Shell shape and genetic variability of Southeast Asian Box Turtles (*Cuora amboinensis*) from Borneo and Sumatra

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Abstract

Distinguishing between species is an essential aspect of animal research and conservation. For turtles, morphology and genetic analysis are potentially valuable tools for identification. Shell shape is an important component of phenotypic variation in turtles and can be easily described and quantified by geometric morphometrics (GM). Here, we focus on carapace and plastron shape discrimination of immature Southeast Asian box turtles (*Cuora amboinensis*) from two of the Greater Sunda Islands with partially distinct faunas. GM analysis identified significant differences in carapace and plastron shape between turtles from Borneo and Sumatra. The discrimination success amounted to 90% and 83.7% for carapace and plastron, respectively. The correlations of carapace and plastron shapes were high for Sumatra (0.846), and less pronounced for Borneo (0.560). We detected no differences in the ontogenetic trajectories of the shell shape between the two islands. We conclude that shell shape can be used for reliable geographic assignment of *C. amboinensis* of unknown origin. In addition to the comparison of shell shapes, turtles from Borneo, Sumatra, Seram, and turtles of unknown origin from two Czech zoos were studied genetically. Analysis of the complete mitochondrial cytochrome *b* gene confirmed the distinctness of turtles from Borneo and Sumatra, with p-distance 2.68 – 4.09% sequence difference. Moreover, we discovered considerable genetic difference in Seram turtles of previously unknown haplogroup (p-distance 6.00 – 8.68%) revealing the need for the revision of the whole species complex of *Cuora amboinensis*.

Key words

Geometric morphometrics, Geoemydidae, Cytochrome b, *Cuora amboinensis*, Conservation biology.

Introduction

The Southeast Asian box turtle *Cuora amboinensis* (Riche *in* Daudin, 1801), belongs to the most diversified and widespread taxon of the genus *Cuora* with a distribution range including a major part of Southeast Asia (Iverson 1992). Unfortunately, it is also the most abundant hard-shelled turtle in Chinese markets and frequently used in traditional Chinese medicine (Cheung & Dudgeon 2006; Chen *et al*. 2009). Thus, it is exploited in huge numbers, especially from Indonesia and Malaysia, despite export quotas and even a total export ban in some regions. As a result, its numbers are rapidly declining and some populations are already extinct (Ives *et al*. 2008; Schoppe 2008, 2009). *Cuora amboinensis* is listed in Appendix II of CITES and globally red-listed as 'Vulnerable' (IUCN 2013). In the face of the current Asian Turtle Crisis (Cheung & Dudgeon 2006) and the
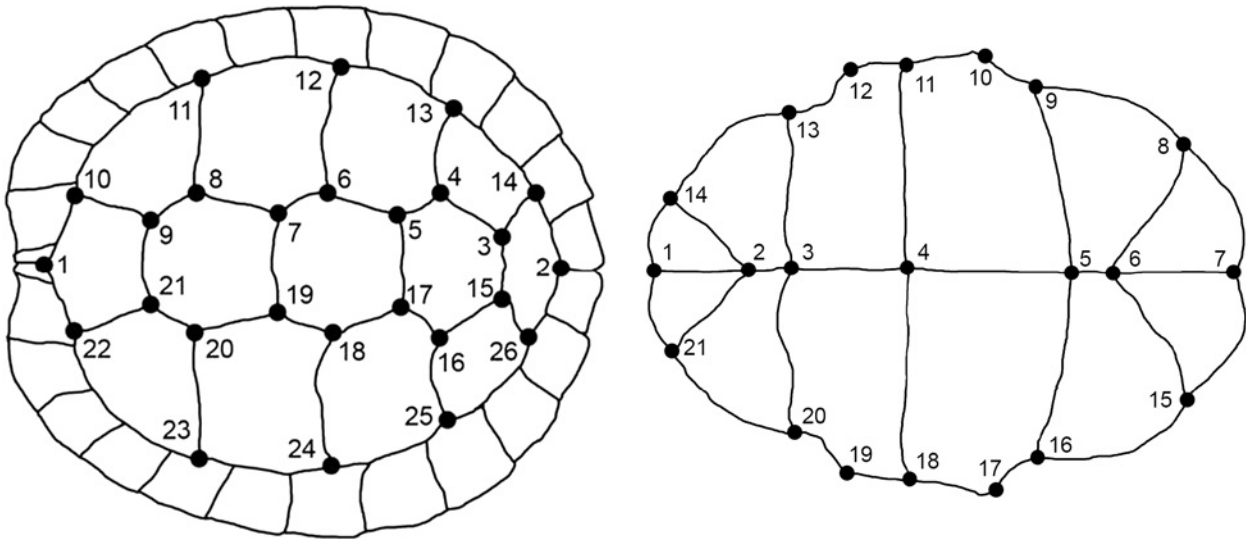
Fig. 1. Carapace and plastron of *Cuora amboinensis* showing the landmarks used in this study.

unsuccessful protection of the species in the wild, ex situ captive breeding programs inside and outside its distribution range are gaining increasing importance. For such captive breeding efforts, the correct identification of subspecies and the geographic provenance of turtles is of paramount importance. Although adults of currently recognized subspecies can be distinguished by standard morphometrics and coloration (RUMMLER & FRITZ 1991; MCCORD & PHILIPPEN 1998), the accurate determination of juveniles still poses serious problems.

Currently, there are four subspecies classified according to morphology and coloration (RHODIN *et al.* 2010). Turtles from Sumatra and Java are considered to belong to *C. a. couro* (Schweigger, 1812). The subspecies *C. a. kamaroma* (Rummler and Fritz 1991) occurs in Borneo, the Malay Peninsula, Cambodia, Laos, Thailand, and Vietnam. Turtles from Myanmar are identified as *C. a. lineata* (McCord and Philippen, 1998) and specimens from the Philippines, Celebes, the Mollucas and Timor are regarded as *C. a. amboinensis* (Riche *in* Daudin, 1801) (RUMMLER & FRITZ 1991; SCHOPPE & DAS 2011). Some authors consider the turtles from Borneo as a distinct form (SCHOPPE 2009). Taxonomy of the Geoemydidae family is still in flux, especially in light of recent molecular genetic studies (e.g. SPINKS *et al.* 2004; STUART & PARHAM 2004; DIESMOS *et al.* 2005; SPINKS & SHAFFER 2006; LE *et al.* 2007; PRASCHAG *et al.* 2006, 2007; FRITZ *et al.* 2008; TIEDEMANN *et al.* 2014), and several species and subspecies have been described as new to science or resurrected from synonymy (e.g. BLANCK *et al.* 2006, PRASCHAG *et al.* 2007, 2009; SPINKS *et al.* 2012; IHLOW *et al.* 2016). A comprehensive genetic study is still lacking for the wide-ranging and polytypic *C. amboinensis*. The situation is further complicated by the frequent hybridization of geoemydid turtles (WINK *et al.* 2001; BUSKIRK *et al.* 2005; STUART & PARHAM 2006; FONG *et al.* 2007; SHI *et al.* 2008; FONG & CHEN 2010) often involving members of the genus *Cuora* (WINK *et al.* 2001; STUART & PARHAM

2004), including *C. amboinensis* (FRITZ & MENDAU 2002; GALGON & FRITZ 2002).

Measuring external morphology using geometric morphometrics (GM) is a practical tool for assessing phenotypic variation of shell shape. This approach is easily applied and yields immediate results, independent from any laboratory work, thus making it highly suitable for taxonomic determination in the field (ZELDITCH *et al.* 2004).

We therefore used GM to analyse the shell shapes of immature *C. amboinensis* box turtles from Borneo and Sumatra. In addition, we used the mitochondrial cytochrome *b* gene to genetically investigate the turtles from these islands, and specimens from other locations, in order to gather more information about these species and to compare the morphological results with the genetic findings.

Materials and Methods

Geometric morphometrics

A total of 195 photographs of *C. amboinensis* were examined (69 turtles from Borneo and 126 from Sumatra) and 132 (69 Borneo, 63 Sumatra) were chosen for further study. These included only immature individuals of unknown sex, with carapace lengths between 70 and 120 mm. Photographs of carapaces or plastra with abnormalities were discarded as well as photos of closed plastra to avoid perspective bias leaving 130 carapaces (68 Borneo, 62 Sumatra) and 98 plastra (69 Borneo, 29 Sumatra) for analysis.

For each turtle, standard dimensions of the shell (carapace length, carapace width, plastron length, plastron width) were measured using a calliper (0.1 mm precision). The digital images of carapace and plastron of each

Table 1. Genetic samples of *Cuora amboinensis* species complex and its closely related species used in this study.

Number of sample	Accession number	Provenance	Taxonomic identification	Source
40		Borneo	*Cuora amboinensis*	this study
41		Borneo	*Cuora amboinensis*	this study
43		Borneo	*Cuora amboinensis*	this study
38		Sumatra	*Cuora amboinensis*	this study
39		Sumatra	*Cuora amboinensis*	this study
55		Sumatra	*Cuora amboinensis*	this study
57		Sumatra	*Cuora amboinensis*	this study
134		Seram	*Cuora amboinensis*	this study
135		Seram	*Cuora amboinensis*	this study
49		unknown	*Cuora amboinensis*	Zoo Prague
50		unknown	*Cuora amboinensis*	Zoo Prague
51		unknown	*Cuora amboinensis*	Zoo Prague
52		unknown	*Cuora amboinensis*	Zoo Prague
53		unknown	*Cuora amboinensis*	Zoo Prague
54		unknown	*Cuora amboinensis*	Zoo Prague
131		unknown	*Cuora amboinensis*	Zoo Ústí nad Labem
132		unknown	*Cuora amboinensis*	Zoo Ústí nad Labem
133		unknown	*Cuora amboinensis*	Zoo Ústí nad Labem
	AY434575	pet trade	*Cuora amboinensis kamaroma*	Spinks *et al.* 2004
	AY434581	pet trade	*Cuora amboinensis couro*	Spinks *et al.* 2004
	AY434580	pet trade	*Cuora amboinensis amboinensis*	Spinks *et al.* 2004
	AY434620	pet trade	*Cuora amboinensis lineata*	Spinks *et al.* 2004
	JN232524	India, Assam	*Cuora amboinensis*	Baruah *et al.* [1]
	AY434570	pet trade	*Cuora flavomarginata sinensis*	Spinks *et al.* 2004
	AY434604	pet trade	*Cuora mouhotii*	Spinks *et al.* 2004
	AY434574	pet trade	*Cuora pani*	Spinks *et al.* 2004
	AY434627	pet trade	*Cuora trifasciata*	Spinks *et al.* 2004

individual were obtained using a digital camera (Canon EOS 30D with Canon 50/1.8 lens) mounted on a tripod. Twenty-one anatomical landmarks of type 1 on plastron and twenty-five landmarks of type 1 and one of type 3 on carapace following the classification of Bookstein (1997) were recorded (Fig. 1.) using TPSdig software (Rohlf 2008). Each set was then symmetrised and one half was removed using the BigFix6 program (Sheets 2003). Statistical examination was performed on half of the landmark sets. We employed the Procrustes superimposition method (Zelditch *et al.* 2004) using the CoordGen6 program (Sheets 2003) to remove the effects of position, orientation and scale, employing sets of x, y coordinates of landmarks from each specimen. We used the standardization on mean carapace length (for each population separately) to remove the size related shell shape differences in the program Standard6 (Sheets 2003). Visualization was performed using CVAGen6 software (Sheets 2003). The vectors of the shell shape ontogeny between turtles from Borneo and Sumatra were compared to the variability of the ontogeny vector inside these two samples using the VecCompare6 program (Sheets 2003) and 400 permutations. When the vector between the samples is bigger than the 95th percentile of the ranges of within-sample angles, we can assume that it is not expected that the samples significantly differ in the shell shape vector of the ontogeny randomly. The correlation between carapace and plastron shape was examined using PLSMaker6 software (Sheets 2003). The partial warp scores for the further statistical analysis were generated using PCAGen6 software (Sheets 2003). The differences in shell shape between turtles from Borneo and Sumatra were tested in the program Statistica 6 (Weiss 2007) using Discriminant Analysis.

DNA samples and mitochondrial DNA sequencing

Nine turtles of known geographical provenance (three from Borneo, four from Sumatra, and two from Seram) were studied genetically. Additionally, nine individuals of unknown geographical provenance from zoological gardens (six samples from Zoological Garden Prague, Czech Republic, three samples from Zoological Garden Ústí nad Labem, Czech Republic) were included in this analysis (Table 1).

For each turtle a claw tip was removed and stored in an Eppendorf tube with 96% ethanol prior to DNA extraction. Total genomic DNA was then isolated using the DNAeasy Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines.

The DNA amplification was performed with the primers suggested by Spinks *et al.* (2004) for a total length of

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[1] Baruah, C., Shanker, K., & Sharma, D.K. (2011): Phylogenetic relationships of Indian freshwater turtles and tortoises based on mitochondrial cytochrome *b* sequences. - unpublished.

1140 bp of cytochrome *b*. The PCR reactions were carried out in 25 µl including 1 µl of each 10 µM primer, 12.5 µl Combi PPP Master Mix (Top-Bio), 1 µl of DNA and 9,5 µl of ddH$_2$O. The PCR protocol started with a denaturation step at 94 ºC for 180 s, followed by 35 cycles composed of denaturation at 94 ºC for 45 s, annealing at 46 ºC for 45 s, and extension at 72 ºC for 1 min and 20 s; and finishing with a further 7 min elongation step at 72 ºC after the last cycle. For some of the samples, the temperature of annealing had to be increased to 50 ºC to obtain usable PCR products. PCR products were purified using a Qiaquick Gel Extraction kit (Qiagen, Hilden, Germany) and directly sequenced in both directions with substantial overlap with the same primers that were used in the PCR reaction. Newly obtained haplotypes of *C. amboinensis* were merged with previously published ones and sequences of additional *Cuora* species and four outgroup species: *Cuora amboinensis* (GenBank accession number: JN232524), *Cuora amboinensis amboinensis* (AY434580), *Cuora amboinensis couro* (AY434581), *Cuora amboinensis kamaroma* (AY434575), *Cuora amboinensis lineata* (AY434620), *Cuora mouhotii* (AY434604), *Cuora flavomarginata sinensis* (AY434570), *Cuora pani* (AY434574) and *Cuora trifasciata* (AY434627).

Chromatograms of newly generated sequence data were manually checked using Chromas Lite 2.01 software (http://www.technelysium.com.au/chromas_lite.html), BioEdit (HALL 1999) and sequences were aligned in the Clustal X 1.81 program (THOMPSON *et al.* 1997).

Phylogenetic analyses

We used our sequence data to construct a bootstrapped maximum likelihood (ML) tree using RAxML software (version 7.2.8-alpha) (STAMATAKIS 2006). The relationship between the subspecies was examined using 1,000 bootstrap replicates and the GTRGAMMA model. The average distances between haplotypes from particular groups were calculated in the mega 7.0.18 program (KUMAR *et al.* 2016) using uncorrected p-distances model.

Results

Geometric morphometrics

We found significant differences in the shape of the carapace (Wilks' Lambda = 0.3764, $F_{(25,104)}$ = 6.8916, p < 0.0001) and plastron (Wilks' Lambda = 0.5815, $F_{(17,80)}$ = 3.3867, p < 0.0001) between the samples from Borneo and Sumatra. In total, 90% of the turtles could be discriminated (97.1% for Borneo, 82.3% Sumatra) by carapacial shape and 83.7% (91.3% for Borneo, 65.5% Sumatra) by plastral shape. The differences are presented in a canonical plot (Fig. 2A) and in a thin plate spline diagram (Fig.

2B) for carapacial shape and in Fig. 3A and B for plastral shape, respectively.

The correlation for carapace length and centroid size (geometric size) differed between the samples from Sumatra and Borneo (Fig. 4). However, we did not find any significant differences with respect to the growth vectors of carapacial (angle between populations 40, angle within Borneo 43.3, angle within Sumatra 26.6) and plastral shape (angle between populations 32, angle within Borneo 45.3, angle within Sumatra 35.3) between the samples from these two islands.

The analysis revealed a weak correlation (0.5597) between carapacial and plastral shape for turtles from Borneo, and a much stronger correlation (0.8458) for turtles from Sumatra.

DNA analysis

We sequenced the mitochondrial cytochrome *b* gene (1140 bp) in 18 individuals and found 14 distinct haplotypes. ML revealed two clearly distinct groups. The first group contained *C. amboinensis* haplotypes from Seram which were deeply divergent from all other haplotypes of *C. amboinensis* (uncorrected p distances 6.00–8.68%). Among the remaining haplotypes, uncorrected p distances ranging from 0.00% to 5.36% were observed. The phylogenetic analyses placed the Seram haplotypes as a sister group of a monophyletic group including the remaining *C. amboinensis* sequences (Fig. 5). The latter group exhibits a clear structure, with sequences from Borneo and Sumatra in distinct parts of the tree. Uncorrected p-distances between haplotypes belonging to the Borneo and Sumatra groups varied within the range of 2.68–4.09% (Table 2.). The haplotypes from the same island were similar (uncorrected p distances: 0.51–1.53% for Borneo and 0.13–0.26% for Sumatra) and formed monophyletic groups (Borneo: bootstrap support 87) and, for the sequences from Sumatra (bootstrap support 98), contained additional sequences of unknown geographic origin. The sister relationship between the "Sumatra clade" and the "132 Zoo Ústí nad Labem and 51 Zoo Prague clade" is moderately supported (bootstrap support 70) with uncorrected p-distances varying within the range of 0.64–1.15%. The relative position of additional sequences from GenBank of *C. amboinensis*, *C. kamaroma*, *C. cuoro* and *C. lineata* in the tree was not resolved because the phylogenetic relationships within this clade were poorly supported.

Discussion

Shell shape variation shows a clear distinctiveness between Borneo and Sumatra populations of *C. amboinensis*, which corresponds with our DNA analyses. Geometric morphometrics therefore provides sufficient
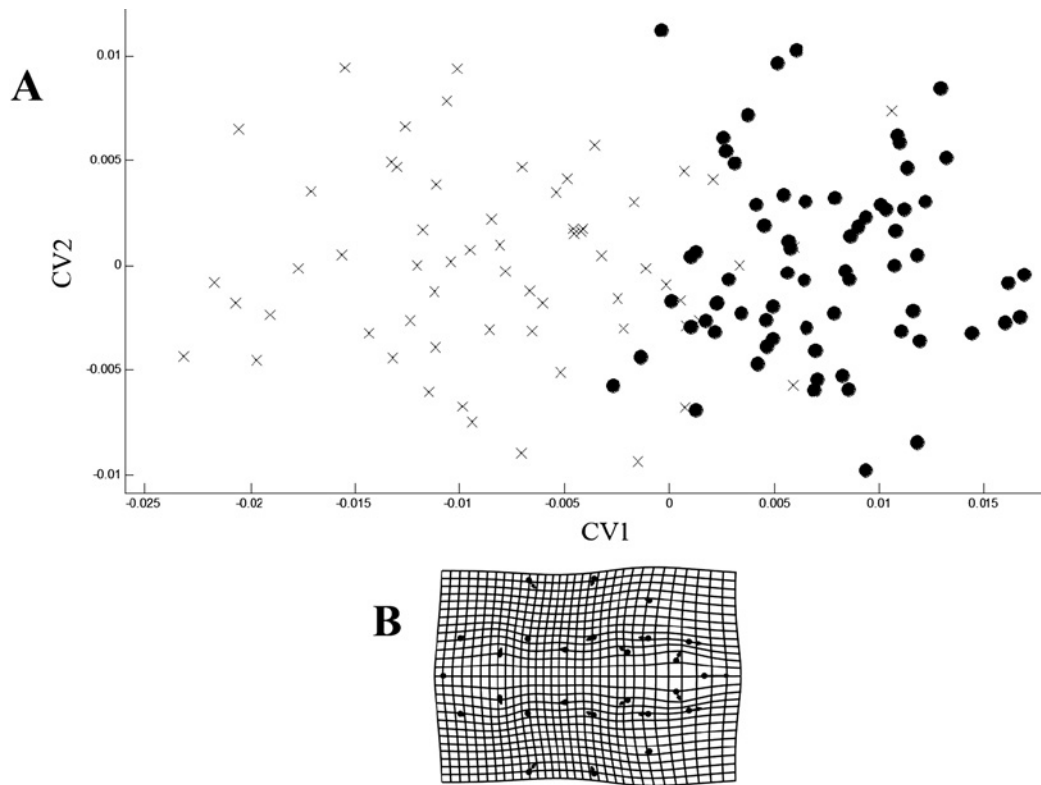
Fig. 2. Canonical plot for carapacial shape. Each carapace is represented by a symbol: × – Sumatra, ● – Borneo (A). The first axis accounts for 79.05% of the total between group variation. The thin plate spline diagram shows a change in carapacial shape along the first axis (in direction of arrows) (B).
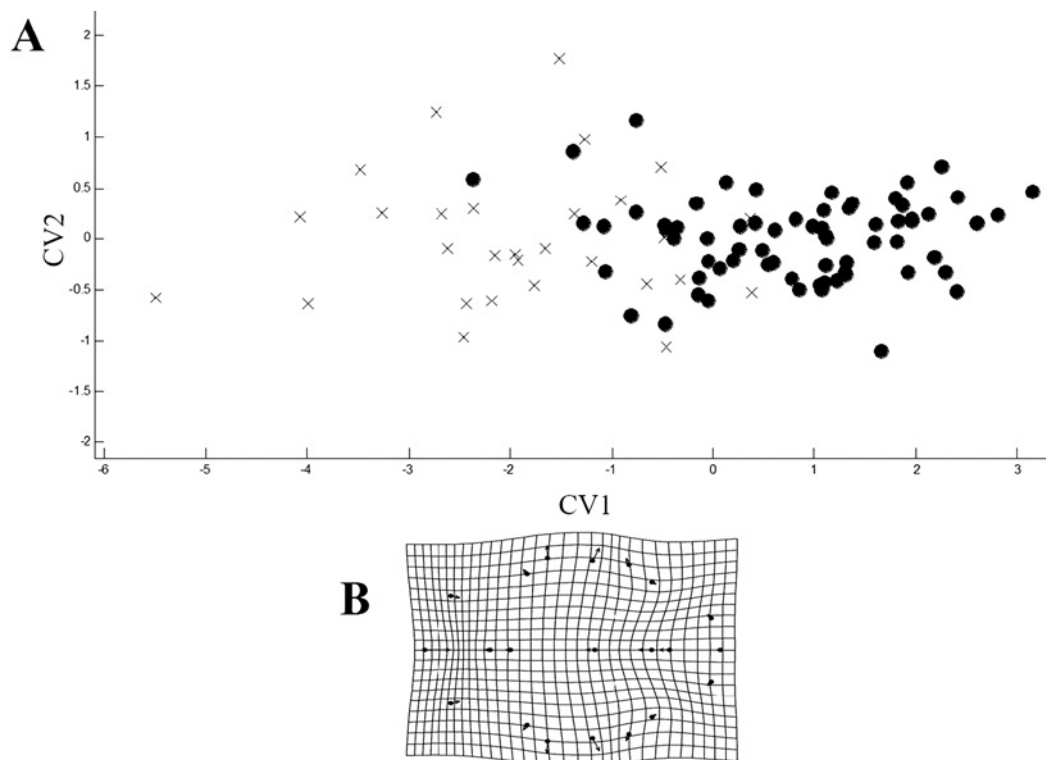

Fig. 3. Canonical plot for plastral shape. Each plastron is represented by a symbol: × – Sumatra, ● – Borneo (A). The first axis accounts for 55.25% of the total between group variation. The thin plate spline diagram shows a change in plastral shape along the first axis (in direction of arrows) (B).
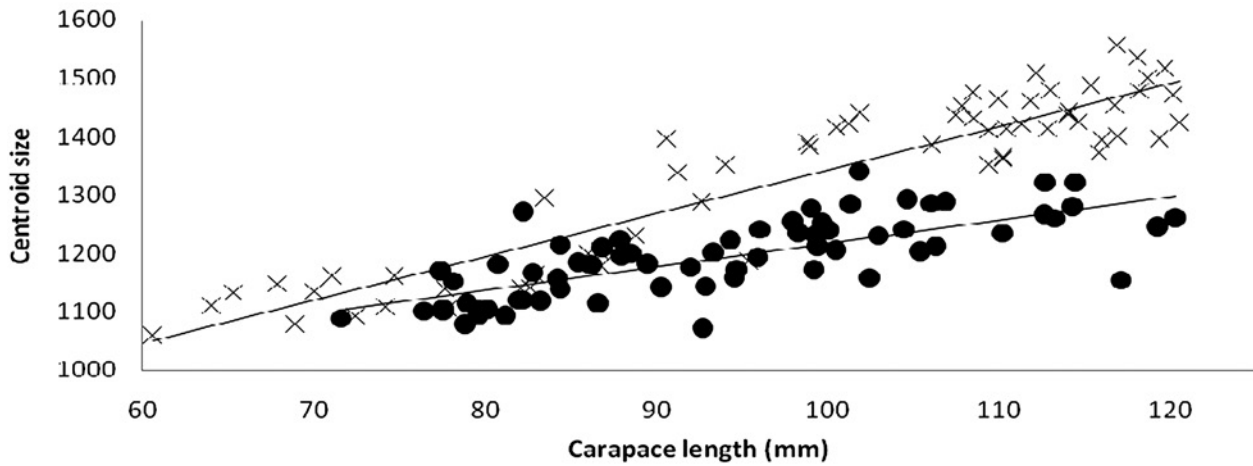
Fig. 4. Correlation of carapacial length and centroid size. × – Sumatra, ● – Borneo
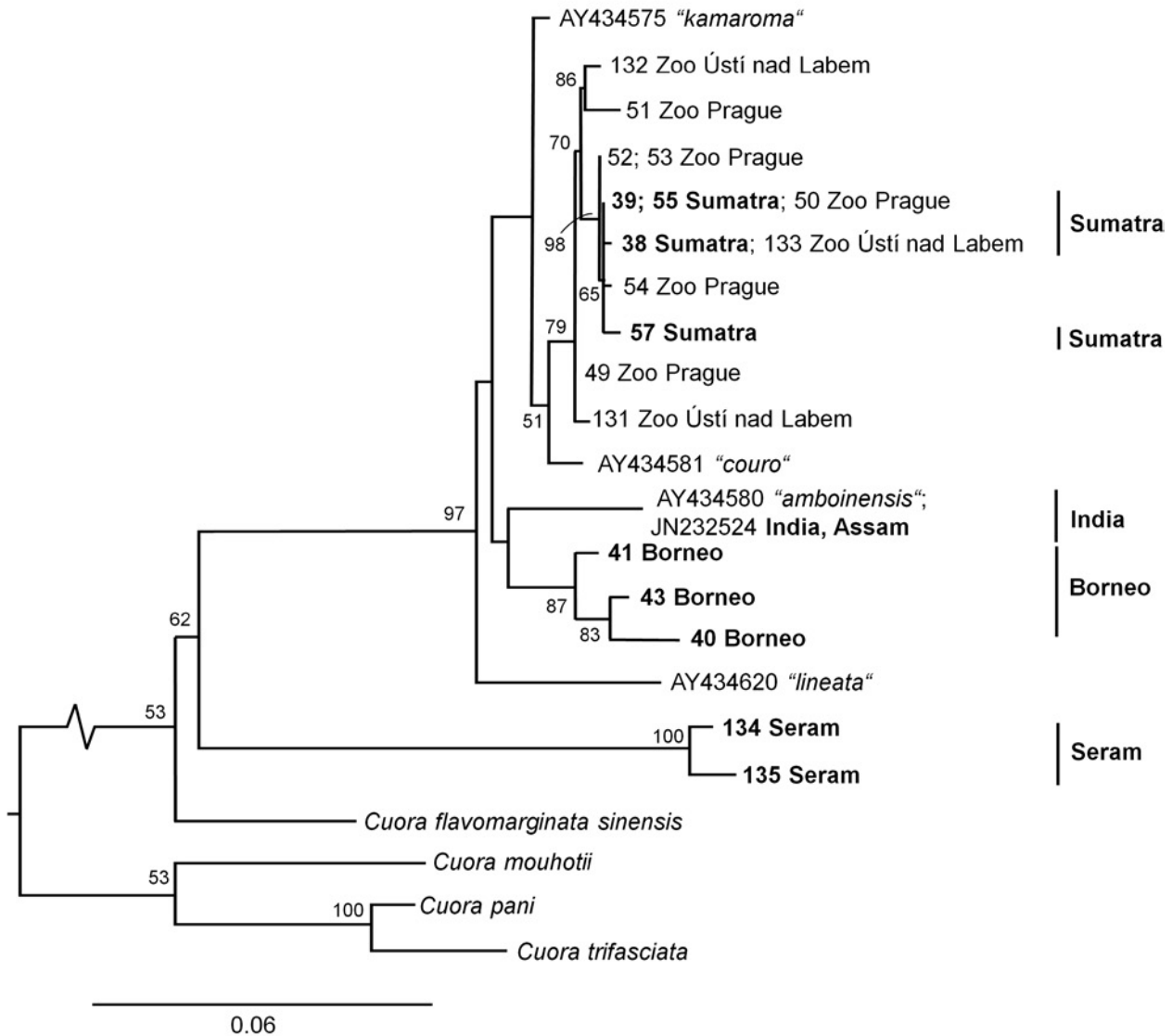

Figure 5. Phylogenetic tree by maximum likelihood (ML) method based on alignment of the complete cytochrome *b* gene (1140 bp). Numbers at branches are bootstrap values > 50. Samples sequenced in this study are labelled with sample numbers and are in bold, remaining samples were sequenced by previous authors and are shown with full taxonomic names or with GenBank accession numbers. For sequences with reliable geographic provenance, the countries are given. Subspecies names in apostrophes are from SPINKS *et al.* (2004). Root length shortened by 75%.

Table 2. Uncorrected p distances (percentages) based on the mitochondrial cytochrome b gene (1140 bp).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	132 Zoo Ústí nad Labem																						
2	51 Zoo Prague	0.64																					
3	52; 53 Zoo Prague	0.51	0.89																				
4	39; 55 Sumatra; 50 Zoo Prague	0.64	1.02	0.13																			
5	38 Sumatra; 133 Zoo Ústí nad Labem	0.77	1.15	0.26	0.13																		
6	54 Zoo Prague	0.64	1.02	0.13	0.13	0.13																	
7	57 Sumatra	0.77	1.15	0.26	0.13	0.26	0.13																
8	49 Zoo Prague	0.38	0.77	0.38	0.51	0.64	0.51	0.64															
9	131 Zoo Ústí nad Labem	0.64	1.02	0.64	0.77	0.89	0.77	0.89	0.26														
10	AY434581 "couro"	1.02	1.15	1.02	1.15	1.28	1.15	1.28	0.64	0.89													
11	AY434575 "kamaroma"	1.40	1.53	1.40	1.53	1.66	1.53	1.66	1.02	1.02	0.89												
12	AY434580 "amboinensis", JN232524 India, Assam	3.58	3.96	3.32	3.45	3.58	3.45	3.32	3.19	3.19	3.45	2.81											
13	41 Borneo	2.68	2.55	2.68	2.81	2.68	2.81	2.68	2.81	2.81	2.43	2.55	3.58										
14	43 Borneo	2.94	2.55	2.94	3.07	2.94	3.07	2.94	3.07	3.07	2.68	2.55	3.58	0.51									
15	40 Borneo	3.96	3.58	3.96	4.09	3.96	4.09	3.96	4.09	4.09	3.70	3.58	4.85	1.53	1.28								
16	AY434620 "lineata"	3.45	3.83	3.45	3.32	3.45	3.32	3.19	3.32	3.32	3.83	3.70	4.47	3.96	4.09	5.36							
17	134 Seram	7.15	7.28	7.41	7.28	7.28	7.28	7.15	7.28	7.28	7.15	7.02	8.17	6.90	7.02	6.00	8.17						
18	135 Seram	7.92	8.05	8.17	8.05	8.05	8.05	7.92	7.79	7.79	7.66	7.54	8.68	7.41	7.54	6.51	8.68	1.02					
19	Cuora flavomarginata sinensis	6.90	7.28	7.15	7.02	6.90	7.02	6.90	7.02	7.02	6.90	6.51	7.02	6.39	6.39	6.39	7.41	6.90	7.28				
20	Cuora mouhotii	6.13	6.13	6.39	6.51	6.39	6.51	6.39	6.00	6.00	6.13	5.49	6.00	6.00	5.62	6.39	7.15	6.39	6.90	5.62			
21	Cuora pani	7.28	7.41	7.54	7.66	7.79	7.66	7.54	7.41	7.41	7.28	6.90	7.41	6.77	6.77	6.90	8.05	7.54	7.92	6.26	5.49		
22	Cuora trifasciata	7.92	8.05	7.92	8.05	7.92	8.05	7.92	7.79	7.79	7.66	7.02	7.79	6.90	6.90	7.02	8.43	7.28	7.66	6.13	4.85	1.66	

geographical specific population recognition according to shell shape. Thus, it is a useful method for accurate turtle classification even in early ontogenetic stages. Similar studies of the other subspecies, populations and ontogenetic stages are needed to map all phenotypic variability in this species. GM can then serve as a simple yet effective practical tool for the determination of specimens of unknown geographical origin, with particular application in fieldwork.

From our genetic analyses, we discovered that the mitochondrial cytochrome *b* sequences of *Cuora amboinensis* turtles from Borneo and Sumatra are clearly distinct, which corresponds with the currently accepted taxonomy of this species (Rummler & Fritz 1991; Rhodin *et al.* 2010), with Bornean turtles being identified with another subspecies (*C. a. kamaroma*) rather than with Sumatran turtles (*C. a. couro*). Yet, in our phylogenetic analyses, previously published sequences identified with these subspecies (Spinks 2004) were distinct from ours, perhaps due to misidentification or geographic variation of these taxa.

A notable finding of our study was that the haplotypes from Seram, geographically corresponding to the subspecies of *C. a. amboinensis*, are highly divergent. However, it was not possible to prove the congruence between morphology and genetic divergences due to the low number of individuals (n=2) for morphometric analysis. We are therefore not yet able to draw taxonomic conclusions for turtles from the Seram provenience. More detailed DNA analyses, including the use of nuclear markers, are needed to clarify the affinities of this population. Moreover, *Cuora amboinensis* could in fact be a species complex with similar patterns as in other turtle species with vast geographical distribution which have been studied recently (Fritz *et al.* 2012; Kindler *et al.* 2012; Kindler *et al.* 2016; Vargas-Ramírez *et al.* 2010; Vargas-Ramírez *et al.* 2013; Petzold *et al.* 2014; Edwards *et al.* 2016).

The shell-shape and genetic differences between Borneo and Sumatra samples are in compliance with the general differences and separation of the majority of these island faunas. The isolation of the Greater Sunda Islands in the Tertiary caused deep genetic divergences between the reptilian fauna (Keogh et al. 2001) and even in mammals (Thinh *et al.* 2010; Nater *et al.* 2011) inhabiting Borneo, Sumatra and the mainland. The similar pattern of distribution in *C. amboinensis* could be the result of a low dispersion ability due to the semiaquatic lifestyle in marshes, ponds and small streams, as seen in the ecologically close genus *Cyclemys* (Fritz *et al.* 2008) and the other *Cuora* species (Spinks & Shaffer 2006) with limited distribution ranges. In contrast, a big river species like the Malaysian giant turtle (*Orlitia borneensis*) with a similar distribution range as *C. amboinensis* could have benefited from geomorphologic conditions during the glacial periods when the Malay Peninsula, Sumatra and Borneo were connected by a system of rivers i.e.the Siam and West Sunda River drainages (Voris 2000). It allowed continuous gene flow between the now isolated areas (Palupcikova *et al.* 2012). The central location of moun-

tains in Borneo may act as an effective natural barrier between the suitable lowland habitats of *C. amboinensis*. A low correlation of the carapace and plastron shape in the Borneo turtles could be a consequence of geographically isolated populations. A similar pattern was observed in the genetic variation found in the Mediterranean turtle (*Mauremys leprosa*) (Fritz *et al*. 2006).

Our results revealing distinct morphological and genetic differences between Sumatra and Borneo box turtles are congruent with the recent findings of Ernst *et al.* (2016), who performed classical morphometric analysis on *C. amboinensis* throughout its distribution range. Their research supported the validity of only two subspecies, *amboinensis* and *kamaroma*. According to these authors, *amboinensis* occupy a range that includes Sumatra, while *kamaroma* turtles inhabit Borneo. Placement of Borneo populations into continental subspecies *C. a. kamaroma* sensu lato is confirmed by the clustering of the examined continental haplotype into the Bornean cluster. Nevertheless, the positions of the additional haplotypes from Genbank in our phylogenetic tree suggest that preliminary taxonomic conclusions made by Ernst *et al.* (2016) need to be confirmed by genetic analyses covering the whole range of the species. In light of our results, the use of the name *C. a. amboinensis* also for Sumatra populations, formerly classified as *C. a. couro*, is especially problematic. Although we analysed just two samples from the Molucca archipelago, where the typical habitat of *C. amboinensis* is found, these haplotypes are strongly different not only from those collected in Sumatra, but also from all other examined ones.

In conclusion, the high identification success of immature *C. amboinensis* specimens based on the phenotypic variation of shell shape using GM clearly demonstrates the usefulness of this method. It could be used on other forms of this species as a practical and effective tool for the determination of *C. amboinensis* of unknown origin thus contributing to the conservation strategies for this taxon as well as benefitting general scientific research.

From our genetic analyses we have discovered not only that the congruence between morphology and genetic distinctness for Borneo and Sumatra box turtles support deep divergent lineages, but moreover, we uncovered previously unknown haplotypes from Seram suggesting that the species status of this population should be reconsidered.

Acknowledgements

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