

Short communication

On the paraphyly of the genus *Kachuga* (Testudines: Geoemydidae)

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1. Introduction

The turtle family Geoemydidae is the most diverse family of living turtles, encompassing about 70 species and 23 genera. Geoemydids are distributed globally in Asia, Europe, North Africa, and Central and South America (Iverson, 1992). The group also occupies a wide range of habitats, from highly aquatic (*Batagur* and *Malayemys*) to highly terrestrial (*Geoemyda*). It is also the most threatened clade of turtles, due to overexploitation to supply the international wildlife trade in Asia (Van Dijk et al., 2000). Despite a surge in the number of molecular phylogenetic studies in this group in recent years (Honda et al., 2002a,b; Barth et al., 2004; Spinks et al., 2004; Diesmos et al., 2005; Praschag et al., 2006; Sasaki et al., 2006), the relationships of many remaining species have not been well resolved. Of these, the relationships among the three giant riverine genera *Kachuga*, *Batagur*, and *Callagur* are the most poorly known Fig. 1.

In the study by Spinks et al. (2004), the maximum likelihood tree using cytochrome *b* alone (Fig. 2) and the tree using combined data (their Fig. 3) supported a identical topology regarding the relationships of the three genera, in which *Kachuga dhongoka* was sister to *Callagur borneoensis*, and *Batagur baska* was sister to both of these species. However, while the sister relationship between *K. dhongoka* and *C. borneoensis* was strongly supported by both the maximum parsimony bootstrap and Bayesian posterior

probability in all analyses of this study, the sister relationship between *B. baska* and the other two taxa was only strongly supported by the Bayesian posterior probability in the combined analysis. In addition, the phylogenetic position of the closely related *Hardella thurjii* was also unclear in this study due to its low support level.

Diesmos et al. (2005) reanalyzed the *cytb* and R35 data from Spinks et al. (2004) with the addition of *Siebenrockiella leytensis*, and found that although *C. borneoensis* and *K. dhongoka* were resolved as sister taxa with strong support in their maximum parsimony tree, *B. baska* was not sister to these two taxa. Instead, the latter species was closely related to members of the genus *Pangshura* with low bootstrap value. Furthermore, *H. thurjii* was weakly placed in the sister position to all other species of *Batagur*, *Callagur*, *Kachuga*, and *Pangshura* in this study, compared to the sister relationship with the genus *Pangshura* supported by Spinks et al. (2004). Praschag et al. (2006) also reanalyzed the *cytb* data from Spinks et al. (2004) with the addition of *Vijayachelys silvatica*. Their Bayesian topology showed an identical set of relationships to that hypothesized by Diesmos et al. (2005) with weak support for the positions of the *Batagur* and *Hardella*. Even though no molecular analysis to date has included *Kachuga trivittata* and *K. kachuga*, on the morphological grounds McDowell (1964) grouped *C. borneoensis* with *K. kachuga* and *K. trivittata*, and hypothesized that the latter was sister to *C. borneoensis*, making the genus *Kachuga* potentially paraphyletic.

Resolution of controversies over the relationships of the genus *Kachuga* and the closely related genera *Callagur* and *Batagur* will require the inclusion of the two remaining species of the genus (i.e., *Kachuga kachuga* and *K. trivittata*). To generate a well-resolved phylogenetic hypothesis for

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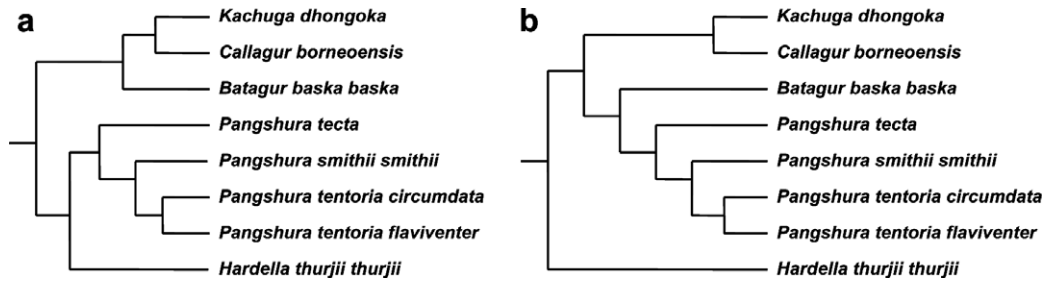


Fig. 1. Previous hypotheses regarding the relationships of *Batagur*, *Callagur*, and *Kachuga* and their relatives. (a) The phylogenetic relationships based on all data combined from Spinks et al. (2004) (Fig. 3). (b) The phylogenetic relationships based on *cytb* and R35 genes from Diesmos et al. (2005).

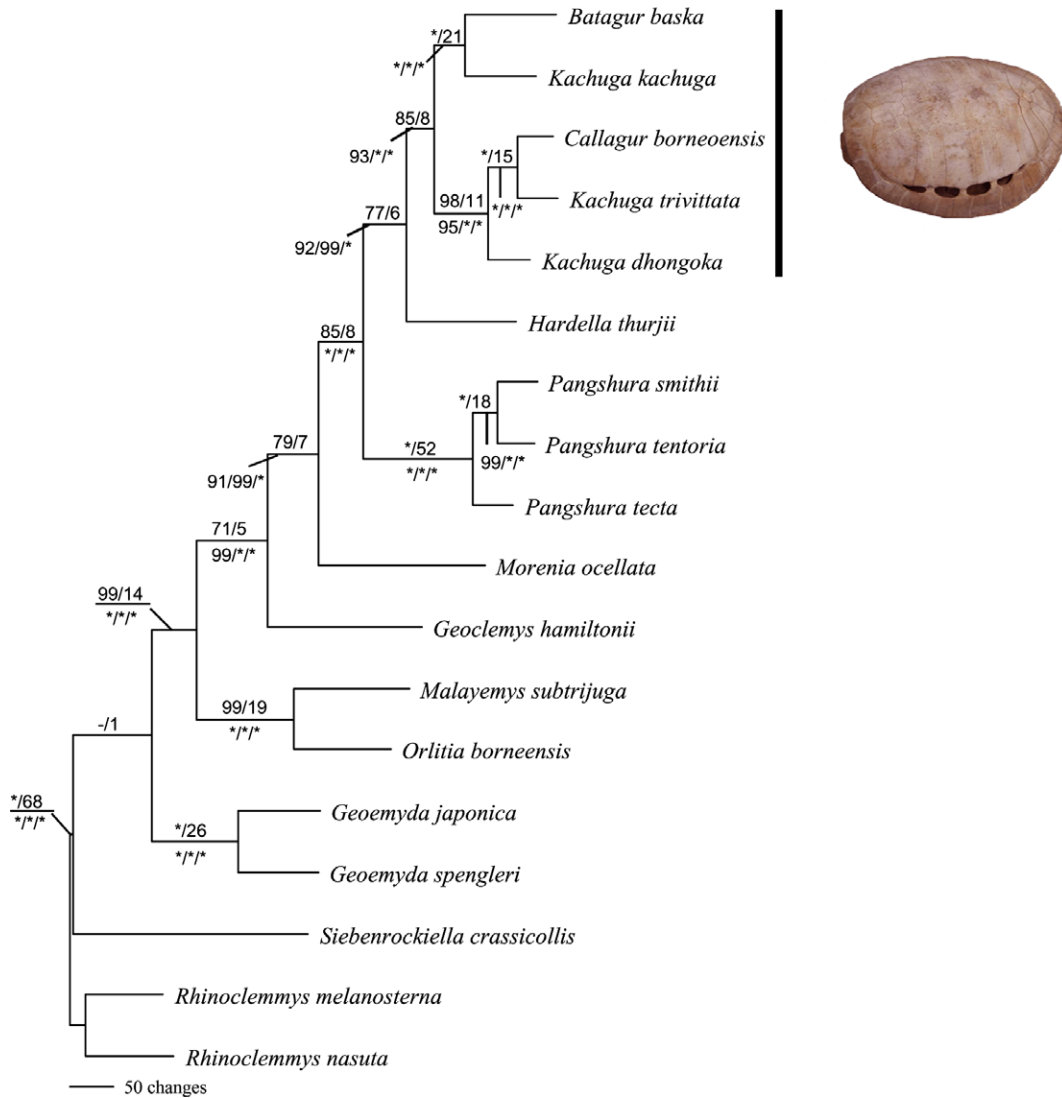


Fig. 2. The maximum parsimony phylogram based on the combined data set. This is the single most parsimonious tree (TL = 2237; CI = 0.53; RI = 0.49). Of 4015 aligned characters, 629 are potentially informative and 3082 are constant. Numbers above branches are MP bootstrap and Bremer values, respectively. Numbers below branches are ML bootstrap, Bayesian single-model posterior probability, and mixed-model posterior probability values, respectively. Asterisk indicates 100% value. The image on the right shows the costal fontanelles, a character found in adult males, shared by the five species left of the vertical bar.

this group, we sequenced six genes, including three mitochondrial (*cytb*, 12S, and 16S) and three nuclear genes (*Cmos*, *Rag1*, and *Rag2*). In addition, to determine the exact phylogenetic positions of *Hardella thurjii* and the

Pangshura, we also included a number of closely related and distantly related outgroups. We also examined morphological features of the genus *Kachuga* and its relatives to determine synapomorphies for this group. We discuss

our molecular results in light of the morphological synapomorphies, and suggest a taxonomic change for this genus based on these results.

2. Materials and methods

2.1. Taxonomic sampling

Two species of the genus *Rhinoclemmys*, *R. melanosterna* and *R. nasuta*, were selected as outgroups based on their close relationship with all other geoemydids (Diesmos et al., 2005; Prasczag et al., 2006). In order to generate a robust phylogeny for this group and to resolve the position of the genera *Pangshura* and *Hardella*, we also included all species of the genus *Kachuga* and other suspected near and far outgroups (Spinks et al., 2004; Prasczag et al., 2006). All taxa and their GenBank numbers are listed in Table 1. The data matrix used in this study was also submitted to TreeBASE (www.treebase.org, Accession No. M3260).

2.2. Molecular data

Both mitochondrial and nuclear DNA were used in this study. Mitochondrial DNA was included because its rates of fixation and substitution make it appropriate to address phylogenetic relationships at the species level (Hillis et al., 1996). In this study, we sequenced three mitochondrial loci, 12S, 16S, and *cytb*. Most prior molecular studies (e.g., Honda et al., 2002a,b; Barth et al., 2004; Prasczag et al., 2006) used only mtDNA. Spinks et al. (2004) also used one nuclear intron, but the authors sequenced only about one third (26) of the ingroup taxa and three outgroup

testudinids for this gene. Since many basal nodes were not well supported in previous studies, in this study, we included three nuclear genes, *Cmos*, *Rag1*, and *Rag2*, which have been useful in addressing the phylogenetic relationships among side-necked turtles, families of turtles, and tortoises (Georges et al., 1999; Krenz et al., 2005; Le et al., 2006). This combined approach of nuclear and mitochondrial genes has been demonstrated to be useful in addressing the relationships of trionychids and testudinids (Engstrom et al., 2004; Le et al., 2006). All primers used for this study are shown in Table 2.

DNA was extracted from tissue or blood samples using the DNeasy kit (Qiagen) following manufacturer's instructions for animal tissues. PCR volume for mitochondrial genes contained 42.2 μ l (18 μ l of water, 4 μ l of buffer, 4 μ l of 20 mM dNTP, 4 μ l of 25 mM MgCl₂, 4 μ l of each primers, 0.2 μ l of *Taq* polymerase (Promega), and 4 μ l of DNA). PCR conditions for these genes were: 95 °C for 5 min to activate the *Taq*; with 42 cycles at 95 °C for 30 s, 45 °C for 45 s, 72 °C for 60 s; and a final extension of 6 min. Nuclear DNA was amplified by HotStarTaq™ mastermix or HotStar *Taq* (Qiagen), since this *Taq* performs well on samples with low-copy targets and the *Taq* is highly specific. For HotStarTaq mastermix, the PCR volume consisted of 21 μ l (5 μ l water, 2 μ l of each primer, 10 μ l of HotStarTaq mastermix, and 2 μ l of DNA or higher depending on the quantity of DNA in the final extraction solution). For HotStar *Taq*, the PCR volume ranges from 21 to 22 μ l (2 μ l of dNTP, 2 μ l of each primer, 2 μ l of buffer 10 \times , 12 μ l of water, and 1 to 2 μ l of DNA depending on the quantity of DNA in the final extraction solution). PCR conditions for nuclear genes were the same as above except the first step (95 °C) was carried out in 15 min, and the

Table 1
GenBank accession numbers and associated samples were used in this study

Species name	GenBank No (12S)	GenBank No (16S)	GenBank No (<i>cytb</i>)	GenBank No (<i>Cmos</i>)	GenBank No(<i>Rag1</i>)	GenBank No(<i>Rag2</i>)	Sample Numbers
<i>Batagur baska</i>	EU030185	EU030199	AY434600	EU030217	EU030233	EU030250	AMCC166654
<i>Callagur borneoensis</i>	EU030186	EU030200	AY434601	EU030218	EU030234	EU030251	AMCC166655
<i>Geoclemys hamiltonii</i>	EU030187	EU030201	AY434573	EU030219	EU030235	EU030252	AMCC166657
<i>Geoemyda japonica</i>	EU030188	EU030202	AY434602	EU030220	EU030236	EU030253	AMCC166658
<i>Geoemyda spengleri</i>	EU030189	EU030203	AY434586	EU030221	EU030237	EU030254	AMCC106625*
<i>Hardella thurjii</i>	AB090025	EU030204	AY434603	EU030222	EU030238	EU030255	AMCC166659
<i>Kachuga dhongoka</i>	EU030190	EU030205	AY434569	EU030223	EU030239	EU030256	AMCC166661
<i>Kachuga kachuga</i>	EU030191	EU030206	EU030215	EU030224	–	–	FMNH224128*
<i>Kachuga trivittata</i>	EU030192	EU030207	EU030216	EU030225	EU030240	EU030257	AMCC164926
<i>Malayemys subtrijuga</i>	EU030193	EU030208	AY434591	EU030226	EU030241	EU030258	FMNH255267*
<i>Morenia ocellata</i>	EU030194	EU030209	AY434605	EU030227	EU030242	EU030259	AMCC166662
<i>Orlitia borneensis</i>	AB090024	EU030210	AY434619	EU030228	EU030243	EU030260	AMCC166663
<i>Pangshura smithii</i>	EU030195	EU030211	AY434589	EU030229	EU030244	EU030261	AMCC166664
<i>Pangshura tecta</i>	EU030196	EU030212	AY434583	EU030230	EU030245	EU030262	AMCC166665
<i>Pangshura tentoria</i>	EU030197	EU030213	AY434610	EU030231	EU030246	EU030263	AMCC166666
<i>Rhinoclemmys melanosterna</i>	DQ497267	DQ497290	AY434590	DQ497359	EU030247	DQ497395	AMCC157821
<i>Rhinoclemmys nasuta</i>	DQ497268	DQ497291	DQ497324	DQ497360	EU030248	DQ497396	AMCC157826
<i>Siebenrockiella crassicollis</i>	EU030198	EU030214	AY434571	EU030232	EU030249	EU030264	AMCC157715

FMNH: Field Museum of Natural History

AMCC: Ambrose Monell Cryo Collection, American Museum of Natural History (<<http://research.amnh.org/amcc>>)

(*: samples with vouchered specimens)

Table 2
Primers used in this study

Primer	Position	Sequence	Reference
L1091 (12S)	491	5'-AAAAAGCTTCAAACCTGGGATTAGATACCCCACTAT-3'	Kocher et al. (1989)
H1478 (12S)	947	5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3'	Kocher et al. (1989)
AR (16S)	1959	5'-CGCCTGTTTATCAAAAACAT-3'	Palumbi et al. (1991)
BR (16S)	2561	5'-CCGGTCTGAACTCAGATCACGT-3'	Palumbi et al. (1991)
CytbG (cytb)	14368	5'-AACCATCGTTGTWATCAACTAC-3'	Spinks et al. (2004)
GLUDGE (cytb)	14358	5'-TGATCTTGAARAACCAACCGTTG-3'	Palumbi et al. (1991)
CytbJSi (cytb)	15011	5'-GGATCAAACAACCCAACAGG-3'	Spinks et al. (2004)
CytbJSr	15030	5'-CCTGTTGGGTTGTTTGTATCC-3'	Spinks et al. (2004)
THR (cytb)	15593	5'-TCATCTTCGGTTTACAAGAC-3'	Spinks et al. (2004)
THR-8 (cytb)	15585	5'-GGTTTACAAGACCAATGCTT-3'	Spinks et al. (2004)
CM1 (Cmos)	163	5'-GCCTGGTGCTCCATCGACTGGGA-3'	Barker et al. (2002)
CM2 (Cmos)	820	5'-GGGTGATGGCAAAGGAGTAGATGTC-3'	Barker et al. (2002)
Cmos1 (Cmos)	163	5'-GCCTGGTGCTCCATCGACTGGGATCA-3'	Le et al. (2006)
Cmos3 (Cmos)	812	5'-GTAGATGTCTGCTTTGGGGGTGA-3'	Le et al. (2006)
Rag1878	1717	5'-GAAGACATCTTGGAAAGGCATGA-3'	This study
Rag2547	2406	5'-TGCATTGCCAATGTCACAGTG-3'	This study
F2 (Rag2)	601	5'-CAGGATGGACTTCTTTCCATGT-3' ^a	Le et al. (2006)
F2-1 (Rag2)	590	5'-TTCCAGAGCTTCAGGATGG-3'	Le et al. (2006)
R2-1 (Rag2)	1312	5'-CAGTTGAATAGAAAGGAACCCAAGT-3' ^b	Le et al. (2006)

^a Modified from F2R2 (Barker et al., 2004).

^b Modified from R2R1 (Barker et al., 2004); Cmos and Rag1 and Rag2 sequences of chicken with GenBank numbers of M19412, M58530, and M58531, respectively; primer positions for mitochondrial genes corresponding to the positions in the complete mitochondrial genome of *Chrysemys picta* (Mindell et al., 1999).

annealing temperatures were 50, 52, and 58 °C for Rag1, Rag2, and Cmos, respectively.

PCR products were visualized using electrophoresis through a 2% low melting-point agarose gel (NuSieve GTG, FMC) stained with ethidium bromide. For reamplification reactions, PCR products were excised from the gel using a Pasteur pipette, and the gel plug was melted in 300 µl sterile water at 73 °C for 10 min. The resulting gel-purified product was used as a template in 42.2 µl reamplification reactions with all PCR conditions similar to those used for mitochondrial genes. PCR products were cleaned using PerfectPrep[®] PCR Cleanup 96 plate (Eppendorf) or using glass milk and 70% ethanol, and cycle sequenced using ABI prism big-dye terminator according to manufacturer recommendation. Sequences were generated in both directions on an ABI 3100 Genetic Analyzer.

In this study, we also sequenced DNA from bone materials from a museum specimen (FMNH 224128) of *Kachuga kachuga*, a very rare species for which we were unable to obtain fresh tissue samples. To minimize the damage to morphological characters of the specimens, we sampled bone from digits of this species. This practice also has the advantage that the bone is small enough for immediate extraction without further manipulation, (e.g., drilling and grinding). Due to the risk of contamination on the surface of the bone, the sample was first cleaned with 10% chlorox, and then placed on a clean surface to dry.

The clean bone was then decalcified by incubation at 55 °C in 1 ml of 0.5 M EDTA for 24 h. After decalcification, the bone was washed with 1 ml of 10 mM Tris to remove remaining EDTA (Austin et al., 2002). At this point the bone was ready for extraction using DNeasy

Kit (Qiagen). The extraction procedure followed the manufacturer's instructions for animal tissues. For the incubation step, the lysis took up to 48 h in order for the bone to become completely digested. During this step, the extraction was checked every 12 h to monitor the progress. If the lysis was occurring slowly, more proteinase K was added (usually in 20 µl increments). A negative control was used in every extraction. DNA obtained from bones was amplified by HotStar Taq (Qiagen) with conditions similar to the ones described above.

2.3. Phylogenetic analysis

We aligned sequence data using ClustalX v1.83 (Thompson et al., 1997) with default settings for complete alignment. Data were analyzed using maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP*4.0b10 (Swofford, 2001) and Bayesian analysis as implemented in MrBayes v3.1 (Huelsenbeck and Ronquist, 2001).

For maximum parsimony analysis, we conducted heuristic analyses with 100 random taxon addition replicates using the tree-bisection and reconnection (TBR) branch swapping algorithm in PAUP, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) (Felsenstein, 1985) was evaluated using 1000 pseudoreplicates and 100 random taxon addition replicates. Bremer indices (BI) (Bremer, 1994) were determined using Tree Rot 2c (Soreson, 1999). All characters were equally weighted and unordered. Gaps in sequence alignments were treated as a fifth character state (Giribert and Wheeler, 1999).

For maximum likelihood analysis the optimal model for nucleotide evolution was determined using Modeltest V3.7 (Posada and Crandall, 1998). Analyses used a randomly selected starting tree, and heuristic searches with simple taxon addition and the TBR branch swapping algorithm. Support for the likelihood hypothesis was evaluated by bootstrap analysis with 100 replications and simple taxon addition. We regard bootstrap values of $\geq 70\%$ as potentially strong support and bootstrap values of $< 70\%$ as weak support (Hillis and Bull, 1993).

For Bayesian analyses we used the optimal model determined using Modeltest with parameters estimated by MrBayes Version 3.1. Analyses were conducted with a random starting tree and run for 5×10^6 generations. Four Markov chains, one cold and three heated (utilizing default heating values), were sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. Two independent analyses were run simultaneously. The posterior probability values (PP) for all clades in the final majority rule consensus tree are reported. We ran analyses on both combined and partitioned datasets to examine the robustness of the tree topology (Nylander et al., 2004; Brandley et al., 2005). In the partitioned analyses, we divided the data into 14 separate partitions, including 12S, 16S and the other twelve based on gene codon positions (first, second, and third) in *cytb*, *Cmos*, *Rag1*, and *Rag2*. Optimal models of molecular evolution for each partition were selected using Modeltest and then assigned to these partitions in MrBayes 3.1. We consider PP values $\geq 95\%$ as strong support for a clade.

3. Results

We obtained a final matrix of 18 species and 4015 aligned characters (12S: 404 characters; 16S: 573 characters; *cytb*: 1140 characters; *Cmos*: 602 characters; *Rag1*: 642 characters; *Rag2*: 654 characters). Only one species, *K. kachuga*, had missing data in *Rag1* and *Rag2* regions as we were unable to sequence these nuclear genes from the bone material. Gaps were present in 12S and 16S datasets, but absent in others.

Using MP bootstrap analysis with the same settings as indicated above, we analyzed the data by gene partitions (see Supplementary Data). In general, separate analyses of mitochondrial genes showed that many basal nodes of the resulting trees are either unresolved or weakly supported. These markers also supported conflicting relationships regarding the positions of *Geoclemys* and *Malayemys*. The tree based on all mitochondrial data combined is better resolved and has higher bootstrap values. However, the positions of *Hardella* and *Siebenrockiella* are still unresolved, and the positions of *Morenia* and *Geoclemys* are weakly supported. Separate analyses of nuclear genes showed little resolution in the trees based on *Rag1*

and *Rag2*. These genes supported conflicting relationships regarding the position of *Rhinoclemmys nasuta*. Similarly, the tree based on all nuclear genes combined is better resolved compared to the trees resulting from analyses of separate nuclear genes and has higher bootstrap values. Nevertheless, the positions of *Batagur*, *Callagur*, *Hardella*, *Kachuga dhongoka*, *K. trivittata*, three species of *Pangshura* are still unresolved. Between tree topologies supported by all mitochondrial and all nuclear data, only the positions of *Geoclemys* are conflicting. Based on the overall poorly resolved trees supported by the partition analyses and the results from the combined analysis (see below), we regard our trees based on all data combined as optimal hypotheses.

The MP analysis of the combined data produced a single tree as shown in Fig. 2. The tree is completely resolved with 93% of its nodes receiving potentially strong support (BP $> 70\%$). Only the relationship between *Siebenrockiella crassicolis* and two species of the genus *Geoemyda* was not well supported (BP $< 50\%$, BI = 1). The phylogenetic results show that the genus *Kachuga* is polyphyletic, and spread across two independent clades. The first clade includes two sister species, *K. kachuga* and *Batagur baska*, and the second clade indicates a sister relationship between *Callagur borneoensis* and *K. trivittata* with *K. dhongoka* being sister to these two species.

We ran the maximum likelihood and single model Bayesian analyses based on all the data combined using the GTR + G + I model of molecular evolution as selected by Modeltest. The parameters estimated by the AIC criterion were: Base frequency $A = 0.2927$, $C = 0.2662$, $G = 0.2100$, $T = 0.2311$. ML $-\ln L = 15288.2607$; rate matrix: $A-C = 4.9124$, $A-G = 18.2245$, $A-T = 4.1045$, $C-G = 1.0530$, $C-T = 62.8545$, $G-T = 1.0000$; proportion of invariable site (I) = 0.5677; gamma distribution shape parameter (G) = 0.5269. For the ML analysis, the total number of rearrangements tried was 2062, and the score of the single best tree found was 15288.261. In the single-model Bayesian analysis (Fig. 2), $-\ln L$ scores reached stationarity after 9000 generations while in the mixed-model Bayesian analysis scores reached equilibrium after 7000 generations in both runs. Only several minor differences were found between the two Bayesian analyses. In the single-model analysis, except for the relationship between *Siebenrockiella* and *Geoemyda*, two nodes received PPs equal to 99% and other nodes had 100% PPs, while in the mixed-model analysis all nodes had PPs equal to 100%. In addition, the PP supporting the relationship between *Siebenrockiella* and *Geoemyda* increased from 63% in the single-model analysis to 75% in the mixed model analysis (Fig. 2).

The topologies of the Bayesian consensus trees, both single and mixed model, and the ML tree were completely resolved and identical. This topology is different from the MP topology in that the positions of *S. crassicolis* and *Geoemyda* are interchanged. Similar to the MP analysis, the relationship between these two taxa was weakly sup-

ported by the ML and the Bayesian analyses (BP = 52%; PP_{single model} = 63%; PP_{mixed model} = 75%). All other nodes received high statistical support values (BP > 70%; PP > 95%) (Fig. 2).

4. Discussion

4.1. Phylogenetic relationships

Using diverse molecular markers, we are able to generate a robust phylogeny with high statistical support values for all nodes, regardless of analysis methods employed, except for the uncertainty in the relationship between *Siebenrockiella* and *Geoemyda*. Our phylogenetic results strongly support the monophyly of the clade consisting of *Batagur*, *Callagur*, *Hardella*, *Kachuga*, and *Pangshura* in all analyses. In Spinks et al. (2004), this clade only received strong support value from the Bayesian posterior probability. In addition, our analyses were able to resolve the phylogenetic position of *Hardella* clearly. Its sister relationship to *Batagur* + *Callagur* + *Kachuga* as supported by this study is novel because previous studies placed it either sister to *Pangshura* (Spinks et al., 2004) or sister to *Batagur* + *Callagur* + *Kachuga* + *Pangshura* (Diesmos et al., 2005; Prasczag et al., 2006) with weak statistical support values.

Our analyses confirm that the sister relationship between *Batagur* and *Pangshura* as weakly supported in Diesmos et al. (2005) and Prasczag et al. (2006) is not recovered. Instead, the arrangement proposed by Spinks et al. (2004) is supported. Especially, with the addition of two important species, *K. kachuga* and *K. trivittata*, the relationships between members of the genus *Kachuga* are resolved with high support level. Notably, our results indicate that the genus *Kachuga*, as traditionally defined, is polyphyletic with regard to *Callagur* and *Batagur*. Although two species *K. kachuga* and *K. trivittata* have not been included in previous molecular studies, their affinity to *Callagur* was suspected by McDowell (1964) based on his morphological analysis. However, while our data support the sister relationship between *Callagur* and *K. trivittata*, *K. kachuga* is not at all closely related to that clade. The sister relationship between *K. kachuga* and *Batagur* is also novel as no previous study has discovered this relationship.

4.2. Taxonomy

Based on our phylogenetic results and on the morphological examination of 27 specimens of *Batagur*, *Callagur*, and *Kachuga* (see Appendix A) we propose that the five species of the three genera *Batagur*, *Callagur*, and *Kachuga* are placed in the genus *Batagur* (Gray, 1855; type species, *B. baska*) because the name *Batagur* has page priority over *Kachuga* (Gray, 1955). All species of this clade share a unique character, presence of the costal fontanelles on the carapace of adult males (pers. obs.; G. Kuchling, pers. comm.) (see Fig. 2).

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Appendix A. Specimens examined for morphological characters in this study

Batagur baska

AMNH 80926, MCZ 29577, MCZ 182565, MCZ 29578, MCZ 31977, CRI 6502, CRI 4390

Callagur borneoensis

AMNH 80933, AMNH R142624, MCZ 42198

Hardella thurjii

AMNH 85774, AMNH 110191, AMNH 82004, AMNH 119006, AMNH 87451

Kachuga trivittata

AMNH 58559, AMNH 58560, AMNH 58565

Kachuga kachuga

FMNH 224152, MCZ 51698, FMNH 224128, FMNH 224127, CRI 2742, CRI 2879

Kachuga dhongoka

RH 1018, UF 103398, UF 107178, FMNH 224154, FMNH 223678, AMNH 80927, AMNH 80928, FMNH 224108

Pangshura smithi

AMNH 85595, FMNH 260384, AMNH 85814

Pangshura tecta

AMNH 4786, AMNH 4793, AMNH 125102

Pangshura tentoria

FMNH 224185, FMNH 259431, FMNH 224109, FMNH 260379

AMNH: American Museum of Natural History; CRI: Chelonian Research Institute; FMNH: Field Museum of Natural History; MCZ: Museum of Comparative Zoology, Harvard University; RH: Ren Hirayama Private Collection;

UF: Florida Museum of Natural History, Florida University.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympev.2007.05.002](https://doi.org/10.1016/j.ympev.2007.05.002).

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