Integrative Taxonomy of Riverine Cyprinids (Genera *Henicorhynchus* and *Labiobarbus*) in the Mekong Delta: Morphological and DNA Barcoding Approaches

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ABSTRACT

Riverine cyprinids, known as "ca linh" in Viet Nam play a vital role in the economic and ecological systems of the Mekong Delta, particularly during the flooding season. These fish include four species from two genera: *Labiobarbus* (*L. leptocheilus* and *L. siamensis*) and *Henicorhynchus* (*H. entmema* and *H. siamensis*). Identifying species within each genus is challenging for non-ichthyologists due to their similar external appearance, particularly at early developmental stages. This study aimed to combine morphological and DNA-based approaches to comprehensively analyze morphological variations and DNA barcoding of riverine cyprinids in the Mekong Delta, Viet Nam. Fish samples were collected along the Mekong River during the flooding seasons. Initially, morphological classification was performed, including the measurement of meristic and morphometric parameters. The results revealed additional morphometric differences between congeneric species. Subsequently, the cytochrome c oxidase subunit I (COI) gene of each species was sequenced. The Kimura 2-parameter (K2P) genetic distances between the two species within each genus, *Labiobarbus* and *Henicorhynchus*, were 0.0878±0.0135 and 0.1005± 0.1230, respectively. Meanwhile, intraspecific genetic distances ranged from 0 to 0.0046. The findings emphasize the importance of integrating traditional morphological taxonomy with molecular methods to overcome challenges in fish species identification.

Keywords: Cyprinidae, Genetic distance, Phylogeny, Species identification

INTRODUCTION

The Mekong River is one of the world's longest rivers, stretching approximately 5,000 km across six countries. It is divided into the Upper Mekong (China and Myanmar) and the Lower Mekong (Thailand, Lao PDR, Cambodia, and Viet Nam) (MRC, 2024). The Mekong River is also one of the world's most important biodiversity hotspots, home to approximately 1,200 fish species, predominantly found within the Lower Mekong Basin (LMB) (Rainboth *et al*., 2012; Sor *et al*., 2023). Annually, the flood season, occurring from June to November, plays a crucial role in shaping regional ecosystems and livelihoods, by enhancing fisheries productivity, influencing river morphology, and improving soil fertility (MRC, 2024).

The Mekong Delta of Viet Nam, located in the LMB at the downstream portion of the Mekong River, has long benefited from seasonal fish migrations originating from the upstream regions during the annual flood season (Renaud and Kuenzer, 2012). Among these migratory species,

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riverine cyprinid fish, locally known as "ca linh" in Vietnamese (referred to as Pla soi khao for *Henicrorhynchus siamensis* and Pla soi look kluay for *Labiobarbus* in Thai, and collectively referred to as mud carp in English) are particularly notable. These species are highly valued for their economic significance, high-quality flesh, and widespread consumer demand.

In the Mekong Delta, these cyprinid fish comprise four species from two genera: *Henicorhynchus* (*Henicorhynchus siamensis* and *H. entmema*) and *Labiobarbus* (*Labiobarbus leptocheilus* and *L. siamensis*) (Tran *et al*., 2013). Morphologically, the two genera can be distinguished by dorsal fin characteristics: *Labiobarbus* species have a longer dorsal fin base (25–30 rays), while *Henicorhynchus* species have a shorter dorsal fin base (8 rays). However, species-level identification within each genus remains challenging due to overlapping morphological traits, as these cyprinids share a slender, silver-grey body with only minor differences in countable features (Tran *et al*., 2013; Nagao Natural Environment Foundation, 2021). While experienced ichthyologists can readily distinguish these species, non-specialists often struggle with species identification based solely on morphology, making misidentifications common.

Advancement of biotechnology have introduced molecular tools, such as DNA barcoding, that significantly improve fish species identification. DNA barcoding complements traditional taxonomic approaches by resolving ambiguity in biodiversity assessments and taxonomic classification (Hebert and Gregory, 2005). Among various molecular markers, the Cytochrome c oxidase subunit 1 (COI) gene, located in the mitochondrial genome, is widely recognized for its reliability in species identification across diverse animal taxa (Hebert *et al*., 2003; Ward *et al*., 2009). The COI gene has been successfully applied of fish species identification in various regions, including Australia (Ward *et al*., 2005), Canada (Hubert *et al*., 2008), India (Lakra *et al*., 2011), Japan (Zhang and Hanner, 2011), the Taiwan Strait (Bingpeng *et al*., 2018), Greece (Tsoupas *et al*., 2022) and others (Bhattacharya *et al*., 2016).

Despite the growing application of DNA barcoding, there remains a lack of updated studies integrating both morphology and molecular approaches for the identification of riverine cyprinid species, particularly in the genera *Henicorhynchus* and *Labiobarbus*. In terms of morphology, previous studies (Rainboth, 1996; Tran *et al*., 2013; Nagao Natural Environment Foundation, 2021) focused on dorsal fin rays and lateral line scales but overlooked other traits like morphometric indices. A recent revision described the morphology of five *Henicorhynchus* species and reclassified *Henicorhynchus lobatus* as *H. entmema* (Ciccotto and Page, 2020). Regarding DNA barcoding, COI sequences of species from these genera are available in the GenBank database. However, only one sequence—listed as *H. lobatus* (a synonym of *H. entmema*) —has been documented in a DNA barcoding study on freshwater fishes in the Mekong Delta (Vu *et al*., 2014). Other COI sequences have been reported in fish diversity studies in Thailand (Panprommin *et al*., 2020; 2021), but these studies did not incorporate morphological data.

Given these gaps, this study aims to integrate morphological and molecular approaches to comprehensively analyze riverine cyprinids (*Henicorhynchus* and *Labiobarbus*) in the Mekong Delta, Viet Nam. The findings will improve fish identification for non-experts in fish taxonomy and contribute to biodiversity research and fisheries management in Southeast Asia.

MATERIALS AND METHODS

Fish sampling

Fish sampling was conducted during the flooding season (August to December) from 2019 to 2022 across several provinces in the Mekong Delta, Viet Nam (Table 1). Fish specimens were obtained directly from local fishermen or purchased at markets along the rivers. The samples were kept fresh on ice and transported to the Genetic Laboratory at the College of Aquaculture and Fisheries, Can Tho University, for morphological and DNA analyses.

Table 1. Summary of riverine cyprinids sampling (genera *Henicorhynchus* and *Labiobarbus*).

Species	Provinces	Latitude	Longitude	Morphological analysis	DNA barcoding analysis
L. leptocheilus	An Giang	10°51'53.3"N	105°11'23.3"E	13	10
	Can Tho	10°04'38.6"N	105°45'13.0"E	17	10
	Long An	10°47'51.3"N	105°43'44.1"E	-	5
L. siamensis	Long An	10°46'45.2"N	105°42'40.0"E	18	8
H. siamensis	An Giang	10°51'53.3"N	105°11'23.3"E	-	5
	Can Tho	10°04'38.6"N	105°45'13.0"E	30	2
	Dong Thap	10°47'47.4"N	105°20'22.1"E	-	10
H. entmema	Can Tho	10°04'38.6"N	105°45'13.0"E	22	8

Morphological analysis

Fish specimens were initially identified based on key characteristics reported by Rainboth (1996), Kottelat (2001), Ciccotto and Page (2020), and Nagao Natural Environment Foundation (2021). Subsequently, each of the identified specimens was assigned unique codes and photographed alongside a ruler.

The total weight (W), meristic characters (countable parameters) and morphometric parameters of the fish were recorded following the guidelines of Kottelat (2001) and Rainboth (1996). Meristic characters included the number of spines and rays in the dorsal, pectoral, ventral, and anal fins, as well as the number of scales along the lateral line and around the caudal peduncle. A total of 22 morphometric parameters were measured, comprising 17 related to the body and five to the head (Figure 1). Measurements were performed using ImageJ software, which convert pixel units from fish photos into length units (Schneider *et al*., 2012).

Genetic analyses

Following morphological analysis, muscle and fin clips of specimens were collected from the specimens and preserved in 96% ethanol for DNA analysis. DNA extraction was performed using the ammonium acetate protocol (Saporito-Irwin *et al*., 1997). Initially, 25 mg of tissue was used to extract DNA based on the principle of using Ammonium acetate for protein precipitation. DNA was then precipitated with cold 100% ethanol and purified through two washes with 70% ethanol. The purified DNA was diluted in TE (Tris-EDTA) solution and stored at -20 ºC for further analysis.

The COI gene was amplified using a mixture of COI primers, including forward primers (Fish F2-t1 and VF2-t1) and reverse primers (Fish R2-t1 and VR1d-t1) (Ward *et al*., 2005; Ivanova *et al*., 2007). The polymerase chain reaction (PCR) was carried out in a total volume of 25 µL, comprising 12.5 µL Master mix (Promega, 2X), 1 µL $MgCl_2$ (25 mM), 0.32 µL of each primer (10 µM) and 2.5 µL DNA (50 $ng \cdot \mu L^{-1}$). The thermal cycling conditions included an initial denaturation at 95 ºC for 2 min, followed by 35 cycles of 94 ºC for 30 s, 52 ºC for 40 s and 72 ºC for 90 s, with a final extension at 72 ºC for 10 min (Ward *et al*., 2005).

An agarose gel electrophoresis (1%) was used to assess the quality of extracted DNA and PCR products at 70 V and 300 mA for 30 min. PCR products displaying a single, clear band under an ultraviolet (UV) transilluminator were considered high quality and sent to Apical Scientific Sdn Bhd, Malaysia, for sequencing. The PCR samples were purified and sequenced using the Sanger method on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems).

Data analysis

Morphological data analysis

The range and mode of meristic characters were calculated and compared with previous studies (Ciccotto and Page, 2020; Nagao Natural Environment Foundation, 2021; Roberts, 1993; Tran *et al*., 2013). Fish size (total length and total weight) and 20 morphometric indices [ratios of morphometric parameters to standard length (SL) and head length (HL)] were compared between the two congeneric species using analysis of covariance (ANCOVA), with HL and SL as covariates. To eliminate the effect of body size on morphometric data, measurements were adjusted using the equation proposed by Elliott *et al*. (1995):

$$M_{adj} = M_0 (L_S/L_0)^b$$

where M_{adj} is the adjusted measurement, M_0 is the original measurement, L_S is the mean standard length of all individuals of each genus, L_0 is the standard length of each fish sample; and b is the slope of the regression $\log M_0$ on $\log L_0$ using all individuals of the same genus. The value of b was calculated for each measurement. Differences in the adjusted data between the two congeneric species were tested using an independent t-test. Data analyses were conducted using IBM SPSS 22 software.

DNA barcoding analysis

The software FinchTV1.4.0 (http://www.geospiza.com) and MEGA X (Kumar *et al*., 2018) were used to view and check the quality of COI sequences. Ambiguous nucleotides with a quality value (Q value) lower than 30 at the two ends of each

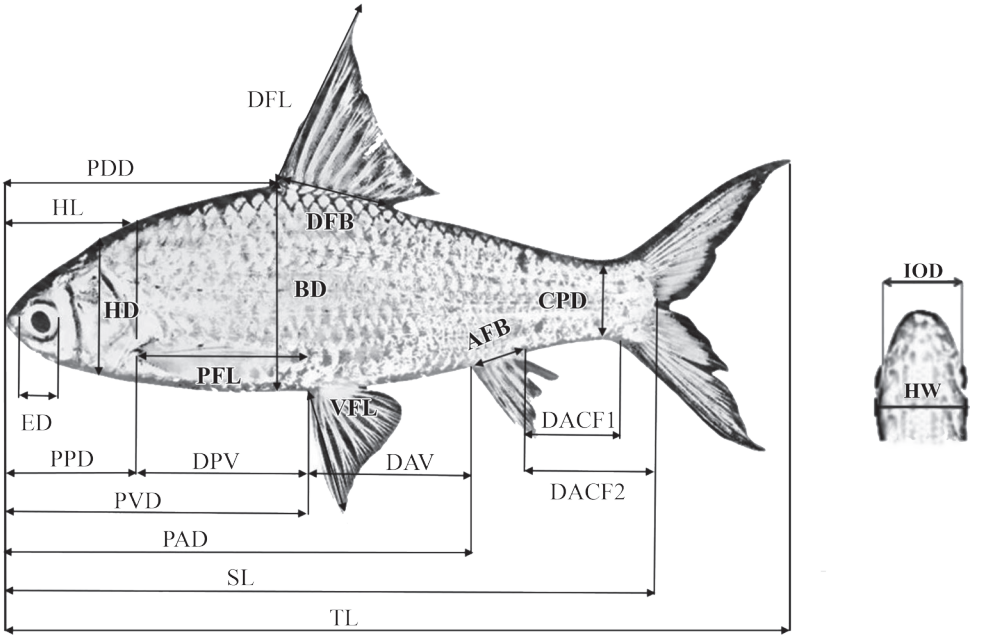

Figure 1. Morphometric parameters of riverine cyprinids (genera *Henicorhynchus* and *Labiobarbus*).
Note: A total of 17 body morphometric parameters include total length (TL), standard length (SL), caudal peduncle depth (CPD), the distance between anal and caudal fin 1 (DACF1), the distance between anal and caudal fin 2 (DACF2), body depth (BD), pre-dorsal distance (PDD), pre-pectoral distance (PPD), pre-ventral distance (PVD), pre-anal distance (PAD), the distance between pectoral and ventral fin (DPV), the distance between ventral and anal fin (DAV), dorsal fin base length (DFB), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin base length (ABL). Five head morphometric parameters consist of eye diameter (ED), head length (HL), head depth (HD), head width (HW), and inter-orbital distance (IOD).

sequence were removed. The trimmed sequences were then aligned in MEGA X and compared with available sequences in the BOLD systems (www.boldsystems.org) and GenBank (GB) through BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sequence similarities. Since the nomenclature of *H. entmema* was recently revised by Ciccotto and Page (2020), GB sequences for *H. lobatus* were used as references.

Genetic divergence was quantified using pairwise distances computed with both uncorrected *p*-distances (nucleotide differences) and the Kimura 2-parameter (K2P) model, with variance estimates obtained via 500 bootstrap replicates in MEGA X. Phylogenetic relationships were reconstructed using the Maximum Likelihood (ML) method implemented in MEGA X software, employing the Tamura-Nei substitution model (Tamura and Nei, 1993). Statistical support for the resulting topology was assessed through 1,000 bootstrap replicates. The analysis incorporated GenBank-derived sequences from representative taxa of closely related genera within the subfamily Labeoninae (Cyprinidae) (Table 2), with *Labeo chrysophekadion* designated as the outgroup taxon to root the phylogenetic tree.

Table 2. GenBank sequences used in this study.

Species	Accession number	References
Labiobarbus leptocheilus	MW147412	Unpublished
Labiobarbu siamensis	MW147419	Unpublished
Henicrorhynchus siamensis	MW147428	Unpublished
Henicrorhynchus entmema	AP012145	Yang *et al*., 2012
Lobocheilos bo	JN646098	Sade and Biun, 2012
Semilabeo obscurus	GU086581	Zheng *et al*., 2010
Mekongina erythrospila	KC631201	Pasco-Viel *et al*., 2014
Bangana tonkinensis	GU086600	Zheng *et al*., 2010
Garra fuliginosa	MK902682	Page *et al*., 2020
Cirrhinus molitorella	MT884504	Chen *et al*., 2021
Epalzeorhynchos frenatum	JF915597	Collins *et al*., 2012
Osteochilus microcephalus	MN342665	Collins *et al*., 2012
Gymnostomus ariza	OR232771	Unpublished
Labeo chrysophekadion	KU568892	van der Walt *et al*., 2017

RESULTS

During the sampling period across provinces in the Mekong Delta, four species were identified, belonging to two genera: *Henicorhynchus* (*H. siamensis* and *H. entmema*); and *Labiobarbus* (*L. leptocheilus* and *L. siamensis*). Based on our observation, *H. siamensis* was the most predominant species, followed by *L. leptocheilus* among riverine cyprinids during the flood season in the Mekong Delta. *Labiobarbus siamensis* was the rarest species. The morphological characteristics and DNA barcoding results are presented below.

Morphological results

External characteristics

The four fish species share common external characteristics, including a slender, elongated body with a silver-gray coloration. The distinguishing features between the genera *Henicorhynchus* and *Labiobarbus* are evident in two key criteria: (1) the presence of two pairs of barbels in *Labiobarbus* species versus the absence of barbels in *Henicorhynchus* species; and (2) a noticeably longer dorsal fin base in *Labiobarbus*

compared to *Henicorhynchus*, which is easily observable with the naked eye (Figure 2).

Species differentiation within the same genus based on external characteristics can be challenging. In the genus *Labiobarbus*, *L. leptocheilus* has shorter barbels that do not extend beyond the eye, whereas in *L. siamensis*, the barbels are longer and extend past the eye. However, accurately determining barbel length is often unreliable due to damage caused by fishing and transportation activities.

The two species of the genus *Henicorhynchus* appear similar, with only slight differences in mouth structure (Figure 3). In both species, the upper jaw is longer than the lower jaw, though this difference is more pronounced in *H. entmema*. Measurements of the upper (UJ) and lower jaw (LJ) lengths reveal that the UJ/LJ ratio of *H. entmema* (1.41±0.06) is significantly higher ($p<0.001$) than in *H. siamensis* (1.20±0.04) (Table 3). Additionally, the rostral cap of *H. entmema* features a medial indent (bottom right, Figure 3), which is not observed in other species of *Henicorhynchus* (Ciccotto and Page, 2020). However, these differences are difficult to discern with the naked eye, particularly in smaller-sized fish.

Table 3. Mouth length (mean±SD) of two *Henicorhynchus* species.

Parameters (cm)	*H. siamensis* (n = 30)	*H. entmema* (n = 22)	p value
Upper jaw (UJ) length	1.04±0.10	0.78±0.11	<0.001
Lower jaw (LJ) length	0.86±0.10	0.56±0.08	<0.001
UJ/LJ	1.20±0.04	1.41±0.06	<0.001

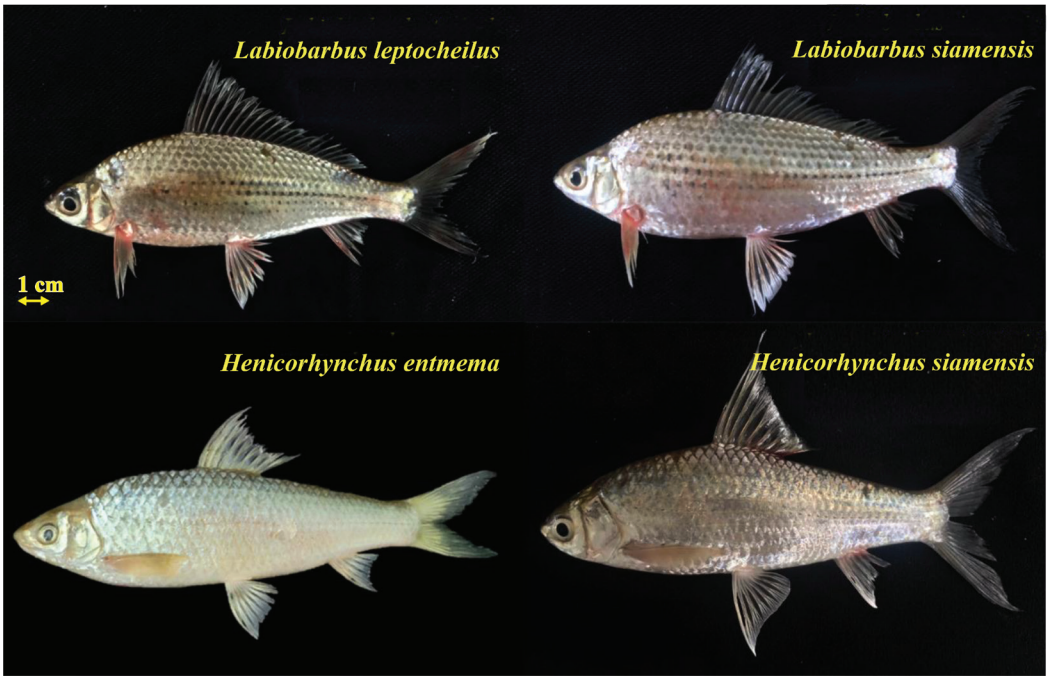

Figure 2. Riverine cyprinids (*Labiobarbus leptocheila*, CTU 188, GB accession number: PQ317425, Standard length SL = 14.73 cm; *Labiobarbus siamensis*, CTU 156, GB accession number: PQ317450, SL = 15.94 cm; *Henicorhynchus entmema*, CTU 44, GB accession number: PQ317476, SL = 16.08 cm; and *Henicorhynchus siamensis*, CTU 25, GB accession number: PQ317468, SL = 16.44 cm) distributed in the Mekong Delta.

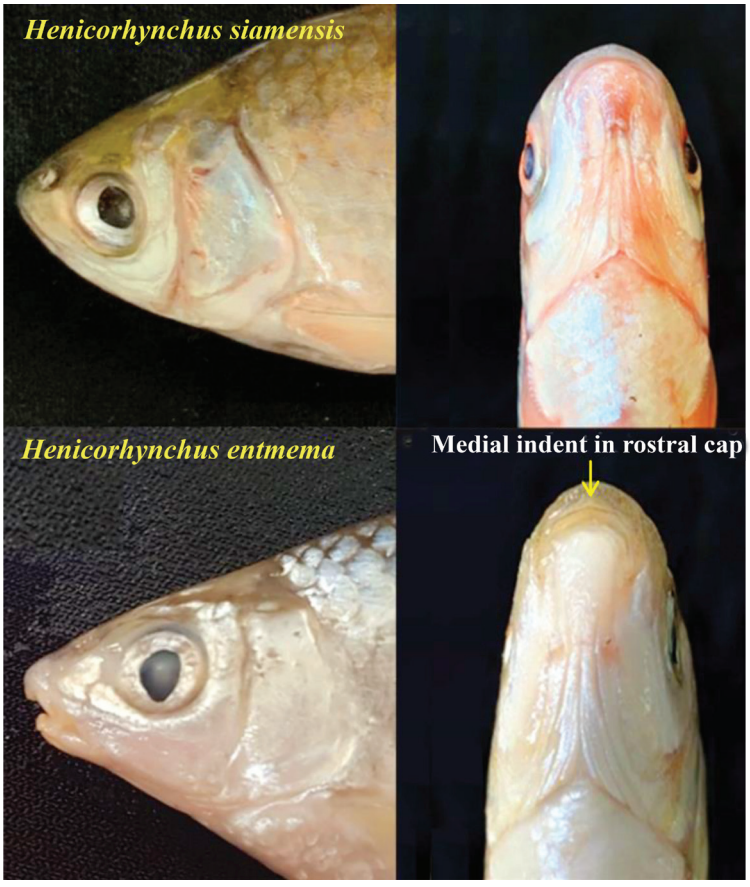

Figure 3. Mouth images of two *Henicorhynchus* species (*H. entmema*, CTU 44, GB accession number: PQ317476, SL = 16.08 cm; and *H. siamensis*, CTU 25, GB accession number: PQ317468, SL = 16.44 cm).

Meristic characters

A comparison of meristic characters between the genera *Labiobarbus* and *Henicorhynchus* reveals differences in two out of six parameters, while the remaining parameters exhibit similar or overlapping ranges of variation (Table 4). Specifically, *Labiobarbus* species have a higher number of dorsal fin rays (23–30) and lateral line scales (35–42) compared to *Henicorhynchus* species which have eight dorsal fin rays and 31–35 lateral line scales. These two meristic characters, therefore, serve as key distinguishing characteristics between the two genera.

Within the genus *Labiobarbus*, *L. leptocheilus* and *L. siamensis* exhibit similar and overlapping ranges in the number of fin rays (Table 4). However, they differ in the number of scales along the lateral line (35 to 38 scales for *L. leptocheilus* and 39 to 41 scales for *L. siamensis*) and around the caudal peduncle (16 to 19 scales in the former and 20 to 21 scales in the latter).

In the genus *Henicorhynchus*, all six meristic characters are similar between the two species (Table 4). As a result, fin ray and body scale counts cannot be used to differentiate these two species.

Morphometric comparison between congeneric species of Labiobarbus and Henicorhynchus

For *Labiobarbus* species, the mean weight and length of the two species showed no significant differences ($p>0.05$), ranging from 7.03 to 86.10 g in total weight and from 7.86 to 20.26 cm in total length (Table 5). Due to their similar body sizes,

both the ratio (index) and adjusted data showed similar results when comparing the two species (adjusted data not shown). The morphometric indices (Table 5) of the two *Labiobarbus* species differed significantly in six out of 16 body indices and three out of four head indices ($p<0.05$). Specifically, *L. siamensis* exhibited a greater caudal peduncle depth (CPD), corresponding to a higher number of caudal peduncle scales (Table 4), and a longer dorsal fin base (DFB). In contrast, *L. siamensis* had a smaller head depth (HD) and eye diameter (ED) ($p<0.01$).

The average body sizes of the two *Henicorhynchus* species were similar ($p>0.05$). Total body weight ranged from 15.20 to 132.90 g, while total length ranged from 11.97 to 21.98 cm (Table 5). The adjusted data (not shown) and morphometric indices (Table 5) followed a similar trend, except for adjusted body depth (BD) and adjusted eye diameter (ED), which were significantly higher in *H. siamensis* (BD: 3.91 vs 3.75; ED: 0.76 vs 0.72). However, the BD/SL and ED/HL indices did not differ between species. Additionally, *H. siamensis* and *H. entmema* exhibited significant differences in four head parameters, except for ED. *Henicorhynchus siamensis* had a larger head length (HL) and head width but a smaller head depth and inter-orbital distance (IOD) when standardized for equal standard length. Regarding body and fin morphology, the species also differed in pectoral fin position (PPD), anal fin position (PAD), the distance between pectoral and ventral fins (DPV), dorsal fin base (DFB) and length (DFL), and anal fin base (AFB). Compared to *H. entmema*, *H. siamensis* exhibited a longer PPD but shorter PAD and DPV, as well as longer DFB, DFL, and AFB.

Genetic analysis of Henicorhynchus and Labiobarbus

The cytochrome c oxidase subunit I (COI) gene sequences obtained in this study were deposited in GenBank under accession numbers PQ317420–PQ317477 (PQ317420–PQ317444 for *L. leptocheilus*, PQ317445–PQ317452 for *L. siamensis*, PQ317453–PQ317469 for *H. siamensis*, and PQ317470 –PQ317477 for *H. entmema*). The final aligned sequences were 647 base pairs (bp) in length. Nucleotide composition analysis revealed distinct species-specific patterns (Table 6), with significant interspecific variation in nucleotide frequencies ($p<0.01$). Intergeneric differences were particularly notable in thymine content, which was lower in *Labiobarbus* species (27.35–28.61%) compared to *Henicorhynchus* species (31.33–31.73%). Similarly, GC content distinguished the genera, with *Labiobarbus* species exhibiting higher

Table 4. Meristic characters of riverine cyprinids (genera *Henicorhynchus* and *Labiobarbus*) distributed in the Mekong Delta.

Meristic characters		***L. leptocheilus*** **(n = 30)**	***L. siamensis*** **(n = 18)**	***H. siamensis*** **(n = 30)**	***H. entmema*** **(n = 22)**
Dorsal fin rays	Range	8	8	8	8
	Mode (%)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)
Pectoral fin rays	Range	14–16	14–16	14–16	14–16
	Mode (%)	15 (59.1)	15 (59.1)	15 (59.1)	15 (59.1)
Ventral fin rays	Range	7–8	7–8	7–8	7–8
	Mode (%)	7 (68.2)	7 (68.2)	7 (68.2)	7 (68.2)
Anal fin rays	Range	5–6	5–6	5–6	5–6
	Mode (%)	5 (81.8)	5 (81.8)	5 (81.8)	5 (81.8)
Lateral line scales	Range	33–35	33–35	33–35	33–35
	Mode (%)	35 (40.9)	35 (40.9)	35 (40.9)	35 (40.9)
Caudal peduncle scales	Range	18–20	18–20	18–20	18–20
	Mode (%)	18 (68.2)	18 (68.2)	18 (68.2)	18 (68.2)

values (45.35–45.67%) than *Henicorhynchus* species (41.90–42.40%).

Comparative COI sequences analysis against GenBank and BOLD Systems databases revealed high sequence homology (99–100%) with conspecific sequences from previous studies (Table 7). Species-specific clustering was observed in both databases, although taxonomic ambiguity was observed between congeneric species pairs, resulting in unresolved species-level identification in BOLD Systems. While *L. leptocheilus*, *L. siamensis*, and *H. siamensis* were well-represented, *H. entmema* had limited representation, with only two sequences in GenBank and nine sequences in BOLD Systems (eight of which were privately held).

Table 5. Body weight (W), total and standard length (TL, SL) and morphometric indices of congeneric species of two genera *Labiobarbus* and *Henicorhynchus*.

Parameters	*L. leptocheilus* (n = 30)	*L. siamensis* (n = 18)	p value	*H. siamensis* (n = 30)	*H. entmema* (n = 22)	p value
W (g)	31.70±20.37^{a}	23.89±24.58^{a}	0.240	44.89±26.98^{A}	48.03±29.1^{A}	0.691
Range (cm)	7.10-86.10	7.03-79.67		15.20-102.9	16.40-132.9	
TL (cm)	13.98±2.82^{a}	12.12±3.93^{a}	0.063	15.33±2.71^{A}	15.76±2.72^{A}	0.573
Range (cm)	8.15-20.26	7.86-19.98		12.99-20.34	11.97-21.98	
SL (cm)	11.27±2.33	9.98±3.26	0.118	12.30±2.29	12.89±2.32	0.357
Range (cm)	6.33-16.53	6.49-16.30		9.46-16.44	9.53-18.62	
Ratios to standard length (%)						
BD	29.49±3.80^{a}	29.23±2.02^{a}	0.362	30.92±1.79^{A}	29.99±2.20^{A}	0.052
CPD	9.73±0.62^{a}	10.94±0.55^{b}	<0.01	11.92±0.37^{A}	11.93±0.51^{A}	0.448
DACF1	10.81±1.50^{a}	10.51±1.16^{a}	0.167	14.88±1.59^{B}	13.55±0.85^{A}	0.023
DACF2	16.75±1.34^{b}	16.04±1.4^{a}	0.016	18.81±1.28^{A}	18.18±1.16^{A}	0.329
PDD	39.26±1.77^{b}	38.06±1.75^{a}	0.031	44.07±1.23^{A}	44.42±1.03^{B}	0.022
PPD	20.18±2.09^{a}	20.81±1.62^{a}	0.078	21.50±2.00^{B}	18.93±1.31^{A}	0.017
PVD	48.63±2.53^{a}	48.96±1.21^{a}	0.110	48.05±1.64^{A}	48.48±2.50^{B}	0.021
PAD	74.80±1.86^{a}	75.56±0.94^{b}	0.036	73.03±1.41^{A}	74.65±1.88^{B}	0.003
DPV	28.51±2.44^{a}	28.27±1.64^{a}	0.491	26.74±2.12^{A}	29.78±1.99^{B}	<0.001
DAV	26.13±1.66^{a}	26.75±1.38^{a}	0.331	25.00±1.64^{A}	25.85±1.60^{A}	0.536
DFL	19.96±1.28^{a}	18.90±2.28^{a}	0.142	24.18±1.90^{B}	21.93±1.28^{A}	<0.01
DFB	43.99±1.95^{a}	46.45±2.52^{b}	0.001	17.25±0.62^{B}	16.27±0.85^{A}	<0.01
PFL	21.00±2.70^{a}	19.76±2.16^{a}	0.326	23.11±1.64^{A}	22.51±2.18^{A}	0.593
VFL	18.96±2.30^{a}	19.67±1.24^{a}	0.154	18.53±1.23^{A}	18.02±1.81^{A}	0.959
AFB	8.77±0.60^{a}	9.20±1.16^{a}	0.103	8.65±0.50^{B}	7.85±0.54^{A}	<0.01
HL	19.49±1.64^{a}	19.86±1.48^{b}	0.035	22.76±2.07^{B}	20.09±1.57^{A}	0.014
Ratios to head length (%)						
HD	99.75±7.15^{b}	90.06±9.57^{a}	<0.01	93.61±5.66^{A}	95.87±7.21^{B}	0.017
ED	35.14±2.04^{b}	32.60±1.08^{a}	<0.01	27.24±1.55^{A}	28.44±2.30^{A}	0.386
HW	77.01±8.72^{a}	74.61±6.16^{a}	0.055	73.08±6.18^{B}	60.35±5.47^{A}	<0.01
IOD	59.58±8.43^{a}	64.40±4.52^{b}	0.015	58.99±6.33^{A}	73.90±5.9^{B}	<0.01

Note: Mean±SD in the same row superscripted with different letters (a, b for *Labiobarbus* species and A, B for *Henicorhynchus* species) are significantly different ($p<0.05$). The abbreviations are the same as in Figure 1.

Intraspecific K2P genetic distances among the four studied species ranged from 0 to 0.0046, with haplotype diversity varying from one to 12 haplotypes per species (Table 8). The lowest intraspecific genetic distance was observed in *H. entmema* (K2P = 0), while *L. siamensis* exhibited the highest value (K2P = 0.0046±0.0018) (Table 8).

Intergeneric pairwise genetic distances (Table 8) revealed substantial divergence between *Labiobarbus* and *Henicorhynchus*, with K2P values ranging from 0.1710 (*L. leptocheilus-H. siamensis*) to 0.1954 (*L. siamensis-H. entmema*), corresponding to nucleotide differences of 14.90–16.46. In contrast, congeneric species pairs showed lower genetic divergence, with K2P distances ranging from 0.0878 to 0.1005.

Table 6. Nucleotide composition (%±SD) of COI sequences of riverine cyprinids (genera *Henicorhynchus* and *Labiobarbus*).

Species	T	C	A	G	CG
L. leptocheilus	28.61±0.08^{b}	26.96±0.08^{c}	26.04±0.05^{b}	18.39±0.05^{d}	45.67±0.14^{d}
L. siamensis	27.35±0.14^{a}	27.74±0.14^{d}	26.98±0.08^{d}	17.93±0.07^{c}	45.35±0.10^{c}
H. siamensis	31.33±0.11^{c}	25.29±0.11^{b}	26.77±0.05^{c}	16.62±0.05^{a}	41.90±0.13^{a}
H. entmema	31.73±0.00^{d}	25.11±0.00^{a}	25.86±0.00^{a}	17.29±0.00^{b}	42.40±0.00^{b}

Note: T = Thymine; C = Cytosin; A = Adenine; G = Guanine; Mean±SD in the same rows superscripted with different lowercase letters are significantly different ($p<0.01$).

Table 7. DNA barcoding data of riverine cyprinids (*Henicorhynchus* and *Labiobarbus*) with reference sequences from GenBank and BOLD Systems.

Species	Sample size	No. Haplotype	GB sequences used to compare	GenBank (% identity)	BOLD Systems (% identity)
L. leptocheilus	25	12	MW147412	99.07–100	99.07–100
L. siamensis	8	4	MW147419	99.55–100	99.55–100
H. siamensis	17	7	MW147428	99.23–100	99.23–100
H. entmema	8	1	AP012145	100	100

Note: Comparison with the first ten sequences in BOLDsystems

Table 8. Pairwise genetic distances within (bold values on the diagonal) and between (K2P values below the diagonal and nucleotide differences above the diagonal) riverine cyprinids (genera *Henicorhynchus* and *Labiobarbus*).

Species	*L. leptocheilus*	*L. siamensis*	*H. siamensis*	*H. entmema*
L. leptocheilus	**0.0030±0.0010**	7.96±1.02	14.90±1.31	15.51±1.31
L. siamensis	0.0878±0.0135	**0.0046±0.0018**	15.51±1.39	16.46±1.40
H. siamensis	0.1710±0.0193	0.1773±0.0194	**0.0033±0.0014**	8.88±1.09
H. entmema	0.1845±0.0192	0.1954±0.0188	0.1005±0.1230	**0**

Phylogenetic relationship

The ML phylogenetic analysis based on COI sequences demonstrated well-defined taxonomic relationships among the four studied species of *Henicorhynchus* and *Labiobarbus*, along with GenBank reference sequences and outgroups representing closely related genera within the subfamily Labeoninae (Cyprinidae). The resulting phylogenetic tree reveals that species within each genus (*Henicorhynchus* and *Labiobarbus*) cluster together in distinct clades with strong bootstrap support (100% and 99%, respectively). These clades are highly divergent from each other and from other genera within Labeoninae. Despite morphological similarities among congeneric species, DNA barcoding analysis effectively resolved species-level distinctions, supporting their taxonomic validity.

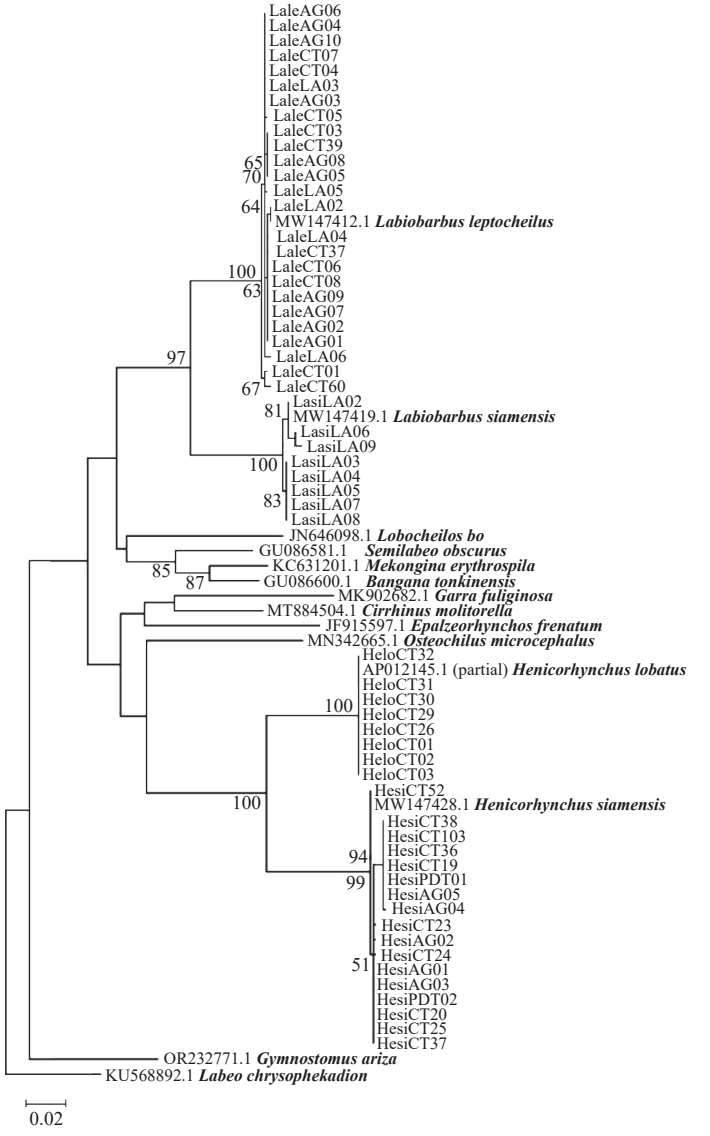

Figure 4. Maximum Likelihood (ML) tree of riverine cyprinids (genera *Henicorhynchus* and *Labiobarbus*) in the Mekong Delta, Viet Nam. Sample codes with LaleCT/LaleAG refer to *Labiobarbus leptocheilus*, LasiLA to *L. siamensis*, HeloCT to *H. lobatus*, and HesiCT/HesiAG/HesiPDT to *H. siamensis*. Other sequences with GenBank number of representative species from other closely related genera within Labeoninae were used as outgroups. Bootstrap values above 50% (based on 1,000 replicates) are shown above the branches.

DISCUSSION

This study provides a comprehensive analysis of the morphological characteristics, including external morphological, meristic characters, and morphometric measurements, complemented by DNA barcoding to accurately differentiate two congeneric species within each genus, *Henicorhynchus* and *Labiobarbus*.

The morphological analyses confirm similarities between species within each genus while highlighting key distinguishing features. In *Labiobarbus*, the number of scales around the caudal peduncle is a primary distinguishing characteristic, with *L. siamensis* having 20–21 scales, compared to 16–19 in *L. leptocheilus*. Additionally, the morphometric index of caudal peduncle depth, which correlates with this meristic character, further supports this distinction as *L. siamensis* exhibits a significantly larger caudal peduncle depth than *L. leptocheilus*. Furthermore, *L. siamensis* possesses a longer dorsal fin base (DFB), which corresponds to a higher number of dorsal fin rays than its sister species. These meristic characters, including dorsal fin rays, caudal peduncle scales, and lateral line scales, are consistent with previous studies (Ciccotto and Page, 2020; Nagao Natural Environment Foundation, 2021; Tran *et al*., 2013).

For *Henicorhynchus* species, previous studies have noted differences in upper and lower jaw lengths (Tran *et al*., 2013; Nagao Natural Environment Foundation, 2021) and the distinctive medial indent of the rostral cap in *H. entmema* (Ciccotto and Page, 2020). This study quantitatively confirms that the ratio of upper to lower jaw length is greater in *H. entmema* than in *H. siamensis*. Additionally, this study identifies additional morphometric differences in head characteristics, fin positioning, and the dimensions of dorsal fin rays, dorsal fin base, and anal fin base, as validated by statistical analysis. These differences were previously undocumented. Ciccotto and Page (2020) examined 19 morphometric indices across five *Henicorhynchus* species, including *H. entmema* (the name *H. entmema* was used in that study) and *H. siamensis*. They reported that most morphometric traits were similar between the two species. However, they found that *H. siamensis* had a smaller interorbital distance (IOD) (42.9% of head length-HL) than *H. entmema* (44.5% of HL). The current study aligns with this pattern, although the absolute values differ (*H. siamensis*: IOD/HL = 59.0%; *H. entmema*: IOD/HL = 73.9%), presumably attributable to variations in the body size of the fish samples examined in each study.

Despite the morphological similarities between congeneric species, their COI sequences exhibit high divergence, with a mean K2P genetic distance of 0.0878 for *Labiobarbus* and 0.1005 for *Henicorhynchus*. These values are comparable to the within-genus mean K2P distance of 0.0993 (ranging from 0 to 20.63) reported for 122 fish genera in Australia (Ward *et al*., 2005) and higher than the mean of 0.0708 (ranging from 0.0247 to 0.1825) reported for 35 cyprinid species from the Yangtze River, China (Shen *et al*., 2016). The interspecific genetic distances between congeneric species of *Labiobarbus* and *Henicorhynchus* greatly exceed their intraspecific genetic distances (ranging from 0 in *H. entmema* to 0.0046 in *L. siamensis*), with inter/intraspecific ratios ranging from 19 to 30 fold. These values are consistent with findings from other studies on diverse fish taxa (Ward *et al*., 2005; Bingpeng *et al*., 2018), demonstrating the reliability of DNA barcoding for species identification.

Analysis of COI sequences from the four investigated species in comparison with GenBank and BOLD systems databases revealed high identity levels (99–100%). While external morphology remains effective for genus-level differentiation and can aid species-level identification, misidentified sequences in public databases underscores the limitations of relying solely on morphology. Within both *Labiobarbus* and *Henicorhynchus*, one instance of sequence misidentification per species resulted in confusion between sister species. Such misidentifications frequently occur in morphologically similar species during data submission to public repositories (Kappel and Schröder, 2020), and have been documented in spiny eel species (Duong

et al., 2020), the silurid catfish *Phalacronotus bleekeri* (Chhorn and Duong, 2022), and other taxa. Consequently, studies that integrate morphological descriptions alongside DNA barcoding provide more robust references for species identification. This combined approach has proven successful in differentiating morphologically similar fish species, such as *Enteromius* (family Cyprinidae) in the Congo Basin, Africa (Van Ginneken *et al*., 2017), groupers of the genus *Epinephelus*, and snappers of the genus *Lutjanus* in Indonesia (Fadli *et al*., 2023; Rahayu *et al*., 2023).

Phylogenetic reconstruction supports the morphological classification of the studied species, with distinct clustering of species within each genus. Figure 4 illustrates the phylogenetic relationships of riverine cyprinids within the broader context of the sub-family Labeoninae. Notably, the analysis reveals that *Labiobarbus* is more closely related to *Cirrhinus*, *Lobocheilos*, and *Gymnostomus* than to *Henicorhynchus*. This finding is particularly interesting given that *Cirrhinus* and *Henicorhynchus* were previously consolidated under *Gymnostomus* based on shared morphological characters, specifically the presence of eight to nine branched dorsal fin rays (Kottelat, 2003; 2013) and post symphyseal knobs. However, molecular evidence from this study, corroborated by previous investigations (Zheng *et al*., 2010; 2012; Yang *et al*., 2012; Ciccotto and Page, 2020), strongly supports their classification as distinct genera.

Our phylogenetic analyses indicate that traditional taxonomic characters, such as dorsal fin ray count (ranging from 8–9 to 23–30 rays) and fin base length, do not consistently align with genetic relationships among riverine cyprinid genera. These findings underscore the importance of integrative taxonomic approaches in resolving species boundaries and systematic relationships, particularly within the species-rich sub-family Labeoninae, which comprises 528 species within Cyprinidae (Fricke *et al*., 2025). The morphological and molecular (COI) data generated in this study provide a valuable reference for future research on cyprinid diversity in the Mekong region.

CONCLUSIONS

This study successfully identified four species from the genera *Henicorhynchus* and *Labiobarbus* by integrating morphological characterization and DNA barcoding. Additional morphometric parameters were provided to enhance species differentiation within each genus. DNA barcoding analysis revealed that interspecific genetic distances were 19 to 30 fold higher than intra specific genetic distances, indicating that the two congeneric species within each genus can be reliably distinguished using molecular data.

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