JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Accepted on 1 August 2011
J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2011.00632.x

Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, Dresden, Germany

Identity of *Pelodiscus sinensis* revealed by DNA sequences of an approximately 180-year-old type specimen and a taxonomic reappraisal of *Pelodiscus* species (Testudines: Trionychidae)

Heiko Stuckas and Uwe Fritz

Abstract

Recent studies identified several distinct genetic lineages within the softshell turtle genus *Pelodiscus* that could represent valid species. Traditionally, *Pelodiscus* was regarded to comprise only a single species (*P. sinensis*). These softshell turtles are economically the most important chelonians of the world, with hundreds of millions of specimens traded as food every year. Moreover, *Pelodiscus* is used as a model organism for embryological and physiological studies, making correct species identification of paramount interest for disciplines beyond taxonomy. However, the understanding of the diversity of *Pelodiscus* was seriously hampered by the unclear taxonomic allocation of the oldest available species name, *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834. To clarify its identity, we reconstructed two mitochondrial DNA fragments of 1013 bp (cyt*b*) and 468 bp (ND4) length of one of the two surviving syntypes and designate this specimen as lectotype (ZMB 38, Museum für Naturkunde Berlin). The sequences obtained from the lectotype represent a previously unknown lineage. Using the phylogenetic placement of all lineages and uncorrected *p* distances of the mitochondrial cyt*b* gene as a yardstick, we suggest that the observed sequence variation is consistent with the existence of at least four distinct species within *Pelodiscus*. The name *P. sinensis* should be restricted to turtles harbouring the mitochondrial lineages B, C, D and the lineage of the lectotype. More divergent lineages are to be identified with *P. axenaria*, *P. maackii* and *P. parviformis*, which are recognized as valid species.

Key words: Asia – softshell turtle – phylogeny – taxonomy

Introduction

Softshell turtles of the genus *Pelodiscus* are distributed over a large native range from the Amur and Ussuri River basins in the Russian Far East and Korea through central and southern China (including Hainan and Taiwan) to Vietnam (Iverson 1992; Ernst et al. 2000). These turtles are economically the most important chelonians of the world, constituting a highly appreciated part of many dishes of the East and South-east Asian cuisine. For this purpose, *Pelodiscus* turtles have been bred and traded in high numbers for centuries, leading to the establishment of many populations outside their native distribution range (see Fritz et al. 2010 for details). Currently, there are more than 300 million turtles sold in China alone, and most of them are farmed *Pelodiscus* (Shi et al. 2008). Whereas most authors recognized over the past fifty years only a single *Pelodiscus* species (*P. sinensis*), a recent paper (Fritz et al. 2010) provided evidence that this genus consists of at least seven genetic lineages, two of which were nomenclaturally treated as distinct species (*P. axenaria* and *P. maackii*). However, for *P. axenaria* doubts remained whether an older name exists, and for the other five lineages, the assignment of one of the many nomenclaturally available names was impossible. Another recent paper (Yang et al. 2011) using mtDNA sequences and morphological characters suggested that *P. parviformis* is another valid species, although contradictory results were obtained with respect to *P. sinensis*. Of outstanding importance is in this context the completely unclear taxonomic allocation of the oldest available name, *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834, rendering the assignment of any younger name highly speculative. The present study aims at clarifying the identity of this taxon using mitochondrial DNA sequences obtained from the historical type specimens. Based on this, a taxonomic reappraisal of all previously identified genetic lineages of *Pelodiscus* is attempted. *Pelodiscus* is used as a model organism for embryological and physiological studies, making correct species identification of paramount interest for disciplines beyond taxonomy (Fritz et al. 2010).

Corresponding author: Uwe Fritz (uwe.fritz@senckenberg.de)
Contributing author: Heiko Stuckas (heiko.stuckas@senckenberg.de)

Materials and Methods

The type specimens of *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834

Trionyx (*Aspidonectes*) *sinensis* was described in 1834 by the German zoologist Arend Friedrich August Wiegmann (2 June 1802–15 January 1841) in his seventh zoological contribution based on the collections of Franz Julius Ferdinand Meyen (28 June 1804–2 September 1840). Meyen, a well-known German botanist and physician, circumnavigated the earth as physician aboard the Princess Louise from 1830 to 1832 and collected animals and plants. Wiegmann's description of '*Trionyx* (*Aspidonectes*) *sinensis*' was based on juvenile softshell turtles that were 'found in the water of a paddy field on a small island in the Tiger River, close to Macao' (Wiegmann 1834: p. 195, literally translated from German). Even though Wiegmann did not mention the number of specimens, the records of the Zoological Museum Berlin (now Museum für Naturkunde) indicate that there were originally three specimens, bearing the catalogue numbers ZMB 37–39. Specimen ZMB 37 is now lost, while ZMB 38 and ZMB 39 are still present and should be regarded as syntypes (Fritz et al. 1994). Both are stuffed dry specimens that shrunk considerably over time. Only ZMB 38 is mounted on a wooden base. The maximum straight line carapace length of ZMB 38 (Fig. 1) is today approximately 75 mm; of ZMB 39, approximately 52 mm.

Chosen markers and primer design

Fritz et al. (2010) have shown that all currently known lineages of *Pelodiscus* are diagnosable by the cytochrome *b* gene (cyt*b*) and

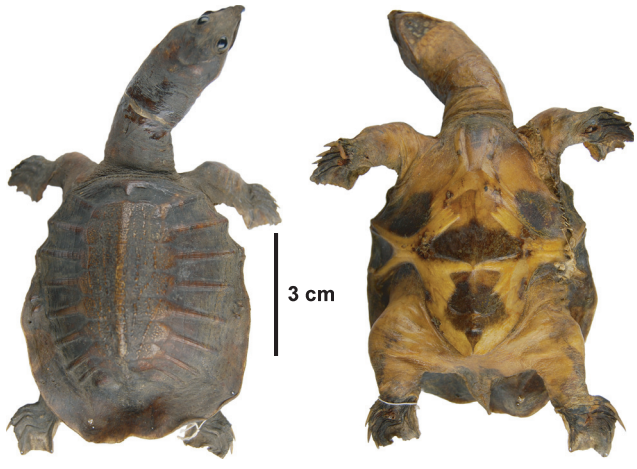

Fig. 1. Dorsal and ventral aspects of the herein designated lectotype of *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834 (ZMB 38, Museum für Naturkunde Berlin), removed from its wooden base

another mitochondrial DNA fragment, corresponding to the second half of the NADH dehydrogenase subunit 4 (ND4) gene plus adjacent DNA coding for tRNAs. To reconstruct these two DNA sequences from the historical type specimens, a first set of primer pairs was developed that targeted the most conserved regions within the cyt*b* and ND4 genes; the sequence data of Fritz et al. (2010; see Table 1 for accession numbers) served as templates. The resulting short non-overlapping DNA fragments were then used for designing a specifically fitting second primer set to fill the gaps. Obtained DNA fragments were of 53–195 bp (cyt*b*) and 36–207 bp length (ND4) after the primer sequences were trimmed (Table S1) and overlapped by 23–104 bp (cyt*b*) and 19–98 bp (ND4) to avoid concatenating authentic and possible contaminant sequences. The resulting contigs were of 1013 bp (cyt*b*) and 468 bp length (ND4).

DNA extraction, PCR and sequencing

Small tissue pieces were carefully removed from the bridge region of the shell of the historical type specimens. DNA was extracted in a clean room using a HeraSafe Safety Cabinet KSP9 (Thermo Fisher Scientific, Waltham, MA, USA) and the AGOWA sbeadex® Forensic Kit (AGOWA, Berlin, Germany) according to the standard protocol recommended by the supplier. This clean room is physically isolated from all other DNA processing facilities, and no *Pelodiscus* samples have been studied there before. Also the PCR set-up was performed in a laminar flow cabinet of the clean room using a final volume of 25 µl containing 1 unit *Taq* polymerase (Bioron, Ludwigshafen, Germany) with the buffer recommended by the supplier, a final concentration of 0.2 mM of each dNTP (Fermentas, St. Leon-Rot, Germany), 0.4 µM of the respective primer pair (Table S1) and 0.4 µg of bovine serum albumin (Fermentas). Workstations and clean room were irradiated with UV light at least 6 h before and after every working step.

Thermocycling was carried out in the main laboratory, and a positive control (containing DNA of a fresh *Pelodiscus* sample, extracted with standard methods in the normal DNA processing facility) and a negative control (all reagents except the DNA template) were always processed downstream along with the type samples. The following cycling conditions were used: 40 cycles with denaturation at 95°C for 45 s, but for 5 min in the first cycle; annealing for 45 s for the cyt*b* but 1 min for the ND4 fragment at a primer-specific temperature (Table S1); and extension at 72°C for 1 min, but 10 min in the final cycle. PCR products were purified using the ExoSAP-IT enzymatic cleanup (1 : 20 dilution; modified protocol: 30 min at 37°C, 15 min at

Table 1. Distinct GenBank haplotypes of *Pelodiscus* species and sequences of the lectotype of *Trionyx* (*Aspidonectes*) *sinensis* used for phylogenetic reconstructions (GenBank accession numbers, in brackets aligned length). Identification of GenBank sequences follows Fritz et al. (2010), except for the new sequences referred to *P. axenaria* and *P. parviformis*. For phylogenetic analyses, only sequences originating from the same individual were merged; missing data were filled in the alignment with Ns

	cyt*b* + tRNA-Thr (1168 bp)	ND4 + tRNA-His + tRNA-Ser + tRNA-Leu (860 bp)	*n*
Pelodiscus sinensis lectotype ZMB 38	FR851459 (partial cyt*b* only, 1013 bp)	FR851460 (partial ND4 only, 468 bp)	1
Pelodiscus axenaria	AY583693 (cyt*b* only, 1140 bp)	n/a	17?
Pelodiscus lineage B	FM999012 (complete, 1168 bp)	FM999020 (complete, 860 bp)	2
Pelodiscus lineage B	FM999013 (complete, 1168 bp)	FM999021 (complete, 860 bp)	1
Pelodiscus lineage B	AY687385[1] (complete, 1168 bp)	AY687385[1] (complete, 860 bp)	1
Pelodiscus lineage B	FM999014 (complete, 1168 bp)	FM999022 (complete, 860 bp)	12
Pelodiscus lineage B	FM999015 (complete, 1168 bp)	FM999023 (complete, 860 bp)	1
Pelodiscus lineage C	FM999016 (complete, 1168 bp)	FM999024 (complete, 860 bp)	3
Pelodiscus lineage D	FM999017 (complete, 1168 bp)	FM999025 (complete, 860 bp)	1
Pelodiscus lineage D	FM999018 (complete, 1168 bp)	FM999026 (complete, 860 bp)	1
Pelodiscus lineage I	AY259553 (cyt*b* only, 1140 bp)	n/a	1
Pelodiscus lineage I	AY583692 (cyt*b* only, 1140 bp)	n/a	17?
Pelodiscus lineage II	n/a	AY259603 (partial ND4 + tRNA-His only, 698 bp)	1
Pelodiscus maackii	FM999011 (complete, 1168 bp)	FM999019 (complete, 860 bp)	8
Pelodiscus maackii	AY962573[1] (complete, 1168 bp)	AY962573[1] (complete, 860 bp)	1
New GenBank sequences (Yang et al. 2011)			
Pelodiscus axenaria	HQ116592 (partial cyt*b* only, 990 bp)	HQ116584 (partial ND4 only, 680 bp)	1
Pelodiscus axenaria	HQ116593 (partial cyt*b* only, 990 bp)	HQ116585 (partial ND4 only, 680 bp)	1
Pelodiscus axenaria	HQ116594 (partial cyt*b* only, 990 bp)	HQ116586 (partial ND4 only, 680 bp)	1
Pelodiscus axenaria	HQ116595 (partial cyt*b* only, 990 bp)	HQ116587 (partial ND4 only, 680 bp)	1
Pelodiscus axenaria	HQ116596 (partial cyt*b* only, 990 bp)	HQ116588 (partial ND4 only, 680 bp)	1
Pelodiscus parviformis	HQ116597 = HQ116599 (partial cyt*b* only, 990 bp)	HQ116589 = HQ116591 (partial ND4 only, 680 bp)	2
Pelodiscus parviformis	HQ116598 (partial cyt*b* only, 990 bp)	HQ116590 (partial ND4 only, 680 bp)	1

The column *n* indicates the number of individuals sharing the respective haplotype (for details, see Fritz et al. 2010; Yang et al. 2011).
[1]Complete mitochondrial genome; sequencing error in AY962573 corrected (see Fritz et al. 2010).

80°C; USB Europe GmbH, Staufen, Germany) and sequenced on an ABI 3130xl Genetic Analyser (Applied Biosystems, Foster City, CA, USA) using the same primers (Table S1) and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Alignment and phylogenetic analyses

The type sequences were aligned in BIOEDIT 7.0.5.2 (Hall 1999) with the data set of Fritz et al. (2010) and new GenBank sequences labelled as *Pelodiscus axenaria* and *P. parviformis* (Yang et al. 2011; for accession numbers, see Table 1). MEGA 4.0.2 (Tamura et al. 2007) was used for exploring sequences and computing uncorrected *p* distances. For phylogenetic purposes, cyt*b* and ND4 sequences were concatenated when it was clear that they originated evidently from the same individual (Fritz et al. 2010; Yang et al. 2011; Table 1), and identical sequences were removed from the alignments. For some GenBank sequences, only one of the two partitions was available. Then, missing positions were filled with Ns for phylogenetic reconstructions.

Phylogenetic relationships of haplotypes were inferred using MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003), with the best evolutionary model for each fragment established in MRMODELTEST (Nylander 2004) by the AIC (Table S2). Evolutionary models were defined without priors for alpha-parameter, points of invariance and rate matrix, and two parallel runs, each with four chains, were conducted. The heating parameter λ was set to 0.2 to obtain convergence. The chains ran for 10 million generations with every 100th generation sampled; the burn-in was set to 2.5 million generations to assure that only the plateau of the most likely trees was sampled. These trees were used for generating a 50% majority rule consensus tree. The posterior probability of any individual clade in this consensus tree corresponds to the percentage of all trees containing that clade and is a measure of clade frequency and credibility. In addition to Bayesian analysis (BA), the maximum-likelihood (ML) approach as implemented in RAxML 7.0.3 (Stamatakis 2006) was applied. Five independent ML searches were performed using different starting conditions and the fast bootstrap algorithm to explore the robustness of the phylogenetic trees by comparing the best-scored trees. Subsequently, 1000 non-parametric thorough bootstrap replicates were calculated and plotted against the best scoring tree with the highest likelihood value obtained from the five independent searches.

Results

Trials to extract and amplify DNA from one of the historical type specimens (ZMB 39) failed completely. The larger specimen ZMB 38, which is designated herein as lectotype of *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834, yielded visible PCR products and clearly readable short DNA sequences of 36–207 bp length (Table S1) that were concatenated and aligned with other sequence data for phylogenetic analyses.

The calculations based on an alignment of 2028 bp total length returned the same topologies under BA and ML, with four well-supported major clades (Fig. 2). The sequences of ZMB 38 are distinct from all previously identified haplotypes of *Pelodiscus* and clustered as sister to the two haplotypes of lineage D. Lineage D and the type sequences together constitute the sister group of lineage C, and lineage B is their successive sister clade. In the calculations were also included new GenBank haplotypes assigned to *P. axenaria* and *P. parviformis* (Yang et al. 2011; Table 1), the latter being a recently described small-sized species of questionable identity (Tang 1997; Fritz et al. 2010; Yang et al. 2011). These new *P. parviformis* haplotypes grouped with high support with earlier published GenBank sequences (cyt*b*: AY259553, AY583692; ND4: AY259603) that were identified by Fritz et al. (2010) with their lineages I and II. The new haplotypes ascribed to *P. axenaria* (Yang et al. 2011) were placed together with an earlier published cyt*b* sequence of *P. axenaria* (AY583693) in

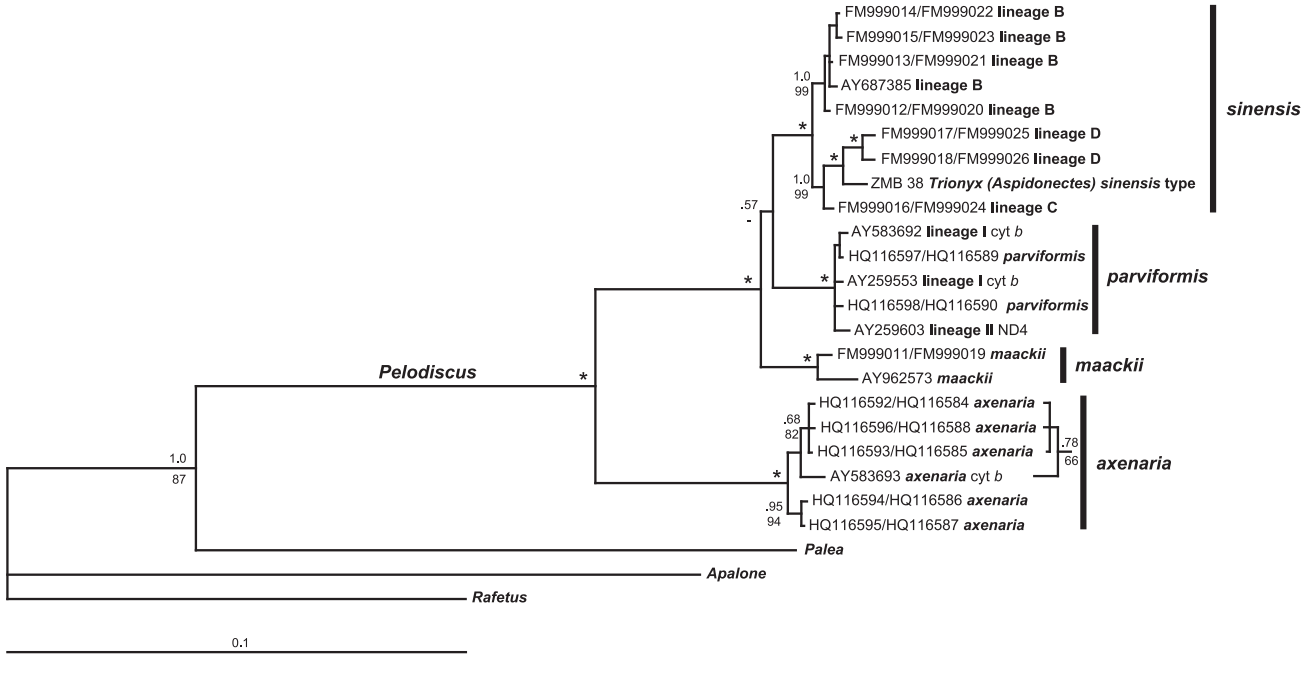

Fig. 2. Bayesian tree for mitochondrial haplotypes of *Pelodiscus*, based on the two mitochondrial partitions concatenated (2028 bp; Table 1). Codes preceding species or lineage names are GenBank accession numbers, and for the type specimen of *Trionyx* (*Aspidonectes*) *sinensis*, the catalogue number. Accession numbers of outgroup sequences: *Apalone spinifera* – AY259557, AY259607; *Palea steindachneri* – AY259552, AY259602; *Rafetus euphraticus* – AY259554, AY259604. Numbers above branches are posterior probabilities; below branches, thorough bootstrap values calculated using RAxML (not shown for some terminal clades with short branch lengths). Asterisks indicate maximum support under both methods. Suggested species delineation on the right

Table 2. Uncorrected *p* distances (means, expressed as percentages) among and within species and lineages of *Pelodiscus* based on haplotype divergences (1168-bp-long alignment of cyt*b* + tRNA-Thr). Haplotypes of lineage I (Fritz et al. 2010) are included in *P. parviformis*. Below the diagonal distances among species and lineages are given; divergences within species and lineages are shown on the diagonal in bold

	n	Type	B	C	D	*P. axenaria*	*P. maackii*	*P. parviformis*	*P. sinensis*
Lectotype	1	–							
Lineage B	3	1.39	**0.11**						
Lineage C	1	1.00	0.68	–					
Lineage D	2	1.19	1.54	1.20	**0.34**				
Pelodiscus axenaria	4	8.68	8.10	7.90	8.66	**0.47**			
Pelodiscus maackii	2	3.28	2.78	2.78	3.47	8.61	**1.20**		
Pelodiscus parviformis	4	2.97	2.36	2.36	3.30	7.75	2.73	**0.26**	
Pelodiscus sinensis (type + B + C + D)	7	–	–	–	–	8.32	3.05	2.71	**1.05**

n = number of distinct haplotypes.

the same well-supported clade. Like in previous analyses (Fritz et al. 2010; Yang et al. 2011), *P. axenaria* turned out as the most distinct lineage being the sister group to all other *Pelodiscus*.

Pelodiscus axenaria is also the most divergent genetic lineage with respect to uncorrected *p* distances (Table 2). The genetic distances among the lectotype and lineages B, C and D are clearly lower than the divergences among *P. axenaria*, *P. maackii* and *P. parviformis* and when these species are compared to lineages B, C, D and the lectotype. The within-lineage divergence of *P. maackii* resembles the value that is observed when the sequences of lineages B, C, D and the lectotype are lumped together.

Discussion

The taxonomy of *Pelodiscus* is considerably blurred by the unclear allocation of the oldest available name, *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834. The new sequences of Yang et al. (2011) clarified that cyt*b* and ND4 sequences from GenBank assigned by Fritz et al. (2010) to their lineages I and II represent the same species. This species is identified by Yang et al. (2011) with *P. parviformis*. Furthermore, Yang et al. (2011) provided evidence for the species status of their *P. parviformis* and *P. axenaria*, because the two taxa occur sympatrically in Quanzhou County, Guangxi, China. However, Yang et al. (2011) failed to rule out that *P. sinensis* could be a senior synonym of *P. axenaria* or *P. parviformis*. Moreover, Yang et al. (2011) obtained contradictory results in phylogenetic analyses. GenBank sequences of '*P. sinensis*' were sometimes paraphyletic with respect to '*P. parviformis*'. This discrepancy is easily explained by the fact that one of the GenBank sequences (AY962573) used by Yang et al. (2011) represents *P. maackii* and not *P. sinensis* (Fritz et al. 2010). This underlines once more the need to disentangle the taxonomic identity of *P. sinensis*.

The mitochondrial sequences of the herein designated lectotype of *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834 are distinct from all previously identified genetic lineages of *Pelodiscus* (Fritz et al. 2010; Yang et al. 2011) and represent another lineage, being closely allied to lineages D, C and B of Fritz et al. (2010). Consequently, *P. sinensis* is neither a senior synonym of *P. axenaria* nor of *P. parviformis*. However, except for the latter two taxa, it remains unclear whether the observed sequence variation represents intraspecific or interspecific variation and whether *P. parviformis* could be conspecific with another lineage except *P. axenaria*.

The sequence divergence of the cyt*b* gene has often been used as a yardstick to assess the species status of chelonians (see the review in Vargas-Ramírez et al. 2010). The divergences among *Pelodiscus* lineages (uncorrected *p* distances of 0.68–8.68%; Table 2) fall in part into the range as observed among distinct species of other chelonian genera (2.8–18.3% for cyt*b*; Vargas-Ramírez et al. 2010; Praschag et al. 2011). Also when *P. axenaria* is excluded, there are still values of 0.68–3.47% obtained, suggesting that yet more than one species is involved.

Unfortunately, except for *P. axenaria* and *P. parviformis* it is unknown whether any lineages occur in syntopy. In such situations, the use of character congruence is advisable for taxonomic classification (Padial et al. 2010). Like in many other softshell turtles, external morphology of *Pelodiscus* is not very helpful, however. Only adult *P. maackii* from the northernmost part of the range of *Pelodiscus* are easily identified by their distinctive colour pattern and large size (Fritz et al. 2010), together with the genetic differences of *P. maackii* qualifying for full species status. For all other *Pelodiscus* lineages, morphological data are deficient or completely lacking (compare also the recent study by Yang et al. 2011). In analogy to the recently suggested candidate species approach for genetically distinct lineages (Vieites et al. 2009), we tentatively regard *P. parviformis* as valid because its genetic divergence resembles the degree of differentiation of *P. maackii* (Table 2). By contrast, the divergences among lineages B, C, D and the lineage represented by the lectotype of *Trionyx* (*Aspidonectes*) *sinensis* are lower, which is why we suggest to treat these differences as intraspecific variation of *P. sinensis*. This view is also supported by the similar within-lineage variation of *P. maackii* and *P. sinensis* sensu stricto.

In conclusion, we propose to recognize the following four *Pelodiscus* species as valid: *P. axenaria* (Zhou, Zhang & Fang, 1991), *P. maackii* (Brandt, 1857), *P. parviformis* Tang, 1997 and *P. sinensis* (Wiegmann, 1834). We cannot exclude that older available names exist for *P. axenaria* and *P. parviformis* (see the list of available names for *Pelodiscus* species in Fritz et al. 2010), but for the time being, these taxa should be treated under the proposed names to enable a nomenclatural distinction.

Acknowledgements

Mark-Oliver Rödel (Museum für Naturkunde Berlin) allowed tissue sampling of the type specimens. Thanks for laboratory work go to Anja Rauh and Christian Kehlmaier.

Zusammenfassung

DNA-Sequenzen eines etwa 180 Jahre alten Typusexemplars klären die Identität von Pelodiscus sinensis *und eine taxonomische Neubewertung von* Pelodiscus-*Arten*

Vor kurzem wurden in der Weichschildkröten-Gattung *Pelodiscus* mehrere verschiedene genetische Linien identifiziert, die unterschiedlichen Arten entsprechen könnten. Traditionell wurde die Gattung *Pelodiscus* mit der einzigen Art *P. sinensis* als monotypisch betrachtet. *Pelodiscus* sind als Nahrungsmittel weltweit die ökonomisch wichtigsten Schildkröten, mit einem jährlichen Handelsvolumen von mehreren hundert Millionen Exemplaren. Außerdem wird *Pelodiscus* als Modellorganismus für embryologische und physiologische Studien genutzt, so dass eine korrekte Artidentifikation auch jenseits der Taxonomie von großer Bedeutung ist. Das Verständnis der Diversität von *Pelodiscus* wurde bislang jedoch massiv durch die unklare taxonomische Identität des ältesten verfügbaren Namens, *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834, behindert. Um dies zu klären, rekonstruierten wir aus einem der beiden heute noch vorhandenen Syntypen zwei mitochondriale DNS-Fragmente mit einer Länge von 1013 bp (cyt*b*) bzw. 468 bp (ND4) und bestimmen dieses Exemplar als Lectotypus (ZMB 38, Museum für Naturkunde Berlin). Die Lectotypus-Sequenzen gehören zu einer bislang unbekannten genetischen Linie. Die Phylogenie und unkorrigierte *p*-Distanzen des cyt*b*-Gens aller Linien lassen darauf schließen, dass die Gattung *Pelodiscus* mindestens vier verschiedene Spezies enthält. Der Name *P. sinensis* sollte nur auf Schildkröten angewendet werden, die die mitochondriale Linie des Lectotypus oder die bereits früher identifizierten Linien B, C oder D aufweisen. Weitere, stärker divergente Linien lassen sich *P. axenaria*, *P. maackii* und *P. parviformis* zuordnen, die als valide Spezies anerkannt werden.

References

Ernst CH, Altenburg RGM, Barbour RW (2000) Turtles of the World. World Biodiversity Database, CD-ROM Series, Windows, Version 1.2. Biodiversity Center of ETI, Amsterdam.

Fritz U, Obst F-J, Günther R (1994) Kritischer Typen-Katalog der Schildkrötensammlung (Reptilia: Testudines) des Zoologischen Museums Berlin. Mitt Zool Mus Berl **70:**157–175.

Fritz U, Gong S, Auer M, Kuchling G, Schneeweiss N, Hundsdörfer AK (2010) The world's economically most important chelonians represent a diverse species complex (Testudines: Trionychidae: *Pelodiscus*). Org Divers Evol **10:**227–242.

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41:**95–98.

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

Nylander JAA (2004) MrModeltest, v2. Evolutionary Biology Centre, Uppsala University, Uppsala, http://www.abc.se/~nylander/ [accessed 17 January 2011].

Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. Front Zool **7:**16.

Praschag P, Stuckas H, Päckert M, Maran J, Fritz U (2011) Mitochondrial DNA sequences suggest a revised taxonomy of Asian flapshell turtles (*Lissemys* Smith, 1931) and the validity of previously unrecognized taxa (Testudines: Trionychidae). Vertebr Zool **61:**147–160.

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

Shi HT, Parham JF, Fan ZY, Hong ML, Yin F (2008) Evidence for the massive scale of turtle farming in China. Oryx **42:**147–150.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22:**2688–2690.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol **24:**1596–1599.

Tang Y (1997) Research on a new species of *Pelodiscus*, Trionychidae in China. Zool Res Kunming Inst Zool **18:**13–17 [in Chinese, with English abstract].

Vargas-Ramírez M, Vences M, Branch WR, Daniels SR, Glaw F, Hofmeyr MD, Kuchling G, Maran J, Papenfuss TJ, Široký P, Vieites DR, Fritz U (2010) Deep genealogical lineages in the widely distributed African helmeted terrapin: evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). Mol Phylogenet Evol **56:**428–440.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. PNAS **106:** 8267–8272.

Wiegmann AFA (1834) Beiträge zur Zoologie, gesammelt auf einer Reise um die Erde, von Dr. F.J.F. Meyen, M.d.A.N. Siebente Abhandlung. Amphibien. Nova Acta Acad Caes Leop-Carol German Nat Curios **17:**183–268.

Yang P, Tang Y, Ding L, Guo X, Wang Y (2011) Validity of *Pelodiscus parviformis* (Testudines: Trionychidae) inferred from molecular and morphological analyses. Asian Herpetol Res **2:**21–29.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers used for PCR and sequencing of the mtDNA fragments containing the cyt*b* and ND4 genes.

Table S2. Best evolutionary models and their parameters selected by MRMODELTEST (AIC).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.