Zootaxa 5839 (2): 345–358
https://www.mapress.com/zt/
Copyright © 2026 Magnolia Press

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5839.2.6
http://zoobank.org/urn:lsid:zoobank.org:pub:E60D62B4-DA3D-4952-9E76-A37922E97A0A

Integrative evidence resolves the taxonomic identity of the Yellow River softshell turtle previously assigned to *Pelodiscus sinensis* (Wiegmann, 1834)

SHIPING GONG[1,5#], QI LAO[1,6#], RUI LIU[1,7], JIAN HONG[1,8], UWE FRITZ[2,3,9], BALÁZS FARKAS[4,10], QIANRU LIANG[1,11*] & JIAXUAN LI[1,12*]
[1]*College of Life Science and Technology, Jinan University, Guangzhou 510632, China*
[2]*Institute of Biology, Leipzig University, 04103 Leipzig, Germany*
[3]*Museum of Zoology, Senckenberg Dresden, A. B. Meyer Building, 01109 Dresden, Germany*
[4]*21 Bercsényi St., 2464 Gyúró, Hungary*
[5] *gsp621@163.com; https://orcid.org/0000-0001-5508-6962*
[6] *lq1030loss@163.com; https://orcid.org/0000-0002-0025-775X*
[7] *15323961072@163.com; https://orcid.org/0009-0003-5342-1001*
[8] *1527752249@qq.com; https://orcid.org/0009-0001-0677-9081*
[9] *uwe.fritz@senckenberg.de; https://orcid.org/0000-0002-6740-7214*
[10] *farkasbalazs@yahoo.com; https://orcid.org/0000-0002-1997-8872*
[11] *1044936731@qq.com; https://orcid.org/0009-0004-7352-4267*
[12] *kim_lijiaxuan@163.com; https://orcid.org/0000-0001-9709-6894*
[#]*These authors contributed equally to this work*
**Corresponding authors*

Abstract

Softshell turtle populations from the Yellow River Basin have traditionally been assigned to *Pelodiscus sinensis*, but their taxonomic identity remained uncertain. Integrating evidence from nuclear SNPs, complete mitochondrial genomes, and data from external morphology and osteology, we reassessed the taxonomic identity of the Yellow River softshell turtle. Phylogenomic analyses consistently place these populations sister to *Pelodiscus maackii*, and external morphology and osteology further demonstrate strong resemblance to *P. maackii* while clearly differentiating them from *P. sinensis*. These results support recognizing the Yellow River softshell turtle as a distinct geographic lineage of *P. maackii*, clarifying its taxonomic identity and refining our understanding of diversity within *Pelodiscus*.

Key words: Geographic lineage, integrative taxonomy, *Pelodiscus maackii*, phylogenomics, Trionychidae, Yellow River Basin

Introduction

The softshell turtle genus *Pelodiscus* (Testudines: Trionychidae) is an important component of the East Asian herpetofauna. For much of the twentieth century, the genus was regarded as monotypic, with *Pelodiscus sinensis* (Wiegmann, 1834) as its only species. Subsequent morphological and molecular phylogenetic studies, however, have demonstrated that species diversity within the genus had been substantially underestimated (Gong *et al.* 2018; TTWG 2025). Over the past two decades, six additional species have been recognized, including *Pelodiscus maackii* (Brandt, 1857), *Pelodiscus axenaria* (Zhou *et al.*, 1991), *Pelodiscus parviformis* Tang, 1997, *Pelodiscus variegatus* Farkas *et al.*, 2019, *Pelodiscus huangshanensis* Gong *et al.*, 2021, and *Pelodiscus shipian* Gong *et al.*, 2022. In addition, *Pelodiscus huike* Liu *et al.*, 2024 was described in recent years and proved to be a synonym of *Pelodiscus axenaria* (Gong *et al.* 2025; TTWG 2025).

Pelodiscus species are widely distributed across East Asia, ranging from the Amur and Ussuri river basins in the Russian Far East through northeastern China, the Korean Peninsula, and Japan, and extending southward to southern Vietnam (Gong *et al.* 2018, 2021; TTWG 2025). Among these, *P. sinensis* has the broadest natural distribution, occurring from southern China northward through several major river systems including the Pearl,

Accepted by A. Bauer: 15 May 2026; published: 29 Jun. 2026

Licensed under Creative Commons Attribution-N.C. 4.0 International https://creativecommons.org/licenses/by-nc/4.0/

Yangtze, Huaihe, and Yellow rivers, and reaching Hebei and Tianjin in northern China (Gong *et al.* 2018). In contrast, *P. maackii* represents the northernmost species of the genus, occurring in northeastern China, the Korean Peninsula, Japan, and the Russian Far East (TTWG 2025). The distribution ranges of *P. sinensis* and *P. maackii* are geographically adjacent, and phylogenetic analyses based on mtDNA sequences have consistently recovered them as sister lineages (Suzuki & Hikida 2014; Gong *et al.* 2018). Notably, the Yellow River Basin lies near the boundary between these two species.

Softshell turtle populations inhabiting the Yellow River Basin have traditionally been assigned to *P. sinensis*. As one of the most economically important freshwater turtle species in East Asia, *P. sinensis* has a long history of domestication and aquaculture (Gong *et al.* 2018). To enhance production traits, multiple domesticated strains with distinct regional characteristics have been selectively bred across its distribution range, including the so-called Yellow River, Huaihe River, Taihu Lake, and Japanese strains (Chen *et al.* 2023; Wang *et al.* 2023). Among these, the Yellow River strain was derived from native softshell turtle populations in the Yellow River Basin (hereafter referred to as the Yellow River softshell turtle). It represents the northernmost cultured strain traditionally assigned to *P. sinensis*. It is characterized by its large body size, a broad carapacial margin, rapid growth, and high reproductive performance (Liang *et al.* 2017). Another widely cultivated strain, the Japanese strain, was introduced into China from Japan and subsequently domesticated through long-term aquaculture (Zhao 2008). This strain is generally believed to originate from softshell turtle populations distributed mainly in regions south of the Kanto region of Japan, including Saga, Oita, and Fukuoka (Zhao 2008; Zhang *et al.* 2014).

The species identity of the Japanese strain provides an important perspective for reassessing the taxonomic status of the Yellow River softshell turtle. Phylogenetic analyses of Japanese *Pelodiscus* populations by Suzuki & Hikida (2014) using mtDNA revealed the coexistence of two distinct lineages in Japan corresponding to *P. maackii* and *P. sinensis*. In that study, *P. maackii* was found to be widely distributed across Japan, whereas *P. sinensis* occurred only sporadically in several localities, including Chiba, Shizuoka, Kyoto, Kumamoto, and Kagoshima. The authors therefore suggested that *P. maackii* represents the native lineage in Japan, whereas *P. sinensis* likely represents an introduced taxon from continental China. If so, the Japanese aquaculture strain imported into China, which is thought to originate primarily from regions south of Kanto, is likely referable to *P. maackii* rather than *P. sinensis*.

Previous studies have further shown that the Yellow River strain and the Japanese strain are highly similar in both molecular data (Zhang *et al.* 2014) and morphological characters (Liang *et al.* 2017; Wang *et al.* 2023), and cluster dendrograms based on external morphology have recovered them as a single clade (Zhang *et al.* 2014; Wang *et al.* 2023). If the Japanese strain indeed belongs to *P. maackii*, then the taxonomic status of the Yellow River softshell turtle, which closely resembles the Japanese strain, warrants reassessment as well. Moreover, based on phylogenetic analyses of mitochondrial 12S rRNA, cyt *b*, and ND4 gene fragments, Hu (2021) found that the Yellow River softshell turtle clustered with *P. maackii*, further raising questions about its taxonomic identity. However, these studies relied primarily on a limited number of mitochondrial markers and lacked nuclear genomic data. Consequently, the evolutionary relationships among the Yellow River softshell turtle, *P. maackii*, and *P. sinensis* have not yet been scrutinized using multilocus datasets, and corresponding morphological evidence remains limited. A comprehensive reassessment integrating genomic and morphological evidence is therefore required.

In the present study, we aim to clarify the taxonomy of the Yellow River softshell turtle. We integrate multiple lines of evidence—genome-wide nuclear single nucleotide polymorphisms (SNPs), complete mitochondrial genome sequences, and external morphological and osteological traits—and combine phylogenetic analyses with morphometric approaches to conduct an integrative assessment of softshell turtle samples collected from the Yellow River Basin (Yulin, Shaanxi Province, and Linyi County, Shanxi Province, China). By synthesizing genomic and phenotypic evidence, this study provides a robust basis for resolving the taxonomic identity of the Yellow River softshell turtle.

Materials and Methods

Sampling.—Thirteen Yellow River softshell turtles were collected from the Yellow River Basin in Yulin City, Shaanxi Province, and Linyi County, Yuncheng City, Shanxi Province, China. In addition, samples representing all seven currently recognized species of *Pelodiscus* were included: *P. axenaria* (n = 12), *P. shipian* (n = 9), *P. huangshanensis* (n = 4), *P. sinensis* (n = 8), *P. parviformis* (n = 8), *P. variegatus* (n = 4), and *P. maackii* (n = 8) (Table

S1). For each specimen, a small tissue sample was clipped from the leathery margin of the carapace and preserved in absolute ethanol at 4 °C. Voucher specimens were preserved in absolute ethanol. All specimens examined in this study are deposited in the Animal Germplasm Resources Repository, College of Life Science and Technology, Jinan University, Guangzhou, Guangdong Province, China. All experimental protocols were approved by the Laboratory Animal Ethics Committee of Jinan University (certificate number: 20220221–49).

Molecular Phylogeny.—Genomic DNA was extracted from carapacial-margin tissue samples using a TIANGEN Blood/Cell/Tissue Genomic DNA Extraction Kit. Library construction and hybridization capture were performed using the AFLP-based genome sequence capture (AFLP Capture) (Li *et al.* 2019). The resulting libraries were sequenced by Annoroad Gene Technology Co., Ltd. (Beijing). Mitochondrial genomes were assembled using GetOrganelle (Jin *et al.* 2020) and MitoZ (Meng *et al.* 2019), with the complete mitochondrial genome of *P. sinensis* (NC_068236.1) used as a reference. The sequencing reads were mapped to the *P. sinensis* reference genome assembly (GCA_049634645.1; GenBank) using BWA (Li & Durbin 2009). The alignment files were converted and sorted with SAMtools (Danecek *et al.* 2021). SNPs were identified and filtered using VCFtools (Danecek *et al.* 2011), followed by linkage disequilibrium (LD) pruning in PLINK (Purcell *et al.* 2007), yielding a high-quality SNP dataset.

For mitochondrial analyses, complete mitochondrial genomes of other *Pelodiscus* species were downloaded from GenBank (Table S1) and aligned with our new data. Following Gong *et al.* (2018), we selected *Apalone spinifera*, *Palea steindachneri*, and *Rafetus euphraticus* as outgroups and downloaded their complete mitochondrial genomes from GenBank. Furthermore, we added two newly generated complete mitochondrial genomes of *Palea steindachneri* as supplementary outgroups. All mitochondrial sequences were compiled in MEGA7 (Kumar *et al.* 2016), aligned using MAFFT v7.313 (Katoh & Standley 2013), and then trimmed with Gblocks v0.91b (Castresana 2000) to generate the final mitochondrial dataset. A maximum likelihood (ML) tree was inferred using IQ-TREE v1.6.8 (Nguyen *et al.* 2015) and the "MERGE+MFP" parameter to select the optimal substitution model, and node support was assessed with 1000 ultrafast bootstraps (UFBoot). In addition, uncorrected pairwise genetic distances (*p*-distances, percentages) between *Pelodiscus* based on ND4 sequences were calculated in MEGA7.

For nuclear SNP analyses, nuclear genome data of *Trionyx triunguis* and *Pelochelys cantorii* were downloaded from GenBank, and nuclear SNP data from two newly sequenced individuals of *Palea steindachneri* were included as additional outgroups. A maximum likelihood (ML) phylogeny based on the nuclear SNP dataset was inferred with IQ-TREE v1.6.8 and the options "-m GTR+ASC -bb 1000 -nt 30".

Morphological Measurements.—Following Zhou *et al.* (1991) and Gong *et al.* (2021), we measured 24 adults of the Yellow River softshell turtle, 22 adults of *P. maackii*, and 11 adults of *P. sinensis* (Table S2) using electronic calipers with a precision of 0.1 mm. The turtles used for morphometric measurements included some individuals that were genetically tested (JNU 20230001–20230008, JNU 20160006–20160010, JNU 20250010–20250017). The morphological characters were as follows: head width (HW; maximum width across the temporal region), snout length (SL), eye diameter (ED), interorbital distance (IOD; minimum distance between the eyes), carapace length (CL), carapace width (CW), plastron length (PL), and posterior carapace width (PCW; distance from the point of maximum carapace width to its posterior margin). Prior to tissue sampling from the carapacial margin, each specimen was photographed and examined, and qualitative characters were recorded, including the shape of carapace and plastron, skin coloration and patterning, and markings on head and gular region. Morphometric data were size-corrected for allometric effects in R using GroupStruct2 (Chan & Grismer 2026), with carapace length (CL) as the standard variable to account for body size variation. Based on the size-corrected dataset, specimens were grouped into three taxa, and one-way ANOVA, principal component analysis (PCA), and linear discriminant analysis (LDA) were conducted in SPSS v27 to evaluate inter-taxon morphological differences and discriminatory power.

Osteology.—Four specimens each of *P. sinensis*, *P. maackii*, and the Yellow River softshell turtle were selected for osteological preparation. Skeletal specimens were prepared following alkaline maceration (Onwuama *et al.* 2012). This was followed by degreasing and bleaching with detergent and sodium carbonate (Rennick *et al.* 2005). Finally, the specimens were assembled and mounted as complete skeletons (Xie *et al.* 2022), yielding skeletal preparations for 12 individuals in total. The skeletons were subsequently photographed. Measurements with a precision of 0.1 mm were taken from skull and plastral elements using tpsDig (Luo 2024), including: (1) the ratio of the minimum to the maximum width of the basisphenoid (Yang *et al.* 2011); (2) the internal angle of the lateral entoplastral processes (Tang 1997); and (3) the curvature formed by the paired xiphiplastra. Diagrams illustrating the measurements of angles are provided in Figure S1.

Results

Maximum likelihood (ML) phylogenies inferred from the nuclear SNP dataset and the mitochondrial genome dataset recovered broadly similar branching patterns (Fig. 1), except for the placement of *P. parviformis* and *P. variegatus* reflecting ancient mitochondrial capture events. Our sequences clustered well with reference sequences, and the seven species of *Pelodiscus* each formed a strongly supported monophyletic clade (UFBoot = 100%). These topologies were concordant with previously reported relationships within *Pelodiscus* and confirmed *P. variegatus* as a deeply divergent evolutionary lineage with introgressed mitochondria (Fritz *et al.* 2010; Stuckas & Fritz 2011; Gong *et al.* 2018, 2021). In the nuclear SNP tree, the individuals of the Yellow River softshell turtle (n = 13) constituted a maximally supported clade that was sister to a clade comprising the *P. maackii* individuals from Heilongjiang (JNU 20250010–20250017). In contrast, in the mitochondrial tree, the Yellow River turtles were embedded among the Heilongjiang *P. maackii*, with the Japanese *P. maackii* sequences (AB904720, AB904722–AB904725) as their shallowly divergent sister clade.

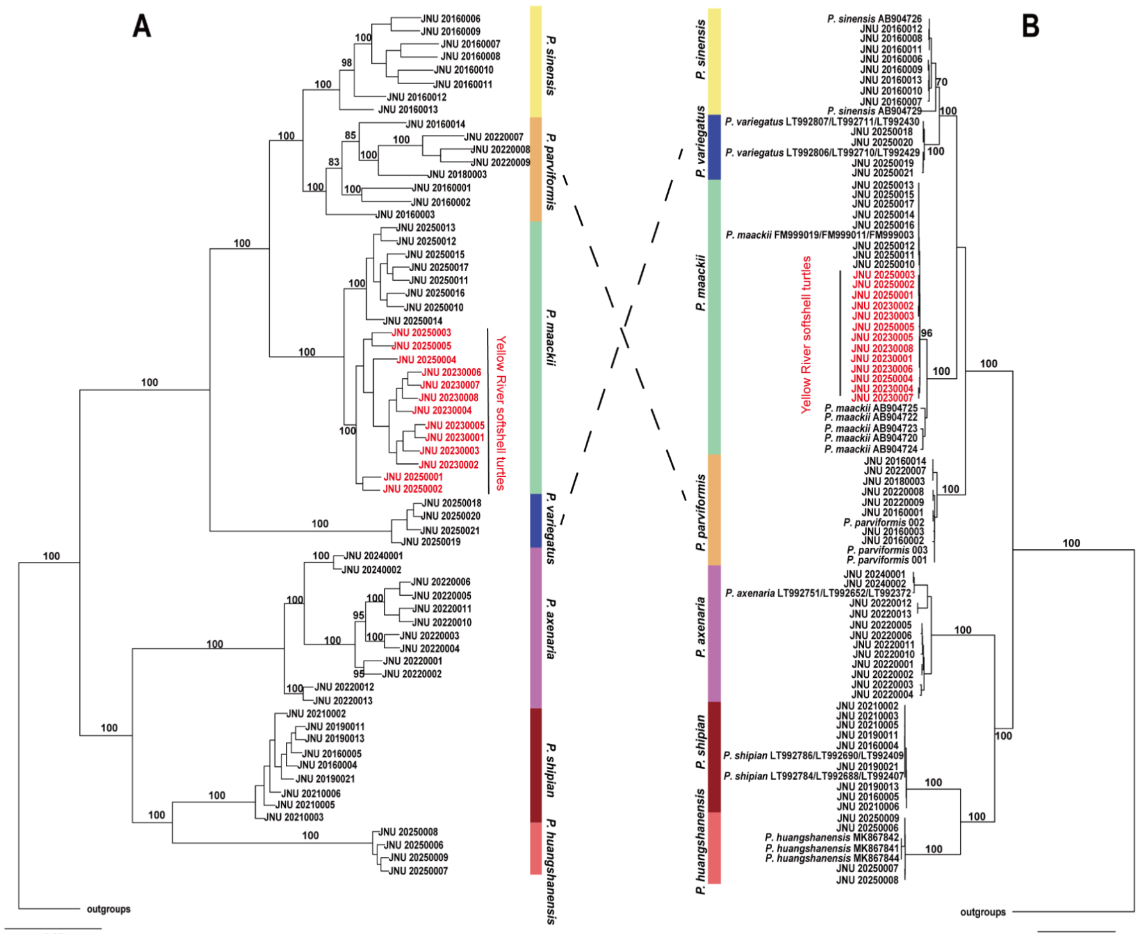

FIGURE 1. Maximum likelihood (ML) trees based on nuclear SNP data (A) and mtDNA sequences (B). Red font indicates Yellow River softshell turtles. Numbers at nodes are bootstrap values, with only major branches displayed. Note the conflicting placements of *Pelodiscus variegatus* and *P. parviformis* (see Discussion).

Uncorrected pairwise genetic distances (*p*-distances, %) based on the mitochondrial ND4 gene are shown in Table 1. Interspecific *p*-distances within *Pelodiscus* ranged from 1.14% to 7.05%, consistent with the results of Gong *et al.* (2021). The Yellow River softshell turtle exhibited the smallest genetic distance to *P. maackii* (0.02%) and the largest to *P. huangshanensis* (7.05%). In contrast, the *p*-distance between the Yellow River softshell turtle and *P. sinensis* (2.61%) was similar to that between *P. maackii* and *P. sinensis* (2.59%), indicating interspecific divergence between the Yellow River softshell turtle and *P. sinensis*. Thus, this pattern is concordant with the phylogenetic results.

TABLE 1. Uncorrected *p*-distances (%) of the mitochondrial ND4 gene for *Pelodiscus* species and the Yellow River softshell turtle.

	1	2	3	4	5	6	7
1—Yellow River softshell turtle							
2—*P. maackii*	0.02						
3—*P. sinensis*	2.61	2.59					
4—*P. variegatus*	3.01	2.99	1.14				
5—*P. parviformis*	3.03	3.06	2.39	2.79			
6—*P. axenaria*	5.79	5.82	5.59	6.08	5.73		
7—*P. huangshanensis*	7.05	7.03	6.51	6.77	6.47	5.16	
8—*P. shipian*	6.92	6.90	6.16	6.24	6.11	5.45	4.30

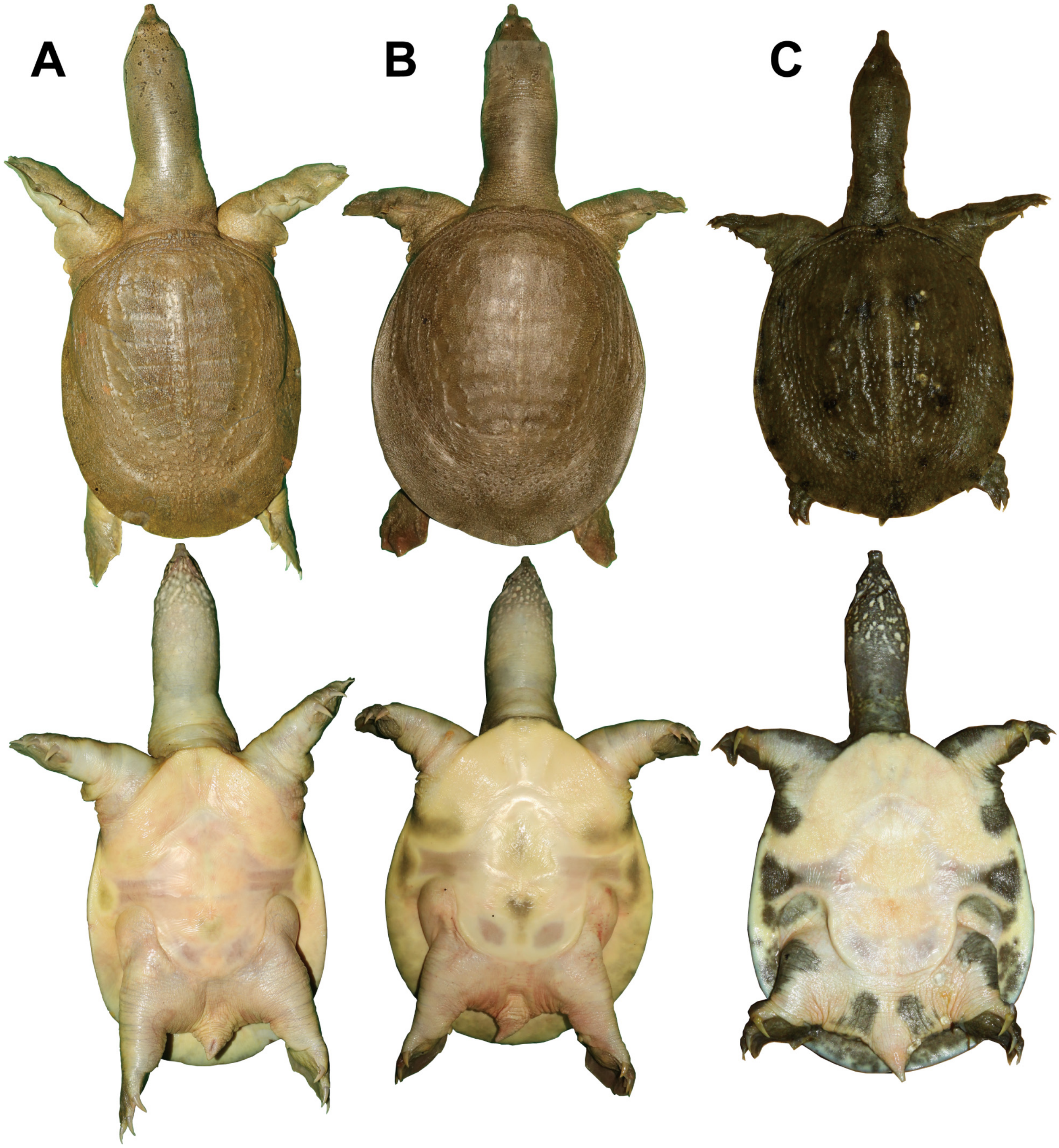

FIGURE 2. Dorsal and ventral aspects of the Yellow River softshell turtle (A, JNU 20230005, female), *Pelodiscus maackii* (B, JNU 20250010, female), and *P. sinensis* (C, JNU 20160009, female).

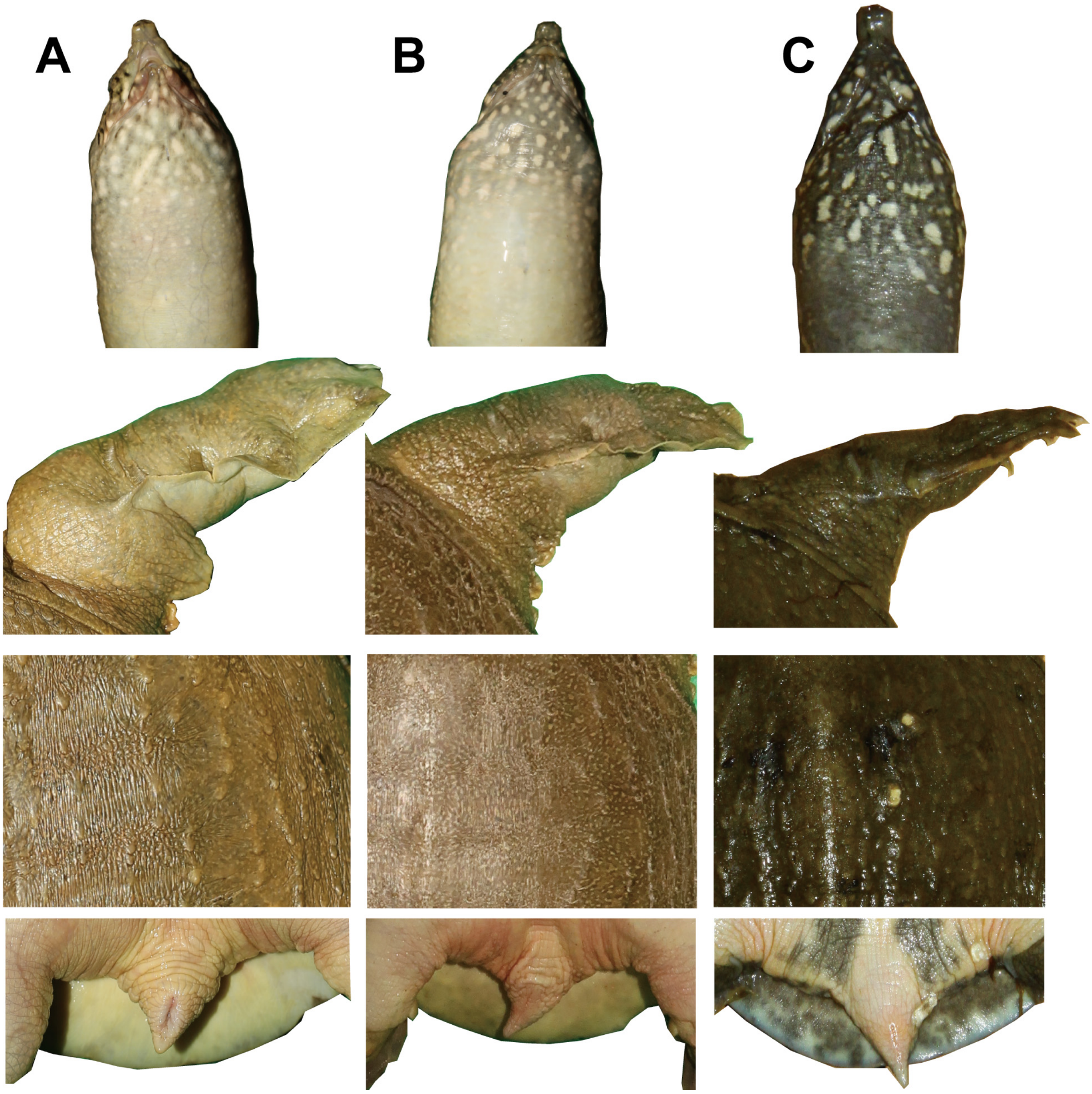

FIGURE 3. Comparative morphological details of the Yellow River softshell turtle (A, JNU 20230005, female), *Pelodiscus maackii* (B, JNU 20250010, female), and *P. sinensis* (C, JNU 20160009, female). From top to bottom: ventral head views, right forelimb, dorsal spots, and underside of the leathery margin of the carapace.

Morphological examination of adult individuals of the Yellow River softshell turtle, *P. maackii*, and *P. sinensis* (Figs. 2–3) revealed clear differences in coloration and pattern. The Yellow River softshell turtle (Figs. 2A, 3A) is generally ochre-yellow overall, with numerous small yellow dots on the dorsum; venter smooth, pale yellow; carapace with faint dark speckling; ventral surface of the leathery carapacial margin clean and lacking obvious pigment deposition; limbs with small yellow dots. *Pelodiscus maackii* (Figs. 2B, 3B) is slightly darker than the Yellow River softshell turtle, overall yellowish-brown, with numerous small yellow dots dorsally but lacking conspicuous dark blotches; venter smooth, pale yellow, with faint dark markings in the axillary region bilaterally and along the mid-venter; ventral surface of the leathery carapacial margin without obvious pigment deposition; limbs with small yellow dots. In contrast, *P. sinensis* (Figs. 2C, 3C) differs markedly from the preceding two taxa, being olive to brownish overall; dorsum lacking small yellow dots but with small, irregular dark blotches; venter milky white, with distinct dark lateral markings; ventral surface of the leathery carapacial margin with conspicuous pigment deposition; limbs lacking yellow dots but exhibiting large dark patches. These morphological observations are broadly consistent with the molecular results in that the Yellow River softshell turtle and *P. maackii* are morphologically more similar.

Morphometric results (Fig. 4) showed that, in females, CW, PCW, PL, and HW differed significantly among the three groups (P < 0.01), exhibiting an overall increasing trend from *P. sinensis* (smallest) over the Yellow River softshell turtle (intermediate) to *P. maackii* (largest). For CL, no significant difference was detected between the Yellow River softshell turtle and *P. maackii*, whereas both differed significantly from *P. sinensis*. SL did not differ significantly among the three groups. ED differed significantly only between the Yellow River softshell turtle and *P. sinensis*, but not in any other pairwise comparisons. For IOD, no significant difference was detected between *P. sinensis* and the Yellow River softshell turtle, whereas the remaining pairwise comparisons were highly significant (P < 0.01). In males (Fig. S2), CW, PL, and IOD differed significantly among the three groups. For CL, PCW, and HW, differences between the Yellow River softshell turtle and *P. maackii* were not significant, whereas both differed significantly from *P. sinensis*. Unlike females, ED did not differ significantly among the three groups, whereas SL showed a significant difference. In both sexes, most morphometric variables showed an overall increasing trend from *P. sinensis* through the Yellow River softshell turtle to *P. maackii*. Principal component analysis (PCA) (Fig. S3) clearly separated *P. sinensis* along PC1, and the Yellow River softshell turtle was placed closer to *P. maackii* on PC1. Linear discriminant analysis, evaluated using both original classification and leave-one-out cross-validation (LOO-CV), showed high accuracy in classifying all samples (Table S3). In the ordinary discriminant analysis results, the overall discriminatory accuracy was 98.2%. Leave-one-out cross-validation yielded consistent discriminatory accuracy results.

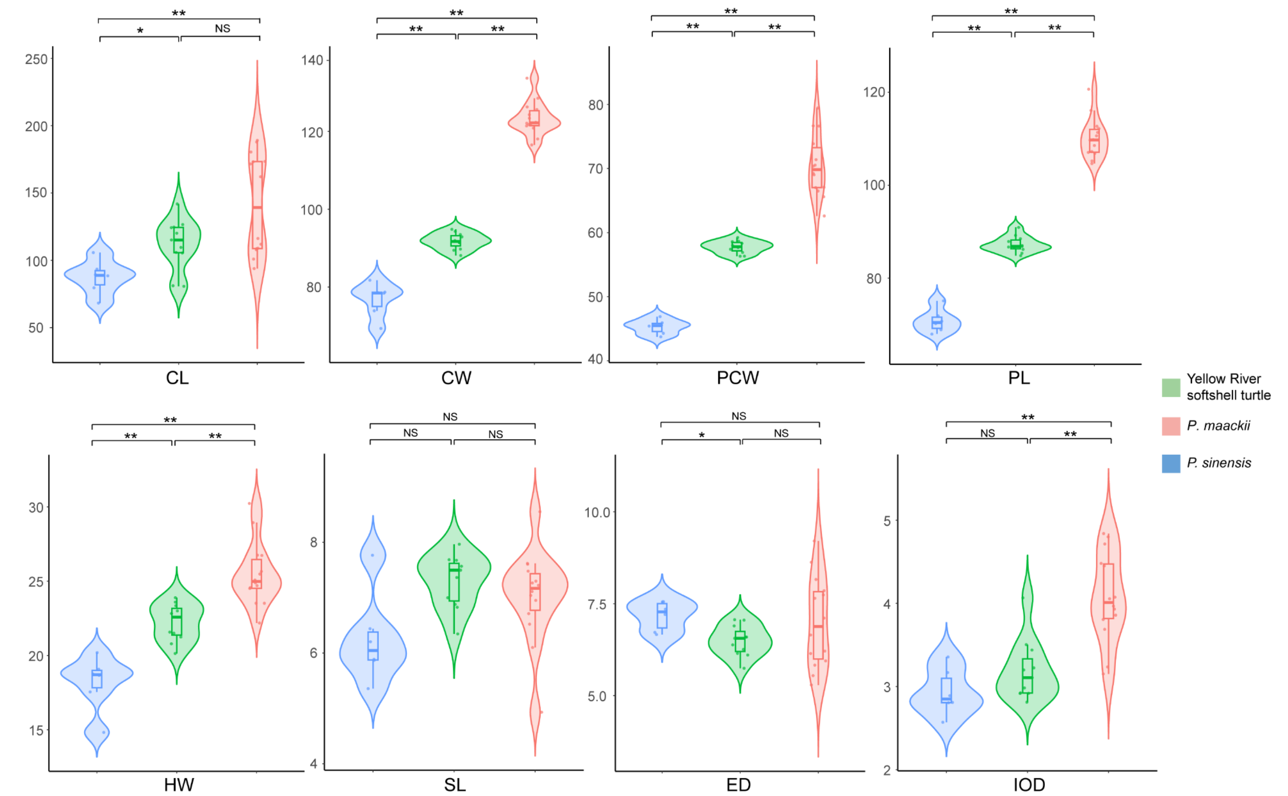

FIGURE 4. Violin plots of size-corrected morphological traits in females of the Yellow River softshell turtle, *Pelodiscus maackii* and *P. sinensis*. NS: not significant; P < 0.05*; P < 0.01**. Horizontal lines within each box represent the median, and boxes encompass the 75th and 25th percentiles; the dots represent the values for each sample.

Osteological comparisons (Fig. 5) revealed that, in lateral view, the parietal-supraoccipital crest was essentially straight in both the Yellow River softshell turtle and *P. maackii* but distinctly arched in *P. sinensis*. The Yellow River softshell turtle and *P. maackii* had relatively flatter, more elongated skulls. Plastral morphology differed in the curvature formed by the paired xiphiplastra: in the Yellow River softshell turtle and *P. maackii*, the angle between the paired xiphiplastra was similar, thus forming a narrow V-shaped configuration, whereas in *P. sinensis* the corresponding structure was U-shaped with a markedly broader curvature. Measurements of the maximum and minimum widths of the basisphenoid and the minimum-to-maximum width ratio indicated that all three groups had ratios < 1/2 (Table 2; Table S4). In addition, measurements of the internal angle of the entoplastron did not differ markedly among the three groups (Table 2; Table S4). In agreement with the qualitative observations, the xiphiplastral angle exceeded 130° in *P. sinensis* but was < 130° in both the Yellow River softshell turtle and *P. maackii* (Table 2). Overall, osteological characters indicate greater cranial similarity between the Yellow River softshell turtle and *P. maackii* than between either of them and *P. sinensis*.

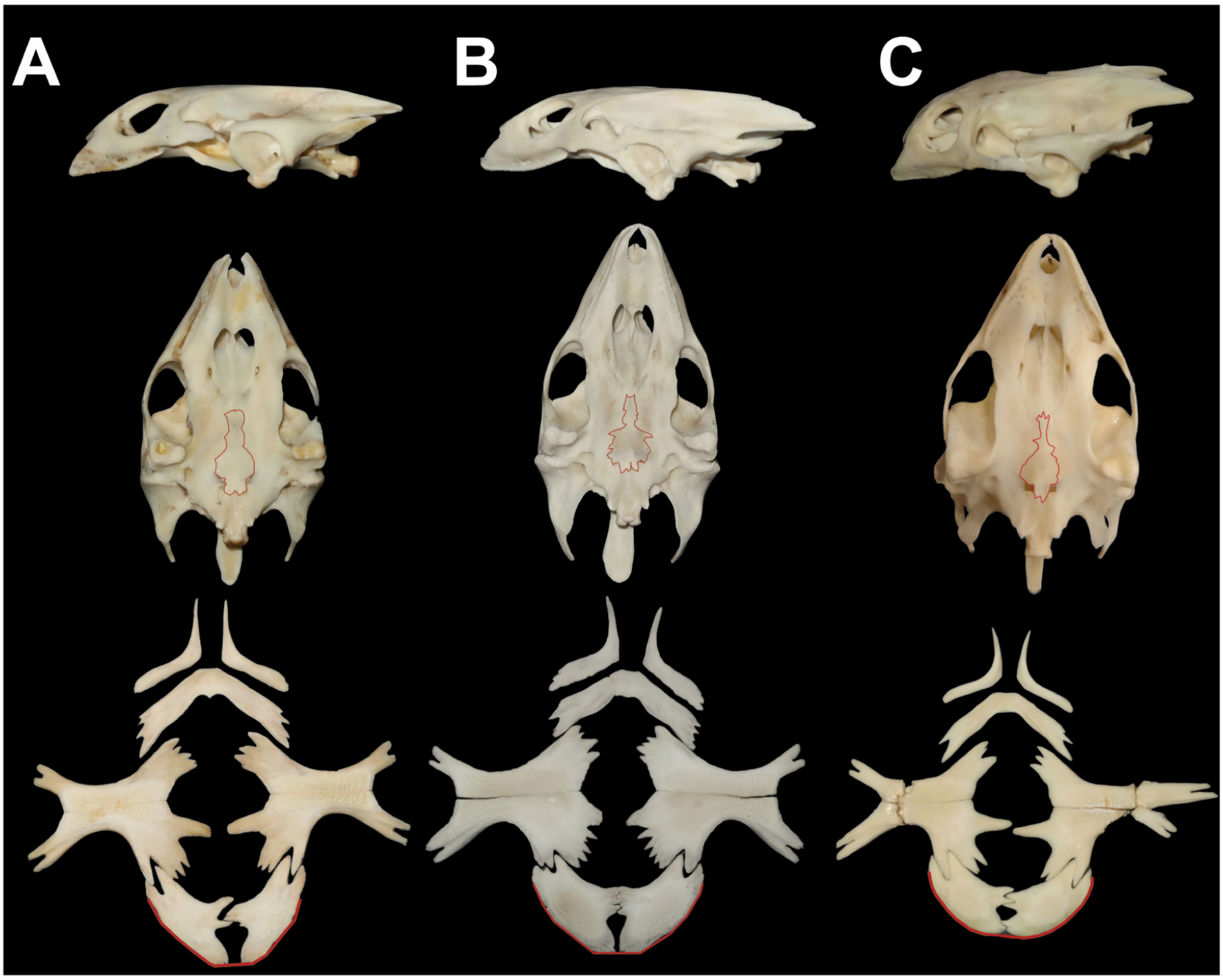

FIGURE 5. Comparative morphology of the skulls and plastral bones of females of the Yellow River softshell turtle (A), *Pelodiscus maackii* (B), and *P. sinensis* (C). From top to bottom: lateral view of the skull, ventral view of the skull, plastral bones. Red boxes in the central images denote the basisphenoids; red markings in the lower row indicate the curvature of the xiphiplastra.

TABLE 2. Skeletal measurements of the Yellow River softshell turtle, *Pelodiscus maackii*, and *P. sinensis*.

	Basisphenoid[1]	Entoplastron[2]	Xiphiplastron[3]
Yellow River softshell turtle	< 1/2	89.57°–102.03°	< 130°
P. maackii	< 1/2	89.10°–108.23°	< 130°
P. sinensis	< 1/2	92.71°–112.54°	> 130°

[1]Ratio of the smallest width to the largest width.

[2]Internal angle of the bone.

[3]Angle formed by the xiphiplastra.

Discussion

The softshell turtle populations from the Yellow River Basin have long been assigned to *P. sinensis*. On this basis, wild softshell turtles harvested from the middle Yellow River were domesticated for aquaculture, giving rise to the commercially important "Yellow River strain" (Hu 2021). However, recent studies have suggested that the Yellow River softshell turtle is more closely related to the Japanese strain, based on both morphological characters (Liang *et al.* 2017; Wang *et al.* 2023; Liang *et al.* 2025) and molecular phylogenetic evidence (Zhang *et al.* 2014; Liang *et al.* 2025). Because the Japanese strain was introduced into China largely from Japan (Zhao 2008), and native Japanese *Pelodiscus* are referable to *P. maackii* (Suzuki & Hikida 2014), the taxonomic placement of the Yellow River softshell turtle warranted reassessment.

In terms of natural distribution, the current understanding was that *P. sinensis* ranges northward from southern China to the Yellow River Basin, whereas populations further north (e.g., in Heilongjiang and Liaoning) were assigned to *P. maackii* (TTWG 2025). Accordingly, the Yellow River Basin lies near the contact zone between the natural ranges of *P. sinensis* and *P. maackii*, underscoring the need for an integrative reassessment of the Yellow River populations using multiple lines of evidence.

In this study, we used genome-wide SNPs, complete mitochondrial genomes, external morphology, and osteology to reassess the taxonomic placement of the Yellow River softshell turtle. The combined results indicate that the Yellow River softshell turtle is more closely related to *P. maackii* and does not represent *P. sinensis*. Nuclear and mitochondrial phylogenies consistently recovered the Yellow River samples as sister to or within the *P. maackii* clade, respectively. External morphology (body coloration, carapace patterning, and external gross morphology) and osteological traits (xiphiplastral curvature) are more similar to *P. maackii* than to *P. sinensis*. Collectively, these data support assigning the Yellow River softshell turtle as a distinct geographic lineage of *P. maackii*.

The nuclear SNP tree placed the Yellow River softshell turtle as the sister clade to *P. maackii*, whereas in the mitochondrial tree sequences of the Yellow River turtles are embedded within *P. maackii*. Such mito-nuclear discordance is common in closely related or recently diverged taxa and has been widely documented across the animal kingdom (Toews & Brelsford 2012) and for turtles (Fritz *et al.* 2024; Kehlmaier *et al.* 2025). Also, the conflicting placements of *P. parviformis* and *P. variegatus* (Fig. 1) illustrate that mito-nuclear discordance occurs multiple times in *Pelodiscus*. Fritz *et al.* (2024) reviewed for turtles many cases of mito-nuclear discordance and found it widespread in several turtle families (Cryptodira: Emydidae, Geoemydidae, Kinosternidae, Trionychidae; Pleurodira: Chelidae), including cases of mitochondrial captures. We assume that the mito-nuclear discordance of *P. variegatus*, deeply divergent in SNP markers, originated from mitochondrial capture of an ancient mitochondrial lineage related to that of *P. sinensis*. Likewise, the mitochondrial genomes of the Yellow River softshell turtle and *P. maackii* sensu stricto were captured from *P. sinensis* and additional introgression between *P. maackii* sensu stricto and the Yellow River softshell turtle, or incomplete sorting, could have caused the observed missing differentiation. Parenthetically it may be noted that the deeply divergent mitochondrial lineage of *P. parviformis*, a species that is sister to *P. sinensis* in the nuclear SNP topology, seems to reflect a distinct evolutionary pattern and needs to be explored separately, which is beyond the scope of the present investigation.

From a regional historical perspective, the genetic pattern observed in the Yellow River softshell turtle and *P. maackii* sensu stricto is consistent with the complex geological and hydrological evolution of the Yellow River Basin and adjacent areas during the Late Pleistocene. Late Pleistocene geological and hydrological changes in northern China (e.g., Bohai Sea formation, river capture, and episodic palaeolake connectivity) have been shown to have restructured lineage divergence and spatial genetic structure in freshwater taxa (Ni *et al.* 2023), and similar processes could have driven initial divergence between Yellow River and Heilongjiang drainages, with subsequent secondary contact leading to mitochondrial introgression. Taken together, these considerations support treating the Yellow River softshell turtle as a geographic lineage of *P. maackii* sensu lato.

In coloration and pattern features, the Yellow River softshell turtle closely resembles *P. maackii*, whereas *P. sinensis* differs markedly, consistent with the molecular phylogenies. Morphometric analyses further indicate that *P. sinensis* is a relatively small-bodied softshell turtle (but larger than *P. huangshanensis*), whereas *P. maackii* is relatively large-bodied (maximum CL up to 35 cm in *P. maackii*, compared to 20 cm in *P. sinensis* and 8.4 cm in *P. huangshanensis*; Brandt 1857; Tang 1997; Fritz *et al.* 2010; Yang *et al.* 2011; Gong *et al.* 2021). According to interviews with local fishermen, the Yellow River softshell turtle specimens collected for this study are relatively small in size, but the largest individuals of this population can weigh up to approximately 6 kg. Based on this, we infer that their body size is comparable to that of *P. maackii* (Brandt 1857).

However, the morphological differences between *P. maackii* sensu stricto and the Yellow River softshell turtle are insufficient to provide unambiguous diagnostic characters separating the two lineages. In osteological comparisons, the shape of the parietal-supraoccipital crest observed in our *P. maackii* and *P. sinensis* material agrees with Chkhikvadze's (1987) observations for *P. maackii* sensu stricto. The crest in the Yellow River softshell turtle is straight, resembling that of *P. maackii* sensu stricto. The basisphenoid width ratio measured for *P. sinensis* agrees with the findings of Yang *et al.* (2011), but we detected no stable differences useful for a taxonomic diagnosis. Overall, the Yellow River softshell turtle closely resembles *P. maackii* in cranial shape and in the curvature formed by the paired xiphiplastra.

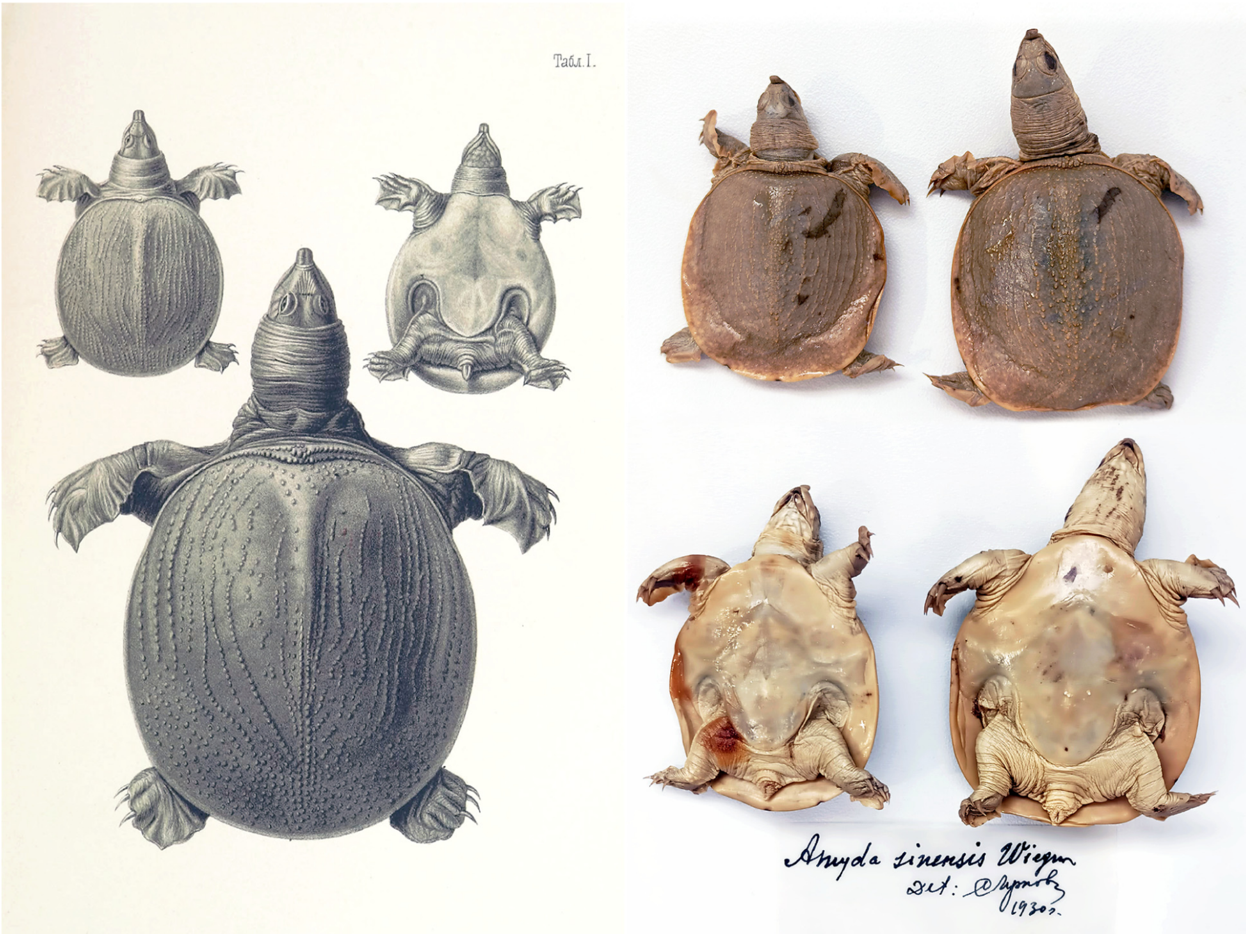

FIGURE 6. Plate I from Strauch (1876) showing two softshell turtles labeled *Trionyx sinensis* collected by Przhevalsky between 1870–1873 in the middle course of the Yellow River and now housed at the Zoological Institute of the Russian Academy of Sciences, St. Petersburg (left), and two juveniles collected by Przhevalsky in their current state, bearing the registration number ZISP 3909 (right; photos: K. D. Milto). The left-hand specimen appears to be identical with the upper individual seen in the plate; the lower individual in the plate is catalogued as ZISP 3906.

In the context of taxonomy, it should be noted that Nikolsky (1915) linked seven softshell turtles brought to Russia by explorer and naturalist Nikolay Mikhaylovich Przhevalsky from the middle course of the Yellow River ("Fl. Chuan-che" = Huanghe) in 1874 (ZISP 3906–3910) with *Trionyx schlegelii* Brandt, 1857. This taxon was based on a specimen obtained by Alexander Georg von Bunge in Peking (= Beijing) and the softshell turtles described and figured by Coenraad Jacob Temminck and Heinrich Schlegel (1834) in Volume III of Philipp Franz von Siebold's first edition of "Fauna japonica". Bunge's specimen, which has to be regarded as a syntype of *Trionyx schlegelii*, was presented to the Imperial Russian Academy (now Zoological Institute of the Russian Academy of Sciences), St. Petersburg, in 1833 (ZISP 177). Plate I from Przhevalsky's travelogue (Strauch 1876) and two preserved juveniles from his collection are shown in our Figure 6. However, Nikolsky (1915) also listed material from "Fl. Chon-Kiang" (= Changjiang/Yangtze River), Shanghai, "Sheché" (Shenzhen?), "Tschun-tschun-tschou" and even Korea as representing *Trionyx schlegelii*, giving reason to Chernov (1930) for lumping them all under *Amyda sinensis*. In its present condition, the strongly faded, virtually unpatterned syntype of *T. schlegelii* (ZISP 177, also bearing a tag with the number "59.a." attached to its left foreleg) cannot be unambiguously referred to any specific *Pelodiscus* taxon, so we refrain from reinstating this name here for the Yellow River softshell turtle or from confirming its synonymization with *P. sinensis*.

Instead, we presently assign the Yellow River softshell turtle to *P. maackii* sensu lato as a distinct geographic lineage. We therefore revise the native range of *P. maackii* as extending south into parts of the Yellow River Basin (i.e., Shaanxi, Shanxi) and north through northeastern China (Heilongjiang, Jilin, Liaoning, Inner Mongolia) to the Korean Peninsula, Japan, and the Russian Far East (Fig. 7). In contrast, *P. sinensis* is likely confined to south-central China, and our data do not support the hypothesis that it is native to the Yellow River Basin (Fig. 7). Based on the available evidence, we consider areas north of the Yellow River to be inhabited primarily by *P. maackii* sensu stricto. Notably, our survey indicates that the Yellow River softshell turtle also occurs in Yinchuan, Ningxia. We hypothesize that the Yanshan Mountains and associated uplands (including the Shanhai Pass region), which separate the North China Plain from the Northeast China Plain, may have subdivided *P. maackii* sensu lato into two geographic populations: the Yellow River population within the respective river basin and a northeastern population across the Northeast China Plain, corresponding to *P. maackii* sensu stricto. Nevertheless, because sampling within this large drainage was geographically limited, populations from the lower Yellow River and the North China Plain (e.g., Beijing, Tianjin, Hebei, Shandong) require further investigations.

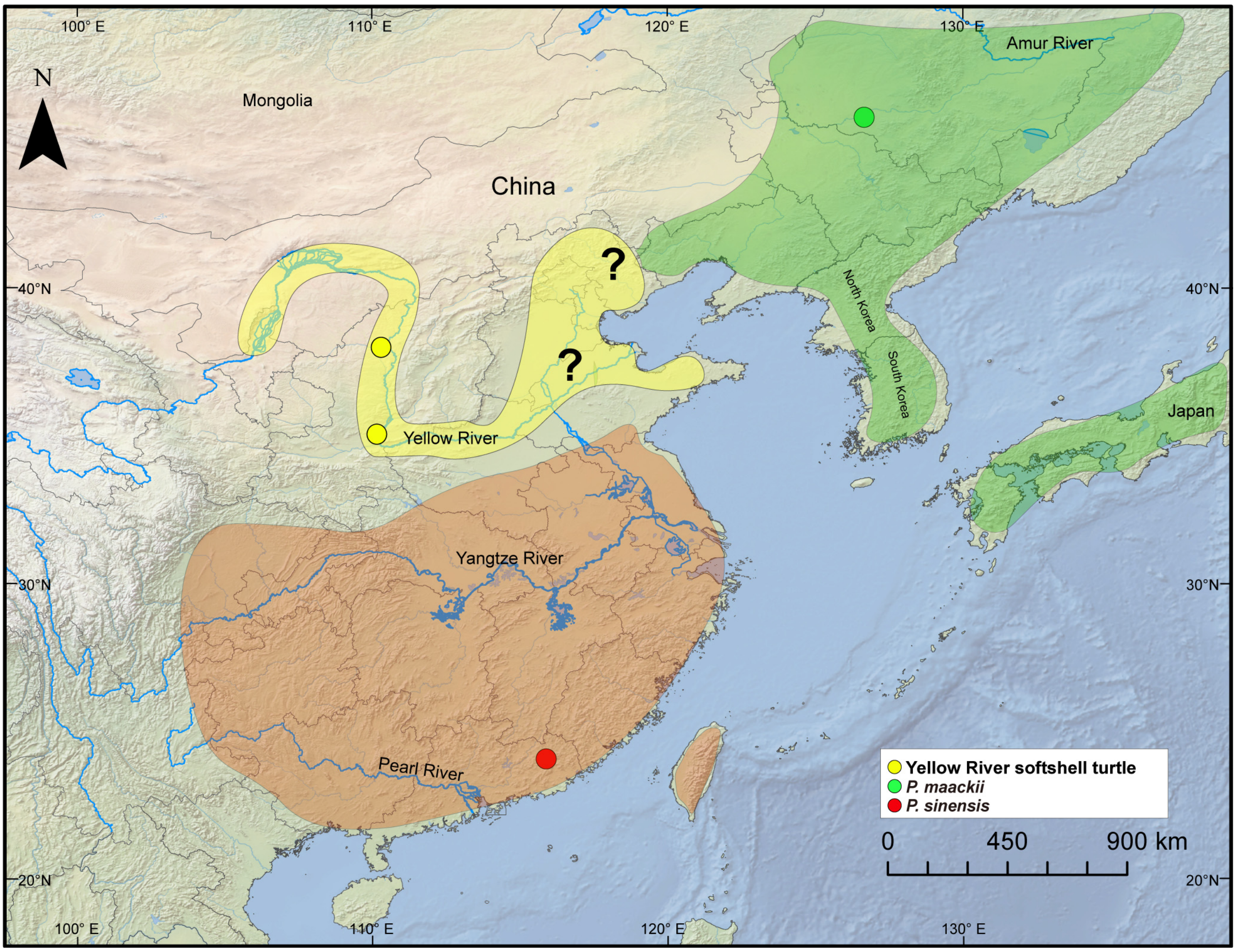

FIGURE 7. Sampling sites of the Yellow River softshell turtle (yellow), *Pelodiscus maackii* (green), and *P. sinensis* (red) in this study, and their natural distribution ranges (TTWG 2025). Question marks denote areas where distribution limits remain uncertain and require confirmation through further sampling.

Our results have important implications for taxonomy, conservation, and aquaculture management. The widely used aquaculture designation "Yellow River strain of *P. sinensis*" is taxonomically misleading, with potential consequences for germplasm management, breeding strategies, and conservation planning. Because native softshell turtles in the Yellow River represent a distinct geographic lineage of *P. maackii* sensu lato, strain nomenclature, germplasm preservation, and stocking practices should explicitly consider its true taxonomic identity and genetic background to prevent admixture between species or lineages. Further work with broader geographic sampling and multiple lines of evidence is needed to refine distributional boundaries and further elucidate the evolutionary relationships of *P. maackii* sensu lato and *P. sinensis*.

This study reassesses the taxonomy of the Yellow River softshell turtle using integrative evidence from nuclear SNPs, complete mitochondrial genomes, external morphology, and osteology. The results provide firm evidence that the Yellow River populations are conspecific with *Pelodiscus maackii* sensu stricto and represent a distinct geographic lineage of that species. These findings clarify the taxonomic identity of the Yellow River populations and the native distribution patterns of *P. maackii* sensu lato and *P. sinensis*, providing a scientific basis for the conservation of the diversity of *Pelodiscus* lineages, the management of aquaculture stocks, and the sustainable use of wild softshell turtle resources.

Acknowledgments

We would like to thank Yan Ge, Liwei Fan, Yangyang Chen, Youqiang Yin, Qiongyu Zhang, and Shihao Peng for assistance with specimen collection and the preparation of softshell turtle skeletal specimens used in this study. We further thank Konstantin D. Milto for sending us photographs of the syntype of *Trionyx schlegelii* and Przhevalsky's specimens housed at ZISP. This work was supported by the National Natural Science Foundation of China (No. 32270542) and the Basic and Applied Research Foundation of Guangzhou, Guangdong, China (2025A04J3510).

References

Brandt, J.F. (1857) Observationes quaedam ad generis Trionychum species duas novas spectantes auctore J.F. Brandt. *Bulletin de la Classe Physico-Mathématique de l'Académie Impériale des Sciences de Saint Pétersbourg*, 16, cols. 110–111.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17 (4), 540–552.
https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chan, K.O. & Grismer, L.L. (2026) GroupStruct2: A user-friendly graphical user interface for statistical and visual support in species diagnosis. *Systematic Biology*, 75 (4), 814–823.
https://doi.org/10.1093/sysbio/syaf090

Chen, J., Jiao, J., Yuan, X., Huang, X., Huang, L., Lin, L., Yin, W., Yao, J. & Zhang, H. (2023) Complete mitochondrial genomes of four *Pelodiscus sinensis* strains and comparison with other Trionychidae species. *Biology*, 12 (3), 406.
https://doi.org/10.3390/biology12030406

Chernov, S.A. (1930) O dal'nevostochnykh i kitayskikh cherepakhakh r. *Amyda*. *Doklady Akademii nauk SSSR*, 1930, 251–255. [in Russian]

Chkhikvadze, V.M. (1987) O sistematicheskom polozhenii dal'nevostochnogo trioniksa. *Bulletin of the Academy of Sciences of the Georgian SSR*, 128, 609–611. [in Russian]

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R. & 1000 Genomes Project Analysis Group (2011) The variant call format and VCFtools. *Bioinformatics*, 27 (15), 2156–2158.
https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M. & Li, H. (2021) Twelve years of SAMtools and BCFtools. *GigaScience*, 10 (2), giab008.
https://doi.org/10.1093/gigascience/giab008

Farkas, B., Ziegler, T., Pham, C.T., Ong, A.V. & Fritz, U. (2019) A new species of *Pelodiscus* from northeastern Indochina (Testudines, Trionychidae). *ZooKeys*, 824, 71–86.
https://doi.org/10.3897/zookeys.824.31376

Fritz, U., Gong, S., Auer, M., Kuchling, G., Schneeweiß, N. & Hundsdörfer, A.K. (2010) The world's economically most important chelonians represent a diverse species complex (Testudines: Trionychidae: *Pelodiscus*). *Organisms Diversity & Evolution*, 10 (3), 227–242.
https://doi.org/10.1007/s13127-010-0007-1

Fritz, U., Herrmann, H.-W., Rosen, P.C., Auer, M., Vargas-Ramírez, M. & Kehlmaier, C. (2024) *Trachemys* in Mexico and beyond: Beautiful turtles, taxonomic nightmare, and a mitochondrial poltergeist (Testudines: Emydidae). *Vertebrate Zoology*, 74, 435–452.
https://doi.org/10.3897/vz.74.e125958

Gong, S.P., Fritz, U., Vamberger, M., Gao, Y. & Farkas, B. (2022) Disentangling the *Pelodiscus axenaria* complex, with the description of a new Chinese species and neotype designation for *P. axenaria* (Zhou, Zhang & Fang, 1991). *Zootaxa*, 5125 (2), 131–143.
https://doi.org/10.11646/zootaxa.5125.2.2

Gong, S.P., Li, J., Fritz, U. & Farkas, B. (2025) When light casts long shadows: The increasingly dark truth about *Pelodiscus axenaria* (Zhou, Zhang & Fang, 1991). *Zootaxa*, 5604 (1), 94–100.
https://doi.org/10.11646/zootaxa.5604.1.11

Gong, S.P., Vamberger, M., Auer, M., Praschag, P. & Fritz, U. (2018) Millennium-old farm breeding of Chinese softshell turtles (*Pelodiscus* spp.) results in massive erosion of biodiversity. *Science of Nature*, 105, 34.
https://doi.org/10.1007/s00114-018-1558-9

Gong, Y., Peng, L., Huang, S., Lin, Y., Huang, R., Xu, Y., Yang, D. & Nie, L. (2021) A new species of the genus *Pelodiscus* Fitzinger, 1835 (Testudines: Trionychidae) from Huangshan, Anhui, China. *Zootaxa*, 5060 (1), 137–145.
https://doi.org/10.11646/zootaxa.5060.1.7

Hu, W. (2021) *Genetic diversity analysis of the Wuwangdu Yellow River softshell turtle population*. Dissertation, Shanxi University, Taiyuan, 61 pp. [in Chinese, with English abstract]
https://doi.org/10.27284/d.cnki.gsxiu.2021.001636

Jin, J.J., Yu, W.B., Yang, J.B., Song, Y., DePamphilis, C.W., Yi, T.S. & Li, D.Z. (2020) GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology*, 21 (1), 241.
https://doi.org/10.1186/s13059-020-02154-5

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30 (4), 772–780.
https://doi.org/10.1093/molbev/mst010

Kehlmaier, C., Fritz, U. & Kuchling, G. (2025) The taxonomic quagmire of northern Australian snake-necked turtles (Testudines: Chelidae): *Chelodina kuchlingi*—Extinct or hiding in plain sight? *Vertebrate Zoology*, 75, 127–145.
https://doi.org/10.3897/vz.75.e150370

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874.
https://doi.org/10.1093/molbev/msw054

Li, H. & Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, 25 (14), 1754–1760.
https://doi.org/10.1093/bioinformatics/btp324

Li, J.X., Zeng, Z.C., Wang, Y.Y., Liang, D. & Zhang, P. (2019) Sequence capture using AFLP-generated baits: A cost-effective method for high-throughput phylogenetic and phylogeographic analysis. *Ecology and Evolution*, 9 (10), 5925–5937.
https://doi.org/10.1002/ece3.5176

Liang, H.W., Cao, L.H., Li, X., Tong, M.M., Jiang, Y.L., Li, Z., Luo, X.Z. & Zou, G.W. (2017) Morphological differences among three cultured strains of the Chinese softshell turtle, *Pelodiscus sinensis*. *Freshwater Fisheries*, 47 (4), 91–96. [in Chinese, with English abstract]
https://doi.org/10.13721/j.cnki.dsyy.2017.04.014

Liang, Y., Huang, C., Wang, P., Xiao, H., Wang, Z.A., Zeng, J., Wang, X., Xiong, S., Hu, Y. & Qin, Q. (2025) Research on SSR genetic molecular markers and morphological differences of different *Pelodiscus sinensis* populations. *Genes*, 16 (3), 318.
https://doi.org/10.3390/genes16030318

Liu, J.Y., Fu, W., Ma, Y.H., Zhou, T., Blanck, T., Zhou, G.J., Xiao, Y.M. & Mo, X.Y. (2024) Shedding light into the confusing history of *Pelodiscus axenaria* (Zhou, Zhang et Fang, 1991), clarifying and correcting its taxonomic position within the *P. axenaria* complex with the description of yet another *Pelodiscus* species. *Russian Journal of Herpetology*, 31 (6), 337–350.
https://doi.org/10.30906/1026-2296-2024-31-6-337-350

Luo, D. (2024) Quantitative analysis of fish morphology through landmark and outline-based geometric morphometrics with free software. *bio-protocol*, 14 (20), e5087.
https://doi.org/10.21769/BioProtoc.5087

Meng, G., Li, Y., Yang, C. & Liu, S. (2019) MitoZ: A toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research*, 47 (11), e63.
https://doi.org/10.1093/nar/gkz173

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32 (1), 268–274.
https://doi.org/10.1093/molbev/msu300

Ni, X., Chen, Y., Deng, G. & Fu, C. (2023) Pleistocene landscape dynamics drives lineage divergence of a temperate freshwater fish *Gobio rivuloides* in coastal drainages of northern China. *Genes*, 14 (12), 2146.

https://doi.org/10.3390/genes14122146
Nikolsky, A.M. (1915) *Faune de la Russie et des pays limitrophes. Reptiles (Reptilia), Volume 1. Chelonia et Sauria*. Académie Impériale des Sciences de Petrograd, 532 pp. [in Russian with additional French title].
Onwuama, K.T., Salami, S.O., Ali, M. & Nzalak, J.O. (2012) Effect of different methods of bone preparation on the skeleton of the African giant pouched rat (*Cricetomys gambianus*). *International Journal of Morphology*, 30 (2), 425–427. https://doi.org/10.4067/S0717-95022012000200011
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J. & Sham, P.C. (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81 (3), 559–575. https://doi.org/10.1086/519795
Rennick, S.L., Fenton, T.W. & Foran, D.R. (2005) The effects of skeletal preparation techniques on DNA from human and non-human bone. *Journal of Forensic Sciences*, 50 (5), JFS2004405-4. https://doi.org/10.1520/JFS2004405
Strauch, A. (1876) Presmykayushchiyasya i zemnovodnyya. *In*: Przhevalsky, N.M. (Ed.), *Mongoliya i strana tangutov. Trekhletnee puteshestvie v vostochnoi nagornoi Azii. Volume II*. V.S. Balasheva, St. Petersburg, pp. 1–55 + pls. I–VIII. [in Russian]
Stuckas, H. & Fritz, U. (2011) Identity of *Pelodiscus sinensis* revealed by DNA sequences of an approximately 180-year-old type specimen and a taxonomic reappraisal of *Pelodiscus* species (Testudines: Trionychidae). *Journal of Zoological Systematics and Evolutionary Research*, 49 (4), 335–339. https://doi.org/10.1111/j.1439-0469.2011.00632.x
Suzuki, D. & Hikida, T. (2014) Taxonomic status of the soft-shell turtle populations in Japan: A molecular approach. *Current Herpetology*, 33 (2), 171–179. https://doi.org/10.5358/hsj.33.171
Tang, Y.Z. (1997) Research on a new species of *Pelodiscus* (Trionychidae) in China. *Zoological Research*, 18 (1), 13–17. [in Chinese, with English abstract]
Temminck, C.J. & Schlegel, H. (1834) Reptilia. I. Les chéloniens. *In*: von Siebold, P.F. (Ed.), *Fauna Japonica, sive Descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batava Imperium tenent, suscepto, annis 1823–1830 collegit, notis observationibus et adumbrationibus illustravit, Vol. III*. J.G. la Lau, Lugduni Batavorum, pp. 1–80 + pls. I–IX.
Toews, D.P. & Brelsford, A. (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, 21 (16), 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x
TTWG [Turtle Taxonomy Working Group] (2025) Turtles of the world. Annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status (10th edition). *Chelonian Research Monographs*, 10, 1–575. https://doi.org/10.3854/crm.10.checklist.atlas.v10.2025
Wang, F., Song, G.T., Chen, Z., Xu, B., Ji, S.F., Zhou, X., Xu, X.N., Hou, G.J., Zhang, Y., Li, D., Zhu, C.J., Su, Y.F., Dong, Z.X. & Jiang, Y.L. (2023) Morphological differences and path analysis among four cultured strains of the Chinese softshell turtle, *Pelodiscus sinensis*. *Chinese Agricultural Science Bulletin*, 39 (20), 147–154. [in Chinese, with English abstract] https://doi.org/10.11924/j.issn.1000-6850.casb2022-0560
Wiegmann, A.F.A. (1834) Beiträge zur Zoologie gesammelt auf einer Reise um die Erde. Siebente Abhandlung. Amphibien. *Nova Acta Physico-Medica Academia Caesarea Leopoldino-Carolina*, 17, 185–268.
Xie, M.M., Hong, X.Y., Li, W., Liu, X.L., Chen, C. & Zhu, X.P. (2022) The skeletal system of the Yangtze giant softshell turtle and comparisons with the Chinese softshell turtle, *Pelodiscus sinensis*. *Journal of Hydrobiology*, 46 (5), 654–663. [in Chinese, with English abstract] https://doi.org/10.7541/2021.2021.074
Yang, P., Tang, Y.Z., Ding, L., Guo, X.G. & Wang, Y.Z. (2011) Validity of *Pelodiscus parviformis* (Testudines: Trionychidae) inferred from molecular and morphological analyses. *Asian Herpetological Research*, 2 (1), 21–29. https://doi.org/10.3724/SP.J.1245.2011.00021
Zhang, C., Zhang, H.Q., Xu, X.J., He, Z.Y. & Wang, C.L. (2014) Comparative analysis of 16S rRNA gene polymorphism in two cultured strains of the Chinese softshell turtle, *Pelodiscus sinensis*. *Journal of Fishery Sciences of China*, 21 (2), 398–404. [in Chinese]
Zhao, C.G. (2008) Biological characteristics and new techniques for breeding and culture of the Japanese softshell turtle (II). *Scientific Fish Farming*, (2), 12–13. [in Chinese]
Zhou, G., Zhang, X. & Fang, Z. (1991) Bulletin of a new species *Trionyx*. *Acta Scientiarum Naturalium Universitatis Normalis Hunanensis*, 14, 379–382. [in Chinese, with English abstract]

Supplementary Materials. The following supporting information can be downloaded from figshare.com using the link https://doi.org/10.6084/m9.figshare.32113177

Figures S1–S3 and Tables S1–S4