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

Authors: Page, Lawrence M., Pfeiffer, John M., Suksri, Siriwan, Randall, Zachary S., and Boyd, David A.

Source: Copeia, 108(2) : 392-402

Published By: The American Society of Ichthyologists and Herpetologists

URL: https://doi.org/10.1643/CI-19-305

Copeia

Copeia 108, No. 2, 2020, 392–402

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

Lawrence M. Page[1], John M. Pfeiffer[1,2], Siriwan Suksri[3], Zachary S. Randall[1], and David A. Boyd[1]

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. troglocataractus*; 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:

---

[1] Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611; Email: (LMP) Lpage1@ufl.edu; (ZSR) zrandall@flmnh.ufl.edu; and (DAB) dboyd@flmnh.ufl.edu.

[2] National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560; Email: PfeifferJ@si.edu.

[3] Inland Fisheries Research and Development Division, Department of Fisheries, 50 Phahon Yothin Road, Chatuchak, Bangkok, 10900, Thailand; Email: siriwan.suksri@gmail.com.

Submitted: 18 October 2019. Accepted: 4 January 2020. Associate Editor: M. P. Davis.

DOI: 10.1643/CI-19-305

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′ (Quenouille et al., 2004), and RAG-RV1 5′–TCCTGRAAGATYTTGTAGAA–3′ (Šlechtová et al., 2007). PCR was conducted using 25 μL solutions containing 1 μL of each primer at 10 mM; 0.25 μL MyTaq Red polymerase and 4 μL buffer (Bioline Reagents); and 2 μL 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) that is near the type locality of *N. masyae* in Nakhon Si Thammarat Province.

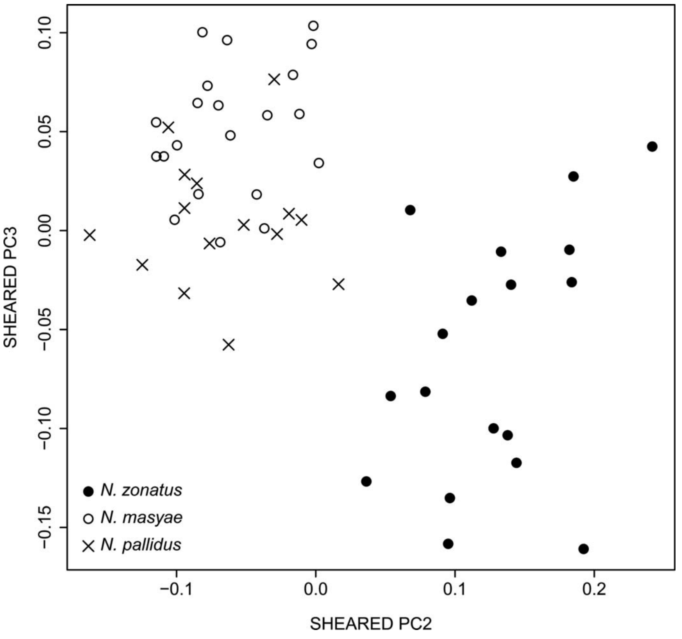

Fig. 1. Principal components analysis. Size accounted for 96.91% of the observed variance. The sheared second and third principal components (PC2 and PC3) accounted for 0.85% and 0.51%, respectively, of the observed variance. Interorbital width (0.52) and pectoral-fin length (–0.42) had the highest loadings 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*.

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

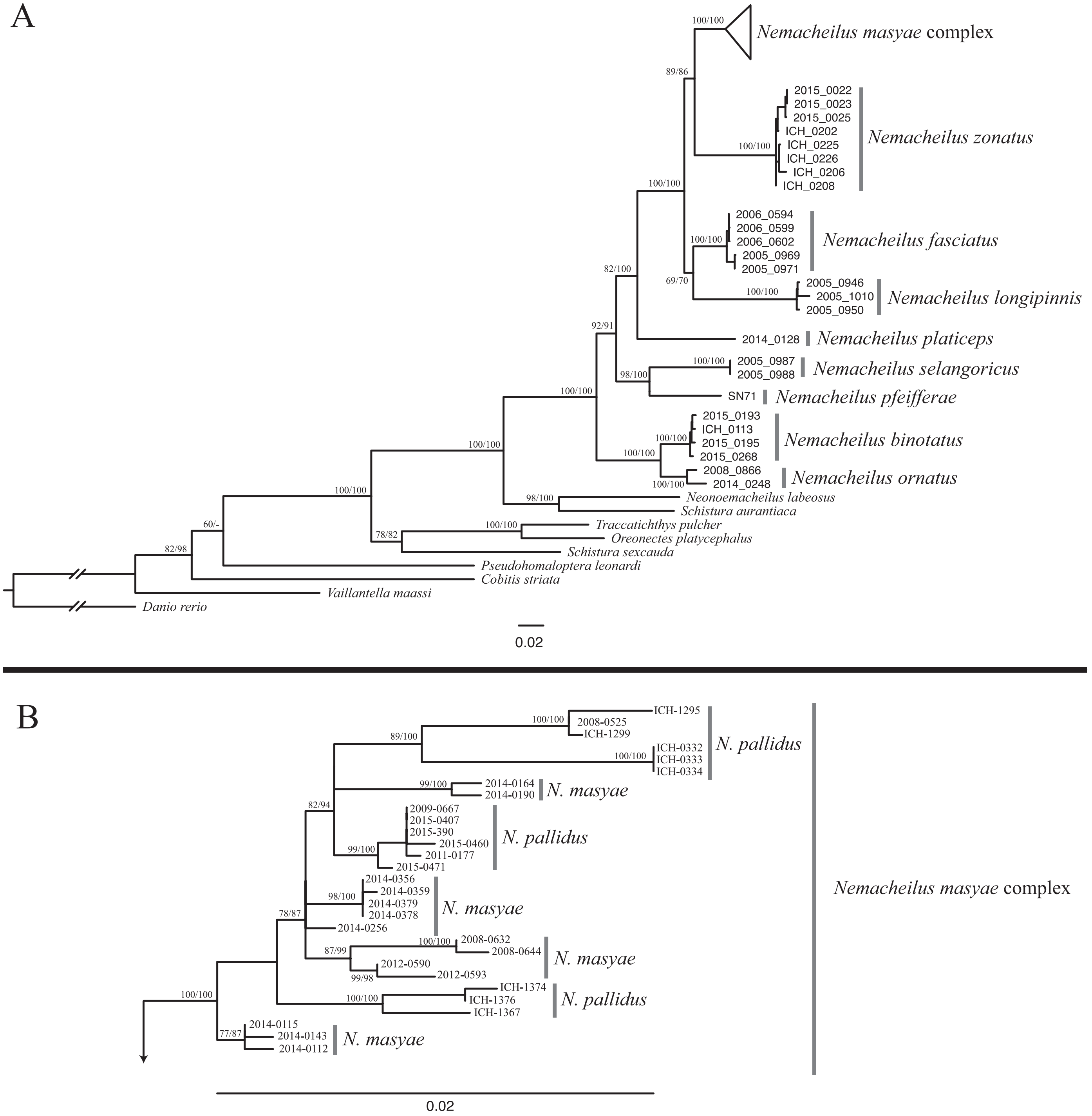

Fig. 2. Combined (CO1+RAG1) maximum likelihood phylogenetic reconstruction of (A) *Nemacheilus* with *N. masyae* clade collapsed and (B) *N. masyae* complex. Samples labeled *N. pallidus* are those from within the range described by Kottelat (1990) for *N. pallidus*, including from the type locality (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
Nemacheilus binotatus	UF 188375	ICH-00113	MN852378	MN861953	genseq-4
Nemacheilus binotatus	UF 237559	2015-0268	MN852372	MN861947	genseq-4
Nemacheilus binotatus	UF 237560	2015-0193	MN852370	MN861945	genseq-4
Nemacheilus binotatus	UF 237560	2015-0195	MN852371	MN861946	genseq-4
Nemacheilus fasciatus	UF 162177	2005-0969	MN852338	MN861913	genseq-4
Nemacheilus fasciatus	UF 162177	2005-0971	MN852339	MN861914	genseq-4
Nemacheilus fasciatus	UF 166880	2006-0594	MN852343	MN861918	genseq-4
Nemacheilus fasciatus	UF 166880	2006-0599	MN852344	MN861919	genseq-4
Nemacheilus fasciatus	UF 166882	2006-0602	MN852345	MN861920	genseq-4
Nemacheilus longipinnis	UF 161603	2005-0946	MN852336	MN861911	genseq-4
Nemacheilus longipinnis	UF 161603	2005-0950	MN852337	MN861912	genseq-4
Nemacheilus longipinnis	UF 161604	2005-1010	MN852342	MN861917	genseq-4
Nemacheilus masyae	UF 173512	2008-0632	MN852348	MN861923	genseq-4
Nemacheilus masyae	UF 173521	2008-0644	MN852349	MN861924	genseq-4
Nemacheilus masyae	UF 235938	2014-0112	MN852355	MN861930	genseq-4
Nemacheilus masyae	UF 235938	2014-0115	MN852356	MN861931	genseq-4
Nemacheilus masyae	UF 235954	2014-0143	MN852358	MN861933	genseq-4
Nemacheilus masyae	UF 235962	2014-0164	MN852359	MN861934	genseq-4
Nemacheilus masyae	UF 235979	2014-0190	MN852360	MN861935	genseq-4
Nemacheilus masyae	UF 236031	2014-0356	MN852363	MN861938	genseq-4
Nemacheilus masyae	UF 236031	2014-0359	MN852364	MN861939	genseq-4
Nemacheilus masyae	UF 236049	2014-0378	MN852365	MN861940	genseq-4
Nemacheilus masyae	UF 236049	2014-0379	MN852366	MN861941	genseq-4
Nemacheilus masyae	UF 236087	2014-0256	MN852362	MN861937	genseq-4
Nemacheilus masyae	UF 236151	2012-0590	MN852353	MN861928	genseq-4
Nemacheilus masyae	UF 236151	2012-0593	MN852354	MN861929	genseq-4
Nemacheilus ornatus	UF 172980	2008-0866	MN852346	MN861921	genseq-4
Nemacheilus ornatus	UF 236094	2014-0248	MN852361	MN861936	genseq-4
Nemacheilus pallidus	UF 172975	2008-0525	MN852347	MN861922	genseq-4
Nemacheilus pallidus	UF 176446	2009-0667	MN852350	MN861925	genseq-4
Nemacheilus pallidus	UF 181189	2011-0177	MN852352	MN861927	genseq-4
Nemacheilus pallidus	UF 188670	ICH-00332	MN852385	MN861960	genseq-3
Nemacheilus pallidus	UF 188670	ICH-00333	MN852386	MN861961	genseq-3
Nemacheilus pallidus	UF 188670	ICH-00334	MN852387	MN861962	genseq-3
Nemacheilus pallidus	UF 190406	ICH-01367	MN852390	MN861965	genseq-4
Nemacheilus pallidus	UF 190406	ICH-01374	MN852391	MN861966	genseq-4
Nemacheilus pallidus	UF 190406	ICH-01376	MN852392	MN861967	genseq-4
Nemacheilus pallidus	UF 190466	ICH-01295	MN852388	MN861963	genseq-4
Nemacheilus pallidus	UF 190466	ICH-01299	MN852389	MN861964	genseq-4
Nemacheilus pallidus	UF 237297	2015-0407	MN852374	MN861949	genseq-4
Nemacheilus pallidus	UF 237298	2015-0460	MN852375	MN861950	genseq-4
Nemacheilus pallidus	UF 237299	2015-0390	MN852373	MN861948	genseq-4
Nemacheilus pallidus	UF 237300	2015-0471	MN852376	MN861951	genseq-4
Nemacheilus pfeifferae	UF 185053	SN71	MN852393	MN861968	genseq-4
Nemacheilus platiceps	UF 235950	2014-0128	MN852357	MN861932	genseq-4
Nemacheilus selangoricus	UF 162174	2005-0987	MN852340	MN861915	genseq-4
Nemacheilus selangoricus	UF 162174	2005-0988	MN852341	MN861916	genseq-4
Nemacheilus zonatus	UF 188240	ICH-00202	MN852379	MN861954	genseq-2
Nemacheilus zonatus	UF 188240	ICH-00206	MN852380	MN861955	genseq-2
Nemacheilus zonatus	UF 188240	ICH-00208	MN852381	MN861956	genseq-2
Nemacheilus zonatus	UF 188447	ICH-00225	MN852382	MN861957	genseq-2
Nemacheilus zonatus	UF 188447	ICH-00226	MN852383	MN861958	genseq-2
Nemacheilus zonatus	UF 237302	2015-0022	MN852367	MN861942	genseq-2
Nemacheilus zonatus	UF 237302	2015-0023	MN852368	MN861943	genseq-2
Nemacheilus zonatus	UF 237302	2015-0025	MN852369	MN861944	genseq-2
Cobitis striata	—	—/—	AB054125	EF458303	
Danio rerio	—	SZYD13031872-A/—	KM207081	U71093	
Pseudohomaloptera leonardi	UF 235746/—	2012-0597/CTOL00177	KR052872	EU711130	
Traccatichthys pulcher	IHB0706008	—/—	JN177214	JN177187	

Table 1. Continued.

Taxon	Catalog no.	Tissue no.	COI	RAG1	GenSeq
Neonoemacheilus labeosus	UF 188371	ICH-00017	MN852377	MN861952	genseq-4
Oreonectes platycephalus	IHB0400270	—/—	JN177215	JN177186	
Schistura aurantiaca	UF 176400	2009-0697	MN852351	MN861926	genseq-2
Schistura sexcauda	UF 192067	ICH-03030	MN852384	MN861959	genseq-4
Vaillantella maassi	—	—/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. troglocataractus,* are larger, at least 40 mm SL, and except for *N. troglocataractus,* 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. paucimaculatus* 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 ⅓ 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

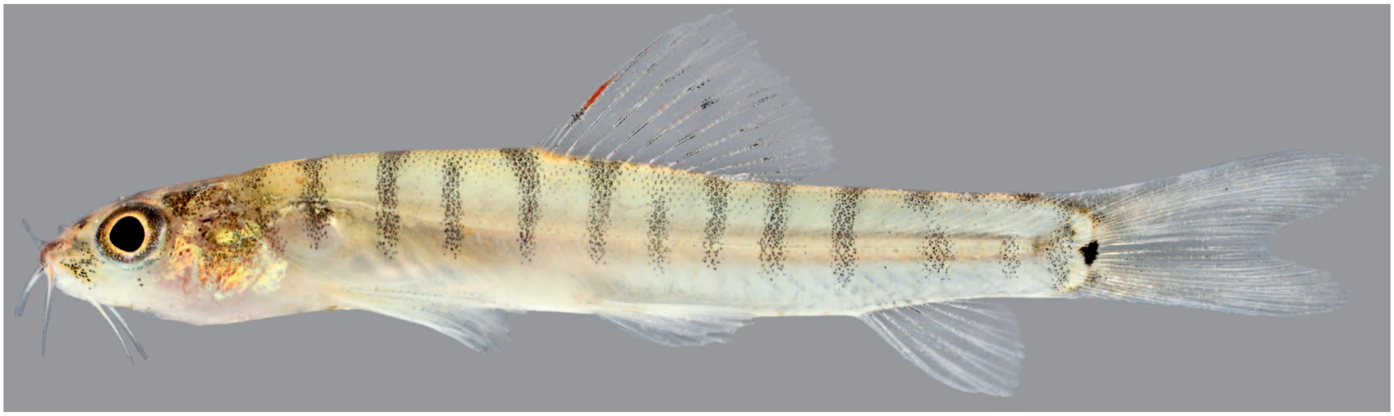

Fig. 3. Lateral view of *Nemacheilus zonatus*, paratype, UF 188240, 25.2 mm SL.

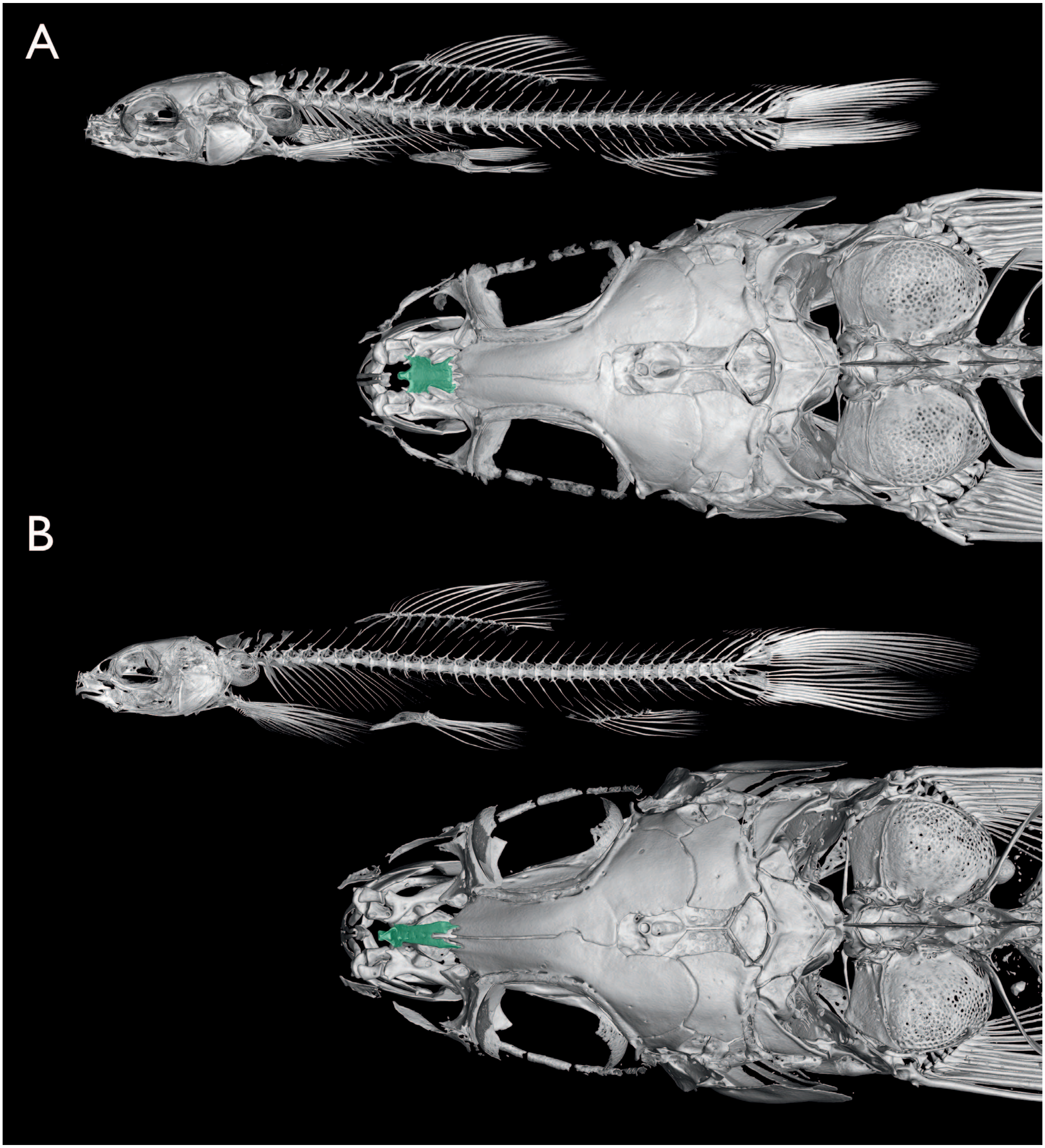

Fig. 4. Lateral and dorsal CT scans of: (A) *Nemacheilus zonatus*, UF 237302, lateral view, 23.2 mm SL, UF 237302 (2nd specimen), dorsal view, 27.0 mm SL; (B) *N. masyae*, UF 188670, lateral and dorsal views, 38.5 mm SL. Dorsal surface of ethmoid complex highlighted.

longer than lower lobe. Small axillary pelvic lobe. Suborbital flap on male (Fig. 5B). Branched dorsal rays 9½–10½, pectoral rays 10–12, pelvic rays 8, branched anal rays 5½, 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, preoperculomandibular 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

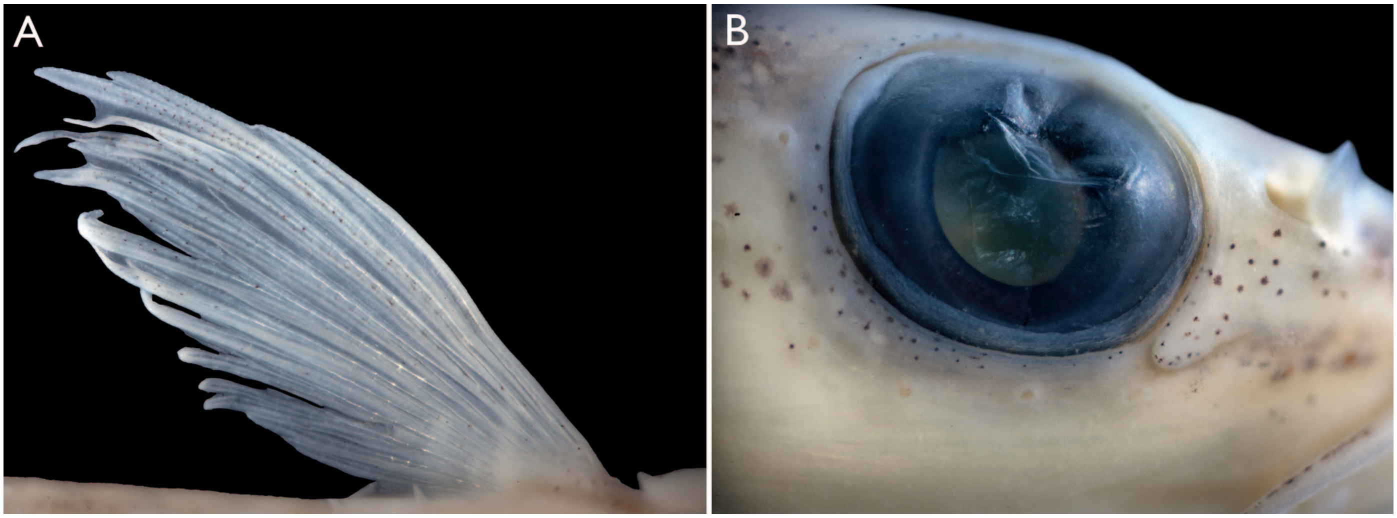

Fig. 5. *Nemacheilus zonatus*: (A) pectoral fin, UF 188447, 24.4 mm SL; (B) suborbital flap, UF 237302, 28.6 mm SL.

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).

	N. zonatus n = 18			*N. masyae 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

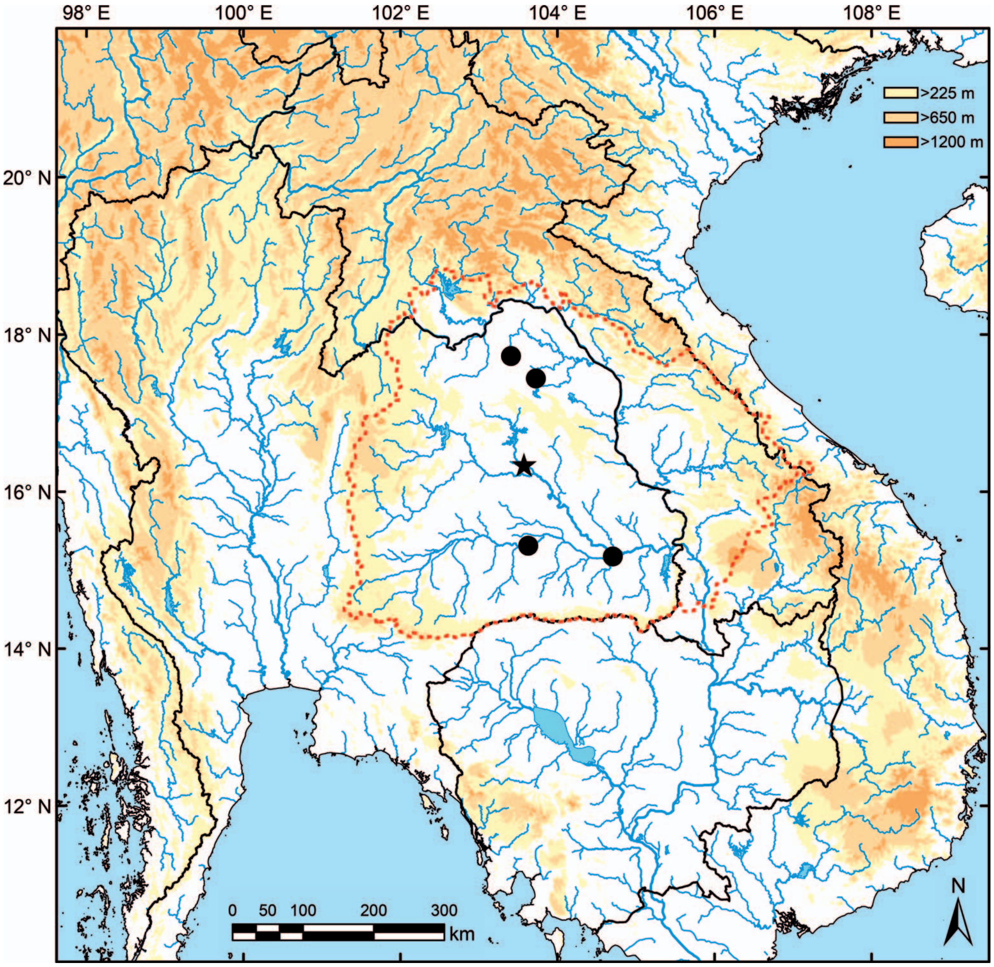

Fig. 6. Distribution of *Nemacheilus zonatus*. Star indicates type locality. Dashed red line outlines Khorat Plateau ecoregion (Abell et al., 2008).

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.

	N. zonatus n = 18			*N. masyae 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

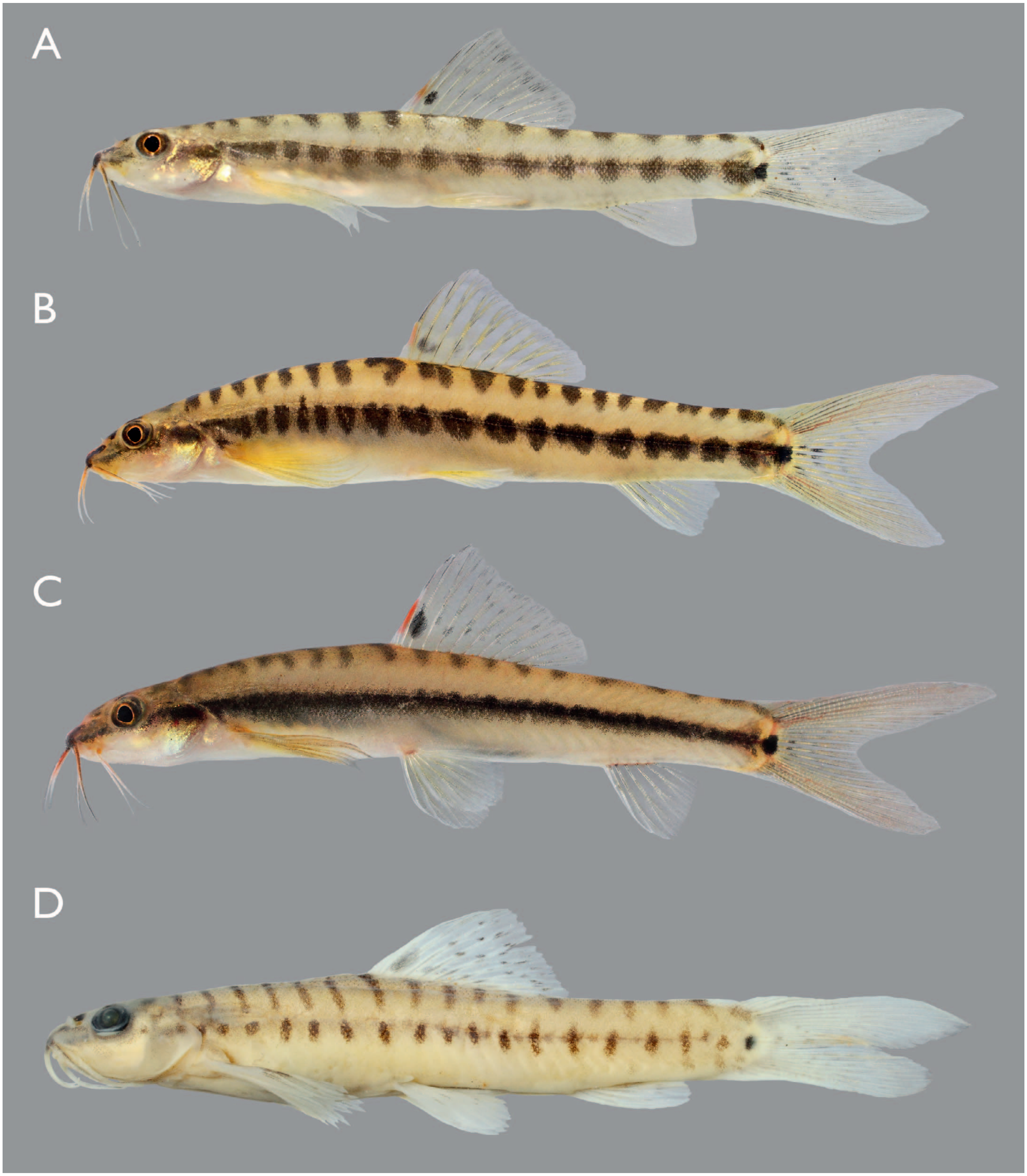

Fig. 7. Color pattern variation in *Nemacheilus masyae*. (A) UF 236087, 44.3 mm SL, Gulf of Thailand drainage; (B) UF 236049, 64.3 mm SL, Panang Tak River drainage; (C) UF 191306, 55.8 mm SL, Mae Klong River drainage; (D) preserved UF 188670, 38.5 mm SL, On River drainage.

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: Chumpon 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.

ACKNOWLEDGMENTS

We greatly appreciate the permissions granted by federal and other governmental agencies to our colleagues, So Nam, Inland Fisheries Research and Development Institute, Fisheries Administration, Phnom Penh, Cambodia; M. Z. Khaironizam, School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia; and Chaiwut Grudpan, Department of Fisheries, Faculty of Agriculture, Ubonrajathanee University, Thailand, to conduct fieldwork and collect fishes necessary for this study. We were assisted in the field by Amir Ahmad, Duangsamorn Boonwun, Chaiwut Grudpan, Jarungjit Grudpan, Ronnachai Itimung, Md. Zain Khaironizam, Zachary Martin, Thach Phanara, Randal Singer, Weerapongse Tangjitjaroen, and Niran Warin. Simon Santorium assisted in the collecting and analysis of morphological data as an undergraduate at the University of Florida. Halle Marchese assisted in preparation of the figures. This project was funded in part by the All Cypriniformes Species Inventory Project (DEB 1022720) and a Rules of Life award (uROL) from the National Science Foundation (NSF 1839915). CT scans were provided by the NSF-funded oVert project (DBI 1701714). Institutional records were searched through iDigBio, funded by NSF's Advancing Digitization of Biodiversity Collections Program (EF 1115210, DBI 1547229). This study was completed under the University of Florida Institutional Animal Care and Use Committee Protocol #201810206.

LITERATURE CITED

Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. C. Balderas, and W. Bussing. 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. Bioscience 58:403–414.

Armbruster, J. W. 2012. Standardized measurements, landmarks, and meristic counts for cypriniform fishes. Zootaxa 3586:8–16.

Baldwin, C. C., J. H. Mounts, D. G. Smith, and L. A. Weigt. 2009. Genetic identification and color descriptions of early life-history stages of Belizean *Phaeoptyx* and *Astrapogon* (Teleostei: Apogonidae) with comments on identification of adult *Phaeoptyx*. Zootaxa 2008:1–22.

Bohlen, J., and V. Šlechtová. 2011. *Nemacheilus paucimaculatus*, a new species of loach from the southern Malay Peninsula (Teleostei: Nemacheilidae). Raffles Bulletin of Zoology 59:201–204.

Bookstein, F. L., B. Chernoff, R. L. Elder, J. M. Humphries, Jr., G. R. Smith, and R. E. Strauss. 1985. Morphometrics in Evolutionary Biology. Special Publication 15, Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.

Chakrabarty, P., M. Warren, L. M. Page, and C. C. Baldwin. 2013. GenSeq: an updated nomenclature and ranking for genetic sequences from type and non-type sources. Zookeys 346:29–41.

Chernomor, O., A. von Haeseler, and B. Q. Minh. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65:997–1008.

Freyhof, J., and D. V. Serov. 2001. Nemacheiline loaches from Central Vietnam with descriptions of a new genus and 14 new species (Cypriniformes: Balitoridae). Ichthyological Exploration of Freshwaters 12:133–191.

Fricke, R., W. N. Eschmeyer, and R. Van der Laan (Eds.). 2019. Eschmeyer's Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed on 30 August 2019.

Hadiaty, R. K., and M. Kottelat. 2010. *Nemacheilus marang*, a new loach (Teleostei: Nemacheilidae) from Sangkulirang karst, eastern Borneo. Zootaxa 2557:39–48.

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35:518–522.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kottelat, M. 1990. Indochinese Nemacheilines. A Revision of Nemacheiline Loaches (Pisces: Cypriniformes) of Thailand, Burma, Laos, Cambodia and Southern Viet Nam. Verlag Dr. Friedrich Pfeil, Munich.

Kottelat, M. 1998. Fishes of the Nam Theun and Xe Bangfai basins, Laos, with diagnoses of twenty-two new species (Teleostei: Cyprinidae, Balitoridae, Cobitidae, Coiidae and Odontobutidae). Ichthyological Exploration of Freshwaters 9:1–128.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X Version 2.0. Bioinformatics 23:2947–2948.

Liu, S., R. L. Mayden, J. Zhang, D. Yu, Q. Tang, X. Deng, and H. Liu. 2012. Phylogenetic relationships of the Cobitoidea (Teleostei: Cypriniformes) inferred from mitochondrial and nuclear genes with analyses of gene evolution. Gene 508:60–72.

Maddison, W. P., and D. R. Maddison. 2018. Mesquite: a modular system for evolutionary analysis. Version 3.5. https://www.mesquiteproject.org

Nguyen, L-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32:268–274.

Quenouille, B., E. Bermingham, and S. Planes. 2004. Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 31:66–88.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61:539–542.

Šlechtová, V., J. Bohlen, and H. H. Tan. 2007. Families of Cobitoidea (Teleostei; Cypriniformes) as revealed from nuclear genetic data and the position of the mysterious genera *Barbucca*, *Psilorhynchus*, *Serpenticobitis* and *Vaillantella*. Molecular Phylogenetics and Evolution 44:1358–1365.