

Taxonomic revision of the critically endangered big-headed turtles (Reptilia: Testudines: Platysternidae Gray, 1869), with description of a new species

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ABSTRACT

The big-headed turtle (*Platysternon megacephalum*), currently the only extant member of the genus *Platysternon* and the family Platysternidae, has undergone severe population declines driven by poaching, illegal trade, and habitat loss, leading to its classification as Critically Endangered (CR) by the International Union for Conservation of Nature (IUCN). Despite its conservation status, persistent taxonomic ambiguities and unresolved phylogenetic relationships have hindered effective protection and management. This study integrated evidence from genome-wide single nucleotide polymorphisms (SNPs), mitochondrial DNA sequences (*COI*, *12S*), and morphological data to reconstruct the phylogeny and phylogeography of *Platysternon* and revise its taxonomy. Results revealed that *Platysternon megacephalum megacephalum* and *Platysternon megacephalum peguense* represent deeply divergent evolutionary lineages, which are herein recognized as distinct species, *Platysternon megacephalum* Gray, 1831 sensu stricto and *Platysternon peguense* Gray, 1870. The subspecies *Platysternon megacephalum shiui* Ernst & McCord, 1987 is taxonomically invalid. In addition, a highly divergent lineage endemic to the Baise-Hechi region of

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Guangxi is described as a third species, *Platysternon baiseensis* sp. nov. This revised taxonomic framework resolves key phylogenetic relationships and delineates species boundaries, establishing a robust systematic foundation for big-headed turtles and providing a critical taxonomic basis for future conservation efforts targeting this endangered group of turtles.

Keywords: Asia; Integrative Taxonomy; Phylogeny; Platysternidae; *Platysternon baiseensis* sp. nov.

INTRODUCTION

Platysternidae Gray, 1869, historically regarded as a monotypic family within the order Testudines, has long been considered to include only a single extant species, *Platysternon megacephalum* Gray, 1831, commonly referred to as the big-headed turtle. This species occurs across Southeast Asia, with a distribution spanning southern China (south of the Yangtze River), Vietnam, Laos, Thailand,

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Myanmar, and Cambodia (TTWG, 2021). Ongoing population collapse, primarily driven by intensive poaching, illegal trade, and widespread habitat destruction, has placed this lineage among the most imperiled Asian freshwater turtles (Gong et al., 2017; Hoang et al., 2021). International concern over its status has led to its classification as Critically Endangered (CR) by the International Union for Conservation of Nature (IUCN) and its inclusion in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which has prohibited international commercial trade since 2013 (UNEP-WCMC, 2021). The Chinese government further reinforced protection in 2021 by elevating its status to Class II under the national system for key protected species. Nevertheless, fundamental taxonomic uncertainties within Platysternidae continue to impede the implementation of evidence-based conservation strategies.

Taxonomic ambiguity surrounding big-headed turtles has persisted since their initial description. Gray (1831) originally assigned *P. megacephalum* to Emydidae based on a specimen from China, later erecting Platysternidae as a separate family (Gray, 1869), acknowledging its distinctive head and skull morphology. However, superficial morphological similarities with Chelydridae generated prolonged debate. Gaffney (1975) proposed collapsing Platysternidae into Chelydridae based on cranial similarities, a view later supported by Gaffney & Meylan (1988) through comparative analyses of cranial, shell, and vertebral morphology.

This hypothesis was later overturned by molecular evidence. Although early 12S rRNA data (Wu et al., 1999) tentatively placed *Platysternon* near Chelydridae, subsequent cytogenetic studies (Bickham & Carr, 1983; Haiduk & Bickham, 1982), nuclear DNA sequence data (Krenz et al., 2005), and multilocus phylogenies incorporating mitochondrial and nuclear markers (Parham et al., 2006; Thomson et al., 2021; Yan et al., 2008) consistently supported the recognition of Platysternidae as a distinct family. On this basis, modern systematics recognizes Platysternidae as a valid, independent family within Testudines (TTWG, 2021).

In contrast, intraspecific taxonomy of *P. megacephalum* has remained controversial. Historically, up to five subspecies have been distinguished based on morphological variation: *P. m. megacephalum* Gray, 1831, *P. m. peguense* Gray, 1870, *P. m. vogeli* Wermuth, 1969, *P. m. tristernalis* Schleich & Gruber, 1984, and *P. m. shiui* Ernst & McCord, 1987. However, traits used in these diagnoses, including carapacial margin serration and postocular stripe patterns, exhibit substantial individual variation, complicating subspecies delimitation. Ernst & Laemmerzahl (2002) conducted a comprehensive morphological reassessment, which synonymized *P. m. vogeli* with *P. m. peguense* and identified *P. m. tristernalis* as a junior synonym of *P. m. megacephalum*, leaving three subspecies tentatively recognized: *P. m. megacephalum*, *P. m. peguense*, and *P. m. shiui* (TTWG, 2021).

More recent genomic studies have challenged the validity of this subspecies framework. Based on mitochondrial genome sequencing, Luo et al. (2019) revealed that divergence between *P. m. peguense* and *P. m. megacephalum* is comparable to interspecific differentiation within the genus *Mauremys*. Gong et al. (2023) extended this finding using genome-wide single nucleotide polymorphisms (SNPs) and mitochondrial markers (*COI*, *12S*) across a broad geographic

range and delineated three deeply divergent clades: one corresponding to the nominotypical subspecies *P. m. megacephalum*, a second matching *P. m. peguense*, and a third previously unrecognized lineage endemic to Guangxi, China (“Baise lineage”). These findings supported recognition of three distinct species and refuted the validity of *P. m. shiui*. This taxonomic framework was independently corroborated by Ngo et al. (2023) through *ND4*-based analyses of specimens from China, Myanmar, and Vietnam. However, despite robust genetic evidence for taxonomic revision, these studies lacked integrative morphological analyses to formally diagnose the Baise lineage or define revised species boundaries.

To resolve these issues, this study expanded sampling across the distribution range of *Platysternon* and applied an integrative framework combining evidence from genome-wide SNPs, mitochondrial DNA sequences (*COI*, *12S*), and comparative morphological data to revise the taxonomy of *Platysternon* and establish a solid scientific foundation for the conservation and management of big-headed turtles.

MATERIALS AND METHODS

Sample collection and molecular data acquisition

A total of 143 samples from 67 localities originally reported by Gong et al. (2023) were re-evaluated for this study. Twelve samples with ambiguous provenance were excluded following rigorous assessment of their provenance. The retained dataset comprised 131 samples, including 90 with genome-wide SNP data. To enhance resolution of the Baise lineage and address sampling gaps, 24 additional specimens were incorporated, including nine newly collected samples from Baise (Guangxi, China), 11 tissue samples collected from four additional regions, Fangchenggang, Wuzhou (Guangxi), Nanchang (Jiangxi), and Jinghong (Yunnan), and four additional DNA samples from Guilin (Guangxi), Lanshan (Hunan), and Quang Nam (Vietnam). The final dataset consisted of 155 samples from 71 localities covering the known distribution range of *Platysternon* (Figure 1B; Supplementary Table S1). Tissue samples were preserved in anhydrous ethanol and stored at -20°C in the Animal Germplasm Resource Bank, College of Life Science and Technology, Jinan University. Genomic DNA was extracted using a TIANamp Genomic DNA Kit (Tiangen Biotech, China). Mitochondrial *COI* and *12S* gene sequences and SNPs were generated following the protocols described in Gong et al. (2023). All experimental protocols were approved by the Ethics Committee of the Institute of Zoology, Guangdong Academy of Sciences (No. GIZ2021317). Specimen collection and animal use in Thailand were approved by the Institutional Ethical Committee of Animal Experimentation of the University of Phayao, Thailand (UP-AE64-02-04-005) and the Institute of Animals for Scientific Purpose Development, Bangkok, Thailand (U1-01205-2558 and UP-AE59-01-04-0022). All procedures and collection protocols were conducted in accordance with the Thailand Animal Welfare Act.

Phylogenetic reconstruction and genetic divergence

Three Emydidae species—*Graptemys ouachitensis*, *Trachemys venusta*, and *Emys orbicularis*—were selected as outgroups based on the global turtle phylogeny of Thomson et al. (2021), representing the sister taxon of Platysternidae. Whole-genome, *COI*, and *12S* sequence data for outgroups were retrieved from the National Center for Biotechnology

Information (NCBI) database (Supplementary Table S2). The mitochondrial DNA sequences were aligned using MAFFT v.7.313 (Kato & Standley, 2013) and trimmed using Gblocks v.0.91b (Castresana, 2000). A concatenated mtDNA matrix comprising 1 011 bp from 158 samples (including 24 new samples and three outgroups) was constructed. A maximum-likelihood (ML) tree was constructed in IQ-TREE v.2 (Minh et al., 2020) using the “MERGE+MFP” model-selection strategy and 10 000 ultrafast bootstrap replicates (UFBoot) to assess nodal support. Nuclear SNP analyses followed the approach of Gong et al. (2023). SNP calling and filtering were performed on 90 samples from Gong et al. (2023), 23 new samples (JNU 20201018 from Jinghong, Yunnan, was not included due to poor data quality), and three outgroups. The final SNP dataset comprised 116 samples and 355 544 high-quality SNPs. An ML tree was reconstructed using IQ-TREE under the command “-m GTR+ASC -bb 1000 -nt 30”.

Genetic differentiation was assessed using pairwise F_{ST} values derived from nuclear SNPs in Arlequin v.3.5.2.2 (Excoffier & Lischer, 2010). Principal component analysis (PCA) and phylogenetic network analysis were carried out using SNPRelater (Zheng et al., 2012) and SplitsTree4 v.4.14.5 (Bryant & Huson, 2023), respectively. Genetic structure was inferred using ADMIXTURE (Alexander et al., 2009) under default parameters, with the number of genetic clusters (K) ranging from two to eight. The lowest cross-validation error was used to define the optimal K value. The genetic clustering results for $K=2-6$ were visualized using PONG (Behr et al., 2016). Pairwise F_{ST} values and uncorrected P -distances based on the mitochondrial dataset were estimated using DnaSP6 (Rozas et al., 2017) and MEGA7 (Kumar et al., 2016), respectively. Four samples with missing *COI* sequences (JNU 20191027, 20191064, 20201008, 20201020) were excluded due to poor data quality, yielding a final mtDNA dataset of 151 samples. A haplotype network was constructed using the TCS algorithm (Clement et al., 2000) in PoPART 1.7 (Leigh & Bryant, 2015) with default settings.

Morphometric and diagnostic trait analysis

A total of 76 big-headed turtles representing the three major genetic clades were subjected to morphometric analysis, corresponding to 32 specimens from clade I (*P. m. megacephalum*), 28 specimens from clade II (*P. m. peguense*), and 16 specimens from clade III (Baise lineage). Seven morphological measurements were taken using vernier calipers to the nearest 0.1 mm: head width (HW), carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), shell height (SH), and tail length (TL). Detailed measurements are illustrated in Supplementary Figure S1. To eliminate the influence of body size on comparative morphological analysis, CL was used as a covariate, and relative values for the remaining six traits (expressed as ratios to CL) were used for statistical comparisons. One-way analysis of variance (ANOVA) was conducted in SPSS v26.0 (Santoso, 2020) to evaluate interclade morphological differentiation, with statistical significance at $P<0.05$ and high significance at $P<0.01$.

In addition to quantitative analyses, qualitative diagnostic features were examined from both physical specimens and high-resolution images. To assess the presence of clade-specific phenotypic characters, evaluated traits included tail extension relative to the lateral plastral margin, coloration,

markings, and patterning of the head, neck, limbs, carapace, and plastron.

RESULTS

Phylogenetic relationships and genetic differentiation

Phylogenetic inference based on genome-wide SNPs (Figure 1A) recovered three strongly supported monophyletic clades (I–III, UFBoot=100), corresponding to the parapatrically distributed taxa *P. m. megacephalum* (clade I), *P. m. peguense* (clade II), and the Baise population from Guangxi, China (clade III; Figure 1B). Clades II and III were well-supported sister taxa, with clade I recovered as the subsequent sister taxon. Mitochondrial phylogeny yielded a congruent topology, recovering the same three monophyletic clades (Figure 1A: mtDNA lineages; Supplementary Figure S2). The distinctness of the three parapatric clades was further confirmed by PCA using pairwise F_{ST} values for both datasets. Pairwise F_{ST} values for SNPs ranged from 0.43 to 0.47 and those for mtDNA sequences (*COI*, *12S*) ranged from 0.90 to 0.93 (Figure 1C).

Phylogenetic network analysis (Supplementary Figure S3) corroborated the genetic differentiation of the three clades. ADMIXTURE analysis of nuclear SNPs further illustrated patterns of genomic structure (Figure 1). At $K=2$, individuals from clades II and III formed a single cluster, while clade I represented a second cluster. At $K=3$, clade III was resolved as a separate genetic cluster. At $K=4$, samples from Hainan, China (JNU 20131005, 20141001, 20151014, 20151015, 20161021) formed a fourth cluster, and several individuals from southern China and northeastern Vietnam showed evidence of admixture. These admixed individuals and the turtles from Hainan were phylogenetically nested within clade I (Figure 1A). Cross-validation analysis (Supplementary Figure S4) indicated that $K=4$ had the lowest cross-validation error (CV=0.51), with $K=3$ yielding a nearly identical score (CV=0.52). Across all models from $K=3-6$, clade III consistently emerged as a genetically distinct cluster (Figure 1A; Supplementary Figure S4). Uncorrected P -distances for mtDNA among the three clades ranged from 2.6% to 5.5% (Supplementary Table S3). Collectively, nuclear and mitochondrial data revealed substantial and concordant genetic divergence among the three major clades of *Platysternon*.

Comparison of measurements and qualitative morphological traits

Using CL as a covariate, proportional values were calculated for the six other morphometric traits across the three clades (Figure 2). The HW/CL ratio of clade II was significantly higher than that of clade III, which was markedly higher than that of clade I (clade II>clade III>clade I). The CW/CL ratio of clade III was significantly higher than that of clade II, which was significantly higher than that of clade I (clade III>clade II>clade I). The PL/CL and PW/CL ratios of clade I were significantly lower than the respective ratios for clades III and II, which did not differ significantly from each other (clade I<clade III≈clade II). The SH/CL ratio of clade III was significantly higher than that of clades I and II, with no significant difference between the latter two (clade III>clade I≈clade II). The TL/CL ratio of clade II was significantly higher than that of clades I and III, which also did not differ significantly from each other (clade II>clade I≈clade III).

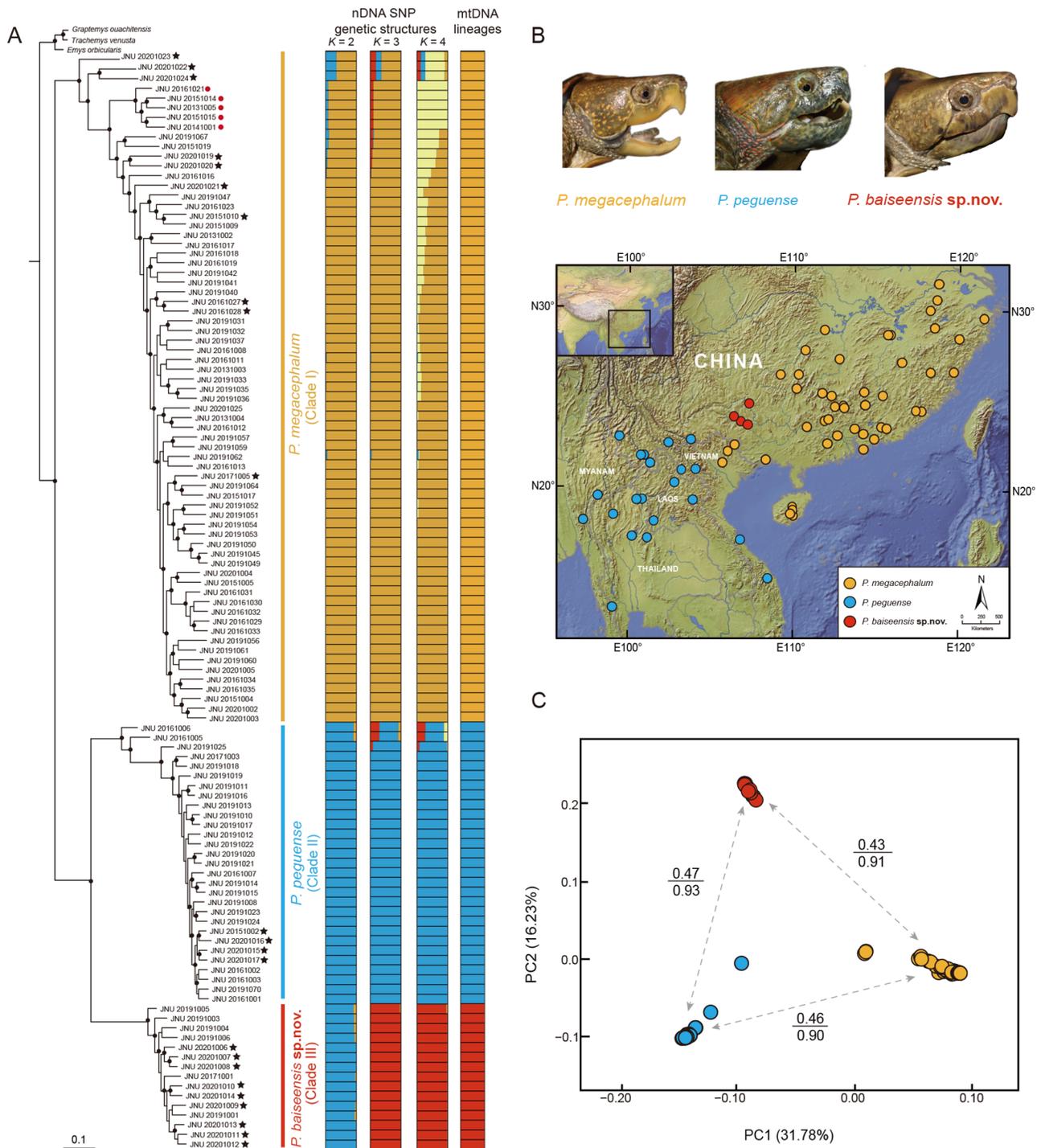


Figure 1 Phylogenetic relationships, genetic structure, collection sites, and genetic differentiation analyses of big-headed turtles (scientific names reflect taxonomy inferred in this study)

A: Maximum-likelihood tree based on SNP data. Stars represent newly sequenced individuals; red dots represent Hainan samples; black dots on nodes indicate UFBoot values ≥ 95 . Right panel: ADMIXTURE results for $K=2-4$ and mitochondrial identity, aligned with sample order in the phylogenetic tree. **B:** Sampling sites and geographic distributions of the three species. **C:** PCA of SNPs based on PC1 and PC2, with pairwise F_{ST} values above lines based on SNPs and below lines based on mtDNA.

Despite statistically significant variation in several morphometric indices, such as significantly longer tails in clade II compared to clades I and III, overlap in measurement ranges across clades (Supplementary Table S4) and the subtlety of these differences rendered them insufficient for diagnostic use at the individual level. Therefore, morphological measurements alone were inadequate for reliable identification among the three clades.

In contrast, qualitative morphological features showed clearer interclade differentiation. (1) Relative tail length varied consistently among clades: when the tail was extended forward along the lateral edge of the plastron, the tip extended beyond the gular scute in clade II but did not reach the gular scute in clades I and III (Figures 3, 5, 7). (2) Plastral pigmentation also differed: adults in clade II retained a distinct dark “丰”-shaped marking on the plastron, a juvenile trait

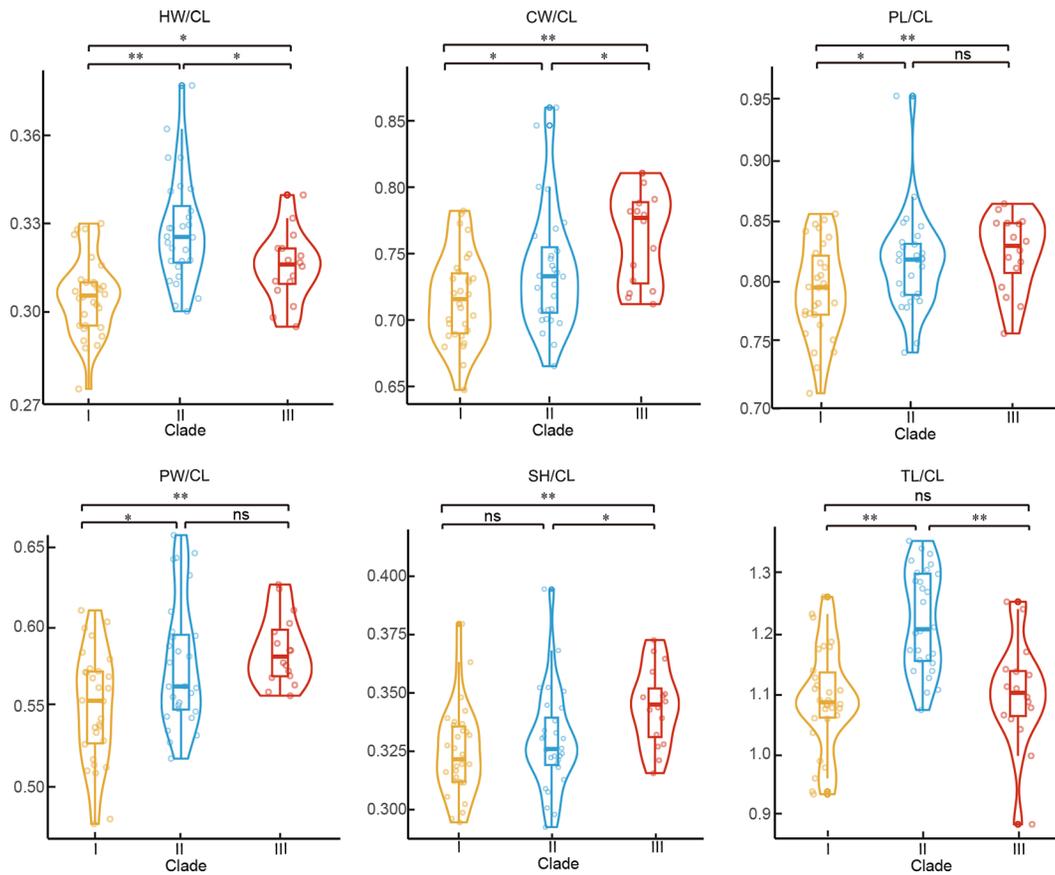


Figure 2 Violin plots of six morphological ratios among three clades of *Platysternon*

Horizontal lines within each box represent median, boxes encompass 75th and 25th percentiles; dots represent values for each sample (32 specimens for clade I, 28 specimens for clade II, 16 specimens for clade III). ns: Not significant. *: $P < 0.05$; **: $P < 0.01$.

absent in adults of clades I and III (Figures 3, 5, 7). Additionally, small orange-red spots were present on the plastron of clades I and II but were entirely absent in clade III. (3) Cranial and appendicular coloration further distinguished clades: adults of clades I and II typically exhibited orange-red spotting or striping on the head, neck, and limbs, while clade III adults lacked this pigmentation and instead displayed a more uniform background coloration, with, at most, pale yellow to whitish markings when patterning was present (Figure 7). In summary, the three clades could be clearly differentiated by a combination of tail characteristics and color patterns of the plastron, head, neck, and limbs.

DISCUSSION

The present study refines and expands upon the findings of Gong et al. (2023), providing robust confirmation for the validity of three distinct taxa corresponding to the three major clades of *Platysternon*. Furthermore, these results offer new insights into the phylogeographic structure and evolutionary diversification of big-headed turtles (Figure 1).

Sources of taxonomic controversies in *Platysternon*

Species delimitation within *Platysternon* has long posed considerable taxonomic challenges. A comprehensive review of earlier taxonomic work revealed two principal factors contributing to longstanding classification controversies.

Variation of putative diagnostic morphological characters: Considerable ontogenetic and individual variation in morphological traits has obscured the identification of taxonomically informative characters in big-headed turtles

(Ernst & Laemmerzahl, 2002). Early subspecies delineations relied on variable traits, including maxillary hook length, distribution of spots or markings on the head, neck, and limbs, carapacial serration, carapacial growth rings, postocular striping, and scute arrangement (Ernst & McCord, 1987; Gray, 1870; Schleich & Gruber, 1984; Wermuth, 1969). For instance, *P. m. peguense* was initially diagnosed based on juvenile traits, including the presence of postorbital yellow stripes and a serrated posterior carapacial margin (Gray, 1870), both of which diminish with maturity. Similarly, *P. m. tristernalis* and *P. m. shiui* were erected based on the presence of additional plastral scutes and orange spotting, traits later shown to fall within the range of individual variation (Ernst & Laemmerzahl, 2002; Gong et al., 2023).

Inadequate geographic representation: Previous molecular studies were limited by sparse or uneven sampling, preventing comprehensive phylogeographic reconstruction. Neither Luo et al. (2019) nor Ngo et al. (2023) included representatives from the Baise-Hechi lineage from Guangxi (clade III sensu Gong et al., 2023 and of the present study). The absence of this lineage, coupled with narrow geographic coverage, hindered comprehensive reconstruction of range-wide phylogeographic patterns and prevented full resolution of the phylogenetic structure, contributing to persistent taxonomic ambiguity.

Taxonomic conclusions

Phylogenetic analyses based on genome-wide nuclear SNPs, mitochondrial markers, and comprehensive geographic sampling revealed three parapatric, deeply divergent, and well-supported clades within *Platysternon* (Figure 1A, C),

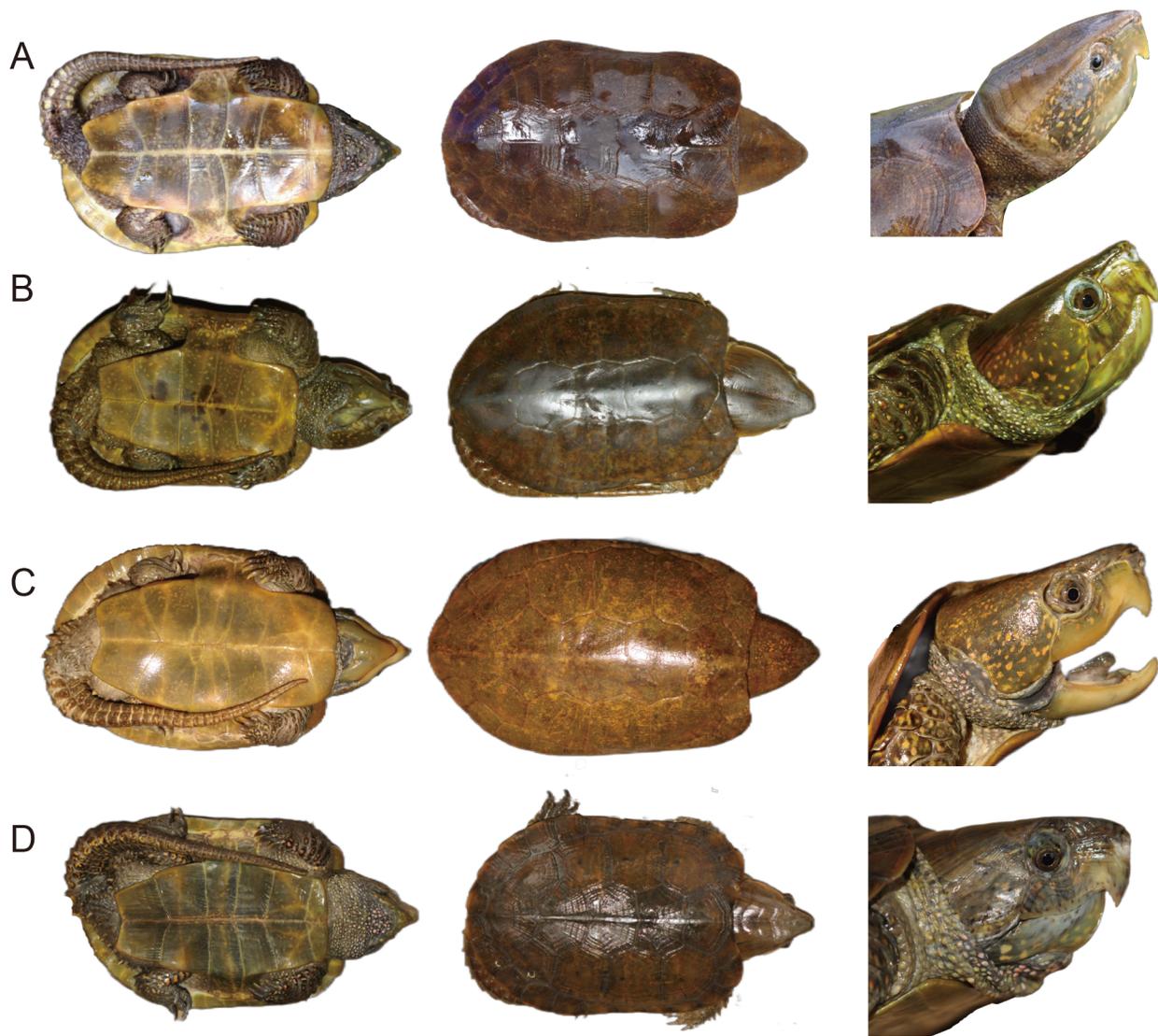


Figure 3 Ventral and dorsal aspects and heads of *Platysternon megacephalum* (clade I)

A: Male, Hainan, China. B: Male, Fujian, China. C: Male, Guangxi, China. D: Male, Guangdong, China. Photos by Shi-Ping Gong.

mirroring the pattern recovered by Gong et al. (2023). Nuclear and mitochondrial datasets independently identified three coherent genetic clusters congruent with the SNP- and mtDNA-based phylogenies (Figure 1A; Supplementary Figure S2). Their genetic differentiation (F_{ST} values and uncorrected P -distances) resembled that of distinct species in *Mauremys* (Zhou et al., 2015) and *Kinosternon* (Hurtado-Gómez et al., 2024). Notably, the mitochondrial haplotype network revealed no shared haplotypes among the three clades, and F_{ST} values for mtDNA exceeded 0.9, indicating pronounced divergence. Concordant differentiation in nuclear and mitochondrial markers, coupled with consistent morphological differences and absence of significant admixture, supports recognition of the three clades as distinct species.

Samples originating from northern Vietnam (JNU 20151019, 20191067, 20191068) and Hainan Island, China (JNU 20131005, 20141001, 20151014, 20151015, 20161021)—localities historically associated with the subspecies *P. m. shiui* (TTWG, 2021)—were phylogenetically nested within clade I in both SNP- and mtDNA-based trees (Figure 1A; Supplementary Figure S2), in line with the conclusion of Gong et al. (2023) that *P. m. shiui* does not represent a valid taxon. However, ADMIXTURE analysis under $K=4$ (Figure 1A, right)

identified Hainan samples as a distinct cluster, grouping them with three samples from Fangchengang, Guangxi (JNU 20201022–20201024). This fourth cluster was connected through many admixed samples primarily distributed across Guangdong, Guangxi, and northern Vietnam (Supplementary Table S1) to more northern and eastern populations within clade I from mainland China. This pattern indicates that the populations within clade I represent a single species with ongoing or historical gene flow during Pleistocene low sea level stands connecting Hainan with the mainland (Ali et al., 2025). It also emphasizes the pronounced contrast between the intraspecific variation of clade I and the deep genetic divergence separating clades I–III as distinct species.

Further research is needed to determine whether the genetic structuring observed within clade I may warrant the future recognition of subspecific units. However, the type locality of *P. m. shiui*—located in northern Vietnam—falls within the admixture zone identified under $K=4$, rendering the name unavailable for formal use. According to the International Code of Zoological Nomenclature (ICZN, 1999: Art. 23.8), a name founded on a hybrid must not be used as the valid name for either of the parental taxa. Therefore, *P. m. shiui* Ernst & McCord, 1987 has to be treated as a junior



Figure 4 Two *Platysternon megacephalum* (clade I) juveniles from Longsheng, Guangxi, China

Top and bottom panels show the same individual. Left: Ventral view. Right: Dorsal view. Photos by Shi-Ping Gong.

synonym of the species *P. megacephalum* Gray, 1831 sensu stricto (clade I), but cannot be used for any of its subspecies, even if in future it should be deemed that *P. megacephalum* sensu stricto (as defined below) is polytypic.

At the species level, all three *Platysternon* lineages exhibited significant differences in multiple morphometric ratios (Figure 2). Despite statistical significance, the degree of overlap among these values limits their suitability for morphological discrimination of individuals (Supplementary Table S4). In contrast, qualitative traits such as relative tail length, coloration, and pattern provide reliable diagnostic features for distinguishing the individual species (see above and diagnoses and key below).

Gong et al. (2023) proposed that geographic barriers, such as river valleys, river basins, and mountainous terrain, restrict gene flow among *Platysternon* lineages. The present data strongly support this geographic isolation hypothesis. Specifically, the Red River in Vietnam serves as a major biogeographical divide between clades I and II, with the adjacent Hoang Lien Mountains likely reinforcing this separation. Additionally, the Guangxi Basin and surrounding high mountains appear to isolate clade III from the other two clades. These patterns align with a model of allopatric speciation driven by historical geographic isolation.

Taken together, the present taxonomic evidence supports the recognition of the three major clades as separate species. Clade III is herein described as a new species, *Platysternon baiseensis* sp. nov., while the lineages represented by clades I and II are elevated to species rank as *Platysternon*

megacephalum Gray, 1831 sensu stricto and *Platysternon peguense* Gray, 1870, respectively.

Taxonomy

Family Platysternidae Gray, 1869

Genus *Platysternon* Gray, 1831

Type species: *Platysternon megacephalum* Gray, 1831 sensu stricto

Diagnosis: Head massive, triangular, with prominent eyes; external eardrums lacking. Upper beak strongly hooked, eagle-like; unparalleled among extant turtles. Head and limbs robust, cannot be retracted into shell. Carapace with median keel; plastral anterior margin nearly rectangular, posterior margin weakly notched. Digits webbed, forefeet with five claws, hindfeet with four. Tail very long, exceeding two-thirds of plastral length; covered with large rectangular scales arranged in rings; spiny scales often present at base (Ernst & Laemmerzahl, 2002; Gray, 1869; Zhang et al., 1998).

Distribution and habitats: Occurs in China (including Zhejiang, Anhui, Jiangxi, Fujian, Hunan, Guizhou, Guangxi, Guangdong, Hong Kong, Hainan, Yunnan, Jiangsu), Vietnam, Thailand, Myanmar, Laos, and Cambodia (TTWG, 2021), typically inhabiting rocky streams in mountainous areas at elevations of 300–2 000 m a.s.l. *Platysternon* turtles are notably nocturnal and exhibit strong climbing abilities (Zhang et al., 1998).

Diet: *Platysternon* species are carnivorous, primarily preying on small fish, crabs, shrimp, mollusks, earthworms, snails, and insects (Zhang et al., 1998).

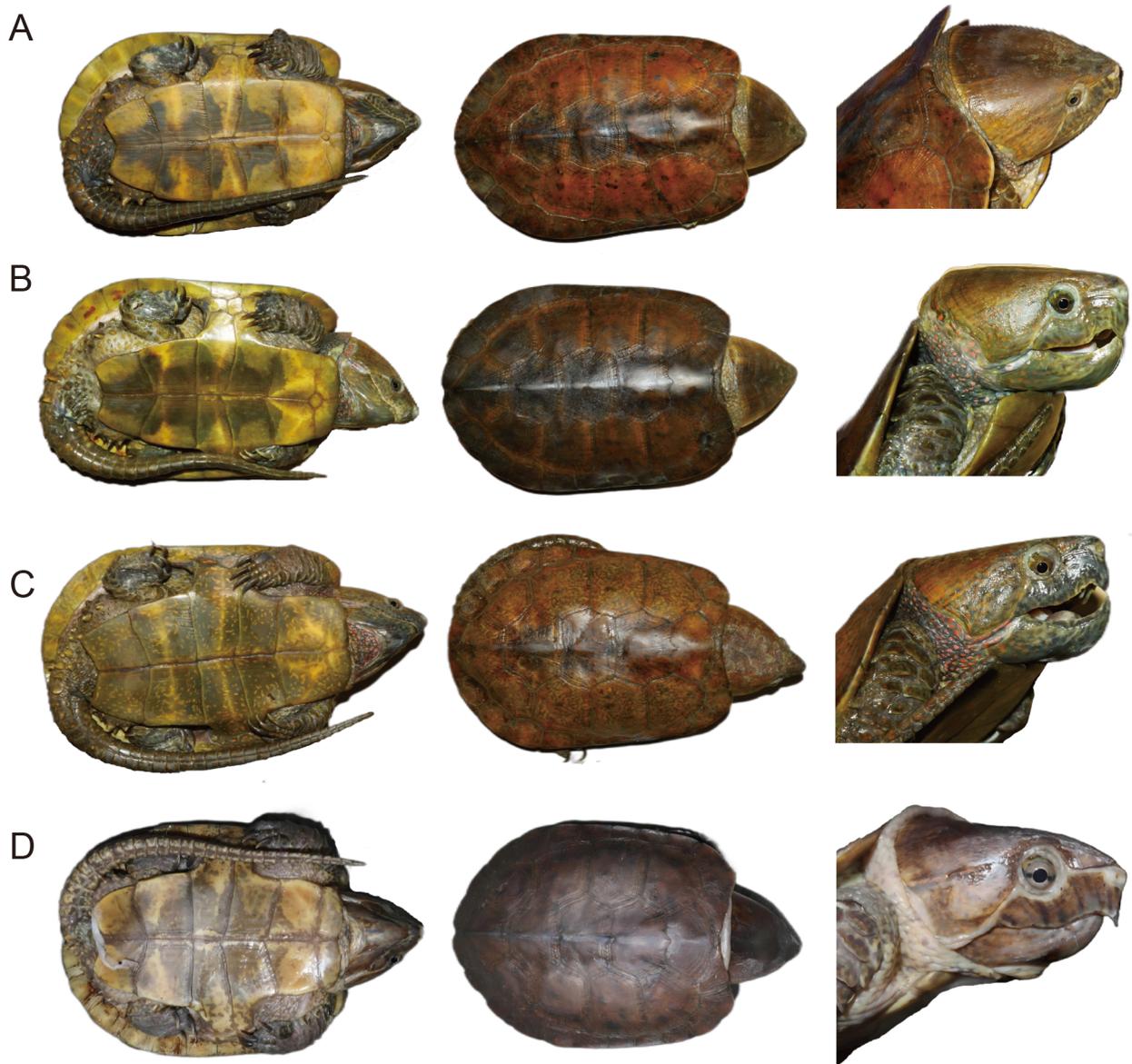


Figure 5 Ventral and dorsal aspects and heads of *Platysternon peguense* (clade II)

A, B: Males, Yunnan, China. C: Female, Yunnan, China. D: Subadult, Thailand. Photos by Shi-Ping Gong.

***Platysternon megacephalum* Gray, 1831 sensu stricto (Figures 3, 4)**

Suggested common name: Big-headed Turtle (English), Píng Xiōng Guī (平胸龟, Chinese)

Holotype: Natural History Museum London, NHM 1946.9.7.42

Type locality: China

Synonyms: *Platysternon megacephalum shiui* Ernst & McCord, 1987; holotype: Smithsonian Institution, National Museum of Natural History, Washington, D.C., USNM 266160; type locality: vicinity of Langson [Lạng Sơn], Vietnam

Diagnosis: Shell moderately flattened. Tail, when bent forward along plastron, reaches humeral or pectoral scute, not beyond gular scute. Carapace without black spots. Plastron without “丰”-shaped dark mark in adults. Head, neck, and limbs with orange-red spots or stripes.

Variation: Juveniles with grass green to brown carapace, orange plastron with fading dark central mark, distinct postorbital stripes, no vocalization when handled. Adults with brown to black-brown carapace; younger individuals with black radiating growth lines. Spots typically lost in aged individuals; plastron uniform dark yellow or brownish black.

Distribution: North of the Red River in Vietnam and south of the Yangtze River in China (including Hainan, Hong Kong, southern Guangxi, Guangzhou, Guizhou, Hunan, Zhejiang, Anhui, Jiangxi, Fujian, Jiangsu; Figure 1B).

***Platysternon peguense* Gray, 1870 (Figures 5, 6)**

Suggested common name: Burmese Big-headed Turtle (English), Miǎn Diàn Píng Xiōng Guī (缅甸平胸龟, Chinese)

Syntypes: Natural History Museum London, NHM 1946.1.22.21–22

Type locality: Pegu [Bago, Myanmar]

Synonyms: *Platysternon megacephalum vogeli* Wermuth, 1969; holotype: Staatliches Museum für Naturkunde Stuttgart, SMNS 4573; type locality: Chiang Mai Province, Thailand

Platysternon megacephalum tristernalis Schleich & Gruber, 1984; holotype: Zoologische Staatssammlung München, ZSM 317/1980/1; type locality: between Mung Lun (Menghanzhen) and Simao, eastern shore of Mekong, southern Yunnan, China

Diagnosis: Shell extremely flattened. Tail very long; tip reaching or extending beyond gular scute when bent forward



Figure 6 Two *Platysternon peguense* (clade II) juveniles from Yunnan, China

Top and bottom panels show the same individual. Left: Ventral view. Right: Dorsal view. Photos by Shi-Ping Gong.

along plastron. Carapace brownish with irregular black spots. Plastron with “丰”-shaped dark central mark. Head, neck, and limbs with orange-red spots and stripes.

Variation: Juveniles with yellow-green to brownish carapace, orange plastron with black “丰”-shaped central mark, distinct postorbital stripes. Emit occasional “squeak-squeak” vocalization when handled. Adults with light brown, reddish brown, or dark brown carapace, often with irregular black spots. Plastron dull yellow with dark “丰”-shaped mark, sometimes with orange spots. Small rhomboid scute between gular and humerus scutes often present.

Distribution: South of the Red River in Vietnam, Thailand, Laos, Myanmar, Cambodia, and southern Yunnan in China (including Xishuangbanna, Jinghong, Pingbian, Cangyuan, Mengla, Honghe; Figure 1B).

***Platysternon baiseensis* sp. nov. (Figures 7, 8)**

Suggested common name: Baise Big-headed Turtle (English), Bǎi Sè Ping Xiōng Guī (百色平胸龟, Chinese)

Etymology: The species nomen *baiseensis* is derived from Baise in Guangxi, China, and the surrounding area, where the new species is primarily distributed.

Holotype: Animal Germplasm Resource Bank, College of Life Science and Technology, Jinan University, Guangzhou City, Guangdong Province, China, JNU 20191003 (Figure 7A), adult female, collected by Shi-Ping Gong on 22 July 2019 from Tianlin County, Baise City, Guangxi, China (E106°14'18", N24°14'19").

Paratypes: JNU 20191001–20191002 (all adult females), JNU 20191004–20191006 (all adult females), and JNU 20201014 (juvenile), collected by Shi-Ping Gong between 2019 and 2020 from Baise City and Hechi City, Guangxi,

China, deposited in the same collection as the holotype.

Diagnosis: Shell moderately flattened. Tail shorter than in *P. peguense*, with tip reaching humeral or pectoral scute but not extending beyond gular scute when bent forward along plastron. Carapace dark brown, unspotted. Plastron uniform brownish-gray, without dark central mark or spots in adults. Head, neck, and limbs usually dull-colored without orange-red spots or stripes; some individuals with pale-yellow spots or stripes.

Holotype: Adult female; HW 48.7 mm, CL 158.4 mm, CW 113.0 mm, PL 134.7 mm, PW 89.3 mm, SH 52.1 mm, TL 180.36 mm. Head large, triangular; head and neck dull-colored. Carapace dark brown without black spots, with slightly raised darker median keel. Plastron dark brown without dark central mark or spots. Limbs strong, covered with large scales, dark-colored without spots. When tail extended forward along lateral edge of plastron, tip reaches humeral or pectoral scute, but not beyond gular scute. Spiny scales at tail base.

Variation (based on type series): HW 29.2–57.1 mm, CL 96.3–171.9 mm, CW 76.3–124.3 mm, PL 77.0–134.7 mm, PW 53.9–98.0 mm, SH 33.4–56.3 mm, TL 100.5–187.1 mm (only for adults). Juvenile (JNU 20201014) with postorbital stripes, brown in color, indistinct black spots on carapace, pale “丰”-shaped central mark on plastron. Tail short, tip reaching humeral scute when tail bent forward along plastron. No sound emitted when handled. Adults lack orange-red spots on head, although some exhibit pale yellow spots or stripes. Chin and neck lack orange-red spots, although some individuals display irregular black longitudinal stripes on upper or lower beak (Figure 7B). Carapace dark brown to brownish-black,

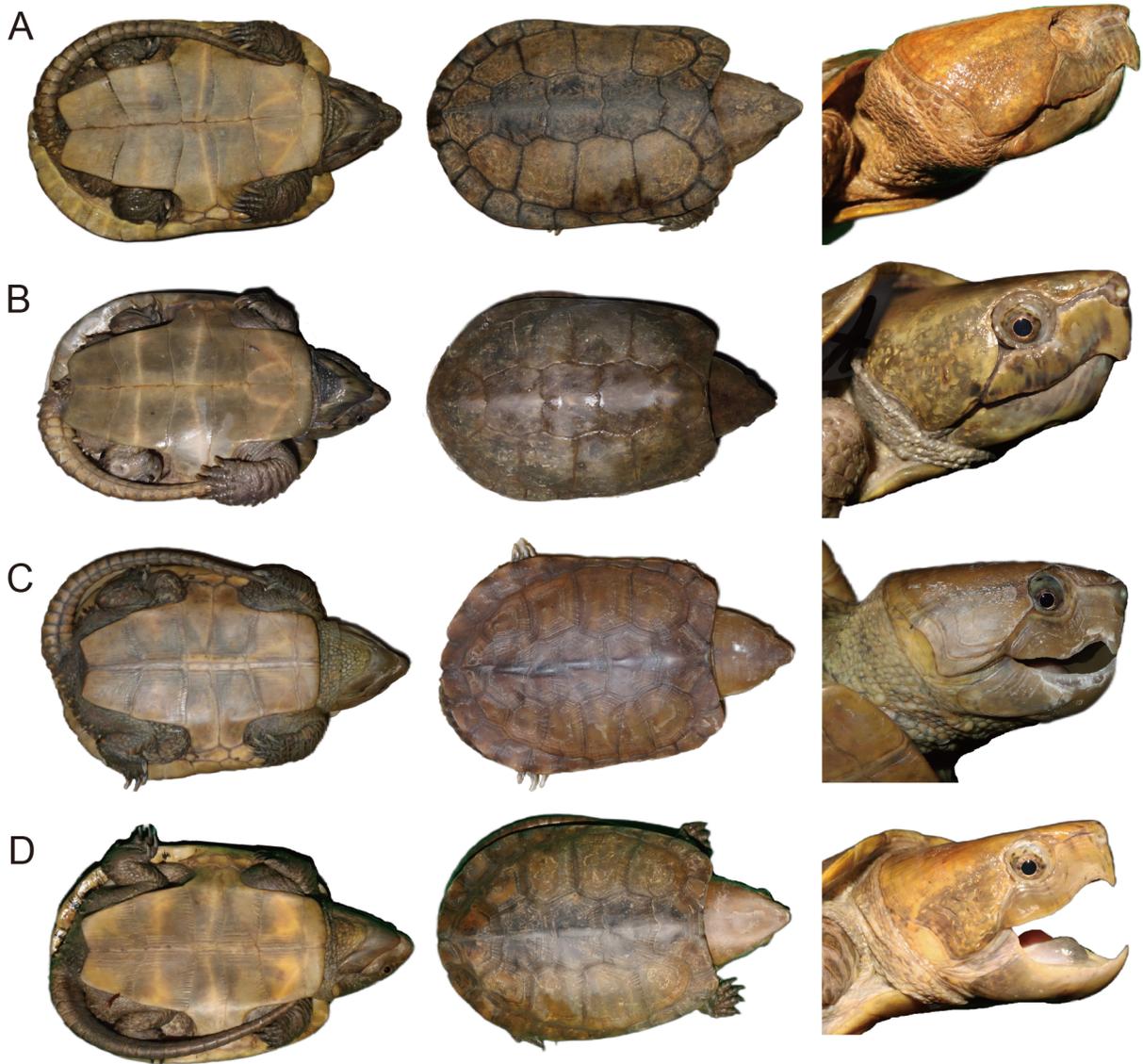


Figure 7 Ventral and dorsal aspects and heads of female *Platysternon baiseensis* sp. nov. (clade III)

A, B: Baise, Guangxi, China (A: Holotype). C, D: Hechi, Guangxi, China. Photos by Shi-Ping Gong.



Figure 8 *Platysternon baiseensis* sp. nov. (clade III) juvenile from Hechi, Guangxi, China

Left: Ventral view. Right: Dorsal view. Photos by Shi-Ping Gong.

unspotted, with distinct keels. Plastron uniformly dark brown, without dark central mark. Limbs dull-colored, without spots or stripes. Tail tip reaches only humeral or pectoral scute when bent forward along plastron.

Distribution: Baise Basin area of Guangxi, China, including Baise and Hechi cities (Figure 1B).

Key to species of *Platysternon*

1) Shell extremely flattened; plastron of adults with central

dark “丰”-shaped mark; when tail bent forward along lateral edge of plastron, tail tip reaches or extends beyond gular scute *Platysternon peguense*

1.1) Shell moderately flattened; plastron of adults without central dark “丰”-shaped mark; when tail bent forward along lateral edge of plastron, tail tip reaches humeral or pectoral scute, not extending beyond gular scute 2

2) Head, neck, and limbs with orange-red spots or stripes;

plastron with orange-red spots
..... *Platysternon megacephalum sensu stricto*
2.1) Head, neck, and limbs without orange-red spots or stripes; plastron dull-colored without orange-red spots..... *Platysternon baiseensis* sp. nov.

CONCLUSION

This study provides a comprehensive taxonomic reassessment of big-headed turtles, integrating genome-wide SNPs, mitochondrial DNA sequence data, and morphological evidence. All datasets showed concordant differentiation patterns and revealed three deeply divergent genealogical lineages. The absence of any substantial admixture, combined with the monophyly and deep genetic divergence of all three lineages, support their recognition as separate species under any widely accepted species concept (Zachos, 2016): *Platysternon megacephalum* Gray, 1831 sensu stricto, *Platysternon peguense* Gray, 1870, and *Platysternon baiseensis* sp. nov.

The present findings resolve long-standing taxonomic ambiguity concerning lineage boundaries, genetic and morphological differentiation, and geographic ranges across the genus. This refined framework provides a sound taxonomic basis for conservation strategies, enabling species-specific assessments and guiding informed management of confiscated individuals, including their reintroduction into appropriate native habitats.

NOMENCLATURE ACTS REGISTRATION

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclature acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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DATA AVAILABILITY

Raw data were deposited in the National Center for Biotechnology Information database (BioProjectID PRJNA1297079; GenBank PV989530–PV989553, PV988198–PV988219), Genome Sequence Archive (<https://ngdc.cncb.ac.cn/gsa/>), (accession number CRA028306), and Science Data Bank (<https://www.scidb.cn/en>) (DOI: 10.57760/sciencedb.j00139.00245, 10.57760/sciencedb.28446). The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.P.G., J.X.L., and Q.R.L. designed the research and methodology. Q.R.L., S.P.G., J.X.L., and U.F. wrote the manuscript. Y.G., J.M.O., Y.Q.Y., C.S.,

M.L., and T.Q.N. reviewed and revised the manuscript. S.P.G., Y.G., C.S., M.L., and T.Q.N. collected the samples. All authors read and approved the final version of the manuscript.

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