National Marine Fisheries Service
NOAA

Fishery Bulletin
established in 1881

Spencer F. Baird
First U.S. Commissioner of Fisheries and founder of *Fishery Bulletin*

New data on the morphology of the Mekong blind sole (*Typhlachirus elongatus*) indicating the need for a revision of the genus

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Abstract—The results of analysis of 88 specimens of the Mekong blind sole (*Typhlachirus elongatus*) from the Mekong River delta in Vietnam indicate that the range of variability in meristic diagnostic characters of this species is broader than previously documented. Comparison of the new morphological data for *T. elongatus* from our analysis with data from the available literature for *T. lipophthalmus* and *T. caecus* reveals no differences between species. This fact confirms Chabanaud's conclusions about the monotypy of the genus *Typhlachirus* and indicates the need for further revision of the genus. Here, molecular data from the genes cytochrome *c* oxidase subunit 1 and 16S rRNA are presented for the first time for the genus *Typhlachirus*.

Manuscript submitted 19 November 2021.
Manuscript accepted 18 March 2022.
Fish. Bull. 120:113–124 (2022).
Online publication date: 1 April 2022.
doi: 10.7755/FB.120.2.2

The soles of the genus *Typhlachirus* (family Soleidae) are found in marine and brackish water and distinguished by the absence of eyes. Three nominal species of the genus—*T. lipophthalmus* (Károli, 1882), *T. caecus* Hardenberg, 1931, and the Mekong blind sole (*T. elongatus*) Pellegrin and Chevey, 1940—have been described from the Indo-West Pacific. Currently, they are all considered valid (Fricke et al., 2021). Until recently, blind sole were poorly represented in ichthyological collections: a total of 15 individuals have been reported (Karoli, 1882; Hardenberg, 1931a, 1931b; Pellegrin and Chevey, 1940; Atack, 2006; Evseenko and Bolshakov, 2018; Tan and Grinang, 2020). Of these individuals, 5 specimens of *T. lipophthalmus* were caught from the coastal waters off the northwestern coast of Kalimantan, the Indonesian territory on the island of Borneo (Karoli, 1882; Atack, 2006; Tan and Grinang, 2020), 5 specimens of *T. caecus* were caught from the mouth of the Rokan River in the Strait of Malacca between Sumatra Island of Indonesia and the Malay Peninsula (Hardenberg, 1931a, 1931b), and 5 specimens of *T. elongatus* were caught from the estuarine part of the Mekong River in Vietnam (Pellegrin and Chevey, 1940; Evseenko and Bolshakov, 2018). However, Abidin and Bintoro (2014) reported about 491 blind sole from the estuarine part of the Indragiri River of Sumatra Island. This circumstance indicates that these species are relatively abundant and are frequently caught as bycatch in local fisheries.

The 3 species of blind sole are very similar in external morphology, meristic features, and body proportions; therefore, distinguishing between them is difficult. The features that the authors of the first descriptions note as diagnostic

are as follows: *T. elongatus* differs from *T. caecus* in a more elongated body shape, a more curved mouth, the shape of the nostril, the absence of pectoral fins, and coloration, and *T. elongatus* differs from *T. lipophthalmus* in coloration and smaller scales (Pellegrin and Chevey, 1940). *Typhlachirus caecus* differs from *T. lipophthalmus* in the presence of pectoral fins (Hardenberg, 1931a). In his revision of blind soles, Chabanaud (1948) carefully studied the external morphology, anatomy, and osteology of all specimens available at that time (8 specimens). He noted that there were no sufficient reasons to distinguish these 3 species and combined them into one monotypic genus *Typhlachirus*. As a species epithet, he proposed to keep the earlier synonym *T. lipophthalmus* (Chabanaud, 1939), suggested by Károli (1882).

This revision (Chabanaud, 1948) had been unknown to subsequent researchers until, in 2018, we published a work on the morphology of blind soles with a historical review of taxonomic changes in the genus *Typhlachirus* (Evseenko and Bolshakov, 2018). At the time of that writing, only 2 species had been recognized as valid: *T. caecus* and *T. elongatus* (Munroe, 2000; Desoutter et al., 2001a; Lapierre, 2007; Kottelat, 2013). However, to identify our specimens, we compared them with all species of the genus *Typhlachirus* known at that time, including the unaccepted *T. lipophthalmus* (Evseenko and Bolshakov, 2018). The purpose of the Evseenko and Bolshakov (2018) article was to discuss the problems of the taxonomy of blind soles and supplement the data on their morphology. However, in Eschmeyer's Catalog of Fishes, the work has been interpreted as a revision of the genus *Typhlachirus*. As a result, all 3 types of blind soles are currently indicated as valid in that database (Fricke et al., 2021), and the Chabanaud (1948) revision mentioned in the article (Evseenko and Bolshakov, 2018) was not taken into account again.

During an expedition to the estuarine part of the Mekong River delta, we caught 85 new specimens of blind soles. When trying to determine the species involved, we found that the diagnostic features from the first descriptions and other available literature on these species did not allow the separation of species of *Typhlachirus*. The range of meristic characters of the new specimens is much more extensive than have been noted for *T. elongatus*. In addition, a pectoral fin was found in all new specimens. Given that the absence of a pectoral fin has been reported as a diagnostic feature for *T. elongatus*, the presence of one in our specimens prompted us to reexamine the specimens of *T. elongatus* studied in our previous work (Evseenko and Bolshakov, 2018), and it also served as a reason for a more thorough study of the new specimens.

The primary purpose of the work reported here was to examine the diagnostic characters used to identify species of the genus *Typhlachirus* and to correct misunderstandings that appeared after the publication of Evseenko and Bolshakov (2018). Because there is no molecular genetic information for the genus *Typhlachirus*, we set out to fill this gap by using DNA barcoding, a tool widely used for identification of species and rapid assessment of their genetic variability (Hebert et al., 2003; Hajibabaei et al., 2007; Crawford et al., 2013; Chambers and Hebert, 2016).

Materials and methods

The materials for this work were 85 specimens of juveniles and adults of the genus *Typhlachirus*, with a range in standard body length (SL) of 14–80 mm (Suppl. Table), collected in the Mekong River delta (Fig. 1): 2 specimens (sp.) 32 mm SL, 32 mm SL, 6 April 2018, trawl no. 1, Co Chien River, 10°15′33″N, 105°57′46″E; 9 sp. 22–36 mm SL, 8 April 2018, trawl no. 5, Co Chien River, 10°10′50″N, 106°10′22″E; 2 sp. 30 mm SL, 52 mm SL, 17 April 2018, trawl no. 4, Tien River, 10°19′48′N, 106°17′38″E; 4 sp. 25–76 mm SL, 17 April 2018, 10°20′17″N, 106°19′60″E; 5 sp. 24–49 mm SL, 17 April 2018, trawl no. 1, Tien River, 10°19′37″N, 106°17′37″E; 1 sp. SL? (a question mark [?] means that the length data were lost), 17 April 2018, 10°19′80″N, 106°17′37″E; 2 sp. SL?, 4 August 2018, Tien River, 10°19′27″N, 106°00′50″E; 1 sp. SL?, 18 April 2018, 10°19′2″N, 106°10′12″E; 4 sp. 14–44 mm SL, 19 April 2018, trawl no. 1, Tien River, 10°19′19″N, 106°00′59″E; 1 sp. SL?, 11 April 2018, 10°17′23″N, 106°34′11″E; 1 sp. 46 mm SL, 24 April 2018, trawl no. 2, 10°16′26″N, 106°43′40″E; 1 sp. SL?, 11 May 2018, Tien River, 10°16′46″N, 10°50′55″E; 3 sp. SL?, 12 May 2018, Ham Luong River, 10°14′22″N, 106°13′40″E; 1 sp. 54 mm SL, 15 May 2018, trawl no. 4, Ham Luong River, 10°06′23″N, 106°24′13″E; 1 sp. 60 mm SL, 17 May 2018, trawl no. 4, Ham Luong River, 9°56′26″N, 106°39′20″E; 2 sp. 23 mm SL, 43 mm SL, 19 May 2018, trawl no. 2, Ham Luong River, 10°02′45″N, 106°27′27″E; 4 sp. 30–58 mm SL, 21 May 2018, trawl no. 2, Ba Lai River, 10°08′50′N, 106°37′57″E; 1 sp. 80 mm SL, 23 May 2018, trawl no. 1, Ba Lai River, 10°08′10″N, 106°39′00″E; 1 sp. SL?, 24 May 2018, 10°07′18″N, 106°48′26″E; 39 sp. 14–64 mm SL (capture data lost).

To study the structure of the shoulder girdle and the degree of reduction of the pectoral fins, the 49- and 53-mm-SL specimens were stained with alizarin and alcian blue and clarified according to the standard procedure (Taylor and Van Dyke, 1985). Radiographs were made and studied for almost all specimens. For comparison, we used X-rays of 2 syntypes of *T. elongatus* 28 and 33 mm SL, MNHN 1939-0270 (provided by the Muséum National d'Histoire Naturelle); 2 paratypes of *T. caecus* 76 and 83 mm SL, MNHN 1942-0080 (in Chabanaud, 1948); and 1 specimen of *T. lipophthalmus* 61 mm SL, ZRC 59653 (in Tan and Grinang, 2020). Additionally, 3 specimens, 59, 67, and 71 mm SL, described in Evseenko and Bolshakov (2018), were studied.

Information for the following features are included in the descriptions: SL, head length (HL), body depth at pectoral fin base (BD), number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in pectoral fin on the ocular (right) side of the body ($P1_d$), number of rays in pectoral fin on the blind (left) side ($P1_s$), number of rays in pelvic fin (P2), number of rays in caudal fin (C), number of vertebrae (V), number of precaudal vertebrae (PrCV),

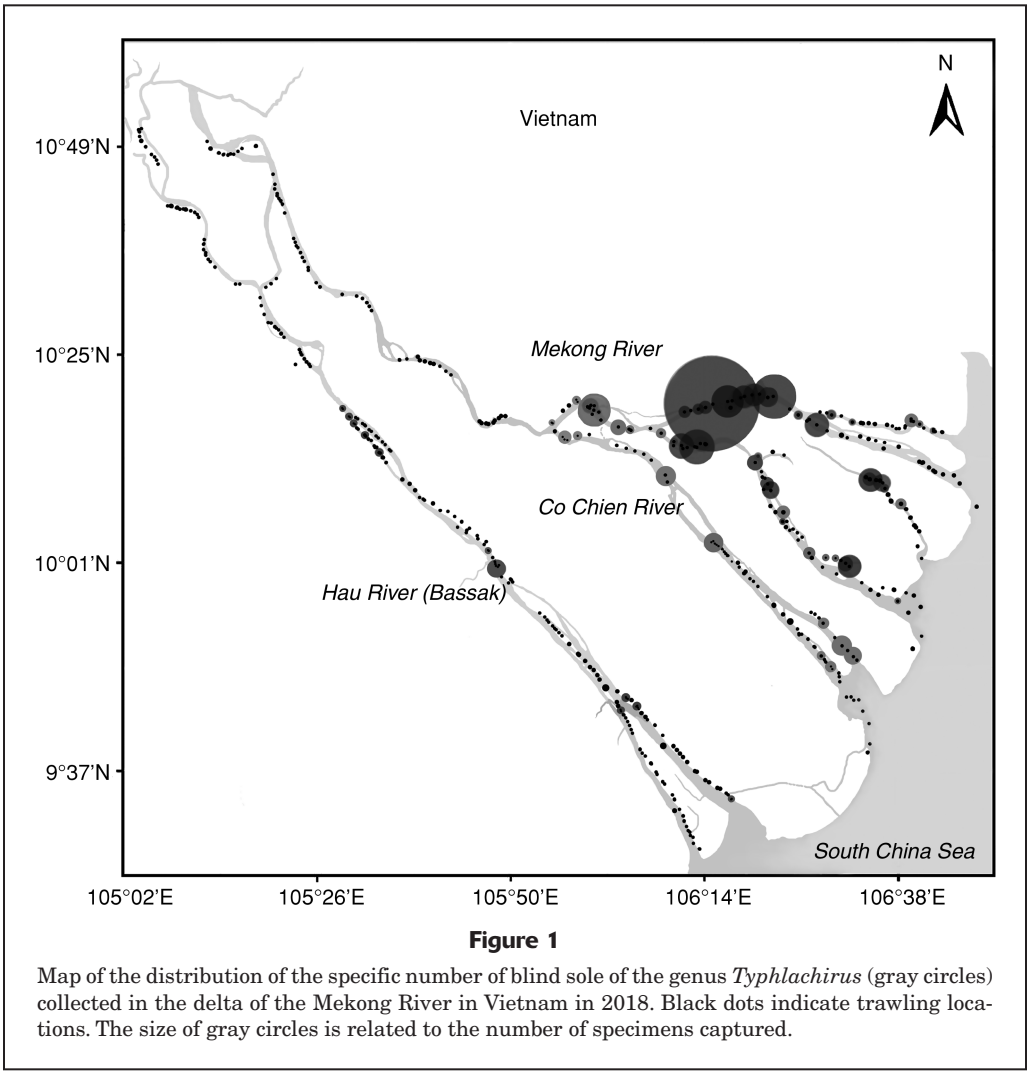

Figure 1

Map of the distribution of the specific number of blind sole of the genus *Typhlachirus* (gray circles) collected in the delta of the Mekong River in Vietnam in 2018. Black dots indicate trawling locations. The size of gray circles is related to the number of specimens captured.

number of caudal vertebrae (CV), and number of pored scales on the horizontal branch of the ocular side lateral line, not including scales on caudal fin (LL).

Statistical analysis

To check the homogeneity of our sample of specimens across all studied features, principal component analysis was performed. On the basis of analysis of combined data (from our work and published reports) on *T. elongatus*, *T. lipophthalmus*, and *T. caecus*, we excluded from further analysis both the number of rays in the pectoral fins (because of the unreliability of data in the original descriptions) and the number of vertebrae (because of the partial lack of data). Results of a preliminary normality test (Shapiro–Wilk test) indicate that all the characters except the number of pores in the lateral line had distributions that were not normal; therefore, we used Spearman's rank correlation coefficients to find linear correlations between SL and other studied features. To ensure that we had not lost any information by using this nonparametric coefficient, we also calculated Pearson correlation coefficients for our data set. To evaluate occurrence frequencies for each feature, the histograms for each character were built (Fig. 2). Principal component analysis was performed in PAST (vers. 2.17c; Hammer et al., 2001), and correlation analysis was done in R (vers. 4.0.3; R Core Team, 2020).

Molecular analysis

We extracted whole genomic DNA from muscle tissue of 14 specimens of *T. elongatus* (Suppl. Table) by using the Diatom DNA Prep 100[1] kit (Izogen Laboratory, Moscow, Russia). In total, 650 base pairs from the barcode region of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene were amplified with the primer set FishF1/FishR2 (Ward et al., 2005). For the subsample of 8 specimens, we amplified the segment (574 base pairs) of the 16S rRNA

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

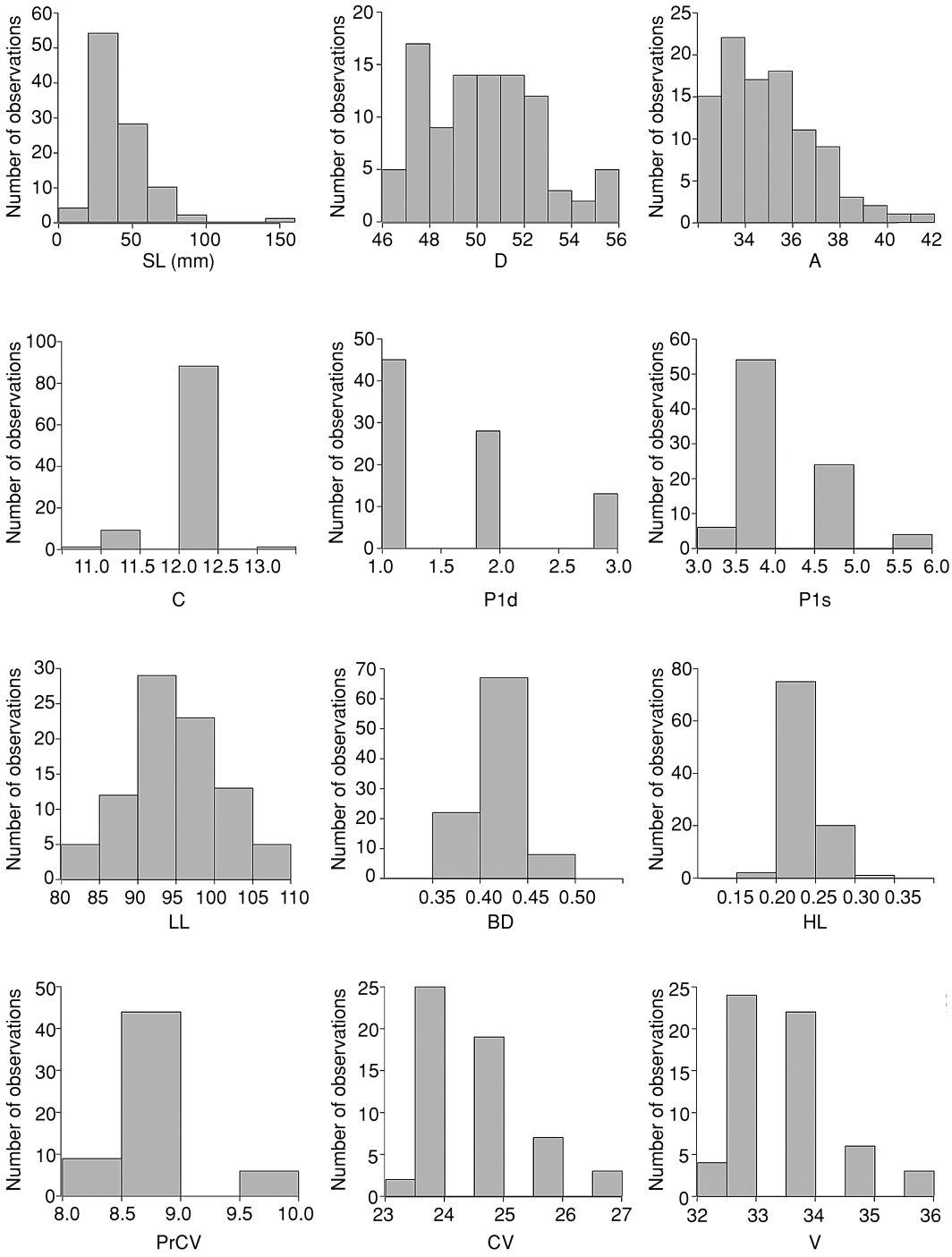

Figure 2

Frequency of occurrence of examined meristic characters and body proportions in 3 nominal species of the genus *Typhlachirus*, based on specimens captured in 2018 in the Mekong River delta of Vietnam and described in the literature (Chabanaud, 1948; Hardenberg, 1931b; Pellegrin and Chevey, 1940; Tan and Grinang, 2020). Features include standard body length (SL), number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in caudal fin (C), number of rays in pectoral fin on the ocular (right) side of the body ($P1_d$) and on the blind (left) side ($P1_s$), number of pored scales on the horizontal branch of the ocular side lateral line not including scales on caudal fin (LL), body depth at pectoral fin base (BD), head length (HL), number of precaudal vertebrae (PrCV), number of caudal vertebrae (CV), and number of vertebrae (V).

gene by using the primer set 16Sar/6Sbr (Palumbi, 1996). The choice of molecular markers was determined by the widest taxonomic representation of Soleidae species in the available databases.

Polymerase chain reaction products were purified and sequenced bidirectionally by using the same primers and an Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, MA) with an Applied Biosystems 3730 Genetic Analyzer (Thermo Fisher Scientific Inc.). All sequences were edited and aligned by using Geneious, vers. 9.1.8 (Kearse et al., 2012), to obtain consensus sequences and check the occurrence of deletions, insertions, and stop codons. Haplotypes were defined by using FaBox (Villesen, 2007) and then were deposited in GenBank (National Institutes of Health, available from website) under the following IDs: MW646924–MW646930 (CO1 haplotypes) and MW648330–MW648332 (16S rRNA haplotypes). Indices of diversity (number of polymorphic [segregating] sites, the nucleotide diversity, and the haplotype diversity; Nei, 1987) were calculated for the mtDNA CO1 gene by using DnaSP software, vers. 6.0 (Rozas et al., 2017). Tajima's D (Tajima, 1989) and Fu and Li's *F* (Fu and Li, 1993) tests of neutrality also were performed with DnaSP.

Distances between mtDNA CO1 sequences were calculated by using MEGA, vers. 6.0 (Tamura et al., 2013), under Kimura's 2-parameter substitution model commonly used for DNA barcoding in studies of fish species (Ward, 2009). A neighbor-joining (Saitou and Nei, 1987) dendrogram with 1000 bootstraps was created by using distances, calculated with Kimura's 2-parameter substitution model, in MEGA to provide a graphic representation of the divergence patterns among *Typhlachirus* and closely related species of Soleidae. We used sequences of 4 species of the genus *Brachirus*—*B. annularis*, *B. harmandi*, the black sole (*B. niger*), and the oriental sole (*B. orientalis*)—the tufted sole (*Dexillus muelleri*), *Aseraggodes kobensis*, the bamboo sole (*Heteromycteris japonicus*, the Atlantic sole (*Pegusa lascaris*), the wavyband sole (*Zebrias japonicus*), the common sole (*Solea solea*), and the tiger sole (*Soleichthys heterorhinos*) from GenBank and the Barcode of Life Data System (BOLD Systems, available from website). Additionally, for measuring intraspecific and interspecific divergence, uncorrected *p* distances, meaning the proportions of nucleotide sites at which 2 sequences being compared are different, were calculated in MEGA by dividing the number of nucleotide differences by the total number of nucleotides compared.

Results

Typhlachirus elongatus Pellegrin and Chevey, 1940

Typhlachirus elongatus Pellegrin and Chevey, 1940:155 (Fig. 1) (first description based on 2 specimens). Mekong River delta. Syntypes (2): MNHN 1939-0270 (2). Rainboth, 1996:222 (listed). Desoutter et al., 2001a:328 (listed). Evseenko and Bolshakov, 2018:553 (description). Tan and Grinang, 2020:7 (fishing localities).

Brachirus elongatus: Munroe in Randall and Lim, 2000:646 (listed). Kottelat, 2013:464 (listed).

The description of the morphology of *T. elongatus* is somewhat scattered; therefore, it is advisable to give a short but complete description here.

Key diagnostic features

D: 46–55, A: 33–40, C: 10–13, $P1_s$: 3–6, $P1_d$: (0)1–3, P_2: 3, V: 32–36, PrCV: 8–10, CV: 23–27, LL: 84–108 (Table 1). The body is right-sided, broad in front, tapered toward the tail, and strongly compressed (Fig. 3). All specimens have a right eye, but it is reduced in varying degrees. Gill rakers are absent from the gill arches. The mouth is curved to

Table 1

Meristic characters and proportional measurements of the Mekong blind sole (*Typhlachirus elongatus*) caught in the Mekong River delta in 2018 (number of specimens [*n*]=85) and from Evseenko and Bolshakov (2018) (*n*=3) in different size classes based on standard body length (SL). Features include number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in caudal fin (C), number of rays in pectoral fin on the ocular (right) side of the body ($P1_d$) and on the blind (left) side ($P1_s$), number of pored scales on the horizontal branch of the ocular side lateral line not including scales on caudal fin (LL), number of precaudal vertebrae (PrCV), number of caudal vertebrae (CV), number of vertebrae (V), body depth at pectoral fin base (BD), and head length (HL).

n	SL (mm)	D	A	C	$P1_d$	$P1_s$	LL	PrCV	CV	V	BD	HL
4	14–20	48–53	35–37	11–12	1–3	3–5	85–92	9	25	34	42–43	24–31
32	21–30	46–54	33–39	11–12	1–3	3–6	85–108	8–10	23–26	32–36	37–46	22–30
18	31–40	47–54	34–39	11–12	1–3	3–5	90–102	9	24–27	33–36	38–46	22–28
16	41–50	47–53	34–38	10–12	1–3	4–6	84–107	8–9	24–26	32–34	38–45	22–26
12	51–60	48–52	34–40	11–13	1–3	4–6	87–107	8–10	23–26	33–34	36–47	21–26
4	61–70	49–53	35–38	12	1–2	4–5	85–105	8–10	24–26	32–35	41–43	20–25
2	71–80	53–55	38–40	11–12	2	4–5	93–99	8–10	24–25	33–34	42–45	23–24

Figure 3

Photograph showing the general view of a Mekong blind sole (*Typhlachirus elongatus*), 71 mm in standard body length, caught on May 2010 in the Mekong River delta of Vietnam. The arrow points to the right pectoral fin. The scale is 1 cm.

varying degrees. The dorsal and anal fins join with the caudal fin. The pectoral fin on the right side of the body is present in all specimens. The pectoral fin on the left side is a transparent membrane; it has a wide base and 3–6 widely spaced rays, half covered by the operculum. The lateral line is straight, extending to the caudal fin on both sides of the body. Both sides of the body are covered with ctenoid scales. The right side of the body is uniformly colored pinkish brown, and the left side is pinkish white.

Morphology

Meristic characters and proportional measurements are presented in Table 1. The body is right-sided, strongly compressed, broad in front, and tapered toward the tail. The caudal fin merges with the dorsal and anal fins; the caudal peduncle is absent. The greatest body depth is 37–47% SL. The head is large, and its length is 20–31% SL. The anterior profile of the head is rounded, and the snout protrudes slightly anterior to the mouth. The epicranial complex consists of 5(6) pterygiophores associated directly with the erisma, the 0(1) pterygiophore between the erisma and second neural spine, and 6(7) pterygiophores attached between the neural spines of the second and third vertebrae. The edge of the isthmus is far behind the vertical through the corner of the mouth. The mouth is terminal, and its anterior half is curved in the ventral direction; the length of the mouth is 33–37% HL. A short dermal fringe surrounds the jaws. The teeth on the jaws are present only on the left side. The teeth on both jaws are long, thin, pointed, and slightly curved. The number of rows is from 1 to 4, and the number of rows increases from front to back.

The left eye is absent (the eye is covered under skin and muscles, and the frontal bone and lateral ethmoid bone limit the eye orbit). Only the lower (right) eye, located just above the corner of the mouth, is visible; the eye is not pigmented. The anterior nostril on the ocular side of the body is tubular and located above the edge of the maxilla; from below it is covered with a valve. The posterior nostril on the ocular side of the body is rounded, located between the eye and the anterior nostril, and covered from above by a valve. The anterior nostril on the blind side of the body is rounded, located above the middle of the maxilla, and surrounded by cilia, and it has a valve. The posterior nostril is tubular and located above the corner of the mouth.

Gill rakers are absent from the gill arches. The gill membranes are covered with skin. The upper end of the gill opens at the level of the eye. Scales are small, are ctenoid on both sides of the body, and have 5–10 cteni. On the ocular side, the largest scales are located in the anteriodorsal part of the body, size of scales decreases in the caudal direction; scales on the blind side are approximately equal to the smallest scales on the ocular side of the body.

The lateral lines on the blind side and ocular side are straight; the number of pored scales on the ocular side to the end of the scale cover is 84–108, and the number on the blind side is 120–159. The lateral line on both sides of the body continues to the caudal fin and runs between 6 and 7 rays; it additionally contains from 23 to 33 perforated scales on the ocular side and from 30 to 50 scales on the blind side. The dorsal fin begins at the most protruding part of the rostrum; the anal fin begins somewhat in front of the level of the base of the right pectoral fin. The length of the rays in the dorsal and anal fins gradually increases in the caudal direction. The caudal fin is long and pointed.

The pectoral fin on the ocular side of the body is present in all specimens; it contains from 1 to 3 unbranched rays (Fig. 4A). The fin size is comparable in size to the scales. The pectoral fin on the blind side is presented in the form of a transparent membrane with a broad base, half covered by the operculum; it contains 3–6 rays (Fig. 4B). Both rays in the pelvic fin are branched and simple. The right pelvic fin is connected to the anal fin by a membrane; the left ventral fin is free. The anus is located in the anterior quarter of the body, between the anal and pelvic fins, and is displaced to the left side. The right side of the body is uniformly colored pinkish brown, each edge of the scales is bordered in black, and the left side is pinkish white.

Biology

Little is known about the biology of this species. *Typhlachirus elongatus* is a benthic fish and lives in muddy brackish waters on soft, silty substrates. In the Mekong River delta, the locations where specimens were captured are concentrated within the estuarine ecotone formed by the 6 branches of the river. In the Hau River, *T. elongatus* were more common in the river's main course, and in the estuarine areas and the coastal sea zone, they were practically

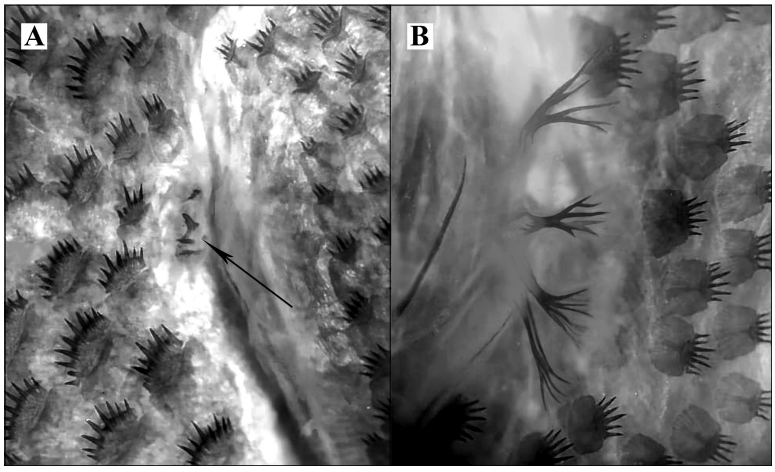

Figure 4

Photographs of the pectoral fin of a Mekong blind sole (*Typhlachirus elongatus*), 71 mm in standard body length, caught on May 2010 in the Mekong River delta of Vietnam, showing (**A**) the right (ocular) side of the body and (**B**) the left (blind) side of the body after staining with alizarin. The arrow in panel A points to the fin.

absent. In the areas where fish were caught in the Mekong River, waters in the bottom layer had temperatures of 28.2–34.1°C and salinity of 0.05–21.0. The early stages of development of this species are unknown, but metamorphosis and sinking to the bottom occur early—the smallest specimens found in collections of fish caught with trawl gear reached a size of 14 mm SL. Our new collections indicate that the species is quite numerous.

Distribution

Typhlachirus elongatus has been found in the estuarine part of the Mekong River (Pellegrin and Chevey, 1940; Evseenko and Bolshakov, 2018). Blind sole were detected in both of the main rivers of the Mekong River delta, the Co Chien (Mekong) and Hau (Bassak) Rivers (Fig. 1).

Statistical analysis

Results of the analysis of the combined sample (based on data from our work and from previous studies for all 3 species of *Typhlachirus*) reveal that all the characters, except the number of pores in the lateral line, have distributions different from normal. In this regard, we used medians and interquartile ranges to describe the average values of diagnostic features and the spread of data, respectively (Table 2). The results of principal component analysis indicate the homogeneity of the studied sample of specimens. The individuals form a single homogeneous group in the space of the first 2 principal components (PCs) (Fig. 5) that is centered around the zero coordinates. The PC1 is almost exclusively associated with the number of pores in the lateral line (loading is 0.9964), and the PC2 is associated with the number of rays in the dorsal and anal fins (loadings were 0.7963 and 0.5990, respectively) (Table 3). The PC1 and PC2 describe 96.1% of the data spread (80.6% and 15.5%, respectively). Individuals of *T. lipophthalmus* and *T. caecus* did not form separate clouds of points but found themselves on the periphery of the cloud for *T. elongatus* in the area of higher PC1 values.

We did not find linear correlations between body length and any other morphological characters: Spearman's rank correlation coefficients did not significantly differ from zero in all the cases. The Pearson's correlation coefficients were the same—all coefficients were zero.

Molecular analysis

Seven haplotypes of the mtDNA CO1 gene were found in species of *Typhlachirus* from the Mekong River delta, with

Table 2

Means, minimums (Min), maximums (Max), medians (Med), and lower interquartile (LQ) and upper interquartile (UQ) ranges of meristic characters and body proportions for the sample of 3 species of the genus *Typhlachirus* that combines data for specimens collected from the Mekong River delta in 2018 and for specimens described in literature (Chabanaud, 1948; Hardenberg, 1931a; Pellegrin and Chevey, 1940; Tan and Grinang, 2020). Features include number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in caudal fin (C), number of rays in pectoral fin on the ocular (right) side of the body ($P1_d$) and on the blind (left) side ($P1_s$), number of pored scales on the horizontal branch of the ocular side lateral line not including scales on caudal fin (LL), number of precaudal vertebrae (PrCV), number of caudal vertebrae (CV), number of vertebrae (V), body depth at pectoral fin base (BD), and head length (HL). *n*=the number of specimens used in the analysis.

Character	Mean	Min–Max	LQ–Med–UQ	*n*
D	50.8	46–56	49–51–52	95
A	36.5	33–43	35–36–38	99
C	11.9	10–13	12–12–12	99
$P1_d$	1.6	1–4	1–1–2	86
$P1_s$	4.3	3–6	4–4–5	88
LL	95.7	84–108	92–95–99	87
PrCV	8.9	8–10	9–9–9	59
CV	24.7	23–27	24–25–25	56
V	33.7	32–36	33–34–34	59
BD	42.0	37–47	41–43–44	98
HL	24.0	20–31	23–24–25	98

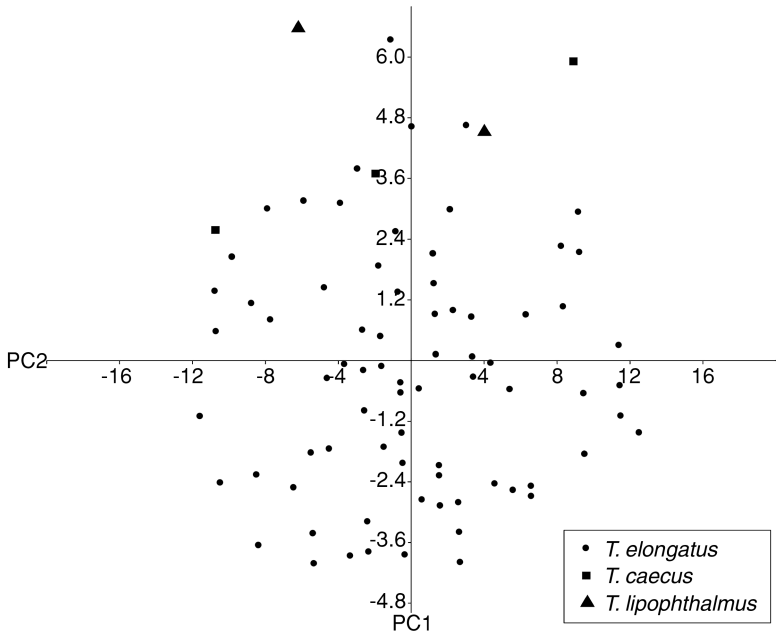

Figure 5

Plot of the principal component (PC) analysis of morphological characters and body proportions of Mekong blind sole (*Typhlachirus elongatus*) (circles), *T. lipophthalmus* (triangles), and *T. caecus* (squares). Each symbol represents a specimen captured in the Mekong River delta of Vietnam in 2018 or a specimen described in the literature (Chabanaud, 1948; Hardenberg, 1931b; Pellegrin and Chevey, 1940; Tan and Grinang, 2020).

only 2 haplotypes shared by more than 1 individual: Hap3 (MW646926) was found in 3 fish, and Hap5 (MW646928) was found in 6 fish. Results of the analysis of genetic diversity in the sample indicate a moderate degree of mtDNA COI variation: haplotype diversity is 0.802, nucleotide diversity is 0.0029, and the number of polymorphic sites is 9 (or 1.4% polymorphic sites). Estimates of Fu and Li's (1993) *F* and Tajima's (1989) D were not significant and hence do not signal selection or demographic expansion.

The final length of the mtDNA CO1 fragment after alignment of 22 sequences of *Typhlachirus* and representatives of Soleidae was 586 base pairs. All haplotypes in *Typhlachirus* were weakly distinguished (0.5% on average) and formed a single clade with high support indices (100%; Fig. 6). *Typlachirus* was obviously most closely related to species of the genus *Brachirus* and monotypic genus *Dexillus*, as predicted from their morphological similarity (Chapleau, 1989; Desoutter and Chapleau, 1997; Desoutter et al., 2001b). The distance between *Typhlachirus* and the closest species, *B. harmandi*, was 14.4%.

Discussion

Although in Eschmeyer's Catalog of Fishes 3 species of blind sole are recognized as valid (Fricke et al., 2021), our new data indicate possible monotypy of the genus *Typhlachirus*. According to the latest review of blind soles (Evseenko and Bolshakov, 2018), based on data from the literature on all known specimens of *T. lipophthalmus*, *T. caecus*, and *T. elongatus*, as well as on the study of 3 specimens of *T. elongatus* (Evseenko and Bolshakov, 2018), the species of the genus *Typhlachirus* differ mainly in meristic characters (Table 4). *Typhlachirus lipophthalmus* differs from *T. caecus* in the number of rays in the dorsal fin and from *T. elongatus* in the number of rays in the anal fin. *Typhlachirus caecus* differs from *T. elongatus* in the number of vertebrae and rays in the anal fin (Table 4). It should be noted that the revision by Chabanaud (1948)

Table 3

Loadings of the first and second principal components (PC) from principal component analysis of morphological characters of *Typhlachirus lipophthalmus*, *T. caecus*, and the Mekong blind sole (*T. elongatus*) from the sample that combines data for specimens collected in 2018 in the Mekong River delta and data from descriptions in the literature (Chabanaud, 1948; Hardenberg, 1931a; Pellegrin and Chevey, 1940; Tan and Grinang, 2020). Features include number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in caudal fin (C), body depth at pectoral fin base (BD), head length (HL), and number of pored scales on the horizontal branch of the ocular side lateral line not including scales on caudal fin (LL).

Component	D	A	C	BD	HL	LL
PC1	−0.0642	−0.0544	−0.0046	−0.0002	−0.0007	0.9964
PC2	0.7963	0.5990	0.0118	0.0013	−0.0016	0.0840

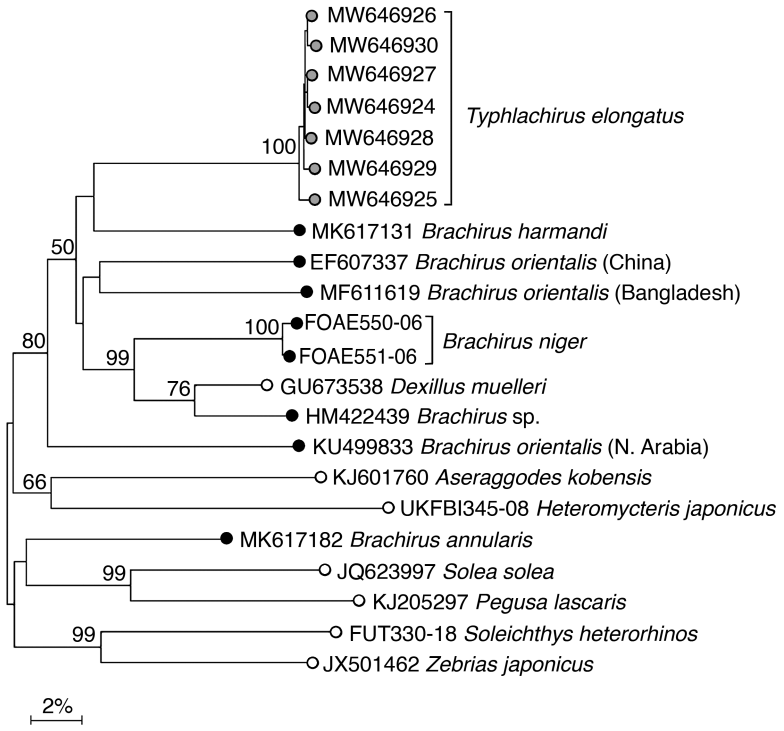

Figure 6

Neighbor-joining dendrogram created by using distances between mtDNA cytochrome *c* oxidase subunit 1 sequences calculated with Kimura's 2-parameter substitution model, showing the discrimination by distance among species of the genus *Typhlachirus*, based on sequences from molecular analysis of specimens captured in the Mekong River delta in 2018 (grey circles), and closely related species of the genus *Brachirus* (black circles) and other representatives of the family Soleidae (open circles), based on sequences available in GenBank and the Barcode of Life Data System (BOLD). Bootstrap values (≥50%) are given above branches. GenBank or BOLD numbers are given for each sequence.

and the review by Evseenko and Bolshakov (2018) were based on a small number of specimens of each species, which, in all probability, explains the registered differences in meristic characters.

We analyzed 88 specimens of *T. elongatus* from the Mekong River delta. The results indicate the homogeneity of our sample (Figs. 5 and 6). In the examined specimens of *T. elongatus*, the range of fluctuations of meristic characters is very wide and can be seen not only from the ranges of minimum and maximum values but also from the interquartile ranges (Table 2). No clear direction in the changes in meristic characters associated with the growth of fish was revealed. Despite the large spread in the number of rays in the dorsal and anal fins (D: 46–56; A: 33–43), the number of precaudal and caudal vertebrae, and the number of pores in the lateral line (PrCV: 8–10; CV: 23–27; LL: 84–108), our individuals form a homogeneous cloud (Fig. 5). Taking into account that PC1 explains a significant proportion of the total variability of the sample and is determined almost exclusively by the number of pores in the lateral line, the introduction of any apriori boundary (or boundaries) for this characteristic between the assumed taxa would automatically lead to a clear division of the sample into 2 (or more) groups. Nevertheless, such a division would be entirely artificial and would hide the objective primary result about the homogeneity of the sample. Results of molecular genetic analysis also indicate the absence of significant differences between specimens from the Mekong River delta.

The range of meristic characters of blind sole from our sample is very broad and includes the values noted in the literature for *T. lipophthalmus*, *T. caecus*, and *T. elongatus*. Several specimens of *T. lipophthalmus* and *T. caecus* turned up on the periphery of the cloud, which is mainly represented by our specimens, in the region of higher PC1 values (the number of pores in the lateral line) (Fig. 5). *Typhlachirus elongatus* from our sample had a pronounced morphological variability; therefore, it is essential to be careful about the taxonomic separation of specimens of the genus *Typhlachirus* with the limiting values of one or another feature. Hence, we explain the extreme position of *T. lipophthalmus* and *T. caecus* in Figure 5 by the small number of specimens used in the analysis. A similar broad range of meristic characters has been reported for some other species of Soleidae. For example, PrCV of 32–44 and LL of 90–133 have been suggested for zebra sole (*Zebrias zebra*) (Stephens, 2011) and PrCV of 32–35 and LL of 82–121 have been suggested for *B. panoides* (Lapierre, 2007). Therefore, such a range of characters without support by other morphological features cannot be a reason for the separation of species. As we noted earlier, the meristic characters of the 3 nominal species of *Typhlachirus* overlap, and extreme values were found even in specimens from the same sample. Accordingly, these characters cannot be used as diagnostic ones.

The presence or absence of a pectoral fin on the right side of the body is noted in several works as an important character and, in fact, the only character that distinguishes *T. lipophthalmus* and *T. elongatus* from *T. caecus* (Hardenberg, 1931a; Pellegrin and Chevey, 1940; Evseenko and Bolshakov, 2018). According to their descriptions, *T. lipophthalmus* (Pellegrin and Chevey, 1940; Chabanaud, 1948; Tan and Grinang, 2020) and *T. elongatus* (Pellegrin and Chevey, 1940; Evseenko and Bolshakov, 2018) lack the pectoral fin on the ocular side of the body. All of our specimens have a pectoral fin on the right side of the body regardless of body length. However, it is not always visible (sometimes presented as a skin outgrowth) or consists

Table 4

Meristic characters and body proportions for 3 species of the genus *Typhlachirus* based on data from the literature on all known specimens of *T. lipophthalmus*, *T. caecus*, and the Mekong blind sole (*T. elongatus*) (Chabanaud, 1948; Hardenberg, 1931a; Pellegrin and Chevey, 1940; Tan and Grinang, 2020). Features include standard body length (SL), number of rays in dorsal fin (D), number of rays in anal fin (A), number of rays in caudal fin (C), number of rays in pectoral fin on the ocular (right) side of the body ($P1_d$) and on the blind (left) side ($P1_s$), number of pored scales on the horizontal branch of the ocular side lateral line not including scales on caudal fin (LL), number of precaudal vertebrae (PrCV), number of caudal vertebrae (CV), number of vertebrae (V), body depth at pectoral fin base (BD), and head length (HL).

Species	SL (mm)	D	A	C	$P1_d$	$P1_s$	LL	PrCV	CV	V	BD	HL
T. lipophthalmus	76	56	41	12	0	5	90	–	–	–	40	25
	38	55	40	11	0	5	–	–	–	–	44	23
	33	56	39	12	0	5	–	–	–	–	39	25
	61	54	39	12	0	0	100	9	25	34	43	26
	150	56	43	12	0	0	–	–	–	–	–	–
T. caecus	93	53	42	12	3	6	105	–	–	35	46	23
	82	53	39	12	4	4	85	9	27	36	45	25
	76	52	41	12	2	4	94	8	27	35	47	23
	74	53	39	12	4	5	–	9	26	35	45	24
T. elongatus	33	56	37	12	0	5	96	–	–	33	45	24
	28	53	34	12	2	5	104	–	–	33	43	25

of only 1 ray immersed under the skin (this ray becomes noticeable only after staining) (Figs. 3 and 4).

This fact prompted us to reexamine 3 specimens of *T. elongatus* described in Evseenko and Bolshakov (2018). On each of these specimens, we found a pectoral fin presented as a skin outgrowth that did not exceed the size of scales found in all specimens (Fig. 4A). The rays in the fins of these specimens became clearly visible only after staining with alizarin. In all likelihood, authors who pointed to the absence of the pectoral fin (Károli, 1882; Pellegrin and Chevey, 1940; Tan and Grinang, 2020) did not notice the fin, or it was reduced to a greater degree than in the specimens we examined. Chabanaud (1939) also noted that the number of rays in the pectoral fin varied even in specimens from one sample. He thought that the degree of development of a pectoral fin depends on the age or sex of the fish. We suggest that the presence or absence of a pectoral fin and the number of rays in it are not species-specific features for blind soles but are demonstrations of individual variability.

We studied and compared all available X-rays of blind sole and found no difference between them. It should be noted here that some of the type specimens have been lost: the location is unknown for the holotype and 2 paratypes of *T. caecus* and the holotype of *T. lipophthalmus* (Fricke et al., 2021). On the basis of the X-ray of the paratype of *T. caecus* and non-type *T. lipophthalmus*, we measured the proportions of the body (BD and HL) and meristic characters (D, A, C, and V). We found that they were within the range of proportional measurements and characters of the studied collection of *T. elongatus*.

The body coloration of these 3 blind sole species varies somewhat but is generally similar (Károli, 1882; Hardenberg, 1931a; Pellegrin and Chevey, 1940; Evseenko and Bolshakov, 2018; Tan and Grinang, 2020). Slight variations in fish colors may be due to phenotypic plasticity or fixation—the difference is visible in photographs between the fresh and fixed specimens (Tan and Grinang, 2020). Additionally, characteristics of the substrate, food items, season, and sex can influence color differences. Noticeable variations in coloration have been reported for *B. aspilos* from different localities, and the authors suggest that these differences do not depend on sex but on the substrate (Okamoto and Motomura, 2021).

It turns out that the only thing that can be used as a diagnostic feature is the place of capture. However, it should be taken into account that all 3 locations where the type material were caught are in coastal waters of the South China Sea. The early developmental stages of blind soles are unknown. However, with a few exceptions, most soles have pelagic eggs and larvae (Ahlstrom et al., 1984), which are freely carried by currents and settle on suitable substrates. There is no geographical boundary between the regions where the type specimens were caught (Morimoto et al., 2000; Qu, 2000). Therefore, the 3 nominal species of blind sole may be local populations of the same species.

Conclusions

We analyzed our materials and found that the features thought to be diagnostic do not work for separating the 3 species of *Typhlachirus*. All the described interspecific differences are within the range of variation found in our specimens from the Mekong River. The pectoral fin on the ocular side of fish of the genus *Typhlachirus* is a reduction feature; results of data analysis for the combined

sample indicate that the fin developed to varying degrees and development did not depend on the size of the fish. Therefore, the presence of the pectoral fin and the number of rays in it cannot serve to identify species. On the basis of this result, we come to the same conclusion as Chabanaud (1948): there are no sufficient causes for saving the independence of the 3 nominal *Typhlachirus* species. Chabanaud's revision was based on a small number of specimens, and in this work, non-type specimens from only one of the type localities were examined. Therefore, to confirm our conclusion about the possible monotypy of the genus, it is necessary to study available type specimens and new specimens of *T. lipophthalmus* and *T. caecus* from the regions where the type material was captured (off the island of Borneo Island and in the Strait of Malacca).

Acknowledgments

The study was supported by the state assignment to the Shirshov Institute of Oceanology of the Russian Academy of Sciences (no. 0149-2019-0009) and work was done on the theme "Ecolan E-3.4—Ecosystem of the Mekong River in the context of global climatic changes and anthropogenic impact." The work was held within the state assignment of the Lomonosov Moscow State University (no 121032500084-6).

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