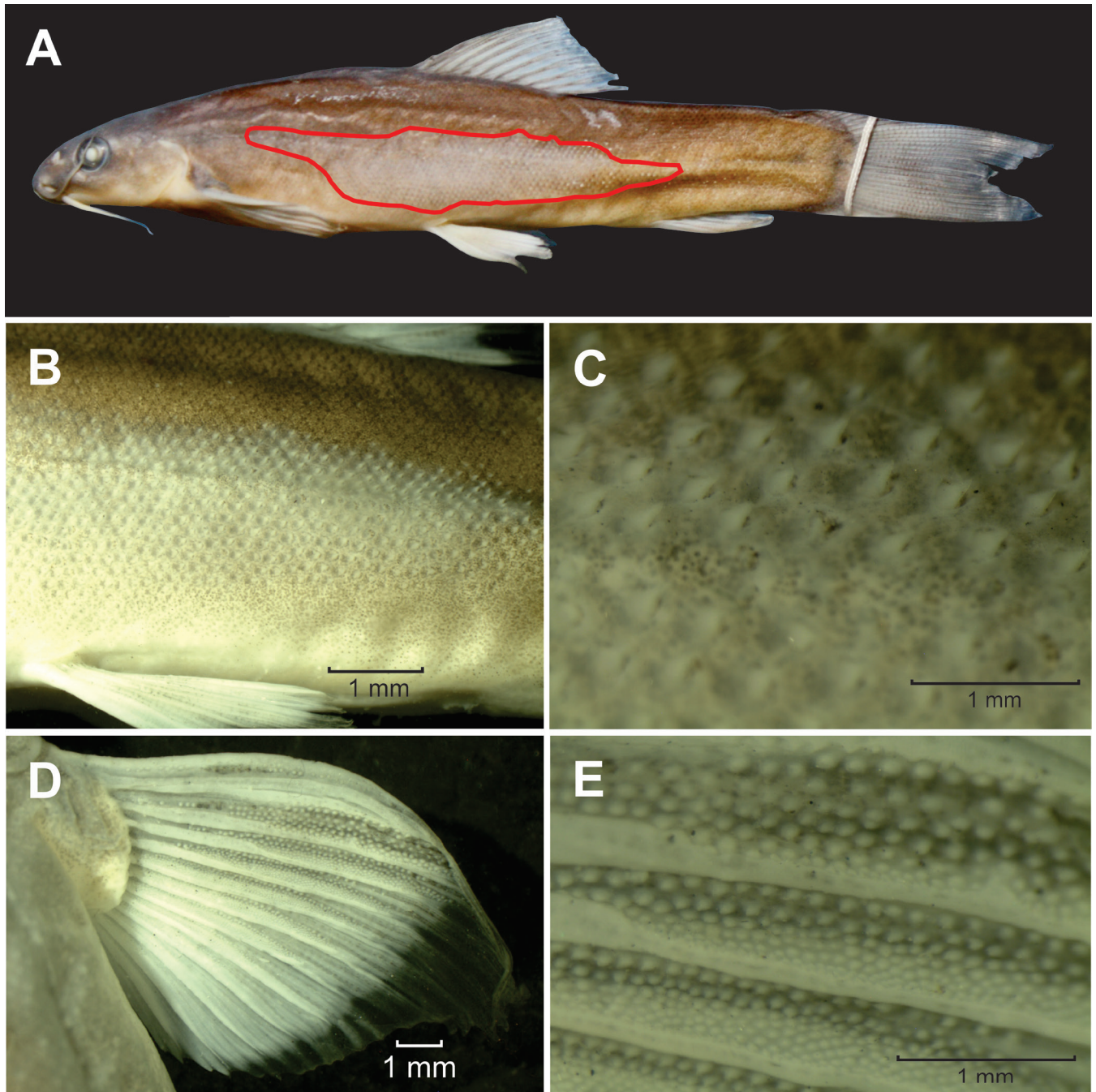


Fig. 5. Tubercles on body side and pectoral fin of large male *Nemacheilus cacao*, new species, ZRC 62553, holotype, 56.9 mm SL. A, red outline depicts field of tubercles on body side; B–C, area of main tuberculation enlarged; D, dorsal view on right pectoral fin; E, tubercles on pectoral fin enlarged.

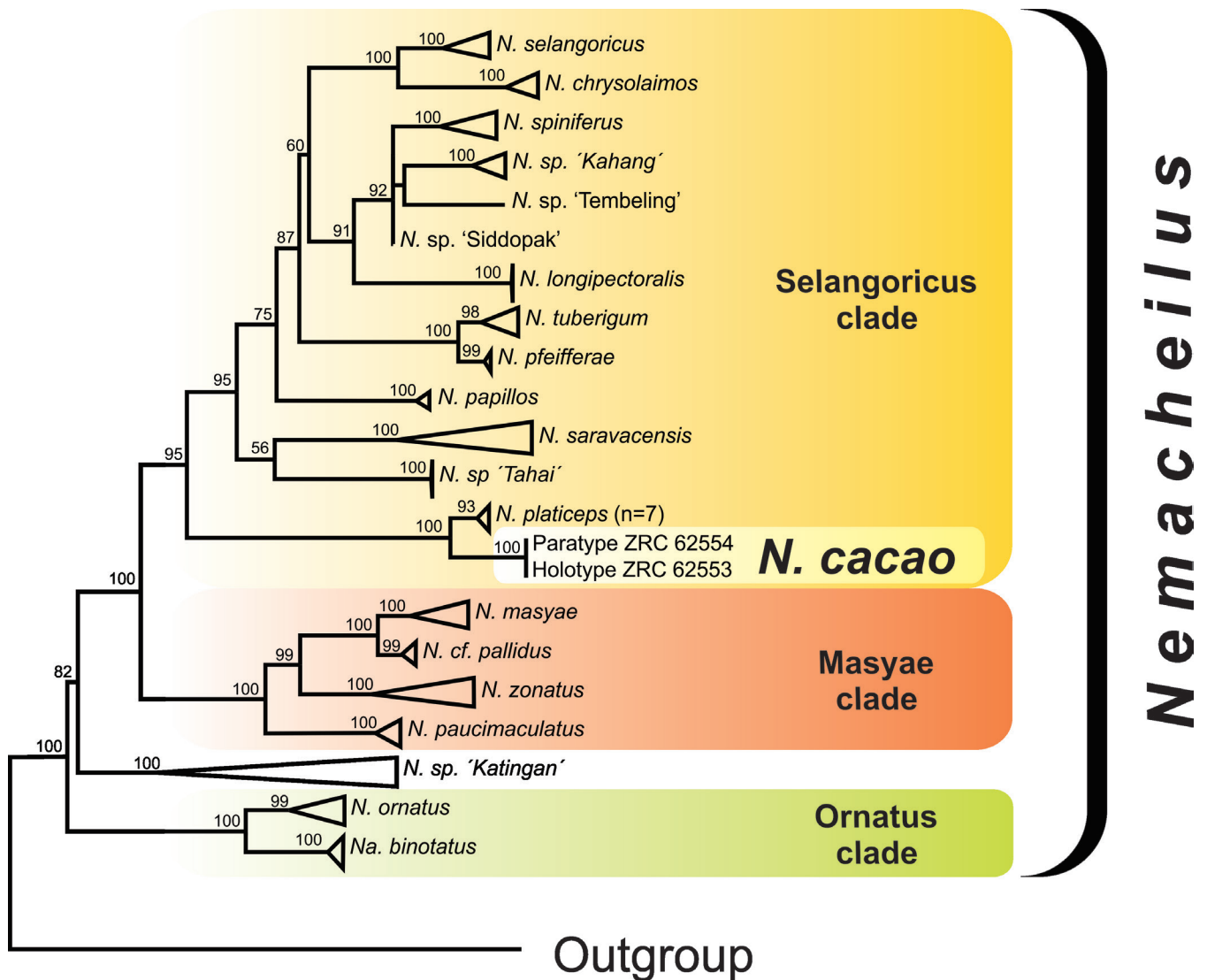


Fig. 6. Bayesian tree of the genus *Nemacheilus* based on the concatenated datasets of mitochondrial cytochrome b and nuclear RAG-1 marker. Datasets from Šlechtová et al. (2021) and Sember et al. (2015) plus two specimens of *Nemacheilus cacao*, new species. The tree shows *N. cacao* to be the sister species to *N. platiceps* and both to be located at the base of the Selangoricus clade within *Nemacheilus*. The outgroup contains 25 nemacheilid species from genera other than *Nemacheilus* and one species from the family Cobitidae.

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