

Morphological Characterization and Taxonomic Validation of *Spinibarbichthys maensis* (Cyprinidae) from the Ma Basin, Lao PDR

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Abstract

This study investigates the morphological variation and taxonomic status of the genus *Spinibarbichthys* based on 100 specimens collected from the Ma Basin (Et District, Houaphanh Province, Lao PDR). A total of 157 characters, comprising 138 morphometric measurements and 19 meristic counts, were analyzed. Statistical comparisons were conducted against the original descriptions of *S. maensis* and *S. denticulatus*, as well as two voucher specimens from Guangxi, China. Our results indicate that the Lao PDR population is conspecific with *S. maensis* and morphologically differs from *S. denticulatus* and the Chinese vouchers. Diagnostic traits supporting this distinction include significantly higher ratios in head length, body depth, caudal peduncle length, and dorsal fin height. Furthermore, *S. maensis* is characterized by a higher lateral line scale count (28–30 + 2 vs. 26–27 + 2 in *S. denticulatus*). These morphometric and meristic data collectively validate *S. maensis* and clarify its taxonomic distribution within the region.

Keywords

Spinibarbichthys, Taxonomic status, Ma River Basin, Lao PDR

Introduction

Lao PDR is a hydrographically significant nation, with 80% of its main watershed situated within the Mekong River Basin, while the remaining 20% drains eastward into the Gulf of Tonkin via the Ma and Neun River systems. The Ma River Basin (MRB) covers 28,400 km², shared between Vietnam (62.4%) and Laos (37.6%). This river originates at an elevation 2,178 m in Dien Bien province of Vietnam, and flows entirely 512 km with 80 km in Laos before discharging into the South China Sea (Doan Van Long 2015; Zuklin *et al.* 2021). Its main tributary, the Nam Et River, drains a catchment of 7,580 km² and originates from the Nam Et-Phou Louey National Protected Area in northern Laos (FAO 2023; Johnson, Krahn, and Seateun 2010). In this region, the dominant species of the genus *Spinibarbichthys* include two species, *S. denticulatus* frequently misidentified as *S. maensis* and *S. nammauensis* known from the Ma and Red River basins in northern Vietnam (Kottela 1989; Kottelat 2013). The genus *Spinibarbus* (Oshima 1919) is characterized by the presence of a small procumbent predorsal spine, 9 branched dorsal rays, 5 ½ branched anal rays, a medially interrupted post-labial groove, and 24–30 lateral line scales. *Spinibarbus* (*sensu stricto*) was previously distinguished from another genus *Spinibarbichthys* (Oshima 1925) in having the last unbranched dorsal ray smooth and slender (vs the rays robust and serrated in the latter) (Van der Laan, R. 2025; Laan, Eschmeyer, and Fricke 2014). Up to date there are 7 species regarded as valid species in *Spinibarbus* (*S. sinensis*, *S. hollandi*, *S. caldwelli*, *S. polylepis*, *S. brevicephalus*, *S. vittatus*, and *S. hoenoti*) and 8 species (*S. denticulatus*, *S. yunnanensis*, *S. nammauensis*, *S. ovalius*, *S. maensis*, *S. nigriventralis*, *S. nguyenhuuuduci*, and *S. nigripinnis*).

Despite the detail morphological description available for species, the taxonomic status of *Italic* is still uncertain, particularly regarding on the populations at the intersection of southern China and northern Vietnam (Endruweit 2014). Molecular divergence studies indicated significant temporal speciation; for example the split between *S. hollandi* and *S. caldwelli* which was estimated to occur in 5.6–4.9 million years ago, suggesting that the former was a relict species in Taiwan while the latter dispersed across the Asian mainland (Tang *et al.* 2005) occurring in southern China and northern Vietnam, was resolved on the basis of molecular and morphological analyses. *Spinibarbus caldwelli* and *Spinibarbus hollandi* have a smooth posterior edge of the last unbranched dorsal fin ray among species in the genus. *Spinibarbus caldwelli* is currently regarded as a junior synonym of *S. hollandi* because of ambiguities in diagnostic characters. In this article, 11 mtDNA cytochrome b sequences of *Spinibarbus* specimens were analyzed together with *Barbodes gonionotus* and *Puntius conchionius* as outgroups. Our results showed that specimens identified as *S. hollandi* from Taiwan were different from those from the Asian mainland at a high level of genetic divergence (0.097–0.112). Furthermore, COI gene sequence analysis has identified distinct genetic differentiation among populations of *S. denticulatus* in the Ha Giang, Tuyen Quang, and Hoa Binh regions (Trang Vu Thi *et al.* 2021). Tuyen Quang, and Hoa Binh were analyzed for genetic diversity based on COI gene sequence analysis. Results showed that the studied populations have relatively high haplotype diversity (Hd). These findings emphasize a critical exigency for updating, integrative taxonomic research that utilizes both morphological data and genetic markers *e.g.* The COI analysis is currently being initiated for *Spinibabichthys* in Ma River Basin, Lao PDR to resolve existing uncertainties. Such clarity is necessary for establishing effective conservation strategies for these species evaluated as the rising threats of habitat modification and climate change in Northern Lao PDR.

Materials and Methods

Study site and sampling collection

Fish sampling was conducted in the Ma Basin (MB) in Houaphanh Province Northern Lao PDR. The first sampling area (20°49'13.39"N, 104°00'20.73"E) is situated at approximately

319 m above sea level and spans roughly 200 km from downstream to upstream (Figure 1). The remaining sites include six river sites (Three sites in the mainstream of Ma River, and three sites in tributary of Et River; one fishpond, and three markets. A total of 100 specimens were obtained during two seasonal periods comprising 64 specimens in the wet season (May 28–31, 2023) and 36 specimens in the dry season (December 6–14, 2023). The standard length (SL) of specimens ranging from 54.57 mm to 325.00 mm SL, represents broad size distribution from juveniles to large adults. Specimen counts by site were accounted as following Ma River: MR-S1 (n = 0), MR-S2 (n = 3), MR-S3 (n = 2); Et River: ER-S4 (n = 17), ER-S5 (n = 37), ER-S6 (n = 7); Fishpond: NKH-FP (n = 2) and market sites of ET-MK (n = 5), SN-MK (n = 12), NN-MK (n = 15), and Guangxi, China (n = 2). Specimens were initially fixed in 10% formalin in the field. After one week, they were transferred to 70% ethanol. Three representative specimens were selected for genetic (DNA) analysis. All specimens were deposited at the Ubon Ratchathani University Natural History Museum of Fisheries (UNMF).

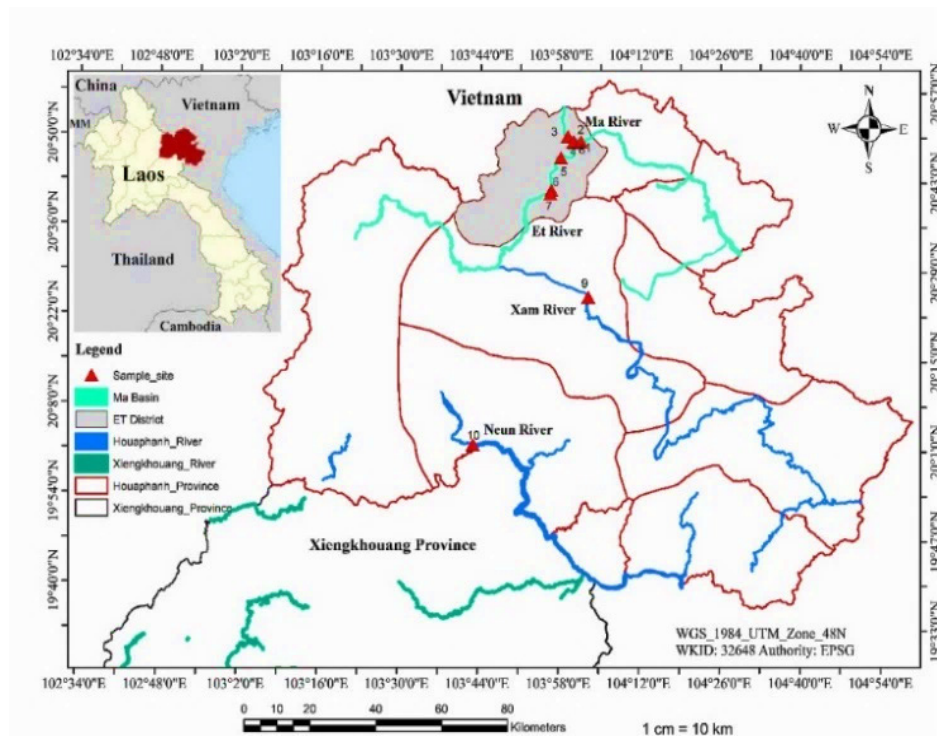


Figure 1. The map shows the locations of collection sites within the Ma River Basin, including the Ma River (MR-S1 at Vang Tha Na, MR-S2 at Pak Nam Et, and MR-S3 at Ban Luu), the Et River (ER-S4 in the Fish Conservation Zone, ER-S5 at Ban Na Mend, and ER-S6 at Ban Na Khouang), the three market sites (ET-MK: Et Market, SN-MK: Sam Neua Market, and NN-MK: Nam Neun Market), and the fishpond (NKH-FP at Ban Na Khouang Fishpond).

Specimens examined:

A total of 100 specimens were collected from Ma River Basin along with two additional specimens obtained from China*. The specimens were classified into four groups according to standard length (SL) of the juvenile group 43 specimens (50–100 mm SL); small-sized group 26 specimens (101–150 mm SL); medium-sized group 22 specimens (151–200 mm SL); and large-sized group 9 specimens (>200 mm SL). Additionally, 2 large-sized specimens from China* (490 mm and 520 mm SL) were included in the analysis (Table1).

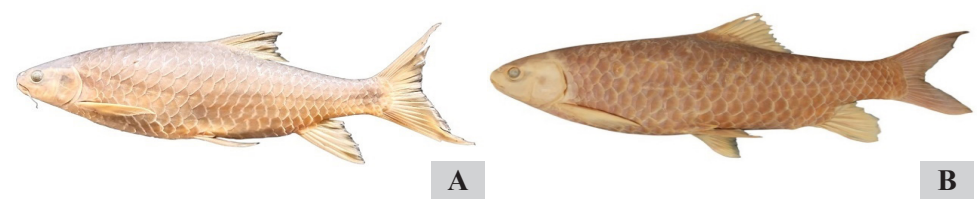


Figure 2. (A) *S. maensis* standard length (325 mm SL), from Ma River basin, northern Laos; (B) *S. denticulatus* (520 mm SL) from Guangxi China* Change: (GN), now specimen in UNMF.

Table 1. The size classes according to standard length and total length (asterisk indicates type specimens of *S. denticulatus*).

Group Size (mm)	N	Standard length (SL)		Total length (TL)	
		Range	Mean±SD	Range	Mean±SD
50-100	43	54.57–99.60	83.78±9.87	70.94–109.10	107.03±13.26
101-150	26	100.50–154.15	120.08±16.13	120.34–190.00	154.15±23.45
151-200	22	151.00–200.00	172.81±13.46	122.00–252.50	212.80±34.90
> 200	9	205.00–325.00	262.59±43.47	262.00–425.00	344.06±56.93
China*	2	490.00–520.00	505.00±15.00	450.00–456.00	453.00±3.00

Counts and measurements

Counts and measurements were taken on the left side of specimens. The size < 200 mm SL (n = 91) were measured to the nearest 0.01 mm by using digital calipers, while the size > 200 mm SL (n = 9) were taken to an accuracy of 0.05 mm. The study analyzed 65 morphometric and meristic traits across four groups: (A) body/fin lengths (31 points); (B) head/mouth lengths (34 points); (C) fin ray and scale counts, and (D) a body network of 15 reference points (a–o) following by (Armbruster 2012; Gernot K et al. 2020; Indochinese Mekhong 2021; Kottelat 2020; Takács et al. 2016)landmark-based (partial truss (Figure 3).

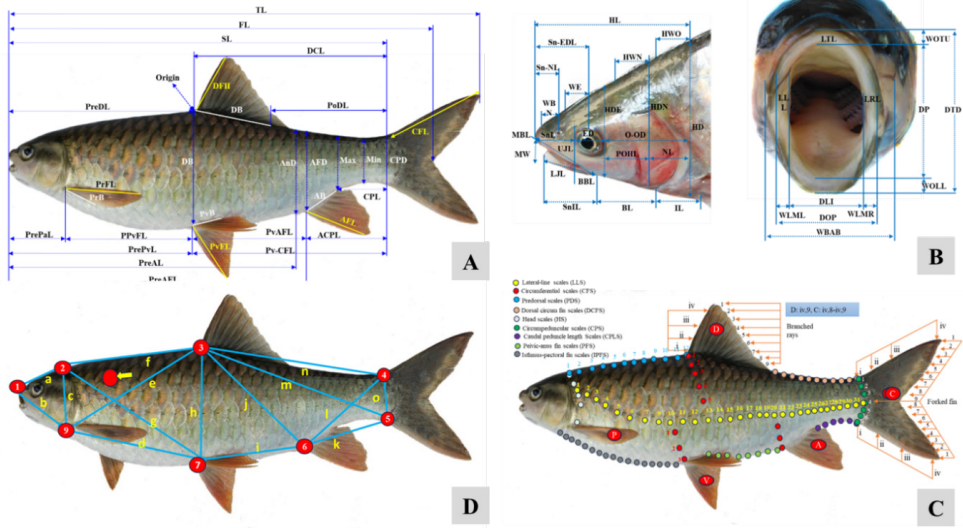


Figure 3. (A) body and fin lengths–31 points (1) SL: standard length; (2) F= fork length, (3) TL= total length; (4) BD= body depth; (5) BW= body width; (6) AnD= anal depth; (7) AFD= anal fin depth; (8) MaCPD= maximum caudal peduncle depth; (9) MiCPD= minimum caudal peduncle depth; (10) CPD= caudal peduncle depth; (11) CPL= caudal peduncle length; (12) CFL= caudal fin length; (13) PreDL= predorsal length; (14) DCL= dorsal caudal length; (15) PoDL= postdorsal length; (16) PrePaL= prepectoral length; (17) PrePvL= prepelvic length; (18) PreAL= preanal length; (19) PreAFL= preanal fin length; (20) PPvFL= pectoral–pelvic fin length; (21) PvAFL= pelvic–anal fin length; (22) ACPL= anal fin–caudal peduncle length; (23) Pv-CFL= prepelvic fin–caudal fin length; (24) DFH= dorsal fin height; (25) PrFL= pectoral fin length; (26) PvFL= pelvic fin length; (27) AFL= anal fin length; (28) DB= dorsal fin base length; (29) PrB= pectoral fin base length; (30) PvB= pelvic fin base length; (31) AB= anal fin base length. (B) (1) HL= head length; (2) SnL= snout length; (3) ED= eye diameter; (4) O-OD= orbit-operculum distance; (5) POHL= post orbital head length; (6) NL= nape length; (7) Sn-EDL= snout–eye diameter length; (8) Sn-NL= snout–nostril length; (9) HDN= head depth at naps; (10) HDE= head depth at eyes; (11) HWO= head width at operculum; (12) HWE= head width at eyes; (13) WBE= width between eye; (14) WBN= width between nostrils; (15) IL= isthmus length; (16) BL= breast length; (17) SnIL= snout–isthmus length; (18) MBL= maxillary barbel length; (19) RBL= rostral barbel length; (20) WBAB= width between anterior barbels; (21) UJL= upper jaw length; (22) LJL= lower jaw length; (23) MW= mouth width; (24) LLT= length of lip type; (25) LLL= left labellum length; (26) RLL= right labellum length; (27) TDD= torus disc depth; (28) PDI= pulvinus depth inside; (29) UTW= upper torus width; (30) LLW= lower labrum width; (31) PDO= pulvinus depth outside; (32) DIL= depth of inside lip; (33) WLML= width of left mouth lip; (34) WRML= width of right mount lip. (C) Meristic counts of fin rays (five fins) of (1) D= dorsal-fin rays; (2) P= pectoral-fin rays; (3) V= pelvic-fi rays; (4) A= anal-fin rays; (5) C= caudal-fin rays, and scales (nine characters) of (1) LLS= lateral–line scales; (2) PrDS= predorsal scales; (3) CFS= circumferential scales; (4) CPS=circumpeduncular scales; (5) PoDS= postdorsal scales; (6) HS= head scales; (7) IPFS= isthmus–pectoral fin scales; (8) PFS= pelvic–anus fin scales, and (9) PoAS= Postanal scales, and (D) 15 body network: (1) a= snout–head; (2) b= snout–pectoral fin; (3) c= head–pectoral fin; (4) d= pectoral fin–pelvic fin; (5) e= pectoral

fin–dorsal fin; (6) f= head–dorsal fin; (7) g= head–pelvic fin; (8) h= dorsal fin–pelvic fin; (9) i= pelvic fin–anal fin, (10) j= dorsal fin–anal fin; (11) k= anal fin–caudal fin low; (12) l= anal fin–caudal fin up; (13) m= dorsal fin–caudal fin low; (14) n= dorsal fin–caudal fin up; (15) o= caudal peduncle up–low flowing method.

Molecular Analysis

The obtained sequences were compared with published data from GenBank (GB) to determine genetic distances and confirm taxonomic relationships. The comparison included four sequences of *S. denticulatus* (Accession numbers: GU086582, JX042168, AP013335, and KJ994631), which showed pairwise identities ranging from 97.2% to 99.4% with the sequences obtained in this study. Two sequences of *S. sinensis* (Accession numbers: MT572009.1 and HM536895) were also included, showing lower identities of 95.3% and 98.2%, respectively. Genetic distance calculations were performed using the Kimura 2-Parameter model (Kimura, 1980) and related methodologies (Kimura 1980; Kumar et al. 2024; Zhou et al. 2024) also, the evolutionary rates when the divergence times are known. This study DNA barcoding is based on the mitochondrial cytochrome oxidase subunit I (COI) gene (Ward et al. 2005) mostly Australian marine fish, were sequenced (barcoded). Three samples of this study were analyzed, with one representative sequence (Accession number: MW446151.1) showing a 99.5% sequence similarity to corresponding records in GenBank. The forward and reverse primers used for COI gene amplification were expressed as following. Used Molecular Evolutionary Genetics Analysis (MEGA12).

FishF1: 5'- CTGAGCCGGAATAGTAGGAAGTGCCTTAAGCCTCCTTATTCGGG
CTG AACTAAGTCAACC-3'

FishR1: 5'- AGGAGGAGACCCAATCCTTTATCAACACTTATTCTGATTCTTTGG
CCA CCCAGAAGT-3'

Statistical analysis

Using OriginPro® 2025 by two-way ANOVA Tukey's test was applied to identify significant differences ($P < 0.05$) at a 95% confidence level in the unction Principal Component Analysis (PCA). The dendrogram clustered info of variable range in function of Hierarchical Cluster Analysis (HCA). The network on the body was applied to compare measurement distances between groups, following the methods (Armbruster 2012; Kumar et al. 2024; Lê, Josse, and Husson 2014; USA 2024; Xiao et al. 2022). we present FactoMineR an R package dedicated to multivariate data analysis. The main features of this package is the possibility to take into account different types of variables (quantitative or categorical, and MEGA12.

Results and Discussion

Morphometrics examined

Description. Selected meristic and morphometrics, including comparative data, expressed as percentages of, are presented in (Table 2):

Fin ray count. Meristic data revealed subtle differences between species. *Spinibarbichthys maensis* is mostly similar with *S. denticulatus* in dorsal and anal fin rays, pharyngeal teeth and gill rakers. However, there is partially an overlapping range in total vertebrae (28-41 in *S. maensis* vs. 38-43 in *S. denticulatus*).

Body proportions. Body depth (BD) and body width (BW) were similar between the two species although *S. maensis* exhibited a wider range. The maximum caudal peduncle depth (MaxCPD) is 14.65% in *S. maensis* and 11.86% in *S. denticulatus*, indicating a deeper peduncle in *S. maensis*. The predorsal, preanal, and prepelvic lengths showed notable differences. For

example, preanal length (PreAL) was greater in *S. maensis* (76.13%) than in *S. denticulatus* (57.72%). In *S. maensis*, the caudal peduncle length (CPL = 30.53%) and anal-fin–caudal peduncle length (ACPL = 30.33%) were the larger proportions, while the pectoral-fin base (PrB = 4.21%) and pelvic-fin base (PvB = 5.13%) were the smaller. The caudal peduncle length (CPL) was a major distinguishing feature of which *S. maensis* is longer than *S. denticulatus* (25.37–40.01%, mean $30.53 \pm 3.10\%$ vs. 19.62–22.04%, mean $20.83 \pm 1.72\%$, respectively). In percentage of caudal peduncle length, the caudal peduncle depth of *S. maensis* is lower than that of *S. denticulatus* (98.43% vs. 96.96%). In contrary, the maximum and minimum of caudal peduncle depth of the former is also higher than the latter (122.29% vs. 110.93% and 110.00% vs. 94.75%, respectively).

Head of morphology. In percentage of the standard length, the head depth at eye (HDE) is rather similar for *S. maensis* and *S. denticulatus* (means $66.32 \pm 4.99\%$ vs. $65.39 \pm 4.27\%$, respectively). Eye diameter (ED) was obviously smaller in *S. maensis* ($27.18 \pm 3.40\%$) than in *S. denticulatus* ($36.35 \pm 1.35\%$), while postorbital head length (POHL) was nearly identical ($27.83 \pm 4.60\%$ vs. $28.25 \pm 1.64\%$). The orbit–operculum distance (O-OD) was greater in *S. denticulatus* (39.05%) than in *S. maensis* (33.83%). Relative to head length, *S. maensis* exhibited longer barbels than *S. denticulatus* (RBL 31.81% vs. 16.26% and MBL 24.16% vs. 9.39%, respectively). The upper- and lower-jaw lengths are also greater in *S. maensis* than in *S. denticulatus* (UJL 29.74% vs. 22.20% and LJL 24.69% vs. 19.15%, respectively).

Mouth and pulvinus features. The depth of torus disc (DTD) and depth of pulvinus in (DPI) were notably larger in *S. maensis* than in *S. denticulatus* (DTD 8.02% vs. 3.95% and DPI 6.18% vs. 3.34%, respectively). The depth of pulvinus out (DPO) was slightly larger in *S. maensis* (5.29%) than in *S. denticulatus* (4.56%). Upper-jaw and lower-jaw lengths were greater in *S. maensis* compared with *S. denticulatus* (7.15% vs. 3.57% and 5.94% vs. 3.07, respectively), suggesting a larger mouth or gape in *S. maensis*. Lip-type measurements (LTL, LLL, LRL) followed the same trend.

Barbel lengths. Barbels were consistently longer in *S. maensis*. The rostral barbel length (RBL) was $7.65 \pm 1.42\%$ in *S. maensis* and $2.60 \pm 1.23\%$ in *S. denticulatus* as well as the maxillary barbel length (MBL) with 5.80% and 1.50%, respectively. The width between anterior barbels (WBAB) was also greater in *S. maensis* (7.75%) than in *S. denticulatus* (4.16%).

Variation and intraspecific differences. *S. maensis* displayed high variability in several traits, notably depth of pulvinus in DPI, $SD=1.1$ and rostral barbel length (RBL, $SD=1.42$). In contrast, *S. denticulatus* exhibited lower standard deviation in the snout-isthmus length (SnIL, $SD=0.04$), likely due to its small sample size ($n=2$) rather than true population uniformity.

Disc and pulvinus proportions. (DTD) were distinctly larger in *S. maensis* than in *S. denticulatus*. The depth of pulvinus (DP) and depth of lips inside (DLI) were relatively greater in *S. denticulatus*, indicating deeper internal lip structures. Lip widths (WLML/WLMR) showed minimal interspecific difference, ranging 3.78–3.98% for *S. maensis* and 4.02–4.52% for *S. denticulatus*.

Lateral-line scales (LLS). *S. maensis* exhibits a range of 28–30+2, with 31 being the modal value (54%). In contrast, *S. denticulatus* shows a narrower range of 26–27+2. This non-overlapping difference in the common count range indicates that LLS may be a key diagnostic character distinguishing the two species. However, limit in the few specimens of *S. denticulatus* is not adequate to clarify this difference. In circumferential scales, *S. maensis* typically possesses 5+3–4 scale rows, whereas *S. denticulatus* consistently exhibits 5+3. In predorsal scales, *S. maensis* shows a range of 9–15 (mode 12), while *S. denticulatus* has fewer predorsal scales, ranging 9. In circumpeduncular scales, *S. maensis* ranges from 12–16 (mode 14), whereas *S. denticulatus* also exhibits 14 but without the broader variation observed in *S. maensis*.

Coloration when fresh. Dorsum scattered dark roseate markings which extend across the body to caudal peduncle; Lateral line scales lined by a distinctly visible stripe along the body length, sometimes appearing yellow with clear black pigment spots (melanophores) running along the line lateral line to extend onto the caudal peduncle; Nape light roseate and with middle black spot and with to the eye; orbit–operculum region dark roseate; pectoral, pelvic, and anal fins with dark roseate markings; Dorsal and caudal fins dark gray; small fish with small dark spots present on both.

Distribution. The species is distributed in the Nam Ma River basin, covering both Laos and Vietnam (specifically in the mainstream and its tributaries). In Laos, its confirmed range includes the tributaries of the Nam Ma system such as Nam Et River, the Xam River (located in Houaphanh provinces), and the Neun River (located in Houaphanh and Xiengkhoung Provinces). The distribution is likely broader, probably extending into other river basins that drain into those three rivers in northern Laos.

Taxonomic significance and diagnostic characters. Extremely large differences in maxillary barbel length (MBL) and rostral barbel length (RBL), regardless of the proportional reference used (standard length, head length, or eye diameter), establish these features as robust primary diagnostic characters distinguishing *S. maensis* from *S. denticulatus*. Significant difference in caudal peduncle depth (CPD) provide the additional, strong secondary distinguishing characters.

Species status. The consistency and magnitude of these differences across multiple morphometric bases strongly support the conclusion that *S. maensis* and *S. denticulatus* are valid, distinct species, each exhibiting unique morphological adaptations likely linked to different ecological niches. Specimens of the genus *Spinibarichthys* collected from the Ma River basin (MRB) show significant morphological divergence when compared to *S. denticulatus* from China*. *S. maensis* exhibits several notable differences, including greater head length and slightly deeper body depth; and larger caudal peduncle length, prepelvic length, dorsal fin height, snout length and eye diameter. The pelvic-fin origin of *S. maensis* is anterior to the dorsal-fin origin, whereas that of *S. denticulatus* posterior the dorsal-fin origin. The combination of these morphological differences provides clear evidence supporting the valid status of *S. maensis* distinguished from *S. denticulatus*.

Principal Component Analysis (PCA) It further supports the morphological separation between *S. maensis* and *S. denticulatus*. By using the correlation matrix, the first two principal components including PC1 (primarily associated with head length, HL) and PC2 (primarily associated with body depth, BD) collectively explained 48.9% of the total variance, with PC1 accounting for 40.6% and PC2 for 8.3% in % SL (Figure 4A).

Polar heatmap with dendrogram of circular hierarchical clustering heatmap illustrates gene expression patterns across samples. The dendrogram demonstrates similarity relationships among genes, while the heatmap colors indicate expression intensity, with red representing higher expression and blue representing lower expression. Distinct clustering patterns were observed, with most genes showing low-to-moderate expression levels and a subset exhibiting relatively higher expression, suggesting differential gene regulation among the studied samples the measured variables % SL (Figure 4B).

PCA–revealed distinct patterns of morphological variation among the four size groups. The first two principal components, PC1 and PC2, explained 26.2% and 11.7% of the total variation, respectively. Although the cumulative variance was moderate, the ordination plot showed meaningful separation among groups. Overall, the PCA demonstrated that morphological variation was structured primarily along PC1, with size-associated traits driving separation among groups. Despite some overlap, the ordination revealed clear tendencies for larger size classes to cluster together and to be associated with specific morphological characters in % HL (Figure 4C).

HCA—was performed by using 64 morphometric variables expressed as percentages of standard length (%SL). Clustering was carried out by applying the unweighted pair group method with arithmetic mean (UPGMA), a standard group-average linkage method, with a distance correlation metric. The resulting dendrogram summarized the grouping patterns across dissimilarity values ranging from 0.001–0.004. The largest cluster by sample size was the “Total” cluster (n=100), whereas the smallest cluster was the China* (n=2) (Figure 4E).

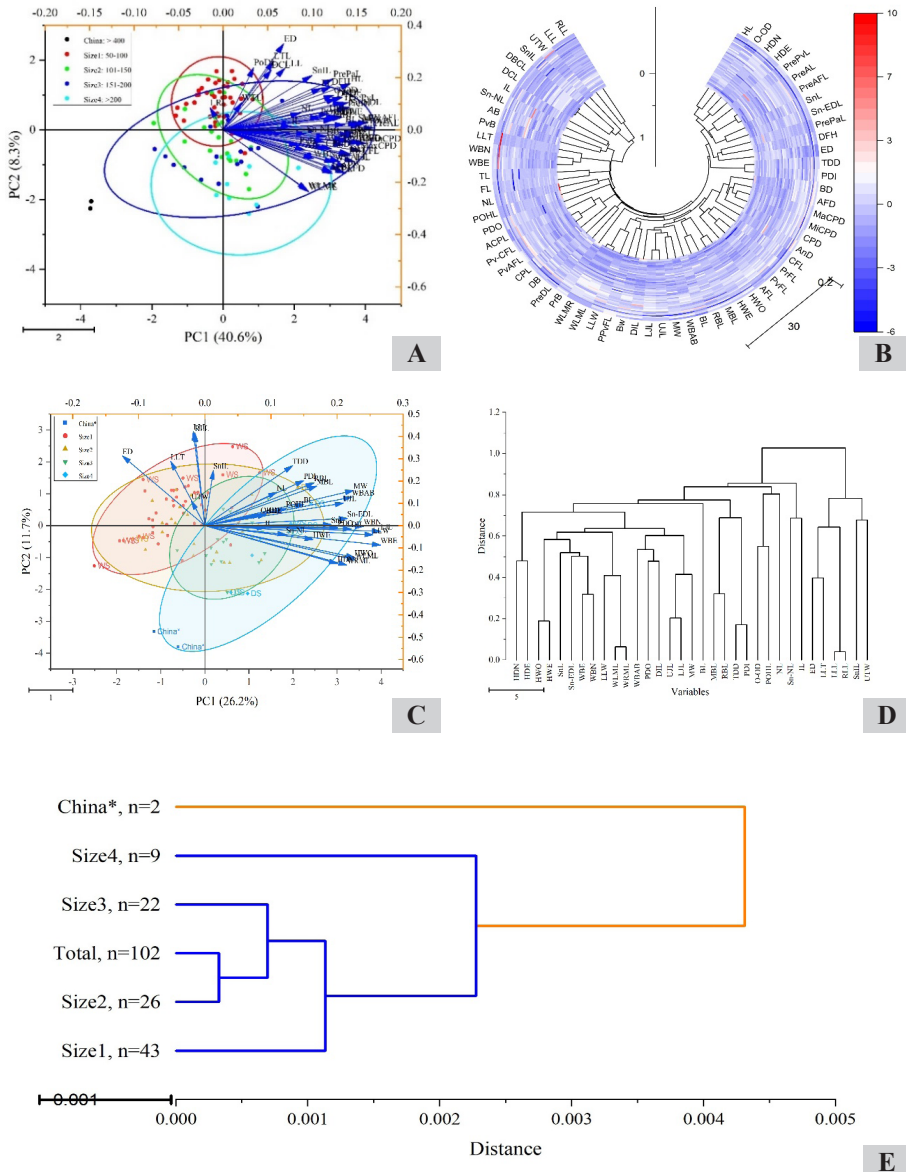


Figure 4. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA): **(A)** PCA–Biplot in percentage standard length (% SL); **(B)** Polar heatmap of 64 variables from 102 specimens in % SL; **(C)** PCA–Biplot of 33 variables in percentage head length (%HL); **(D)** HCA–clustering of 33 variable in % HL, and **(E)** HCA–cluster variable in groups average with correlation distance of *Spinibarbichthys maensis* and *S. denticulatus*.

Table 2. Morphometric data for examined of *Spinibarbichthys maensis* from Ma Basin Laos and *Spinibarbichthys denticulatus* from Guangxi, China* (type specimens) Continued.

Character states	<i>S. maensis</i>					<i>S. denticulatus</i>				
	n	Min	Max	Mean	SD	n	Min	Max	mean	SD
Standard length (mm SL)	100	54.57	325.00	105.06	56.93	2	490.00	520.00	505.00	21.21
Head length (mm HL)	100	14.18	74.69	28.57	13.03	2	77.50	85.00	81.16	3.75
Percent of standard length (%)										
Head length (HL)	100	19.47	30.62	24.13	1.78	2	15.82	16.35	16.08	0.37
Body depth (BD)	100	25.11	44.87	32.36	3.28	2	24.04	24.69	24.37	0.46
Body width (BW)	100	12.04	23.82	16.34	2.48	2	11.54	12.24	11.89	0.50
Total length (TL)	100	61.65	156.25	127.47	12.25	2	87.69	91.84	89.76	2.93
Fork length (FL)	100	67.50	135.75	112.83	8.41	2	80.77	89.80	85.28	6.38
Min caudal peduncle depth (MiCPD)	100	9.99	16.48	13.00	1.09	2	9.23	9.59	9.41	0.26
Max caudal peduncle depth (MaCPD)	100	12.63	18.68	14.65	1.10	2	11.22	12.50	11.86	0.90
Anal depth (AnD)	100	18.58	32.92	23.76	2.19	2	18.27	18.98	18.62	0.50
Anal fin depth (AFD)	100	17.70	27.06	21.03	1.97	2	17.12	18.78	17.95	1.17
Caudal peduncle depth (CPD)	100	10.45	15.84	12.51	0.92	2	9.80	11.54	10.67	1.23
Caudal fin length (CFL)	100	10.65	18.38	13.30	1.35	2	9.62	9.80	9.71	0.13
Pre-dorsal length (PreDL)	100	43.38	63.71	53.85	3.24	2	40.38	40.82	40.60	0.31
Dorsal caudal length (DCL)	100	29.23	59.52	40.34	8.41	2	24.49	35.58	30.03	7.84
Postdorsal length (PoDL)	100	11.38	41.93	25.07	8.28	2	11.22	24.04	17.63	9.06
Pre-pectoral length (PrePaL)	100	19.83	34.59	25.31	2.34	2	14.69	15.77	15.23	0.76
Pre-pelvic length (PrePvL)	100	44.72	74.02	54.49	3.90	2	36.53	38.46	37.50	1.37
Pre-anal length (PreAL)	100	60.50	93.95	76.13	5.40	2	57.69	57.76	57.72	0.04
Pre-anal fin length (PreAFL)	100	62.45	94.31	79.19	5.42	2	59.18	59.62	59.40	0.31
Pectoral-pelvic fin length (PPvFL)	100	19.50	37.71	30.47	3.27	2	21.22	23.27	22.25	1.45
Pelvic fin-caudal fin length (Pv-CFL)	100	39.25	64.09	49.63	4.45	2	41.02	41.92	41.47	0.64
Pelvic-anal fin length (PvAFL)	100	21.66	35.08	28.01	2.81	2	22.86	24.04	23.45	0.84
Anal fin-caudal peduncle length (ACPL)	100	8.63	30.33	21.52	2.56	2	17.88	18.16	18.02	0.20
Caudal peduncle length (CPL)	100	25.37	40.01	30.53	3.10	2	19.62	22.04	20.83	1.72
Dorsal fin height (DFH)	100	16.57	33.56	23.12	2.29	2	12.24	13.46	12.85	0.86
Pectoral fin length (PrFL)	100	11.20	25.79	20.38	2.22	2	15.58	16.94	16.26	0.96
Pelvic fin length (PvFL)	100	9.50	24.01	18.78	1.99	2	14.42	15.31	14.86	0.62
Anal fin length (AFL)	100	12.01	31.33	18.65	2.77	2	14.42	15.51	14.97	0.77
Dorsal fin base length (DB)	100	12.14	19.82	15.27	1.46	2	11.54	13.27	12.40	1.22
Pectoral fin base length (PrB)	100	2.87	5.78	4.21	0.65	2	3.06	3.46	3.26	0.28
Pelvic fin base length (PvB)	100	3.75	7.60	5.13	0.78	2	3.46	3.67	3.57	0.15

Table 2. Morphometric data for examined of *Spinibarbichthys maensis* from Ma Basin Laos and *Spinibarbichthys denticulatus* from Guangxi, China* (Continued).

Character states	<i>S. maensis</i>					<i>S. denticulatus</i>				
	n	Min	Max	Mean	SD	n	Min	Max	mean	SD
Anal fin base length (AB)	100	5.27	10.73	7.74	1.02	2	6.54	7.96	7.25	1.00
Snout length (SnL)	100	6.86	11.98	8.71	0.98	2	5.81	5.92	5.86	0.08
Eye diameter (ED)	100	4.46	8.56	6.56	0.96	2	3.06	3.08	3.07	0.01
Orbit-operculum distance (O-OD)	100	8.05	12.91	10.57	1.01	2	5.38	7.14	6.26	1.24
Post orbital head length (POHL)	100	3.55	10.84	6.71	1.20	2	4.29	4.81	4.55	0.37
Nape length (NL)	100	2.30	10.65	4.09	0.84	2	2.31	2.45	2.38	0.10
Snout- eye diameter length (Sn-EDL)	100	8.18	16.21	11.05	1.09	2	7.35	7.69	7.52	0.24
Snout-nostril length (Sn-NL)	100	3.78	10.99	5.47	0.92	2	3.85	3.88	3.86	0.02
Head depth at naps (HDN)	100	13.22	24.68	19.97	2.16	2	14.29	15.00	14.64	0.51
Head depth at eyes (HDE)	100	11.66	21.46	16.00	1.58	2	10.19	10.82	10.50	0.44
Head width of operculum (HWO)	100	12.42	21.31	15.45	1.61	2	10.41	10.58	10.49	0.12
Head width at eyes (HWE)	100	10.13	19.13	13.81	1.48	2	8.65	9.39	9.02	0.52
Width of eye (WE)	100	7.53	19.53	10.60	1.66	2	7.69	7.96	7.83	0.19
Width between nostrils (WBN)	100	5.19	16.16	6.79	1.19	2	4.18	4.42	4.30	0.17
Isthmus length (IL)	100	3.48	9.58	5.25	0.99	2	3.67	4.23	3.95	0.39
Maxillary barbels length (MBL)	100	3.01	8.21	5.80	1.03	2	0.96	2.04	1.50	0.76
Rostral barbels length (RBL)	100	3.88	10.62	7.65	1.42	2	1.73	3.47	2.60	1.23
Width between anterior barbels (WBAB)	100	5.97	12.84	7.75	1.00	2	4.08	4.23	4.16	0.11
Upper jaw length (UJL)	100	5.49	9.21	7.15	0.86	2	3.46	3.67	3.57	0.15
Lower jaw length (LJL)	100	3.91	7.92	5.94	0.93	2	2.88	3.27	3.07	0.27
Mouth width or interorbital width (MW)	100	5.69	10.64	7.46	0.92	2	3.46	3.88	3.67	0.29
Disc of torus depth (DTD)	100	5.34	13.24	8.02	1.13	2	3.67	4.23	3.95	0.39
Depth pulvinus in (DPI)	100	3.62	11.43	6.18	1.16	2	2.45	4.23	3.34	1.26
Depth of pulvinus out (DPO)	100	5.29	10.15	7.48	0.94	2	4.23	4.90	4.56	0.47
Depth lip inside (DLI)	100	4.34	8.18	5.67	0.75	2	4.29	4.81	4.55	0.37
Snout isthmus length (SnIL)	100	2.57	14.86	8.50	1.44	2	4.23	4.29	4.26	0.04
Breast length (BL)	100	4.43	11.21	6.35	0.97	2	2.24	4.81	3.53	1.81
length of lip type (LLT)	100	1.22	4.47	3.11	0.70	2	1.02	1.92	1.47	0.64
Labellum left length (LLL)	100	1.54	4.06	2.86	0.55	2	0.82	0.96	0.89	0.10
Labellum right length (LRL)	100	1.39	3.96	2.83	0.55	2	0.96	8.57	4.77	5.38
Width of torus upper (WTU)	100	0.07	2.03	0.80	0.38	2	0.38	0.41	0.40	0.02
Width of labrum lowe (WLL)	100	0.32	1.74	0.94	0.24	2	0.38	0.51	0.45	0.09
Width lip mouth left (WLML)	100	0.18	1.89	0.93	0.40	2	0.58	0.71	0.65	0.10
Width lip mouth right (WLMR)	100	0.20	1.75	0.92	0.38	2	0.58	0.71	0.65	0.10
Percent of head length (%)										
Head depth at naps (HDN)	100	55.41	101.83	82.86	8.08	2	90.32	91.76	91.04	1.02
Head depth at eyes (HDE)	100	53.13	79.59	66.32	4.99	2	62.35	68.39	65.37	4.27

Table 2. Morphometric data for examined of *Spinibarbichthys maensis* from Ma Basin Laos and *Spinibarbichthys denticulatus* from Guangxi, China* (Continued).

Character states	<i>S. maensis</i>					<i>S. denticulatus</i>				
	n	Min	Max	Mean	SD	n	Min	Max	mean	SD
Head width of operculum (HWO)	100	53.01	85.40	64.12	6.08	2	64.71	65.81	65.26	0.78
Head width at eyes (HWE)	100	40.41	79.85	57.27	5.06	2	52.94	59.35	56.15	4.54
Snout length (SnL)	100	26.92	49.43	36.14	3.53	2	35.53	37.42	36.47	1.34
Eye diameter (ED)	100	20.15	33.38	27.18	3.40	2	18.82	19.35	19.09	0.38
Orbit-operculum distance (O-OD)	100	33.68	50.11	43.83	3.37	2	32.94	45.16	39.05	8.64
Post orbital head length (POHL)	100	14.96	43.41	27.83	4.60	2	27.10	29.41	28.25	1.64
Nape length (NL)	100	11.54	26.87	16.81	2.17	2	14.12	15.48	14.80	0.97
Snout- eye diameter length (Sn-EDL)	100	34.88	55.14	45.72	3.11	2	46.45	47.06	46.76	0.43
Snout-nostril length (Sn-NL)	100	14.97	43.77	22.72	3.64	2	23.53	24.52	24.02	0.70
Width between eye (WBE)	100	33.94	55.69	43.45	5.14	2	47.06	50.32	48.69	2.31
Width between nostrils (WBN)	100	19.36	35.93	27.86	3.21	2	26.45	27.06	26.76	0.43
Isthmus length (IL)	100	13.68	37.21	21.82	4.06	2	23.23	25.88	24.55	1.88
Breast length (BL)	100	18.10	39.25	26.33	3.63	2	14.19	29.41	21.80	10.76
Snout isthmus length (SnIL)	100	26.40	50.45	35.73	4.45	2	25.88	27.10	26.49	0.86
Maxillary barbels length (MBL)	100	12.79	33.15	24.16	3.97	2	5.88	12.90	9.39	4.96
Rostral barbels length (RBL)	100	16.47	47.29	31.81	5.43	2	10.59	21.94	16.26	8.02
Width between anterior barbels (WBAB)	100	23.48	43.61	32.13	3.38	2	25.81	25.88	25.84	0.05
Upper jaw length (UJL)	100	20.95	41.04	29.74	3.75	2	21.18	23.23	22.20	1.45
Lower jaw length (LJL)	100	15.70	34.54	24.69	3.87	2	17.65	20.65	19.15	2.12
Mouth width or interorbital width (MW)	100	25.07	44.73	30.95	3.50	2	21.18	24.52	22.85	2.36
length of lip type (LLT)	100	5.39	18.76	12.86	2.71	2	6.45	11.76	9.11	3.76
Left labellum length (LLL)	100	6.65	18.34	11.89	2.29	2	5.16	5.88	5.52	0.51
Right labellum length (RLL)	100	6.23	16.88	11.74	2.22	2	5.42	5.88	5.65	0.33
Torus disc depth (TDD)	100	21.96	44.97	33.27	4.20	2	23.23	25.88	24.55	1.88
Pulvinus depth inside (PDI)	100	14.60	40.31	25.60	4.42	2	15.48	25.88	20.68	7.35
Upper torus width (UTW)	100	1.01	5.91	3.22	1.19	2	2.35	2.58	2.47	0.16
Lower labrum width (LLW)	100	2.34	5.55	3.94	0.80	2	2.35	3.23	2.79	0.62
Pulvinus depth outside(PDO)	100	23.55	38.83	30.98	3.35	2	25.88	30.97	28.43	3.60
Depth of inside lip (DIL)	100	17.50	36.67	23.88	3.29	2	27.10	29.41	28.25	1.64
Width of left mouth lip (WLML)	100	1.06	7.99	3.88	1.65	2	3.53	4.52	4.02	0.70
Width of right mount lip (WRML)	100	1.24	7.16	3.78	1.52	2	3.53	4.52	4.02	0.70
Percent of caudal peduncle length (%)										
Caudal peduncle depth (CPD)	100	73.92	126.17	98.43	10.07	2	96.00	97.92	96.96	1.36
Max caudal peduncle depth (MaCPD)	100	88.66	141.83	110.93	10.67	2	114.58	130.00	122.29	10.90
Min caudal peduncle depth (MiCPD)	100	73.23	120.16	94.75	9.13	2	100.00	120.00	110.00	14.14
Percent of eye horizontal diameter (%)										
Maxillary barbels length (MBL)	100	47.52	161.28	90.17	21.04	2	31.25	66.67	48.96	25.04

Rostral barbels length (RBL)	100	57.44	245.23	119.32	29.57	2	56.25	113.33	84.79	40.36
Percent of disc of torus depth (%)										
Upper jaw length (UJL)	100	66.44	144.04	90.43	14.58	2	81.82	100.00	90.91	12.86
Lower jaw length (LJL)	100	47.20	128.11	75.08	14.08	2	68.18	88.89	78.54	14.64
Mouth width or Interorbital width (MW)	100	68.77	153.01	94.12	13.98	2	81.82	105.56	93.69	16.78
Length of lip type (LLT)	100	16.98	66.36	39.15	8.89	2	27.78	45.45	36.62	12.50
Labellum left length (LLL)	100	22.81	56.27	36.04	7.22	2	22.22	22.73	22.47	0.36
Labellum right length (LRL)	100	20.91	56.36	35.64	7.30	2	22.73	233.33	128.03	148.92
Depth pulvinus in (DPI)	100	55.56	99.02	76.72	7.51	2	66.67	100.00	83.33	23.57
Width of torus upper (WTU)	100	0.87	31.85	10.23	5.28	2	9.09	11.11	10.10	1.43
Width of labrum lowe (WLL)	100	4.71	21.95	11.87	3.18	2	9.09	13.89	11.49	3.39
Depth of pulvinus Out (DPO)	100	2.87	227.36	94.65	24.48	2	100.00	133.33	116.67	23.57
Depth lip inside (DLI)	100	49.41	126.24	72.41	12.57	2	113.64	116.67	115.15	2.14
Width lip mouth left (WLML)	100	2.69	24.64	11.72	5.06	2	13.64	19.44	16.54	4.11
Width lip mouth right (WLMR)	100	2.91	21.30	11.50	4.71	2	13.64	19.44	16.54	4.11
Meristic	Minimum-Maximum					Minimum-Maximum				
Gill rakers	4+10–12					4+10-12				
Pharyngeal teeth	2.3.5–5.3.2					2.3.5–5.3.2				
Total vertebrae	41 (n=3)					-				
Dorsal-fin rays (D)	iii,9 (n=3), iv,9 (n=97)					iv,9 (n=2)				
Anal-fin rays (A)	iii,5 (n=6), iii, 6 (n=94)					iii,5 (n=2)				
Pectoral-fin rays (P)	i,14 (n=9), i,15 (n=56), i,16 (n=35)					i,15 (n=2)				
Pelvic-fin rays (V)	i,9 (n=65)–i,10 (n=35)					i,9 (n=2)				
Caudal-fin rays (C)	2+17 (n=100)					2+17 (n=2)				
Lateral-line scales (LLS)	28 (n=10), 30+2 (n=90)					26+2 (n=1) or 27+2 (n=1)				
Circumferential scales (CFS)	5+3 (n=85), 5+4 (n=15)					5+3 (n=2)				
Predorsal scales (PrDS)	9 (n=12), 10 (n=25), 11 (n=35), 12 (n=18), 13 (n=10)					9 (n=2)				
Postdorsal scales (PoDS)	12 (n=9), 13 (n=45), 14 (n=37), 15 (n=3) or 16 (n=6)					12 (n=2)				
Circumpeduncular scales (CPS)	12 (n=70), 13 (n=10), 14 (n=12) or 16 (n=8)					14 (n=2)				
Postanal scales (PoAS)	5 (n=43), 6 (n=40), 7 (n=15) or 8 (n=2)					7 (n=2)				
Naps scales (NS)	12 (n=15), 14 (n=68), 16 (n=12) or 18 (n=5)					18 (n=2)				
Isthmus-pectoral fin scales (IPFS)	14 (n=12), 17 (n=30), 18 (n=47), 19 (n=10)					17 (n=2)				
Pelvic-anus fin scales (PFS)	6 (n=8), 7 (n=5), 8 (n=68), 9 (n=10) or 10 (n=9)					8 (n=2)				

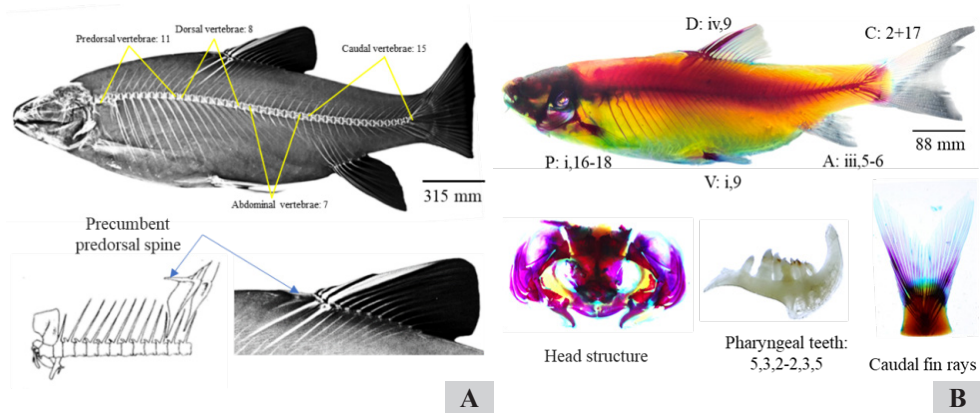


Figure 5. Specimens of *Spinibarbichthys maensis* from Ma Basin, Lao PDR: (A) Standard length is 315 mm SL (CR-L9661, March 24, 2025).

Morphometric Ratios

Principal Component Analysis (PCA) was bi-plotted based on morphometric ratios. The first Principal Component Analysis (PCA) was bi-plotted based on morphometric ratios. The first two principal components explained 44.4% of the total variation (PC1 = 25.6%, PC2 = 18.8%). PC1 was mainly associated with SL/BD, SL/BW, SL/CPL, and SL/Pv-CFL, whereas PC2 was influenced by SL/HL, SL/DFH, and SL/PreDL. The scatter plot indicated partial separation among size groups along PC1 and PC2, with overlapping clusters, suggesting size-related morphological variation (Table 3; Figure 6A).

Table 3. Results of morphometric ratio of 18 variables of *Spinibarbichthys* (n=102).

Code	Mean	SD	Min	Max	Eigenvalue	% PV	CP1	CP2
SL/BD	3.14	0.30	2.34	4.16	4.61	25.62	0.40	0.05
SL/HL	4.20	0.39	3.27	6.32	3.39	18.82	0.14	0.43
SL/CPL	7.64	0.75	6.14	10.40	2.33	12.97	0.24	0.22
SL/PreDL	1.87	0.11	1.57	2.48	1.58	8.79	0.21	0.34
SL/Pv-CFL	2.03	0.15	1.56	2.44	1.40	7.80	0.30	0.11
SL/BW	6.29	0.89	4.25	8.67	1.05	5.82	0.34	0.02
SL/DFH	4.43	0.63	2.98	8.17	0.82	4.53	0.17	0.42
HL/ED	3.77	0.54	3.00	5.31	0.62	3.46	-0.09	0.33
HL/HDE	1.52	0.12	1.26	1.88	0.57	3.18	0.19	-0.09
HL/HDN	1.22	0.13	1.02	1.80	0.40	2.23	0.23	-0.25
HL/SnL	2.79	0.27	2.02	3.72	0.37	2.05	0.19	-0.17
HL/MW	3.29	0.38	2.24	4.72	0.25	1.41	0.26	0.08
HL/O-OD	2.30	0.20	2.00	3.04	0.23	1.29	0.18	0.01
BD/HL	1.35	0.13	1.02	1.74	0.20	1.11	-0.29	0.33
BD/CPD	2.58	0.20	1.86	3.19	0.15	0.86	-0.31	0.07
BD/MiCPD	2.49	0.19	2.16	3.29	0.01	0.03	-0.19	0.18
O-OD/ED	1.65	0.27	1.22	2.44	0.00	0.01	-0.19	0.28
CPL/CPD	1.06	0.11	0.83	1.37	0.00	0.01	-0.09	-0.11

Note: SD= Standard deviation; Min = Minimum, Max = Maximum; %PV = % of Variance, CP= Principal Component.

In the Pearson correlation matrix among morphometric ratios, most ratios related to standard length (SL) showed significant positive correlations with each other ($p \leq 0.05$), indicating strong interdependence among size-related variables. Several ratios related to head length (HL) also exhibited significant correlations, both positive and negative. Negative correlations were mainly observed from body depth-related ratios (BD/HL, BD/CPD, BD/MiCPD) and SL and HL-based variables, suggesting contrasting growth patterns. Significant correlations were indicated by asterisks ($p \leq 0.05$), while color intensity reflected the strength and direction of relationships (Figure 6B).

Network on the Fish Body

The chord diagram (Figure 6C) illustrated the interrelationships among morphometric and meristic variables (a–o) across four size groups (Size1–Size4) and the comparative “China” population. The populations in the Size1–Size4 groups displayed extensive interconnections among variables, indicating consistent proportional growth and overall morphological homogeneity. Strong linkages were observed in the Size2 and Size3 groups (12.7% and 12.5% of total associations, respectively), suggesting high morphometric coherence. In contrast, Size1 (10%) and Size4 (13%) exhibited moderate connection densities. The “China” population showed markedly fewer linkages and lower association intensity (2.8%) with the other groups, reflecting clear morphometric differentiation. Among the examined variables, characters c, m, n, and j exhibited the highest association frequencies (5.6%, 5.6%, 5.1%, and 4.2%, respectively).

The Principal Component Analysis (PCA) comparison (Figure 6D) further rarefied these patterns. The first two principal components (PC1 and PC2) accounted for 96.28% and 1.20% of the total variance, respectively, indicating that most of the morphological variation is captured along PC1. There was a clear separation between the “China” population and the four size groups (Size1–Size4) along the PC1 axis. Despite partial overlaps among the size classes, a subtle ontogenetic gradient was visible along PC1: Size1 and Size2 cluster on the negative side, whereas Size3 and Size4 shifted toward intermediate to positive positions. This distribution reflected continuous shape changes associated with growth.

Comparative

Based on a comparative analysis of morphometric and meristic characters among five *Spinibarbichthys* taxa, the results reveal significant interspecific and intraspecific variation (Table 6). Meristic counts exhibit clear differentiation among species and populations. Total vertebral counts separate the taxa into two groups. *S. maensis* (Vietnam) and *S. ovalius* (40–43 vertebrae), together with *S. nammauensis* and *S. sinensis* (41–43 vertebrae), generally showed higher vertebral numbers than *S. denticulatus* in China and *S. maensis* in Laos (38–41). Variation is also observed in lateral line scale counts. Specimens of *S. maensis* in Laos have fewer scales (29–30+2) compared with *S. sinensis* (31–32/6+5) and *S. ovalius* (29–32). Notably, have (IPFS) and the nuphar spots (NS) were recorded exclusively in *S. denticulatus* (China) and *S. maensis* (Laos), indicating their diagnostic value for these taxa.

Morphometric ratios highlighted pronounced differences in body shape, particularly in *S. ovalius*. This species exhibited the deepest body relative to standard length (BD/SL = 2.26–2.51) and the shortest head (HL/SL = 2.9–3.9) among the examined taxa. In contrast, *S. nammauensis* showed the longest head relative to standard length (HL/SL = 4.2–4.3), whereas *S. maensis* (Laos) displayed intermediate values (3.27–4.97). Comparisons of eye-related proportions (O–OD/ED) indicated that *S. maensis* (Laos) had smaller values (1.58–2.44) than *S. denticulatus* (China) (1.75–2.33), suggesting interspecific differences in orbital morphology.

The primary morphological character distinguishing *Spinibarbus* (Oshima, 1919) from *Spinibarbichthys* (Oshima, 1926) is the structure of the last simple dorsal-fin ray. Species of *Spinibarbus* possess a smooth, non-serrated dorsal spine and typically exhibit a recumbent spine anterior to the dorsal fin. In contrast, *Spinibarbichthys* is characterized by a serrated dorsal spine (Kottelat 2001; Oshima 1919, 1925; Tuan and Binh 2019). Both genres share the presence of five branched anal-fin rays. Subsequent morphological and geographical analyses confirmed that species with serrated dorsal spines, such as *S. denticulatus*, belong to *Spinibarbichthys*, whereas species with smooth spines remain in *Spinibarbus*. Two vertebral

lineages are recognized within this complex: a low-count group (40–43 vertebrae), including *S. sinensis* and *S. denticulatus*, and a high-count group (44–46 vertebrae), represented by *S. yunnanensis* (Yang Junxing Cen Yinrui 1994).

Spinibarbichthys maensis is distinguished from congeners by its high, laterally compressed body (2.59–2.68 in SL), short and elevated head, posterior position of the dorsal-fin origin relative to the pelvic-fin origin, fewer lateral line scales (27–28), 19 paired serrae on the last simple dorsal ray, 10 gill rakers on the first branchial arch, and 14 circumpeduncular scales. Compared with *S. denticulatus*, *S. maensis* exhibits fewer lateral line scales and a deeper body profile (Marco 2014); this study overall, this study confirms that dorsal spine serration is the primary diagnostic character separating *Spinibarbichthys* from *Spinibarbus*. Within *Spinibarbichthys*, species-level differentiation is achieved through a combination of vertebral counts, predorsal scale counts, and key morphometric traits such as body depth and the relative position of the dorsal fin (Table 5).

Table 4. Comparison between data of Yang (1994) and this study reveals distinct species-level patterns in predorsal scales and vertebral counts among *Spinibarbichthys* species.

Character	<i>S. sinensis</i>	<i>S. denticulatus</i>	<i>S. yunnanensis</i>	<i>S. maensis</i> (this study)
Predorsal scales	6–10	9–12	13–15	9–13
Caudal vertebrae	14–15	13–14	16–17	15
Total vertebrae	41–43	40–42	44–46	38–41
Lateral line Scales	29–35	26–33	29–35	28–30+2

The cluster analysis based on morphological variables revealed two primary lineages with a significant morphological distance of approximately 0.63. The first major cluster positioned at the top of the dendrogram includes populations of *S. denticulatus* from China (n=2)*, *S. ovalius* from Vietnam, and *S. sinensis* from Vietnam. Within this group, *S. ovalius* and *S. sinensis* exhibited the highest similarity, clustering at less than 0.1. The second major cluster comprised the remaining populations, primarily dominated by *S. maensis* (Laos), *S. nammauensis* (Vietnam), and a large cohort of *S. denticulatus* (China, n=50). Notably, populations of *S. maensis* (Vietnam) and *S. denticulatus* (Vietnam) showed very high morphological affinity (distance < 0.05). Interestingly, the *S. denticulatus* populations from China* were split between the two main clusters, suggesting significant morphological divergence within the species based on the specific individuals sampled (n=2* vs. n=50) (Table 5; Figure 6E).

Table 5. The cluster analysis of *Spinibarbichthys* spp. based on morphological variables from references and this study. Abbreviations indicate as Figure 6.

Code of species	Sden VN, n=1	Snam VN, n=3	Ssin VN, n=1	Sova VN, n=1	Smae VN, n=2	Sden CN, n=50	Sden CN, n=2	Same this study n=100
Sden VN, n=1	0	0.10	0.53	0.57	0.01	0.06	0.59	0.15
SnamVN, n=3	0.10	0	0.76	0.74	0.06	0.10	0.88	0.17
Ssin VN, n=1	0.53	0.76	0	0.08	0.61	0.57	0.52	0.65
Sova VN, n=1	0.57	0.74	0.08	0	0.61	0.58	0.53	0.64
Smae VN, n=2	0.01	0.06	0.61	0.61	0	0.05	0.66	0.15
Sden CN, n=50	0.06	0.10	0.57	0.58	0.05	0	0.60	0.12
Sden CN, n=2*	0.59	0.88	0.52	0.53	0.66	0.60	0	0.41
This study n=100	0.15	0.17	0.65	0.64	0.15	0.12	0.41	0

* *S. denticulatus* from Guangxi China. The specimens in UNMF (type specimens to compare).

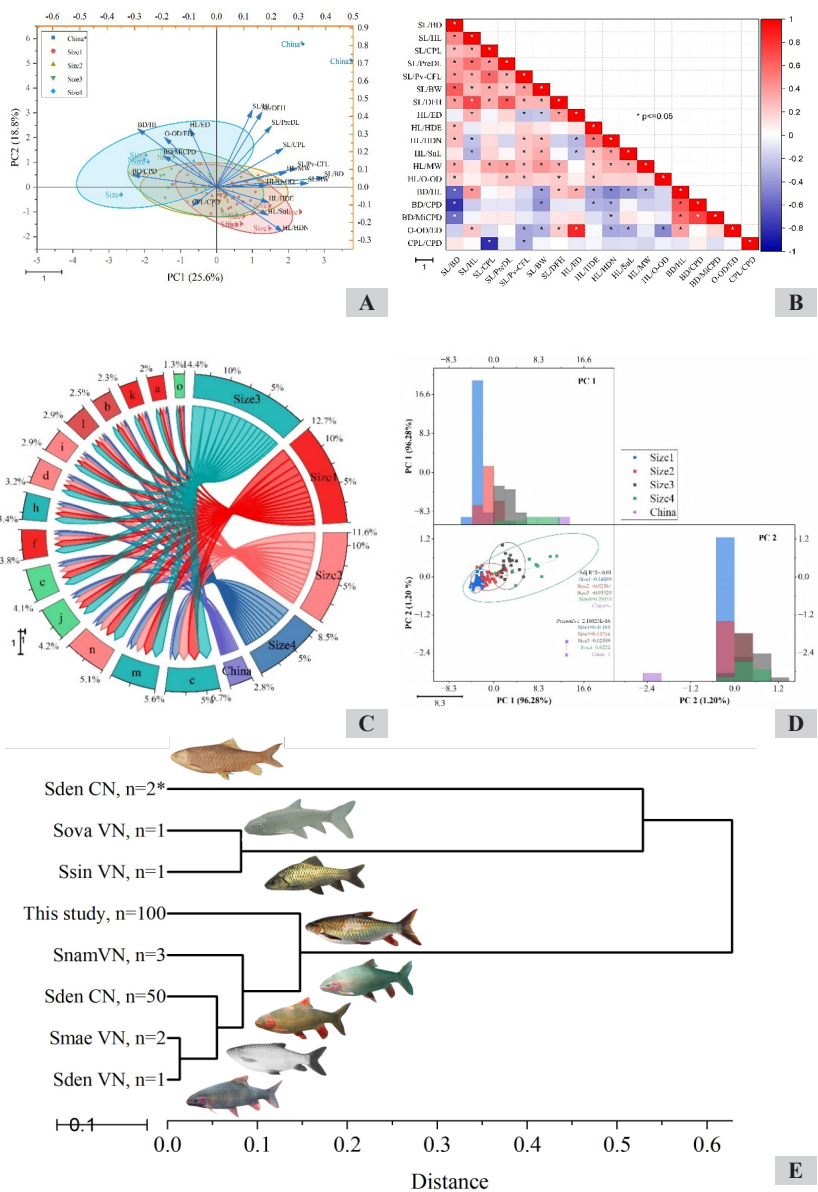


Figure 6. (A) Principal Component Analysis (PCA) in biplot on the 18 variables; (B) correlation plot showed significant positive correlation with each other ($p \leq 0.05$); (C) network on the body by chord diagram on the 15 networks in body; (D) scatter matrix on the scores of CPA in next work in body; (E) Hierarchical Cluster Analysis (HCA) of phylogenetic relationships among *Spinibarbus* different localities from Lao, Vietnam, and China. Sden = *S. denticulatus*; Sova = *S. ovalius*, Ssin = *S. sinensis*; Snam = *S. nammauensis*; Same = *S. maensis*; VN = Vietnam; CH = China. a) snout–head; (b) snout–pectoral fin; (c) head–pectoral fin; (d) pectoral fin–pelvic fin; (e) pectoral fin–dorsal fin; (f) head–dorsal fin; (g) head–pelvic fin; (h) dorsal fin–pelvic fin; (i) pelvic fin–anal fin; (j) dorsal fin–anal fin; (k) anus fin–caudal fin low; (l) anal fin–caudal fin up; (m) dorsal fin–caudal fin low; (n) dorsal fin–caudal fin up; (o) caudal peduncle up–low1.

Table 6. Comparison of meristics and morphometrics of five species of subgenus *Spinibarbichthys* in China, Vietnam, and Laos

Meristics and morphometrics	References (Duong Quang Ngoc 2007; Tuan and Binh 2019)					UNMF	This study
	<i>S. nannmauensis</i> VN, n=3 ^a	<i>S. sinensis</i> VN, n=1 ^a	<i>S. ovalius</i> VN, n=1 ^a	<i>S. maensis</i> VN, n=2 ^a	<i>S. denticulatus</i> CN, n=50 ^b	<i>S. denticulatus</i> CN, n=2 [*]	<i>S. maensis</i> LA, n=100
Gill rakers	6–8	12–14	–	10	8–15	10–12	10–12 (+4)
Pharyngeal teeth	2.3.4–4.3.2	2.3.5–5.3.2	–	2.3.5–5.3.2	5.3.2; 5.3.2	2.3.5–5.3.2	2.3.5–5.3.2
Total vertebrae	41–43	–	–	40–43	38–43	–	38–41
D	iii–iv,8–9	–	–	iv, 9	iii–vi,8–9	iv,9	iii–iv,9
A	–	–	–	3,5	3,5–9	ii,5	iii,5–6
P	1,14–16	–	–	1,14	i,14–16	i,15	i,14–i16
V	1,8–9	–	–	1,8	i,8–9	i,9	i,9–i,10
C	2+16–18	–	–	2+18	2+16–18	2+17	2+17
LLS	29–35	31–32/6+5	29–32	27–28	27–33	26–27+2	28–30+2
CFS	6+3	6+5	4+5–6	5+3	3+4–5	5+3	5+3–4
PrDS	13	–	–	8+5	8–11;9–12	9	9–12
CPS	14–15	–	–	14	12–13;12–14	14	14
PoDS	–	–	–	–	12	12	12–16
PoAS	–	–	–	–	–	7	5–8
NS	–	–	–	–	–	18	14–20
IPFS	–	–	–	–	–	17	15–18
PFS	–	–	–	–	–	8	6–10
SL/BD	3.5–4.1	3.5–3.8	2.26–2.51	2.59–2.68	3.00–3.21	4.05–4.16	2.34–3.98
SL/HL	4.2–4.3	4.26–4.40	2.9–3.9	4.26–4.40	3.92–4.18	6.12–6.32	3.27–4.97
SL/CPL	–	–	–	–	7.15–7.86	8.67–10.21	6.14–8.89
SL/PreDL	–	–	–	–	1.25–1.39	2.45–2.48	1.57–2.08
SL/BW	–	–	–	–	–	8.17–8.67	4.25–8.30
SL/Pv-CFL	–	–	–	–	–	2.39–2.44	1.56–2.34
SL/DFH	–	–	–	–	–	7.43–8.17	2.98–6.03
HL/ED	5.1–5.4	4.1–4.2	4.6–5.3	4.25–4.29	4.33–5.63	5.17–5.67	3.00–4.96
HL/HDE	2.2–2.3	2.2–2.3	2.2–2.6	1.87–2.0	2.18–2.35	1.46–1.60	1.26–1.88
HL/HDN	1.23–1.27	1.35	1.48–1.7	1.07–1.13	1.29–1.43	1.09–1.11	1.02–1.80
HL/O-OD	2.5–3.1	2.71	2.55–2.9	2.06–2.22	–	2.21–3.04	2.00–3.97
HL/SnL	–	–	–	–	–	2.67–2.81	2.02–3.72
HL/MW	–	–	–	–	–	4.08–4.72	2.24–3.99
BD/HL	–	–	–	–	–	1.47–1.56	1.02–1.74
BD/CPD	2.2–2.4	2.42	2.88–3.37	2.8–3.0	2.38–2.58	2.50–2.52	1.86–3.19

Meristics and morphometrics	References (Duong Quang Ngoc 2007; Tuan and Binh 2019)					UNMF	This study
	<i>S. nammauensis</i> VN, n=3 ^a	<i>S. sinensis</i> VN, n=1 ^a	<i>S. ovalius</i> VN, n=1 ^a	<i>S. maensis</i> VN, n=2 ^a	<i>S. denticulatus</i> CN, n=50 ^b	<i>S. denticulatus</i> CN, n=2 [*]	<i>S. maensis</i> LA, n=100
BD/MicPD	1.2–1.5	1.77	1.6	2.01–2.25	1.25–1.39	2.57–2.60	2.16–3.29
O-OD/ED	–	–	–	–	–	1.75–2.33	1.22–2.44
CPL/CPD	2.3–2.4	1.3–1.4	1.03–1.13	1.1–1.4	1.00–1.15	1.00–1.20	1.00–1.37

D = dorsal fin rays; P = pectoral fin rays; V = peivic fin rays; A = anal fin rays, C = caudal fin rays, C = caudal fin rays; LLS = lateral–line scales; CFS = circumferential scales; PrDS = predorsal scales; PoDS = postdorsal scales; PoAS = postanal scales; CPS = circumpeduncular scales; NS = nape scales; IPFS = isthmus-pectoral fin scales; PFS = pelvic-anal fin scales; UNMF: Ubon Ratchathani University Natural History Museum of Fisheries (comparative materials).

DNA barcoding and sequence analysis

The data clearly supports the three groupings as distinct species based on the barcoding gap concept, which states the genetic distances within a species are much smaller than distances between species. The lowest and highest distances within three species groups were <0.007 and ≥0.024, respectively. *S. maensis* and *S. denticulatus* (0.024–0.028) appear to be more closely related to each other than either is to *S. sinensis* (0.042–0.058), based on having the smallest interspecific distances.

Table 7. Average pairwise Kimura-2-Parameter (K2P) genetic distances between *Spinibarbichthys maensis*, *S. denticulatus* and *Spinibarbus sinensis* based COI gene.

No	Species	1	2	3	4	5	6	7	8
1	<i>S. sinensis</i> **								
2	<i>S. sinensis</i> **	0.005							
3	<i>S. maensis</i>	0.052	0.057						
4	<i>S. maensis</i>	0.052	0.057	0.000					
5	<i>S. maensis</i>	0.054	0.058	0.002	0.002				
6	<i>S. denticulatus</i> *	0.043	0.047	0.025	0.025	0.027			
7	<i>S. denticulatus</i> *	0.042	0.046	0.024	0.024	0.026	0.001		
8	<i>S. denticulatus</i> *	0.044	0.049	0.026	0.026	0.028	0.004	0.002	
9	<i>S. denticulatus</i> *	0.044	0.048	0.026	0.026	0.028	0.006	0.005	0.007

Note: (*) Three COI sequences of *S. maensis* and (**) two COI sequences of *S. sinensis* from GenBank with accession numbers indicated. Values in the diagonal are genetic distances of within-species in this study and GenBank sequences.

Note: (*) Three COI sequences of *S. maensis* and (**) two COI sequences of *S. sinensis* from GenBank with accession numbers indicated. Values in the diagonal are genetic distances of within-species in this study and GenBank sequences.

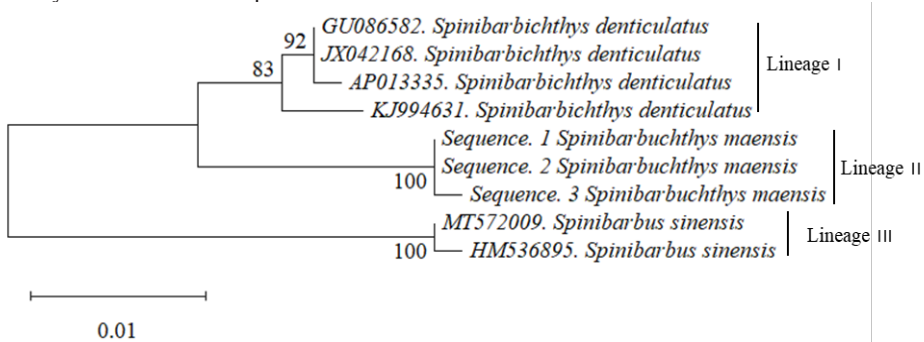


Figure 8. K2P distance neighbor-joining tree of *S. maensis* Ma Rever Basin, Houaphanh province northern Lao PDR, and congeneric species published in GenBank based on the DNA COI gene sequences.

Conclusions

The present study conclusively clarifies that *Spinibarbichthys maensis* and *S. denticulatus* are separated and valid species. Despite some superficial similarities, they possess distinct evolutionary lineages supported by morphometric, meristic, and molecular evidence.

The key diagnostic characters distinguished them comprises with three characters such as the barbel length, the position of the dorsal-fin origin and the number of lateral-line scales

Among them, the barbel length is the most distinct feature. *S. maensis* possesses significantly longer rostral and maxillary barbels compared to *S. denticulatus*, either the comparative ratio used by the standard length or head length. In case of the dorsal-fin origin, *S. maensis* possesses the origin posterior to the pelvic-fin origin. In contrast, *S. denticulatus* has the origin anterior or parallel to the pelvic fin. In lateral-line scales, it is not overlapped in the counts of which *S. maensis* is higher than *S. denticulatus* (28–30+2 vs. 26–27+2, respectively).

The secondary morphological differences are the lengths of caudal peduncle and head, the width of jaw and the depth of pulvinus (mouth cushion) of which *S. maensis* are higher ratios than those of *S. denticulatus*.

Statistic and genetic results support the species separation between *S. maensis*, and *S. denticulatus* DNA barcoding confirms the species status with a genetic distance of 0.024–0.028 between them, which is significantly higher than the intraspecific variation (<0.007). Principal Component Analysis (PCA) shows clear morphological separation, primarily driven by size-associated traits such as the head length and body depth, explaining nearly 49% of the variance.

Spinibarbichthys maensis is endemic to the Nam Ma River basin in Laos and Vietnam including tributaries like the Nam Et, Nam Xam, and Nam Neun. Its morphology suggests unique adaptations to this specific river system, diverging from the *S. denticulatus* populations found in China.

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