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A new species of the genus *Pelodiscus* (Testudines, Trionychia) from Jiangxi China

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Abstract

A new species of softshell turtle (*Pelodiscus jiangxiensis* **sp. nov.**) is described based on five specimens from Jiangxi, China. Phylogenetic analysis of mitochondrial sequences indicated that the new species is the sister lineage to a *Pelodiscus* clade that includes two currently recognized species, namely *P. axenaria* (Zhou, Zhang & Fang, 1991) from Hunan and *P. huangshanensis* Gong *et al.* 2021 from Anhui. Morphologically, the new species is most similar to *P. axenaria* but can be distinguished by its plain, yellowish white plastron, the absence of the single dark gray central figure, and a distinct carapace pattern. The description of this species increases the number of recognized *Pelodiscus* species to seven, of which six are distributed in China and four are endemic.

Key words: morphology, *Pelodiscus jiangxiensis* **sp. nov.**, phylogeny, soft-shelled turtle

Introduction

The genus *Pelodiscus* Fitzinger is widely distributed in East Asia, with a distribution ranging from the Amur and Ussuri Rivers region of far eastern Russia through the Korean Peninsula, Japan, and eastern and southern China to southern Vietnam (Fritz *et al.* 2010; Prashag *et al.* 2011; TTWG 2017; Gong *et al.* 2018). The genus was first described in 1834 by the German zoologist August Wiegmann, based on juvenile specimens collected from a small island in the Tiger River, close to Macao (Wiegmann 1834). The genus *Pelodiscus* was long considered to include a single species, *P. sinensis*, despite a high degree of morphological variation within its distribution (van Dijk *et al.* 2000; Ernst *et al.* 2000; Zhao and Adler 1993), until Chkhikvadze (1987) discovered another species, *P. maackii*, in the northernmost part of its range by examining osteological features. Two additional species from central China, *P. axenaria* (Zhou, Zhang & Fang, 1991) and *P. parviformis* (Tang, 1997), were described in the 1990s based on morphological characters. However, the validity of the three species was repeatedly questioned and not accepted by many researchers for a long time (Ernst *et al.* 2000; TTWG 2007), until Fritz *et al.* (2010) first confirmed the validity of *P. maackii* and *P. axenaria* using a combined analysis of three mitochondrial DNA (mtDNA) fragments and one nuclear locus. Yang *et al.* (2011) arrived at the same conclusion with respect to *P. parviformis* based on molecular and morphological data.

Through broad sampling, Gong *et al.* (2018) discovered four previously unknown mitochondrial lineages, including one deeply divergent lineage from Jiangxi named *Pelodiscus sp* clade I, which forms the sister clade to the *P. axenaria* lineage. The analysis of nuclear loci (R35, Rag2, P26S4) also supported the species-level status of *Pelodiscus sp* clade I. However, no in-depth studies on this lineage were conducted owing to a lack of specimens and morphological data. Two other *Pelodiscus* species were recently described on the basis of morphology and molecular analyses, namely *P. variegatus* (Farkas *et al.* 2019) and *P. huangshanensis* (Gong *et al.* 2021). These recent discoveries of novel *Pelodiscus* spp. suggest that the taxonomic richness of the genus is highly underestimated. Furthermore, the phylogenetic analysis performed by Gong *et al.* (2021) revealed that *P. huangshanensis* is a sister group to the cryptic species *Pelodiscus sp* clade I, and cluster with *P. axenaria*.

In this study, we collected five specimens of suspected cryptic species *Pelodiscus sp* clade I from two cities in Jiangxi Province, China, and performed phylogenetic analysis using *cyt b* and *ND4* to confirm its uniqueness and species-level taxa. Its morphological features and distinction from other *Pelodiscus* spp are described in detail. Based on these analyses, we believe it is a new species.

Materials and methods

Sampling. Five softshell turtle specimens were collected from Jiangxi, China, in August 2021—two (one adult female and one subadult male) from Yingtan city and three (two adult males and one subadult female) from Yichun city. Tissue (muscle) samples were collected from the two Yingtan specimens (JY20220001, JY20220002) and subsequently fixed in metformin substances, and then transferred to the Turtle Museum of Hainan Normal University for permanent storage (Figs. 2 and 3). Meanwhile, the three Yichun specimens were kept in captivity at the College of Life Sciences of Hainan Normal University (Fig. 4), and small tissue samples were taken from the edge of the turtles' soft shells, preserved in 95% ethanol, and stored at -40 °C prior to DNA extraction and PCR. Tissue samples were only collected from the two adult Yichun specimens (JYC20220001, JYC20220002) in order to avoid unnecessary harm to the smallest one. This species is highly sensitive to disturbance, and tissue sample collection could elicit a stress response, resulting in refusal to feed, shell rot, or even death, especially in younger individuals.

Morphometrics. Morphological analysis was performed following Zhou *et al.* (1991), Tang (1997), Farkas *et al.* (2019), and Gong *et al.* (2021) and included the following morphological characters: tubercles on carapace surface; head length (HL, from snout tip to posterior margin of parietal); head width (HW, at widest part of temporal region); snout length (SL); distance between eyes (DEL, minimum distance between eyes); carapace length (CL); carapace width (CW); plastron length (PL); body length (BL, from snout tip to tail end); carapace and plastron color; and presence or absence of dark spot(s) on plastron. All measurements were obtained using electronic calipers and were made to the nearest 0.01 mm.

DNA extraction, polymerase chain reaction (PCR), and sequencing. Genomic DNA was extracted from muscle or soft shell edge tissue samples using a DNA extraction kit from Tiangen Bio-tech Co., Ltd. (Beijing, China). Partial segments of the mitochondrial genes *cyt b* and *ND4* were amplified using specific primers (*cyt b*: C-F and C-R, Chen 2006; *ND4*: ND4-672 and H-Leu, Engstrom *et al.* 2004, Stuart & Parham 2004). The PCR for *cyt b* was performed using the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 50 s, 52 °C for 45 s, and 72 °C for 80 s, and a final extension at 72 °C for 10 min. The PCR for *ND4* was performed in the following conditions: initial denaturation at 94 °C for 3 min; 35 cycles at 94 °C for 41 s, 55 °C for 41 s, and 72 °C for 60 s, and a final extension at 72 °C for 3 min. The resulting PCR products were sequenced using Guangzhou Ai Ji Bio-tech Co., Ltd. (Guangzhou, China)

Phylogenetic analyses. The raw sequences in the DNA sequence file (.seq) provided by Guangzhou Ai Ji Bio-tech were first aligned using CodonCode Aligner (DHL 2014) and manually stitched. The sequences were submitted to GenBank (accession numbers: OM328074-OM328077 for *cyt b*; OM328078-OM328081 for *ND4*). Sequences from the other six *Pelodiscus* species and from one outgroup taxon *Palea steindachneri* Siebenrock 1906 were downloaded from GenBank (Engstrom *et al.* 2004; Fritz *et al.* 2010; Stuckas & Fritz 2011; Yang *et al.* 2011; Suzuki & Hikida 2014; Dong *et al.* 2016; Gong *et al.* 2018; Yu *et al.* 2019) and aligned to sequences from *P. jiangxiensis* **sp. nov.** using BioEdit (Borland 1983). Sequences (*cyt b* and *ND4*) from the *P. sinensis* lectotype (FR851459 and FR851460, respectively), and the complete mtDNA sequence of *P. huangshanensis* (MK867844; Yu *et al.* 2019; Gong *et al.* 2021) were used. MEGA 7.0.21 (Kumar *et al.* 2016) was used to construct a maximum likelihood (ML) tree using the Kimura 2-parameter model with 500 bootstrap (BS) replicates and to calculate pairwise distances (*p*-distances) among the taxa.

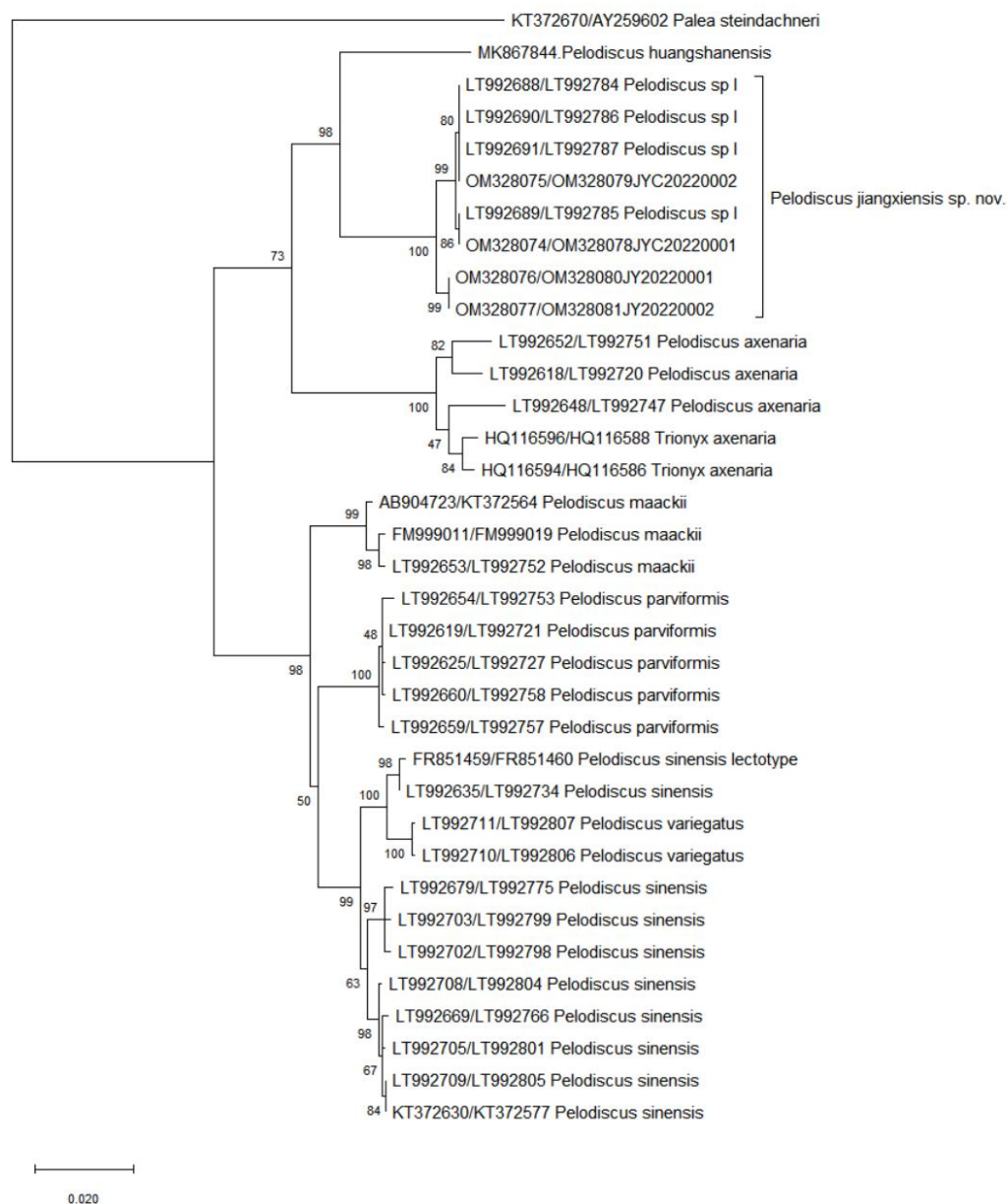


Figure 1. Maximum likelihood (ML) phylogenetic tree based on concatenated *cyt b* and *ND4* fragments. Node values indicate the bootstrap support.

Results

The *cyt b* and *ND4* sequences from four specimens shared two and three haplotypes, respectively. The four *P. jiangxiensis* **sp. nov.** specimens collected in this study formed two clades, with the two collected from Yingtan city diverging from the two collected from Yichun city, which clustered together with the cryptic *Pelodiscus* *sp.* clade I (Gong *et al.* 2018) that were also collected from Yichun city, Jiangxi province. Thus, the mysterious clade described by Gong *et al.* (2018) should be considered synonymous with *P. jiangxiensis* **sp. nov.** Furthermore, *P. jiangxiensis* **sp. nov.** formed a sister group with *P. huangshanensis*, and this clade was sister to *P. axenaria*, which is mainly distributed in Hunan and the adjacent Guangxi area. Phylogenetic analysis of the remaining *Pelodiscus* species yielded results that were similar to those described by Fritz *et al.* 2010, Prashag *et al.* 2011; Stuckas & Fritz 2011, Yang *et al.* 2011, and Gong *et al.* 2018, i.e., the *p*-distances, based on concatenated *cyt b* and *ND4* sequences, between *P. jiangxiensis* **sp. nov.** and six other species ranged from 0.045 (*P. huangshanensis*) to 0.074 (*P. sinensis*) (Table 1). Combined with morphological data, the specimens from Jiangxi Province China should be considered as a new species.

Table 1. Uncorrected pairwise distances of concatenated *cyt b* and *ND4* sequences among the genus *Pelodiscus*.

	1	2	3	4	5	6	7
1 <i>Pelodiscus_jiangxiensis</i> <i>sp. nov.</i> (n=8)	0.001-0.007						
2 <i>P._maackii</i> (n=3)	0.068-0.071	0.002-0.005					
3 <i>P. parviformis</i> (n=5)	0.066-0.070	0.025-0.030	0.001-0.004				
4 <i>Pelodiscus axenaria</i> (n=5)	0.059-0.067	0.074-0.082	0.069-0.078	0.006-0.024			
5 <i>P. sinensis</i> (n=10)	0.064-0.074	0.023-0.031	0.021-0.029	0.073-0.085	0.000-0.006		
6 <i>P. huangshanensis</i> (n=1)	0.045-0.046	0.073-0.075	0.073-0.075	0.057-0.064	0.069-0.076	0	
7 <i>P. variegatus</i> (n=2)	0.070-0.073	0.030-0.032	0.030-0.031	0.079-0.085	0.009-0.017	0.075	0.001

Suggested English name: Jiangxi softshell turtle

Suggested Chinese name: 江西鳖

Holotype. JY20220001, an adult female (Fig. 2), was collected from an upstream sandy bottom river in PingDing Town, YuJiang District, Yingtan City, Jiangxi Province, China. The specimen was collected during a field survey on August 5, 2021, and was deposited at the Turtle Museum of Hainan Normal University (voucher: JY20220001).

Paratypes. JY20220002, subadult male (Fig. 3); JYC20220001, adult male; JYC20220002, subadult female; JYC20220003, adult male (Fig. 4).

Description of holotype. Holotype an adult female, carapace length 93.45 mm; plastron length 69.12 mm; body length 143.37 mm (Table 2). Back and sides of head uniformly olive brown; pale yellow spots with fine edge on both upper and lower jaws and adjacent areas of the throat; faint yellow band on each side of the neck; no black pinstripes around eyes. Carapace oval with a high keel, olive brown with small black dots and markings dispersed, dark golden mottling more or less scattered, and underside of leathery margin pigmented. Tubercles on dorsal surface indistinct. Plastron yellowish-white with a dark patch on each side of the armpit and no other markings. Entoplastron “ \cap ” shaped.

Table 2. Morphometrics of *Pelodiscus jiangxiensis* **sp. nov.** specimens collected from Jiangxi, China. For abbreviations, see Materials and Methods.

	Sex	HL	HW	SL	DEL	CL	CW	PL	BL
JY20220001	female	33.09	16.68	7.05	3.59	93.45	80.46	69.12	143.37
JY20220002	male	30.9	14.4	4	2.53	72.47	68.09	58.07	127.03
JYC20220001	male	-	-	6.75	-	90.68	78.57	62.1	-
JYC20220002	female	-	-	4.56	-	70.6	67.81	55.93	-
JYC20220003	male	-	-	6.59	-	87.91	79.2	65.22	-

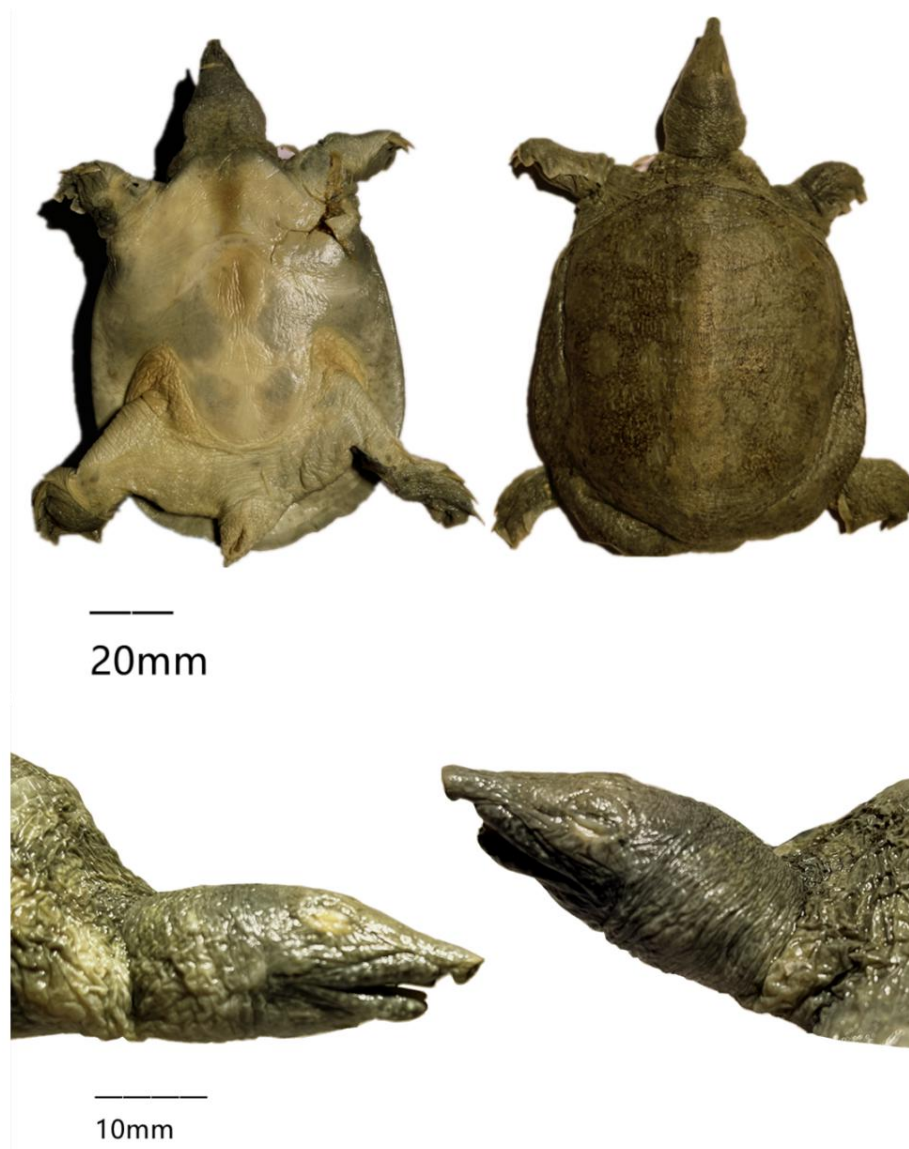


Figure 2. Dorsal and ventral aspects of the head and neck of the *Pelodiscus jiangxiensis* **sp. nov.** holotype (JY20220001, adult female).

Distribution. This species is currently known only from two localities, the cities of Yingtan and Yichun, Jiangxi, China. The species may also inhabit nearby areas but owing to lack of time and personnel, a wider survey was not performed in this study.

Morphological Comparisons.

Pelodiscus jiangxiensis **sp. nov.** can be distinguished from *P. sinensis* and *P. maackii* by: (1) small size; adult CL of 8–10 cm (vs. *P. sinensis* 23 cm and *P. maackii* 35 cm); (2) keel high (vs. keel low); (3) entoplastron “∩” shaped (vs. entoplastron “^” shaped).

Pelodiscus jiangxiensis **sp. nov.** can be distinguished from *P. variegatus* by: (1) small size; adult CL of 8–10 cm (vs. *P. variegatus* 23 cm); (2) plastron yellowish-white with no markings, only a dark patch on each side of the armpit, (vs. *P. variegatus* pinkish white to pale reddish orange with distinct, large dark blotches).

Pelodiscus jiangxiensis **sp. nov.** can be distinguished from *P. parviformis* by: (1) small size; adult CL of 8–10 cm (vs. *P. parviformis* 16 cm); (2) tubercles on dorsal surface indistinct (vs. dorsal tubercles in

longitudinal series more or less discrete, central tubercle in front of marginal ridge of carapace small); (3) entoplastron “∩” shaped (vs. entoplastron “^” shaped).

Pelodiscus jiangxiensis **sp. nov.** can be distinguished from *P. axenaria* by: (1) small size; adult CL of 8–10 cm (vs. *P. axenaria* 20 cm); (2) *P. jiangxiensis* **sp. nov.** plastron yellowish-white with no markings, only a dark patch on each side of the armpit, underside of leathery margin of carapace pigmented (vs. *P. axenaria* a single dark gray central figure enclosed by hypo- and xiphiplastra, underside of leathery margin of carapace unmarked (Fig 4)).

Pelodiscus jiangxiensis **sp. nov.** can be further distinguished from *P. huangshanensis* by: (1) back and sides of head uniformly olive brown green; yellowish white spots with fine edge on upper and lower jaw and adjacent areas of the throat; faint yellow band marking on each side of the neck (vs. *P. huangshanensis* back and side of head with many fine dark brown to black spots; the neck shows no white longitudinal striae bilaterally); (2) carapace pattern is olive brown green with small black dots and markings dispersed, dark golden mottling pattern more or less scattered. Tubercles on dorsal surface indistinct (vs. *P. huangshanensis* carapace olive to dark brown. The dorsal surface has many tubercles, but it is indistinct in the center).

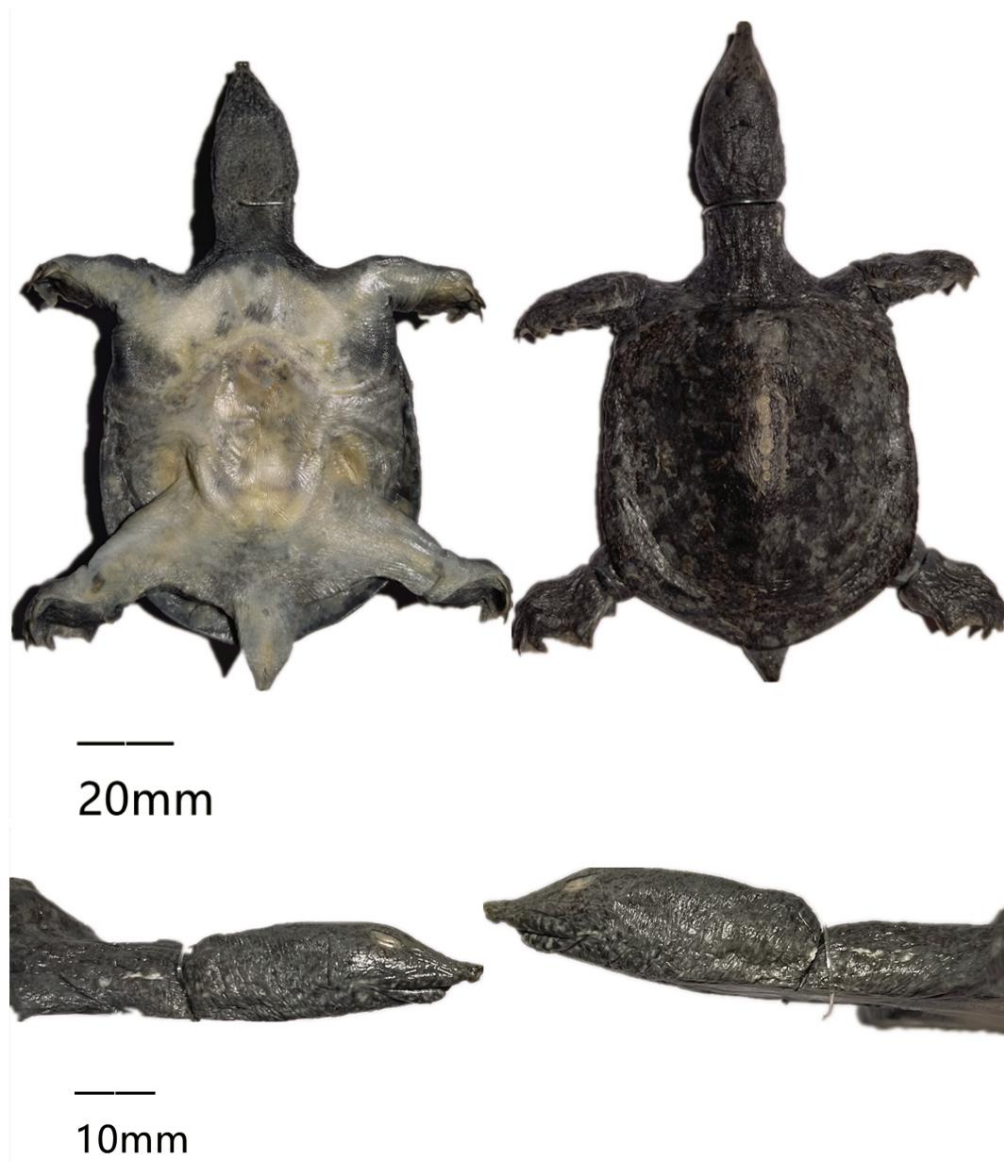


Figure 3. Dorsal and ventral aspects of the head and neck of a *Pelodiscus jiangxiensis* **sp. nov.** paratype (JY20220002, subadult male).



Figure 4. Dorsal and ventral aspects of *Pelodiscus jiangxiensis* sp. nov. paratypes (JYC20220001, adult male; JYC20220002, subadult female; JYC20220003, adult male) and a single *P. axenaria* specimen (HBC20220001, adult male) collected from Changde City, Hunan, China.

Discussion

China is the center of the *Pelodiscus* species distribution and harbors six of the seven currently recognized species, four (*P. parviformis*, *P. huangshanensis*, *P. axenaria*, and *P. jiangxiensis* **sp. nov.**) being endemic. This study described a new species *Pelodiscus jiangxiensis* **sp. nov.**, increasing the total number of species in this genus to seven, further proving the underestimated diversity of this genus. The discovery of *P. jiangxiensis* **sp. nov.** also provides an identity for the cryptic *Pelodiscus* sp clade I described by Gong *et al.* (2018).

The genus *Pelodiscus* has been traditionally exploited for food in China since the Spring and Autumn Period, over 2400 years ago (Kang and Yan 2000). Although the most widely hunted and consumed species is *P. sinensis* (Fritz 2010; Gong *et al.* 2018), the high morphological similarity among *Pelodiscus* species and lack of regulation inevitably puts other species with narrow ranges under the same hunting pressure. As far as we know, *P. maackii*, a once common species in northeast China, has now shrunk to only a few small isolated populations along China's borders with North Korea and Russia mainly as a consequence of over hunting for food from the late 1970s to early 1990s. Although their current population is low, they are not guaranteed any protection. During our survey we found that the two rare species *P. axenaria* and *P. jiangxiensis* **sp. nov.** are regarded as highly valuable food resources in their native ranges, with a price of 500–1000 yuan per kilogram, almost 2.5–5 times the price of the sympatric species *P. sinensis*. Owing to the lure of profit, both *P. axenaria* and *P. jiangxiensis* **sp. nov.** are widely hunted by local villagers without any restrictions or regulations. Furthermore, based on our observations of wild and captive individuals, we found that *Pelodiscus jiangxiensis* **sp. nov.**, unlike the generalist *P. sinensis*, only inhabits sandy bottom river segments with clear, highly oxygenated water, avoids deep water with silt substrate, and is highly sensitive to water quality and anthropogenic disturbance. This strict habitat preference makes *Pelodiscus jiangxiensis* **sp. nov.** far less common than the more adaptive *P. sinensis* in their range, resulting in a high probability of their population being imperiled by immoderate overhunting.

To date, most *Pelodiscus* species have not been subjected to in-depth ecological studies and less is known about their population status and habitats, especially for those that are newly discovered, although some of them may actually be endangered or even critically endangered. In conclusion, we call for a comprehensive reassessment of the conservation status of *Pelodiscus* species and prioritizing enforcement of appropriate conservation laws if necessary.

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