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A revision of the *Paracanthocobitis zonalternans* (Cypriniformes: Nemacheilidae) species complex with descriptions of three new species

RANDAL A. SINGER[1,2], JOHN M. PFEIFFER[1] & LAWRENCE M. PAGE[1]
[1]*Florida Museum of Natural History, Gainesville, FL 32611*
[2]*Corresponding author. E-mail: rsinger@flmnh.ufl.edu*

Abstract

The *Paracanthocobitis zonalternans* species complex is revised based on analysis of morphological and molecular data. Three new species, *P. nigrolineata, P. marmorata,* and *P. triangula* are described, and *P. phuketensis* is removed from synonymy. All species are described morphologically, geographic ranges are delimited, and relationships are discussed for those for which molecular data (*cytochrome c oxidase* subunit 1 - *COI*) are available. In view of the morphological similarities of some of the species, a surprising result of this study was the moderately large genetic distances among species. Uncorrected *COI* p-distances between geographic clades of *P. zonalternans* ranged from 7.6–9.3%, suggesting that the species are reproductively isolated from one another even though morphological changes are minor. *Paracanthocobitis phuketensis,* distributed in several rivers draining to the Gulf of Thailand and to the Andaman Sea, shows considerable intraspecific variation that should be explored in detail for historical and ecological explanations. *Cobitis chlorosoma* McClelland, 1839 from Assam, India, is a synonym of *P. botia*, not *P. zonalternans.*

Key words: Cypriniformes, Southeast Asia, zipper loaches, *Acanthocobitis*

Introduction

The zipper loaches (Nemachelidae: *Paracathocobitis* and *Acanthocobitis*) are a geographically widespread and species-rich group of fishes in Southeast Asia. Recent systematic studies on *Paracanthocobitis* and *Acanthocobitis* have dramatically improved our taxonomic understanding of the group and have resulted in a 3-fold increase in recognized species-level diversity (Kottelat 2012, Singer & Page 2015). At present, there are 14 recognized species of *Paracanthocobitis* ranging from the Indus drainage in Pakistan to the Mekong drainage of Cambodia and Laos. Although species boundaries within *Paracanthocobitis* have recently been more clearly delimited, uncertainties regarding the most diminutive representative of the group, *P. zonalternans* remain (Kottelat 1990, Singer & Page 2015). *Paracanthocobitis zonalternans* traverses several large drainages from India to Peninsular Malaysia including the Brahmaputra Meghna, Irrawaddy, Sittang, Salween and Mae Khlong. Our objective herein is to describe the taxonomic diversity found within populations currently identified as *P. zonalternans*.

Paracanthocobitis zonalternans was originally described as *Cobitis zonalternans* Blyth 1860, and has subsequently been assigned to *Nemacheilus* Bleeker, 1863, *Acanthocobitis* Peters, 1861, and *Paracanthocobitis* Grant, 2007. Two syntypes of *P. zonalternans* at ZSI were redescribed by Day (1869) and by Hora (1921), but subsequently were reported to be lost by Hora (1929). Kottelat (1990) selected a specimen (ZSM 27468) from the Salween drainage in Tak Province, Thailand, as a neotype noting that the population in the Salween is geographically proximal to the Tenasserim region from which Blyth (1860) described *P. zonalternans.*

Paracanthocobitis zonalternans is easily distinguishable from other species of *Paracanthocobitis* in being diminutive (less than 45 mm SL), having an incomplete lateral line (usually ending at or just beyond the dorsal-fin insertion), and having 10½ or fewer branched dorsal-fin rays (Singer & Page 2015). Kottelat (1990) described *P. zonalternans* as ranging from Manipur, India, to the Salween drainage of Thailand and south to peninsular Thailand close to the border of Malaysia, in the Brahmaputra, Chindwin, Sittang, Salween, Tapi, and Mae Khlong drainages, with remarks that it might also be in the Irrawaddy drainage. Singer & Page (2015) confirmed the species

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distribution in the Irrawaddy and added localities from the Meghna drainage. *Paracanthocobitis zonalternans* tends to avoid high gradient mountain streams and prefers shallow riffles with large pebbles and stones (Kottelat 1990), often with slower to moderate flow and with woody debris or leaf litter, at least in the Salween, Mae Khlong, and southern Thailand populations (Singer & Page 2015).

Kottelat (1990) noted that *P. zonalternans* populations varied in color pattern, presence or absence of an axillary pelvic lobe, depth of caudal peduncle, number of caudal and pelvic-fin rays, and position of the anus, but chose not to recognize taxonomic units due to what he hypothesized to be introgression of the various forms, and treated *Noemacheilus phuketensis* Klausewitz as a synonym of *P. zonalternans*. Kottelat (2012) hypothesized that several species were confused under the name *P. zonalternans*. Singer & Page (2015) suggested that rather than being a highly variable species distributed across several freshwater ecoregions, *P. zonalternans* was a collection of several more geographically restricted, taxonomically diagnosable populations. With more recent collections and morphological and molecular data, the *P. zonalternans* species complex herein is recognized as consisting of five species, three of which are new, bringing the total known species of *Paracanthocobitis* to 18. *Paracanthocobitis phuketensis*, previously considered a synonym of *P. zonalternans*, is removed from synonymy, and three new species, *P. triangula*, *P. marmorata* and *P. nigrolineata* are described from material previously identified as *P. zonalternans*.

Materials and methods

The mitochondrial protein-coding gene *cytochrome c oxidase* (*COI*) was used to reconstruct the phylogeny and measure genetic diversity in populations of the *P. zonalternans* species complex (Table 1). Fin clips were preserved in 95% ethanol, and DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Inc.). Primers for polymerase chain reaction (PCR) and sequencing were: FISH_BCL—TCAACYAATCAYAAAGATATYGGCAC and FISH_BCH—ACTTCYGGGTGRCCRAARAATCA (Baldwin *et al*. 2009). PCR was conducted using 25µl reactions using the following reagents and volumes: H_20 (16.75µl), 5X Mytaq Red Buffer (4µl) (Bioline Reagents Ltd.), primer (1µl@10mM), Taq (0.25ul@5U/µl), and template (1µl). Unpurified PCR product was sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida for bidirectional Sanger sequencing. Chromatograms were assembled and edited using Geneious v6.1.2 (Kearse *et al.* 2012).

Consensus sequences were aligned in Mesquite v3.04 (Maddison & Maddison 2015) using ClustalW (Larkin *et al*. 2007). The best-fit partitioning scheme and model of molecular evolution were selected via PartitionFinder v1.1.0 (Lanfear 2012) using BIC as the model selection metric, searching all possible partitioning schemes and each model of nucleotide evolution available in MrBayes v3.2.6 (Ronquist *et al*. 2012). Maximum Likelihood (ML) analyses were executed in RAxMLGUI v1.5 (Stamatakis 2014, Silvestro & Michalak 2012) using 100 tree inferences. Nodal support was measured using thorough bootstrapping and the MRE-based stopping criterion. Bayesian inference (BI) analyses were conducted in MrBayes v3.2.6 using the CIPRES Science Gateway (Miller *et al.* 2010). BI analyses were implemented using two runs of four chains for 24 X 10^6 generations sampling every 1000 trees and omitting the first 8000 as burn-in. Convergence of the two runs was monitored using the average standard deviation of split frequencies, the Potential Scale Reduction Factor (PSRF), and Effective Sample Size (ESS) of the parameters from the Markov chain Monte Carlo (MCMC) algorithm.

An S-H test (Shimodaria & Hasegawa 1999) and Bayes factors were used to test whether several topological constraints were significantly different from the optimal topology. Bayes factors were measured using two times the difference of the –ln likelihood (2lnBf) and interpreted following the methods of Kass & Raferty (1995) as modified by Nylander *et al.* (2004); i.e. 2lnBf 0–2: "not worth a bare mention", 2–6: "positive" support, 6–10: "strong" support, >10: "very-strong" support.

The haplotype network was generated in PopART using TCS (Clement *et al*. 2002) as the network inference method. Mean uncorrected p-distances were measured within and between groups of *P. zonalternans* using MEGA 7.0.16 (Kumar *et al*. 2016).

Fieldwork was conducted in Thailand using backpack electroshockers, seines, and dip nets. Specimens were euthanized in MS-222, preserved in 10% formalin, and transferred to 70% ethanol for storage. Live color photos were taken using a Canon Powershot digital camera.

TABLE 1. Information on samples of *Paracanthocobitis* included in the molecular analyses. GenSeq designations follow Chakrabarty et al. (2013).

Genus	Species	Catalog no. (UF unless listed)	Tissue sample no.	GenBank accession no.	GenSeq	Drainage
Paracanthocobitis	*nigrolineata*	NIFI 5080	2015–0384	MF289063	genseq-1	Mae Khlong
Paracanthocobitis	*nigrolineata*	188054	2015–0404	MF289065	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	188054	2015–0408	MF289066	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	188055	2015–0448	MF289067	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	188055	2015–0465	MF289068	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	188053	2015–0386	MF289064	genseq-2	Mae Khlong
Paracanthocobitis	*nigrolineata*	172979	2008–0589	MF289048	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	176389	2009–0637	MF289050	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	176411	2009–0696	MF289052	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	176449	2009–0673	MF289051	genseq-3	Mae Khlong
Paracanthocobitis	*nigrolineata*	181149	2011–0246	MF289053	genseq-3	Mae Khlong
Paracanthocobitis	*zonalternans*	188197	ICH–0038	MF289073	genseq-4	Salween
Paracanthocobitis	*phuketensis*	182833	2012–0132	MF289057	genseq-4	Phang–nga
Paracanthocobitis	*phuketensis*	183331	2012–0152	MF289058	genseq-4	Tapi
Paracanthocobitis	*phuketensis*	236043	2014–0389	MF289062	genseq-4	Kraburi
Paracanthocobitis	*phuketensis*	236051	2014–0380	MF289061	genseq-4	Panang Tak
Paracanthocobitis	*zonalternans*	188197	ICH–0033	MF289069	genseq-4	Salween
Paracanthocobitis	*zonalternans*	188197	ICH–0036	MF289070	genseq-4	Salween
Paracanthocobitis	*zonalternans*	188198	ICH–0081	MF289071	genseq-4	Salween
Paracanthocobitis	*zonalternans*	188198	ICH–0082	MF289072	genseq-4	Salween
Paracanthocobitis	*pictilis*	172927	2008–0624	MF289049	genseq-4	Ataran
Paracanthocobitis	*mandalayensis*	181110	2011–0271	MF289056	genseq-4	Wang
Paracanthocobitis	*mandalayensis*	181110	2011–0273	MF289055	genseq-4	Wang
Paracanthocobitis	*mandalayensis*	181110	2011–0277	MF289054	genseq-4	Wang
Paracanthocobitis	*maekhlongensis*	NIFI 4853	2012–0032	MF289060	genseq-1	Mae Khlong
Paracanthocobitis	*maekhlongensis*	182864	2012–0033	MF289059	genseq-2	Mae Khlong

Measurements and counts follow Kottelat (1990) and were taken on up to 10 specimens from each sample (specimens from one locality and date). The origin of a median fin is the anterior-most point at which the fin connects to the body. Insertion is the posterior-most point at which a median fin connects to the body or the point of connection to the body of a paired fin. Measurements were made to the nearest 0.1 mm using digital calipers and a Leica M8 dissection microscope. All measurements are in millimeters standard length (SL) unless indicated otherwise. Juveniles (< 15 mm) were excluded from counts and measurements because of potential ontogenetic variation. The number of dark bands on the caudal fin seems to increase ontogenetically in at least some species. However, there was not a sufficient number of juveniles to rigorously quantify this pattern. In Material Examined, the catalog number is followed by the number of specimens (ex. = examined), size range (mm SL), and locality with geographic coordinates. Species were recognized using the phylogenetic species concept (Cracraft 1987).

Morphometric (= mensural) data were analyzed using a sheared principal component analysis (PCA) in which the correlation matrix was factored (Humphries *et al.* 1981; Bookstein *et al.* 1985) to examine differences among populations. Differences among populations were then examined in Microsoft Excel 2013 by plotting the sheared second principal component of the meristic data against the sheared third principal component of the morphometric data.

Photographs were taken of preserved specimens using a Visionary Digital (Palmyra, Virginia) with Canon 40D and 5D cameras, and edited using Adobe Photoshop CS5.1. Geographic coordinates for specimen records lacking coordinates were estimated using maps and Google Earth. Maps were constructed using Arc GIS 3.3 and Adobe Photoshop CS3.

Specimens were examined from the following institutions; abbreviations follow Eschmeyer & Fricke (2015): Academy of Natural Sciences (ANSP), Philadelphia, U.S.A.; California Academy of Sciences (CAS), San Francisco, U.S.A.; The Field Museum of Natural History (FMNH), Chicago, U.S.A; Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, U.S.A.; National Inland Fisheries Institute (NIFI), Bangkok, Thailand; Royal Ontario Museum (ROM), Department of Natural History, Toronto, Canada; Senckenberg Forschungsinstitut und Naturmuseum (SMF), Frankfurt, Germany; Florida Museum of Natural History (UF), Gainesville, U.S.A.; University of Michigan Museum of Zoology (UMMZ), Ann Arbor, U.S.A.; Smithsonian Institution National Museum of Natural History (USNM), Washington D.C., U.S.A.; Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, Singapore; Zoological Museum Amsterdam (ZMA), Leiden, Netherlands. Requests to examine specimens from the Naturhistoriska Riksmuseet, Stockholm, Sweden (NRM) and the Zoological Survey of India, Kolkata, India (ZSI), were declined.

Results

A *COI* matrix of three genera and 28 terminals was generated. The *COI* alignment contained no stop codons or indels, and the average sequence length was 645 nt. *Paracanthocobitis zonalternans* individuals were from the Salween (n=5), Mae Khlong (n = 11), and four drainages in peninsular Thailand: Panang Tak, Kraburi, Thung Maphrao, and Tapi (n = 1 from each). Molecular data included sequences from the type locality of *P. zonalternans* and from several localities near the type locality of *P. phuketensis*. *Schistura fasciolata* (JN177233-Liu *et al.* 2012) and *Nemacheilus pallidus* (MF289074; UF 176446) were used as an outgroup.

The best-fit partition scheme divided the dataset into two partitions: Partition 1—*COI* codon positions 1+2; Partition 2—codon position 3. The best-fit models of molecular evolution for each partition (Partition 1= SYM+I; Partition 2 = GTR+GAMMA) were implemented in the BI analyses. ML analyses used GTR+GAMMA for each partition given the models available in RAxML and the recommendations in the program manual. Convergence of the BI runs was supported by the average of the standard deviation of split frequencies (0.002), average PSRF value (1.000), and high ESS values (>9000) for all parameters of the MCMC.

The optimal topology (i.e., with the greatest likelihood) was generated using BI (Table 2) and resolved three geographic clades within the *P. zonalternans* species complex. The ML reconstruction resolved a similar topology to the BI reconstruction except that *P. phuketensis* was sister to *P. nigrolineata,* and *P. zonalternans* was resolved as paraphyletic with respect to *P. nigrolineata* and *P. phuketensis*. However, constraining *P. zonalternans* as monophyletic resulted in a topology that was not significantly different (S-H Test: >.05) from the unconstrained analysis (Table 2). BI analysis requiring the non-monophyly of *P. zonalternans* (i.e., a negative constraint) resolved

a topology identical to the unconstrained ML analysis and was significantly worse than the optimal BI topology (2lnBF=6.9).

TABLE 2. Statistical comparisons of topological constraints and the optimal topologies generated in ML and BI reconstructions.

	ML			BI		
	–Lnl	p–value	Worse?	–Lnl	BF	Worse?
Unconstrained topology	–2606.28	–	–	–2427.07	–	–
P. zonalternans monophyletic	–2605.78	>0.05	No	NA	NA	NA
P. zonalternans not monophyletic	NA	NA	NA	–2430.52	6.9	Yes

Individuals of the *P. zonalternans* species complex were resolved in three primary geographic lineages (Fig. 1): the Salween clade, Mae Khlong clade, and peninsular Thailand clade. These clades have strong (Mae Khlong and peninsular Thailand) or moderate (Salween clade) molecular support (Fig. 1) and show high between-clade sequence divergence (Table 3). Data on individuals from the type locality of *P. zonalternans* and near-topotypic material for *P. phuketensis* allow unambiguous assignment of these names to the Salween and peninsular Thailand clades, respectively. The Mae Khlong clade represents a new species, described herein.

The *COI* haplotype network depicted three distinct clusters corresponding to individuals from the Salween drainage (*P. zonalternans*), peninsular Thailand (*P. phuketensis*), and the Mae Khlong drainage (*P. nigrolineata*) (Fig. 2). The three clusters are separated by a minimum of 26 *COI* nucleotide substitutions. Mean *COI* p-distances within and between the three species are reported in Table 3.

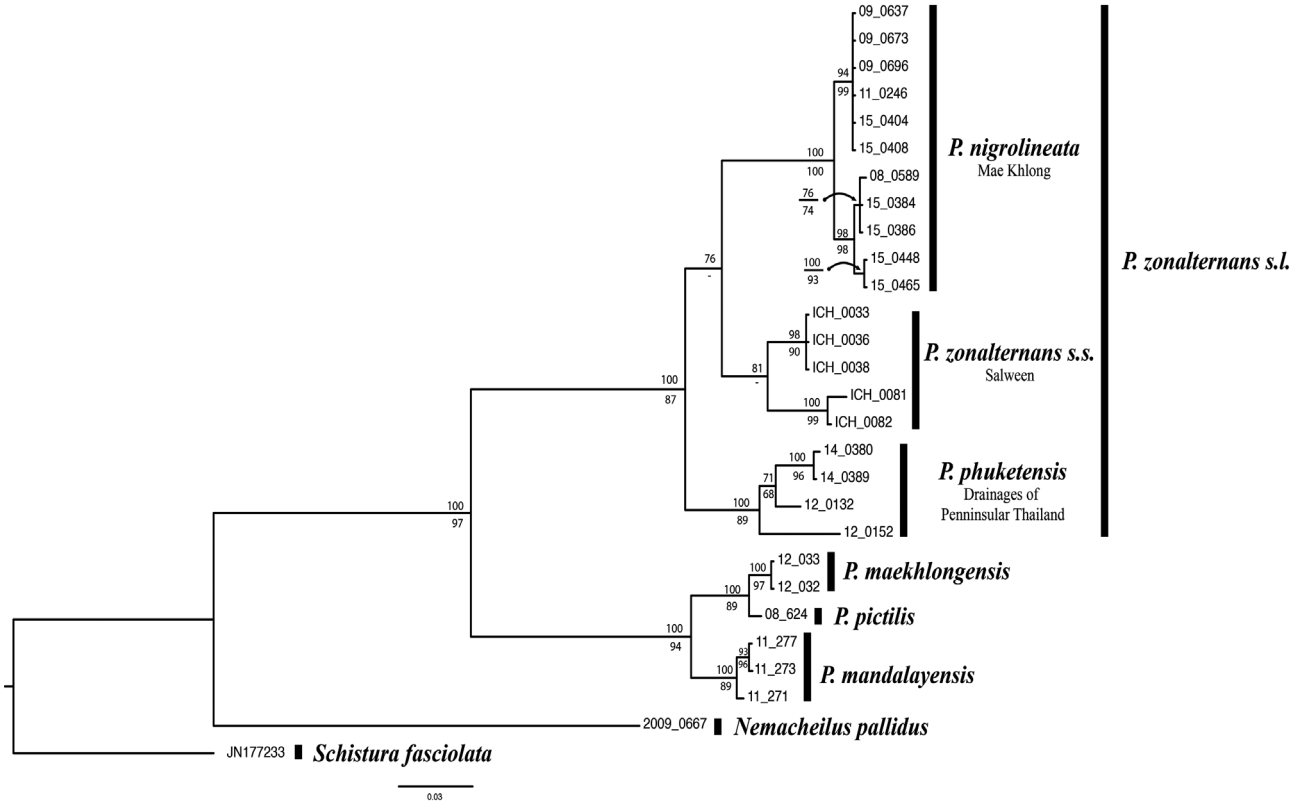

FIGURE 1. Most likely topology generated in the BI reconstruction. Values above and below branches correspond to BI posterior probabilities and ML bootstrap support, respectively. Drainages are provided for clades in the *P. zonalternans* species complex.

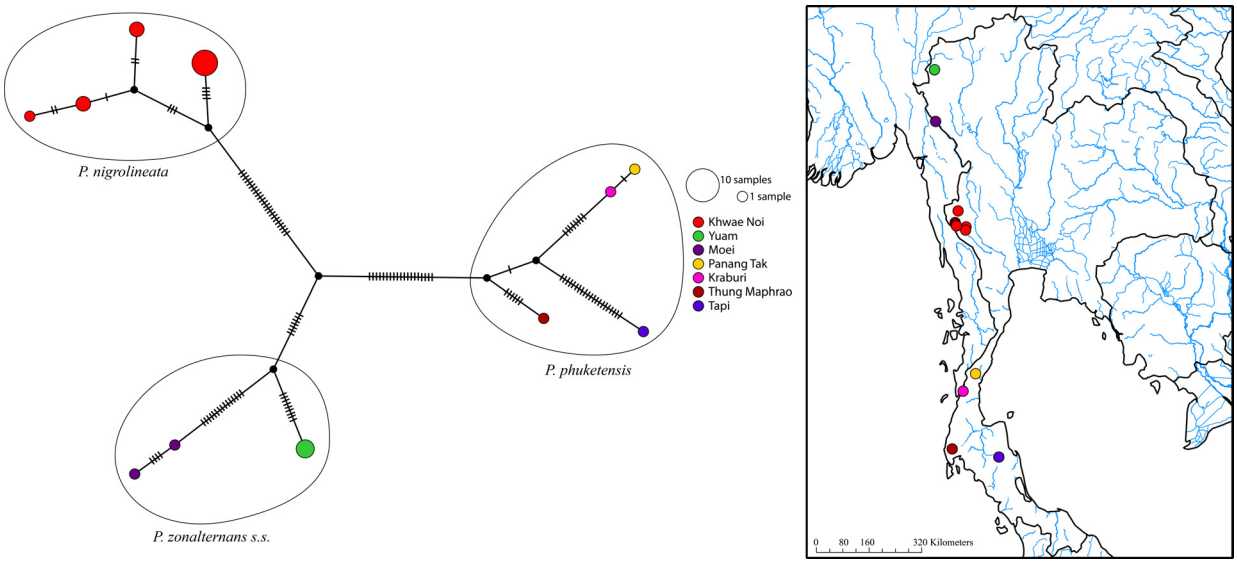

FIGURE 2. Haplotype network and map of localities of the 20 individuals of the *P. zonalternans* species complex included in the analyses.

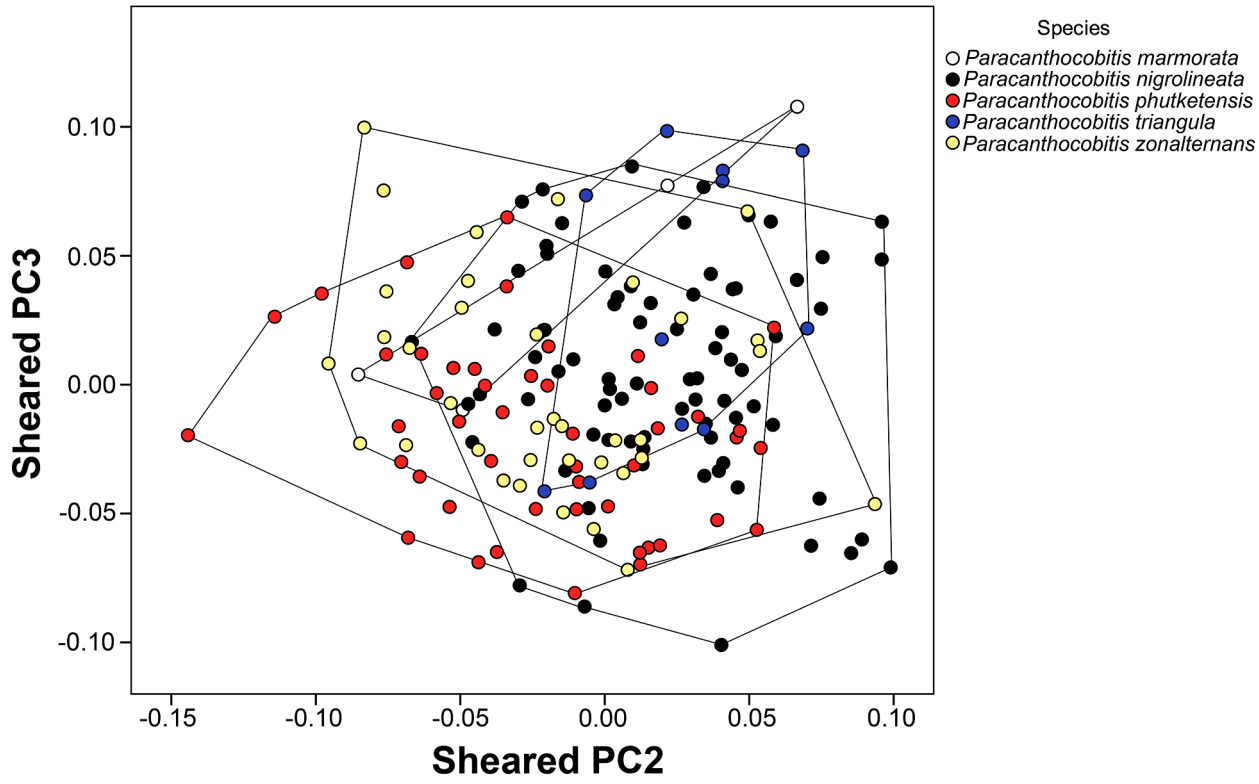

FIGURE 3. Plot of the sheared second principal component (PC2) of the meristic data and sheared third principal component (PC3) of morphometric data on specimens of the *P. zonalternans* species complex.

Two hundred and four individuals were examined for morphological variation, including type specimens of *P. zonalternans* and *P. phuketensis*. An analysis of 11 morphometric and seven meristic characters (Tables 4 and 5) was used to differentiate the species recognized herein. Lateral pigmentation, coloration on pectoral-fin base, shape of black spot on upper margin of caudal-fin base, axillary pelvic lobe presence or absence, and number of caudal-fin rays proved to be the most informative morphological characters to distinguish species. Fin-ray counts were mostly conserved across species. A combination of morphological characters was required to distinguish most species, which may explain why these species were not diagnosed in previous studies.

TABLE 3. Mean *COI* p-distances within and between groups of the *P. zonalternans* species complex.

Species	Within Group Mean p–distance (%)	Between Group Mean p–distance (%)		
		P. zonalternans	*P. phuketensis*	*P. nigrolineata*
P. zonalternans	2.61	–		
P. phuketensis	3.29	7.88	–	
P. nigrolineata	0.88	7.59	9.26	–

Principal component analysis (Fig. 3) of the morphometric data show substantial overlap in characters. As a result, the PCA failed to separate species based on clustering. This was indicative of low variation in overall body shape as well as overlap in meristic counts among the populations sampled. As a result, a combination of morphological characters and genetic data was used to separate species.

***Paracanthocobitis zonalternans* (Blyth, 1860)**

Dwarf Zipper Loach

Fig. 4

Cobitis zonalternans Blyth, 1860:172. Type locality: Tenasserim provinces, Burma. Two syntypes (Day, 1869:551; Day, 1872:186), presumed lost (Hora, 1929:319). Neotype, ZSM 27468, designated by Kottelat, 1990:42, Huai Mae Charno, 4 km south Amphoe Mae Ramat on road 1085 [approximately 16.967N, 98.567E], Tak Province, Salween drainage, Thailand.

Nemacheilus zonalternans.—Day, 1869:551.

Nemachilus zonalternans.—Day, 1889:232.

Noemacheilus zonalternans.—Sen, 1985:119, fig. 63.

Acanthocobitis zonalternans.—Kottelat, 1990:13.

Acanthocobitis (Paracanthocobitis) zonalternans.—Grant, 2007:3.

Paracanthocobitis zonalternans.—Singer & Page, 2015:396.

Diagnosis. *Paracanthocobitis zonalternans* is distinguished from all other species of *Paracanthocobitis* by combination of incomplete lateral line ending near dorsal-fin insertion; 9½–10½ branched dorsal-fin rays; axillary pelvic lobe present; series of black blotches, usually without black stripe but with faint dusky stripe, along side of body, usually ending near dorsal-fin insertion; circular black spot on upper margin of caudal-fin base; black pigment extending from lateral stripe onto pectoral-fin base; no marmorated pattern anterior to dorsal-fin origin; dorsal saddles short, rarely connecting to lateral blotches; 8 (occasionally 7) branched upper caudal-fin rays.

Description. Meristic and morphometric data are in Tables 4 and 5. Body deepest just anterior to dorsal-fin origin; slightly compressed before dorsal fin, strongly compressed postdorsally. Head depressed, snout gently rounded to slightly pointed when viewed dorsally or laterally. Dorsal-fin origin far in front of pelvic-fin origin; distal margin convex. Pectoral fin 0.17–0.26 SL, not reaching pelvic fin; pelvic fin 0.16–0.22 SL, not reaching anal fin; caudal fin emarginate.

Lateral line incomplete, reaching at most to dorsal-fin insertion; 19–50 pores. Axillary pelvic lobe present. Mouth arched with heavily papillated lips; upper lip with 3–5 rows of papillae, continuous with large pads on lower lip. Three pairs of barbels; inner rostral barbel extends to or slightly past base of maxillary barbel, maxillary barbel and outer rostral barbel extend to or slightly past eye. Body covered with scales; 9½ (12 specimens)–10½ (43 specimens) branched dorsal-fin rays; 9–12, usually 10, pectoral-fin rays; 6 pelvic-fin rays; 5½ branched anal-fin rays; 8 (occasionally 7) branched upper caudal-fin rays; 8 branched lower caudal-fin rays. Suborbital flap in male. Maximum SL 41.8 mm female.

Body yellow-brown with 10–15 dark brown to black blotches along lateral line sometimes overlain with faint dusky stripe usually ending near dorsal-fin insertion; 14–18 small dark brown dorsal saddles. Dark spots and blotches on head; uninterrupted black bar from eye to tip of snout. Ocellus with black center near upper margin of caudal peduncle; 3–6 concentric rows of dark spots in dorsal fin, 2–3 rows of dark spots in anal fin, 4–6 dark bands on caudal fin. Dusky lateral stripe extending onto pectoral-fin origin.

TABLE 4. Morphometric data for species of the *P. zonalternans* complex. Numbers in parentheses numbers of specimens.

Species	*P. zonalternans* (n=55)	x̄±SD	*P. phuketensis* (n=55)	x̄±SD	*P. marmorata* (n=4)	x̄±SD	*P. triangula* (n=11)	x̄±SD	*P. nigrolineata* (n=79)	x̄±SD
Standard length	20.5–41.8		17.1–33.3		28.2–33.9		24.6–29.2		21.5–42.4	
% SL										
Pre-dorsal length	0.42–0.50	0.5 ± 0.02	0.39–0.48	0.5 ± 0.02	0.43–0.49	0.5± 0.03	0.43–0.48	0.5 ± 0.01	0.42–0.50	0.5 ± 0.02
Head length	0.17–0.25	0.2 ± 0.02	0.16–0.22	0.2 ± 0.01	0.20–0.22	0.2 ± 0.01	0.19–0.22	0.2 ± 0.01	0.17–0.25	0.2 ± 0.02
Snout length	0.07–0.11	0.1 ± 0.01	0.07–0.10	0.1 ± 0.01	0.07–0.10	0.1 ± 0.02	0.07–0.10	0.1 ± 0.01	0.06–0.11	0.1 ± 0.01
Pre-pelvic length	0.47–0.56	0.5 ± 0.02	0.47–0.54	0.5 ± 0.01	0.51–0.55	0.5 ± 0.02	0.50–0.53	0.5 ± 0.01	0.48–0.56	0.5 ± 0.02
Pre-anal length	0.73–0.81	0.8 ± 0.02	0.73–0.81	0.8 ± 0.02	0.76–0.78	0.8 ± 0.01	0.77–0.80	0.8 ± 0.01	0.74–0.81	0.8 ± 0.01
Body depth	0.14–0.25	0.2 ± 0.02	0.14–0.22	0.2 ± 0.02	0.17–0.18	0.2 ± 0.03	0.14–0.17	0.2 ± 0.01	0.13–0.25	0.2 ± 0.02
Caudal-peduncle depth	0.08–0.14	0.1 ± 0.01	0.09–0.14	0.2 ± 0.01	0.11–0.12	0.1 ± 0.01	0.09–0.12	0.1 ± 0.01	0.08–0.14	0.1 ± 0.02
Pectoral-fin length	0.17–0.26	0.2 ± 0.02	0.18–0.28	0.2 ± 0.02	0.19–0.22	0.2 ± 0.01	0.18–0.23	0.2 ± 0.02	0.19–0.26	0.2 ± 0.02
Pelvic-fin length	0.16–0.22	0.2 ± 0.01	0.16–0.22	0.2 ± 0.01	0.15–0.17	0.2 ± 0.01	0.16–0.19	0.2 ± 0.01	0.15–0.23	0.2 ± 0.02
Eye diameter	0.26–0.38	0.3 ± 0.03	0.26–0.43	0.3 ± 0.04	0.28–0.32	0.3 ± 0.02	0.32–0.37	0.4 ± 0.02	0.26–0.42	0.3 ± 0.03
Interorbital width	0.27–0.43	0.4 ± 0.03	0.29–0.46	0.4 ± 0.04	0.32–0.40	0.4 ± 0.04	0.29–0.36	0.3 ± 0.02	0.26–0.41	0.3 ± 0.03

TABLE 5. Meristic data for species of the *P. zonalternans* complex. Numbers in parentheses numbers of specimens.

Species	*P. zonalternans* (n=55)	mode	*P. phuketensis* (n=55)	mode	*P. marmorata* (n=4)	mode	*P. triangular* (n=11)	mode	*P. nigrolineata* (n= 79)	mode
Dorsal rays	9½–10½	10½	9½–10½	10½	9½	9½	9½–10½	10½	9½–10½	10½
Pectoral rays	9–12	10	9–10	9	9–10	10	9–10	9	9–13	10
Pelvic rays	6	6	6	6	5(1)–6	6	6	6	6	6
Anal rays	5–6(1)	5	5	5	5	5	5	5	5	5
Upper branched caudal-fin rays	7(2)–8	8	7–8	8	8	8	8	8	8	8
Lower branched caudal-fin rays	8	8	8	8	8	8	8	8	8	8
Lateral–line pores	19–50	33	20–48	31	25–31	28	20–37	26	21–56	37

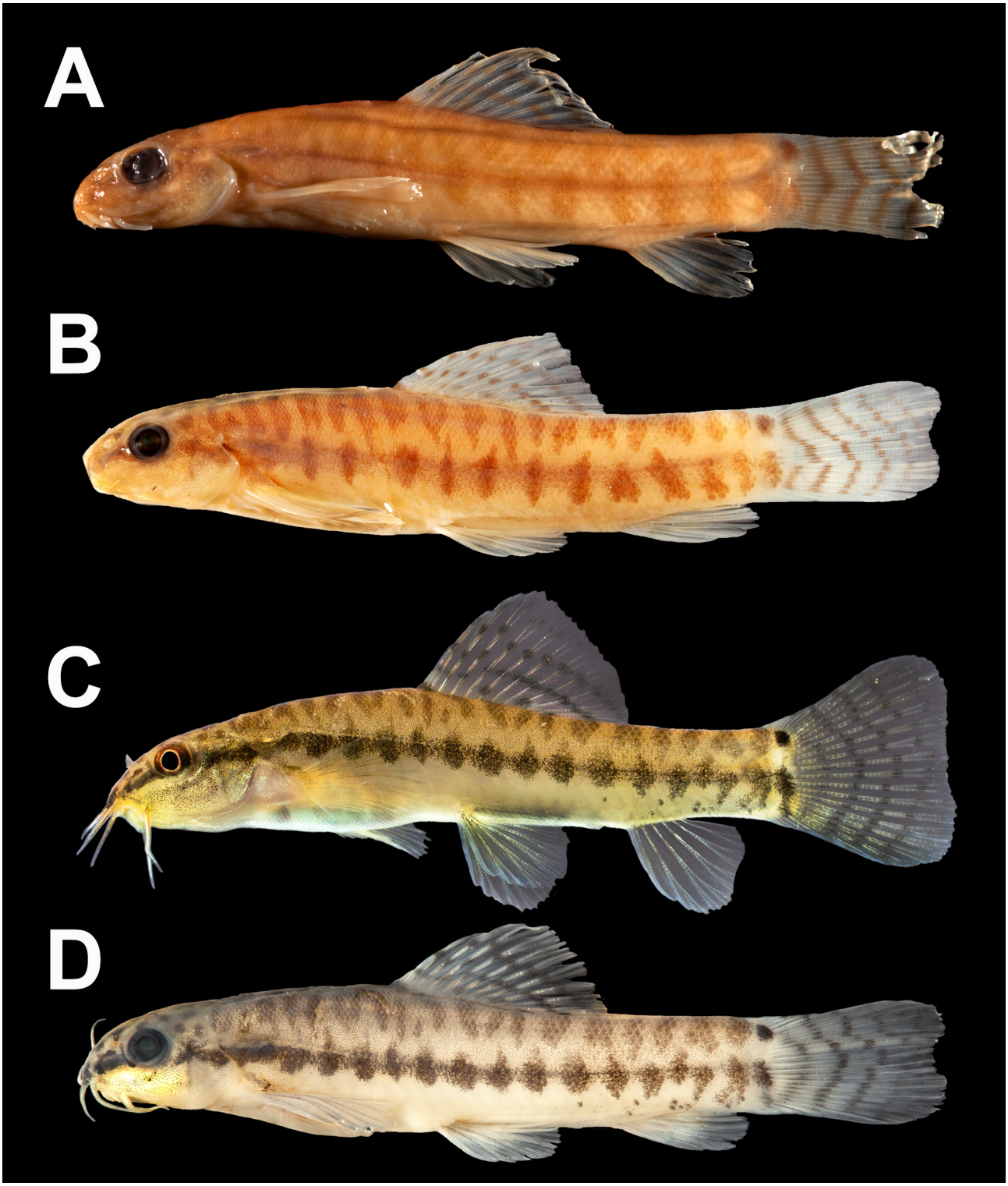

FIGURE 4. *Paracanthocobitis zonalternans* from the Salween drainage, Thailand. (A) ZSM 27468, neotype of *P. zonalternans,* Tak Province, 33.1 mm SL; (B) ZRC 41258, Tak Province, 36.6 mm SL; UF 188197, Mae Hong Son Province, live (C), and preserved (D), 29.5 mm SL. Live photo by Z. S. Randall (UF).

Comparisons. *Paracanthocobitis zonalternans* differs from all other species of *Paracanthocobitis* except *P. phuketensis, P. nigrolineata*, *P. triangula*, and *P. marmorata* in having an incomplete lateral line ending near the dorsal-fin insertion (vs. lateral line to the end of the caudal peduncle) and 9½–10½ (vs. >10½) branched dorsal-fin rays. *Paracanthocobitis zonalternans* is most similar morphologically to *P. nigrolineata* but differs in having a series of black blotches, usually black stripe along the side of the body absent or dusky and ending near the dorsal-

fin insertion (vs. having a series of black blotches overlain by a black stripe ending near the caudal fin), and sometimes having 7 (vs. always 8) upper branched caudal-fin rays. *Paracanthocobitis zonalternans* differs from *P. phuketensis* in having black pigment extending from a black lateral stripe onto the pectoral-fin base, an axillary pelvic lobe (vs. lobe usually absent, occasionally rudimentary) and in having short (not extending ventrally to dorsal stripe) dorsal saddles in the majority of specimens that rarely connect to lateral blotches (vs. dorsal saddles longer than interspaces, frequently connecting to lateral blotches). *Paracanthocobitis zonalternans* differs from *P. triangula* in having a circular (vs. triangular) black spot on the upper margin of the caudal-fin base and in having black pigment extending from the black blotches along the side of the body onto the pectoral-fin base. *Paracanthocobitis zonalternans* differs from *P. marmorata* in having an axillary pelvic lobe and a circular (vs. tear-drop shaped) black spot on the upper margin of the caudal-fin base and in not having a marmorated pattern between the dorsal saddles and lateral blotches.

Distribution. *Paracanthocobitis zonalternans* is known from the Salween River drainage in Thailand and Myanmar (Fig. 5).

Material Examined. Thailand: Salween drainage: CAS 35775, 6, 23.5–42.6 mm, Nam Mesarieng, near Mesarieng (Mae Sariang), northern Thailand, 18.151N, 97.934E; CAS 35776, 2, 41.3–41.8 mm, same locality as CAS 35775; CAS 36042, 2, 38.8–40.8 mm, same locality as CAS 35775; FMNH 124763, 4, 19.6–27.7 mm, Nam Mae Yuan River, rte. 108, 103 km N of Mae Sariang, 18.807N, 97.931E; FMNH 124764, 1, 29.1 mm, Mae Samat River, rte. 108, 15 km S of Mae Hong Son, 19.189N, 97.984E; NIFI 00874, 14, 28.0–32.5 mm, Tak Province, Huai Mae Kamui, Amphoe Tha Song Yang, 17.230N, 98.229E; NIFI 6393, 1, 35.7 mm, Mae Hong Son Province, Mae Sanga River, 18.238N, 97.961E; ROM 51139, 5, 23–31.9 mm, Tak Province, N of Mae Charao, 16.967N, 98.567E; UF 188197, 7, 20.5–29.5 mm, Mae Hong Son Province, Khun Yuam County, Pai River near Surin River, approx. 1.5 km N of Ban Mae Surin on rte. 108, 18.928N, 97.937E; UF 188198, 12, 23.3–35.7 mm, Tak Province, Amphoe Tha Song Yang, Moei River at rte. 105, approx. 7 km SE of Nam Tha Song Yang, 17.516N, 97.966E; USNM 288452, 26 (10 ex.) 33.5–42.4 mm, Mae Hong Son Province, mountain stream, 5 km W of Mae Sariang, 18.191N, 97.878E; ZRC 41258, 8, 26.5–36.6 mm, Tak Province, Mae Nam Moi, S of Phop Phra, 16.290N, 98.706E; ZSM 27468, neotype of *Cobitis zonalternans*, 33.1 mm, Huay Me Charno, 4 km S of Amphoe Mae Romat on road 1085.

***Paracanthocobitis phuketensis* (Klausewitz, 1957)**
Phuket Zipper Loach
Figs. 6 & 7

Noemacheilus phuketensis Klausewitz, 1957:195, fig. 1. Type locality: Phuket Island, Thailand.
Holotype: SMF 3966.
Acanthocobitis phuketensis.—Bănărescu & Nalbant, 1964:160.
Noemacheilus (Acanthocobitis) phuketensis.—Bănărescu & Nalbant, 1968:322.

Diagnosis. *Paracanthocobitis phuketensis* is distinguished from all other species of *Paracanthocobitis* by combination of incomplete lateral line ending near dorsal-fin insertion; 9½–10½ branched dorsal-fin rays; axillary pelvic lobe usually absent, occasionally rudimentary; series of black blotches, sometimes overlain with faint dusky stripe along side of body not obscuring lateral blotches, ending near caudal-fin; circular black spot on upper margin of caudal-fin base; black pigment not extending from black blotches along side of body onto pectoral-fin base; no marmorated pattern anterior to dorsal-fin origin; dorsal saddles longer than interspaces, frequently connecting to lateral blotches; 7–8 branched upper caudal-fin rays.

Description. Meristic and morphometric data are in Tables 4 and 5. Body deepest just anterior to dorsal-fin origin; slightly compressed before dorsal fin, strongly compressed postdorsally. Head depressed, snout gently rounded to slightly pointed when viewed dorsally or laterally. Dorsal-fin origin far in front of pelvic-fin origin; distal margin convex. Pectoral fin 0.18–0.28 SL, not reaching pelvic fin; pelvic fin 0.16–0.22 SL, not reaching anal fin; caudal fin emarginate. Lateral line incomplete, reaching just beyond dorsal-fin insertion; 20–48 pores. Axillary pelvic lobe usually absent, occasionally rudimentary. Mouth arched with heavily papillated lips; upper lip with 3–5 rows of papillae, continuous with large pads on lower lip. Three pairs of barbels; inner rostral barbel extends to or slightly past base of maxillary barbel, maxillary barbel and outer rostral barbel extend to or slightly past eye. Body covered with scales; 9½ (5 specimens)–10½ (50 specimens) branched dorsal-fin rays; 9–10, usually 9, pectoral-fin

rays; 6 pelvic-fin rays; 5½ branched anal-fin rays; 7–8 branched upper caudal-fin rays; 8 branched lower caudal-fin rays. Suborbital flap in male. Maximum SL 33.3 mm female.

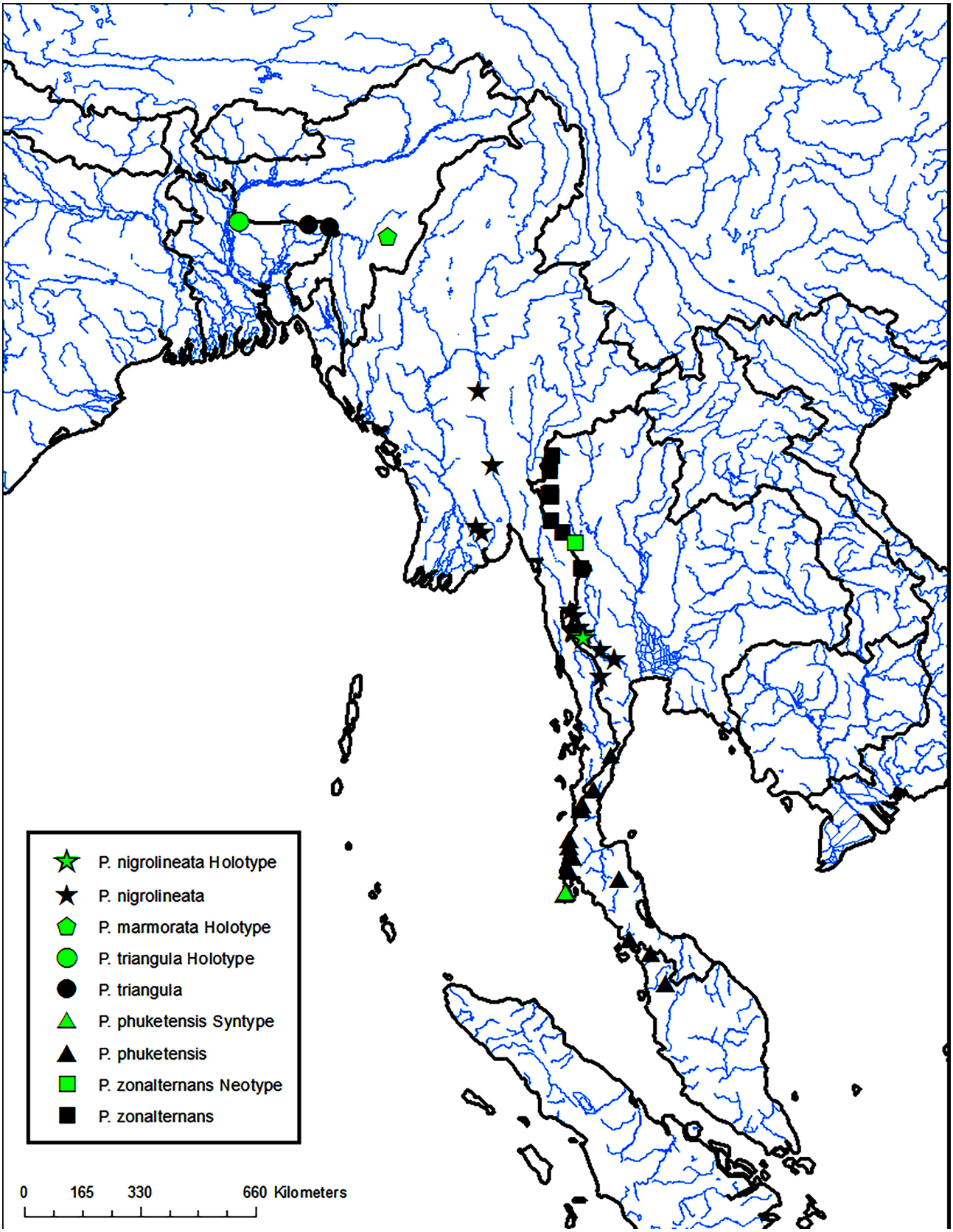

FIGURE 5. Localities of examined specimens of *Paracanthocobitis*.

FIGURE 6. Holotype of *Paracanthocobitis phuketensis,* SMF 3966, Phuket Island, Thailand, 29.4 mm SL. Photo by F. Wicker (SMF).

Body yellowish-brown with 10–12 dark brown to black blotches along lateral line sometimes overlain with faint dusky stripe usually ending near caudal fin; 12–15 small dark brown dorsal saddles; dorsal saddles longer than interspaces, frequently connecting to lateral blotches. Dark spots and blotches on head; uninterrupted black bar from eye to tip of snout. Ocellus with black center near upper margin of caudal peduncle; 3–6 concentric rows of dark spots in dorsal fin, 2–3 rows of dark spots in anal fin, 4–6 dark bands on caudal fin. Dusky lateral stripe not extending onto pectoral-fin origin.

Comparisons. *Paracanthocobitis phuketensis* differs from all other species of *Paracanthocobitis* except *P. zonalternans, P. nigrolineata*, *P. triangula*, and *P. marmorata* in having an incomplete lateral line ending near the dorsal-fin insertion (vs. lateral line to the end of the caudal peduncle) and 9½–10½ (vs. >10½) branched dorsal-fin rays. *Paracanthocobitis phuketensis* differs from *P. zonalternans* in not having black pigment extending from a black lateral stripe onto the pectoral-fin base; axillary pelvic lobe usually absent, occasionally rudimentary (vs. always present); and in having dorsal saddles longer than interspaces that frequently connect to lateral blotches vs. short (not extending ventrally to dorsal stripe) dorsal saddles that rarely connect to lateral blotches. *Paracanthocobitis phuketensis* differs from *P. nigrolineata* in not having a distinct black stripe along the side of the body obscuring the lateral blotches and black pigment extending from the lateral stripe onto the pectoral-fin base; and axillary pelvic lobe usually absent, occasionally rudimentary (vs. always present). *Paracanthocobitis phuketensis* differs from *P. triangula* in having a circular (vs. triangular) black spot on the upper margin of the caudal-fin base, and axillary pelvic lobe usually absent, occasionally rudimentary (vs. always present). *Paracanthocobitis phuketensis* differs from *P. marmorata* in having a dusky lateral stripe along the side of the body (vs. no stripe); axillary pelvic lobe usually absent, occasionally rudimentary (vs. always absent); a circular (vs. tear-drop shaped) black spot on the upper margin of the caudal-fin base; and in not having a marmorated pattern between the dorsal saddles and lateral blotches. *Paracanthocobitis phuketensis* from Phuket Island and Phang-Nga river drainages further differs from *P. nigrolineata, P. triangula*, and *P. marmorata* in sometimes having 7 (vs. always 8) upper branched caudal-fin rays.

Distribution. *Paracanthocobitis phuketensis* is known from Peninsular Thailand in the Panang Tak drainage and on Phuket Island, south to the Perlis, Padang Sarai, and Ketil drainages of Peninsular Malaysia (Fig. 5).

Material Examined. Malaysia: Malay Peninsula: MCZ 47226, 1, 26.9 mm, Satun Malay Peninsula, Boripatra River, on road from Satun to Hat Tai., 6.815N, 99.974E; ZRC 41103, 1, 17.1 mm, Perlis Sintok-Pdg., Senai Road, 6.456N, 100.491E; ZRC 46671, 3, 22.9–27.8 mm, Kedah Kg. Tandop about 6–7 mi from Baling on road to Weng, 5.665N, 100.901E. Thailand: Phang-Nga drainage: UF 182833, 4, 23.6–27.3 mm, near Phang Nga, off rte. 4090, 8.571N, 98.418E; Tapi (Tapee) drainage: UF 183331, 2, 31.3–33.3 mm, Nakhon Si Thammarat Province, near rte. 4015, 8.349N, 99.692E; NIFI 02181, 2, 27.0–27.1 mm, Surat Thani Province, Suratthani Tapi (Tapee) drainage, Khlong Sok at Ban Kheng Sok, 8.907N, 98.522E; Kraburi drainage: UF 236043, 16 (10 ex.), 23.3–31.3 mm, Ranong, Kraburi River at Khlong Bangsikim, trib. of Laoun River, km 3/537 near Ban Kreo Noi, 10.1505N,

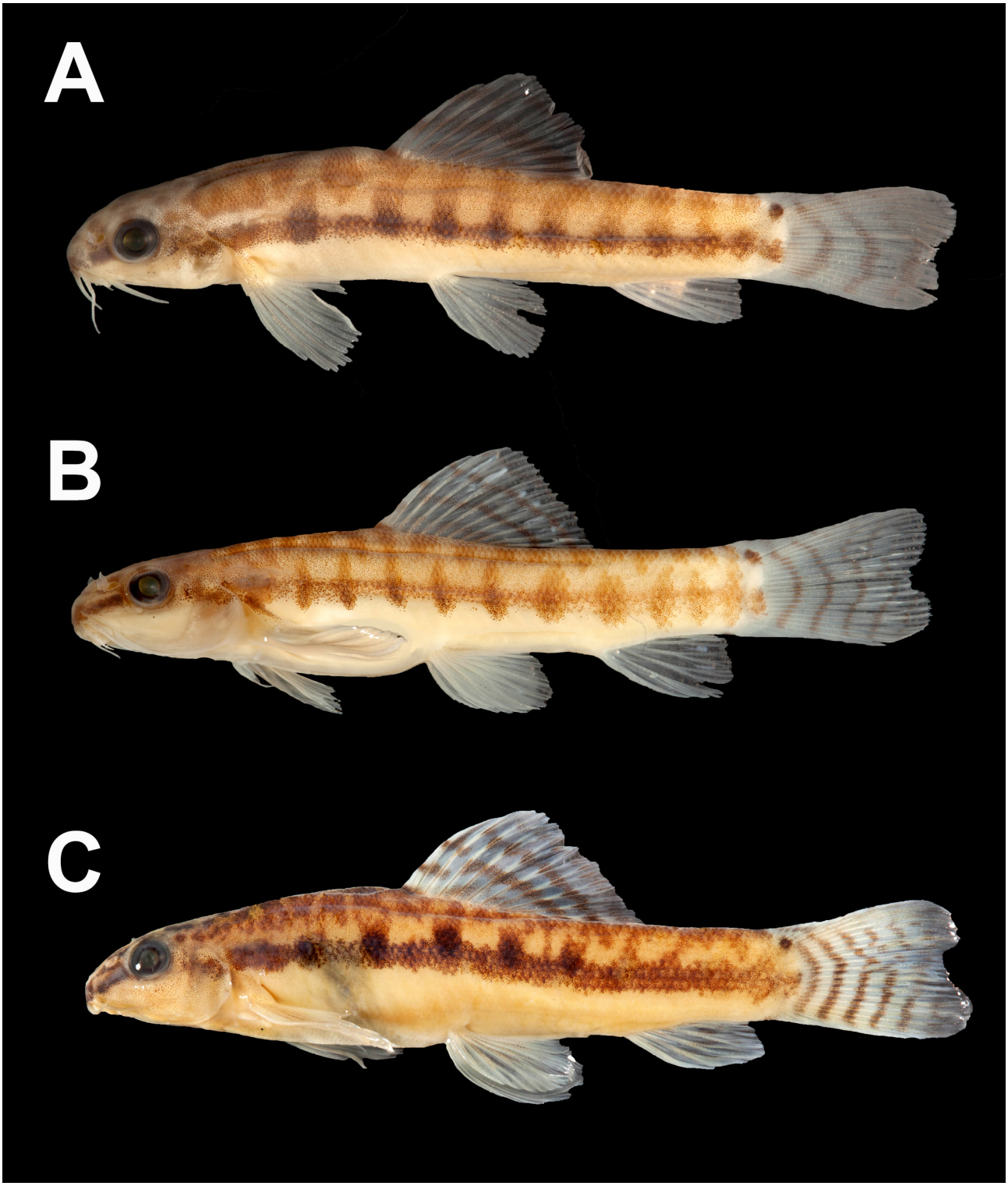

FIGURE 7. Geographic variation in color pattern in *Paracanthocobitis phuketensis*. (A) ZRC 45717, Phuket Island, Thailand, 26.7 mm SL; (B) ZRC 46671, Nahkon Si Thammarat Province, Thailand, 27.8 mm SL; (C) ZRC 41974, Ranong Province, Thailand, 36.4 mm SL.

98.717E; ZRC 42198, 3, 24.7–30.9 mm, Ranong Province, Khlong Phrae, Sai Ban Kraeo Noi (Bang Kaeo), km 8 on road branching E, 32 km of Ranong on rte to Kra Buri, 10.184N, 98.720E; Songkhla drainage: ZRC 41974, 5 (1 adult, 4 juveniles), 36.4 mm, Ranong King, Amphae Suk Sam Lan (Ran), Ton Koi Waterfall, 9.353N, 98.430E; ZRC 42184, 8 (5 adult, 3 juveniles), 18.5–23.1 mm, Ranong stream, N of Khura Buri, 100 km S of Ranong, 9.199N, 98.413E; Panang Tak drainage: UF 236051, 4, 27.9–32 mm, Chumphon Province, Tha Sae Co., Panang

Tak River at Khlong Rap Ro, 10.627N, 99.057E. Phuket Island: FMNH 124765, 2, 25.5–28.4 mm, Klong Vang In Nevi, (trib., Kra River), rte. 4, 18 km from Kra Buri (52 km SW of Chumphon), 10.272N, 98.758E; FMNH 124766, 1, 29.0 mm, Klong Chuitt, rte. 4090, 18 km NW of Pling, 8.588N, 98.398E; ROM 48972, 2, 27.7–31.3 mm, Ranong Province, N of Bang Kaeo, off highway 4 close to border of Burma, 10.250N, 98.750E; SMF 3966, 29.4 mm, holotype of *Noemacheilus phuketensis*, Phuket Island, off west coast of south peninsula Thailand, 7.992N, 98.343E; SMF 3967, 22.2 mm, paratype of *Noemacheilus phuketensis*, same locality as SMF 3966; SMF 4244, 37.5 mm, paratype of *Noemacheilus phuketensis*, same locality as SMF 3966; UMMZ 238962, 2, 22–24.4 mm, Phuket, Nam Tok Ton Sai, 8.026N, 98.363E; ZRC 45717, 8, 21–26.7 mm, Phuket, 7.957N, 98.317E. Gulf of Thailand: ZRC 42016, 1, 23.6 mm, Prachaup Khiri Khan Province, Thaup Sakae District, W of Ang Thong, 11.449N, 99.483E.

***Paracanthocobitis nigrolineata*, new species**
Blacklined Zipper Loach
Figs. 8 & 9

Holotype. NIFI 5080, 33.1 mm SL, Thailand, Kanchanaburi Province, Thong Pha Phum, Mae Khlong drainage, unnamed tributary of Khwae Noi just S of rte. 323, 14.540N, 98.780E, 27 January 2015.

Paratypes. Thailand, Kanchanaburi Province: ANSP 179829, 2, 35.6–36 mm SL, Ulong River, tributary of Khwae Noi, at rte. 323 bridge, 5–10 km from Thong Pha Phum, 14.782N, 98.669E, 4 March 2001; NIFI 3087, 4, 27–29.4 mm SL, Thong Pha Phum, Huay Ulong, 14.760N, 98.638E, no date; UF 188053, 9, 24.9–35.8 mm SL, same locality as holotype, 27 January 2015; UMMZ 209461, 7, 29.9–35.5 mm SL, Khwae Yai, 14.227N, 99.234E, 28 February 1975; Ratchaburi Province: ZRC 42002, 2, 29.7–42.4 mm SL, Suan Pheng District, Suan Pheng Waterfall, 13.524N, 99.239E, no date.

Diagnosis. *Paracanthocobitis nigrolineata* is distinguished from all other species of *Paracanthocobitis* by combination of incomplete lateral line ending near dorsal-fin insertion; 9½–10½ branched dorsal-fin rays; axillary pelvic lobe present; black stripe along side of body, ending near caudal-fin, usually overlying black lateral blotches extending ventrally from lateral stripe; circular black spot on upper margin of caudal-fin base; black pigment extending from lateral stripe onto pectoral-fin base; no marmorated pattern anterior to dorsal-fin origin; black dorsal saddles short, not connecting to lateral blotches; 8 branched upper caudal-fin rays.

Description. Meristic and morphometric data are in Tables 4 and 5. Body deepest just anterior to dorsal-fin origin; slightly compressed before dorsal fin, strongly compressed postdorsally. Head depressed, snout gently rounded to slightly pointed when viewed dorsally or laterally. Dorsal-fin origin far in front of pelvic-fin origin; distal margin convex. Pectoral fin 0.18–0.23 SL, not reaching pelvic fin; pelvic fin 0.16–0.19 SL, not reaching anal fin; caudal fin emarginate. Lateral line incomplete, reaching just beyond dorsal-fin insertion; 21–56 pores. Axillary pelvic lobe present. Mouth arched with heavily papillated lips; upper lip with 3–5 rows of papillae, continuous with large pads on lower lip. Three pairs of barbels; inner rostral barbel extends to or slightly past base of maxillary barbel, maxillary barbel and outer rostral barbel extend to or slightly past eye. Body covered with scales; 9½ (13 specimens)–10½ (66 specimens) branched dorsal-fin rays; 9–13, usually 10, pectoral-fin rays; 6 pelvic-fin rays; 5½ branched anal-fin rays; 8 branched upper caudal-fin rays; 8 branched lower caudal-fin rays. Suborbital flap in male. Maximum SL 42.4 mm female.

Body yellow-brown with 10–13 dark brown to black blotches along lateral line overlain with black stripe usually obscuring lateral blotches and ending at caudal-fin origin; 13–24 small dark brown dorsal saddles. Dark spots and blotches on head; uninterrupted black bar from eye to tip of snout. Ocellus with black center near upper margin of caudal peduncle; 3–6 concentric rows of dark spots in dorsal fin, 2–3 rows of dark spots in anal fin, 4–6 dark bands on caudal fin. Black lateral stripe extending onto pectoral-fin origin.

Comparisons. *Paracanthocobitis nigrolineata* differs from all other species of *Paracanthocobitis* except *P. zonalternans, P. phuketensis, P. triangula*, and *P. marmorata* in having an incomplete lateral line ending near the dorsal-fin insertion (vs. lateral line to the end of the caudal peduncle) and 9½–10½ (vs. >10½) branched dorsal-fin rays. *Paracanthocobitis nigrolineata* is most similar morphologically to *P. zonalternans* but differs in having a black stripe along the side of the body, usually overlying black lateral blotches, ending near the caudal fin (vs. stripe absent or dusky and ending near the dorsal-fin insertion), and in always having 8 (vs. sometimes 7) upper branched caudal-fin rays. *Paracanthocobitis nigrolineata* differs from *P. phuketensis* in having a black stripe along

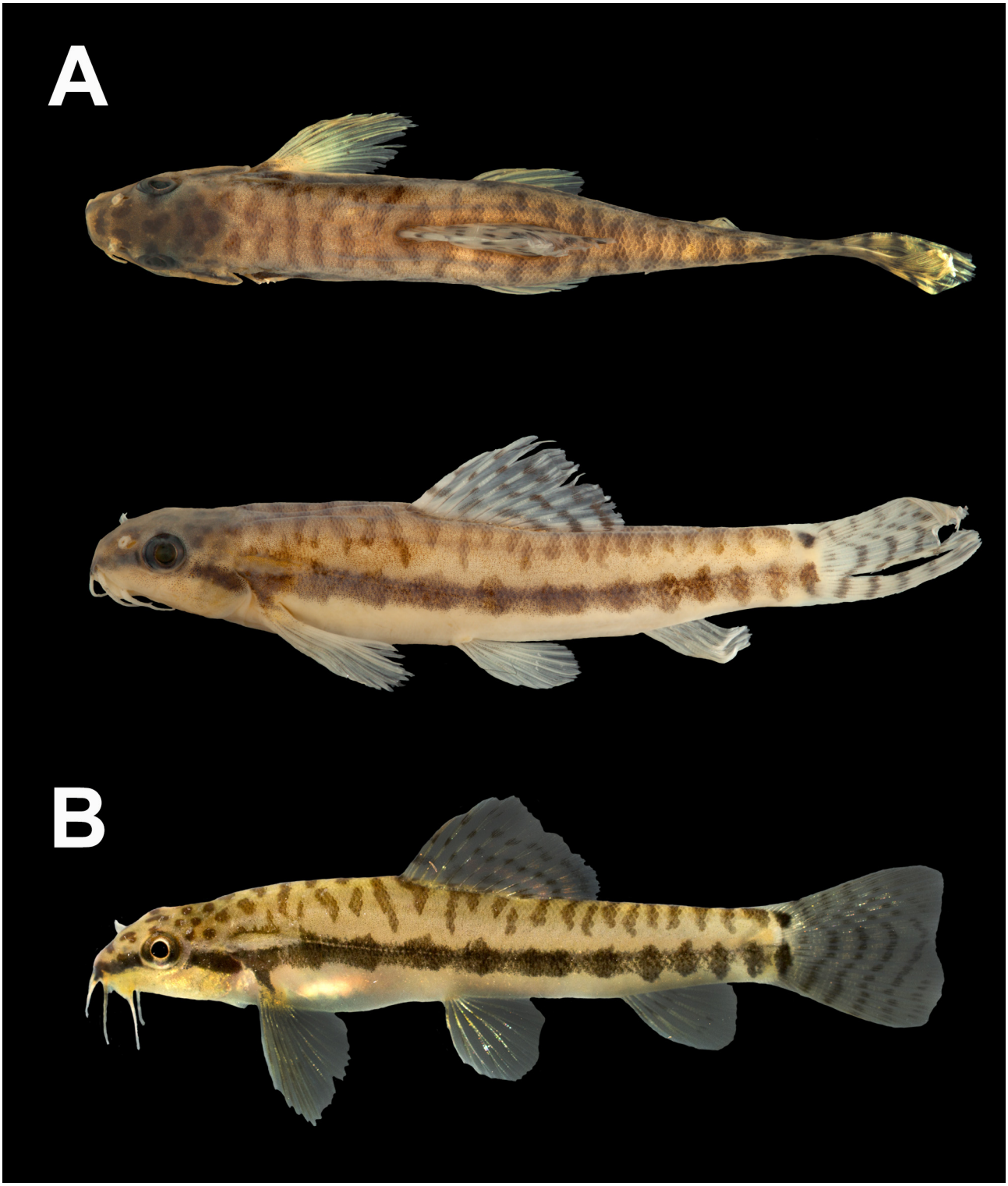

FIGURE 8. (A) Dorsal and lateral views of holotype of *Paracanthocobitis nigrolineata*, NIFI 5080, Kanchanaburi Province, Thailand, 33.1 mm SL; (B) live photo of holotype. Live photo by Z. S. Randall.

the side of the body, usually obscuring black lateral blotches (vs. a series of black blotches, sometimes overlain with a faint dusky stripe not obscuring the black blotches); black pigment extending from the lateral stripe onto the pectoral-fin base; axillary pelvic lobe present (vs. absent, occasionally rudimentary), and 8 (vs. sometimes 7) branched upper caudal-fin rays. *Paracanthocobitis nigrolineata* differs from *P. triangula* in having a circular (vs. triangular) black spot on the upper margin of the caudal-fin base and in having black pigment extending from the lateral stripe onto the pectoral-fin base. *Paracanthocobitis nigrolineata* differs from *P. marmorata* in having a

circular (vs. teardrop-shaped) black spot on the upper margin of the caudal-fin base, black pigment extending from the lateral stripe onto the pectoral-fin base, an axillary pelvic lobe, and in not having a marmorated pattern between the dorsal saddles and lateral blotches.

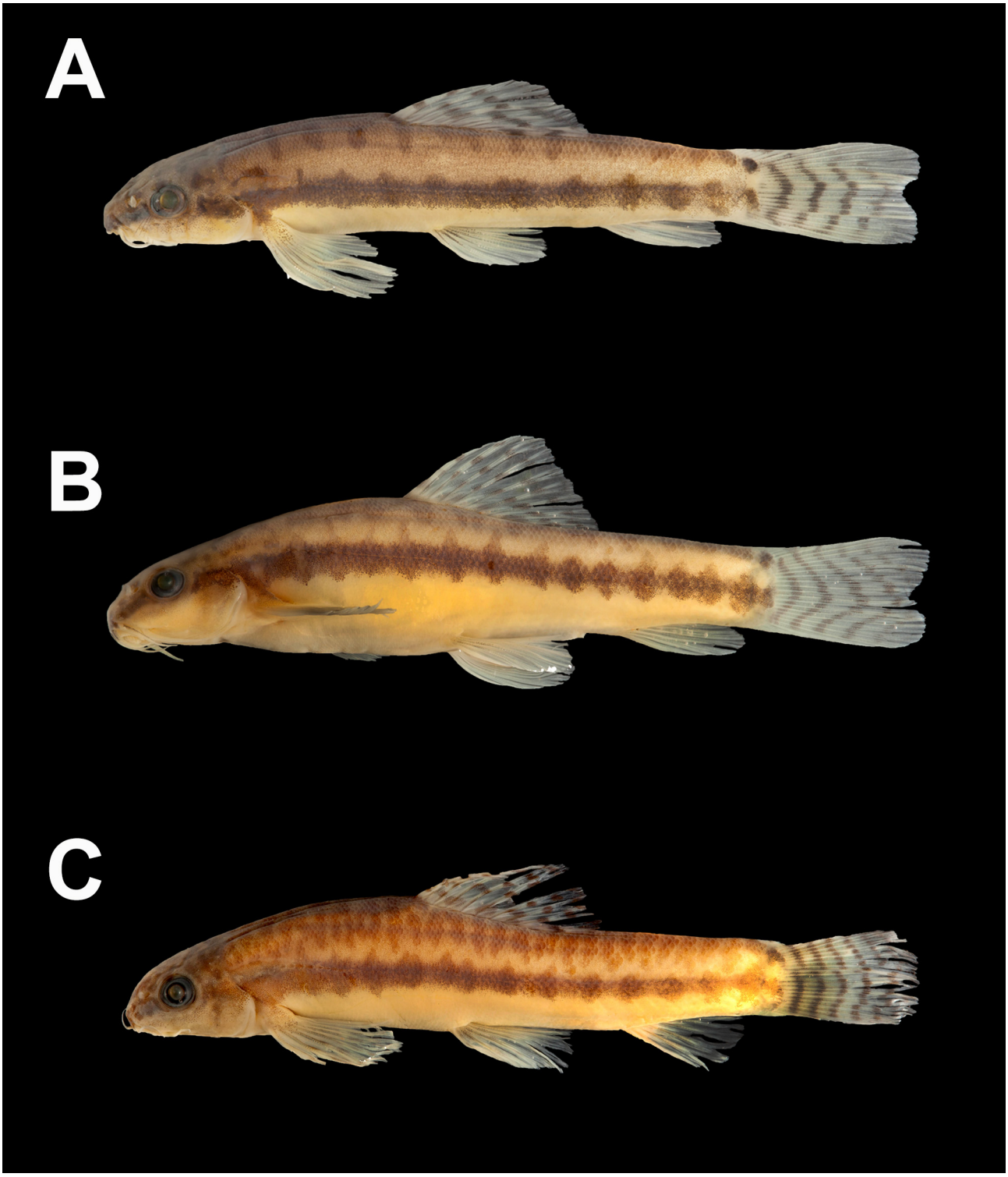

FIGURE 9. Geographic variation in color pattern in *Paracanthocobitis nigrolineata*. (A) UF 188053, Kanchanaburi Province, Thailand, 33.4 mm SL; (B) USNM 378439, Taungoo, Myanmar, 37.6 mm SL; (C) ZRC 43453, Shan State, Myanmar, 38.0 mm SL.

Distribution. *Paracanthocobitis nigrolineata* is known from Irrawaddy and Sittang drainages of Myanmar and the Mae Khlong drainage of Thailand with a large geographic gap in-between (Fig. 5). This gap is likely due to the paucity of sampling in Myanmar.

Etymology. The epithet *nigrolineata* refers to the black stripe along the side of the body.

Material Examined (excluding types). Myanmar: Irrawaddy drainage: CAS 32368, 13, 19.0–26.6 mm, near Indaw, Chaung and Karnaing, 24.226N, 96.143E; CAS 34752, 2, 23.5–31.3 mm, Ngamoeyeik Creek, 9 mi NW Hlegu, 17.219N, 96.201E; UF 30191, 2, 36.3–35.7 mm, Gyobu Reservoir, 9 km N of Taik-Kyi Taik-Kyi township, Rangoon, 16.831N, 96.250E; ZRC 43453, 10, 17.3–38.0 mm, Shan State, Ma Gawe River, along Kalaw-Thazi Hwy between Mandalay and Nampantet, 21.397N, 96.245E. Sittang drainage: USNM 378439, 10, 31.5–39.7 mm, Kayin Stream near the Sittang River, east of Taungoo, 18.958N, 96.478E. Thailand: Kanchanaburi Province: Mae Khlong drainage: NIFI 01435, 13, 24.6–32.0 mm, Khao Noi, 13.967N, 99.590E; NIFI 02057, 7, 19.9–33.9 mm, same locality as NIFI 01435; UF 172979, 1, 31.3 mm, Lichia River, at 323 bridge, 15.071N, 98.581E; UF 176389, 2, 29.1–34.8 mm, Huay Ka Yeng County, Kring Ta Ko, 14.753N, 98.501E; UF 176411, 1, 22.8 mm, Huay Ka Yeng, at 3272 bridge, 14.660N, 98.534E; UF 176449, 35 (10 ex.), 22–30.1 mm, Mae Nam Khwae, Huay Ban Rai, 14.7193N, 98.506E; UF 176546, 2, 24.9–25.5 mm, Huay Ka Yeng, trib. of Mae Nam Khwae at Huay Pok Kok, 14.680N, 98.527E; UF 176554, 23 (10 ex.), 25.4–24.4 mm, same locality as UF 176449; UF 181138, 2, 34.5–36.4 mm, Mae Nam Khwae Noi at Ban Huay Pakkok, 14.380N, 98.480N; UF 181149, 7, 29–36.4 mm, Pakkok River—Mae Nam Khwae Noi at Ban Huay Paousa, 14.633N, 98.800E; UF 182821, 13, 27.7–37.4 mm, Thong Pha Phum, near rte. 3272, 14.690N, 98.521E; UF 188054, 3, 33.8–38.8 mm, Thong Pha Phum, Mae Klong River, 14.650N, 98.530E; UF 188055, 4, 25.7–31.6 mm, Sangkhla Buri Co., Song Karia River at rte. 323, 15.220N, 98.440E; UF 188056, 1, 25.4 mm, Sangkhla Buri Co., unnamed trib of Lake Vajiralongkorn, 15.060N, 98.560E.

Paracanthocobitis triangula, new species

Wedged Zipper Loach

Fig. 10

Holotype. UMMZ 250272, 28.6 mm SL, female, Bangladesh, Meghna drainage, 25.183N, 89.983E, 21 February 1978.

Paratypes. Bangladesh: Meghna drainage: CAS 235899, 1, 23.4 mm SL, Sylhet, Nur Nody Stream, upstream from Lubha River at Nihalpur Village, 25.036N, 92.306E, 24 January 1998; UMMZ 208784, 13 (10 ex.), 25.8–29.2 mm SL, same locality and date as holotype; UMMZ 208629, 1, 24.6 mm SL, Sylhet, Dauki/Piyain Gang River, 25.101N, 91.753E, 19 February 1978; UF 188249, 3, 26.1–28.4 mm SL, same locality and date as holotype.

Diagnosis. *Paracanthocobitis triangula* is distinguished from all other species of *Paracanthocobitis* by combination of incomplete lateral line ending near dorsal-fin insertion; 9½–10½ branched dorsal-fin rays; axillary pelvic lobe present; series of black blotches, sometimes overlain with faint dusky stripe along side of body not obscuring lateral blotches, ending just beyond dorsal-fin insertion; small black triangular blotch in ocellus on upper margin of caudal-fin base; black pigment of the midlateral stripe not extending onto pectoral-fin base; no marmorated pattern between dorsal saddles and lateral blotches; dorsal saddles usually extending ventrally just past faint lateral stripe, usually connecting to lateral blotches; 8 branched upper caudal-fin rays.

Description. Meristic and morphometric data are in Tables 4 and 5. Body deepest just anterior to dorsal-fin origin; slightly compressed before dorsal fin, strongly compressed postdorsally. Head depressed, snout gently rounded to slightly pointed when viewed dorsally or laterally. Dorsal-fin origin far in front of pelvic-fin origin; distal margin convex. Pectoral fin not reaching pelvic fin; pelvic fin not reaching anal fin; caudal fin emarginate. Lateral line incomplete, reaching at most to just past dorsal-fin insertion with 20–37 pores. Axillary pelvic lobe present. Mouth arched with heavily papillated lips; upper lip with 3–5 rows of papillae, continuous with large pads on lower lip. Three pairs of barbels; inner rostral barbel extends to or slightly past base of maxillary barbel, maxillary barbel and outer rostral barbel extend to or slightly past eye. Body covered with scales; 9½ (1 specimens)–10½ (10 specimens) branched dorsal-fin rays; 9–10, usually 9, pectoral-fin rays; 6 pelvic-fin rays; 5½ branched anal-fin rays; 8 branched upper caudal-fin rays; 8 branched lower caudal-fin rays. Suborbital flap in male. Maximum SL 29.2 mm female.

Body yellow-brown with 10–12 dark brown to black blotches along lateral line sometimes overlain with faint dusky stripe not obscuring lateral blotches, ending just beyond dorsal-fin insertion; 12–14 small dark brown dorsal saddles uniform, equal to or wider than interspaces, usually extending ventrally just past lateral stripe and connecting to lateral blotches. Dark spots and blotches on head; uninterrupted black bar from eye to tip of snout.

Ocellus with small black triangular blotch in center, near upper margin of caudal peduncle; 3–6 concentric rows of dark spots in dorsal fin, 2–3 rows of dark spots in anal fin, 4–6 dark bands on caudal fin. Black lateral stripe not extending onto pectoral-fin base.

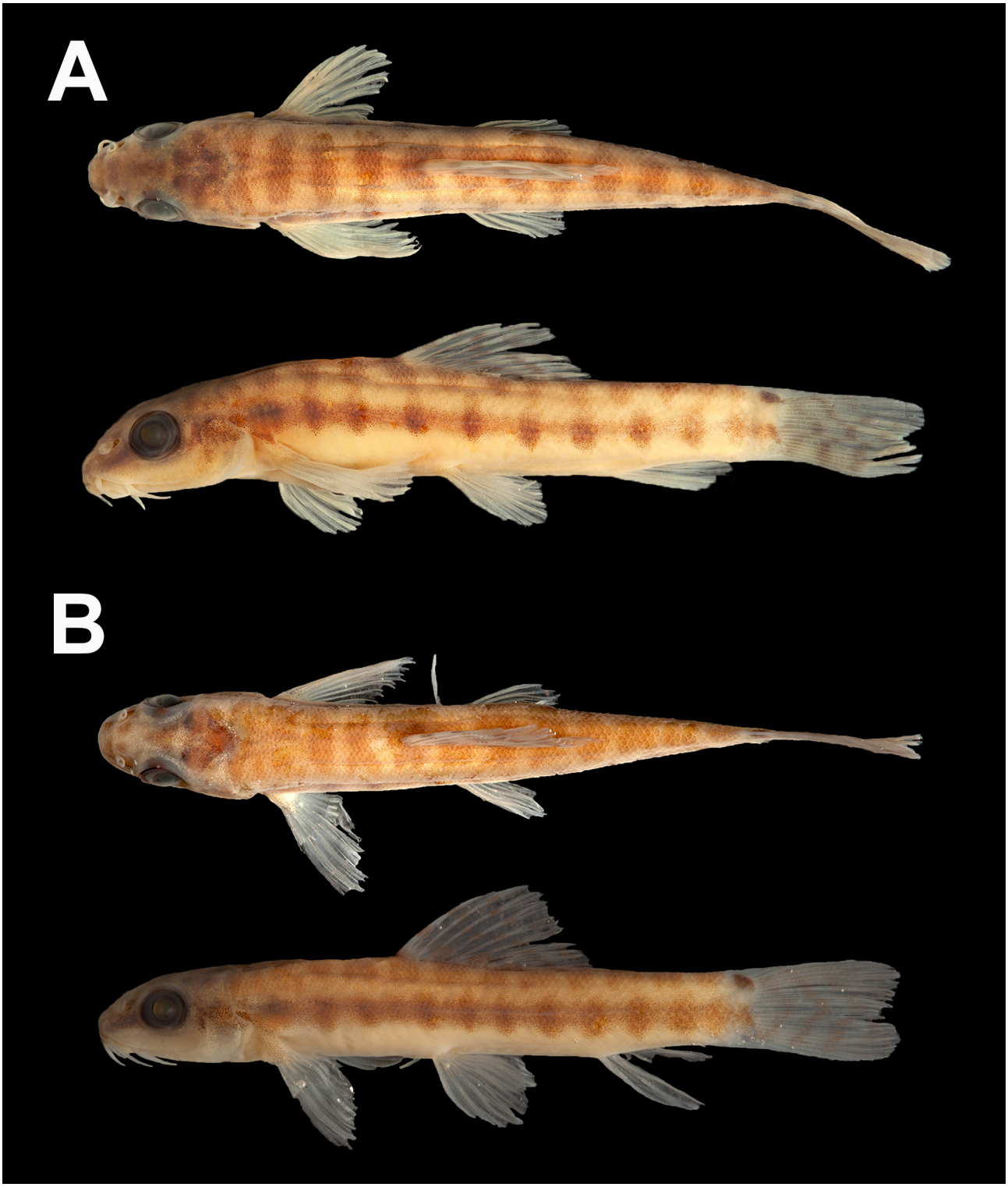

FIGURE 10. Type specimens of *Paracanthocobitis triangula*. (A) Dorsal and lateral views of holotype, UMMZ 250272, Meghna drainage, Bangladesh, 28.6 mm SL; (B) Dorsal and lateral views of paratype, CAS 235899, Meghna drainage, Bangladesh, 23.4 mm SL.

Comparisons. *Paracanthocobitis triangula* differs from all other species of *Paracanthocobitis* except *P. zonalternans, P. phuketensis, P. nigrolineata*, and *P. marmorata* in having an incomplete lateral line ending near the dorsal-fin insertion (vs. lateral line to the end of the caudal peduncle) and 9½–10½ (vs. >10½) branched dorsal-fin rays. *Paracanthocobitis triangula* differs from *P. zonalternans, P. phuketensis, P. nigrolineata*, and *P. marmorata* in having a small black triangular (vs. circular or teardrop-shaped) blotch on the upper margin of the caudal-fin base. *Paracanthocobitis triangula* further differs from *P. zonalternans* in lacking black pigment extending from the lateral stripe onto the pectoral-fin base, from *P. phuketensis* in having an axillary pelvic lobe (vs. usually absent, occasionally rudimentary), and from *P. nigrolineata* in not having a black stripe along the side of the body with black pigment from the stripe extending onto the pectoral-fin base. *Paracanthocobitis triangula* further differs from *P. marmorata* in having an axillary pelvic lobe and in not having a marmorated pattern between the dorsal saddles and lateral blotches.

Distribution. *Paracanthocobitis triangula* is known from the Brahmaputra (Jamuna) and Meghna drainages of Bangladesh (Fig. 5).

Etymology. The epithet *triangula* refers to the usual presence of a small black triangular blotch in the ocellus on the upper margin of the caudal peduncle.

***Paracanthocobitis marmorata*, new species**
Marmorated Zipper Loach
Fig. 11

Holotype. ZMA 119.972, 29.4 mm SL, female, India, Barak drainage, 24.799N, 93.782E, date unknown.

Paratypes. India: Barak drainage, same locality as holotype: ZMA 250.182, 2, 28.2–31.9 mm SL, UF 188251, 1, 33.9 mm SL.

Diagnosis. *Paracanthocobitis marmorata* is distinguished from all other species of *Paracanthocobitis* by combination of incomplete lateral line ending just beyond dorsal-fin insertion; 9½–10½ branched dorsal-fin rays; no axillary pelvic lobe; no black stripe along side of body; marmorated pattern of dark lines and blotches between irregularly shaped dorsal saddles dark blotches along side of body; teardrop-shaped black spot on upper margin of caudal-fin base; 8 branched upper caudal-fin rays.

Description. Meristic and morphometric data are in Tables 4 and 5. Body deepest just anterior to dorsal-fin origin; slightly compressed before dorsal fin, strongly compressed postdorsally. Head depressed, snout gently rounded to slightly pointed when viewed dorsally or laterally. Dorsal-fin origin far in front of pelvic-fin origin; distal margin convex. Pectoral fin 0.19–0.22 SL, not reaching pelvic fin; pelvic fin 0.15–0.17 SL, not reaching anal fin; caudal fin emarginate. Lateral line incomplete, reaching at most to just past dorsal-fin insertion with 25–31 pores. No axillary pelvic lobe. Mouth arched with heavily papillated lips; upper lip with 3–5 rows of papillae, continuous with large pads on lower lip. Three pairs of barbels; inner rostral barbel extends to or slightly past base of maxillary barbel, maxillary barbel and outer rostral barbel extend to or slightly past eye. Body covered with scales; 9½ branched dorsal-fin rays; 9–10 pectoral-fin rays; 5–6 pelvic-fin rays; 5½ branched anal-fin rays; 8 branched upper caudal-fin rays; 8 branched lower caudal-fin rays. Suborbital flap in male. Maximum SL 33.9 mm female.

Body yellow-brown with numerous, irregular and poorly defined dark brown dorsal saddles; dark blotches along lateral line extending onto lower side of body; dark brown marmorated pattern between saddles and lateral blotches, usually ending after dorsal-fin insertion. No dark stripe along side. Dark spots and blotches on head; uninterrupted black bar from eye to tip of snout. Ocellus near upper margin of caudal peduncle, small teardrop-shaped black spot in center; 3–6 concentric rows of dark spots in dorsal fin, 2–3 rows of dark spots in anal fin, 4–6 dark bands on caudal fin.

Comparisons. *Paracanthocobitis marmorata* differs from all other species of *Paracanthocobitis* except *P. zonalternans, P. phuketensis, P. nigrolineata*, and *P. triangula*, in having an incomplete lateral line ending near the dorsal-fin insertion (vs. lateral line to the end of the caudal peduncle) and 9½–10½ (vs. >10½) branched dorsal-fin rays. *Paracanthocobitis marmorata* differs from *P. zonalternans, P. phuketensis, P. nigrolineata*, and *P. triangula* in having a dark marmorated pattern between the dorsal saddles and lateral blotches and a teardrop-shaped (vs. circular or triangular) black spot on the upper margin of the caudal-fin base, and in not having an axillary pelvic

lobe (rarely present in *P. phuketensis*). *Paracanthocobitis marmorata* further differs from *P. zonalternans* and *P. nigrolineata* in not having black pigment extending onto the pectoral-fin base.

Distribution. *Paracanthocobitis marmorata* is known from the Barak drainage of Assam, India (Fig. 5).

Etymology. The epithet *marmorata* refers to the marmorated pattern on the nape and between the dorsal saddles and lateral blotches in lieu of the black stripe along the side of the body typical of other members of the *P. zonalternans* complex.

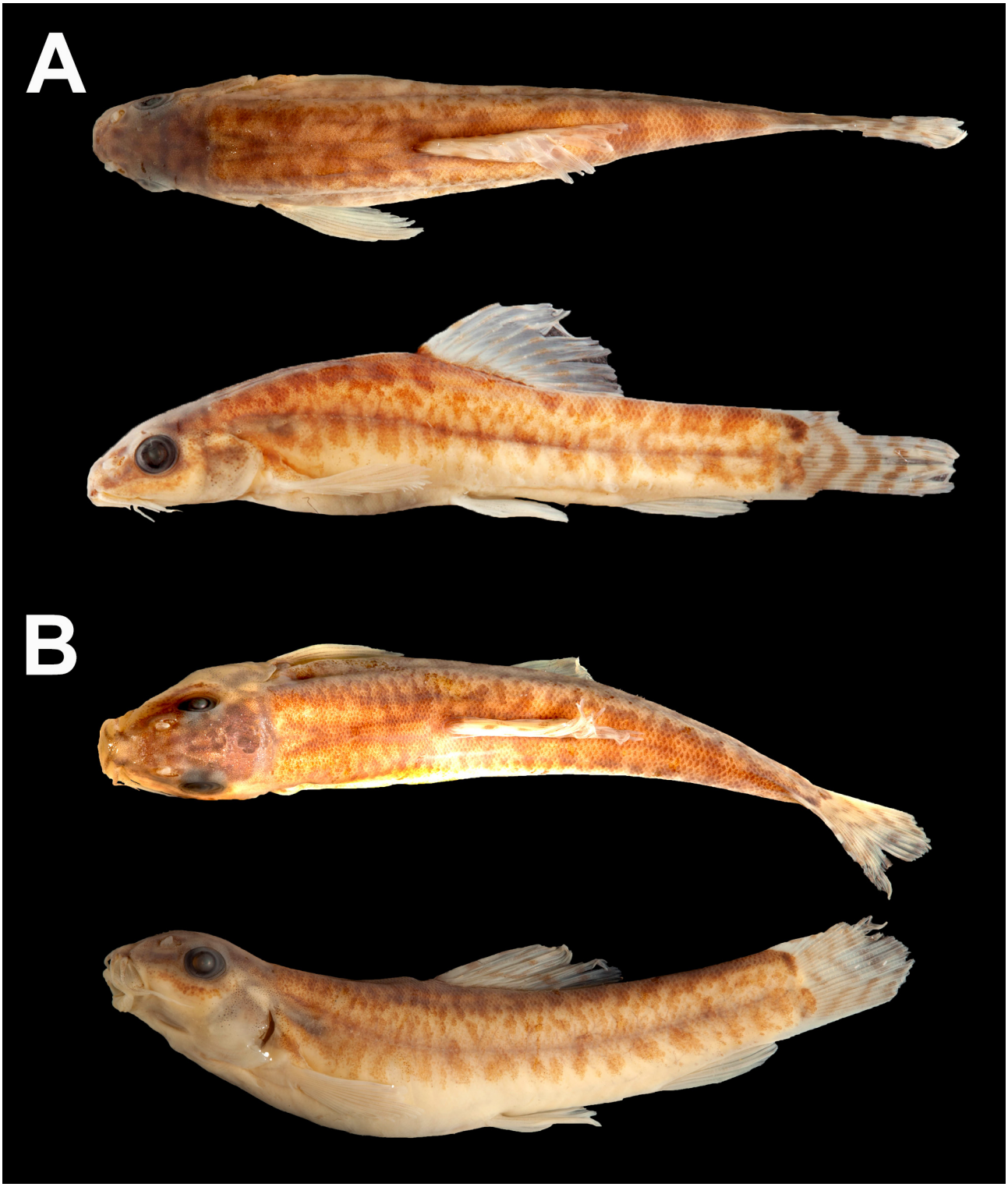

FIGURE 11. Type specimens of *Paracanthocobitis marmorata*. (A) Dorsal and lateral views of holotype, ZMA 119.972, Barak drainage, India, 29.4 mm SL; (B) Dorsal and lateral views of paratype, UF 188251, Barak drainage, India, 33.9 mm SL.

TABLE 6. Distinguishing characters for species of the *P. zonalternans* complex.

Species	*P. zonalternans*	*P. phuketensis*	*P. nigrolineata* n.sp.	*P. triangula* n.sp.	*P. mamorata* n.sp.
Dark lateral pigment	Series of spots	Series of spots occasionally connecting to dorsal saddles	Black stripe	Series of spots occasionally connecting to dorsal saddles	Marmorated pattern
Black onto pectoral–fin base	Yes	No	Yes	No	No
No. branched caudal–fin rays	7+8, 8+8	7+8, 8+8	8+8	8+8	8+8
Axillary pelvic Lobe	Yes	Variable	Yes	No	Yes
Black spot on caudal base	Circular	Circular	Circular	Thin/triangular	Teardrop–shaped
Maximum SL	41.8	26.3	42.4	29.2	33.9

Discussion

Two of the five species in the *P. zonalternans* complex, *P. marmorata* and *P. triangula*, are quite distinctive morphologically. The other three species, *P. zonalternans, P. nigrolineata,* and *P. phuketensis*, are similar to one another morphologically and distributed in a narrow geographic area, north to south in the Salween, Mae Khlong, and peninsular drainages (Fig. 5). Despite the high degree of morphological similarities found across these three species, the genetic distances in the mitochondrial marker are moderately large, ranging from 7.6% between *P. zonalternans* and *P. phuketensis* to 9.3% between *P. nigrolineata* and *P. phuketensis* (Table 3), suggesting that the species are reproductively isolated from each other even though morphological changes are less pronounced. *Paracanthocobitis phuketensis,* distributed in several rivers draining to the Gulf of Thailand and to the Andaman Sea, shows the greatest intraspecific genetic variation of the group. This variation should be explored in more detail for historical and ecological explanations.

Molecular data agree with the hypothesis proposed by Singer & Page (2015) that the *P. zonalternans* species complex forms a clade separate from the *P. botia* clade, represented here (Fig. 1) by *P. maekhlongensis*, *P. pictilis,* and *P. mandalayensis*. In addition, the genetic distances among species of the *P. botia* clade, which are very distinct morphologically from one another, are less than those found between *P. zonalternans* and *P. nigrolineata* and *P. phuketensis*.

Šlechtová *et al*. (2007) found *Acanthocobitis* to be sister to *P. zonalternans* plus *P. botia* (identifications of the *Paracanthocobitis* species are uncertain, but almost certainly represent the *P. zonalternans* and *P. botia* clades). However, more specimens and tissues from both major clades within *Paracanthocobitis* and from the sister genus *Acantocobitis* are needed to confirm relationships among the species in these genera as well as relationships of these genera to other nemacheilids.

Kottelat (2012) listed *Cobitis chlorosoma* McClelland, 1839 as a synonym of *Acanthocobitis zonalternans*. The original description for *C. chlorosoma* is very short: "Light green, clouded with a darker green, caudal and dorsal marked with spots in transverse rows. D.11: P.12: V.8: A.6: C.18. Hab. Upper Assam." Text with a drawing (plate 52, fig. 3) of the species accompanying the description states: "Light green, clouded with dark olive green above the lateral line, fins tinged with red, dorsal and anal slightly barred, snout short, six cirri." The only species of *Paracanthocobitis* known from Assam is *P. botia*, which has counts that agree with those given by McClelland for *C. chlorosoma* (Singer & Page 2015), and a pigment pattern very similar to that shown in the drawing of *C. chlorosoma* (McClelland, 1839: pl. 52). Most species of *Paracanthocobitis* have a greenish cast and often have fins tinged with red. *Paracanthocobitis zonalternans* is an exception, being light brown or yellowish with bold black spots or a black stripe along the lateral line. *Acanthocobitis pavonacea*, also described by McClelland (1839) from Assam and shown on the same plate as *C. chlorosoma*, has a distinctly pointed caudal fin and 17 or more branched dorsal-fin rays, unlike that shown for *C. chlorosoma*, which is here considered a synonym of *P. botia*.

Key to species of *Paracanthocobitis* in the *P. zonalternans* complex

1. Dark marmorated pattern between dorsal saddles and lateral blotches; teardrop-shaped (vs. circular or triangular) black spot on upper margin of caudal-fin base . . . . . *P. marmorata*
- No dark marmorated pattern between dorsal saddles and lateral blotches; circular or triangular black spot on upper margin of caudal-fin base. 2
2. No black pigment extending from black lateral stripe or blotches onto pectoral-fin base 3
- Black pigment extending from black lateral stripe or blotches onto pectoral-fin base. 4
3. Axillary pelvic lobe present; triangular black spot on upper margin of caudal-fin base . . . . . *P. triangula*
- Axillary pelvic lobe usually absent, occasionally rudimentary; circular black spot on upper margin of caudal-fin base . . . . . *P. phuketensis*
4. Series of black blotches, usually with stripe absent or dusky and ending near the dorsal-fin insertion; minimum HL 17% of SL . . . . . *P. zonalternans*
- Black stripe along side of body ending near caudal fin and overlaying lateral blotches; minimum HL 19% of SL . . . . . *P. nigrolineata*

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