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Variation in the Arrow Loach, *Nemacheilus masyae* (Cypriniformes: Nemacheilidae), in Mainland Southeast Asia with Description of a New Species

Lawrence M. Page¹, John M. Pfeiffer^{1,2}, Siriwan Suksri³, Zachary S. Randall¹, and David A. Boyd¹

Analyses of morphological and molecular data from recently collected specimens of *Nemacheilus* from Cambodia, Malaysia, and Thailand indicate that *N. pallidus* is a junior synonym of *N. masyae*, and an undescribed species of *Nemacheilus* occurs in large tributaries of the Mekong River in Thailand. The new species, described herein, is small—with a maximum-known standard length of 28.6 mm—and has a distinctive color pattern of dusky black bars along the side of the body that cross over the back and join the bars on the other side. Molecular phylogenetic analyses suggest that the new species is most closely related to *N. masyae*, which reaches a much larger size—to 66.2 mm SL—and otherwise is easily distinguished from the new species. The new species is known from the Songkhram and Mun river drainages in Thailand and appears to be restricted to the Khorat Plateau ecoregion of the Mekong River basin. *Nemacheilus masyae* occurs throughout mainland southeast Asia, including in the Chao Phraya, Mae Klong, Mekong, and coastal drainages of the Malay Peninsula.

TWELVE species of *Nemacheilus* currently are recognized in mainland southeast Asia: *N. arenicolus*, *N. banar*, *N. binotatus*, *N. cleopatra*, *N. longistriatus*, *N. masyae*, *N. ornatus*, *N. pallidus*, *N. paucimaculatus*, *N. platiceps*, *N. selangoricus*, and *N. troglodactylus*; another 13 species occur in Indonesia, and five occur in India (Fricke et al., 2019). Recently collected specimens from several drainages in Cambodia, Malaysia, and Thailand could not confidently be assigned to either *N. masyae* or *N. pallidus* based on the descriptions in Kottelat (1990) even though some specimens were from, or collected close to, the type localities of both species. Analyses of morphological and molecular data from across the hypothesized distributions of these two nominal species were conducted to test their monophyly as well as examine their relationships to other species of *Nemacheilus* for which molecular data were available. In the process, we discovered an undescribed species of *Nemacheilus*, described herein, in large tributaries of the Mekong River in the Khorat Plateau ecoregion (Abell et al., 2008) of Thailand.

MATERIALS AND METHODS

Specimens were collected in Cambodia, Malaysia, and Thailand using nets of various sizes, euthanized in MS-222, fixed in 10% formalin, and transferred to 70% ethanol for storage. Tissue samples for DNA analysis were taken from one or more specimens at most sites by excising fins and placing them in 95% ethanol. Measurements and counts follow Armbruster (2012) except for caudal-peduncle length, which was measured from the rear of the anal-fin base to the middle of the caudal-fin base, and prepectoral, prepelvic, and preanal lengths, which were taken from the anterior tip of the snout to the origin of each fin. Fin measurements are of depressed fins. All measurements were taken to the nearest 0.1 mm

using digital calipers and from the left side when possible. Total ray counts are given for paired fins; branched ray counts are given for unpaired fins. The last branched ray of the dorsal and anal fins, sharing a pterygiophore with the penultimate ray, is given as ½. Counts of vertebrae were made on micro-CT (computed tomography) scans. Counts of abdominal vertebrae include the Weberian complex ($n = 5$ vertebrae), with the first caudal vertebra being the first one with its hemal spine posterior to the anteriormost anal-fin pterygiophore, and counts of caudal vertebrae including the urostyle complex. Dorsal-saddle count was taken posterior to the opercle. Lateral blotch count was taken along the length of the body; it does not include the prominent black spot at the caudal-fin base.

A sheared principal components analysis (PCA), based on methods adapted from Bookstein et al. (1985), was conducted in R (R Core Team, 2019). The second and third principal components were plotted to visualize variation in morphology independent of size. Photographs of live and preserved specimens were taken with a Canon 7D camera or a 5D camera on a Visionary Digital system (Palmyra, VA). All images of live specimens were taken immediately after capture. Figures were edited using Adobe Photoshop CC 2018 (San Jose, CA). CT scans were generated using a GE Phoenix v|tome|x M scanner (GE Measurement and Control, Boston, MA) at the University of Florida's Nanoscale Research Facility. X-ray data were processed using GE's proprietary datos|x reconstruction software v. 2.3 (GE Measurement and Control), and segmented and visualized using VG StudioMax v. 3 (Volume Graphics, Heidelberg, Germany). The scan data are freely available for download at MorphoSource (UF 188670, 38.5 mm SL, MorphoSource numbers: M19872, M19871; UF 237302, 23.2 mm SL, MorphoSource numbers:

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M26456, M26457). The map was created with ArcMap 10.5 in ArcGIS (ESRI, Redlands, CA).

DNA was extracted from tissue samples using the QIAamp DNA mini kit (Qiagen). Mitochondrial cytochrome c oxidase subunit 1 (COI) and nuclear recombination activating (RAG1) genes were amplified by polymerase chain reaction (PCR) and sequenced using the following primers: FISH-BCL 5′-TCAACYAATCAYAAAGATATYGGCAC-3′, FISH-BCH 5′-ACTTCYGGGTGRCCRAARAATCA-3′ (Baldwin et al., 2009), RAG1-F 5′-AGCTGTAGTCAGTAYCACAARATG-3′ (Que-nouille et al., 2004), and RAG-RV1 5′-TCCTGRAAGATYTTG-TAGAA-3′ (Šlechtová et al., 2007). PCR was conducted using 25 μ L solutions containing 1 μ L of each primer at 10 mM; 0.25 μ L MyTaq Red polymerase and 4 μ L buffer (Bioline Reagents); and 2 μ L DNA template. Thermocycling parameters followed Liu et al. (2012). Purification and bidirectional Sanger sequencing were performed by Eurofins Genomics (Louisville, KY).

Chromatograms were assembled and edited with Geneious 8.1.9 (<https://www.geneious.com>). Alignment and concatenation of the two genes were implemented with Mesquite 3.5 (Maddison and Maddison, 2018) using Clustal W 2.1 (Larkin et al., 2007). Partitioning and phylogenetic analyses were carried out on the concatenated two-gene dataset using the high-performance computing cluster (HiPerGator) at the University of Florida. ModelFinder (Kalyaanamoorthy et al., 2017) as implemented in IQ-TREE (Nguyen et al., 2015) was used to determine the best partition scheme and model of nucleotide evolution. A partitioned maximum-likelihood (ML) analysis was conducted with IQ-TREE (Nguyen et al., 2015; Chernomor et al., 2016) using 1,000 ultrafast bootstraps (Hoang et al., 2018). Bayesian-inference (BI) analysis was conducted with MrBayes 3.2.6 (Ronquist et al., 2012) using two independent runs of eight chains and 24 million generations, sampling trees every 1,000 generations and discarding the first 25% as burn-in.

RESULTS

In the sheared principal components analysis, size accounted for 96.91% of observed variance; the second and third principal components accounted for 0.85 and 0.51%, respectively (Fig. 1). Interorbital width (0.52) and pectoral-fin length (−0.42) had the highest loadings on sheared PCII, which best revealed dissimilarity between *N. masyae* and *N. zonatus*, new species. Gape width (0.57) and body depth (−0.47) had the highest loadings on sheared PCIII, which failed to clearly distinguish between the two species.

Our two-gene molecular dataset included 64 terminal taxa representing 18 species, nine of which belong to *Nemacheilus* (Table 1). The total aligned dataset was 1,530 nucleotides (nt) in length, and each terminal was represented by both COI (avg. 648 nt) and RAG1 (avg. 875 nt). The best tree recovered in our ML analysis is presented in Figure 2, which includes nodal support reported as ML ultrafast bootstraps (UFBS) and BI posterior probabilities (PP).

All specimens that would have been identified as *N. pallidus* based on the original description of the species' range (Kottelat, 1990), including three specimens from the type locality (UF 188670), were nested within a large clade of *N. masyae*. Samples of *N. masyae* included a specimen from Surat Thani Province in peninsular Thailand (UF 191954)

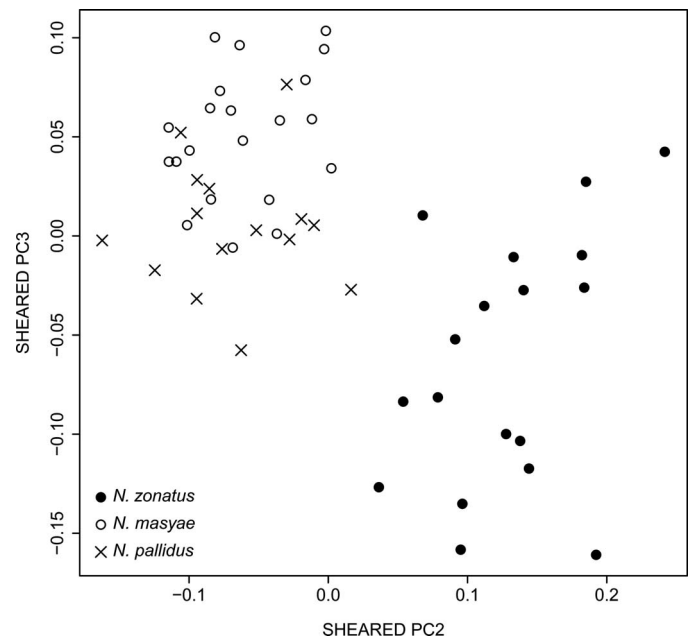


Fig. 1. Principal components analysis. Size accounted for 96.91% of the observed variance. The sheared second and third principal components (PC2 and PC3) accounted for 0.85% and 0.51%, respectively, of the observed variance. Interorbital width (0.52) and pectoral-fin length (−0.42) had the highest loadings on sheared PCII; gape width (0.57) and body depth (−0.47) had the highest loadings on sheared PCIII. Filled circle = individuals of *N. zonatus*, empty circle = individuals within the range described by Kottelat (1990) for *N. masyae*, and X = individuals within the range described by Kottelat (1990) for *N. pallidus*.

that is near the type locality of *N. masyae* in Nakhon Si Thammarat Province.

The sister species to *N. masyae* (Fig. 2) is *N. zonatus*, new species, from the Mekong River basin in Thailand, described below. Sister to these two species among those included in the phylogenetic analysis is a clade containing *N. fasciatus*, native to Java and Sumatra, and *N. longipinnis*, native to Sumatra. This clade is sister to *N. platiceps*, found in the lower Mekong basin of Cambodia, Laos, Thailand, and Vietnam, and this larger clade is sister to *N. selangoricus* of peninsular Malaysia and Indonesia and *N. pfeifferae* of Sumatra. The sister to all other species of *Nemacheilus* in the analysis is a clade containing *N. binotatus* of the Chao Phraya and Mae Klong basins of Thailand and *N. ornatus* of peninsular and southeast Thailand.

Nemacheilus zonatus, new species

urn:lsid:zoobank.org:act:4981B890-66D2-4034-A83E-3B6C42E33F8A

Banded Arrow Loach

Figures 3–5, Tables 1–2

Holotype.—NIFI 5114, 24.1 mm SL, Thailand, Kalasin Province, Mekong River basin, Mun River drainage, Pao River (tributary of Chi River), at route 214 bridge, Kamalasai District, 16.34022°N, 103.57584°E, L. M. Page, Z. S. Randall, J. M. Pfeiffer, and D. A. Boyd, 6 January 2015.

Paratypes.—All Thailand, Mekong River basin. UF 237302, 5, 23.2–28.6 mm SL, same data as holotype; ZRC 61125, 1, 23.2

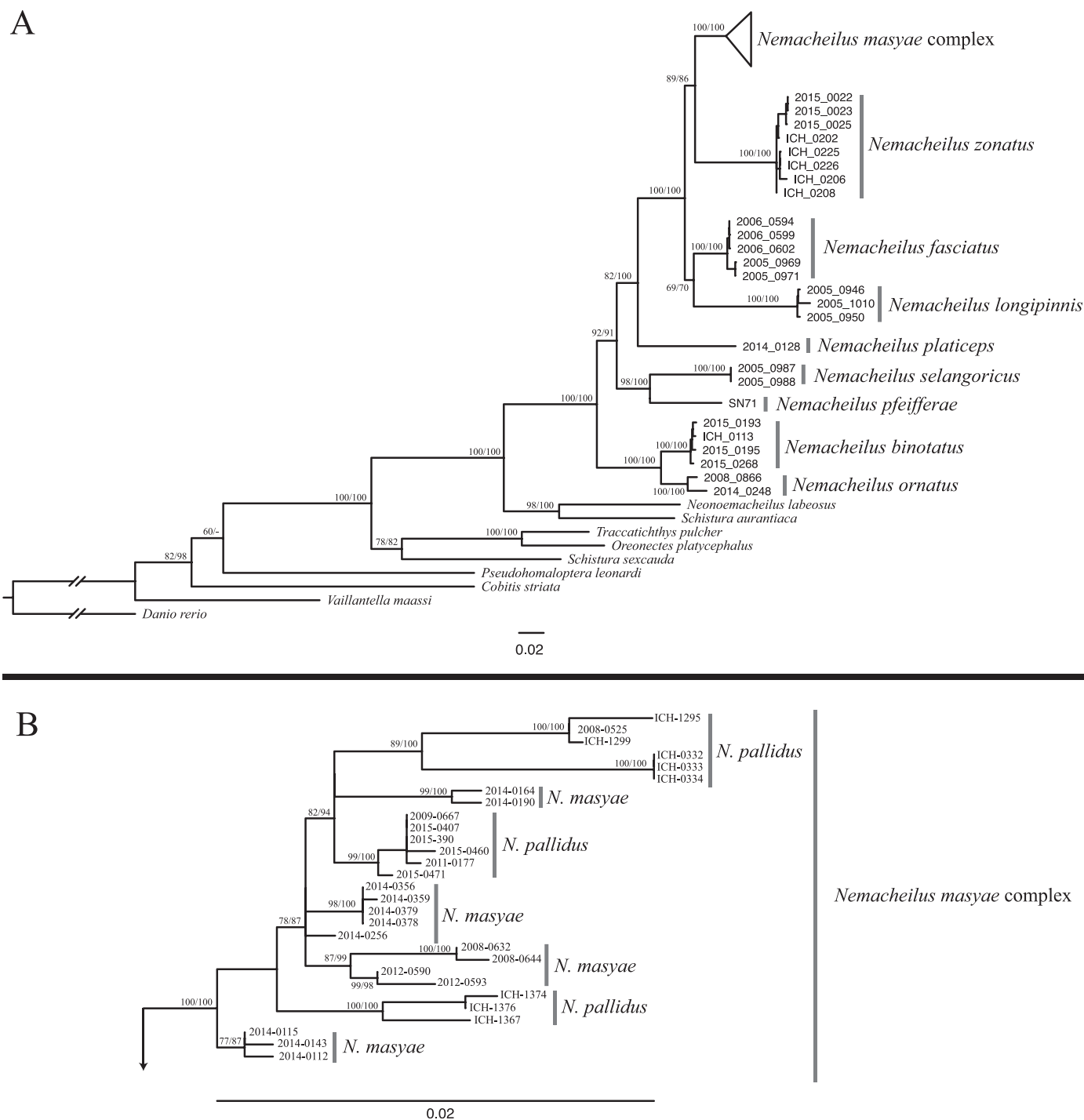


Fig. 2. Combined (CO1+RAG1) maximum likelihood phylogenetic reconstruction of (A) *Nemacheilus* with *N. masyae* clade collapsed and (B) *N. masyae* complex. Samples labeled *N. pallidus* are those from within the range described by Kottelat (1990) for *N. pallidus*, including from the type locality (UF 188670). ML ultrafast bootstraps and BI posterior probabilities values are plotted on the branches. See Data Accessibility for tree files.

mm SL, same data as holotype. Mun drainage: UF 170212, 1, 22.6 mm SL, Ubon Ratchathani Province, Mun River at Warinchumraj, 15.17862°N, 104.70665°E, W. Tangjitjaroen, J. Grudpan, S. Udduang, and L. M. Page, 16 November 2007; UF 237301, 4, 15.3–18.3 mm SL, Surin Province, Mun River, Tha Tum District, 15.31534°N, 103.62985°E, J. M. Pfeiffer, L. M. Page, D. A. Boyd, and Z. S. Randall, 6 January 2015. Songkhram drainage: UF 188240, 3, 20.0–25.2 mm SL, Sakon Nakhon Province, Songkhram River at bridge west of Ban Na

Si Nuan on route 2281, Charoen Sin District, 17.72947°N, 103.41073°E, Z. S. Randall, J. M. Pfeiffer, M. Tan, D. A. Boyd, N. Warin, and R. Itimung, 25 January 2016; UF 188447, 3, 20.9–24.4 mm SL, Sakon Nakhon Province, Un River (tributary of Songkhram River), at route 222 bridge, Phang Khon District, 17.44263°N, 103.72763°E, D. A. Boyd, Z. S. Randall, J. M. Pfeiffer, R. Itimung, N. Warin, and M. Tan, 25 January 2016.

Diagnosis.—*Nemacheilus zonatus* is distinguished from all other species of *Nemacheilus* in southeast Asia by the

Table 1. Specimens included in the molecular phylogeny, their associated GenBank accession numbers, and GenSeq designations (Chakrabarty et al., 2013). Samples labeled *N. pallidus* are those from within the range described by Kottelat (1990) for *N. pallidus*, including from the type locality (UF 188670).

| Taxon | Catalog no. | Tissue no. | COI | RAG1 | GenSeq |
|-----------------------------------|-------------|---------------------|----------|----------|----------|
| <i>Nemacheilus binotatus</i> | UF 188375 | ICH-00113 | MN852378 | MN861953 | genseq-4 |
| <i>Nemacheilus binotatus</i> | UF 237559 | 2015-0268 | MN852372 | MN861947 | genseq-4 |
| <i>Nemacheilus binotatus</i> | UF 237560 | 2015-0193 | MN852370 | MN861945 | genseq-4 |
| <i>Nemacheilus binotatus</i> | UF 237560 | 2015-0195 | MN852371 | MN861946 | genseq-4 |
| <i>Nemacheilus fasciatus</i> | UF 162177 | 2005-0969 | MN852338 | MN861913 | genseq-4 |
| <i>Nemacheilus fasciatus</i> | UF 162177 | 2005-0971 | MN852339 | MN861914 | genseq-4 |
| <i>Nemacheilus fasciatus</i> | UF 166880 | 2006-0594 | MN852343 | MN861918 | genseq-4 |
| <i>Nemacheilus fasciatus</i> | UF 166880 | 2006-0599 | MN852344 | MN861919 | genseq-4 |
| <i>Nemacheilus fasciatus</i> | UF 166882 | 2006-0602 | MN852345 | MN861920 | genseq-4 |
| <i>Nemacheilus longipinnis</i> | UF 161603 | 2005-0946 | MN852336 | MN861911 | genseq-4 |
| <i>Nemacheilus longipinnis</i> | UF 161603 | 2005-0950 | MN852337 | MN861912 | genseq-4 |
| <i>Nemacheilus longipinnis</i> | UF 161604 | 2005-1010 | MN852342 | MN861917 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 173512 | 2008-0632 | MN852348 | MN861923 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 173521 | 2008-0644 | MN852349 | MN861924 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 235938 | 2014-0112 | MN852355 | MN861930 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 235938 | 2014-0115 | MN852356 | MN861931 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 235954 | 2014-0143 | MN852358 | MN861933 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 235962 | 2014-0164 | MN852359 | MN861934 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 235979 | 2014-0190 | MN852360 | MN861935 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236031 | 2014-0356 | MN852363 | MN861938 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236031 | 2014-0359 | MN852364 | MN861939 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236049 | 2014-0378 | MN852365 | MN861940 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236049 | 2014-0379 | MN852366 | MN861941 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236087 | 2014-0256 | MN852362 | MN861937 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236151 | 2012-0590 | MN852353 | MN861928 | genseq-4 |
| <i>Nemacheilus masyae</i> | UF 236151 | 2012-0593 | MN852354 | MN861929 | genseq-4 |
| <i>Nemacheilus ornatus</i> | UF 172980 | 2008-0866 | MN852346 | MN861921 | genseq-4 |
| <i>Nemacheilus ornatus</i> | UF 236094 | 2014-0248 | MN852361 | MN861936 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 172975 | 2008-0525 | MN852347 | MN861922 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 176446 | 2009-0667 | MN852350 | MN861925 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 181189 | 2011-0177 | MN852352 | MN861927 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 188670 | ICH-00332 | MN852385 | MN861960 | genseq-3 |
| <i>Nemacheilus pallidus</i> | UF 188670 | ICH-00333 | MN852386 | MN861961 | genseq-3 |
| <i>Nemacheilus pallidus</i> | UF 188670 | ICH-00334 | MN852387 | MN861962 | genseq-3 |
| <i>Nemacheilus pallidus</i> | UF 190406 | ICH-01367 | MN852390 | MN861965 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 190406 | ICH-01374 | MN852391 | MN861966 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 190406 | ICH-01376 | MN852392 | MN861967 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 190466 | ICH-01295 | MN852388 | MN861963 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 190466 | ICH-01299 | MN852389 | MN861964 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 237297 | 2015-0407 | MN852374 | MN861949 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 237298 | 2015-0460 | MN852375 | MN861950 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 237299 | 2015-0390 | MN852373 | MN861948 | genseq-4 |
| <i>Nemacheilus pallidus</i> | UF 237300 | 2015-0471 | MN852376 | MN861951 | genseq-4 |
| <i>Nemacheilus pfeifferae</i> | UF 185053 | SN71 | MN852393 | MN861968 | genseq-4 |
| <i>Nemacheilus platiceps</i> | UF 235950 | 2014-0128 | MN852357 | MN861932 | genseq-4 |
| <i>Nemacheilus selangoricus</i> | UF 162174 | 2005-0987 | MN852340 | MN861915 | genseq-4 |
| <i>Nemacheilus selangoricus</i> | UF 162174 | 2005-0988 | MN852341 | MN861916 | genseq-4 |
| <i>Nemacheilus zonatus</i> | UF 188240 | ICH-00202 | MN852379 | MN861954 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 188240 | ICH-00206 | MN852380 | MN861955 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 188240 | ICH-00208 | MN852381 | MN861956 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 188447 | ICH-00225 | MN852382 | MN861957 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 188447 | ICH-00226 | MN852383 | MN861958 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 237302 | 2015-0022 | MN852367 | MN861942 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 237302 | 2015-0023 | MN852368 | MN861943 | genseq-2 |
| <i>Nemacheilus zonatus</i> | UF 237302 | 2015-0025 | MN852369 | MN861944 | genseq-2 |
| <i>Cobitis striata</i> | — | —/— | AB054125 | EF458303 | |
| <i>Danio rerio</i> | — | SZYD13031872-A/— | KM207081 | U71093 | |
| <i>Pseudohomaloptera leonardi</i> | UF 235746/— | 2012-0597/CTOL00177 | KR052872 | EU711130 | |
| <i>Traccaticthys pulcher</i> | IHB0706008 | —/— | JN177214 | JN177187 | |

Table 1. Continued.

| Taxon | Catalog no. | Tissue no. | COI | RAG1 | GenSeq |
|---------------------------------|-------------|-------------|----------|----------|----------|
| <i>Neonoemacheilus labeosus</i> | UF 188371 | ICH-00017 | MN852377 | MN861952 | genseq-4 |
| <i>Oreonectes platycephalus</i> | IHB0400270 | —/— | JN177215 | JN177186 | |
| <i>Schistura aurantiaca</i> | UF 176400 | 2009-0697 | MN852351 | MN861926 | genseq-2 |
| <i>Schistura sexcauda</i> | UF 192067 | ICH-03030 | MN852384 | MN861959 | genseq-4 |
| <i>Vaillantella maassi</i> | — | —/CTOL00197 | AB242173 | EU711132 | |

presence of 8–12 thin dusky black bars along the side of the body that cross over the back and join the bars on the other side and the small body size—to 29 mm SL.

Molecular data indicate that *N. zonatus* is most closely related to *N. masyae* (Fig. 2), which reaches a much larger size (to 66 mm SL), has black blotches rather than uniformly thin bars along the side of the body, black saddles along the dorsal midline, and a conspicuous black spot on the anterior dorsal-fin rays. *Nemacheilus zonatus* also has a shorter snout (24–32 vs. 35–44% HL), a smaller gape (12–18 vs. 17–26% HL), an ethmoid complex that is broad with large anterolateral flanges and narrowest at its middle then widening to its contact with the frontal (vs. ethmoid complex narrow with small anterolateral flanges, narrowest anteriorly then widening slightly to its contact with frontal; Fig. 4), and 34 total vertebrae ($n = 2$ specimens) vs. 36 in *N. masyae* ($n = 1$).

The other ten species of *Nemacheilus* known from mainland southeast Asia, *N. arenicolus*, *N. banar*, *N. binotatus*, *N. cleopatra*, *N. longistriatus*, *N. ornatus*, *N. paucimaculatus*, *N. platiceps*, *N. selangoricus*, and *N. troglotataractus*, are larger, at least 40 mm SL, and except for *N. troglotataractus*, which is a cave-inhabiting species that lacks dark pigment, have much more dark brown or black pigment on the back and side of the body (Kottelat, 1990, 1998; Freyhof and Serov, 2001; Bohlen and Šlechtová, 2011). *Nemacheilus arenicolus* has 8–13 large brown blotches along the side and 7–11 brown blotches along the back, *N. banar* has 8–13 dark blotches along the side and 9–15 dark blotches along the back, *N. binotatus* has a black stripe along the side of the body and a black stripe along the back, *N. cleopatra* has 9–13 vertically elongated dark blotches along the side and 8–11 dark blotches along the back, *N. longistriatus* has 8–12 dark brown to black blotches along the side overlain with a black stripe and 9–14 dark brown blotches along the back, *N. ornatus* has 8–17 black blotches along the side and irregular black blotches along the back, *N. paucima-*

culatus has 8–12 wide dark brown bars along the side and 8–10 dark brown blotches along the back, *N. platiceps* has 14–16 irregular brown bars, usually split ventrally, on the side that cross over the back and join the bars on the other side, and *N. selangoricus* has 8–12 dark brown bars much wider than the interspaces along the side of the body that cross over the back and join the bars on the other side. *Nemacheilus longipectoralis*, restricted to Borneo, is similar to *N. zonatus* in that it has dark bars on the side of the body that cross over the back (see Hadiaty and Kottelat, 2010: fig. 8), but the bars are wider—as wide or wider than interspaces—and more numerous (16–20 vs. 8–12 in *N. zonatus*).

Description.—Morphometric data in Table 2; meristic data in Table 3. *Nemacheilus zonatus* has slender, slightly compressed body; slenderest at caudal peduncle, deepest at nape. Snout pointed; mouth slightly subterminal, slightly arched; lips thin; upper lip with shallow furrows, no median notch; lower lip with slightly deeper furrows, shallow median indentation. Anterior nostril a short tube. Processus dentiformis (a bony projection at the symphysis of the upper jaw) present. Three pairs of barbels; maxillary barbel reaches slightly past posterior margin of eye, inner rostral barbel reaches almost to anterior margin of eye; outer rostral barbel reaches to posterior margin of eye. Eye slightly elliptical horizontally, dorsolaterally positioned, about equal to snout length, to $\frac{1}{3}$ head length. Pectoral fin somewhat falcate, with fimbriate distal margin; inner rays short, outermost 2nd to 4th rays longest (Fig. 5A). In juveniles and females, pectoral fin reaches about half-way from origin of pectoral fin to origin of pelvic fin; in large males, it reaches to pelvic fin. Pelvic fin originates under 4th to 5th dorsal ray, reaches slightly past dorsal fin insertion, does not reach anus. Anus about length of eye in front of anal fin. Distal margin of dorsal fin straight, of anal fin convex. Caudal fin deeply forked with upper lobe

**Fig. 3.** Lateral view of *Nemacheilus zonatus*, paratype, UF 188240, 25.2 mm SL.

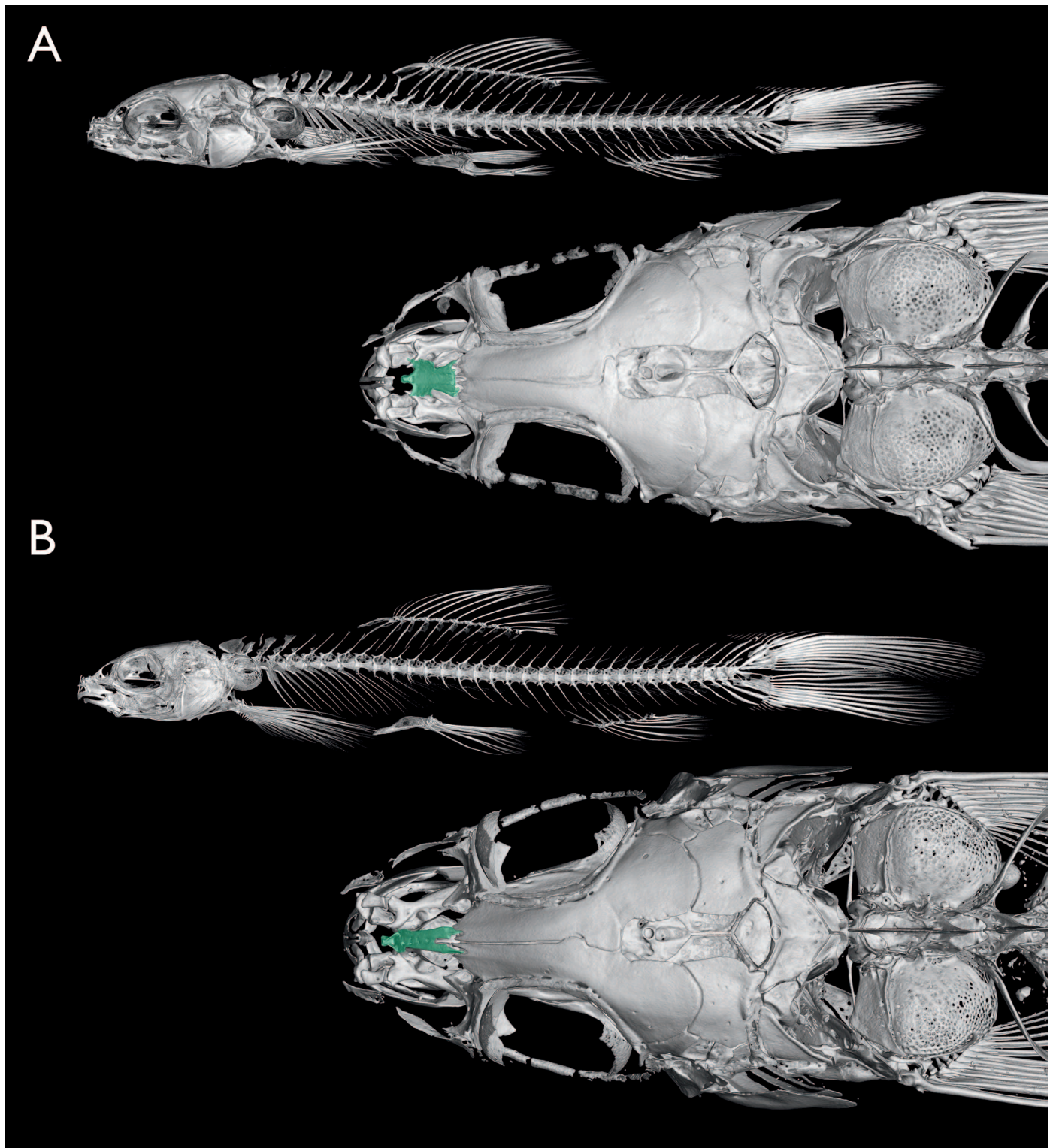


Fig. 4. Lateral and dorsal CT scans of: (A) *Nemacheilus zonatus*, UF 237302, lateral view, 23.2 mm SL, UF 237302 (2nd specimen), dorsal view, 27.0 mm SL; (B) *N. masyae*, UF 188670, lateral and dorsal views, 38.5 mm SL. Dorsal surface of ethmoid complex highlighted.

longer than lower lobe. Small axillary pelvic lobe. Suborbital flap on male (Fig. 5B). Branched dorsal rays $9\frac{1}{2}$ – $10\frac{1}{2}$, pectoral rays 10–12, pelvic rays 8, branched anal rays $5\frac{1}{2}$, upper caudal lobe 9 rays, lower caudal lobe 8 rays. Body covered with embedded scales. Lateral line complete. Supraorbital pores 6, infraorbital pores 4 + 10, preoperculo-mandibular pores 9, supratemporal pores 3. Vertebrae (2 specimens): 34

(22 abdominal including Weberian complex + 12 caudal). In dorsal view, ethmoid complex broad with long, broad anterolateral flanges, ethmoid narrowest at middle then widening steeply to its contact with frontal (Fig. 4A).

Color in life.—Body nearly translucent with light yellow back and upper side; 8–12 thin, dusky bars on side of body and

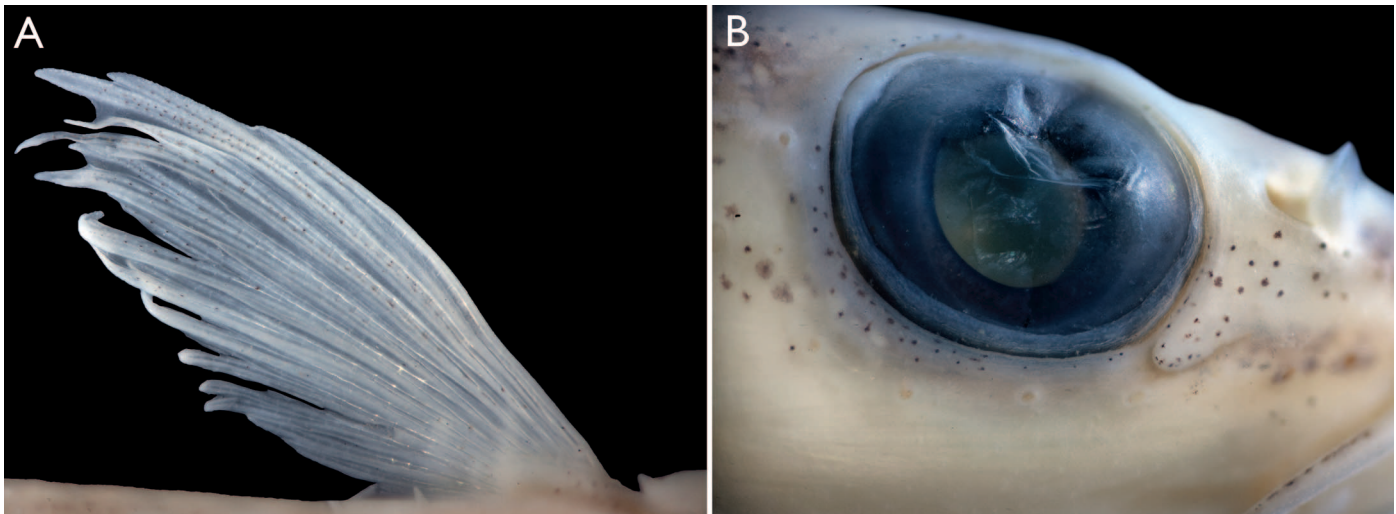


Fig. 5. *Nemacheilus zonatus*: (A) pectoral fin, UF 188447, 24.4 mm SL; (B) suborbital flap, UF 237302, 28.6 mm SL.

over back. Bars much thinner than interspaces. Black bar on nape at posterior margin of head, another bar between eyes, interrupted bar immediately behind nostrils, dusky black patch on side of snout. Bright yellow sheen on cheek, opercle, and anterior part of lateral line. Posterior end of caudal peduncle white with bold, black, somewhat triangular spot. Bright red spot about half-way up most anterior ray of dorsal fin; melanophores in 2 faint bands across fin. Other fins mostly clear; scattered melanophores on leading edge of pectoral fin and proximal portion of anal fin. Caudal fin with scattered melanophores on proximal half, pale white on upper and lower lobes.

Sexual dimorphism.—All specimens were collected in November and January, and sexual dimorphism, if present, may not have been well developed during this period. The largest male, 28.6 mm SL and collected on 6 January 2015, has a

well-developed suborbital flap indicating that it was an adult. A smaller male, 27.0 mm SL, from the same collection has a developing flap (Fig. 5B). About half of the specimens, including the two males with a suborbital flap, had tubercles along the rays on the lower surface of the pectoral fin.

Etymology.—The epithet *zonatus* is a Latin adjective meaning “banded” or “barred” in reference to the bars along the side of the body that cross over the back and meet the bars on the opposite side.

Distribution and habitat.—*Nemacheilus zonatus* is known from the Mun and Songkhram river drainages (Fig. 6) on the Korat Plateau of Thailand. These large drainages have expansive areas of sandy substrate where this species is found in moderate flow near the banks.

Table 2. Morphometric data for *Nemacheilus zonatus* (all types) and *N. masyae* (see Material Examined).

| | <i>N. zonatus</i> n = 18 | | | <i>N. masyae</i> n = 36 | | |
|-------------------------------|--------------------------|------|------|-------------------------|------|------|
| | Range | Mean | SD | Range | Mean | SD |
| Standard length (mm) | 15.3–28.6 | 22.2 | 3.69 | 29.3–66.2 | 45.3 | 9.62 |
| In percent of standard length | | | | | | |
| Head length | 23.1–27.5 | 25.4 | 1.79 | 18.9–23.9 | 22.1 | 1.17 |
| Pectoral-fin length | 19.6–27.0 | 23.5 | 1.79 | 14.7–22.2 | 19.0 | 2.07 |
| Prepelvic-fin length | 46.6–54.2 | 50.4 | 1.87 | 46.6–52.2 | 49.5 | 1.42 |
| Predorsal length | 44.0–51.6 | 47.2 | 2.13 | 45.6–52.6 | 48.0 | 1.50 |
| Preanal-fin length | 72.6–81.5 | 77.4 | 2.11 | 73.6–82.3 | 78.1 | 1.89 |
| Anal-fin length | 16.6–20.8 | 18.4 | 1.31 | 14.7–19.2 | 17.0 | 0.94 |
| Pelvic-fin length | 16.1–19.8 | 17.7 | 1.23 | 11.4–18.7 | 16.4 | 1.12 |
| Dorsal-fin length | 25.1–32.6 | 29.4 | 1.71 | 24.9–29.8 | 27.8 | 1.05 |
| Caudal-peduncle length | 11.5–15.7 | 13.6 | 1.35 | 14.7–18.5 | 16.5 | 1.00 |
| Caudal-peduncle depth | 9.9–13.3 | 10.1 | 0.98 | 8.5–11.6 | 10.0 | 0.65 |
| Body depth | 11.0–15.8 | 13.1 | 1.38 | 12.9–17.5 | 15.4 | 1.04 |
| In percent of head length | | | | | | |
| Snout length | 23.7–31.8 | 27.0 | 2.43 | 34.6–44.1 | 38.4 | 2.13 |
| Gape width | 12.3–17.5 | 15.7 | 1.58 | 16.7–26.1 | 21.2 | 2.55 |
| Eye diameter | 23.3–29.1 | 25.8 | 1.64 | 20.2–29.1 | 24.1 | 2.51 |
| Interorbital width | 15.0–22.6 | 18.4 | 2.06 | 19.5–32.3 | 27.4 | 3.00 |

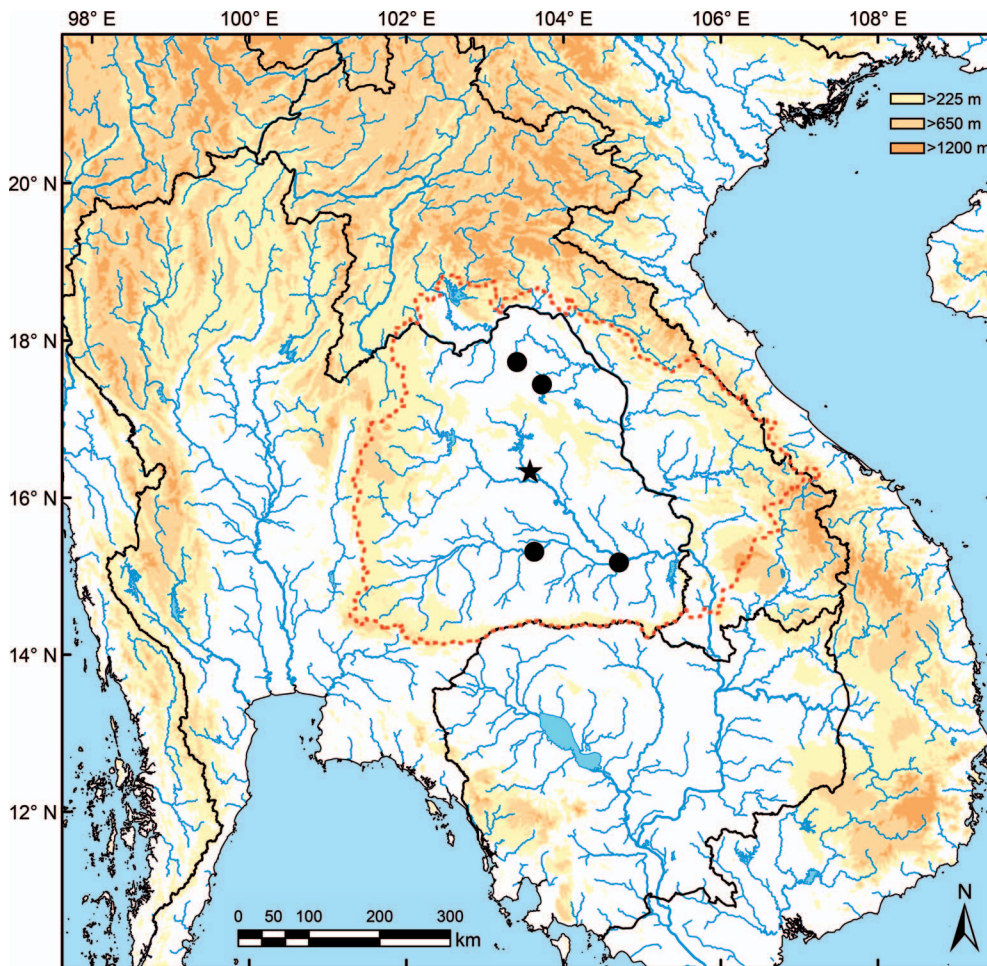


Fig. 6. Distribution of *Nemacheilus zonatus*. Star indicates type locality. Dashed red line outlines Khorat Plateau ecoregion (Abell et al., 2008).

DISCUSSION

Nemacheilus pallidus was diagnosed from other Indochinese species of *Nemacheilus* by the combination of 14–19 dark blotches along the side of the body and 13–16 dark saddles along the back, a black spot at the posterior end of the lateral line, and a black spot on the anterior dorsal rays at about one fourth the height of the ray (Kottelat, 1990). This basic color pattern is shared with *N. masyae*, from which it was said to differ by having a deeper body (14.8–19.1, mean 16.9% SL, vs. 12.6–17.6, mean 14.7% SL), eyes situated more on the sides of the head (interorbital width 5.7–8.0 vs. 4.9–6.8% SL), and spots and saddles lighter and thinner than interspaces (vs. darker and wider than interspaces in *N. masyae*). *Nemacheilus pallidus* was described by Kottelat (1990) as

occurring in the Mekong basin in Cambodia, Laos, Thailand, the upper tributaries of the Chao Phraya, and the Mae Klong basin. *Nemacheilus masyae* was described as occurring in peninsular Malaysia and Thailand, and in the Mae Klong and Chao Phraya basins; however, no locality records were provided or shown on the map (Kottelat, 1990: 55–57, 59) for the Mae Klong or the Chao Phraya and, contrary to the textual description, locality records were given for streams in Chantaburi Province in southeast Thailand.

The putative differences in color pattern, with spots and saddles thinner than interspaces in *N. pallidus* vs. wider in *N. masyae*, do not hold up when specimens are examined from across the range of the putative species (Fig. 7) or in the figures provided by Kottelat (1990: figs. 29, 36). Although body proportions were reported to separate *N. masyae* and *N.*

Table 3. Meristic data for *Nemacheilus zonatus* and *N. masyae*. Specimens same as in Table 2.

| | <i>N. zonatus</i> n = 18 | | | <i>N. masyae</i> n = 36 | | |
|----------------------|--------------------------|------|------|-------------------------|------|------|
| | Range | Mean | SD | Range | Mean | SD |
| Branched dorsal rays | 9–10 | 9.1 | 0.24 | 8–10 | 9.0 | 0.24 |
| Branched anal rays | 5 | 5 | — | 5 | 5 | — |
| Branched caudal rays | 17 | 17 | — | 17 | 17 | — |
| Pectoral rays | 10–12 | 11.2 | 0.64 | 10–13 | 12.1 | 0.70 |
| Pelvic rays | 8–8 | 8 | — | 8–8 | 8 | — |
| Dorsal saddles | 8–12 | 10.5 | 1.28 | 10–20 | 13.9 | 2.25 |
| Lateral blotches | 9–13 | 11.2 | 1.30 | 11–18 | 14.4 | 1.77 |

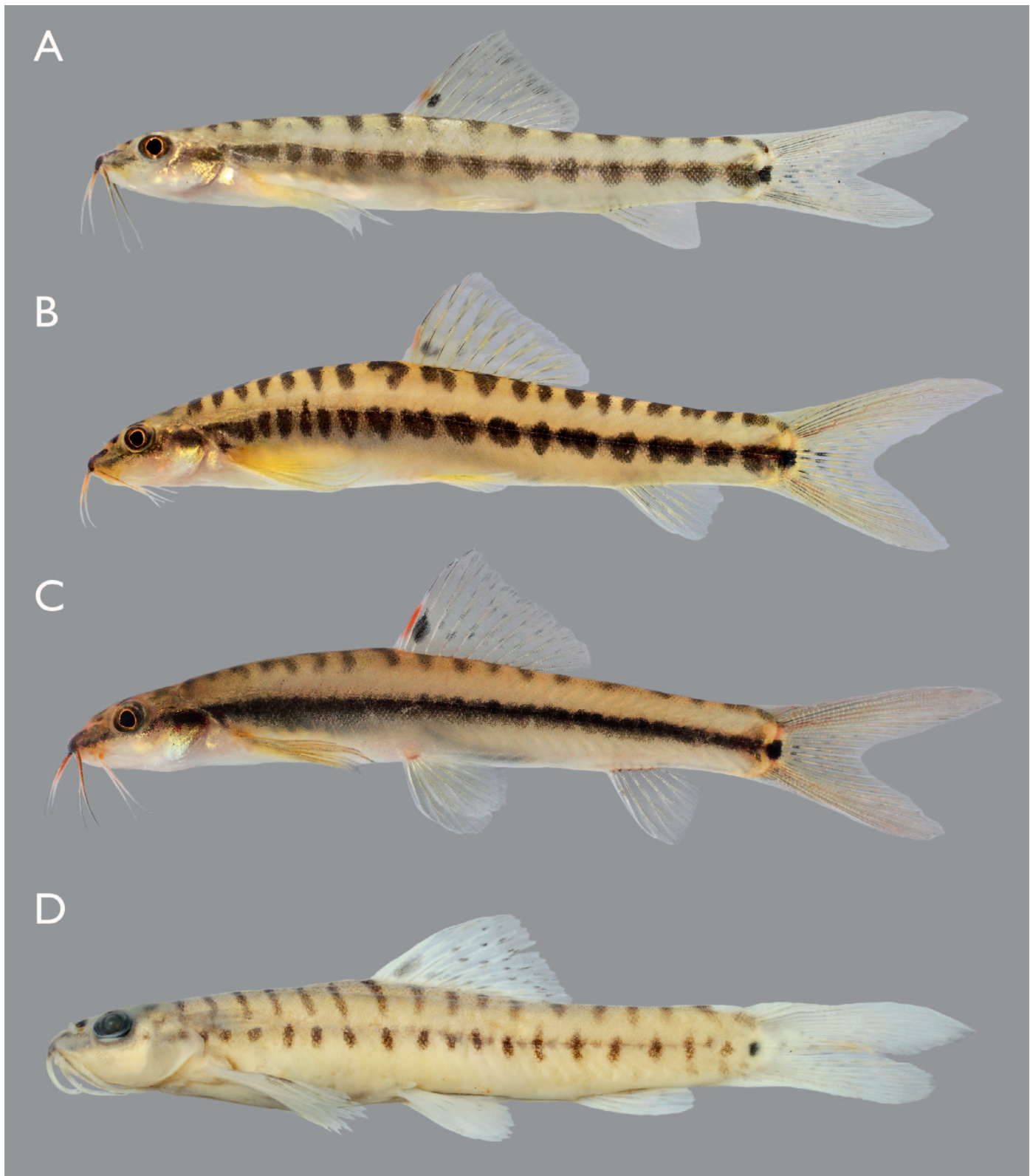


Fig. 7. Color pattern variation in *Nemacheilus masyae*. (A) UF 236087, 44.3 mm SL, Gulf of Thailand drainage; (B) UF 236049, 64.3 mm SL, Panang Tak River drainage; (C) UF 191306, 55.8 mm SL, Mae Klong River drainage; (D) preserved UF 188670, 38.5 mm SL, On River drainage.

pallidus (Kottelat, 1990: 57), the separation was small and not tested statistically. The lack of morphological distinctions, including in body shape (Fig. 1), and the non-monophyly of *N. pallidus* (Fig. 2B) indicate that *N. pallidus* is a junior

synonym of *N. masyae*. *Nemacheilus pallidus* is relegated to the synonymy of *N. masyae*, which occurs in the Mae Klong, Chao Phraya, Mekong, and many coastal drainages of peninsular Thailand and Malaysia.

MATERIAL EXAMINED

Nemacheilus masyae: Thailand. Chao Phraya basin: Lampang Province: UF 188670, 1, 38.5 mm SL, Phlueng River, 18.71394°N, 99.92719°E, 23 January 2016. Mae Klong basin: Kanchanaburi Province: UF 176446, 2, 29.3–36.5 mm SL, Huay Ban Rai, 14.71933°N, 98.50583°E, 2 January 2010; UF 176560, 1, 33.0 mm SL, same data as UF 176446; UF 181189, 1, 53.0 mm SL, Huay Ulong, 14.75883°N, 98.64705°E, 23 April 2011; UF 191306, 1, 55.8 mm SL, Malai River, 15.13507°N, 98.36954°E, 7 January 2017; UF 237297, 3, 35.4–36.2 mm SL, Pracham Mai River, 14.65983°N, 98.53422°E, 28 January 2015; UF 237299, 1, 41.9 mm SL, unnamed stream, 14.54356°N, 98.78667°E, 27 January 2015; UF 237300, 2, 33.3–43.9 mm SL, unnamed stream, 15.06996°N, 98.56254°E, 29 January 2015. Mekong basin: Ubon Ratchathani Province: UF 172975, 1, 48.3 mm SL, mountain stream, 15.74720°N, 105.45043°E, 9 June 2008; UF 172976, 1, 51.9 mm SL, same data as UF 172975; UF 185102, 2, 32.3–34.5 mm SL, Huay Ta Wang, 15.79191°N, 105.37609°E, 3 January 2013; UF 237298, 1, 49.3 mm SL, Song Karia River, 15.22318°N, 98.44648°E, 29 January 2015. Trat basin: Trat Province: UF 235938, 2, 40.0–50.4 mm SL, Klong Sung, 12.46667°N, 102.63333°E, 29 January 2014. Welu basin: Chanthaburi Province: UF 235962, 1, 44.9 mm SL, Trok Nong, 12.53662°N, 102.24427°E; UF 235979, 1, 52.3 mm SL, Klong Philo, 12.52602°N, 102.17583°E, 30 January 2014. Peninsular Thailand: Gulf of Thailand basin: Chumphon Province: UF 236031, 3, 52.0–66.2 mm SL, Khlong Tasae, 10.87258°N, 99.23755°E, 2 February 2014; UF 236049, 3, 55.8–64.3 mm SL, Khlong Rap Ro, 10.62748°N, 99.05725°E, 3 February 2014. Tapi basin: Nakhon Si Thammarat Province: UF 236087, 2, 42.6–44.3 mm SL, Tapi River, 8.50732°N, 99.50870°E, 5 February 2014. Malaysia: Sungai Siam basin: Johor Province: UF 173512, 3, 43.7–49.1 mm SL, small stream, 1.8°N, 103.5°E, 2 August 2008. Sungai Sayong basin, Johor Province: UF 173521, 3, 34.1–40.9 mm SL, small stream, 1.84804°N, 103.48267°E, 2 August 2008. Kelantan basin: Kelantan Province: UF 236151, 3, mm SL, 43.1–54.1 mm SL, Go Lok River, 5.75145°N, 101.98066°E, 9 February 2014.

DATA ACCESSIBILITY

Supplemental material is available at <https://www.copeiajournal.org/ci-19-305>.

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