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New data on the diversity of the Southeast Asian leaf turtle genus *Cyclemys* BELL, 1834. Molecular results (Reptilia: Testudines: Geoemydidae*)

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With 2 Tables and 4 Figures

Kurzfassung. **Neue Daten zur Diversität der südostasiatischen Dornschildkröten-Gattung *Cyclemys* BELL, 1834. Molekulare Ergebnisse (Reptilia: Testudines: Geoemydidae). –** Um die intragenerische Diversität von *Cyclemys* aufzuklären, wurden 38 Proben untersucht, die alle fünf derzeit anerkannten Arten der Gattung umfassen. Es wurde ein 982 nt langes Fragment des mitochondrialen Cytochrom *b*-Gens sequenziert und bei 28 Proben wurden erfolgreich Fingerprint-Untersuchungen des Genoms mit ISSR-PCR durchgeführt. Beide Methoden erbrachten weitestgehend kongruente Resultate und deckten eine wesentlich stärkere Differenzierung auf, als durch morphologische Merkmale bzw. die derzeit anerkannte Taxonomie widergespiegelt wird. Hinter der bislang anerkannten Art *Cyclemys oldhamii* verbergen sich drei distinkte Arten (*Cyclemys oldhamii* GRAY, 1863 sensu stricto, *Cyclemys shanensis* ANNANDALE, 1918, *Cyclemys* n. sp. 1). Eine dieser Arten, *Cyclemys shanensis*, ist mit *Cyclemys tcheponensis* (BOURRET, 1939) konspezifisch, die zu einer Unterart von *Cyclemys shanensis* abgewertet wird. Eine weitere kryptische Art (*Cyclemys* n. sp. 2) wurde im *Cyclemys atripons-pulchristriata*-Komplex enttarnt. Trotz der morphologischen Ähnlichkeit zu *Cyclemys atripons* IVERSON & MCCORD, 1997 und *Cyclemys pulchristriata* FRITZ, GAULKE & LEHR, 1997 scheint *Cyclemys* n. sp. 2 nach den Cytochrom *b*-Daten *Cyclemys oldhamii* sensu stricto von der Malaiischen Halbinsel und *Cyclemys dentata* (GRAY, 1831) näher zu stehen.

Abstract. To elucidate intrageneric diversity, 38 samples representing all five currently recognised species of *Cyclemys* were analysed by sequencing a 982 nt fragment of the mitochondrial cytochrome *b* gene and genomic fingerprinting with ISSR-PCR. The latter technique yielded data for 28 samples. Both methods produced highly congruent results and detected a much higher differentiation than reflected by morphology and current taxonomy. Behind the hitherto recognised species *Cyclemys oldhamii*, three distinct species are hidden (*Cyclemys oldhamii* GRAY, 1863 sensu stricto, *Cyclemys shanensis* ANNANDALE, 1918, *Cyclemys* n. sp. 1). One of these species, *Cyclemys shanensis*, is conspecific with *Cyclemys tcheponensis*

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* Geoemydidae is used as BOUR & DUBOIS (1986) demonstrated that this name has nomenclatural priority over Bataguridae.

(BOURRET, 1939), which is downgraded as a subspecies of *Cyclemys shanensis*. A further cryptic species (*Cyclemys* n. sp. 2) was uncovered in the *Cyclemys atripons-pulchristriata* complex. Despite the morphological similarity between *Cyclemys* n. sp. 2, *Cyclemys atripons* IVERSON & MCCORD, 1997 and *Cyclemys pulchristriata* FRITZ, GAULKE & LEHR, 1997, the cytochrome *b* data suggest that *Cyclemys* n. sp. 2 is more closely related to *Cyclemys oldhamii* sensu stricto from the Malay peninsula and *Cyclemys dentata* (GRAY, 1831).

Key words. Reptilia, Testudines, Geoemydidae, *Cyclemys*, *Cyclemys atripons*, *Cyclemys dentata*, *Cyclemys dhor shanensis*, *Cyclemys oldhamii*, *Cyclemys pulchristriata*, *Cyclemys shanensis shanensis* n. comb., *Cyclemys shanensis tcheponensis* n. comb., *Cyclemys tcheponensis*, new species, taxonomy, ISSR-PCR, cytochrome *b* sequences, Southeast Asia.

Introduction

Until recently, it was believed that the Southeast Asian leaf turtle genus *Cyclemys* BELL, 1834 contains only one or two species: *Cyclemys dentata* (GRAY, 1831) and possibly *C. tcheponensis* (BOURRET, 1939). In 1997 two papers appeared around the same time increasing the number of *Cyclemys* species to five. Based on external morphology, FRITZ et al. (1997) published a revision of the genus and recognised four species differing significantly in coloration and scute seam proportions on the plastron: (1) *Cyclemys dentata* (GRAY, 1831) sensu stricto, spread over the Malay peninsula (including southernmost Thailand), Sumatra, and Java; it also occurs on Borneo and some islands of the Palawan and Sulu regions of the Philippines; (2) *Cyclemys pulchristriata* FRITZ, GAULKE & LEHR, 1997, known only from its type locality in central Vietnam (Annam); (3) *Cyclemys oldhamii* GRAY, 1863, distributed over NE India, Myanmar (Burma), Thailand, and the Malay peninsula as well as Borneo, Sumatra, and Java; (4) *Cyclemys tcheponensis* (BOURRET, 1939) from Chiang Mai (Thailand), Tonkin (Vietnam), and the border region between Laos and Vietnam.
In describing *C. atripons* from southeastern Thailand and Cambodia, IVERSON & MCCORD (1997) added a fifth species to the list. *C. atripons* is similar to *C. pulchristriata*, and J. IVERSON (pers. comm.) supposed that it represents the same taxon. Moreover, *C. dentata* resembles *C. atripons* and *C. pulchristriata* somewhat in that all have more or less uniform yellow plastra. In contrast, *C. tcheponensis* and *C. oldhamii* possess dark brown to black plastra. With the exception of the uniformly coloured *C. oldhamii*, the head and neck of all taxa are conspicuously striped (FRITZ et al. 1996, 1997, IVERSON & MCCORD 1997).
Later, FRITZ & ZIEGLER (1999) extended the known range of *C. pulchristriata* to southern and central Vietnam. FRITZ et al. (2001) compared *C. atripons* and *C. pulchristriata* and found out that slight but statistically highly significant coloration differences exist. Further, they detected in a 982 nt fragment of the mitochondrial cytochrome *b* gene a sequence divergence of 0.9-1%, arguing for a separation between *C. atripons* and *C. pulchristriata* 2.5 million years ago.
To find out whether the other morphological units defined by FRITZ et al. (1997) represent different taxa, we decided to use molecular methods here too. As a marker for phylogenetic distinctiveness, we analysed, as did FRITZ et al. (2001), a 982 nt fragment of the mitochondrial cytochrome *b* gene. If all five taxa listed in FRITZ et al. (1997) and IVERSON & MCCORD (1997) represent distinct species, a similar or congruent differentiation pattern displaying five major mitochondrial lineages should be detected; this should reflect a closer relationship between the morphologically similar *C. oldhamii* and *C. tcheponensis* on the one hand and (*C. atripons* + *C. pulchristriata*) and *C. dentata* on the other. To test whether nuclear markers support the mitochondrial data and whether hybridisation events may have played a role among *Cyclemys*, as known for some other Asiatic chelonians (PARHAM et al. 2001, WINK et al. 2001), we used genomic fingerprinting with Inter-Simple-Sequence-Repeat (ISSR) profiles; the latter is a powerful tool for detecting hybrids (WINK et al. 2001). We will report elsewhere on our current morphological investigations and further taxonomic consequences.
This study is a further step towards a better understanding of the taxonomy of the Southeast Asian turtle genus *Cyclemys* (FRITZ et al. 1996, 1997, 1999, 2001, FRITZ & OBST 1999, FRITZ & ZIEGLER 1999).

Materials and methods

Blood and tissue sampling: In total, blood or tissue samples from 38 *Cyclemys* were examined (Tab. 1). With the exception of *C. dentata*, the locality of the specimens we studied was known at least in regard to the region in which they were collected (Fig. 1). Taxa were determined by the morphological key characters outlined by FRITZ et al. (1997). Cambodian specimens of the *Cyclemys atripons-pulchristriata* complex were defined as *C. atripons* according to the presumed distribution of this taxon (Cambodia, SE Thailand) by IVERSON & MCCORD (1997). Vietnamese specimens fall into the range of *C. pulchristriata* (FRITZ & ZIEGLER 1999) and are treated here as such. One specimen from Cambodia, however, morphologically resembles *C. pulchristriata*; we will discuss this case later. As outgroups we used blood samples from two subspecies of *Pyxidea mouhotii*, a species long thought to be congeneric with *Cyclemys* (SMITH & JAMES 1958, WERMUTH & MERTENS 1961), and of *Cuora trifasciata* (Tab. 1).

Most specimens were obtained by P. VALENTIN (Vienna) in Myanmar (Burma) and by E. LEHR, mainly in Cambodia and Vietnam. The latter are now, in part, in the collection of the Museum für Tierkunde Dresden; some of them have been kept alive. In addition, tissue samples from specimens that stem from central Thailand and belong to the private collection of M. REIMANN (Braunweiler) were used (now MTD 40652, MTD 41615-41617), as well as *C. oldhamii* specimens, allegedly from Kachin province (Myanmar), obtained through the pet-trade. As no specimens of *C. dentata* with reliable origins were available, we studied tissue and blood samples from four pet-trade turtles said to come from Indonesia. Currently, large numbers of turtles are being exported from Sumatra and the Indonesian part of Borneo and sold to the Chinese food markets and the international pet-trade (FRITZ & GAULKE 1997, M. AULIYA and F. YUWONO pers. comm.). Thus, our *C. dentata* may have originated on one of these two islands. Moreover, the locality data of our *C. oldhamii* from Kachin province, Myanmar have to be treated with caution as the specimens were also acquired via the international pet-trade. Many cases are known where locality data of pet-trade specimens are wrong or maybe even intentionally falsified (FRITZ & OBST 1998, 1999, PARHAM et al. 2001).

Taxon	Number of Specimens	Origin	Voucher Numbers (MTD)
Cyclemys atripons	1	Orasey market in Phnom Penh, Cambodia (perhaps originating at Lake Tonle Sap, Cambodia)	42516
Cyclemys atripons	7	Northeastern Cambodia, mountain region of Siem Reap	42549, 42550, 42551, 42552, other specimens alive at the MTD
Cyclemys dentata	4	Pet-trade, Indonesia	42597, 42912, 43735
Cyclemys oldhamii	7	Myanmar (Burma), Lake Inlé	
Cyclemys oldhamii	5	Pet-trade, Myanmar (Burma), Kachin province (?)	41611, 41612, 41613, 41614, 42578
Cyclemys oldhamii	2	Malaysia, Penang	
Cyclemys oldhamii	4	Central Thailand	40652, 41615, 41616, 41617
Cyclemys oldhamii	1	Cambodia, Ratanakiri province, Srai	42509
Cyclemys pulchristriata	4	Market in Saigon, South Vietnam (most likely originating in South Vietnam)	43785, other specimens alive at the MTD
Cyclemys tcheponensis	3	Market in Lao Bao, Vietnam (most likely originating in neighbouring Laos)	42537, 44389, other specimen alive at the MTD
Cuora trifasciata	2	Pet-trade	
Pyxidea mouhotii mouhotii	2	Pet-trade	
Pyxidea mouhotii obsti	2	Central Vietnam	

Tab. 1: Specimens used in this study. Voucher numbers refer only to preserved or living specimens at the Museum für Tierkunde Dresden (MTD).

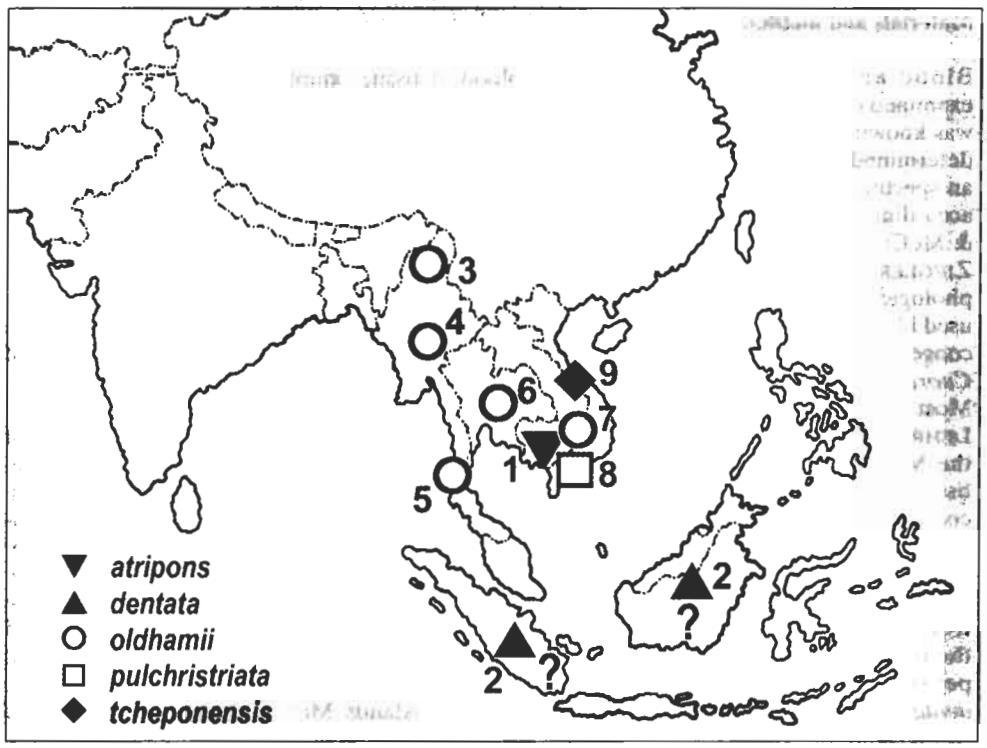

Fig. 1: Geographic origin of samples (taxa in alphabetical order, exact localities see Tab. 1). ***Cyclemys atripons***: (1) Cambodia; ***Cyclemys dentata***: (2) Indonesia, perhaps Borneo or Sumatra; ***Cyclemys oldhamii***: (3) Myanmar, Kachin province; (4) Myanmar, Lake Inlé; (5) Malaysia, Penang; (6) Central Thailand; (7) Cambodia, Ratanakiri province; ***Cyclemys pulchristriata***: (8) South Vietnam; ***Cyclemys tcheponensis***: (9) Border region of Laos and Vietnam.

Blood samples were obtained by coccygeal vein puncture as described in HASKELL & POKRAS (1994) and tissue samples (thigh muscle) by the dissection of frozen or fresh dead turtles. Samples were stored in EDTA-buffer (10% EDTA, 0.5% sodium fluoride, 0.5% thymol, 1% tris, pH 7.0; ARCTANDER 1988). Total genomic DNA was extracted from small aliquots following standard proteinase K and phenol chloroform protocols (SAMBROOK et al. 1989).

PCR and sequencing: Polymerase chain reaction (PCR) was used to amplify a fragment containing the target sequence (982 nt of the mitochondrial cytochrome *b* gene) as described in WINK et al. (2001).
PCR products were sequenced directly using the dideoxy chain termination method (SANGER et al. 1977) with the same primers and conditions chosen as in WINK et al. (2001). All sequences were aligned manually.

Phylogenetic and statistical analysis: Phylogenetic relationships among taxa and haplotypes were analysed under different criteria using the computer programmes MEGA (KUMAR et al. 2001) and PAUP version 4.0b8 (SWOFFORD 2001). For outgroup rooting, *Cuora trifasciata* and *Pyxidea mouhotii* were employed. Maximum parsimony and maximum likelihood searches were conducted with the heuristic search approach using the tree-bisection-and-reconnection swapping algorithm. For maximum parsimony the default settings were applied. Bootstrap analyses (1000 replicates) were performed under the maximum parsimony criterion to examine the robustness of tree furcations. For maximum likelihood, Modeltest Version 3.06 (POSADA & CRANDALL 1998) was used to conduct likelihood ratio tests to determine an appropriate model of sequence evolution. The TVM+G model with the settings specified by Modeltest was then used in the maximum likelihood search.

Taxon and origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *C. dentata* Indonesia	0.0152													
2 *C. oldhamii* Malaysia, Penang	0.0142	0.0000												
3 *C. pulchristriata* Vietnam, Saigon (*Cyclemys* n. sp. 2)	0.0244	0.0285	-											
4 *C. atripons* Cambodia (*Cyclemys* n. sp. 2)	0.0254	0.0295	0.0000	-										
5 *C. pulchristriata* Vietnam, Saigon	0.0437	0.0438	0.0449	0.0458	0.0020									
6 *C. atripons* Cambodia	0.0437	0.0438	0.0449	0.0458	0.0020	0.0111								
7 *C. oldhamii* Myanmar, Lake Inle (*C. shanensis*)	0.0682	0.0621	0.0693	0.0703	0.0693	0.0693	0.0020							
8 *C. oldhamii* Thailand (*C. shanensis*)	0.0691	0.0631	0.0703	0.0712	0.0702	0.0702	0.0000	0.0010						
9 *C. tcheponensis* Vietnam, Lao Bao (*C. shanensis*)	0.0721	0.0661	0.0733	0.0743	0.0712	0.0712	0.0031	0.0031	0.0000					
10 *C. oldhamii* Cambodia, Ratanakiri (*C. shanensis*)	0.0681	0.0621	0.0693	0.0702	0.0092	0.0671	0.0031	0.0031	0.0061	-				
11 *C. oldhamii* Myanmar, Kachin (?)(*Cyclemys* n. sp. 1)	0.1016	0.0936	0.1001	0.1017	0.1007	0.1016	0.0946	0.0965	0.0986	0.0965	0.0010			
12 *Pyxidea mouhotii obsti*	0.1260	0.1200	0.1283	0.1292	0.1241	0.1251	0.1242	0.1250	0.1270	0.1260	0.1148	0.0010		
13 *Pyxidea m. mouhotii*	0.1204	0.1134	0.1217	0.1226	0.1195	0.1204	0.1226	0.1234	0.1255	0.1225	0.1159	0.0153	0.0102	
14 *Cuora trifasciata*	0.1263	0.1193	0.1235	0.1244	0.1233	0.1242	0.1223	0.1232	0.1252	0.1242	0.1181	0.0703	0.0662	0.0000

Tab. 2: Genetic distances between *Cyclemys* and related species. Genetic distances (uncorrected p distances) are presented as minimum values between the lineages in the lower left section. The diagonal presents maximum distances within the lineage. Preliminary new species names suggested in the text are given in brackets.

ISSR-PCR: ISSR-PCR (inter simple sequence repeats) is a PCR-based fingerprint that uses single primers binding to microsatellite loci. Optimisation reactions were run with different primers and under variable PCR conditions. Best results (clear and polymorphic patterns) were yielded with the primer $(GACA)_4$ at an annealing temperature of 55°C and primer $(CA)_{10}$ at 42°C annealing temperature. Further conditions were chosen as described in WINK et al. (2001). PCR products were separated on a denaturing Sequagel matrix at 65W for 4 hours and visualised autoradiographically. Fragment patterns were analysed by eye and transferred into a 1/0 matrix scoring presence/absence of each particular fragment in all samples. Only bands that were unambiguously scorable were included in the analysis. The 1/0 matrix was used to calculate an UPGMA phylogenetic tree as implemented in PAUP* (SWOFFORD 2001).

Results

Our working hypothesis was that five genetic lineages in two major branches exist, reflecting morphological similarities: (*Cyclemys oldhamii* + *Cyclemys tcheponensis)* + (*Cyclemys dentata* + (*Cyclemys atripons* + *Cyclemys pulchristriata*)). However, the variation found matches only in part the five nominal species and the presumed relationships. Unexpected variation was detected especially among specimens identified as *Cyclemys oldhamii* and as *C. atripons* or *C. pulchristriata.*

Nucleotide variation: New nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank Nucleotide Sequence Database.

In *C. oldhamii* we found 9 haplotypes that belong to three distinct genetic clusters.

Specimens from Myanmar (allegedly Kachin province) are represented by two closely related haplotypes that differ from all other *Cyclemys* by an approximate 10% sequence divergence (termed *Cyclemys* n. sp. 1 in Tab. 2). Genetic distances between *C. oldhamii* from Penang, Malaysia (one haplotype) and *C. oldhamii* from Lake Inlé in Myanmar (three haplotypes), central Thailand (two haplotypes), and Cambodia are more than 6% (Tab. 2).

In the *C. atripons-pulchristriata* complex, eight haplotypes were detected. Whereas most *C.*

pulchristriata and *C. atripons* are closely related to each other, a completely different haplotype (termed *Cyclemys* n. sp. 2 in Tab. 2) separated by genetic distances of 4.5% from other *C. pulchristriata* and *C. atripons* was found in one sample of each species.
The samples of *C. dentata* contained three similar haplotypes close to the haplotype of *C. oldhamii* from the Malay peninsula. No variation was found among *C. tcheponensis* as all three specimens possess the same haplotype, which is very similar to the haplotypes of *C. oldhamii* from Lake Inlé, central Thailand, and Cambodia.

Phylogeny: Maximum parsimony, maximum likelihood, and neighbour-joining (not shown) analyses produced highly concordant trees (Fig. 2 and 3) that are supported by high bootstrap values in most furcations (Fig. 2). In all trees, *C. "oldhamii"* show up at three very different positions: Specimens allegedly from Kachin province (northern Myanmar; *Cyclemys* n. sp. 1) consistently appear as sister taxon to all other *Cyclemys*. *C. "oldhamii"* from central Thailand, Cambodia, and Lake Inlé (central Myanmar) cluster with *C. tcheponensis*, whereas *C. "oldhamii"* from the Malay peninsula appear at a remote and entirely unexpected position close to *C. dentata* and a *Cyclemys* species (*Cyclemys* n. sp. 2) identified by morphological means as *C. pulchristriata* or *C. atripons*.
This situation is further complicated by the fact that the *Cyclemys atripons-pulchristriata* complex is also consistently divided into three groups instead of two: Except for *Cyclemys* n. sp. 2, the haplotypes of the samples from Vietnam, which were identified as *C. pulchristriata*, appear as direct sister group of nearly all *C. atripons* from Cambodia, as predicted. However, one sample from Cambodia clusters together with the specimens from Vietnam. It belongs to a turtle morphologically resembling *C. pulchristriata* based on its wide head and neck stripes and will be discussed below.
In any case, *C. atripons* and *C. pulchristriata* compose the sister group of a unit including *C. dentata*, which supports our original hypothesis. However, this clade also includes *C. "oldhamii"* from the Malay peninsula, as previously mentioned, and two samples from Cambodia and Vietnam (*Cyclemys* n. sp. 2) we originally determined as *C. atripons* or *C. pulchristriata*. This means that behind the *C. atripons-pulchristriata* complex a third taxon is hidden that is morphologically highly similar to *C. atripons* and *C. pulchristriata*. However, according to mitochondrial cytochrome *b* sequences it is more closely related to *C. dentata* and *C. "oldhamii"* from the Malay peninsula. Remarkably, this cryptic species (*Cyclemys* n. sp. 2) was found in Vietnam and Cambodia together with *C. pulchristriata* and *C. atripons*.

Divergence times: Despite considerable variation between different taxa (Avise et al. 1992, Walker & Avise 1998), for many chelonians a molecular clock of 0.4% sequence divergence per 1 million years is accepted between pairs of lineages (Avise et al. 1992, Bowen et al. 1993, Lamb & Lydeard 1994, Walker & Avise 1998, Lenk et al. 1999). If the same evolution rate is applied to *Cyclemys*, the differentiation of *Cyclemys* from *Pyxidea* dates back 30 million years. Even within *Cyclemys*, differentiation ages up to 25 million years between different taxa occur, referring to the divergence time of *C. "oldhamii"* (Myanmar, allegedly Kachin province = *Cyclemys* n. sp. 1) from all other *Cyclemys*. With a genetic distance of 4.5%, the third taxon of the *C. atripons-pulchristriata* complex mentioned above (*Cyclemys* n. sp. 2) would have separated from the other two taxa 11 million years ago.

ISSR-PCR: Due to bad DNA quality, especially from some tissue samples, not all specimens yielded results. As ISSR-PCR amplifies DNA fragments of 100 to 2000 nt in length, defragmented DNA cannot be used for this method. 28 of the 38 *Cyclemys* samples were successfully amplified, and these included at least one specimen from all major groups and localities. Obtained ISSR-PCR profiles produced several polymorphic bands, 45 of which were scored and included in the phylogenetic analysis. The UPGMA unrooted phylogram is shown in Figure 4. Several genetic lineages can be distinguished that strongly support the results of the cytochrome *b* gene analyses. *C. "oldhamii"* appears on three clearly differentiated branches, representing the samples from Myanmar (allegedly Kachin province) on one branch, the samples from Malaysia (Penang) on the second, and the remaining *C. "oldhamii"* together with

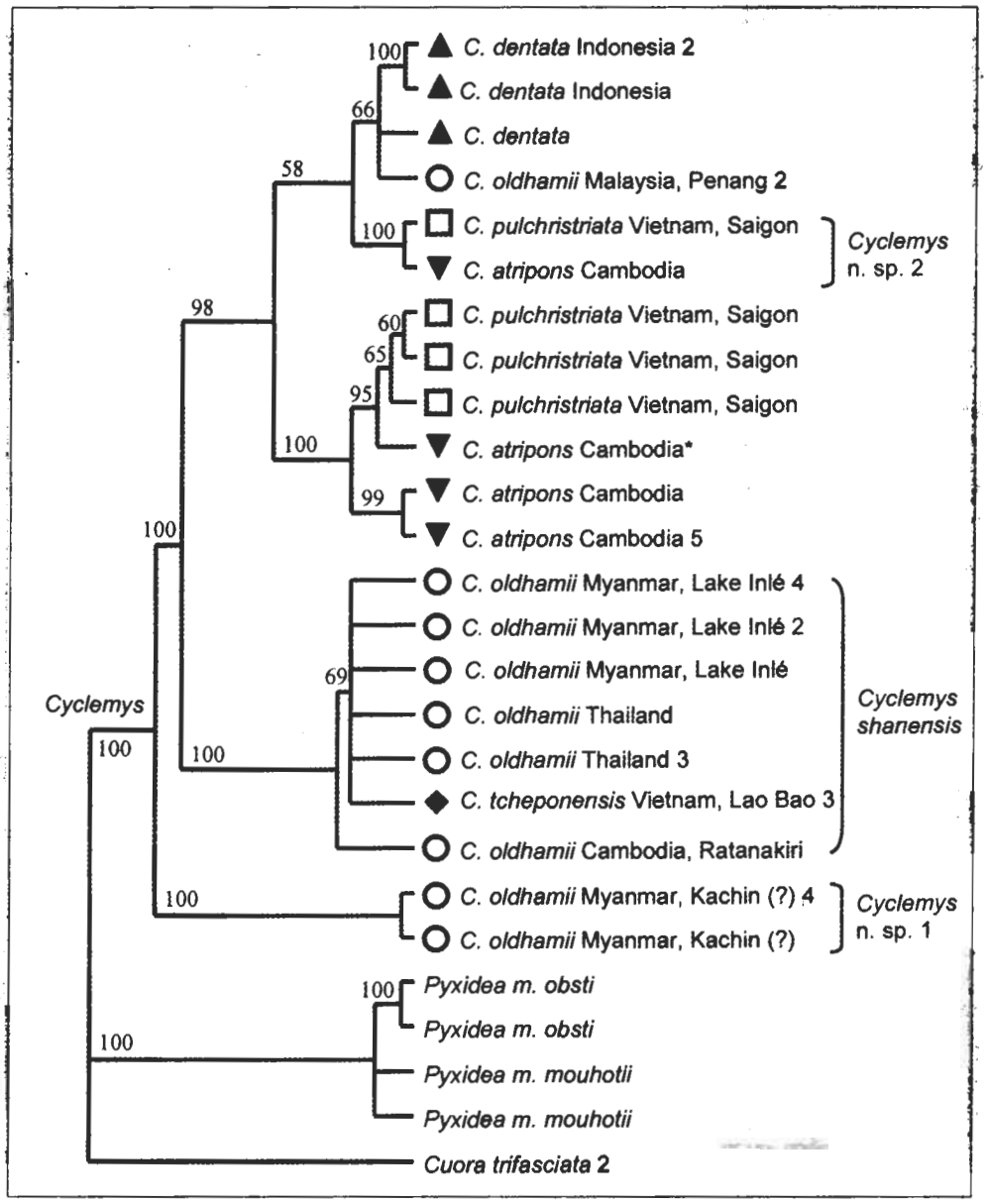

Fig. 2: Maximum parsimony strict consensus cladogram of the 8 most parsimonious trees, tree length: 1291 steps, CI = 0.2022, HI = 0.7978, RI = 0.4835, bootstrap values are based on 1000 replicates. Symbols as in Figure 1. Numbers after localities or taxa indicate more than one sample with identical sequences. On the right, preliminary new species names are given (see text). The sample marked with an asterisk (*) is from a specimen morphologically resembling *C. pulchristriata*.

C. tcheponensis on the third branch. The *C. pulchristriata* and *C. atripons* samples also occur in three groups, corroborating the findings in cytochrome *b* gene differentiation. The only difference to the cytochrome *b* trees is that the Cambodian sample that morphologically resembles *C. pulchristriata* appears on the ISSR-UPGMA tree together with the other Cambodian samples and not with the Vietnamese *C. pulchristriata*.

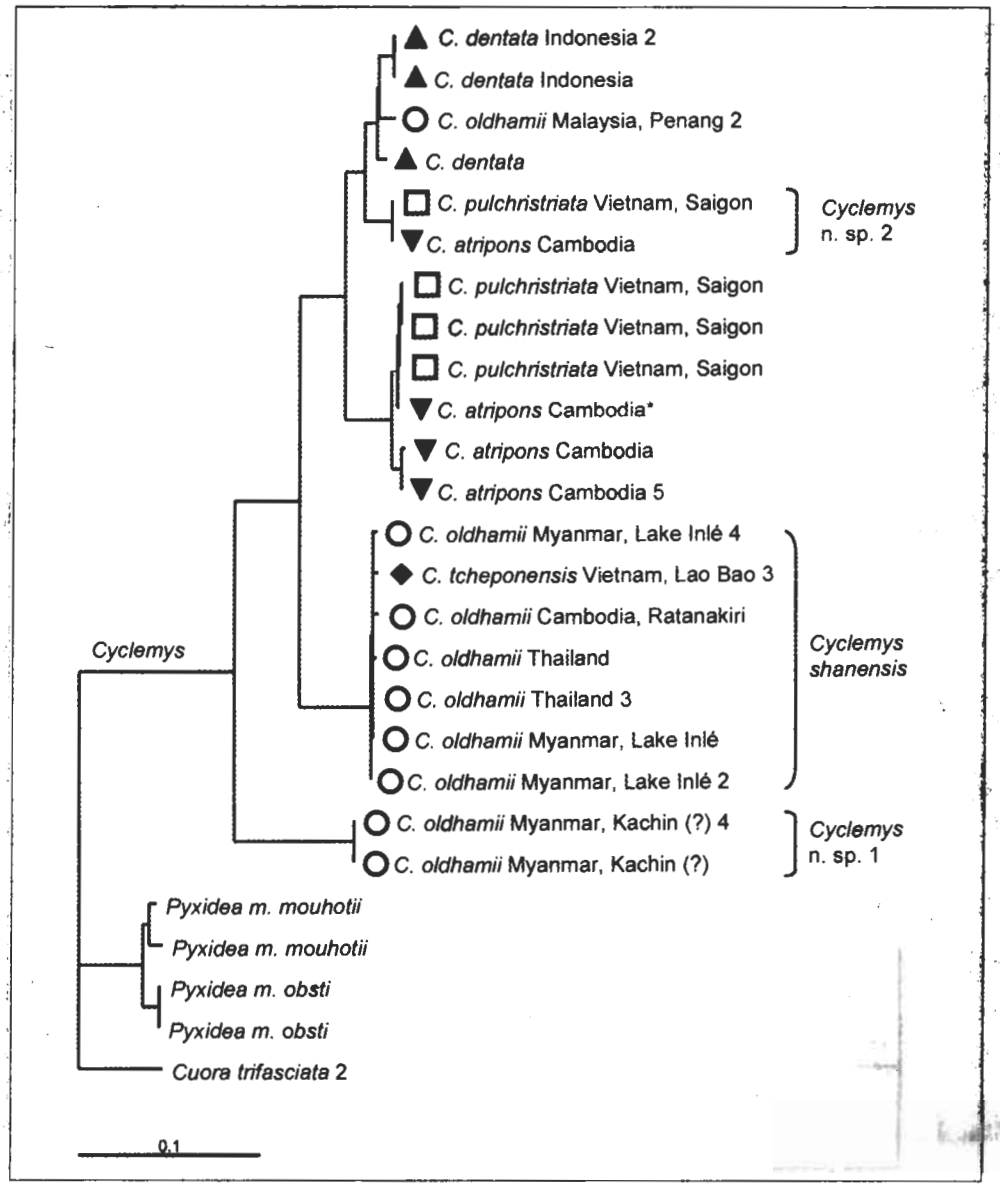

Fig. 3: Maximum likelihood phylogram calculated with the TVM+G model as selected by hierarchical likelihood ratio tests in Modeltest Version 3.06 (POSADA & CRANDALL 1998), heuristic search approach, branch-swapping algorithm: Tree-bisection-reconnection. Symbols, names, etc. as in Figure 2.

ISSR-PCR also allows testing for hybridisation events that are known to have played a role in different mammals (GYLLENSTEN & WILSON 1987, LEHMAN et al. 1991, THULIN et al. 1998) as well as among chelonians (PARHAM et al. 2001, WINK et al. 2001). This could explain the unexpected positions of the Cambodian sample of the *C. atripons-pulchristriata* complex and of *C. "oldhamii"* from the Malay peninsula in the mitochondrial cytochrome *b* trees mentioned above. If hybridisation had occurred, one would expect most ISSR-PCR fragments from the putative hybrid to occur in either of the two parental species as well. However, this is not the case.

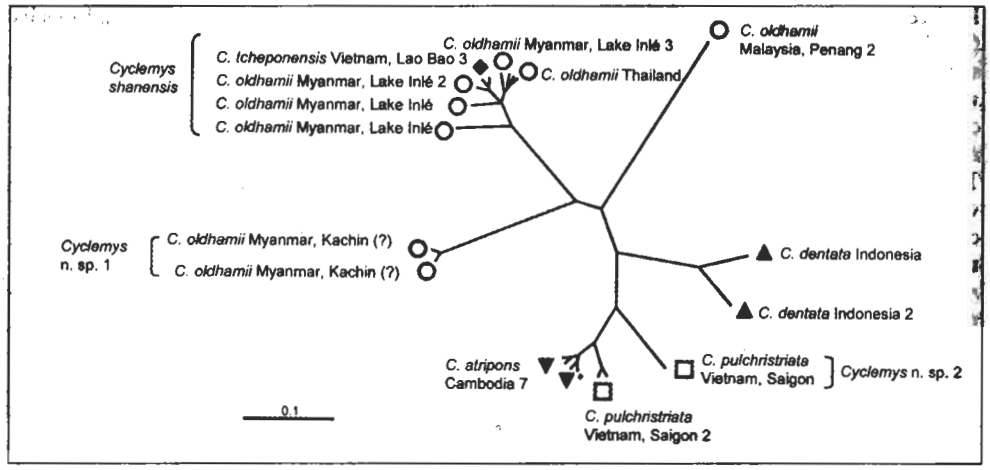

Fig. 4: UPGMA unrooted phylogram based on 45 polymorphic ISSR characters. Symbols, names, etc. as in Figure 2.

Discussion and preliminary taxonomic consequences

Our preliminary results demonstrate that *Cyclemys* is a highly diverse genus containing more taxa than previously believed. As we are far away from a complete sampling of the whole range of the genus, the diversity is suspected to be even greater than demonstrated now. In any case, our data illustrate convincingly that traditional morphological characters alone, such as body proportions or colour and pattern characters (see FRITZ et al. 1996, 1997, IVERSON & MCCORD 1997), are not sufficient for understanding the taxonomic diversity found among *Cyclemys*.

According to our molecular data, the species previously recognised as *Cyclemys oldhamii* sensu FRITZ et al. (1997) is, without doubt, a conglomerate of three distinct species that are not closely related but are morphologically very similar. One of these species, possibly originating in Kachin province, Myanmar (Burma), is even the sister taxon of all other *Cyclemys*. It is an undescribed new species that, if a molecular clock rate of 0.4% sequence divergence per million years is applied, has separated from all other *Cyclemys* some 25 million years ago, i. e. in the Late Oligocene. Unfortunately, the distribution of this taxon remains doubtful. The specimens available to us originated from the international pet-trade via Hong Kong (M. REIMANN pers. comm.). In this paper, we refer to this taxon provisionally as "*Cyclemys* n. sp. 1".

Our original hypothesis on the systematics of *Cyclemys* predicted that *C. tcheponensis* and *C. oldhamii* sensu FRITZ et al. (1997) are closely related and form their own clade. FRITZ & ZIEGLER (1999) even suggested that *C. tcheponensis* might be a subspecies of *C. oldhamii* as in Myanmar (Burma) and Thailand specimens with intermediate characters occur. This postulate has been corroborated insofar as all three specimens of *C. tcheponensis* analysed proved to be very close to *C. "oldhamii"* from central Thailand, Lake Inlé (central Myanmar), and a specimen from Ratanakiri province in Cambodia. The low sequence divergence detected argues for a recent separation of this taxon from *C. tcheponensis* and for conspecifity. The very close relationship of both is further supported by the fact that *C. "oldhamii"* specimens from central Thailand exhibit as juveniles a striped facial pattern virtually identical to *C. tcheponensis*. This pattern is lost with increasing age (FRITZ & ZIEGLER 1999, P. P. VAN DIJK pers. comm.). Otherwise there are no differences known between both taxa (FRITZ et al. 1997, FRITZ & ZIEGLER 1999). Therefore, *Cyclemys tcheponensis* (BOURRET, 1939) is tentatively regarded as conspecific here. It may be noted that the name *Cyclemys dhor shanensis* ANNANDALE, 1918 is available as species name. We have been fortunate to study specimens (FRITZ & ZIEGLER 1999) and blood samples from the type locality of *Cyclemys dhor shanensis* (Lake

Inlé, central Myanmar, this study) and can confirm that it represents the taxon which loses its facial stripes at adulthood. Hence, the subspecies from the western and central part of the species' range that loses facial stripes during ontogeny should be called *Cyclemys shanensis shanensis* ANNANDALE, 1918, whereas *Cyclemys shanensis tcheponensis* (BOURRET, 1939) n. comb. refers to the subspecies in the eastern part of the area that retains a striped head and neck throughout life.

The third species formerly hidden behind the name *C. oldhamii* is currently known to occur with certainty only on the Malay peninsula. Mergui, the type locality of *C. oldhamii*, is also located on this peninsula (FRITZ et al. 1997) so that the name *C. oldhamii* has to be restricted to this species. In contrast to the morphological similarity to *Cyclemys* n. sp. 1 and *C. shanensis*, the species from the Malay peninsula is, according to the cytochrome *b* data, much more closely related to other taxa (Figs. 2-3). In the future, it has to be investigated whether the *Cyclemys* attributed to *C. oldhamii* from Sumatra, Borneo, and Java by FRITZ et al. (1997) belong to this species too or whether they represent other taxa.

A further cryptic taxon was discovered in the *Cyclemys atripons-pulchristriata* complex, termed "*Cyclemys* n. sp. 2" here. According to mitochondrial sequence data it is more closely related to *C. dentata* and *C. oldhamii* sensu stricto from the Malay peninsula than to *C. atripons* or *C. pulchristriata*. *Cyclemys* n. sp. 2 is separated from (*C. atripons* + *C. pulchristriata*) by a genetic distance of approx. 4.5% (Tab. 2). This corresponds to an assumed divergence time of 11 million years (Late Miocene) and a distinctly longer separation than between *C. atripons* and *C. pulchristriata*. Remarkably, one specimen of *Cyclemys* n. sp. 2 was hidden among our samples from Cambodia and another among the samples from Vietnam, which suggests a wide-spread and perhaps even sympatric occurrence with the morphologically similar *C. atripons* and *C. pulchristriata*.

The discovery of *Cyclemys* n. sp. 2 raises the question to which species the names *atripons* and *pulchristriata* apply as there is now a new candidate for being identical either with the name-bearing type of *C. atripons* or *C. pulchristriata*. This problem will be addressed in detail elsewhere. We only wish to point out here the provisional character of the nomenclature in these three species.

The sole sample of the *C. atripons-pulchristriata* complex that does not appear perfectly concordant with the division into three distinct taxa (*C. atripons*, *C. pulchristriata*, *Cyclemys* n. sp. 2) is from Cambodia and marked with an asterisk in Figures 2-4. It is from a live specimen in our care that resembles *C. pulchristriata* morphologically despite the fact that it was obtained in Cambodia where *C. atripons* should occur. Regarding mitochondrial cytochrome *b* sequences, this specimen clusters indeed with *C. pulchristriata*; but, according to ISSR profiles, it is more closely related to *C. atripons*. This specimen is the reason why our genetic distances between *C. atripons* and *C. pulchristriata* (Tab. 2) differ significantly from the data of FRITZ et al. (2001).

Several factors complicate the interpretation of these results. First, it is possible that the turtle did not originate in Cambodia and represents a *C. pulchristriata* from Vietnam as indicated by the wide head and neck stripes as well as the cytochrome *b* data. Today in Southeast and East Asia, trade in chelonians is extensive (VAN DIJK et al. 2000) and translocations by man have evidently taken place in some areas (D. HENDRIE pers. comm.). Second, mitochondrial genes as the studied cytochrome *b* gene can be subject to introgression. Third, ISSR-PCR does not provide a completely accurate picture as the scoring of profiles is not secured against faulty interpretation of homology of the fragments between different samples. Thus, specimens belonging to closely related taxa differing only in a few fragments might appear in false positions within a tree. It is obvious that we need additional data to resolve this problem.

All things considered, *Cyclemys* appears to be a highly diverse, phylogenetically old genus, which separated in Middle Oligocene times from other lineages according to the molecular clock calculations presented in this study. Yet its species retained a great morphological similarity possibly due to a conservative mode of life in a tropical habitat that changed little over time. The persistence of certain morphological characters in distantly related species of the genus could be an example of a mosaic evolution according to MAYR (1963). Characters, ei-

ther plesiomorphic or homoplastic, occurring in different combinations in several taxa include a striped head and neck pattern, a dark plastral coloration, a wide anal notch, and a long seam between the femoral scutes. We are currently investigating additional morphological markers, among others osteological characters to find further clues for allowing morphological diagnoses for the cryptic *Cyclemys* species.

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