



A new subspecies of *Batagur affinis* (Cantor, 1847), one of the world's most critically endangered chelonians (Testudines: Geoemydidae)

PETER PRASCHAG¹, ROHAN HOLLOWAY², ARTHUR GEORGES², MARTIN PÄCKERT³,
ANNA K. HUNSDÖRFER³ & UWE FRITZ^{3,4}

¹The Turtle Conservancy, Behler Chelonian Institute, P.O. Box 1289, Ojai, CA 93024, USA

²Institute of Applied Ecology, Research Group, University of Canberra, Canberra 2601, Australia

³Museum of Zoology, Senckenberg Dresden, A.B. Meyer Building, D-01109 Dresden, Germany

⁴Corresponding author. E-mail: uwe.fritz@senckenberg.de

Abstract

Estuarine *Batagur* are among the most critically endangered chelonian species. We assess the taxonomic status of the recently discovered Cambodian relic population of *Batagur* by phylogenetic analyses of three mitochondrial (2096 bp) and three nuclear DNA fragments (1909 bp) using sequences from all other *Batagur* species and selected allied geoemydids. Furthermore, we calculated haplotype networks of the mitochondrial cytochrome *b* gene for Cambodian terrapins, *B. affinis*, *B. baska*, and *B. kachuga* and compare external morphology of estuarine *Batagur* populations. Genetically, Cambodian *Batagur* are closely related with, but distinct from *B. affinis* from Sumatra and the west coast of the Malay Peninsula. Morphologically, Cambodian *Batagur* resemble the distinctive *B. affinis* populations from the eastern Malay Peninsula that were not available for genetic study. We suggest that the *Batagur* populations from the eastern Malay Peninsula and Cambodia represent a new subspecies of *B. affinis* that once was distributed in estuaries surrounding the Gulf of Thailand (*Batagur affinis edwardmolli* subsp. nov.). Its patchy extant distribution is most probably the result of large-scale habitat alteration and century-long overexploitation. In addition, our phylogenetic analyses suggest repeated switches between riverine and estuarine habitats during the evolution of the extant *Batagur* species.

Key words: Southeast Asia, South Asia, *Batagur affinis affinis*, *Batagur affinis edwardmolli* subsp. nov., *Batagur baska*, *Batagur kachuga*, endangered species

Introduction

Batagur baska (Gray, 1830) is one of the world's most critically endangered terrapins. Its range was recently restricted to a region extending from coastal north-easternmost India and adjacent Bangladesh southwards to at least the Ayeyarwady and Bago estuaries in Myanmar (Praschag *et al.* 2007, 2008). Populations from the Malay Peninsula and Sumatra, traditionally treated as conspecific, turned out to represent the distinct species *B. affinis* (Cantor, 1847). Both *B. affinis* and *B. baska* are large terrapin species, reaching a maximum shell length of approximately 60 cm (Moll 1980; Ernst *et al.* 2000). Their distribution is more or less confined to brackish water; they occur in estuaries, mangrove belts and inshore beds of marine vegetation (Kalyar *et al.* 2007). Phylogenetically, *B. affinis* and *B. baska* together are sister to a riverine inland species, *B. kachuga* (Gray, 1831) from northern India (Praschag *et al.* 2007), that was placed for a long time in the genus *Kachuga* (Le *et al.* 2007; Praschag *et al.* 2007).

Batagur affinis and *B. baska* declined dramatically throughout their ranges as a result of overharvesting of adults and eggs coupled with habitat degradation (Das 1997; Moll 1997; Kalyar *et al.* 2007; Platt *et al.* 2008). Estuarine *Batagur* once also occurred in southern Vietnam and Cambodia, but until now, nothing has been

known about their taxonomic identity. According to Bourret (1941), '*B. baska*' was common in the late 19th Century in Cochinchina (southern Vietnam) and Cambodia, and eggs were collected along the Prek Tap Chéang River, now known as Sre Ambel, a coastal river in the province of Koh Kong, Cambodia (Platt *et al.* 2003). The latter authors added that '*B. baska*' also occurred in the early 20th Century in the Tonle Sap, a large freshwater lake in the inland of Cambodia. While Platt *et al.* (2003) failed to confirm the species for the Tonle Sap, an unexpected habitat for an estuarine species, they were able to provide evidence for the existence of a relic population in the Sre Ambel River system. In the present study we compare specimens of this Sre Ambel population genetically with *B. baska* and *B. affinis* using sequence data of three mitochondrial (2096 bp) and three nuclear DNA fragments (1909 bp). Further, we compare external morphology and natural history of estuarine *Batagur* populations, correlate these data with observed genetic variation, and describe the populations from the Gulf of Thailand as a new subspecies.

Material and methods

Tissue samples comprising skin and a small quantity of underlying muscle were collected from seven *Batagur* caught in the Sre Ambel River system in Cambodia's south-west. Samples were taken as a small slither from the trailing edge of the outer toe of the rear right foot. The samples were preserved in 75% ethanol for shipment and storage at -20°C. These samples are housed in the tissue collection of the Institute for Applied Ecology, University of Canberra (voucher numbers AA1001-AA1004, AA1014-AA1016). For one of these samples, three mitochondrial and three nuclear genomic fragments were sequenced on an ABI 3130 Genetic Analyzer, following the procedures and using the primers described in Le *et al.* (2007) and Fritz *et al.* (2008). Mitochondrial sequences were for the cytochrome *b* gene (*cyt b*) plus the adjacent portion of the tRNA-Thr gene (1169 bp), and the partial 12S rRNA (392 bp) and 16S rRNA genes (535 bp). Nuclear sequences corresponded to the partial C-mos (590 bp), Rag1 (647 bp), and Rag2 genes (672 bp). Sequences of the 12S, 16S, C-mos, Rag1, and Rag2 fragments were also produced for *B. baska*, using a sample from the tissue collection of the Museum of Zoology Dresden (MTD T 3088, Sunderbans, West Bengal, India), for which the *cyt b* gene was previously sequenced (Praschag *et al.* 2007). For the other six Cambodian samples and additional samples of *B. affinis* (MTD T 5673-5675) and *B. baska* (MTD T 5672), only the phylogeographically highly informative mtDNA fragment including the *cyt b* gene was generated. The new samples of *B. affinis* were from confiscated terrapins of Indonesian origin; the new *B. baska* sample was obtained from a market specimen from Mongla, Bangladesh. GenBank accession numbers of the 21 new sequences are: FN256231-FN256247 and FN313567-FN313570.

Sequences from previous studies (Le *et al.* 2007; Praschag *et al.* 2007, 2008) were downloaded from GenBank and aligned with our data in MEGA 4.0 (Tamura *et al.* 2007). To elucidate phylogenetic relationships, the six-gene data set of Le *et al.* (2007) was supplemented with sequences of *B. baska* and the Cambodian *Batagur* and their analyses were repeated (it should be noted that sequences labelled by Le *et al.* 2007 as '*B. baska*' are actually *B. affinis*). The appropriate substitution model for the concatenated sequence data was estimated using MODELTEST 3.04 (Posada & Crandall 1998) and MrMODELTEST (Nylander 2004). According to the Akaike information criterion, the best fit model was GTR+I+G with empirical base frequencies: $\pi A=0.2943$, $\pi C=0.2670$, $\pi G=0.2074$, $\pi T=0.2313$; proportion of invariable sites $I=0.5723$; gamma shape parameter $\alpha=0.5496$; rate matrix: R(a)[A-C]=3.5980, R(b)[A-G]=11.9253, R(c)[A-T]=4.9027, R(d)[C-G]=1.5067, R(e)[C-T]=36.1455, R(f)[G-T]=1.0000. Phylogenetic trees were reconstructed using Maximum Parsimony (MP) and Maximum Likelihood (ML) as implemented in PAUP* 4.0b10 (Swofford 2002) and Bayesian inference of phylogeny as implemented in MrBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). Two *Rhinoclemmys* species (Le *et al.* 2007) were used as outgroups. Both MP and ML analyses were performed in a heuristic search with TBR branch swapping option (10^5 rearrangements) and best-fit model settings applied to the data set in ML. Under MP, gaps were coded as fifth character state; 3155 of 4015 aligned characters

(including gaps) were constant in the ingroup sequences; 560 characters were variable and parsimony-informative; 300 variable characters were singletons. When the two outgroup sequences were considered, 3093 sites were constant; 629 were variable and parsimony-informative and 293 variable characters were parsimony-uninformative. Clade support for MP trees was estimated using 1000 bootstrap replicates (Felsenstein 1985) in a fast heuristic search with all characters unordered and equally weighted and gaps treated as fifth character state. ML bootstrap support was obtained by 100 bootstrap replicates in GARLI 0.951 (Zwickl 2006) with the model parameter raw string defined within a separate GARLI nexus block file. Using default settings, branch lengths were optimized with a threshold of 2×10^4 generations per bootstrap replicate. Constant $-\ln L$ values were reached after a few hundred generations and the allowed minimum value of optimization precision was reached after approximately 5500 generations in different replicates. Therefore, the parameter `<genthreshfortopoterm>` was set to 6000 in order to speed up the analysis. Two independent search replicates per bootstrap replicate were performed by default settings `<searchreps=2>` to increase the chance of finding the best tree per bootstrap replicate.

Bayesian analyses (BA) were performed using the Metropolis-coupled Markov chain Monte Carlo algorithm with two parallel runs, each with one cold and three heated chains. In a first BA the concatenated sequence data set was divided into six partitions corresponding to the three mitochondrial and three nuclear fragments and the GTR+I+G model was applied to each partition. The overall rate was allowed to vary between partitions by setting the priors `<ratepr=variable>` and model parameters (gamma shape, proportion of invariable sites, etc.) unlinked across partitions, so that for each partition a separate set of parameters was estimated. In addition, a second unpartitioned analysis was run. For both analyses, the heating parameter λ was set to 0.1 to obtain convergence. The chains ran for 10^6 generations with every 100th generation sampled (burn-in=5000). The remaining trees of each analysis were used for generating 50% majority rule consensus tree. The posterior probability of any individual clade in such a consensus tree corresponds to the percentage of all trees containing that clade, and is by thus a measure for clade frequency and credibility.

To explore differentiation of mitochondrial haplotypes, parsimony networks were constructed for two data sets of *cyt b* using TCS 1.21 (Clement *et al.* 2000). The first data set comprised short aDNA sequences (320 bp) of historical type and museum specimens of *B. affinis* and *B. baska* (Praschag *et al.* 2008) combined with the corresponding fragments of sequences generated in the present study and GenBank sequences for *B. affinis*, *B. baska*, and *B. kachuga*. The second, 1067-bp-long data set (1038 bp *cyt b* + 29 bp tRNA-Thr) included only the GenBank sequences of this minimum length and sequences of the present study. Sequences AY434600 (*B. affinis*) and EU030215 (*B. kachuga*), which lacked the terminal 29 bp of tRNA-Thr, were placed in the last two positions of the alignment. For this second data set, uncorrected *p* distances were calculated in MEGA 4.0.

External morphology of Cambodian *Batagur* was compared with museum and live specimens and photos of *B. baska* from the Sundarbans (India, Bangladesh) and Myanmar, with *B. affinis* from Indonesia (confiscated animals) and the west coast of peninsular Thailand and Malaysia, and with *B. affinis* from the east coast of peninsular Malaysia. In addition, one subadult male from the questionable locality 'Indus Delta, Sindh' (Pakistan) from the collection of the Natural History Museum Vienna was examined. For catalogue numbers of studied museum specimens, see Praschag *et al.* (2008), if not given below. Live *B. affinis* and photos of live terrapins were from the Klong La-ngu River (Satun Province), Thailand and from the Dungun, Perak, and Terengganu Rivers, Malaysia. The Klong La-ngu and Perak Rivers are on the west coast, the Dungun and Terengganu Rivers on the east coast of the Malay Peninsula.

Results

Phylogeny. The topologies of our BA, ML and MP trees were broadly concordant. The BA and ML trees were identical, and the single most parsimonious tree (2282 steps; CI=0.521, RI=0.517, RC=0.270) differed only in the placement of *Siebenrockiella crassicollis*. In the MP analysis, this species was basal to all other

geoemydids of the ingroup, albeit with weak bootstrap support (<50%), whereas in the BA and ML analyses the two *Geoemyda* species were basal (Fig. 1). Our phylogeny, based on the same three mitochondrial and three nuclear genes, had the same general topology as the phylogeny previously reported by Le *et al.* (2007) but with the addition of true *B. baska* and the Cambodian *Batagur*.

With respect to *Batagur*, our phylogenetic analyses demonstrate, with high statistical support, that the riverine *B. kachuga* is basal to a terminal clade comprising the estuarine *B. baska*, *B. affinis*, and the Cambodian *Batagur*. This clade is sister to another clade consisting of the other three *Batagur* species, *B. dhongoka* + (*B. borneoensis* + *B. trivittata*).

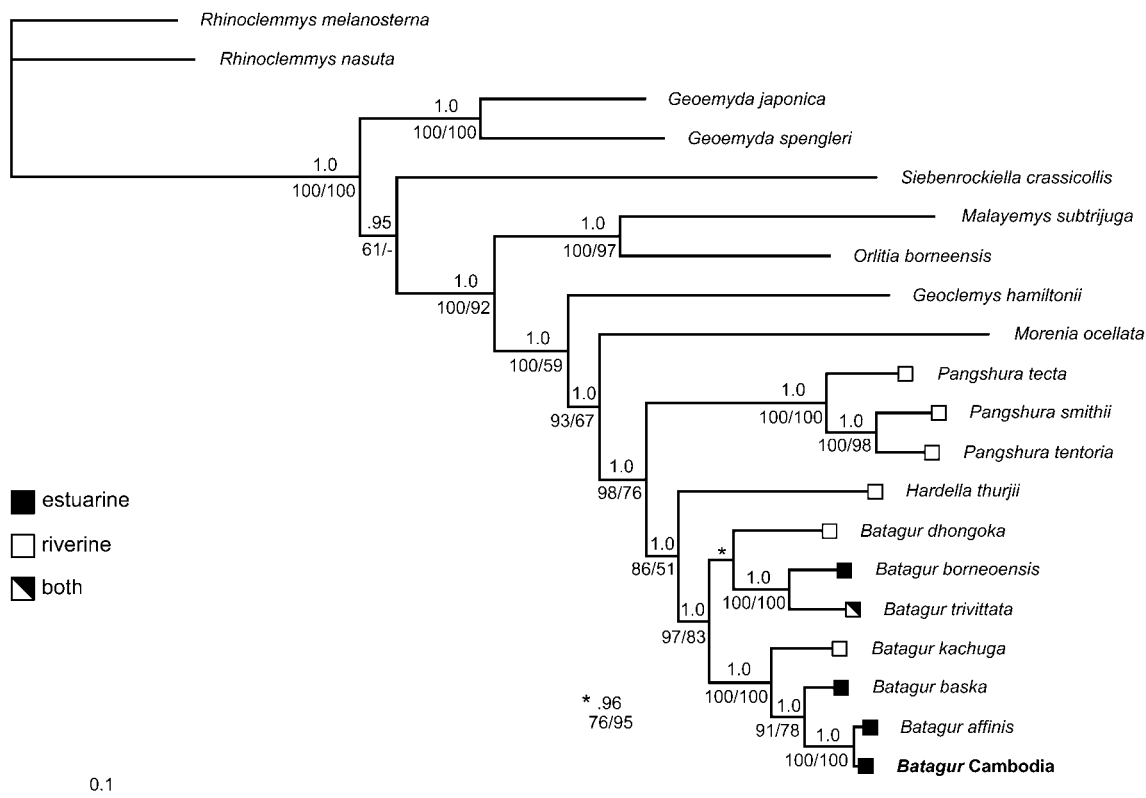


FIGURE 1. Bayesian reconstruction of the phylogeny of *Batagur* and allied geoemydid taxa, based on the expanded data set of Le *et al.* (2007). Numbers above nodes are posterior probabilities (partitioned analysis). Posterior probabilities are identical for unpartitioned analysis, except for the clade comprising *B. dhongoka* + (*B. borneoensis* + *B. trivittata*) and the basal clade of all taxa except *Geoemyda* and *Rhinoclemmys* (.97 and .81, respectively). Numbers below nodes, ML and MP bootstrap values. For *Pangshura* + (*Hardella* + *Batagur*) the habitat is coded.

Haplotype networks and uncorrected *p* distances. For the 320 bp data set and 90%-95% connection limits, the parsimony network analyses yielded three unconnected networks—one for each of *Batagur baska*, *B. kachuga*, and *B. affinis* plus the sequences of the Cambodian *Batagur* (not shown). The haplotype of the Cambodian *Batagur* was separated by seven mutational steps from the more frequent haplotype of *B. affinis* with the latter differing from the rarer haplotype of *B. affinis* by one step. Within the other two species haplotypes differed by a maximum of three steps each. When a connection was enforced, the number of mutational steps did not change in each of the resulting three subnets. However, haplotypes of *B. affinis* were joined then via a loop, so that the Cambodian haplotype was alternatively also connected via nine mutational steps with the rarer haplotype of *B. affinis* (Fig. 2a).

Using the 1067 bp data set, four unconnected networks or single haplotypes were obtained under the 95% threshold (one for each of *Batagur baska*, *B. kachuga*, *B. affinis*, and the Cambodian *Batagur*; not shown). Compared to the 320 bp data set, one additional mutational step occurred within the net of *B. kachuga*, so that an ancestral haplotype was separated by two steps from each of its descendants. Under the 90% threshold, the

Cambodian haplotype was connected via 18 steps with each of the *B. affinis* haplotypes, while the haplotype of *B. baska* and the net of *B. kachuga* remained separate. When a connection was enforced, haplotypes of *B. baska* and *B. kachuga* differed by a minimum of 50 mutational steps and a maximum of 54 steps; haplotypes of *B. baska* and *B. affinis* were separated by 44 to 47 steps; and haplotypes of *B. affinis* and *B. kachuga* by a minimum of 54 and a maximum of 61 steps. The Cambodian haplotype differed from the closest other haplotypes, the two haplotypes of *B. affinis* by 18 to 19 steps (Fig. 2b). This pattern is also echoed by the uncorrected *p* distances (Table 1). The Cambodian *Batagur* sequences differed on average from those of *B. affinis* by 1.692% and from *B. baska* and *B. kachuga* by 4.592% and 5.635%, respectively. The latter values resemble the uncorrected *p* distances occurring between *B. affinis*, *B. baska* and *B. kachuga* (4.220%-5.085%).

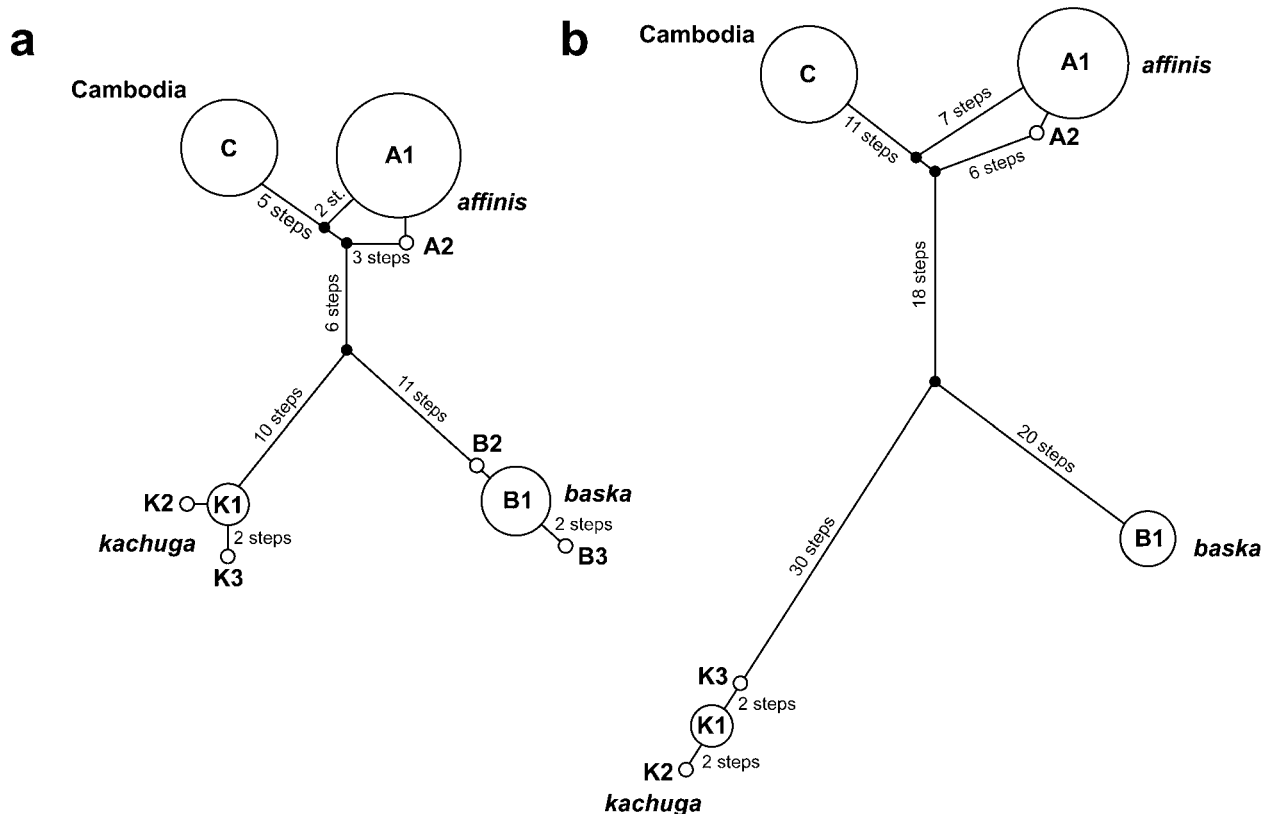


FIGURE 2. Parsimony networks for mitochondrial haplotypes of *Batagur affinis*, *B. baska*, *B. kachuga*, and the Cambodian *Batagur* (connection enforced). Symbol size corresponds to haplotype frequency; missing node haplotypes black. Lines joining haplotypes, one mutational step except otherwise indicated. (a) Network based on a 320-bp-long alignment of *cyt b*. Haplotypes and their frequencies (see Appendix): *B. affinis* – A1 (*n*=9), A2 (*n*=1); *B. baska* – B1 (*n*=5), B2 (*n*=1), B3 (*n*=1); *B. kachuga* – K1 (*n*=3), K2 (*n*=1), K3 (*n*=1); Cambodian *Batagur* – C (*n*=7). Haplotypes A1 and B1 include the lectotype of *Tetraonyx affinis* Cantor, 1847 and topotypic specimens of *Emys baska* Gray, 1830, respectively (Praschag *et al.* 2008). Haplotypes K1 and K2 are from topotypic specimens of *Emys kachuga* Gray, 1831. (b) Network based on a 1067-bp-long alignment of *cyt b*. Haplotypes and their frequencies: *B. affinis* – A1 (*n*=8), A2 (*n*=1); *B. baska* – B1 (*n*=4); *B. kachuga* – K1 (*n*=3), K2 (*n*=1), K3 (*n*=1); Cambodian *Batagur* – C (*n*=7).

External morphology and natural history. Morphologically, *Batagur baska* is highly distinct from *B. affinis* (Praschag *et al.* 2007, 2008). *Batagur affinis* from the east and west coasts of the Malay Peninsula are known also to differ morphologically (Moll 1980; pers. observ.; see also Figs 3-4; Table 2); our sequence data of *B. affinis* all correspond to the west coast form that seems also to occur in Sumatra (cf. the identical mtDNA sequences of confiscated Indonesian terrapins from Praschag *et al.* 2007 and this study; Sumatra is the only part of Indonesia where *B. affinis* occurs). Cambodian *Batagur* resemble *B. affinis* from the east coast, but males slightly differ in coloration, so that most adult males can be reliably distinguished.



FIGURE 3. (a) *Batagur baska*, male, Sundarbans, Bangladesh – photo: S.M.A. Rashid; (b) *B. baska*, semiadult female (the pointed, upturned snout develops only with increasing age), Sundarbans, Bangladesh – photo: P. Praschag; (c) west coast form of *B. affinis*, male, Klong La-ngu River, Satun Province, Thailand – photo: B. Horne; (d) west coast form of *B. affinis*, female, Perak River, Malaysia – photo: E.O. Moll; (e) east coast form of *B. affinis*, male, Dungun River, Malaysia – photo: E.H. Chan; (f) east coast form of *B. affinis*, female, Terengganu River, Malaysia – photo: E.O. Moll; (g) Cambodian *Batagur* male, Sre Ambel River system, Cambodia – photo: R. Holloway; (h) Cambodian *Batagur* female, Sre Ambel River system, Cambodia – photo: B. Horne. Note differences in head shape, soft part and iris coloration.

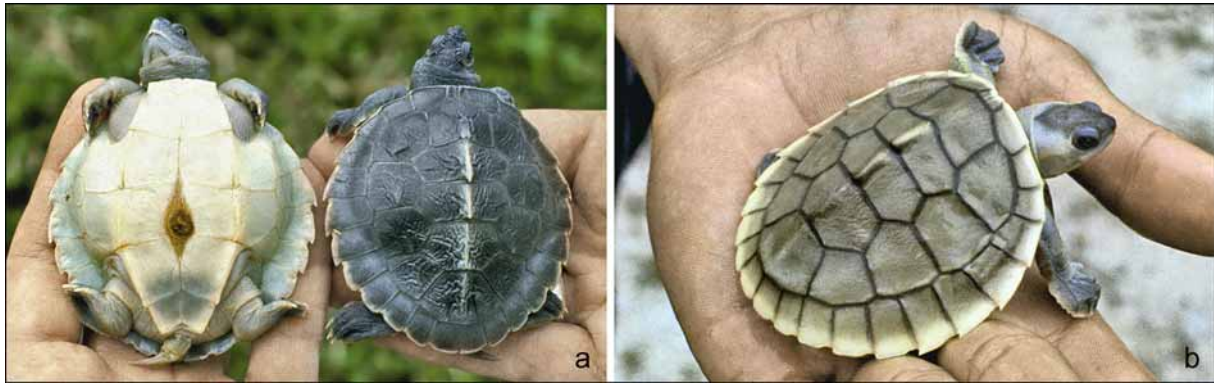


FIGURE 4. Hatchlings of *Batagur affinis*, (a) west coast form, Perak River, Malaysia; (b) east coast form, Terengganu River, Malaysia – photos: E.O. Moll. Note yellow marginal scutes and silvery blotches in temporal and parietal region in the east coast hatchling.

TABLE 1. Uncorrected *p* distances (percentages) within and between *Batagur affinis*, *B. baska*, *B. kachuga*, and the Cambodian *Batagur* based on a 1067-bp-long alignment of *cyt b*. *n*, number of haplotypes. Below diagonal, average between-group differences; on diagonal, average within-group differences in bold. In brackets, standard error estimates (500 bootstrap replicates).

	<i>n</i>	<i>affinis</i>	<i>baska</i>	<i>kachuga</i>	Cambodia
<i>affinis</i>	2	0.021 (0.020)			
<i>baska</i>	1	4.220 (0.635)	0 (0)		
<i>kachuga</i>	3	5.085 (0.703)	4.937 (0.667)	0.153 (0.071)	
Cambodia	1	1.692 (0.379)	4.592 (0.634)	5.635 (0.720)	0 (0)

These morphological differences are paralleled by differences in nesting behaviour. *Batagur baska* nests along the sea shore or on sandy islands in the brackish estuaries, while *B. affinis* and Cambodian terrapins swim far upstream for nesting to reach beaches that are often located well above tidal influence. Some *B. affinis* females may swim over 80 km to these sites (Moll 1980; Ernst *et al.* 2000; Kalyar *et al.* 2007). On the west coast of peninsular Malaysia, females lay each clutch in a single nest and then make a false nest (‘body pit’) nearby. In the Terengganu River population (east coast), by contrast, the females often divide the clutch into two or three separate nests (Moll 1980 and pers. comm.).

Taxonomic conclusions

Our genetic and morphological data demonstrate the distinctness of the disjunct Cambodian population of *Batagur*. However, the degree of genetic differentiation is clearly lower than that between *B. affinis*, *B. baska*, and *B. kachuga* (Fig. 2; Table 1). The Cambodian *Batagur* is undoubtedly very closely related to *B. affinis*, which we interpret as subspecific rather than interspecific level variation.

Although slight coloration differences exist between the two (Table 2), the Cambodian *Batagur* resembles morphologically populations of *B. affinis* from eastern Malaysia (Dungun and Terengganu Rivers) that were unfortunately not available for genetic study. We suggest that these populations and the Cambodian *Batagur* represent the same taxon and became isolated owing to human activity. We contend that this taxon was distributed originally in estuaries surrounding the Gulf of Thailand.

The type locality of Penang for *B. affinis* is along the west coast of peninsular Malaysia which, together with mtDNA sequence data of the lectotype (Praschag *et al.* 2008), provides evidence that the name *Tetraonyx affinis* Cantor, 1847 refers to the west coast taxon and that no name is available for the east coast form, also

distributed in Cambodia (cf. Fritz & Havaš 2007; Präsachag *et al.* 2008). Consequently, we describe the eastern form of *B. affinis* as a subspecies new to science.

TABLE 2. External morphology and current distribution ranges of estuarine *Batagur* based on personal observations and data from Anderson (1879), Rashid & Swingland (1997), and Moll (1980 and pers. comm.).

	<i>Batagur baska</i>	<i>Batagur affinis</i> west coast	<i>Batagur affinis</i> east coast	Cambodian <i>Batagur</i>
Male	Head elongated with pointed, upturned snout; head black, area around nostrils pale bluish, rest of head and distal part of neck deep black, passing into rich crimson on base of neck; iris matte greenish yellow; forelimbs brilliant rosy carmine, hind limbs, tail and thighs dull reddish purple. Carapace during mating season rich brown to reddish, in some individuals slightly marbled with darker lines; plastron having a rosy yellow tint	Head distinctly shorter than in <i>B. baska</i> , with blunt snout and shorter distance from nostril to eye; head jet black or very dark grey, never brownish; iris during mating season immaculate white. Carapace black or dark grey, during mating season black	Head elongated with pointed, upturned snout and in comparison to west coast males longer distance between nostril and eye; head and soft parts chocolate brown to almost black, light coloured individuals with brown and never grey skin; edges of mouth orange; iris golden or bright yellow. Carapace dark brown to black	Closely resembling <i>B. affinis</i> from the east coast of Malaysia. However, head often rusty brown to reddish, distal portion of neck turns proximally into greyish; limbs greyish as well. Carapace dark grey
Female	Head elongated with pointed, upturned snout; light grey with waxy-blue nostrils and yellowish jaws, temporal region paler, almost white; other soft parts grey, never brownish	Head short with blunt snout; distance from nostril to eye very short (resembling the riverine <i>B. kachuga</i>); head dark grey or brownish, jaws dirty yellow or light brown; other soft parts dark grey or brownish	Head elongated with pointed, upturned snout like in <i>B. baska</i> ; head greyish to brownish with whitish grey to silvery blotches in temporal and parietal region and brown jaws	Look exactly like females from east coast of Malaysia
Hatchling	Unknown	Overall dark grey, without light temporal blotches	Overall brown, with whitish grey to silvery temporal and parietal blotches and distally yellow marginal scutes	Look exactly like hatchlings from east coast of Malaysia
Range	Northeastern India from Orissa to West Bengal, Sundarbans of Bangladesh and coastline of Myanmar, perhaps northernmost west coast of peninsular Thailand	Southwest coast of peninsular Thailand, west coast of peninsular Malaysia, Sumatra (Indonesia)	East coast of peninsular Malaysia and of peninsular Thailand	Sre Ambel River system, Cambodia

***Batagur affinis edwardmollii* subsp. nov.**

Holotype. Natural History Museum Vienna, NMW 38903, juvenile in alcohol (hatched and died in captivity), Sre Ambel River system, Koh Kong Province, Cambodia; don. Head Start Centre Sre Ambel, July 2009.

Paratypes. Museum of Zoology Dresden, MTD 47538, juvenile in alcohol, same data as holotype. Field Museum of Natural History, Chicago, FMNH 224093 (ex EOM 2398; specimen figured on Plate IIIA in Moll 1980), broken shell of adult female, Terengganu River, Malaysia; leg. Edward O. Moll, 7 July 1976.

Etymology. The new subspecies is named in recognition of Professor Edward O. Moll, one of the foremost experts on river turtles, who substantially contributed to the knowledge of *Batagur affinis* and its natural history.

Diagnosis. Adults differ from nominotypical subspecies of *Batagur affinis* by their distinctly more elongated head with upturned snout; males with chocolate brown to almost black head (east coast of

peninsular Malaysia) or sometimes rusty brown to reddish head (Sre Ambel River system, Cambodia), edges of mouth orange; iris golden or bright yellow. Females and juveniles with conspicuous whitish grey to silvery blotches in temporal and parietal region; hatchlings with distally yellow marginal scutes. For corresponding characters of *B. a. affinis*, see Table 2.

Description of holotype. Specimen slightly macerated; some epidermal scutes detached from shell. Carapace roundish when viewed from above, with weakly serrated central and posterior marginal scutes; medial keel distinct, with posteriorly directed, slightly pointed spines. Plastron anteriorly truncated, posteriorly with anal notch. Straight line carapace length approximately 86 mm, carapace width 84 mm; medial plastron length 74 mm, maximum plastron length (to tips of anal scutes) 78 mm.

Range: East coast of peninsular Malaysia and adjacent Thailand; Sre Ambel River system, Cambodia (Fig. 5).

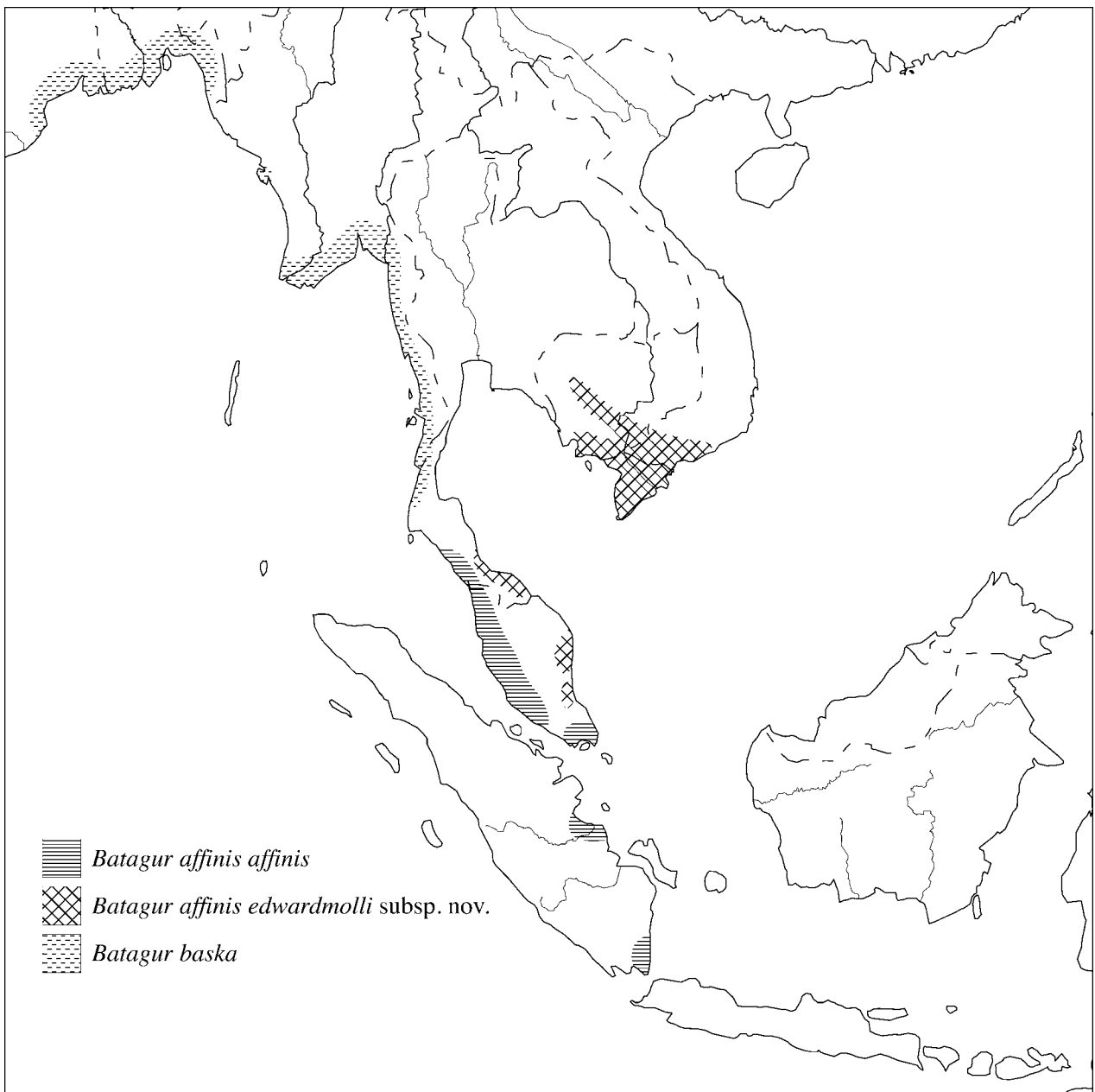


FIGURE 5. Historical distribution of *Batagur affinis affinis*, *B. a. edwardmollii*, and *B. baska* (modified from Praschag *et al.* 2008). Note that the species are extirpated in most of their former ranges.

Discussion

Our phylogenetic analyses suggest repeated switches of habitat during the evolution of the extant *Batagur* species. The vast majority of the some 60 geoemydid species (Fritz & Havaš 2007) are freshwater terrapins, while *B. affinis*, *B. baska*, and *B. borneoensis* are the only species living in tidal, brackish areas of the estuaries of medium and large rivers (Ernst *et al.* 2000). In addition, the nearly extinct *B. trivittata* once used this habitat, but also lives and nests until today far upstream in the inland of Myanmar (Maxwell 1911; Kuchling *et al.* 2006). When the natural history of the closely related genera *Hardella* and *Pangshura* is considered, the most parsimonious view has the ancestor of *Pangshura*, *Hardella*, and *Batagur* as a riverine freshwater terrapin (Fig. 1). If this is true, the ancestor of the extant *Batagur* taxa may well have been riverine, in which case an estuarine mode of life has been independently acquired in each subclade of *Batagur* by *B. affinis*, *B. baska*, *B. borneoensis*, and *B. trivittata*. Consequently, the riverine mode of life of the other species would represent the ancestral ecological adaptation. Alternatively, the ancestral *Batagur* could have been an estuarine terrapin and *B. kachuga*, *B. dhongoka*, and *B. trivittata* may have returned independently to the riverine freshwater habitat.

Most *Batagur* species exhibit conspicuous sexual and seasonal dichromatism (Anderson 1879; Moll 1980; Moll *et al.* 1981; Praschag *et al.* 2007)—an extremely rare trait among chelonians. According to Klingelhöffer & Mertens (1944) and Moll *et al.* (1981), the bright coloration of males is associated with reproduction. A function as premating isolation mechanism seems plausible given the capability of many chelonians to hybridize (cf. Fritz *et al.* 2008) and that, where the similar-sized *Batagur* species occur syntopically, they differ considerably in breeding coloration. For instance, *B. affinis* often occurs together with *B. borneoensis*. During the mating season, the coloration of males of the two species is highly distinct. Breeding males of *B. borneoensis* have a light coloured carapace with three broad black longitudinal stripes; the skin of the head gets bright white with a conspicuous black-edged scarlet stripe running between the dark eyes from the nose to the occiput (Moll *et al.* 1981). On the west coast of the Malay Peninsula, males of *B. a. affinis* have instead a black carapace and a jet black head with immaculate white eyes; on the east coast, the head of males of *B. a. edwardmollii* gets chocolate brown to black, with orange edges of the mouth and a golden iris. These contrasting differences are suggestive of character displacement (Moll *et al.* 1981). The coloration differences of the two *B. affinis* subspecies and of *B. baska* (Table 2) could also act as isolating mechanism between these taxa. While the two *B. affinis* subspecies are entirely allopatrically distributed, there is at least the possibility of a historical sympatric occurrence of *B. baska* and *B. a. affinis* near the border region of Myanmar and peninsular Thailand.

Also with respect to nesting behaviour, character displacement seems to occur between different *Batagur* species. *Batagur affinis*, which occurs sympatrically with the estuarine *B. borneoensis*, nests far upstream, whereas *B. borneoensis* nests on the same sea beaches as marine turtles (Dunson & Moll 1980). By contrast, *B. baska*, occurring farther northwest where *B. borneoensis* is absent, uses sea beaches for nesting (Ernst *et al.* 2000). However, in the Ayeyarwady Delta, large numbers of *B. baska* and *B. trivittata* nested together until about 100 years ago (Maxwell 1911; Kuchling *et al.* 2006).

Acknowledgements

Colin Poole, Mark Gately (Wildlife Conservation Society), and Rick Hudson (Turtle Survival Alliance) assisted to get all Cambodian permits for exporting the holotype and the Cambodian paratype of *Batagur affinis edwardmollii*. Richard Gemel (Natural History Museum of Vienna) and Alfred Engl (Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management) facilitated their import into the EU. For providing pictures we thank S.M.A. Rashid, Eng Heng Chan, Martin Gilbert and Colin Poole, Steven Platt, Ed Moll, and Brian Horne. Steven Platt, Brian Horne and most of all Ed Moll shared valuable information collected in the field and were always open for helpful discussions. We are particularly grateful to

Nao Thuok and Sovannara Heng for their assistance and encouragement in the early phases of the PhD project of RH. His field work was supported by a grant from the Wildlife Conservation Society, the Chelonian Research Foundation and the University of Canberra; the Department of Agriculture Forestry and Fisheries of the Kingdom of Cambodia provided logistic support and assistance in arranging the appropriate permits.

References

- Anderson, J. (1879 [1878]) *Anatomical and Zoological Researches, Comprising an Account of the Zoological Results of the Two Expeditions to Western Yunnan in 1868 and 1875; and a Monograph of the Two Cetacean Genera Platanista and Orcella*. Bernard Quaritch, London, text: (3), xxxv, (1), 985 pp., atlas: (4), 29 pp., 85 plates.
- Bourret, R. (1941) *Les Tortues de l'Indochine*. Institut Océanographique de l'Indochine, Station Maritime de Cauda, Nhatrang, 235 pp.
- Cantor, T. (1847) Catalogue of reptiles inhabiting the Malayan peninsula and islands. *Journal of the Asiatic Society of Bengal*, 16, 607–656, 897–952, 1026–1078.
- Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657–1660.
- Das, I. (1997) Conservation problems of tropical Asia's most-threatened turtles. In: van Abbema, J.V. (Ed.), *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles—An International Conference*. New York Turtle and Tortoise Society, New York, N.Y., pp. 295–301.
- Dunson, W.A. & Moll, E.O. (1980) Osmoregulation in sea water of hatchling emydid turtles, *Callagur borneoensis*, from a Malaysian sea beach. *Journal of Herpetology*, 14, 31–36.
- Ernst, C.H., Altenburg, R.G.M. & Barbour, R.W. (2000) *Turtles of the World. World Biodiversity Database, CD-ROM Series, Windows, Version 1.2*. Biodiversity Center of ETI, Amsterdam.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
- Fritz, U., Ayaz, D., Buschbom, J., Kami, H.G., Mazanaeva, L.F., Aloufi, A.A., Auer, M., Rifai, L., Šilić, T. & Hundsdoerfer, A.K. (2008) Go east: Phylogeographies of *Mauremys caspica* and *M. rivulata*—Discordance of morphology, mitochondrial and nuclear genomic markers and rare hybridization. *Journal of Evolutionary Biology*, 21, 527–540.
- Fritz, U. & Havaš, P. (2007) Checklist of chelonians of the world. *Vertebrate Zoology*, 57, 149–368.
- Gray, J.E. (1830) *Illustrations of Indian Zoology, chiefly selected from the collection of Major-General Hardwicke. Vol. I, Part 4*. Treuttel, Wurtz, Treuttel, Jun. and Richter, London, plates 75 and 78.
- Gray, J.E. (1831) *Illustrations of Indian Zoology, chiefly selected from the collection of Major-General Hardwicke. Vol. I, Part 5*. Treuttel, Wurtz, Treuttel, Jun. and Richter, London, plate 74.
- Kalyar, Thorbjarnarson, J. & Thirakhupt, K. (2007) An overview of the current population and conservation status of the critically endangered river terrapin, *Batagur baska* (Gray, 1831) in Myanmar, Thailand and Malaysia. *The Natural History Journal of Chulalongkorn University*, 7, 51–65.
- Klingelhöffer, W. & Mertens, R. (1944) Bemerkungen über die Umfärbung von *Callagur borneoensis*. *Wochenschrift für Aquarien- und Terrarienkunde*, 41, 35–36.
- Kuchling, G., Ko, W.K., Min, S.A., Lwin, T., Myo, K.M., Khaing, T.T. (1), Khaing, T.T. (2), Mar, W.W. & Win, N.N. (2006) Two remnant populations of the roofed turtle *Kachuga trivittata* in the upper Ayeyarwady River system, Myanmar. *Oryx*, 40, 176–182.
- Le, M., McCord, W.P. & Iverson, J.B. (2007) On the parphyly of the genus *Kachuga* (Testudines: Geoemydidae). *Molecular Phylogenetics and Evolution*, 45, 398–404.
- Maxwell, F.D. (1911) *Reports on Inland and Sea Fisheries in the Thongwa, Myaungmya, and Bassein Districts and the Turtle-banks of the Irrawaddy Division*. Government Printing Office, Rangoon, Burma, 57 pp.
- Moll, E.O. (1980) Natural history of the river terrapin, *Batagur baska* (Gray) in Malaysia (Testudines: Emydidae). *Malaysian Journal of Science*, 6(A), 23–62.
- Moll, E.O. (1997) Effects of habitat alteration on river turtles of tropical Asia with emphasis on sand mining and dams. In: van Abbema, J.V. (Ed.), *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles—An International Conference*. New York Turtle and Tortoise Society, New York, N.Y., pp. 37–41.
- Moll, E.O., Matson, K.E. & Krehbiel, E.B. (1981) Sexual and seasonal dichromatism in the Asian river turtle *Callagur borneoensis*. *Herpetologica*, 37, 181–194.
- Nylander, J.A.A. (2004) *MrMODELTEST v2*. Evolutionary Biology Centre, Uppsala University, Uppsala.
- Platt, K., Platt, S.G., Thirakhupt, K. & Rainwater, T.R. (2008) Recent records and conservation status of the critically endangered Mangrove terrapin, *Batagur baska*, in Myanmar. *Chelonian Conservation Biology*, 7, 195–204.

- Platt, S.G., Stuart, B.L., Sovannara, H., Kheng, L., Kalyar & Kimchhay, H. (2003) Rediscovery of the critically endangered river terrapin, *Batagur baska*, in Cambodia, with notes on occurrence, reproduction and conservation status. *Chelonian Conservation and Biology*, 4, 691–695.
- Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
- Praschag, P., Hundsdörfer, A.K. & Fritz, U. (2007) Phylogeny and taxonomy of endangered South and South-east Asian freshwater turtles elucidated by mtDNA sequence variation (Testudines: Geoemydidae: *Batagur*, *Callagur*, *Hardella*, *Kachuga*, *Pangshura*). *Zoologica Scripta*, 36, 429–442.
- Praschag, P., Sommer, R.S., McCarthy, C., Gemel, R. & Fritz, U. (2008) Naming one of the world's rarest chelonians, the southern *Batagur*. *Zootaxa*, 1758, 61–68.
- Rashid, S.M.A. & Swingland, I.R. (1997) On the ecology of some freshwater turtles in Bangladesh. In: van Abbema, J.V. (Ed.), *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles—An International Conference*. New York Turtle and Tortoise Society, New York, N.Y., pp. 225–242.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Swofford, D.L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, ver. 4. Sinauer Associates, Sunderland, MA.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
- Zwickl, D.J. (2006) *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion*. PhD dissertation, The University of Texas, Austin, Texas.

Appendix. Mitochondrial *cyt b* haplotypes of *Batagur* taxa from Fig. 2 (GenBank accession numbers; short sequences of 320 bp asterisked). For new sequences the collection vouchers are given in brackets (AA – Institute for Applied Ecology, University of Canberra; MTD T – Museum of Zoology Dresden, Tissue Collection).

Batagur affinis—A1 (*n*=9): AM691750-AM691754, FN313568-FN313570 (MTD T 5673-5675), AM922509*; A2 (*n*=1): AY434600.

Batagur baska—B1 (*n*=5): AM495267-AM495269, FN313567 (MTD T 5672), AM922507*; B2 (*n*=1): AM922510*; B3 (*n*=1): AM922508*.

Batagur kachuga—K1 (*n*=3): AM495284-AM495285, AM495287; K2 (*n*=1): AM495286; K3 (*n*=1): EU030215.

Cambodian *Batagur* —C (*n*=7): FN256231-FN256237 (AA1001-AA1004, AA1014-AA1016).