# Diversity of the Southeast Asian leaf turtle genus *Cyclemys*: how many leaves on its tree of life?

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In the present study, we use mtDNA sequence data (cyt *b* gene) in combination with nuclear DNA sequences (C-mos, Rag2 genes, R35 intron), nuclear genomic fingerprints (ISSR) and morphological data to reveal species diversity within the Southeast Asian leaf turtle genus *Cyclemys*, a morphologically difficult group comprising cryptic species. Two morphologically distinct major groupings exist, a yellow-bellied species group with three taxa (*Cyclemys atripons*, *C. dentata*, *C. pulchristriata*) and a dark-bellied species group. The latter contains besides the morphologically variable *C. oldhamii* three additional new species (*C. enigmatica* n. sp., *C. fusca* n. sp., *C. gemeli* n. sp.). According to mtDNA data, *C. fusca* and *C. gemeli* constitute with high support the sister group of a clade comprising all other species, indicating that the dark-bellied species are not monophyletic, despite morphological similarity. mtDNA sequences of *C. enigmatica*, being highly distinct in nuclear genomic markers, do not differ from the sympatric *C. dentata*, suggesting that the original mitochondrial genome of *C. enigmatica* was lost due to introgressive hybridization. Morphological discrimination of *Cyclemys* species is possible using multivariate methods. However, gross morphology of most dark-bellied species on the one hand and of *C. atripons* and *C. pulchristriata* on the other is so similar that reliable species determination is only possible when genetic markers are used. The high diversity within *Cyclemys* requires revision of the IUCN Red List Categories for leaf turtles because the former assessment was based on the wrong assumption that in the entire range of the genus occurs only a single species.

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## Introduction

Leaf turtles (genus *Cyclemys* Bell, 1834; Geoemydidae) are small to medium-sized semiaquatic terrapins distributed over most of Southeast Asia (Iverson 1992; Fritz *et al.* 1997). Both their scientific and common names (*Cyclemys*; leaf turtles) were coined in allusion to shell shape, resembling in juveniles a serrated, ovoid to roundish leaf. A distinctive genus-diagnostic morphological character of leaf turtles is the ontogenetic development of a plastral hinge, resulting in a secondary division of the abdominal scutes (Fritz *et al.* 1997). While it was long thought that the genus contains only one or two species (Wermuth & Mertens 1977; King & Burke 1989; Iverson 1992), it turned out that it is more speciose (Fritz *et al.* 1997; Iverson & McCord 1997; Guicking *et al.* 2002; Stuart & Fritz 2008). The most recent investigation using mtDNA sequences from historical type specimens suggested that five species exist, one of which is undescribed yet (Stuart & Fritz 2008). Their distribution ranges remain unclear, however. According to morphology and preliminary nuclear fingerprinting data, a sixth species, occurring sympatrically

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with *Cyclemys dentata*, could exist. The only two individuals of this putative sixth species for which mtDNA sequence data are available yielded haplotypes closely resembling *C. dentata* (Guicking *et al.* 2002), a finding suggestive of introgression or hybridization.

The world's chelonian fauna currently faces a unique overkill scenario, caused by massive overexploitation for food and Traditional Chinese Medicine acting in accord with large-scale habitat destruction, and this is especially true for Southeast Asia (van Dijk *et al.* 2000). Leaf turtles are currently placed in the Red List Category 'Lower Risk' by the IUCN (2007) and are not covered by CITES. However, this assessment is based on the assumption that all leaf turtles are conspecific. It is obvious that species with smaller distribution ranges are likely to be more vulnerable which is why an exact understanding of the diversity of *Cyclemys* is in dire need, being the necessary prerequisite for any successful conservation effort.

The current study aims to clarify the taxonomy of leaf turtles and presents a revision of the genus, based on a rangewide sampling. We use for the first time nuclear DNA sequences (C-mos, Rag2 genes, R35 intron) combined with mtDNA sequence data (cyt *b* gene), nuclear genomic fingerprint data and a morphological data set. In addition to samples of fresh specimens, we also use mtDNA data from historical museum specimens to find out whether the very similar mitochondrial haplotypes of *C. dentata* and the above-mentioned putative species (Guicking *et al.* 2002) reflect a geographically widespread and old introgression, or occur only localized, suggestive of recent and sporadic hybridization. Special efforts were undertaken to pinpoint species-specific morphological key characters because previous studies (Guicking *et al.* 2002; Stuart & Fritz 2008) concluded that some *Cyclemys* species are not reliably identifiable solely on the basis of morphology ('cryptospecies'). Within the frame of the present study, we describe three species as new for science.

## Materials and methods

### *Morphology and sampling*

The complete *Cyclemys* holdings of most major European natural history museums were reviewed (BMNH, The Natural History Museum London; MNHG, Muséum d'histoire naturelle, Genève; MNHN, Muséum National d'histoire naturelle, Paris; MTD, Museum für Tierkunde Dresden; NHMW, Naturhistorisches Museum Vienna; RMNH, Nationaal Natuurhistorisch Museum Leiden = Naturalis; SMF, Senckenberg Museum Frankfurt am Main; SMNS, Staatliches Museum für Naturkunde Stuttgart; ZMA, Zoologisch Museum Amsterdam; ZMB, Zoologisches Museum Berlin; ZMH, Zoologisches Museum Hamburg; ZMUC, Zoological Museum Copenhagen; ZSM, Zoologische Staatssammlung München). In addition, selected specimens from the collections of the American Museum of Natural History, New York (AMNH), the California Academy of Sciences, San Francisco (CAS), the Field Museum of Natural History, Chicago (FMNH), the Museum of Comparative Zoology, Cambridge, MA (MCZ), the Museum of Vertebrate Zoology, Berkeley (MVZ), the Oxford University Museum of Natural History (OUMNH), the United States National Museum of Natural History, Smithsonian Institution, Washington D.C. (USNM), and known-locality leaf turtles kept alive by Petr Petras, Prague, and Peter Valentin, Vienna, were examined. Altogether, more than 400 leaf turtles were studied morphologically; notes and photographs were taken to document colouration and pattern. Individuals were considered adult when they could be unambiguously sexed. For the shell of adult specimens landmarks were defined and, based on these landmarks, 71 measurements (Fig. 1) were taken using a calliper (accuracy: 1 mm). For adult turtles, discriminant analyses were calculated with SPSS 7.5 with default parameters. In the discriminant analyses only specimens were included for which complete sets of measurements were available and that could be either morphologically unambiguously assigned to a certain species or, if this was impossible (as in the case of the 'cryptospecies' *C. atripons* and *C. pulchristriata*), when additional genetic data were available allowing species identification. To avoid a bias of sex-specific

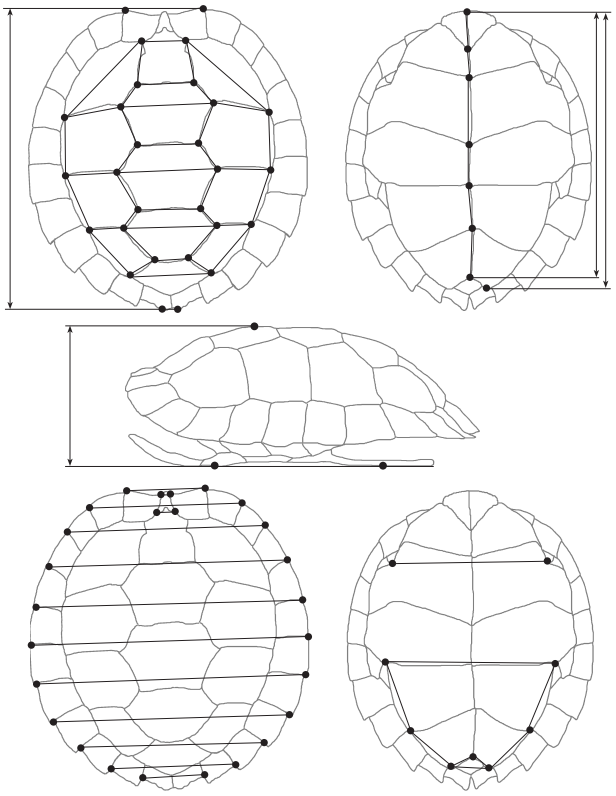

**Fig. 1** Location of landmarks and measurements of the shell as used for discriminant analyses of Asian leaf turtles (*Cyclemys*).

characters, males and females were treated separately. As *Cyclemys* males are much rarer than females, statistics could only be performed for females. Of juvenile and subadult specimens only basic straight line measurements were recorded (carapace length and width, plastron length, shell height). For genetic investigations, alcohol preserved tissue or blood samples were obtained from the CAS, FMNH, MTD, NHMW and USNM collections, including topotypic samples of *Cyclemys dhor shanensis* and *Geoemyda tcheponensis*. In addition, dried muscle tissue of the shells of some crucial historical museum specimens from the NHMW and ZSM collections was sampled (see Appendix I).

***Laboratory procedures***

*DNA-extraction*. Total genomic DNA from fresh samples was extracted by overnight incubation at 55 °C in lysis buffer (6% DTAB, 1.125 M NaCl, 75 mM Tris–HCl, 37.5 mM EDTA, pH 8.0) including 0.5 mg of proteinase K (Merck, Whitehouse Station, NJ), and subsequent purification following the DTAB method (Gustincich *et al*. 1991). DNA was precipitated from the supernatant with 0.2 volumes of 4 M LiCl and 0.8 volumes of isopropanol, centrifuged, washed, dried and resuspended in TE buffer.

Isolation and amplification of mtDNA from historical museum specimens (Appendix I) were performed in ultraviolet-sterilized laminar flow PCR enclosures (HeraSafe KSP9, Thermo, Waltham, MA) in a clean room for ancient DNA. The room and working stations were irradiated with UV light at least 6 h before and after every working step. In this ancient DNA laboratory no *Cyclemys* specimens were studied before, and a negative control containing all reagents but no tissue was always processed. Tissue residues from dried shells of historical museum specimens were washed three times (at intervals of 2, 2 and 12 h) with 1.5 mL of GTE buffer (100 mM glycine, 10 mM Tris–HCl, pH 8.0, 1 mM EDTA) and for 1 min in 100% ethanol, 5 min in 70% ethanol and 10 min in sterile water. Samples were gently vortexed for 5 s after placing into new washing medium. Washed tissues were dried and incubated at 56 °C for 15 h in 300 µL of TNES buffer (10 mM Trizma Base, 100 mM NaCl, 10 mM EDTA, 2% sodium lauryl sulphate {SDS, 39 mM} DTT) and 60 µL of proteinase K (20 mg/mL). The remaining extraction procedure followed the DNeasy Tissue Kit blood and tissue 50 (Qiagen, Venlo, the Netherlands, Cat. No. 69564) protocol for animal tissues, with modifications after Kearney & Stuart (2004).

*Polymerase chain reaction (PCR) and sequencing*. PCR was used to amplify an mtDNA fragment containing approximately 1000 bp of the cyt *b* gene and 40 bp of the adjacent tRNA-Thr gene from the fresh samples listed in Appendix I. The primers mt-A, H-15909, CR12H (Lenk *et al*. 1999), CytbG (Spinks *et al*. 2004), mt-c-For2, mt-a-neu3 and mt-E-Rev2 (Praschag *et al*. 2007) were used. PCR amplification, PCR product purification and sequencing followed either Wink *et al*. (2001) or Praschag *et al*. (2007). None of the sequences contained internal stop codons, and nucleotide frequencies corresponded to those of coding mtDNA; therefore, we conclude to have amplified and sequenced mtDNA and not nuclear copies of mitochondrial genes.

For each historical sample, 11.25 µL of water, 0.25 µL of Ampli *Taq* Gold (Applied Biosystems, Foster City, CA), 2.5 µL of Ampli *Taq* Gold buffer (including $MgCl_2$), 4 µL BSA (10 mg/mL), 1 µL dNTPs (10 mmol/mL), 1 µL of the forward and reverse primers (10 pmol/µL) and 4 µL of DNA template were used for PCR. The negative controls containing all PCR reagents except the DNA template were always included. A total of 340–360 bp of the most informative part of the cyt *b* gene was amplified in three fragments overlapping by 64 and 51 bp after primer sequences were trimmed. This measure ensured that fragments of contaminant DNA were not concatenated into chimeric sequences that might be erroneously judged to be authentic (Olson & Hassanin 2003). The three primer pairs used were the ones from Stuart & Fritz (2008). Initial 5 min denaturing at 95 °C was followed by 39 cycles of 95 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were sequenced directly in both directions using 2 µL Big Dye sequencing buffer (5×), 5 µL water, 1 µL of primer (5 pmol/µL), 1 µL Big Dye and 1–5 µL of the PCR product. The cycle sequencing program and product purification again followed Praschag *et al*. (2007). Sequencing was performed on an ABI 3130 (Applied Biosystems).

Nuclear genomic sequences of the C-mos and Rag2 genes and from an intron from the RNA fingerprint protein 35 (R35) were obtained via PCR amplification using PCR protocols and primers Cmos1, Cmos3, F2 and R2-1 from Le *et al*. (2006), and R35Ex1 and R35Ex2 from Fujita *et al*. (2004); PCR product purification and sequencing followed the same protocols as for mtDNA for fresh samples (Praschag *et al*. 2007).

***Phylogenetic and haplotype network analyses***

Based on the findings of Spinks *et al*. (2004), *Heosemys spinosa* and *Leucocephalon yuwonoi* were used as outgroups. Their cyt *b* sequences were downloaded from GenBank (AY434578, AY434608; Spinks *et al*. 2004) and the C-mos, Rag2 and R35 fragments were sequenced using voucher specimens from the collection of the Museum of Zoology Dresden (*H. spinosa*: MTD D 43910; *L. yuwonoi*: MTD D 45165; accession numbers: AM931602–AM931603, AM931621–AM931622, AM931707–AM931708).

Mitochondrial and nuclear sequences were analysed separately; in the analyses of the cyt *b* gene, the sequences of six historical type specimens from Stuart & Fritz (2008) and four GenBank sequences were included (Appendix I). Data were analysed under the optimality criteria Maximum Parsimony (MP; equal weighting; PAUP* 4.0b10, Swofford 2002, command: hs add = cl) and Maximum Likelihood (ML; GARLI 0.95,

Zwickl 2006; mtDNA settings: genthreshfortopoterm = 10 000 ratematrix = 6 rate statefrequencies = estimate ratehetmodel = gamma numratecats = 4 invariantsites = estimate and nDNA settings identical except for ratehetmodel = none numratecats = 1) as well as with Bayesian inference of phylogeny (BA; MRBAYES 3.1; Ronquist & Huelsenbeck 2003; settings: ngen = 10 000 000 nchains = 4 nrun = 2 sample = 500 temp = 0.2 mcmcdiagn = yes Diagnfreq = 1000 Swapfreq = 1 Nswaps = 1 printfreq = 500 Savebrlens = yes Startingtree = random and the burn-in was set to sample only the plateau of the most likely trees [Correction added on 17 June 2008, after first online publication. The term mcmcdiagn has been corrected]). The best evolutionary model for all four fragments was established by hierarchical likelihood testing using MODELTEST 3.06 (best-fit model after AIC: TVM + I + Γ for mtDNA and TrN + I for nDNA; Posada & Crandall 1998). In BA, the three genes were set to have their own independent evolutionary model with these settings: charset C-mos 1–568; charset Rag2 = 569–1193; charset R35 = 1194–2255; lset applyto = (1, 2) nst = 6; lset applyto = (3) nst = 6 rates = propinv. Under parsimony, 705 of 984 aligned sites of cyt *b* were constant and 188 characters were variable and parsimony-informative; 91 variable characters were parsimony-uninformative. For the ingroup taxa, 781 characters were constant, 170 variable characters were parsimony-informative, and 33 variable characters were parsimony-uninformative. The consensus of 1 210 894 equally most parsimonious trees (tree length = 476; CI = 0.6744, RI = 0.9477) showed that these trees differed only by the position of individuals on short terminal branches. The alignment of the three nuclear fragments comprised 2255 sites of which 2186 were constant; of 69 variable sites were 24 parsimony-informative. For the ingroup taxa, 11 variable characters were parsimony-informative and six were singletons; five sites were heterozygotic and coded as mixed bases. Because invariant sites have little effect on bootstrap support under parsimony (Felsenstein 2004), MP bootstrap values were calculated with data sets that had all constant sites removed; ML bootstrap values were calculated with complete data sets (MP: nreps = 1000 maxtre = 1000 for mtDNA and nreps = 10 000 with no maxtre setting for nDNA; ML: bootstrapreps = 100 genthreshfortopoterm = 5000).

For mtDNA sequences of *C. dentata* and of the morphologically distinctive turtles clustering with *C. dentata*, a parsimony haplotype network using TCS 1.21 (Clement *et al.* 2000) was also constructed. The alignment for the network comprised 684 bp, offering the best compromise between a maximum of informative sites and minimizing ambiguities caused by short sequences of historical museum specimens.

*Inter-Simple-Sequence-Repeats (ISSR).* ISSR is a powerful fingerprinting technique for species-level investigations. It employs a single PCR primer, binding to di- or trinucleotide repeat motifs (microsatellites) that are abundant in eukaryotic genomes (Tautz & Renz 1984; Condit & Hubbell 1991). Since sequences of microsatellites are conserved over a wide range of organisms, universal primers can be applied. Amplified regions correspond to the nucleotide sequence between two inverted simple sequence repeat (SSR) priming sites (Wolfe *et al.* 1998; Bornet & Branchard 2001). SSR regions appear to be scattered evenly throughout the genome (Tautz & Renz 1984; Condit & Hubbell 1991), yielding a large number of polymorphic bands. ISSR markers are inherited in a dominant or co-dominant Mendelian fashion (Gupta *et al.* 1994; Tsumura *et al.* 1996) and are interpreted as dominant markers, scored as diallelic with 'band present' or 'band absent'. The absence of a band is interpreted as primer divergence or loss of a locus through the deletion of the SSR site or chromosomal rearrangement (Wolfe & Liston 1998; Wolfe *et al.* 1998). ISSR fingerprints are usually diagnostic for species-level taxa (e.g. Gupta *et al.* 1994; Zietkiewicz *et al.* 1994; Bornet & Branchard 2001; Fritz *et al.* 2005a,b) and hybrids share with both parental taxa diagnostic bands, while distinct taxa typically possess unique bands. Thus, limited or non-existing gene flow is reflected by distinct banding patterns for reproductively isolated taxa, while shared bands that are otherwise diagnostic for parental taxa are indicative for gene flow (Wolfe *et al.* 1998; Wink *et al.* 2001; Nagy *et al.* 2003; Fritz *et al.* 2005a).

In this study, the primers $(GACA)_4$, $(GATA)_4$ or $(CA)_{10}$ were applied for PCRs using samples of five of the six terminal clades as revealed by phylogenetic analyses of mtDNA sequences plus the morphologically distinctive leaf turtles with mtDNA haplotypes resembling *C. dentata* (Appendix I). The sixth terminal clade, represented by only one sample (NHMW 37153), could not be studied because this sample was used up for mtDNA sequencing.

Each PCR was performed in a volume of 25 μL; 25 ng of total DNA served as template for amplifications and 6 pmol PCR primer, 1.5 mM $MgCl_2$, 0.1 mM of dGTP, dCTP, and dTTP, 0.075 mM dATP, 1 μCi $^{33}$P- α-dATP, 2.5 μL 10× amplification buffer and 0.8 units *Taq* polymerase (Amersham, Uppsala, Sweden) were added. After an initial denaturation (2 min at 94 °C), 33 cycles of 60 s at 94 °C, 120 s at 55 °C and 120 s at 72 °C were performed on a thermocycler (Biometra, Göttingen, Germany). After 33 cycles the reaction temperature was maintained at 72 °C for 4 min and then lowered to 4 °C for further storage. PCR products were separated electrophoretically on a denaturing Sequagel matrix (length 40 cm) at 65 W for 3.5 h. After drying, the gel was exposed to an X-ray film (Hyperfilm-MP, Amersham) for 2–6 days, and developed (X-ray developer and fixer, Kodak, Rochester, NY).

The three PCR primers yielded together 45 unambiguously scorable bands that were transferred in a presence–absence matrix (1/0; Appendix II). Using this matrix, cluster analyses (Neighbor-Joining = NJ trees) were calculated as implemented

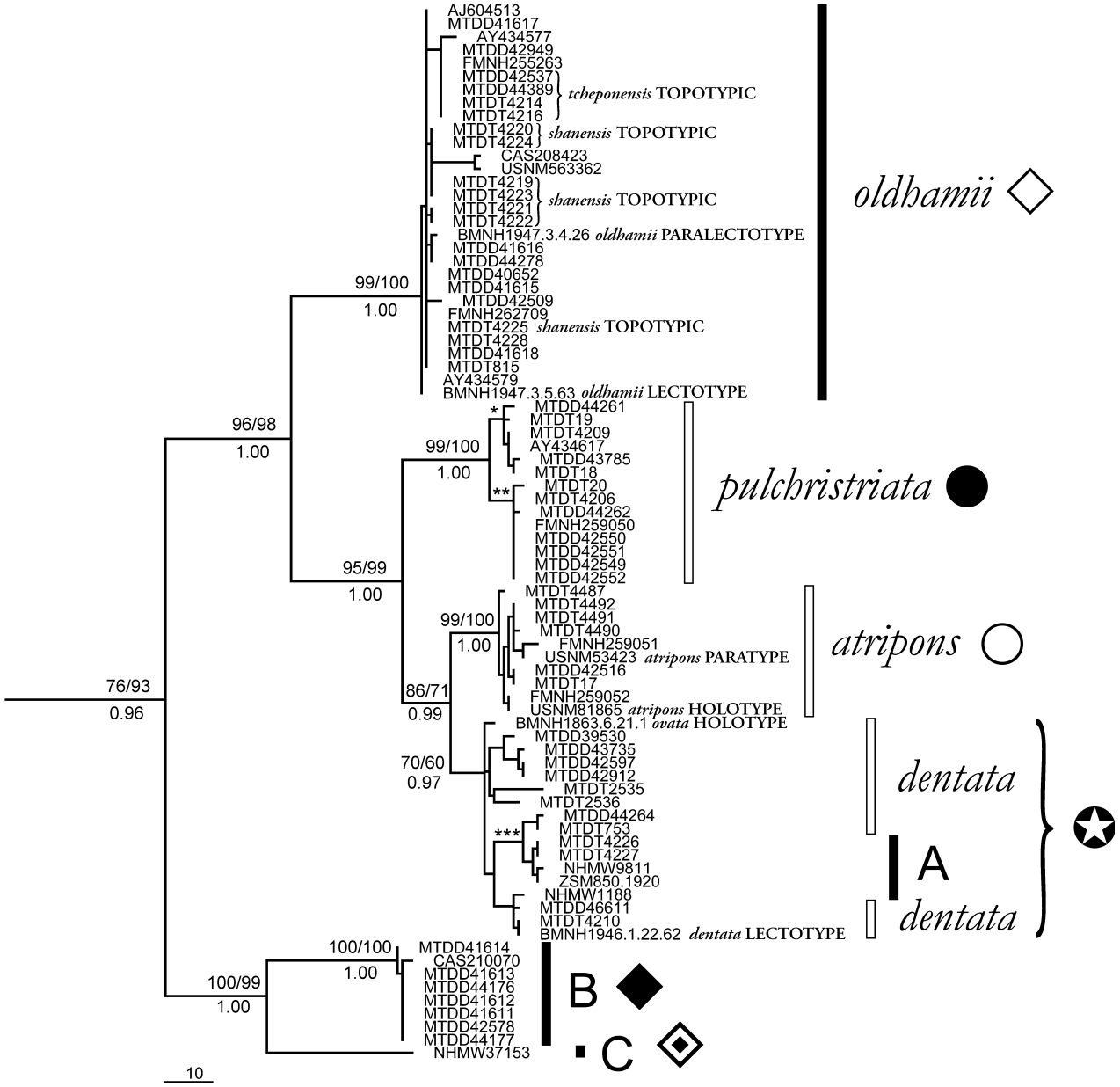


**Fig. 2** Maximum Likelihood phylogram based on a 984-bp fragment of the mitochondrial cyt *b* gene of Asian leaf turtles (*Cyclemys*). Maximum Parsimony and Bayesian analysis recovered the same general phylogenetic hypothesis (differences occurred only in the position of short terminal branches). Outgroups (*Heosemys spinosa*, *Leucocephalon yuwonoi*) removed for clarity. Support values above crucial nodes are ML and MP bootstraps (100 and 1000 replicates); below nodes, Bayesian posterior probabilities (*92/94/0.99; **97/100/1.00; ***64/74/1.00). Species names according to Stuart & Fritz (2008) are indicated on the right; letters designate additional putative species. Black bars indicate dark plastral colouration; white bars, mainly yellow plastron colouration. Symbols stand for terminal mtDNA clades. Note the dark-bellied *Cyclemys* (A) embedded within *C. dentata*. For sample codes and accession numbers, see Appendix I.

in PAUP* 4.0b10 (Swofford 2002). In addition, NJ trees were calculated with TREECON (van de Peer & de Wachter 1994) using Nei and Li distances (Nei & Li 1979) instead of pairwise distances; the robustness of the obtained branching patterns was tested using the bootstrap (2000 or 500 replicates, respectively; Felsenstein 1985).

## Results

### *Phylogenetic analyses*

*mtDNA data.* The monophyly of *Cyclemys* is moderately to well-supported, depending on the tree-building method (bootstrap ML: 76, MP: 93; posterior probability BA: 0.96; Fig. 2). Within *Cyclemys*, all phylogenetic analyses revealed

the same general topology and yielded in most cases well-supported major branching patterns (ML/MP bootstrap and BA posterior probability values of at least 86/98 and 0.99 for most species-level and more basal branches; for weaker support values see below). The most basal split separates sequences from the north-westernmost part of the range of *Cyclemys* (Assam, India; northern and central Myanmar) from all other leaf turtles that occur in a considerably structured clade. On the terminal level six clades occur, five of which correspond with the species as delineated by Stuart & Fritz (2008). These six clades differ by uncorrected average *p* distances of 2.82% (*C. atripons* and *C. dentata*) to 11.70% (*C. atripons* and the sequence NHMW 37153 from Assam; Table 1).

The mentioned considerably structured basal clade contains sequences of the dark-bellied *C. oldhamii* and the three *Cyclemys* species with yellow or mainly yellow plastra, *C. atripons*, *C. dentata* and *C. pulchristriata*. Nested among *C. dentata* are sequences from the Malay Peninsula (MTD T 4226–4227), Sumatra (NHMW 9811), Java (NHMW 1188) and Borneo (ZSM 850.1920) that belong to dark-bellied terrapins (A in Fig. 2); monophyly of this mixed clade with respect to plastral colouration is only weakly supported by the bootstrap (60) under parsimony; ML and BA yielded somewhat higher support values of 70 and 0.97, respectively. In the parsimony network the haplotypes of these dark-bellied *Cyclemys* are embedded within *C. dentata* (Fig. 3). *Cyclemys atripons* and *C. dentata* are sister species in all phylogenetic analyses; *C. pulchristriata* is sister of *C. atripons* + *C. dentata*. However, monophyly of the *C. atripons* + *C. dentata* clade is only moderately supported by the bootstrap under parsimony (71). Within *C. pulchristriata* two well-supported clades occur, suggestive of intraspecific variation. *Cyclemys oldhamii* is the sister taxon of *C. pulchristriata* + (*C. atripons* + *C. dentata*).

Sister to *C. oldhamii* + (*C. pulchristriata* + (*C. atripons* + *C. dentata*)) is the second basal clade comprising sequences from the north-westernmost part of the range of *Cyclemys* (Assam, India; northern and central Myanmar; dark-bellied turtles). Within this Assam-Myanmar clade all sequences from Myanmar (B in Fig. 2) are suggested as sister group of one highly distinct sequence from Assam (NHMW 37153; C in Fig. 2). This Assamese sequence differs by 6.00% sequence divergence (uncorrected *p* distance) from the samples from Myanmar and by 9.94–11.70% from other *Cyclemys* species. These high values suggest that NHMW 37153 represents a distinct species, in particular when the within species divergences of the other *Cyclemys* species are considered (average values of 0.08–1.42%; Table 1).

*Nuclear DNA data.* Phylogenetic trees were computed for two data sets. First, for a data set including an alignment consisting of concatenated sequences of the two nuclear genes

**Table 1** Uncorrected pairwise distances (mean, minimum, maximum; percentages) of the mitochondrial cyt *b* gene within and between *Cyclemys* species, *Heosemys spinosa* and *Leucocephalon yuwonoi*. *Cyclemys* species in same order as in Fig. 2. On the diagonal the within species divergences are given in bold.

| | *oldhamii* | *pulchristriata* | *atripons* | *dentata* | A | B | C | *spinosa* | *yuwonoi* |
|---|---|---|---|---|---|---|---|---|---|
| *Cyclemys oldhamii* | **0.39 (0–2.26)** | | | | | | | | |
| *Cyclemys pulchristriata* | 7.47 (6.72–9.09) | **0.60 (0–1.24)** | | | | | | | |
| *Cyclemys atripons* | 7.62 (6.81–9.54) | 4.92 (4.17–6.52) | **0.39 (0–1.27)** | | | | | | |
| *Cyclemys dentata* | 7.11 (6.30–9.41) | 4.74 (4.27–6.94) | 2.82 (2.27–4.42) | **1.42 (0–2.56)** | | | | | |
| A | 7.08 (6.21–8.50) | 4.88 (4.12–6.57) | 3.47 (2.75–4.74) | 1.47 (0.56–2.35) | **0.91 (0–2.00)** | | | | |
| B | 9.91 (9.45–11.88) | 10.34 (10.06–10.52) | 10.77 (10.03–12.56) | 10.36 (9.41–12.54) | 10.61 (9.22–12.00) | **0.08 (0–0.31)** | | | |
| C | 9.94 (9.52–10.95) | 10.25 (10.10–10.40) | 11.70 (11.19–13.35) | 10.81 (10.10–11.94) | 11.31 (10.11–12.58) | 6.00 (5.87–6.19) | — | | |
| *Heosemys spinosa* | 12.26 (11.58–12.70) | 13.01 (12.72–13.19) | 13.21 (12.82–14.14) | 13.07 (12.60–14.38) | 13.49 (12.31–14.65) | 12.51 (12.46–12.60) | 12.46 | — | |
| *Leucocephalon yuwonoi* | 13.05 (12.65–14.41) | 13.39 (13.21–13.52) | 13.34 (13.01–14.45) | 13.44 (13.01–14.64) | 14.03 (13.13–15.20) | 13.44 (13.29–13.52) | 12.77 | 13.72 | — |

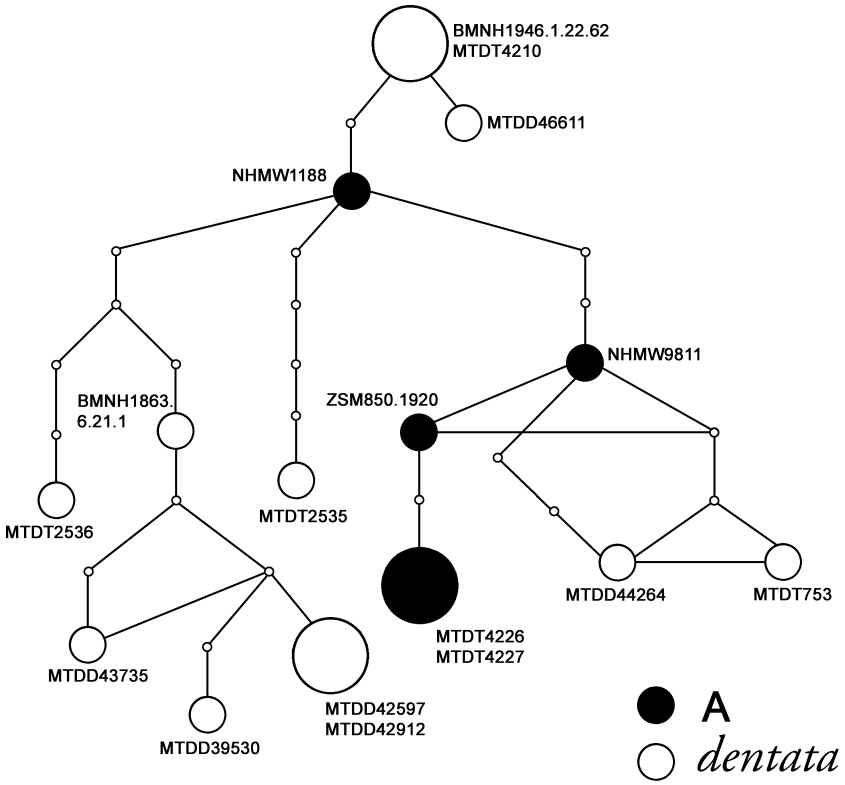


**Fig. 3** Parsimony network for mtDNA haplotypes of *Cyclemys dentata* and sympatric dark-bellied *Cyclemys* (A) based on a 684-bp long alignment (cyt *b* fragment). The haplotype with the biggest outgroup probability of 0.1667 on the top. Symbol size corresponds to haplotype frequency; for sample codes see Appendix I. Missing haplotypes are represented by small circles. Each line between symbols represents one mutation step.

(C-mos: 568 bp, Rag2: 625 bp) and the R35 intron (1062 bp) for two representatives of all terminal clades revealed by analysis of mtDNA except clade C (only sample used up); and second, for an expanded data set including some additional concatenated sequences of two loci for the following species, the lacking third locus being replaced by Ns: *C. atripons* (C-mos + Rag2 of FMNH 259051), *C. dentata* (Rag2 of MTD T 42597 + R35 of MTD T 42912), *C. pulchristriata* (C-mos + Rag2 of MTD T 4206), and *C. oldhamii* (C-mos + Rag2 of FMNH 255263). Using the expanded data set (not shown), the topologies of the BA and ML trees remained unchanged but most support values for the branching patterns were lower. Using parsimony, 60 equally parsimonious trees (73 steps; CI = 0.9589, RI = 0.9375) were found instead of three (72 steps; CI = 0.9722, RI = 0.9444) with the smaller but complete data set, and the resolution of the strict and majority rule consensus trees was much worse.

The monophyly of *Cyclemys* is well-supported by the nuclear genomic data. Using the smaller data set, the branching patterns of the BA and ML trees and the MP 50% majority rule consensus are identical (Fig. 4). The MP strict consensus tree places the sample CAS 208423 in the basal polytomy of *Cyclemys*, however, while this sample is otherwise suggested as sister of *C. oldhamii* (MTD T 4219, MTD T 4223). In agreement with the mtDNA trees, the three yellow-bellied species *C. atripons*, *C. dentata* and *C. pulchristriata* are monophyletic. However, while the mtDNA trees suggest *C. atripons* + *C. dentata* as sister species, the nuclear genes favour a weakly supported sister group relationship of the morphologically very similar *C. atripons* + *C. pulchristriata*. Another difference to the mitochondrial data set is the position of the dark-bellied *Cyclemys* with mtDNA haplotypes resembling *C. dentata* (A in Fig. 4) that are consistently placed in a weakly supported clade with the dark-bellied *Cyclemys* from northern and central Myanmar (B in Fig. 4).

### *ISSR*

The branching patterns of both NJ trees were congruent; the tree obtained with PAUP serves as example (Fig. 5). With the two exceptions detailed below, the clusters correspond perfectly with the terminal mtDNA clades revealed by phylogenetic analyses (Fig. 2).

The first exception refers to two samples (MTD T 4226–4227) of dark-bellied *Cyclemys* with haplotypes embedded within *C. dentata*. These samples are highly distinct from *C. dentata* and all other species, and occur in a long branch with 100% bootstrap support. The underlying fingerprints of both samples have six unique bands that are absent in all other samples (Appendix II: fragments G, H, I, P, S, AF). These distinctive bands, not occurring in any other *Cyclemys* species, rule out that MTD T 4226–4227 could represent hybrids.

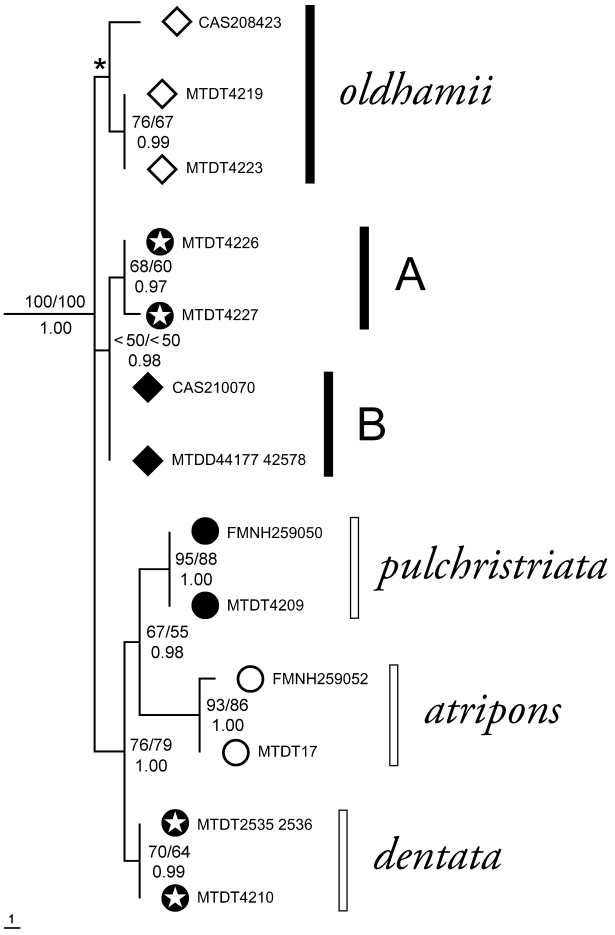


**Fig. 4** Maximum Likelihood phylogram based on three nuclear DNA fragments (C-mos, Rag2, R35; 2255 bp) of Asian leaf turtles (*Cyclemys*). Outgroups (*Heosemys spinosa*, *Leucocephalon yuwonoi*) removed for clarity. Bayesian analysis recovered the same branching pattern; the asterisk indicates the only difference of the Maximum Parsimony strict consensus tree (branch not found). Support values above nodes are ML and MP bootstraps (100 and 10 000 replicates) greater than 50 except one case where lower values are shown; below nodes, Bayesian posterior probabilities. Black bars indicate dark-bellied leaf turtles; white bars, yellow-bellied turtles. Symbols preceding sample codes, mtDNA clades; for further explanation, see Fig. 2.

The second difference is the position of a sample of a juvenile turtle from Bago Yoma, Myanmar (CAS 208423; large arrow in Fig. 5), whose mtDNA and nDNA sequences suggest identity with *C. oldhamii*. In the ISSR trees it is located basally to the leaf turtles from northern and central Myanmar being highly distinct in mtDNA. The geographical origin of CAS 208423 as well as its position in the ISSR tree and its ISSR banding pattern could indicate a hybrid origin. Two bands present in the leaf turtles from northern and central Myanmar (Appendix II: fragments AB, AC) occur in CAS 208423 together with a band present in all *C. oldhamii* samples (fragment AG); AB is a unique band occurring only in turtles from northern and central Myanmar.

**Table 2** Parameters of the discriminant analysis for 84 adult leaf turtle females; non-standardized discriminant coefficients available upon request.

| Test of function | Wilk's λ | $\chi^2$ | df | *P* |
|---|---|---|---|---|
| 1–5 | 0.000 | 541.134 | 355 | 0.000 |
| 2–5 | 0.000 | 375.248 | 264 | 0.000 |
| 3–5 | 0.005 | 254.962 | 195 | 0.003 |
| 4–5 | 0.044 | 148.466 | 128 | 0.104 |
| 5 | 0.229 | 70.062 | 63 | 0.253 |

| Function | Eigen value | % Variance | Cumulative % | Canonical correlation |
|---|---|---|---|---|
| 1 | 31.862 | 53.6 | 53.6 | 0.985 |
| 2 | 11.583 | 19.5 | 73.1 | 0.959 |
| 3 | 8.412 | 14.2 | 87.2 | 0.945 |
| 4 | 4.201 | 7.1 | 94.3 | 0.899 |
| 5 | 3.371 | 5.7 | 100.0 | 0.878 |

***Morphology***

Discriminant analyses were run for 84 adult leaf turtle females (6 *C. atripons*, 20 *C. dentata*, 24 *C. oldhamii*, 7 *C. pulchristriata*, 16 species A, 11 species B) represented by 71 measurements each (Fig. 6; Table 2). Species C could not be included because not all measurements were available for the only genetically verified specimen.

One-hundred percent of the leaf turtles were correctly reclassified to their *a priori* groups. Stepwise discriminant analysis revealed that seven variables allow a correct reclassification of 75% of the turtles (66% when cross-validated). The most important morphological character contributing to group discrimination is the mid-seam length of the femoral scutes, a character already stressed by Fritz *et al.* (1997) using univariate statistics, followed successively by the length of the femoral scute rim, the seam length between the 1st and 2nd vertebral scute, the seam length between the 3rd and 4th costal scute, the length of the anal scute rim, the carapace width measured at the seam between the 7th and 8th marginal scute, and the length of the contact of the 4th costal and the marginal scutes. These characters refer to the shape of the carapace and of the plastral hind lobe.

The same perfect group discrimination of 100% correct reclassification was achieved when only yellow- or dark-bellied species were processed. A stepwise analysis of only the data sets of *C. atripons* and *C. pulchristriata* showed that two characters, the length of the anal scute rim and the seam length between the 3rd and 4th costal scutes, were the most important characters for group discrimination in these two species (Wilk's λ: 0.032, $\chi^2$: 20.688, df = 12, *P* = 0.055; Eigen value: 30.347, % variance: 100, canonical correlation: 0.055).

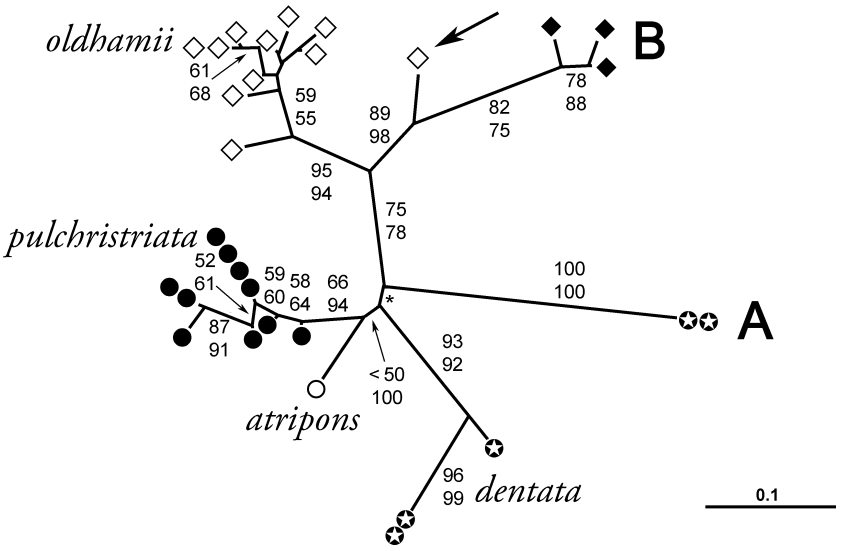


**Fig. 5** Unrooted Neighbor-Joining tree based on ISSR fingerprints of *Cyclemys* using pairwise evolutionary distances. For sample codes, see Appendix I. Symbols indicate mtDNA clades (see Fig. 2); large arrow, putative hybrid; numbers at branches, bootstrap values for pairwise evolutionary distances (2000 replicates, top) and Nei and Li distances (500 replicates, bottom); asterisk, bootstrap support values of < 50 and 60.

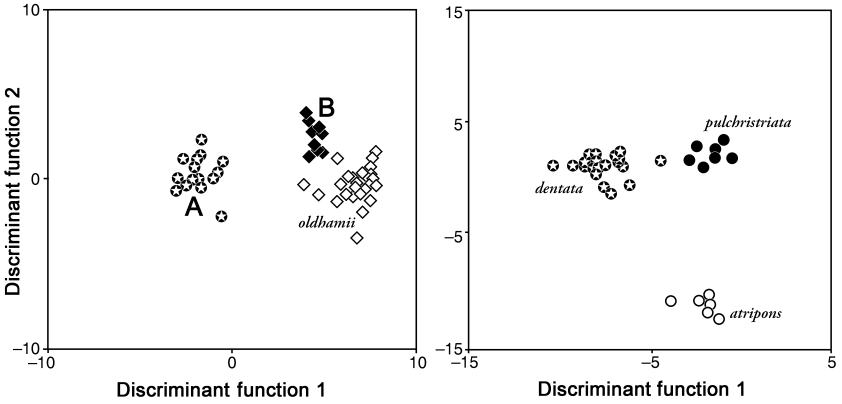


**Fig. 6** Scatter diagrams for the canonical discriminant function of Asian leaf turtles (6 *Cyclemys atripons*, 20 *C. dentata*, 24 *C. oldhamii*, 7 *C. pulchristriata*, 16 species A, 11 species B) based on 71 shell measurements. For clarity, dark-bellied and yellow-bellied species shown in two diagrams. Symbols correspond to mtDNA clades (see Fig. 2).

Using only these two characters, 100% of the *C. atripons* and *C. pulchristriata* were correctly regrouped. A comparison of quotients of the maximum plastral length/average anal rim length and of the carapace length/average seam length between the 3rd and 4th costal scutes using *t*-tests revealed no significant differences, however, suggesting that the discriminating power of these characters might be due to small sample size and random individual differences.

Using colouration and pattern, yellow- and dark-bellied species are highly distinct. Furthermore, *C. dentata* is easily differentiated from the other two yellow-bellied species (Table 3). The comparison of gross morphology of genetically confirmed *C. atripons* and *C. pulchristriata* revealed that they slightly differ in shell patterning. The radiating pattern of *C. atripons* is, especially on the plastron, finer and the dark lines tend to be longer than in *C. pulchristriata* (Figs 7 and 8). However, distinction of the two species using these characters is difficult. Moreover, we also studied some unpatterned museum specimens for which no genetic data were available, indicating that these characters are not helpful at all in such cases. Among the dark-bellied *Cyclemys* species, *C. oldhamii* is easily identified by its mottled crown of the head (see colour photo in Fritz & Ziegler 1999). All other dark-bellied species have a uniformly coloured top of the head. However, it needs to be mentioned parenthetically that in stuffed old museum specimens of *C. oldhamii* the soft part colouration may fade (e.g. in BMNH 1947.3.4.26, paralectotype of *C. oldhamii*).

Size differences are not much pronounced between the different species although *C. dentata* seems to be somewhat smaller than the other species (Table 3). Despite *C. dentata* is one of the most common *Cyclemys* species in museum collections, we never measured a specimen exceeding 210 mm carapace length, while we studied many larger individuals of other, in part much rarer species.

In summary, dark-bellied *Cyclemys* species can be reliably distinguished from yellow-bellied species using colouration and pattern; within the yellow-bellied species, determination of *C. dentata* is also reliably possible, while distinction of

**Table 3** Gross morphology, maximum size, colouration and pattern of Asian leaf turtle species. Throat colouration of hatchlings matches adults.

| Character | *Cyclemys atripons* | *Cyclemys dentata* | *Cyclemys pulchristriata* | A = *Cyclemys enigmatica* | B = *Cyclemys fusca* | C = *Cyclemys gemeli* | *Cyclemys oldhamii* |
|---|---|---|---|---|---|---|---|
| Hatchling | | | | | | | |
| Head and neck stripes | Present, wide | Present, narrow | Present, wide | Absent | ? | ? | Present, narrow |
| Prevalent plastral colour | Yellow | Brownish | Yellow | Brownish | ? | ? | Brownish |
| Plastral pattern | Few large dark specks, ocellate pattern along submarginal seams | Mottled with small dark specks | Few large dark specks, ocellate pattern along submarginal seams | Mottled with small dark specks | ? | ? | Large dark central plastral figure, ocellate pattern along submarginal seams |
| Adult | | | | | | | |
| Carapace length (maximum; mm) | 236 | 210 | 227 | 235 | 242 | 231 | 254 |
| Shell outline | Ovoid to elongated | Ovoid | Ovoid to elongated | Ovoid | Ovoid | Elongated to rectangular | Rectangular |
| Femoral mid-seam | ≤ anal mid-seam | < anal mid-seam | ≤ anal mid-seam | ≥ anal mid-seam | ≥ anal mid-seam | ≥ anal mid-seam | ≥ anal mid-seam |
| Anal notch | Narrow to wide, acute-angled to obtuse-angled | Narrow, acute-angled | Narrow to wide, acute-angled to obtuse-angled | Wide, obtuse-angled | Wide, obtuse-angled | Wide, obtuse-angled | Wide, obtuse-angled |
| Carapacial primary colour | Chestnut | Dark brown | Chestnut | Dark brown, often with reddish tinge | Dark brown | Dark brown | Dark brown |
| Carapacial pattern (if present) | Fine radiating black lines or lacking | Fine radiating black lines or lacking | Wide radiating black lines or stout black specks | Usually lacking in older individuals | Usually lacking in older individuals | Lacking in older individuals | Usually lacking in older individuals |
| Prevalent plastral colour | Yellow | Yellow | Yellow | Dark brown to black | Dark brown to black | Dark brown | Dark brown to black |
| Plastral pattern | Fine radiating black lines or lacking | Fine radiating black lines or lacking | Radiating pattern of short and robust lines or specks or lacking | Dense black radiating lines or lacking | Dense black radiating lines or lacking | Lacking | Dense black radiating lines or lacking |
| Bridge | Yellow with black radiating lines or entirely black | Entirely yellow or with black radiating lines | Entirely yellow or with black radiating lines | Dark brown to black | Dark brown to black | Dark brown | Dark brown to black |
| Crown of head | Speckled | Speckled | Speckled | Uniform copper to brownish, lighter than temporal region | Uniform greenish yellow, lighter than temporal region | Uniform brown, not lighter than temporal region | Speckled |
| Temporal region and neck | Distinctly striped | Distinctly striped | Distinctly striped | Uniform dark | Uniform dark | Uniform dark | In western populations uniform dark, in eastern populations distinctly striped |
| Throat | Yellow | Dark-yellow striped or vermiculated | Yellow | Dark | Dark | Dark | Dark |
| Palate and tongue of specimens in alcohol | Grey | Grey | Grey | Pure white | Grey | ? | Grey |

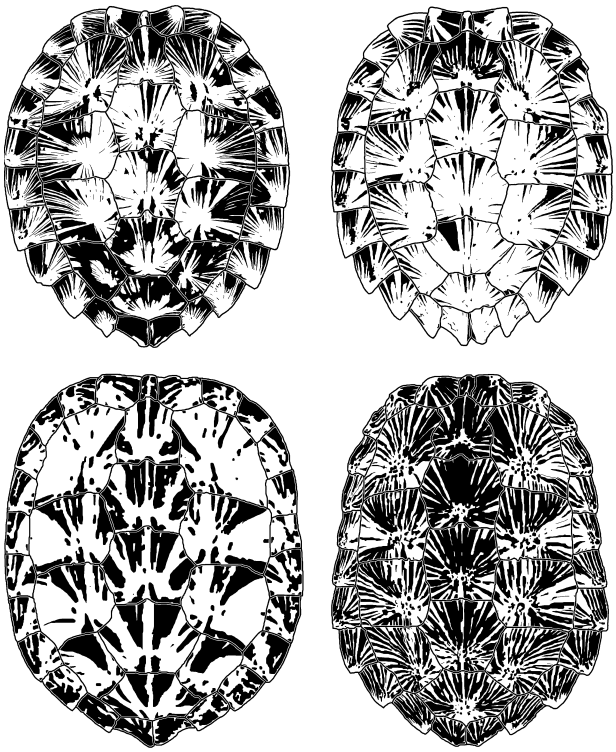

**Fig. 7** Carapacial pattern of *Cyclemys atripons* (top: MTD D 42516, female; FMNH 259052, subadult male) and *C. pulchristriata* (bottom: MTD T 18, live collection, female; MTD D 43785, female). For collection data, see Appendix I. — Drawings: Ch. Schmidt.

*C. atripons* and *C. pulchristriata* is very difficult to impossible. Within the dark-bellied species, *C. oldhamii* is easily identified; determination of the remaining species is difficult without statistical treatment.

## Discussion

Our data indicate a high diversity within *Cyclemys*. The concordance of mitochondrial and nuclear genomic markers provide evidence for five distinct species; the highly distinct mtDNA of the Assamese specimen NHMW 37153, for which no nuclear data are available, suggests the existence of a sixth species. Three of these six species fit each into the two major morphological groupings proposed by Fritz *et al*. (1997) that comprise yellow-bellied (*C. atripons*, *C. dentata*, *C. pulchristriata*) or dark-bellied species (*C. oldhamii*, *Cyclemys* species B and C). However, the topology of all mtDNA trees indicates that the dark-bellied species are not monophyletic, despite their morphological similarity. Rather, the two dark-bellied species B and C from the north-westernmost part of the range are suggested as sister group of a clade comprising the three yellow-bellied species as well as the dark-bellied *C. oldhamii* (Fig. 2).

According to nuclear genomic markers a fourth dark-bellied species exists (*Cyclemys* species A), sharing its mitochondrial genome with *C. dentata*. Considering that these haplotypes cluster with both other yellow-bellied species (*C. atripons*, *C. pulchristriata*), it seems likely that the mitochondrial genome of the dark-bellied species A was replaced by the mitochondrial genome of the sympatrically occurring yellow-bellied *C. dentata* and not vice versa. Using mtDNA data of typical dark-bellied leaf turtles from the Malay Peninsula (MTD T 4226–4227; fresh samples), Sumatra, Java and Borneo (NHMW 9811, NHMW 1188, ZSM 850.1920; historical museum specimens), we demonstrate for four very remote regions that the mitochondrial genome of *C. dentata* occurs in this dark-bellied species. This indicates (i) that the *dentata*-like mtDNA haplotypes are geographically widespread; and (ii) that the original mitochondrial genome of the dark-bellied species was perhaps entirely lost. Because we found *dentata*-like haplotypes in historical museum specimens from the late 19th or early 20th century, it can be further concluded that the introgression is old and not caused by recent hybridization due to an anthropogenically disturbed environment as suggested for other cases in chelonians (breakdown of ecological isolation; Fritz *et al*. 2008).

In our nDNA trees (Fig. 4), *Cyclemys* species A is not clearly differentiated from species B. One interpretation of this finding could be that species A represents hybrids between *C. dentata* and species B. However, this seems not likely when it is considered that the genetically verified records of species A and species B are separated by at least 1000 km air line. This great geographical distance and the highly distinct ISSR fingerprints of species A (Fig. 5) favour instead the hypothesis that incomplete lineage sorting is responsible for the similarity of species A and B in the investigated slowly evolving nuclear DNA fragments.

In the last comprehensive revision of leaf turtles (Fritz *et al*. 1997), entirely based on morphological characters, only two dark-bellied species were distinguished, *C. oldhamii* and *C. tcheponensis*, differing by the presence or absence of head and neck stripes and hatchling colour pattern. Using morphological data from additional specimens (Fritz & Ziegler 1999) and mtDNA sequence data (cyt *b*; Guicking *et al*. 2002; Stuart & Fritz 2008), it turned out that these simple morphological characters are not sufficient for delineating species borders. Considering our new data it is obvious that many previous difficulties in species-delineation of the dark-bellied *Cyclemys* were due to the fact that several very similar species are involved and that one of these species shares its mitochondrial genome with the yellow-bellied species *C. dentata*. Further factors contributing to confusion are colour pattern changes during growth and that within *C. oldhamii* two morphologically distinctive morphs occur. In the east of the range of this species, adult turtles retain the juvenile head and neck stripes throughout life, while in the west of the range the stripes are

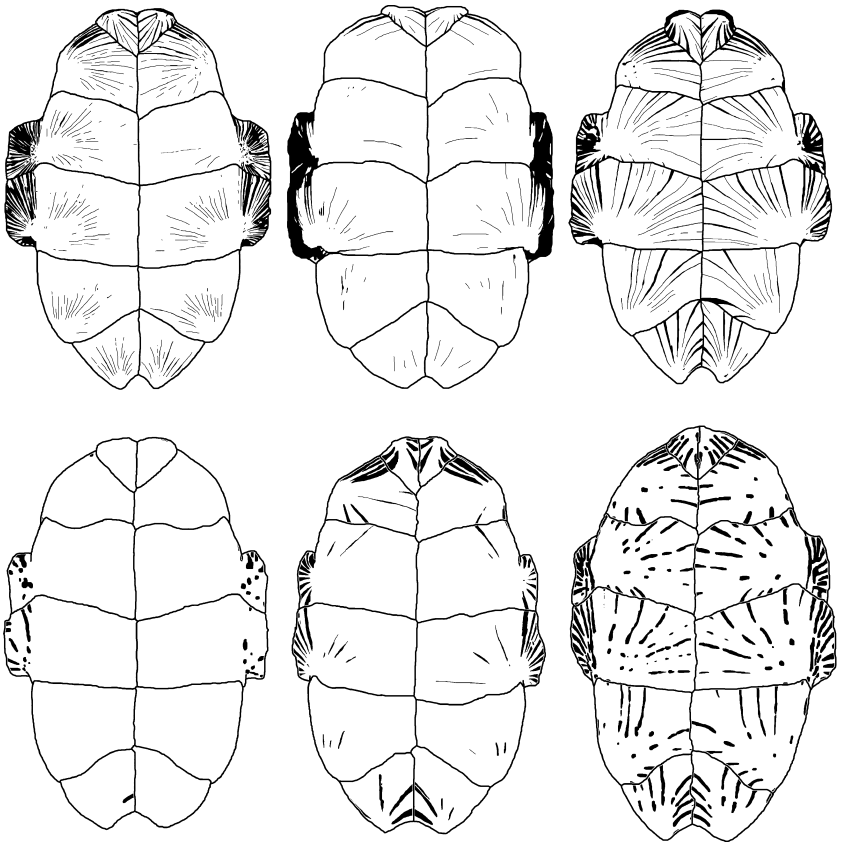

**Fig. 8** Plastral pattern of *Cyclemys atripons* (top: FMNH 259052; MTD T 17, live collection; MTD D 42516) and *C. pulchristriata* (bottom: MTD T 18, live collection; MTD D 43785; MTD D 42550). All specimens except FMNH 259052 (subadult male) are adult females. Note the pattern of finer and longer lines in *C. atripons*. For collection data, see Appendix I. — Drawings: Ch. Schmidt.

lost during ageing. When only dark plastral colouration plus presence or absence of and neck head stripes is used for species determination, the results are inevitably misleading.

Fritz *et al*. (1997) placed dark-bellied turtles bearing head and neck stripes into the species *C. tcheponensis* and assigned the dark-bellied turtles without head and neck stripes to *C. oldhamii*. Later, Guicking *et al*. (2002) provided evidence for the existence of two further dark-bellied species and tentatively restricted the name *C. oldhamii* to the dark-bellied species with introgressed *dentata* mtDNA, because the type locality of *C. oldhamii* lies on the Malay Peninsula from where their studied specimens originated. Guicking *et al*. (2002) found that mtDNA sequences of *C. tcheponensis* and certain dark-bellied *Cyclemys* without head and neck stripes were not distinct and concluded that these turtles are conspecific. They proposed to use the name *C. shanensis* for this species and recognized the unstriped western morph as subspecies *C. s. shanensis* and the striped eastern morph as *C. s. tcheponensis*. A third, genetically highly distinct dark-bellied species was discovered by Guicking *et al*. (2002), but they refrained from a description as only specimens originating from the international turtle-trade were known then. Using an additional known-locality museum specimen, we describe this species (our *Cyclemys* species B) below as new for science.

Unfortunately it turned out that the preliminary species delineation of Guicking *et al*. (2002) contributed to further confusion. Using mtDNA sequences of historical type specimens, Stuart & Fritz (2008) provided evidence that the name *C. oldhamii* refers to the same species for which Guicking *et al*. (2002) proposed the name *C. shanensis*, leading to the unsatisfactory situation that no scientific name is available for the species with the introgressed mitochondrial genome of *C. dentata*. Also this species, as well as species C, is described here as a new species.

As for the yellow-bellied group, our study confirms the distinctness of three species, *C. atripons*, *C. dentata* and *C. pulchristriata*. While *C. atripons* is suggested as sister species of the morphologically distinctive *C. dentata* by mtDNA data (Stuart & Fritz 2008; this study), the new nuclear genomic sequence data agree better with gross morphology in that the very similar species *C. atripons* and *C. pulchristriata* are proposed as sister taxa.

## Conclusions and taxonomy

Although novel diagnostic characters were found in our investigation and at least females of all species can be discriminated morphologically using multivariate statistics, determination of *C. atripons* and *C. pulchristriata* and of most dark-bellied species remains difficult. This implies that morphology

alone should be used only for a first assignment to the yellow- or dark-bellied species group and for excluding the two easily determinable species (*C. dentata*, *C. oldhamii*). For a reliable determination of the remaining species, the application of genetic markers is recommended.

From a conservation aspect, the complex diversity requires a revision of the IUCN Red List Categories for *Cyclemys*. The previous IUCN assessment was based on the assumption that a single species occurs in the entire range of the genus. The ranges of the seven species recognized in the present study are all smaller, in part considerably smaller, implying a higher risk from local overexploitation and habitat destruction. The high diversity of *Cyclemys* argues also for caution with releasing confiscated leaf turtles into the wild, as practised in several Southeast Asian countries (D. Hendrie, T. Nadler, pers. comm.). Such actions could result in the loss of biodiversity by hybridization, an imminent and serious threat when the many well-known hybrids between chelonian species are considered (reviews in Schilde *et al*. 2004; Buskirk *et al*. 2005; Bowen & Karl 2007; see also Stuart & Parham 2007).

**Key for adult *Cyclemys***

**1** Plastron entirely yellow or prevalent plastral colour yellow; femoral mid-seam shorter than or equal to anal mid-seam; anal notch small to wide and acute-angled to obtuse-angled.....2

**1′** Plastron entirely dark (brown or black) or prevalent plastral colour dark (brown or black); femoral mid-seam approximately equal to or longer than anal mid-seam; anal notch wide, obtuse-angled.....4

**2** Femoral mid-seam shorter than anal mid-seam; anal notch small and acute-angled; throat striped or with light and dark vermiculations, light head and neck stripes narrow (in live specimens reddish); in aged individuals bridge never covered by massive black bar.....*Cyclemys dentata* (Gray, 1831)

**2′** Femoral mid-seam shorter than or equal to anal mid-seam; anal notch small to wide and acute-angled to obtuse-angled; throat uniformly light coloured, light head and neck stripes wide (in live specimens yellow or salmon); in aged individuals bridge may be covered by massive black bar.....3

**3** If black radiating pattern is present on plastron, radiating lines long and thin.....*Cyclemys atripons* Iverson & McCord, 1997

**3′** If black radiating pattern is present on plastron, radiating lines short and stout .....*Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997

**4** Crown of the head speckled; shell rectangular when viewed from above; all juveniles and adults from the eastern part of the range with conspicuous head and neck stripes, adults from the western part of the range unstriped.....*Cyclemys oldhamii* Gray, 1863

**4′** Crown of the head uniform.....5

**5** Crown of the head brown, but not lighter than temporal region; shell elongated to rectangular.....*Cyclemys gemeli* n. sp.

**5′** Crown of the head lighter than temporal region; shell ovoid when viewed from above.....6

**6** Crown of the head uniformly greenish yellow to light brown; palate and tongue of specimens in alcohol grey, sometimes with darker grey spots; northern and central Myanmar.....*Cyclemys fusca* n. sp.

**6′** Crown of the head uniformly copper or light brown; palate and tongue of specimens in alcohol pure white; southern Malay Peninsula and Greater Sunda Islands.....*Cyclemys enigmatica* n. sp.

This key cannot be used for hatchlings and many juveniles. For the distinctive colouration and pattern of hatchlings, see Fig. 9 and Table 3. The characters of juveniles are intermediate between hatchlings and adults, making species determination often extremely difficult.

Genus *Cyclemys* Bell, 1834

Yellow-bellied *Cyclemys* species

*Cyclemys atripons* Iverson & McCord, 1997

1997 *Cyclemys atripons* Iverson & McCord, *Proceedings of the Biological Society Washington*, *110*, 629; Fig. 3. — Type locality: Kao [Mt.] Kuap (= Khao Kuap), Krat [Trat], Thailand. Holotype: United States National Museum of Natural History (USNM 81865, stuffed female, skull separate).

*Diagnosis.* Adult: Shell ovoid to heart-shaped; in aged adults elongated. Femoral mid-seam shorter than or equal to anal mid-seam; anal notch small to wide and acute-angled to obtuse-angled. Plastron mainly or entirely yellow; if a radiating dark plastral pattern is present, it consists of few thin black lines on each scute. A similar radiating pattern may occur on the light brown carapace. Bridge in some aged adults solid black. Top of the head with small dark specks; temporal region and neck with wide yellow to salmon stripes; underside of the neck with wide yellow to salmon stripes or unpatterned light coloured; throat unpatterned light coloured. Morphological distinction from *C. pulchristriata* is difficult. Hatchling (Fig. 9): Plastron mainly yellow, with few large

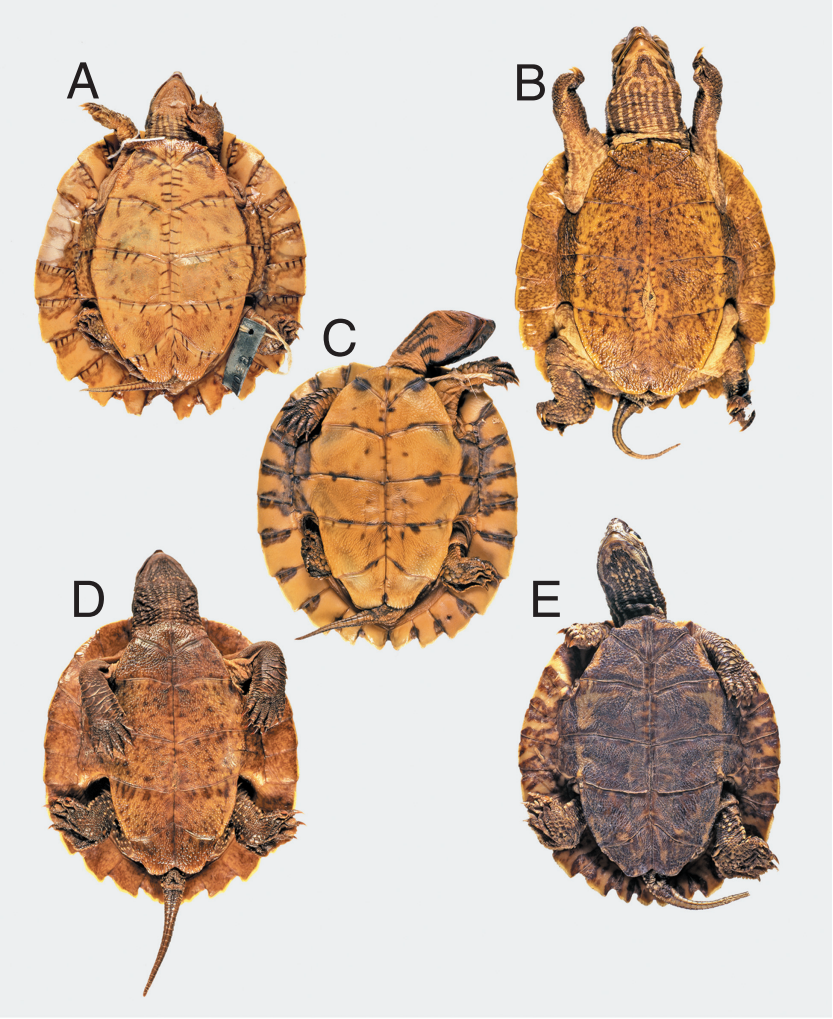


**Fig. 9** A–E. Ventral aspects of hatchlings of (A) *Cyclemys atripons* (USNM 94745, Khao Sabap, Chanthaburi, Thailand; paratype); (B) *C. dentata* (ZMUC E56, Palawan, Philippines); (C) *C. pulchristriata* (Phuc Son, central Vietnam; holotype); (D) *C. enigmatica* (ZMA 19029:2, western Nias, Indonesia; paratype); (E) *C. oldhamii* (SMNS 5355:1, Thailand). Not to scale. Juvenile pattern (radiating lines along scute seams) already developing in USNM 94745. Hatchlings of *C. fusca* and *C. gemeli* unknown. — Photos: F. Höhler.

black spots; ocellate pattern at submarginal seams; light head and neck stripes wide, throat light coloured. Hatchlings of *C. atripons* and *C. pulchristriata* are very similar and cannot be reliably distinguished. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Range*. South-eastern Thailand, including the Ko Chang and Ko Kut islands; south-western Cambodia (Fig. 10).

*Cyclemys dentata* (Gray, 1831)

1826 *Emys hasseltii* Boie in Fitzinger (nomen nudum), *Neue Classification der Reptilien*, 45.

1831 *Emys dhor* Gray in Griffith & Pidgeon, *A Classified Index and Synopsis of the Animal Kingdom Arranged in Conformity with its Organization, by the Baron Cuvier, Vol. 9, A Synopsis of the Species of the Class Reptilia*, 8. — Restricted type locality (by lectotype designation; Fritz *et al*. 1997): Java. Lectotype (Fritz *et al*. 1997): The Natural History Museum London (BMNH 1946.1.22.62, hatchling in alcohol).

1831 *Emys dentata* Gray (nomen novum pro *Emys dhor* Gray, 1831), *Synopsis Reptilium or Short Descriptions of the Species of Reptiles, Part 1, Cataphracta, Tortoises, Crocodiles & Enaliosaurians*, Errata.

1834 *Cyclemys orbiculata* Bell, *Proceedings of the Zoological Society London, 1834*, 17. — Designated type locality (Gray 1863): Java. Syntypes: Oxford University Museum of Natural History (OUMNH 8512–8513, shells of adult females).

1835 *Cistudo diardii* Duméril & Bibron (nomen novum pro *Emys dhor* Gray, 1831), *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles, Vol. 2*, 227.

1863 *Cyclemys bellii* Gray, *Proceedings of the Zoological Society London, 1863*, 179. — Type locality: Madras or Bombay? (in error). Holotype: Oxford University Museum of Natural History (OUMNH 8513, shell of adult female).

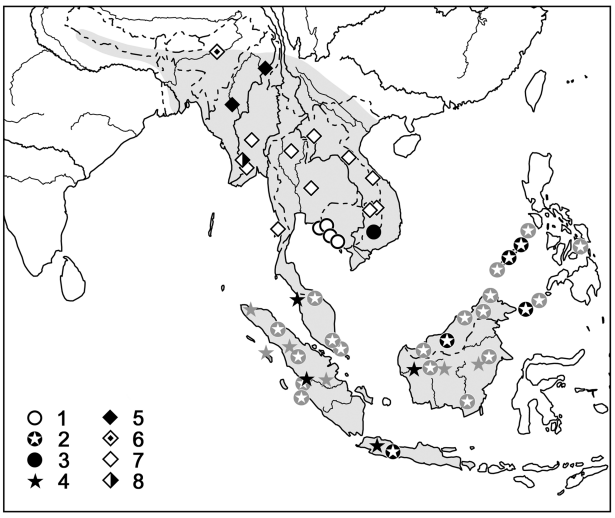


**Fig. 10** Range of the genus *Cyclemys* (shaded) and genetically verified records of (1) *C. atripons*, (2) *C. dentata*, (3) *C. pulchristriata*, (4) *C. enigmatica*, (5) *C. fusca*, (6) *C. gemeli*, (7) *C. oldhamii* and (8) putative *C. fusca* × *C. oldhamii* hybrid. For *C. dentata* and *C. enigmatica* genetically not verified records are also shown (grey symbols).

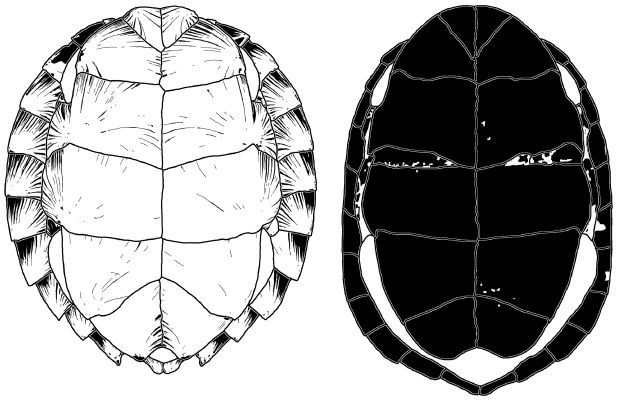

**Fig. 11** Ventral aspect of the shells of adult *Cyclemys dentata* (left; ZMUC E145, Tawitawi, Philippines) and *C. oldhamii* (right; MNHN 1997-4296, Ban Toup, Bokeo, Laos). Note distinct shell shape, ratios of femoral and anal mid-seam lengths, anal notches, and plastral pattern. In the *C. dentata* specimen the abdominal scutes are not yet secondarily divided. — Drawings: Ch. Schmidt.

1863 *Cyclemys ovata* Gray, *Proceedings of the Zoological Society London*, *1863*, 178; unnumbered figure on page 179. — Type locality: Sarawak. Holotype: The Natural History Museum London (BMNH 1863.6.21.1, shell of adult female).

*Diagnosis.* Adult: Shell ovoid to heart-shaped. Femoral mid-seam shorter than anal mid-seam; anal notch narrow and acute-angled. Colouration and pattern of shell (Fig. 11), head and neck resemble *C. atripons* but carapace on average darker brown and bridge never black; head and neck stripes narrower and reddish coloured; throat distinctly striped or mottled with dark pattern, never uniform light. Hatchling (Fig. 9): Ventral shell brownish-yellow with mottled dark pattern; light head and neck stripes narrow, throat striped. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Range.* Southern Malay Peninsula, Sumatra, Java, Borneo and nearby small islands; Palawan Islands and Sulu Archipelago, Philippines; introduced in Leyte and some other islands of the Philippines (Fig. 10).

*Remarks.* Fritz *et al.* (1997) and Stuart & Fritz (2008) demonstrated that *C. orbiculata* Bell, 1834, *C. bellii* Gray, 1863 and *C. ovata* Gray, 1863 are junior synonyms of *C. dentata* (Gray, 1831).

*Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997

1997 *Cyclemys pulchristriata* Fritz, Gaulke & Lehr, *Salamandra*, *33*, 183; Figs 1c, 2c, 3c, 7c. — Type locality: Phuc-Son, Annam. Holotype: Naturhistorisches Museum Vienna (NHMW 29525 : 4, hatchling in alcohol).

*Diagnosis.* Adult: Very similar to *C. atripons*, from which it is difficult to distinguish. If a dark plastral pattern is present in *C. pulchristriata*, it consists rather of short wide black lines or stout black specks; a black bridge is not known to occur. Hatchling (Fig. 9): Cannot be distinguished reliably from hatchling of *C. atripons*. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Range.* Central and southern Vietnam; easternmost Cambodia (Fig. 10).

*Remarks.* Four genetically verified adult males of *C. pulchristriata* have straight-line carapacial lengths of 139–175 mm (mean 152 mm); the carapaces of eight females measure 171–227 mm (mean 200 mm). This suggests that males are on average distinctly smaller. According to the studied museum specimens, a sexual size dimorphism does not occur in *C. dentata*, *C. enigmatica* and *C. oldhamii* (males of *C. fusca* and *C. gemeli* unknown). Of *C. atripons* was only one genetically verified male available (FMNH 259052); its shell length of 183 mm resembles the shell lengths of six females (type series and genetically verified individuals: 172–236 mm; mean: 207 mm).

Dark-bellied *Cyclemys* species

*Cyclemys enigmatica* n. sp.

*Holotype.* Naturhistorisches Museum Vienna (NHMW 9811, Padang, Sumatra, coll. Consul J. Schild 1901, stuffed adult female).

**Table 4** Basic straight line measurements (to the nearest mm) of adult type specimens of *Cyclemys enigmatica* (females). Carapace width measured at seam between 7th and 8th marginal.

| | NHMW 1188 | NHMW 9811 | NHMW 29524 | NHMW 29528:1 | RMNH 6066 | RMNH 6068 | RMNH 27828 | ZSM 850.1920 |
|---|---|---|---|---|---|---|---|---|
| Carapace length | 208 | 235 | 211 | 193 | 210 | 193 | 213 | 198 |
| Carapace width | 152 | 157 | 143 | 130 | 146 | 138 | 151 | 147 |
| Shell height | 74 | 85 | 79 | 72 | 76 | 68 | 72 | 81 |
| Plastron length (maximum) | 198 | 232 | 199 | 183 | 201 | 182 | 207 | 194 |
| Plastron length (mid-seam) | 192 | 222 | 189 | 174 | 194 | 173 | 194 | 185 |
| Mid-seam length gularia | 26 | 30 | 43 | 23 | 25 | 24 | 27 | 22 |
| Mid-seam length humeralia | 19 | 25 | 22 | 21 | 16 | 18 | 24 | 20 |
| Mid-seam length pectoralia | 48 | 50 | 22 | 39 | 50 | 41 | 40 | 44 |
| Mid-seam length abdominalia | 38 | 48 | 49 | 33 | 40 | 30 | 40 | 40 |
| Mid-seam length femoralia | 36 | 40 | 39 | 32 | 31 | 30 | 31 | 25 |
| Mid-seam length analia | 35 | 34 | 29 | 27 | 30 | 30 | 32 | 30 |

*Paratypes*. Nationaal Natuurhistorisch Museum Leiden (RMNH 3838, Padang, Sumatra, leg. Hoiner, juvenile in alcohol; RMNH 6066, 6088, Sumatra, coll. Müller, stuffed adult females; RMNH 27828, Java, leg. Kuhl & van Hasselt, skeleton of adult female); Naturhistorisches Museum Vienna (NHMW 1188, Java, old collection, pre 1900, stuffed adult female; NHMW 29524, Java, leg. R. Werner 1924, don. Regart, adult female in alcohol; NHMW 29528 : 1, Indragiri, Sumatra, don. Steindachner February 1905, adult female in alcohol); Zoologisch Museum Amsterdam (ZMA 19029 : 1–2, western Nias, leg. J. P. Kleiweg de Zwann, hatchlings in alcohol); Zoologische Staatssammlung München (ZSM 850.1920, Kapoeas River Region, western Borneo, leg. Bruegel 1907, shell of adult female).

*Etymology*. The species name *enigmatica*, Latinized adjective from the Greek noun aínigma (enigma, conundrum or riddle) refers to the long-lasting confusion caused by the distinct morphology of the new species and its introgressed mitochondrial genome.

*Diagnosis*. Adult: Dark coloured, with mainly or entirely dark brown to black shell. Carapace ovoid. Femoral mid-seam longer than or equal to anal mid-seam; anal notch wide and obtuse-angled. Young adults with dense black radiating pattern on carapace and plastron; aged adults often with uniform dark brown carapace and black plastron. Head and neck stripes absent. Crown of the head uniform brown, in life distinctly lighter coloured than temporal region. Palate and tongue in alcohol pure white. Hatchling (Fig. 9): Underside of the shell brownish-yellow, mottled with small darker brown specks; head and neck uniform dark, without stripes. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Description of the holotype*. Shell flat; carapace ovoid, uniform brown; plastron dark brown with indistinct pattern of black radiating lines. Carapacial rear margin weakly serrated, with healed injuries. Hyo-hypoplastral hinge well developed, abdominal scutes secondarily divided by hinge. Femoral mid-seam longer than anal mid-seam; anal notch wide and obtuse-angled (Fig. 12). For measurements, see Table 4.

**Fig. 12** Holotype of *Cyclemys enigmatica* n. sp. (NHMW 9811, adult female, Padang, Sumatra); lateral, dorsal and ventral aspect. Scale bar = 5 cm. — Photos: A. Schumacher.

*Range*. Southern Malay Peninsula and Greater Sunda Islands (including satellite islands; Fig. 10).

*Remarks*. Data of *C. enigmatica* are labelled as 'A' in Figs 2–6.

The allocation of the mtDNA sequences of *C. enigmatica* in two distinct subclades comprising sequences of *C. dentata*

(Fig. 2) could indicate that multiple hybridization events lead to the replacement of the original mitochondrial genome of *C. enigmatica*. The intermediate morphology of some adult museum specimens from Java (RMNH 6062–6065, 27829 with yellow plastra; see Fritz *et al*. 1997) suggests that hybridization still happens.

*Cyclemys fusca* n. sp.
*Holotype.* Museum of Zoology Dresden (MTD D 42578, Kachin State, Myanmar, obtained 2000 from M. Reimann via international pet-trade, adult female).

*Paratypes.* California Academy of Sciences (CAS 210070, Pwedon Chaung at Payawa Sakah = Elephant Camp, Alaungdaw Kathapa National Park, Sagaing Division, Myanmar, 22°19.204N 94°29.113E, leg. J. B. Slowinski, K. D. Wiseman, J. M. Lovette and J. V. Vindum 6 July 1999, juvenile), Museum of Zoology Dresden (MTD D 40842–40843, MTD D 41611–41614, MTD D 42596, MTD D 44176–44177, Kachin State, Myanmar, obtained 1998–2001 from M. Reimann via international pet-trade, adult females). All type specimens are in alcohol. Although only CAS 210070 has exact locality data, the adult specimen MTD D 42578 is chosen as holotype because the juvenile turtle does not yet display important characters (shell shape, colouration and pattern).

*Etymology.* The Latin species name *fusca*, brown, is chosen in allusion to the general colouration of adult specimens.

*Diagnosis.* Adult: Dark coloured, resembling *C. enigmatica* but light crown of the head in life with distinct greenish-yellow tinge. Palate and tongue of specimens in alcohol grey. Hatchling: Unknown. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Description of the holotype.* Shell moderately domed; carapace ovoid, uniform brown; plastron uniform blackish brown. Carapacial rear margin distinctly serrated; soft parts uniformly beige. Hyo-hypoplastral hinge present; abdominal scutes secondarily divided by hinge. Femoral mid-seam longer than anal mid-seam; anal notch wide and obtuse-angled. Healed injury on top of head (Fig. 13). For measurements, see Table 5.

*Range.* Northern and central Myanmar (Burma) and perhaps adjacent India and Bangladesh (Fig. 10).

**Fig. 13** Holotype of *Cyclemys fusca* n. sp. (MTD D 42578, adult female, Kachin State, Myanmar); lateral, dorsal and ventral aspect. Scale bar = 5 cm. — Photos: B. Bastian.

**Table 5** Basic straight line measurements (to the nearest mm) of adult type specimens of *Cyclemys fusca* (females). Carapace width measured at seam between 7th and 8th marginal.

| | MTD D 40842 | MTD D 40843 | MTD D 41611 | MTD D 41612 | MTD D 41613 | MTD D 41614 | MTD D 42578 | MTD D 42596 | MTD D 44176 | MTD D 44177 |
|---|---|---|---|---|---|---|---|---|---|---|
| Carapace length | 223 | 188 | 230 | 242 | 193 | 179 | 241 | 224 | 199 | 240 |
| Carapace width | 149 | 138 | 156 | 164 | 142 | 126 | 174 | 153 | 138 | 170 |
| Shell height | 89 | 78 | 82 | 96 | 80 | 72 | 102 | 83 | 68 | 99 |
| Plastron length (maximum) | 211 | 184 | 215 | 232 | 191 | 172 | 243 | 212 | 189 | 239 |
| Plastron length (mid-seam) | 202 | 175 | 201 | 223 | 182 | 165 | 235 | 196 | 178 | 226 |
| Mid-seam length gularia | 31 | 25 | 23 | 45 | 25 | 40 | 31 | 20 | 28 | 31 |
| Mid-seam length humeralia | 22 | 18 | 22 | 34 | 20 | 25 | 25 | 26 | 17 | 18 |
| Mid-seam length pectoralia | 49 | 43 | 51 | 26 | 48 | 18 | 57 | 50 | 41 | 60 |
| Mid-seam length abdominalia | 34 | 33 | 36 | 52 | 30 | 43 | 48 | 33 | 30 | 49 |
| Mid-seam length femoralia | 32 | 27 | 39 | 38 | 26 | 33 | 41 | 39 | 30 | 34 |
| Mid-seam length analia | 34 | 29 | 33 | 40 | 36 | 27 | 37 | 29 | 31 | 36 |

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*Remarks*. Data of *C. fusca* are labelled as 'B' in Fig. 2 and Figs 4–6.

The juvenile paratype CAS 210070 (carapace length 93 mm) has faint neck and head stripes and a slightly mottled top of the head, suggesting that these characters occur in hatchling *C. fusca* and are lost during growth. Also the juveniles AMNH R-58423 (Singkaling HKamti, Burma, leg. H. C. Raven; carapace length 90 mm) and BMNH 1929.12.1.15 (Lake Indawgyi, Myitkyina District, Upper Burma, presented by the Indian Museum 1929; 105 mm) have a similar colouration while the slightly larger BMNH 1930.6.8.4 (Naga Hills, India, presented by the Bombay Natural History Society; 123 mm) has a uniformly coloured head and neck. As no genetic data are available for the AMNH and BMNH specimens, we refrain from assigning them to *C. fusca*; according to the collection site BMNH 1930.6.8.4 could also represent a juvenile *C. gemeli*.

*Cyclemys gemeli* n. sp.

*Holotype*. Naturhistorisches Museum Vienna (NHMW 37153, street from Tezpur to Arunachal Pradesh, 5 km to border of Arunachal Pradesh, Jia Bhoroli River Region, Assam, India, coll. R. Gemel 7 November 2003, incomplete bony shell of adult, sex unknown).

*Etymology*. The species is named in honour of the collector of the holotype, Richard Gemel (Vienna), in recognition of his work in the Herpetological Collection of the Natural History Museum Vienna.

*Diagnosis*. Adult: Dark coloured, resembling the other dark-bellied *Cyclemys* species but carapace elongated to rectangular and crown of the head uniform brown, not lighter coloured than temporal region. Hatchling: Unknown. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Description of the holotype*. Carapace and plastral hind lobe without epidermal scutes. Carapace elongated; rear margin nearly smooth. Measurements (in mm) — carapace: length 231, width between 7th and 8th marginal 157; shell height: approximately 80; plastral hind lobe: mid-seam length of abdominal scutes 41, of femoral scutes 35, of anal scutes 32. Anal notch wide, obtuse-angled (Fig. 14).

*Range*. North-eastern India (Fig. 10).

*Remarks*. Data of *C. gemeli* are labelled as 'C' in Fig. 2.

The holotype of *C. gemeli* was caught and consumed by local Assamese villagers near the border of Arunachal Pradesh. It was preserved as bony shell after removal of tissue for genetic investigation. The description of colouration and pattern (Table 3) is based on colour photos of a topotypic live female of 23 cm carapacial length, provided by Peter Praschag (Jia Bhoroli River Region, Nameri National Park, 35 km to Tezpur, Assam, India; voucher photos deposited in the MTD collection). Due to the presence of epidermal scutes, the carapacial rear margin of this turtle is distinctly more serrated than in the holotype.

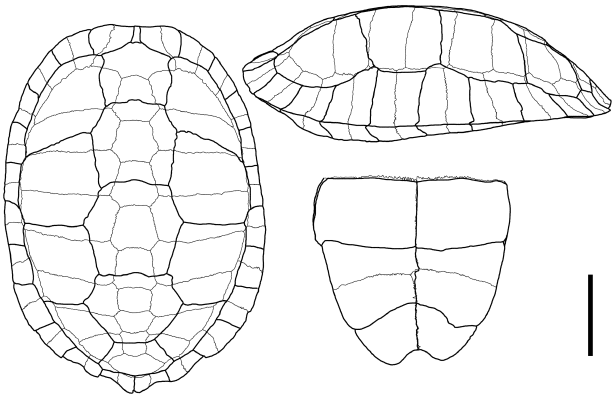

**Fig. 14** Holotype of *Cyclemys gemeli* n. sp. (NHMW 37153, adult of unknown sex, street from Tezpur to Arunachal Pradesh, 5 km to border of Arunachal Pradesh, Jia Bhoroli River Region, Assam, India); dorsal and lateral aspect of carapace and ventral aspect of plastral hind lobe. Scale bar = 5 cm. — Drawings: Ch. Schmidt.

It seems possible that the juvenile carapace USNM 293726 (Sukhna, Mahanada Sanctuary, Darjeeling, West Bengal, India, leg. John G. Frazier 3 April 1987) and the subadult female ZMH R00288 (subadult female, Nishangara, 28°15′N 81°13′E, Bahraich, Uttar Pradesh, India, approximately 5 km S Nepali border, leg. G. A. von Maydell, German India Expedition 1955/1957) represent *C. gemeli* as well. The soft part and shell colouration of ZMH R00288 match the above-mentioned photos of the live Assamese leaf turtle. For another juvenile specimen (BMNH 1930.6.8.4) possibly representing *C. gemeli*, see above under *C. fusca* (Remarks).

*Cyclemys oldhamii* Gray, 1863

1863 *Cyclemys oldhamii* Gray, *Proceedings of the Zoological Society London*, *1863*, 178. — Restricted type locality (by lectotype designation; Fritz *et al.* 1997): Mergui. Lectotype (Fritz *et al.* 1997): The Natural History Museum London (BMNH 1947.3.5.63, shell of adult male).

1918 *Cyclemys dhor shanensis* Annandale, *Record of the Indian Museum*, *14*, 67; Plate 20: Figs 1 and 2a. — Type locality: Fort Stedman (3000 feet), Lake Inlé, Burma. Lectotype (Fritz *et al.* 1997): Zoological Survey of India, Calcutta (ZSI 18594, shell of adult male; only photos studied).

1939 *Geoemyda tcheponensis* Bourret, *Annexe*, *Bulletin Général de l'Instruction Publique*, *1939 (6)*, 7; Fig. 1. — Type locality:

Upper Sé Bang Hiên River, central Annam. Holotype: Centre for Natural Resources Management and Environmental Studies, Hanoi (CRES T-43, juvenile in alcohol; see Fritz *et al*. 1999).

1989 *Cyclemys tiannanensis* Kou, *Current Herpetology of East Asia*, 193; Figs 1–5. — Type locality: 'Nanliang, Mengla Co., Xishuangbanna of Yunnan Province, China' [based on a trade specimen of unknown provenance; Fritz *et al*. 1997). Holotype: Department of Biology, Yunnan University (RT 8311002, subadult male; only photos studied).

*Diagnosis*. Adult: Dark coloured, resembling the other dark-bellied species but carapace rectangular (Fig. 11) and crown of the head light brown with distinctive small dark spots. Head and neck stripes absent in adults from the western part of the range, present in adults from the eastern part of the range. Palate and tongue in alcohol grey. Hatchling (Fig. 9): Brownish plastron covered by extensive dark central figure; ocellate pattern at submarginal seams; distinctive head and neck stripes present, throat dark. For maximum size and comparison with diagnostic characters of other species, see Table 3.

*Range*. Central and southern Myanmar (Burma), central and northern Thailand, Laos, northern Cambodia, northern and central Vietnam; perhaps neighbouring southern China (Fig. 10).

*Remarks*. The adult male BMNH 1974.2594 from Tasan (leg. M. A. Smith March 1919) represents *C. oldhamii*, and not *C. fusca* as suggested by the map in Fritz & Ziegler (1999), because these authors confused the collection site with Tasan, Myanmar. BMNH 1974.2594 was actually collected at Tasan, Thailand (approximately 25 miles SW Chumphon, 10°10′N 98°50′E).

When a subspecific distinction between the unstriped western and striped eastern turtles is desired, the names *C. o. oldhamii* Gray, 1863 and *C. o. tcheponensis* (Bourret, 1939), respectively, should be applied.

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## References

Bornet, B. & Branchard, M. (2001). Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter*, *19*, 209–215.

Bowen, B. W. & Karl, S. A. (2007). Population genetics and phylogeography of sea turtles. *Molecular Ecology*, *16*, 4886–4907.

Buskirk, J. R., Parham, J. F. & Feldman, C. R. (2005). On the hybridisation between two distantly related Asian turtles (Testudines: *Sacalia* × *Mauremys*). *Salamandra*, *41*, 21–26.

Clement, M., Posada, D. & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1660.

Condit, R. & Hubbell, S. P. (1991). Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome*, *34*, 66–71.

van Dijk, P. P., Stuart, B. L. & Rhodin, A. G. J. (2000). Asian Turtle Trade. Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia, Phnom Penh, Cambodia, 1–4 December 1999. *Chelonian Research Monographs*, *2*, 1–164.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*, 783–791.

Felsenstein, J. (2004). *Inferring Phylogenies*. Sunderland, Massachusetts: Sinauer Associates.

Fritz, U. & Ziegler, T. (1999). Contribution to the knowledge of *Cyclemys tcheponensis* (Bourret, 1939) and the distribution of *Cyclemys* in the Indochinese region. *Revue française d'Aquariologie*, *26*, 71–78.

Fritz, U., Gaulke, M. & Lehr, E. (1997). Revision der südostasiatischen Dornschildkröten-Gattung *Cyclemys* Bell, 1834, mit Beschreibung einer neuen Art. *Salamandra*, *33*, 183–212.

Fritz, U., Niekisch, M. & Ziegler, T. (1999). Rediscovery of the holotype of *Geoemyda tcheponensis* Bourret, 1939. *Zoologische Abhandlungen*, *50*, 243–247.

Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M. G., Lenk, P., Joger, U. & Wink, M. (2005a). A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys*. *Zoologica Scripta*, *34*, 351–371.

Fritz, U., Široký, P., Kami, H. & Wink, M. (2005b). Environmentally caused dwarfism or a valid species — Is *Testudo weissingeri* Bour, 1996 a distinct evolutionary lineage? New evidence from mitochondrial and nuclear genomic markers. *Molecular Phylogenetics and Evolution*, *37*, 389–401.

Fritz, U., Ayaz, D., Buschbom, J., Kami, H. G., Mazanaeva, L. F., Aloufi, A. A., Auer, M., Rifai, L., Šilić, T. & Hundsdörfer, 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.

Fujita, M. K., Engstrom, T. N., Starkey, D. E. & Shaffer, H. B. (2004). Turtle phylogeny: insights from a novel nuclear intron. *Molecular Phylogenetics and Evolution*, *31*, 1031–1040.

Gray, J. E. (1863). Observations on the box tortoises, with the description of three new Asiatic species. *Proceedings of the Zoological Society London*, *1863*, 173–179.

Guicking, D., Fritz, U., Wink, M. & Lehr, E. (2002). New data on the diversity of the Southeast Asian leaf turtle genus *Cyclemys* Bell, 1834. Molecular results. *Faunistische Abhandlungen*, *23*, 75–86.

Gupta, M., Chyi, Y.-S., Romero-Severson, J. & Owen, J. L. (1994). Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, *89*, 998–1006.

Gustincich, S., Manfioletti, G., del Sal, G., Schneider, C. & Carninci, C. (1991). A fast method for high-quality genomic DNA extraction from whole human blood. *Bio Techniques*, *11*, 298–302.

IUCN [International Union for the Conservation of Nature and Natural Resources]. (2007). *IUCN Red List of Threatened Species*. <www.iucnredlist.org> [Downloaded on 28 October 2007].

Iverson, J. B. (1992). *A Revised Checklist with Distribution Maps of the Turtles of the World*. Richmond, Indiana: Privately Printed.

Iverson, J. B. & McCord, W. P. (1997). A new species of *Cyclemys* (Testudines: Bataguridae) from Southeast Asia. *Proceedings of the Biological Society of Washington*, *110*, 629–639.

Kearney, M. & Stuart, B. L. (2004). Repeated evolution of limblessness and digging heads in worm lizards revealed by DNA from old bones. *Proceedings of the Royal Society B*, *271*, 1677–1683.

King, F. W. & Burke, R. L. (1989). *Crocodilian, Tuatara, and Turtle Species of the World*. Washington, DC: Association of Systematic Collections.

Le, M., Raxworthy, C. J., McCord, W. P. & Mertz, L. (2006). A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, *40*, 517–531.

Lenk, P., Fritz, U., Joger, U. & Wink, M. (1999). Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Molecular Ecology*, *8*, 1911–1922.

Nagy, Z. T., Joger, U., Guicking, D. & Wink, M. (2003). Phylogeography of the European whip snake *Coluber (Hierophis) viridiflavus* as inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene and ISSR genomic fingerprinting. *Biota*, *3*, 109–118.

Nei, M. & Li, W.-H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, *76*, 5269–5273.

Olson, L. E. & Hassanin, A. (2003). Contamination and chimerism are perpetuating the legend of the snake-eating cow with twisted horns (*Pseudonovibos spiralis*). A case study of the pitfalls of ancient DNA. *Molecular Phylogenetics and Evolution*, *27*, 545–548.

van de Peer, Y. & de Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in the Biosciences*, *10*, 569–570.

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.

Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Schilde, M., Barth, D. & Fritz, U. (2004). An *Ocadia sinensis* × *Cyclemys shanensis* hybrid. *Asiatic Herpetological Research*, *10*, 120–125.

Spinks, P. Q., Shaffer, H. B., Iverson, J. B. & McCord, W. P. (2004). Phylogenetic hypotheses for the turtle family Geoemydidae. *Molecular Phylogenetics and Evolution*, *32*, 164–182.

Stuart, B. L. & Fritz, U. (2008). Historical DNA from type museum specimens clarifies diversity of Asian leaf turtles (*Cyclemys*). *Biological Journal of the Linnean Society*, doi: 10.1111/j.1095-8312.2008.00966.x.

Stuart, B. L. & Parham, J. F. (2007). Recent hybrid origin of three rare Chinese turtles. *Conservation Genetics*, *8*, 169–175.

Swofford, D. L. (2002). PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sunderland, Massachusetts: Sinauer Associates.

Tautz, D. & Renz, M. (1984). Simple sequences are ubiquitous repetitive components of eukaryontic genomes. *Nucleic Acids Research*, *12*, 4127–4138.

Tsumura, Y., Ohba, K. & Strauss, S. H. (1996). Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics*, *92*, 40–45.

Wermuth, H. & Mertens, R. (1977). Testudines, Crocodylia, Rhynchocephalia. *Das Tierreich*, *100*, I–XXVII, 1–174.

Wink, M., Guicking, D. & Fritz, U. (2001). Molecular evidence for hybrid origin of *Mauremys iversoni* Pritchard et McCord, 1991, and *Mauremys pritchardi* McCord, 1997. *Zoologische Abhandlungen*, *51*, 41–49.

Wolfe, A. D. & Liston, A. (1998). Contributions of PCR-based methods to plant systematics and evolutionary biology. In D. E. Soltis, P. S. Soltis & J. J. Doyle (Eds) *Molecular Systematics of Plants II. DNA Sequencing* (pp. 43–86). Boston, Massachusetts: Kluwer Academic Publishers.

Wolfe, A. D., Xiang, Q.-Y. & Kephart, S. R. (1998). Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Molecular Ecology*, 7, 1107–1125.

Zietkiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (ISSR)-anchored polymerase chain reaction amplification. *Genomics*, *20*, 176–183.

Zwickl, D. J. (2006). *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion*. PhD Dissertation, Austin, Texas: The University of Texas. [Genetic Algorithm for Rapid Likelihood Inference Software available from http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html].

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**Appendix I** *Cyclemys* samples used for genetic investigations, including sequences downloaded from GenBank. For mitochondrial and nuclear genomic sequences, GenBank accession numbers are given. MTD D, Museum of Zoology Dresden, Herpetological Collection; MTD T, Museum of Zoology Dresden, Tissue Collection; for other abbreviations of sample codes (museum acronyms), see Materials and methods.

| Species | Locality | Sample | Cyt *b* | C-mos | Rag2 | R35 | ISSR | Remarks/Reference |
|---|---|---|---|---|---|---|---|---|
| *atripons* | Cambodia: Koh Kong Province: Sre Ambel District: Sre Ambel Town; 11°07′20″N 103°44′45″E | FMNH 259051 | AM931624 | AM931585 | AM931604 | – | – | Obtained from local turtle-trader |
| *atripons* | Cambodia: Koh Kong Province: Sre Ambel District: Sre Ambel Town; 11°07′20″N 103°44′45″E | FMNH 259052 | AM931625 | AM931586 | AM931605 | AM931623 | – | Obtained from local turtle-trader |
| *atripons* | SW Cambodia: Koh Kong Province: coastal forests, Southern Cardamon Mts, near Trapeang Rung Estuary | MTD T 4491 | AM931626 | – | – | – | – | Obtained from local fisherman |
| *atripons* | SW Cambodia: Koh Kong Province: coastal forests, Southern Cardamon Mts, near Trapeang Rung Estuary | MTD T 4492 | AM931627 | – | – | – | – | Obtained from local fisherman |
| *atripons* | SW Cambodia: Koh Kong Province: mid-reaches of the Tatai River, Central Cardamon Mts | MTD T 4490 | AM931628 | – | – | – | – | Obtained from local fisherman |
| *atripons* | SW Cambodia: Koh Kong Province: Upper Tatai River, Central Cardamon Mts | MTD T 4487 | AM931629 | – | – | – | – | Obtained from local hunter |
| *atripons* | Thailand: Trat Province: Kao Kuap | USNM 81865 | DQ444271 | – | – | – | – | Historical museum specimen, collected 1929. Holotype of *Cyclemys atripons*; Stuart & Fritz (2008) |
| *atripons* | Thailand: Trat Province: Koh Chang | USNM 53423 | DQ444270 | – | – | – | – | Historical museum specimen, collected 1914. Paratype of *Cyclemys atripons*; Stuart & Fritz (2008) |
| *atripons* | Unknown | MTD D 42516 | AM931630 | – | – | – | – | Obtained at Orasey market in Phnom Penh |
| *atripons* | Unknown | MTD T 17 | AM931631 | AM931587 | AM931606 | AM931694 | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *dentata* | Indonesia | MTD D 42597 | AM931632 | – | AM931607 | – | + | International pet-trade |
| *dentata* | Indonesia | MTD D 42912 | AM931633 | – | – | AM931695 | + | International pet-trade |
| *dentata* | Indonesia | MTD D 43735 | AM931634 | – | – | – | | International pet-trade |
| *dentata* | Indonesia | MTD T 4210 | AM931635 | AM931588 | AM931608 | AM931696 | + | International pet-trade |
| *dentata* | Indonesia: Java | BMNH 1946.1.22.62 | DQ444272 | – | – | – | – | Historical museum specimen, collected by 1828. Lectotype of *Cyclemys dentata*; Stuart & Fritz (2008) |
| *dentata* | Indonesia: Java | MTD D 44264 | AM931636 | – | – | – | – | International pet-trade |
| *dentata* | Malaysia: Borneo: Sarawak | BMNH 1863.6.21.1 | DQ444275 | – | – | – | – | Historical museum specimen, collected by 1863. Holotype of *Cyclemys ovata*; Stuart & Fritz (2008) |
| *dentata* | Philippines: Palawan: Malinao River 27 km south of Puerto Princesa | MTD T 2535 | AM931637 | AM931589 | AM931609 | – | – | Field-collected; voucher photos in MTD T collection |
| *dentata* | Philippines: Palawan: vicinity of Taytay, small creek to Lake Manguao | MTD T 2536 | AM931638 | – | – | AM931697 | – | Field-collected; voucher photos in MTD T collection |
| *dentata* | Philippines: Tawitawi: Languyan | MTD D 39530 | AM931639 | – | – | – | – | Field-collected |
| *dentata* | Unknown | MTD D 46611 | AM931640 | – | – | – | – | International pet-trade |
| *dentata* | Unknown | MTD T 753 | AM931641 | – | – | – | – | International pet-trade |
| *enigmatica* | Indonesia: Borneo: Kapoeas River Region | ZSM 850.1920 | AM931642 | – | – | – | – | Historical museum specimen, collected 1907 |
| *enigmatica* | Indonesia: Java | NHMW 1188 | AM931643 | – | – | – | – | Historical museum specimen, collected by 1900 |
| *enigmatica* | Indonesia: Sumatra: Padang | NHMW 9811 | AM931644 | – | – | – | – | Historical museum specimen, collected by 1901. Holotype of *Cyclemys enigmatica* |
| *enigmatica* | Malaysia: Penang | MTD T 4226 | AM931645 | AM931590 | AM931610 | AM931698 | + | Obtained from local turtle-trader; voucher photos in MTD T collection |

**Appendix I** *Continued.*

| Species | Locality | Sample | Cyt *b* | C-mos | Rag2 | R35 | ISSR | Remarks/Reference |
|---|---|---|---|---|---|---|---|---|
| *enigmatica* | Malaysia: Penang | MTD T 4227 | AM931646 | AM931591 | AM931611 | AM931699 | + | Obtained from local turtle-trader; voucher photos in MTD T collection |
| *fusca* | Myanmar: Kachin State | MTD D 41611 | AM931647 | – | – | – | – | International pet-trade |
| *fusca* | Myanmar: Kachin State | MTD D 41612 | AM931648 | – | – | – | – | International pet-trade |
| *fusca* | Myanmar: Kachin State | MTD D 41613 | AM931649 | – | – | – | + | International pet-trade |
| *fusca* | Myanmar: Kachin State | MTD D 41614 | AM931650 | – | – | – | + | International pet-trade |
| *fusca* | Myanmar: Kachin State | MTD D 42578 | AM931651 | – | – | AM931700 | – | International pet-trade. Holotype of *Cyclemys fusca* |
| *fusca* | Myanmar: Kachin State | MTD D 44176 | AM931652 | – | – | – | – | International pet-trade |
| *fusca* | Myanmar: Kachin State | MTD D 44177 | AM931653 | AM931592 | AM931612 | – | – | International pet-trade |
| *fusca* | Myanmar: Sagaing Division: Alaungdaw Kathapa National Park: Pwedon Chaung at Payawa Sakah (Elephant Camp); 22°19.204N 94°29.113E | CAS 210070 | AM931654 | AM931593 | AM931613 | AM931701 | + | Field-collected |
| *fusca* × *oldhamii?* | Myanmar: Bago Yoma; 18°81.670N 96°08.504E | CAS 208423 | AM931655 | AM931594 | AM931614 | AM931702 | + | Field-collected |
| *gemeli* | India: Assam: Jia Bhoroli River Region, street from Tezpur to Arunachal Pradesh, 5 km to border of Arunachal Pradesh | NHMW 37153 | AM931656 | – | – | – | – | Obtained from local villagers. Holotype of *Cyclemys gemeli* |
| *oldhamii* | Cambodia: Ratanakiri Province: Srai Chhouk | MTD D 42509 | AM931657 | – | – | – | – | Obtained from local villagers |
| *oldhamii* | Cambodia: Stung Treng Province: Siem Pang District | FMNH 262709 | AM931658 | AM931595 | – | – | – | Field-collected |
| *oldhamii* | Central Thailand | MTD D 40652 | AM931659 | – | – | – | – | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Central Thailand | MTD D 41615 | AM931660 | – | – | – | + | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Central Thailand | MTD D 41616 | AM931661 | – | – | – | – | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Central Thailand | MTD D 41617 | AM931662 | – | – | – | – | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Central Thailand | MTD D 41618 | AM931663 | – | – | – | – | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Central Thailand | MTD D 42949 | AM931664 | – | – | – | – | Obtained from local turtle-trader in 1970s |
| *oldhamii* | Laos: Khammouan Province: Nakai District: Khammouan Limestone (= Phou Hin Poun) National Biodiversity Conservation Area; 17°53′N 104°52′E, 570 m | FMNH 255263 | AM931665 | AM931596 | AM931615 | – | – | Field-collected |
| *oldhamii* | Laotian/Vietnamese border region: vicinity of Lao Bao (close to type locality of *Geoemyda tcheponensis*) | MTD D 42537 | AM931666 | – | – | – | + | Obtained from local turtle-trader |
| *oldhamii* | Laotian/Vietnamese border region: vicinity of Lao Bao (close to type locality of *Geoemyda tcheponensis*) | MTD D 44389 | AM931667 | – | – | – | + | Obtained from local turtle-trader |
| *oldhamii* | Laotian/Vietnamese border region: vicinity of Lao Bao (close to type locality of *Geoemyda tcheponensis*) | MTD T 4214 | AM931668 | – | – | – | + | Obtained from local turtle-trader; live collection of MTD |
| *oldhamii* | Laotian/Vietnamese border region: vicinity of Lao Bao (close to type locality of *Geoemyda tcheponensis*) | MTD T 4216 | AM931669 | – | – | – | – | Obtained from local turtle-trader; live collection of MTD |
| *oldhamii* | Myanmar: Bago District: Dawe; 17°44′52″N 96°14′1″E | USNM 563362 | AM931670 | – | – | – | – | Field-collected |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4219 | AM931671 | AM931597 | AM931616 | AM931703 | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4220 | AM931672 | – | – | – | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4221 | AM931673 | – | – | – | – | Field-collected; voucher photos in MTD T collection |

**Appendix I** *Continued.*

| Species | Locality | Sample | Cyt *b* | C-mos | Rag2 | R35 | ISSR | Remarks/Reference |
|---|---|---|---|---|---|---|---|---|
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4222 | AM931674 | – | – | – | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4223 | AM931675 | AM931598 | AM931617 | AM931704 | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4224 | AM931676 | – | – | – | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Shan State: Lake Inle (type locality of *Cylcemys dhor shanensis*) | MTD T 4225 | AM931677 | – | – | – | + | Field-collected; voucher photos in MTD T collection |
| *oldhamii* | Myanmar: Tanintharyi (Tenasserim): Mergui | BMNH 1947.3.5.63 | DQ444274 | – | – | – | – | Historical museum specimen, collected by 1856. Lectotype of *Cyclemys oldhamii*; Stuart & Fritz (2008) |
| *oldhamii* | Northern Thailand | MTD D 44278 | AM931678 | – | – | – | – | International pet-trade |
| *oldhamii* | Thai/Laotian border region: Luang Prabang Mts | BMNH 1947.3.4.26 | DQ444273 | – | – | – | – | Historical museum specimen, collected by 1862. Paralectotype of *Cyclemys oldhamii*; Stuart & Fritz (2008) |
| *oldhamii* | Unknown | MTD T 4228 | AM931679 | – | – | – | – | International pet-trade; voucher photos in MTD T collection |
| *oldhamii* | Unknown | MTD T 815 | AM931680 | – | – | – | – | International pet-trade; voucher photos in MTD T collection |
| *oldhamii* | Unknown | – | AJ604513 | – | – | – | – | Schilde *et al.* (2004) |
| *oldhamii* | Unknown | – | AY434577 | – | – | – | – | Spinks *et al.* (2004) |
| *oldhamii* | Unknown | – | AY434579 | – | – | – | – | Spinks *et al.* (2004) |
| *pulchristriata* | Cambodia: Mondolkiri Province: Pichrada District: Phnom Nam Lyr Wildlife Sanctuary; near 12°32′16″N 107°32′00″E (type locality of *Cyclemys pulchristriata* lies in the same mountain range) | FMNH 259050 | AM931681 | AM931599 | AM931618 | AM931705 | – | Field-collected |
| *pulchristriata* | Unknown | MTD D 42549 | AM931682 | – | – | – | + | Cambodian turtle-trade |
| *pulchristriata* | Unknown | MTD D 42550 | AM931683 | – | – | – | + | Cambodian turtle-trade |
| *pulchristriata* | Unknown | MTD D 42551 | AM931684 | – | – | – | + | Cambodian turtle-trade |
| *pulchristriata* | Unknown | MTD D 42552 | AM931685 | – | – | – | + | Cambodian turtle-trade |
| *pulchristriata* | Unknown | MTD D 43785 | AM931686 | – | – | – | + | Obtained at Cau-Mong market, Saigon |
| *pulchristriata* | Unknown | MTD D 44261 | AM931687 | – | – | – | – | International pet-trade |
| *pulchristriata* | Unknown | MTD D 44262 | AM931688 | – | – | – | – | International pet-trade |
| *pulchristriata* | Unknown | MTD T 18 | AM931689 | – | – | – | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *pulchristriata* | Unknown | MTD T 19 | AM931690 | – | – | – | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *pulchristriata* | Unknown | MTD T 20 | AM931691 | – | – | – | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *pulchristriata* | Unknown | MTD T 4206 | AM931692 | AM931600 | AM931619 | – | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *pulchristriata* | Unknown | MTD T 4209 | AM931693 | AM931601 | AM931620 | AM931706 | + | Obtained at Cau-Mong market, Saigon; live collection of MTD |
| *pulchristriata* | Unknown | – | AY434617 | – | – | – | – | Spinks *et al.* (2004) |

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**Appendix II** Data matrix for ISSR marker bands of *Cyclemys* samples.

| | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | AA | AB | AC | AD | AE | AF | AG | AH | AI | AJ | AK | AL | AM | AN | AO | AP | AQ | AR | AS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MTD T 17 | *atripons* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD D 42597 | *dentata* | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| MTD D 42912 | *dentata* | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| MTD T 4210 | *dentata* | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| MTD T 4226 | *enigmatica* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| MTD T 4227 | *enigmatica* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| CAS 210070 | *fusca* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| MTD D 41613 | *fusca* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD D 41614 | *fusca* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| CAS 208423 | *fusca* × *oldhamii?* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| MTD D 41615 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD D 42537 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD D 44389 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD T 4214 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD T 4219 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| MTD T 4220 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD T 4222 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| MTD T 4223 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| MTD T 4224 | *oldhamii* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| MTD T 4225 | *oldhamii* | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| MTD D 42549 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD D 42550 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD D 42551 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD D 42552 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD D 43785 | *pulchristriata* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD T 18 | *pulchristriata* | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD T 19 | *pulchristriata* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD T 20 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD T 4206 | *pulchristriata* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| MTD T 4209 | *pulchristriata* | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |