Phylogenetic hypotheses for the turtle family Geoemydidae ☆

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Abstract

The turtle family Geoemydidae represents the largest, most diverse, and most poorly understood family of turtles. Little is known about this group, including intrafamilial systematics. The only complete phylogenetic hypothesis for this family positions geoemydids as paraphyletic with respect to tortoises, but this arrangement has not been accepted by many workers. We compiled a 79-taxon mitochondrial and nuclear DNA data set to reconstruct phylogenetic relationships for 65 species and subspecies representing all 23 genera of the Geoemydidae. Maximum parsimony (MP) and maximum-likelihood (ML) analyses and Bayesian analysis produced similar, well-resolved trees. Our analyses identified three main clades comprising the tortoises (Testudinidae), the old-world Geoemydidae, and the South American geoemydid genus *Rhinoclemmys*. Within Geoemydidae, many nodes were strongly supported, particularly based on Bayesian posterior probabilities of the combined three-gene dataset. We found that adding data for a subset of taxa improved resolution of some deeper nodes in the tree. Several strongly supported groupings within the Geoemydidae demonstrate non-monophyly of some genera and possible interspecific hybrids, and we recommend several taxonomic revisions based on available evidence.
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1. Introduction

Currently, the turtle family Geoemydidae is composed of 23 genera and approximately 73 species. It is the largest turtle family in the world, accounting for about 25% of the total species-level diversity of turtles (Iverson, 1992). Geoemydids are predominantly freshwater aquatic and semi-aquatic turtles, and are widely distributed from Europe and North Africa, to India and southern Russia, to Indonesia, and the Philippines. Although geoemydids are often referred to as "Old World pond turtles," one genus, *Rhinoclemmys*, is found in the New World from Mexico south to Ecuador, Venezuela, and Brazil (Ernst and Barbour, 1989; Iverson, 1992).

The Geoemydidae (the names Batagurinae/Bataguridae are junior synonyms of Geoemydidae (Bour and Dubois, 1986; McCord et al., 2000)) has been the subject of several recent morphological and molecular phylogenetic studies, and its taxonomy is in flux. Based on seven morphological characters, McDowell (1964) subdivided what was then the Emydidae into two subfamilies, Emydinae and Batagurinae, and further subdivided the Batagurinae into four implicitly monophyletic generic complexes: *Batagur*, *Geoemyda*, *Hardella*, and *Orlitia* (Table 1a). Based on a similar mechanism for closing the anterior part of the shell, Bramble (1974) hypothesized that *Cuora*, *Cyclemys*, and *Pyxidea* form a closely related phyletic assemblage (his *Cyclemys* group). Bramble further postulated that the *Cyclemys* group was probably derived from a *Heose-*

Table 1
Generic complexes after McDowell (1964), Bramble (1974), and Carr and Bickham (1986)

Generic complexes	Batagur	Geoemyda	Hardella	Orlitia		
(a) Generic complexes of McDowell (1964)						
Genera in each	Batagur	Geoemyda	Hardella	Orlitia		
	Callagur	Cuora	Geoclemys	Siebenrockiella		
	Chinemys	Cyclemys	Morenia			
	Hieremys	Heosemys				
	Kachuga	Mauremys				
	Malayemys	Melanochelys				
	Ocadia	Notochelys				
		Rhinoclemmys				
		Sacalia				
		Pyxidea[a]				
		Leucocephalon[b]				
Generic complexes	**Batagur**	**Geoemyda**	**Hardella**	**Heosemys**	**Orlitia**	
(b) Generic complexes of Bramble (1974)						
Genera in each	Batagur	Geoemyda	Hardella	Heosemys	Orlitia	
	Callagur	Mauremys	Geoclemys	Cuora	Siebenrockiella	
	Chinemys	Melanochelys	Morenia	Cyclemys		
	Hieremys	Notochelys		Pyxidea		
	Kachuga	Rhinoclemmys				
	Malayemys	Sacalia				
	Ocadia	Leucocephalon				
Generic complexes	**Batagur**	**Geoemyda**	**Hardella**	**Heosemys**	**Malayemys**	**Orlitia**
(c) Generic complexes of Carr and Bickham (1986)						
Genera in each	Batagur	Geoemyda	Hardella	Heosemys	Malayemys	Orlitia
	Callagur	Mauremys	Geoclemys	Cuora		Siebenrockiella
	Chinemys	Melanochelys	Morenia	Cyclemys		
	Hieremys	Notochelys		Pyxidea		
	Kachuga	Rhinoclemmys				
	Ocadia	Sacalia				
		Leucocephalon				

McDowell's complexes are based on 15 morphological characters, Bramble's are based on 20 morphological characters while Carr and Bickham based their arrangement on karyotypes.

[a] McDowell (1964) considered *Pyxidea mouhotii* a junior synonym of *Geoemyda*; thus, *Pyxidea* would be included in his *Geoemyda* complex.

[b] *Leucocephalon,* would be in the *Geoemyda* complex since the type species was redescribed from *Geoemyda yuwonoi* (McCord et al., 2000).

mys-like ancestor and therefore all four genera could be united into a *Heosemys* complex (Table 1b). Using chromosomal data, Carr and Bickham (1986) concluded that the genus *Malayemys* was distinct enough to warrant elevating it to its own generic-level complex along with the other five complexes (Table 1c). The combined complexes of McDowell (1964) and Bramble (1974) are further supported by chromosomal data (Bickham, 1975), while weak support for Carr and Bickham's (1986) *Malayemys* complex comes from allozyme data (Sites et al., 1984).

Hirayama (1984) produced the first fully resolved generic level phylogenetic hypothesis for the Geoemydidae (Fig. 1) based on 82 morphological and four chromosomal characters. Hirayama proposed a novel phylogenetic hypothesis for the group that recognized a basal, sister-group relationship between two previously unrecognized clades. One, equivalent to the *Batagur*, *Hardella*, and *Orlitia* complexes, was highly aquatic, including herbivorous turtles with an extensive secondary palate (his broad-jawed group). The other, equivalent to McDowell's *Geoemyda* complex plus the tortoises, were relatively terrestrial turtles with a less extensive secondary palate (the narrow-jawed group) (Hirayama, 1984). Gaffney and Meylan (1988) elevated the Batagurinae and Emydinae to family status (Bataguridae and Emydidae, respectively), and recognized Hirayama's broad-jawed and narrow-jawed clades at the subfamilial level (Geoemydinae and Batagurinae, respectively).

Recently, both morphological and molecular analyses have been conducted on various subsets of the Geoemydidae. Yasukawa et al. (2001) completed a morphological (35 characters) phylogenetic analysis of 28 species of the subfamily Geoemydinae, and their results were largely in agreement with those of Hirayama (1984). Like Hirayama (1984), Yasukawa et al. (2001) found that *Rhinoclemmys* was not monophyletic and therefore partitioned it into two genera: *Rhinoclemmys*, which included *R. areolata*, *R. diademata*, *R. funerea*, *R. melanosterna*, *R. nasuta*, *R. pulcherrima*, and *R. punctularia*, and *Chelopus*, which was resurrected for
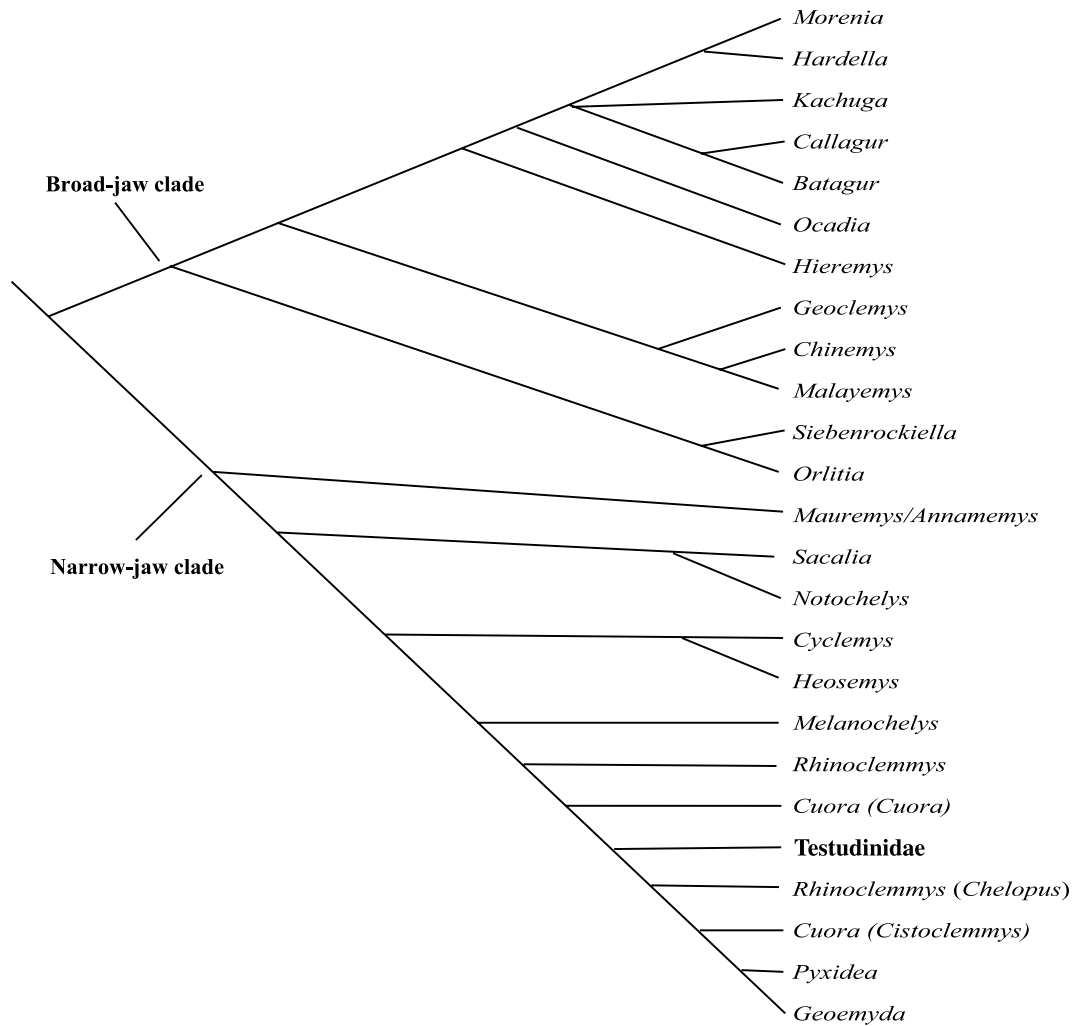
Fig. 1. Phylogenetic hypothesis of Hirayama (1984) (after Gaffney and Meylan, 1988). Hirayama's hypothesis is based on 86 characters (82 morphological and 4 chromosomal), from 37 species of geoemydids, 24 emydids, and an undisclosed number of testudinids. Notice the placement of the Testudinidae.

annulata and *rubida*. Hirayama (1984) and Yasukawa et al. (2001) also recognized the division of *Cuora* into *Cuora* (containing *C. amboinensis, C. aurocapitata, C. mccordi, C. pani, C. trifasciata, C. yunnanensis,* and *C. zhoui*) and *Cistoclemmys* (containing *flavomarginata* and *galbinifrons*; reviewed in Ernst and Barbour, 1989).

Honda et al. (2002a) analyzed phylogenetic relationships among 17 geoemydine (*sensu* Hirayama, 1984; including all genera except *Melanochelys* and *Rhinoclemmys*) and four batagurine genera (four species) based on 882 base pairs (bp) of combined 12S and 16S ribosomal mitochondrial DNA (mtDNA). The primary goal of their study was to reconstruct phylogenetic relationships within the genus *Cuora* (the Asian box turtles). Based on their discovery that the monotypic *Pyxidea* was nested within *Cuora*, Honda et al. (2002a) recommended synonymizing *Cistoclemmys* and *Pyxidea* with *Cuora*, and this recommendation has been followed by some recent authors (Stuart and Parham, in press). Honda et al. (2002a,b) further noted that *Mauremys*

appeared to be paraphyletic with respect to *Chinemys* and *Ocadia*, but made no taxonomic recommendations.

Additional intrageneric phylogenetic analyses have been completed for four geoemydid genera. Sites et al. (1981) produced a phylogeny for a subset of *Rhinoclemmys* (five out of eight species) based on isozyme data. In their results, *R. pulcherrima* is the sister taxon to the group (*R. rubida* (*R. punctularia* (*R. funerea, R. areolata*))). Iverson et al. (1989) (using morphometric data) and Barth et al. (2003) (using 871 bp of cytochrome *b* [cyt*b*] mtDNA sequence data), produced phylogenies for the three species of *Chinemys* (*C. megalocephala, C. nigricans* [= *kwangtungensis*], and *C. reevesii*). Both analyses found *C. reevesii* paraphyletic with respect to *C. megalocephala*. Guicking et al. (2002) produced a phylogeny for all five species of *Cyclemys* based on 982 bp cyt*b* and anonymous nuclear (inter simple sequence repeats [ISSR]) DNA data. They found strong support for the non-monophyly of three species including *C. pulchristriata, C. atripons,* and *C. oldhamii*.

They also identified two genetically distinct lineages within *Cyclemys* that may represent undescribed species. Finally, Stuart and Parham (in press) analyzed phylogenetic relationships within *Cuora* and found support for elevating all three subspecies of *C. galbinifrons* (*C. g. boureti*, *C. g. galbinifrons*, and *C. g. picturata*) to full species status. They also found paraphyly of *Cuora* with respect to *Pyxidea*, and followed Honda et al. (2002a) in subsuming *Pyxidea* within *Cuora*. Here, we follow the taxonomic revisions proposed by Honda et al. (2002a) and Stuart and Parham (in press) in considering *mouhotii* a species of *Cuora*.

In spite of these analyses, phylogenetic relationships and the taxonomy derived from those relationships within the Geoemydidae remain uncertain. The widespread confusion regarding the phylogenetic content and relationships of the Geoemydidae stems from at least three issues. First, no analyses have included a broad enough sampling of geoemydid turtles and appropriate outgroups to draw firm conclusions on intrafamilial relationships. Second, there is a lack of even the most rudimentary knowledge of the natural history, distribution and ecology of most species in the wild (Ernst and Barbour, 1989; Lau et al., 2000; Lau and Shi, 2000; Thirakhupt and van Dijk, 1994). Third, a number of studies have identified potential widespread hybridization among species and genera, which has greatly confounded recent efforts to clarify species boundaries and taxonomic status of several taxa (Parham et al., 2001; Stuart and Parham, in press; Wink et al., 2001). In part, all of these stem from the same potential cause—many key species of Asian turtles have been commercially over-exploited in the food and medicine trade during the last several decades (Engstrom et al., 2002; Stuart and Parham, in press; van Dijk et al., 2000), forcing systematists to rely on specimens derived solely from market vendors as a source of material. Recent economic change in China has led to a staggering increase in the numbers of turtles imported for food and traditional Chinese medicine (TCM) (Gibbons et al., 2000; IUCN Asian Turtle Workshop, 2001; van Dijk et al., 2000), and wild populations of many geoemydid species have been over-harvested to the point where they are commercially extinct. The extremely high demand and value of turtles and turtle products for food and TCM has led to a large and growing turtle-farming industry in China and southeast Asia. Turtle farmers typically keep turtles of many species in multi-species ponds (Shi and Parham, 2001), and Parham et al. (2001) asserted that these conditions produced hybrids that went to markets and were purchased and described as new species.

To work toward a stronger resolution of the phylogeny of the diverse, poorly known, and frequently endangered geoemydid turtles, we present a comprehensive molecular phylogeny for almost the entire family (and appropriate outgroups) based on cyt*b* and 12S ribosomal mtDNA as well as nuclear DNA sequence data from a novel intron (Fujita et al., in press). Using the resultant phylogenetic trees, we address three key issues for geoemydid turtles. First, we derive a new phylogeny for almost the entire group (59 of 73 species and all 23 genera), and use rigorous statistical tests to compare our tree with those proposed by previous, primarily morphological analyses. Second, we briefly address the origin and validity of several potentially hybrid species. We note that clearly-identified hybrid taxa do provide important insights into the hybridization potential between long-recognized species and genera, but they should not be considered valid species. Finally, we propose several taxonomic revisions within this diverse group of turtles to reflect the emerging consensus on their phylogenetic relationships.

2. Materials and methods

2.1. Choice of taxa and genes

Due to the rarity of many geoemydid turtles in the wild, much of the material currently used in phylogenetic studies (including ours) comes from turtles collected from food markets in Asia and from the pet trade (Guicking et al., 2002; Hirayama, 1984; Honda et al., 2002a,b; Parham et al., 2001; Stuart and Parham, in press; Yasukawa et al., 2001, and see below). Our tissue samples were obtained from live animals (66 geoemydids and four tortoises) from the private collection of WPM and five species from other sources (see Appendix A). As has been the case in the past, WPM specimens will be deposited in museums upon the death of the animal. Species from the WPM collection were identified by WPM and JBI. Blood samples were drawn from these species and shipped to UC Davis for DNA analysis, where they are stored in the HBS tissues collection (see Appendix A for catalogue numbers). Requests for tissue samples from specimens used herein should be made to either PQS or HBS.

Included in our analyses are 65 geoemydid species and subspecies as well as five tortoise species. We also include nine emydid turtles (GenBank sequences) as outgroups since Emydidae is believed to be the sister taxon to the (Geoemydidae + Testudinidae) clade (Shaffer et al., 1997). Because our analysis includes fairly complete taxon sampling at the species level within the Geoemydidae, we can explicitly test the phylogenetic hypotheses of McDowell (1964), Bramble (1974), Carr and Bickham (1986), Hirayama (1984), Wu et al. (1998), Yasukawa et al. (2001) and Honda et al. (2002a).

The choice of molecular data is crucial for phylogenetic analyses, and molecular studies can now be tailored specifically for particular phylogenetic groups and/

or questions (Lamb and Lydeard, 1994). Ideally, the chosen nucleotides are variable enough to be phylogenetically informative yet not so variable as to be excessively homoplastic (Sanderson and Shaffer, 2002). For our analyses we used the protein-coding cyt*b* mtDNA, 12S ribosomal (rDNA) mtDNA, and a ∼1 kb intron from the R35 neural transmitter gene (Friedel et al., 2001; Fujita et al., in press). We chose cyt*b* because previous analyses indicated that this gene should evolve at a rate appropriate for both inter- and intrafamilial phylogenetic studies of turtles (Bowen et al., 1993; Caccone et al., 1999; Shaffer et al., 1997; Weisrock and Janzen, 2000). Our cyt*b* data set comprises the entire 1140 bp gene for 80 individuals (we included two *Mauremys iversoni*). We included the 12S rDNA and nuclear intron sequence data for two reasons. First, increasing evidence indicates that single gene partitions sometimes reflect idiosyncrasies of individual genes rather than trees of species (Maddison, 1991; Ruvolo, 1997). Thus, we include sequence data from two unlinked data partitions (mtDNA and nDNA) in order to reconstruct a more robust species-level phylogeny. Second, recent studies have shown that rDNA and nuclear intron sequences often evolve more slowly than cyt*b* mtDNA in vertebrates (Alfaro and Arnold, 2001; Giannasi et al., 2001; Prychitko and Moore, 2000), including turtles (Engstrom et al., unpublished; Fujita et al., in press; Palkovacs et al., 2002; Shaffer et al., 1997), suggesting that the rDNA and nDNA data may provide increased resolution for the deeper nodes of geoemydid phylogeny. Most of the 12S sequences are from the analysis of Honda et al. (2002a) and represent a fairly broad sampling of the Geoemydinae (*sensu* Hirayama, 1984). We supplemented these sequences with 13 additional sequences in order to assemble complete 12S sampling of all geoemydid genera except *Leucocephalon yuwonoi*, which was unavailable at the time of analysis.

Our primary goals in compiling the R35 data set were to include unlinked DNA sequence data and to provide greater resolution deep in the geoemydid tree, particularly for nodes that are poorly supported based on mtDNA data alone. We therefore compiled a 29-taxon nDNA data set consisting of one representative from each geoemydid genus except *Cuora*, for which we included three species. We also included three distantly related tortoises as outgroups to the Geoemydidae. In compiling our R35 data set, choice of representative geoemydids was not straightforward, due to the taxo-

nomic confusion regarding the content of *Mauremys* and *Cuora*, and also the possibility of hybridization between a number of taxa (see below). For example, previous analyses have suggested that *Mauremys* is paraphyletic with respect to *Chinemys* (Honda et al., 2002a,b), and *Cuora* may be paraphyletic with respect to *Geoemyda* and *Rhinoclemmys* (Hirayama, 1984; Yasukawa et al., 2001). We solved this dilemma by using our initial cyt*b* analysis (Fig. 2) and results from the literature to choose representatives of *Mauremys* and *Cuora* that should capture the overall generic divergence and relationships within the Geoemydidae.

For *Mauremys* we chose *M. mutica* since it is, according to our mitochondrial data, phylogenetically nested within a clade containing all members of the genus. For *Cuora* we chose three species, *C. aurocapitata*, *C. flavomarginata*, and *C. serrata*. Based on our mtDNA data, *Cuora aurocapitata* is also phylogenetically nested within a clade containing all members of *Cuora*, whereas *Cuora flavomarginata* (and *C. galbinifrons*) are sometimes placed in the genus *Cistoclemmys* (i.e., Hirayama, 1984; Yasukawa et al., 2001). We also attempted to include *Cuora serrata*, *M. iversoni*, and *M. pritchardi* because of their recently proposed hybrid status (Parham et al., 2001; Stuart and Parham, in press; Wink et al., 2001). However, of these three we were able to acquire high-quality R35 sequence data only from *C. serrata*.

2.2. DNA extraction, amplification, and sequencing

Our cyt*b* and nuclear intron data sets consist of sequences we have generated for this study, augmented by nine emydid cyt*b* sequences downloaded from GenBank. Our 12S data set consists of 47 sequences, 34 from GenBank and 13 from this study (for Accession numbers see Appendix A). Tissue samples consisted of whole blood from live turtles or muscle tissue from preserved specimens. Blood was either frozen and maintained at −80 °C, or preserved in a lysis buffer composed of 100 mM Tris (pH 8), 100 mM EDTA, 10 mM NaCl, and 1% SDS and stored at −20 °C. Muscle tissue was preserved in 95% ethanol and stored at −20 °C.

Genomic DNA was obtained from blood and muscle tissue via proteinase K digestion followed by phenol/chloroform extraction. The entire 1140 nucleotide cyt*b* gene was sequenced for 66 geoemydids and three tortoises, and shorter sequences were generated for the

Fig. 2. Maximum-likelihood reconstruction based on the 79-taxon cyt*b* data set (1140 bp). Estimated model parameters conform to the GTR + G + I model of nucleotide sequence evolution. $-\ln L = 19174.7845$, rate matrix: A–C = 0.456, A–G = 10.1546, A–T = 0.4458, C–G = 0.4822, C–T = 8.1373, G–T = 1. Base frequencies: A = 0.36, C = 0.36, G = 0.06, T = 0.21. Proportion of invariable sites (I) = 0.403. γ-Shape parameter = 0.9412. Numbers above and below branches are bootstrap proportions and decay indices (respectively) recovered from a MP analysis of this data set (9 most parsimonious trees, not shown) length = 4225 steps, CI = 0.219, RI = 0.600. * Indicates posterior probabilities ⩾95% from clades recovered from Bayesian analysis of this data set. Potential hybrid species are enclosed in quotation marks. We follow Stuart and Parham (in press) in the use of the name *C. picturata*, although this is controversial.
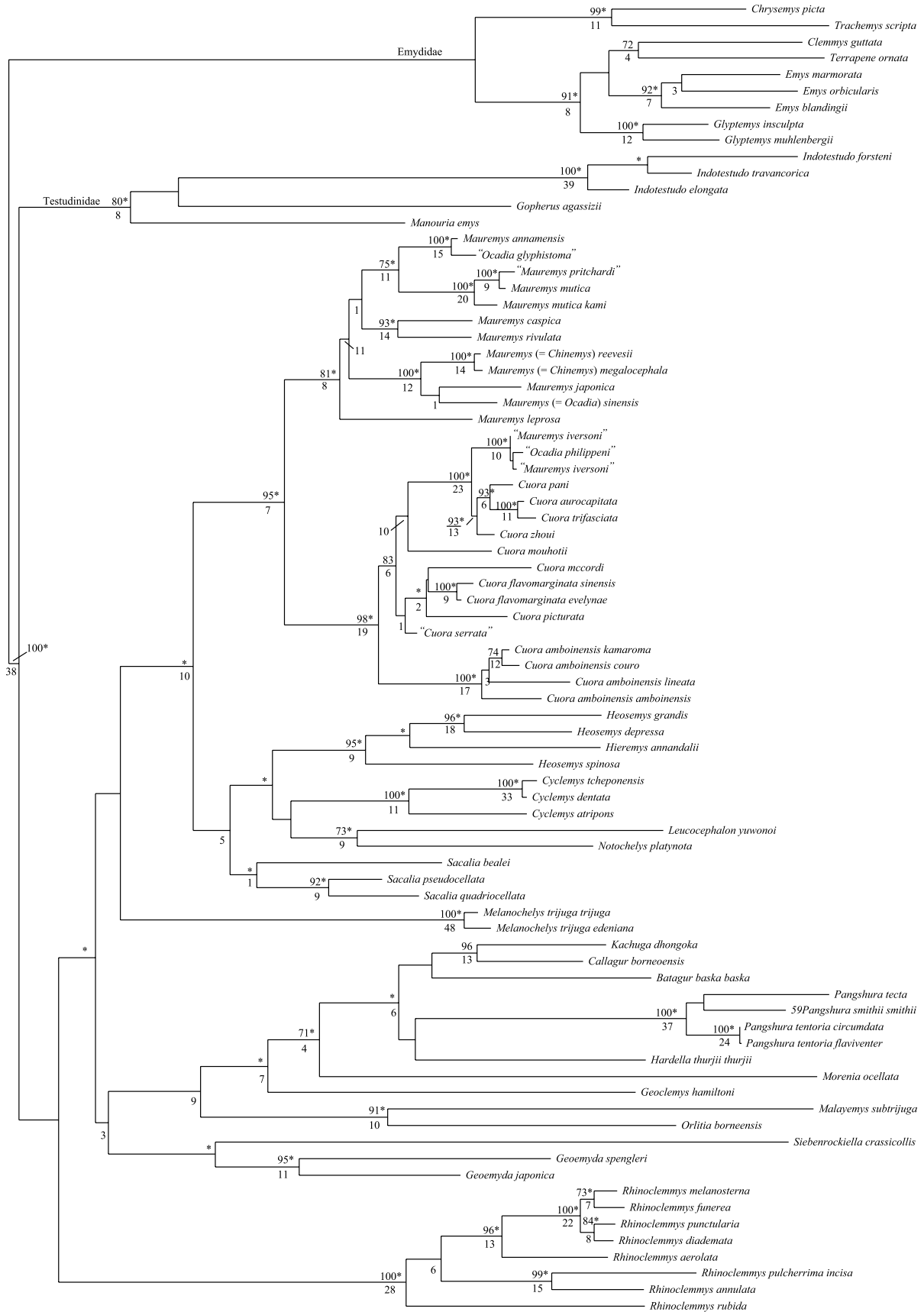
Table 2
Oligonucleotide primers for amplification and sequencing of turtle mitochondrial and nuclear DNA

Primer	Sequence (5′ to 3′)[a]	Position[b]	Gene	Source
CytbG	AACCATCGTTGTWATCAACTAC	14368–14389	Cytb	Shaffer lab
CytbJSi	GGATCAAACAACCCAACAGG	15011–15030	Cytb	Shaffer lab
CytbJSr	CCTGTTGGGTTGTTTGATCC	15030–15011	Cytb	Shaffer lab
GLUDGE	TGATCTTGAARAACCAYCGTTG	14358–14378	Cytb	Palumbi et al. (1991)
THR	TCATCTTCGGTTTACAAGAC	15593–15574	Cytb	Shaffer lab
THR-8	GGTTTACAAGACCAATGCTT	15585–15566	Cytb	Shaffer lab
12SA	AAACTGGGATTAGATACCCCACTAT	501–525	12S	Kocher et al. (1989)
12SB	GAGGGTGACGGGCGGTGTGT	939–920	12S	Kocher et al. (1989)
R35EX1	ACGATTCTCGCTGATTCTTGC	—	R35	Shaffer lab
R35EX2	GCAGAAAACTGAATGTCTCAAGG	—	R35	Shaffer lab

[a] Redundancy codes R = A and G, W = A and T, Y = C and T.
[b] Position refers to the 5′ to 3′ location of the primer relative to the complete mitochondrial genome sequence of the turtle *Chrysemys picta* (Mindell et al., 1999).

remaining two tortoises (1115 and 1126 bp, respectively). Eight of the nine emydid cyt*b* sequences obtained from GenBank are complete (the remaining sequence was 1131 bp). The 12S sequences from GenBank and from this study (41 geoemydids, four emydids and two tortoises) consisted of about 400 bp. However, the final nine nucleotide positions at the 3′ end of the sequence were difficult to align so we excluded these from our analyses. Our nuclear data come from intron number one of the RNA fingerprint protein (R35). The function of this protein is unknown but the gene is thought to behave as a single locus (Friedel et al., 2001). Exon priming intron crossing (EPIC) primers for R35 were developed in the Shaffer lab by Matt Fujita (Fujita et al., in press) and our R35 data set consists of 712 nucleotides for 29 taxa (26 geoemydids and three tortoises).

Gene products were amplified using *Taq*-mediated PCR, and the PCR products were sequenced on ABI 377 or ABI 3100 automated sequencers in the UC Davis Division of Biological Sciences DNA sequencing facility. Initial cyt*b* sequences were amplified and sequenced using universal primers. Some geoemydid taxa did not amplify, or did not amplify well, using these primers, so we designed primers specifically for the geoemydids. Our primers allowed us to amplify and sequence both light and heavy strands of the entire cyt*b* gene (Table 2). Cytochrome *b* sequences were aligned within individual turtles using SeQed (Applied Biosystems) and converted into amino acid sequences using GeneJockey (Biosoft, Cambridge, England). Alignments across taxa were made by eye in PAUP* V4.0b10 (Swofford, 2001). No insertions or deletions were detected and all nucleotide sequences translated into amino acid sequences. The 12S rDNA sequence data was generated using the 12SA and 12SB universal primers of Kocher et al. (1989). For our nuclear data, we used sequence data from a ~1 kb intron from the R35 neural transmitter gene. The best sequencing results for this study were obtained with the R35EX2 primer, so the intron was sequenced in only one direction. All primers are listed in Table 2. 12S and

R35 sequences were aligned using ClustalX v1.64b (Thompson et al., 1997) using default settings. Minor adjustments were made to the 12S alignment by eye and indels were treated as missing data (coded as "-" in PAUP*4.0b10). Our aligned sequence file is available from TreeBASE (www.treebase.org, accession number S1002). Sequences were deposited in GenBank (Accession numbers in Appendix A).

2.3. Phylogenetic analysis

Phylogenetic relationships were estimated with maximum parsimony (MP) and maximum-likelihood (ML) using PAUP*4.0b10 (Swofford, 2001) and Bayesian analysis using MrBayes v3.0B4 (Huelsenbeck and Ronquist, 2001). Heterogeneity of the three data sets (cyt*b*, 12S, and R35) was assessed using the incongruence length difference (ILD) test (Farris et al., 1994) (partition homogeneity test in PAUP*4.0b10). Data sets were combined by concatenating sequences, with missing data coded as "?". Third codon position saturation of cyt*b* data was assessed by plotting transitions and transversions against uncorrected "*p*" distances. Third codon positions appear saturated (results not shown), but we nonetheless include these characters, because recent work has shown that third codon positions can contain phylogenetic information regardless of the perceived degree of saturation (Broughton et al., 2000; Källersjö et al., 1999).

For each MP analysis, we ran 100 replicate random stepwise heuristic searches with tree-bisection-reconnection (TBR) branch swapping and searches constrained to one million rearrangements each. We then bootstrapped the MP trees 100 times to assess their statistical reliability (Felsenstein, 1985). We consider bootstrap proportions of <50% as not supported, proportions between 50 and ≥70% as weakly supported, and proportions 70% as potentially well supported (Georges et al., 1998; Hillis and Bull, 1993). Decay indices (DI) were calculated using AutoDecay 4.0.2'PPC (Eriksson, 1998) and visualized using Treeview 1.5

Table 3
Maximum-likelihood model parameters, for data sets compiled for testing previous hypotheses, estimated using Modeltest (Posada and Crandall, 1998)

Parameter	Data Set			
	Hirayama (1984)	Wu et al. (1998)	Yasukawa et al. (2001)	Honda et al. (2002a)
Number of taxa	50	13	34	28
Nucleotides	2243	2243	2243	2243
Model	GTR + G + I	GTR + G	GTR + G + I	GTR + G + I
Base frequencies				
A	0.34	0.33	0.32	0.33
C	0.30	0.29	0.29	0.29
G	0.14	0.14	0.14	0.14
T	0.22	0.24	0.24	0.24
Rate matrix				
A–C	1.5128	2.1169	1.2091	1.6517
A–G	9.6847	9.154	8.8317	8.1731
A–T	1.5026	1.9421	1.0251	1.4857
C–G	0.5096	0.381	0.4576	0.4722
C–T	20.3875	22.2061	17.2281	18.9182
G–T	1	1	1	1
Gamma (G)	0.8406	0.1807	1.0123	0.6304
Invariable sites (I)	0.4854	0	0.5338	0.4317
Unconstrained $-\ln L$ score	20783.2652	8929.8764	13624.4513	13505.0099
$-\ln L$ score constrained to previous hypothesis	22506.0518	8952.5282	14214.0329	13565.8346
$-\ln L$ score constrained to Fig. 3	20792.5004	8932.136	13624.9559	13509.0175

In cases where multiple hypotheses were tested from the same paper (e.g., Wu et al., 1998), we show only their best $-\ln L$ score.

(Page, 1996). Uninformative characters were excluded for calculations of consistency indices (CI) and retention indices (RI). Maximum-likelihood reconstructions employed model parameters for each data set estimated with Modeltest V3.06PPC (Posada and Crandall, 1998). Model parameters are shown in Figure legends and in Table 3. Due to computational limitations, we constrained the topology of the outgroups and used the nearest-neighbor-interchange (NNI) branch swapping algorithm for initial ML searches. These trees were then used as starting trees for subsequent ML searches employing the subtree-pruning-regrafting (SPR) branch swapping algorithm. For Bayesian analysis, we partitioned the data into five partitions, three for cyt*b* (first, second, and third codon positions), 12S, and R35. We ran three analyses starting from random trees and employed Metropolis-coupled Markov chain Monte Carlo (Huelsenbeck and Ronquist, 2001) with one cold and three heated chains (using default heating values). Each analysis was run for 10^6 generations, sampling the chains every 100 generations. Log-likelihood values of sample points were plotted against generation time (not shown) and stationarity of the Markov chains was determined to be attained when the values reached a stable equilibrium. Sample points prior to equilibrium were discarded as burn-in and the remaining values were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) for a clade are then the proportion of samples recovering that particular clade.

To test previous phylogenetic hypotheses, we constructed data sets containing all or nearly all of the same geoemydid taxa used in the original analyses of McDowell (1964), Bramble (1974), Carr and Bickham (1986), Hirayama (1984), Wu et al. (1998), Yasukawa et al. (2001), and Honda et al. (2002a). Using the appropriate data set, we constructed constraint trees equivalent to the generic complexes and phylogenetic hypotheses in each of these papers as well as pruned versions of our optimal tree (the combined data ML tree). Next, we obtained likelihood scores based on model parameters estimated from these data sets (Table 3), and then reconstructed likelihood trees using the same data sets and parameters, but without imposing any constraints. We then tested constrained trees vs. unconstrained trees using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) with RELL optimization implemented in PAUP*4.0b10.

3. Results

3.1. 80-taxon phylogenetic results

Given our goal of producing a comprehensive tree for all geoemydid taxa, we first asked whether there was any significant conflict within our mtDNA or between our mtDNA and nDNA data partitions. Currently, the ILD test is often used to assess data combinability, although

recent work indicates that this test has limited ability to detect incongruence (Darlu and Lecointre, 2002; Dolphin et al., 2000; Dowton and Austin, 2002), and at least some authors (Yoder et al., 2001) go so far as to suggest that the ILD test should not be used as a measure of data partition combinability. Nevertheless, we used the ILD test in an attempt to gain some insight regarding congruence of our data partitions. To assess the mtDNA data partitions, we compiled a data set consisting of combined cyt*b* and 12S sequence data for 47 taxa and we compiled a combined mtDNA and R35 data set for 25 taxa to examine congruence between the mtDNA and nuclear data partitions. Results of the ILD test indicated a conflict within the mtDNA (cyt*b* vs 12S, $p = 0.01$) but no conflict between the nuclear and mtDNA (cyt*b* vs R35, $p = 1.0$; 12S vs R35, $p = 0.97$). For the 47-taxon data set, the major discrepancies between the cyt*b* and 12S trees were the relative positions of three emydid outgroups, both tortoises and two geoemydids (*S. crassicollis* and *H. annandalii*). In a subsequent ILD test with all the emydids and tortoises (six taxa) removed from the analysis the incongruence disappeared ($p = 0.20$), suggesting that the incongruence was largely due to our outgroups. We further explored this by running seven more ILD tests with the six emydids and tortoises included, but sequentially removing blocks of six different geoemydid taxa for each run (we removed the last five taxa only in the final run). In six of seven runs the data were incongruent ($p \leqslant 0.02$) and in only one case did the results approach congruence ($p = 0.04$). We take these results to indicate that most of the apparent conflict between our mtDNA data partitions is within the outgroups/tortoises or between the outgroups/tortoises and ingroup and should not greatly impact our ingroup reconstructions.

We present our phylogenetic results as two trees, a cyt*b*-only tree and a combined mitochondrial/nuclear DNA tree based on cyt*b*, 12S and R35 sequence data. The cyt*b* only tree has data for all species, whereas the combined tree attempts to gain additional resolution for the deeper levels of the geoemydid tree by providing sequence data for key representatives spanning the major clades of the tree. Our cyt*b*-only tree had 1140 bp of cyt*b* sequence for 80 taxa. Of the 1140 bp, 550 were parsimony-informative. Maximum parsimony analysis recovered nine trees (not shown) while ML analysis recovered a single tree (Fig. 2). In all three Bayesian analyses, stationarity was reached and $-\ln L$ scores

converged to approximately the same value after 31,000 generations (results not shown). Fig. 2 shows the cyt*b*-only ML reconstruction with DIs, BPs and PPs on branches recovered from the MP and Bayesian analyses. Our combined 80-taxon mtDNA/R35 data set had 1140 bp of cyt*b* for 80 taxa, 391 bp of 12S data for 47 taxa and 712 bp of R35 data for 29 taxa. Of the combined 2243 bp, 712 bp are parsimony-informative. Maximum parsimony analysis recovered three trees (not shown) and ML analysis recovered a single tree (Fig. 3). In all three Bayesian analyses stationarity was reached and $-\ln L$ scores converged to approximately the same value after 40,000 generations (results not shown). Fig. 3 shows the 80-taxon combined data ML reconstruction with DIs, BPs, and PPs on branches recovered from the MP and Bayesian analyses.

3.2. Testing previous hypotheses of geoemydid relationships

To test previous phylogenetic hypotheses explicitly, we compared $-\ln L$ scores recovered from trees constrained to previous hypotheses with $-\ln L$ scores recovered from unconstrained trees using the SH test. For testing the generic complexes of McDowell (1964), Bramble (1974), and Carr and Bickham (1986), we used the combined mtDNA/R35 data set. We assumed that the intent of these previous authors was that each generic complex was monophyletic, and we constrained ML searches to trees compatible with each generic complex hypothesis. We did not impose any phylogenetic structure among the generic complexes, or among taxa within complexes. Next, we compiled a tree file in PAUP* V4.0b10 containing the constrained trees as well as the tree in Fig. 3 and compared $-\ln L$ scores of all trees using the SH test. The unconstrained tree (Fig. 3) always had the best $-\ln L$ score, which was significantly better than any of the three generic complex hypotheses (SH test, $p = 0.000$ in all cases).

We used a similar strategy to test the phylogenetic hypotheses of Hirayama (1984), Wu et al. (1998), Yasukawa et al. (2001), and Honda et al. (2002a). In these cases, we compiled combined mtDNA and nDNA data sets containing all of the geoemydid species that they used in their respective analyses. In some cases, we had no sequence data for a few geoemydid or tortoise species. These geoemydid species were eliminated from the

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Fig. 3. Maximum-likelihood reconstruction based on the combined mtDNA/R35 data set (2243 bp). Estimated model parameters conform to the GTR + G + I model of nucleotide sequence evolution. $-\ln L = 25141.579$, rate matrix: A–C = 1.225, A–G = 9.1189, A–T = 1.1447, C–G = 0.5409, C–T = 16.1548, G–T = 1. Base frequencies: A = 0.34, C = 0.31, G = 0.12, T = 0.23 Proportion of invariable sites (I) = 0.4541. γ-Shape parameter = 0.7535. Numbers above and below branches are bootstrap proportions and decay indices (respectively) recovered from a MP analysis of this data set (3 most parsimonious trees, not shown) length = 5025 steps, CI = 0.239, RI = 0.594. * Indicates posterior probabilities $\geqslant 95\%$ from clades recovered from Bayesian analysis of this data set. Potential hybrid species are enclosed in quotation marks. Numbers to the right of clades are maximum uncorrected "*p*" sequence divergences for that clade based only on cyt*b*.
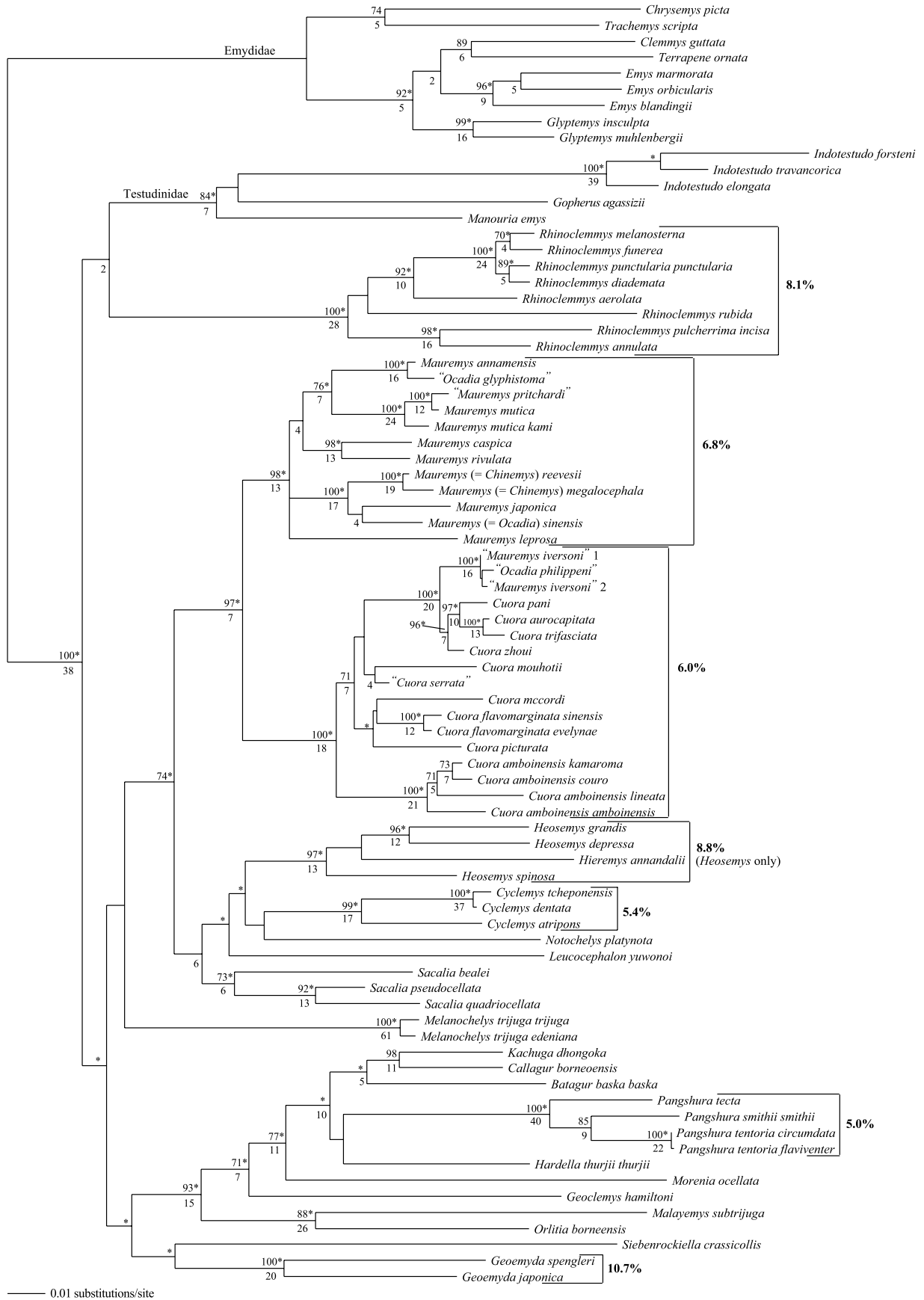
analysis, but in tests involving tortoises, we used some of our tortoise sequences. We estimated model parameters for each data set using Modeltest, and used them in recovering likelihood trees from constrained and unconstrained searches (Table 3). For each previous hypothesis, we again compiled a tree file in PAUP* V4.0b10 containing trees constrained to the hypothesis under consideration, as well as trees constrained to a pruned version of Fig. 3 and trees recovered from unconstrained searches. The $-\ln L$ scores from the resulting trees were then tested against one another using the SH test in PAUP* V4.0b10.

For testing the hypothesis of Hirayama (1984), we compiled a 50-taxon data set that contained all of the geoemydid taxa used in his original analyses except for *Kachuga trivittata* and the fossil taxon *Echmatemys*. For outgroups, Hirayama included *Echmatemys*, 24 species of emydids (we have nine) and an undisclosed number of tortoise species (we included our five tortoises). With our data, the ML tree resulting from an unconstrained search had a significantly better $-\ln L$ score than the ML tree constrained to Hirayama's (1984) hypothesis (SH test, $p = 0.00$). The $-\ln L$ score for the ML tree constrained to Fig. 3 was not significantly different than the $-\ln L$ score for the unconstrained ML tree (SH test, $p = 0.61$).

For testing the hypotheses of Wu et al. (1998) we compiled a data set containing all 13 taxa used in their analyses including 12 geoemydid species as well as *Chelus fimbriata* (from GenBank), a chelid turtle that they used as an outgroup. Wu et al. (1998) produced a neighbor-joining (NJ) and MP tree based on 393 bp of 12S rDNA sequence data. The tree resulting from an unconstrained ML search of this data set had a significantly better $-\ln L$ score than the trees constrained to either the NJ or MP topology of Wu et al. (1998) ($p \leqslant 0.03$). The $-\ln L$ score for the ML tree constrained to a pruned version of Fig. 3 was not significantly different from the $-\ln L$ score for the unconstrained ML tree (SH test, $p = 0.62$).

The data set we compiled for testing the hypothesis of Yasukawa et al. (2001) contained 34 geoemydids including all of the taxa used in their original analysis, although some ambiguity exists over material attributed to the genus *Cyclemys*. Yasukawa et al. (2001) examined skeletal remains for eleven specimens of *Cyclemys*, but these specimens were not identified to the species level. We included our three *Cyclemys* (*C. atripons*, *C. dentata*, and *C. tcheponensis*), which probably includes the species studied by Yasukawa et al. (2001). The unconstrained ML tree recovered from this data set had a significantly better $-\ln L$ score than the ML tree constrained to the hypothesis of Yasukawa et al. (2001) (SH test, $p = 0.00$), and the $-\ln L$ score for the ML tree constrained to Fig. 3 was not significantly different from that of the unconstrained ML tree (SH test, $p = 0.48$).

Finally, the data set we compiled for testing the hypothesis of Honda et al. (2002a) contained all of the geoemydid taxa used in their original analysis except for *Cuora f. flavomarginata* and perhaps *Cyclemys*. Honda et al. (2002a) included *Cuora f. flavomarginata*, but we included *C. f. sinensis* since we do not have a representative of the former subspecies. In addition, Honda et al. (2002a) included "*Cyclemys* sp." in their analyses but they did not indicate which species were included. As before, we included our three *Cyclemys* in order to represent the genus. Honda et al. (2002a) also included two emydid turtles (*Emys orbicularis* and *Trachemys scripta elegans*) and two tortoises (*Testudo horsfieldii* and *Geochelone carbonaria*). We have cyt*b* sequence data for the first two emydid turtles, but we substituted *Manouria emys* and *Gopherus agassizii* as representative tortoises. For outgroups, Honda et al. (2002a) followed Gaffney and Meylan (1988) and included a musk turtle (*Staurotypus triporcatus*—Family Kinosternidae) and a softshell turtle (*Pelodiscus sinensis*—Family Trionychidae), because according to Gaffney and Meylan (1988) these turtle families are basal to the Emydidae/Geoemydidae/Testudinidae clade. Therefore, in order to replicate their data set most accurately, we included an 892 bp cyt*b* *S. triporcatus* sequence and a complete (1140 bp) *P. sinensis* cyt*b* sequence from GenBank (see Appendix A). With our mtDNA data, the tree resulting from an unconstrained ML search had a significantly better $-\ln L$ score than any of the trees constrained to the ML, MP, and NJ hypotheses of Honda et al. (2002a) (SH test, $p \leqslant 0.003$). Once again, the $-\ln L$ score from the tree constrained to Fig. 3, was not significantly different from that of the unconstrained ML tree (SH test, $p = 0.55$).

4. Discussion

As in other studies, our cyt*b* sequence data are more variable than the 12S or nuclear intron data (Engstrom et al., unpublished; Giannasi et al., 2001; Palkovacs et al., 2002; Prychitko and Moore, 2000; Shaffer et al., 1997). For the 41 geoemydid taxa with complete cyt*b* and 12S data, cyt*b* has a mean uncorrected pairwise sequence divergence of 13.7% while the corresponding 12S data are 8.3% divergent (S1, 2). For the 24 geoemydid taxa with complete mitochondrial and nuclear sequence data, mean uncorrected pairwise sequence divergence of cyt*b* = 14.7%, 12S = 8.7%, and R35 = 1.8% (S1–3).

Including the 12S and R35 data did not have a profound affect on our reconstructions, although it did help resolve a few problematic nodes. Posterior probabilities increased for the (*Batagur/Callagur/Kachuga*) clade as well as for the positions of the (*Malayemys/Orlitia*) and the (*Geoemyda/Siebenrockiella*) clades but MP bootstrap

support was significantly greater for the position of the (*Malayemys*/*Orlitia*) clade only. In addition, *Leucocephalon* and *Notochelys* are sister taxa with reasonably strong support in the cyt*b*-only tree but, with the added data, *Leucocephalon* shifts to assume a sister-group position to a clade containing (*Heosemys*/*Hieremys*/*Cyclemys*/*Notochelys*) in Fig. 3. Several relationships that were poorly resolved in the cyt*b* only analysis, including the position of *Rhinoclemmys*, *Hardella*, and *Melanochelys*, remained unstable in the combined analysis (compare Figs. 2 and 3).

We view the topology in Fig. 3 as our best current hypothesis of geoemydid relationships because it is based on our most complete dataset, reflects generally strong agreement between all three analytical methods that we employed, and displays no major conflicts between it and the cyt*b*-only topologies. On the combined mtDNA/R35 ML tree, 64% of nodes were well supported under MP with BPs of $\geqslant 70\%$, while 69% of nodes had Bayesian posterior probabilities $\geqslant 95\%$. Overall, we have made much progress toward a resolution of the phylogeny of the Geoemydidae, although unresolved issues remain, particularly deep in the tree.

At the family level, our combined data analyses support a monophyletic Emydidae as the sister taxon to Geoemydidae plus Testudinidae (BP = 100%, DI = 38, PP = 100%, Fig. 3). We included nearly all geoemydid species, including all but one *Rhinoclemmys* (*R. nasuta*), and the tortoises included in our analysis represent a broad sampling of testudinid phylogenetic diversity (Gerlach, 2001). Given the diversity of taxa included in our analyses, we are confident that the Testudinidae and the New World geoemydid genus *Rhinoclemmys* are each monophyletic. The remaining members of Geoemydidae (exclusive of *Rhinoclemmys*) may form a monophyletic group, although statistical support for this is based solely on Bayesian posterior probabilities. Within the Geoemydidae, key results include the sister-group relationship of *Cuora* and *Mauremys* (among which several hybridization events have been proposed), the close relationship of *Heosemys* and *Hieremys*, the consistent close relationships among *Kachuga*, *Callagur*, *Batagur*, *Pangshura*, and *Hardella*, and the identification of a series of monotypic genera as phylogenetically basal branches with no close living relatives. Most of these genera, including *Siebenrockiella*, *Orlitia*, *Malayemys*, and *Geoclemys*, have long been recognized as distinctive, monotypic taxa based on morphological criteria, whereas *Leucocephalon* (McCord et al., 2000) has only recently been so recognized.

4.1. Previous hypotheses

Within the Geoemydidae, our reconstructions have little in common with most previous phylogenetic hypotheses. Under a likelihood framework, we were able to reject the generic complex hypotheses of McDowell (1964), Bramble (1974), and Carr and Bickham (1986) as well as the morphology-based hypotheses of Hirayama (1984) and Yasukawa et al. (2001). While the phylogeny in Fig. 3 has a statistically significantly better $-\ln L$ score than the DNA-based hypotheses of Honda et al. (2002a), there are some similarities between their hypotheses and our own. For example, in our analyses, as well as that of Honda et al. (2002a), *Mauremys* is paraphyletic with respect to *Chinemys*. The discrepancies between our phylogenetic hypotheses and Honda et al. (2002a) might be due to taxon sampling and slower rates of nucleotide substitution within 12S compared to cyt*b*. For taxon sampling, Honda et al. (2002a) included 22 species/subspecies from 12 genera. At the time of their analyses, phylogenetic relationships within the Geoemydidae were largely unstable. Thus, the sampling of Honda et al. (2002a) is somewhat haphazard, whereas ours is nearly complete and includes relatively large amounts of sequence data.

4.2. Hybridization

Within the last two decades, 14 new species of geoemydid turtles have been described from China (Kou, 1989; Parham et al., 2001, and references therein), and most of these taxa have been described from animals culled from the large food markets of China and Hong Kong. These taxa form a vexing, but potentially important aspect of our understanding of the evolution and biodiversity of the Geoemydidae. Many of these species have unconfirmed locality data, have not been found by in the wild by researchers, are sometimes unfamiliar to people living in the regions from which they are purportedly derived, and sometimes have phenotypes that appear intermediate between those of other recognized species (Parham et al., 2001). Thus, some of these new ''species,'' including *Mauremys iversoni* and *Cuora serrata* may be of recent, human-mediated, hybrid origin (Parham et al., 2001; Stuart and Parham, in press). Conversely, Wink et al. (2001) proposed that *Mauremys iversoni* and *M. pritchardi* might be the result of ancient hybridization events, based on molecular clock estimates of taxon age. Which, if any, of these species are the products of human-mediated hybridization events is of considerable importance since some are known from few specimens and are presumed to be in grave danger of extinction (van Dijk, 2000). Thus, if they are valid evolutionary taxa, these species may require immediate, potentially costly intervention to prevent extinction. Alternatively, if they are hybrids produced during captive farming efforts, they are not valid species, and are not candidates for protection (although they may still be of value in the pet /TCM trade). In either case, these forms may provide important insights into the evolution of intrinsic reproductive isolating mechanisms in turtles.

A thorough examination of hybridization within the Geoemydidae is beyond the scope of our data, and requires much deeper sampling of both the purported hybrid taxa and their postulated parental forms for nuclear and mitochondrial gene trees. However, phylogenetic relationships, even for haploid, maternally inherited mtDNA can provide some insights into hybridization (Perry et al., 2002). In using our phylogeny to make inferences regarding potential hybrid species, we rely on the following criteria. First, recent hybrids (that is, those generated by turtle farmers) should have cyt*b* haplotypes that are very similar, or identical, to their maternal parental species. Second, if a hybrid is a cross between species from different genera, and those genera are monophyletic, then some fraction of the time a hybrid will fall in the "wrong" genus, and those cases are identifiable phylogenetically. If the cross is equally successful regardless of which sex is the mother, then one prediction is that about half the time a hybrid species will fall in the "correct" genus, and half the time it will not. Thus, if species fall in the "wrong" place in our phylogeny, and particularly if there are very short branch lengths between these misplaced taxa and their sister species, then they become candidates for hybrid origin. With this criterion, we can distinguish recent, anthropogenically derived and ancient, natural hybridization only by the amount of divergence between taxa, and this is difficult to interpret absolutely. In addition, mtDNA is maternally inherited, and hybridization could go undetected in our analyses if successful crosses were always between females of the genus to which a hybrid species was originally assigned and males from the "wrong" genus. However, nuclear sequences may help in these cases.

Our phylogeny, together with inferences from previous authors, illustrates that hybridization between *Mauremys* and *Cuora* is a plausible explanation for some of the taxonomic inconsistencies in our results. In our analyses, *Mauremys* and *Cuora* are closely related suggesting that they have retained the ability to hybridize from their shared common ancestor. In our analyses, *M. iversoni*, *O. glyphistoma*, and *O. philippeni* appear to be hybrids, and other work indicates that *M. iversoni* as well as *M. pritchardi* and *C. serrata* may be hybrid taxa (Parham et al., 2001; Stuart and Parham, in press; Wink et al., 2001). Below we discuss these putative hybrid species.

4.3. Ocadia glyphistoma

In our results, *Ocadia* as currently recognized is polyphyletic (Fig. 3). It includes the well-established species *O. sinensis* (Gray, 1870), and the recently described *O. glyphistoma* (McCord and Iverson, 1994) and *O. philippeni* (McCord and Iverson, 1992). The type species, *O. sinensis*, was described from "China" over

130 years ago and is closely related to *Chinemys reevsii*, *C. megalocephala*, and *Mauremys japonica* (BP = 100%, DI = 16, PP = 100%, Fig. 3). Thus, *O. glyphistoma* falls in the "wrong" place in our phylogeny (Fig. 3). Rather than grouping with *O. sinensis*, *O. glyphistoma* is well-nested within *Mauremys*, and is relatively similar (1.2% uncorrected cyt*b* sequence divergence) to *M. annamensis*. The description of *Ocadia glyphistoma* was based on ten specimens (nine living and one preserved) reportedly from North Vietnam and Southeast China (McCord and Iverson, 1994). Our specimen of *O. glyphistoma* is most similar morphologically to *O. sinensis* yet falls on a short branch in the clade with *Mauremys*, a result consistent with it being a hybrid between a male *O. sinensis* and a female *M. annamensis*.

4.4. Ocadia philippeni

Ocadia philippeni was described from nine specimens (seven living and two preserved) reportedly from Hainan Island, China (McCord and Iverson, 1992). The interpretation of *O. philippeni* as a potential hybrid is somewhat clouded by its close relationship with *Mauremys iversoni*, which is itself a potential hybrid species (see below). The *O. philippeni* in our analysis appears to be a hybrid because it falls on a very short branch with a non-congeneric species (*M. iversoni*), and both of these species are nested well within the genus *Cuora* (BP = 100%, DI = 16, PP = 100%, Fig. 3). However, the *Mauremys iversoni/O. philippeni* clade is reasonably well-differentiated from all other *Cuora* (3.6% average cyt*b* divergence between *O. philippeni* and the *Cuora pani/aurocapitata/trifasciata/zhoui* clade), a result we would not expect if *M. iversoni* and *O. philippeni* are both recent hybrids between recognized taxa.

4.5. Mauremys iversoni

Mauremys iversoni was described from 29 specimens reportedly from Fukien Province, China (Pritchard and McCord, 1991). As with *O. philippeni*, the status of *M. iversoni* remains open to interpretation. Both specimens of *M. iversoni* in our analysis appear to be hybrids because (1) they fall on very short branches with respect to *O. philippeni* and (2) they are deeply nested within the larger genus *Cuora* with strong support (BP = 100, DI = 16, PP = 100%, Fig. 3). There is very little cyt*b* sequence divergence between either specimen of *M. iversoni* and that of *O. philippeni* (0.26–0.61%), less divergence than within, for example, the polytypic geoemydid species *Cuora amboinensis* (*C. a. couro*, *C. a. amboinensis*, *C. a. lineata*, and *C. a. kamaroma*) which ranges from 1.1 to 5.1%. This very low level of divergence is consistent with the interpretation that our specimens of *M. iversoni* and *O. philippeni* are either recent hybrids between the same female species of *Cuora*

and male *Mauremys* and *Ocadia*, respectively, or perhaps that these individuals represent the result of hybridization between an undescribed species of *Cuora* and either a species of *Mauremys* or *Ocadia*. Other work indicates that some specimens assigned to *M. iversoni* are hybrids between *Mauremys mutica* and *Cuora trifasciata*. Parham et al. (2001) analyzed allozyme and mtDNA (ND4 and COI) data from a known hybrid (purchased from a turtle farm in China) morphologically assignable to *M. iversoni* and proposed that their individual was a hybrid between *M. mutica* and *C. trifasciata*. In addition, Wink et al. (2001) analyzed mtDNA (cyt*b*) and anonymous nuclear DNA (ISSR) from two *M. iversoni* and also proposed that their individuals were crosses between *M. mutica* and *C. trifasciata*. Somewhat consistent with this interpretation, our *M. iversoni* specimens form a distinct group within *Cuora* that is the sister taxon to the group (((*C. aurocapitata*, C.*trifasciata*), *C. pani*), *C. zhoui*) (Fig. 3), implicating one of these taxa as the potential female parental species. However, the cyt*b* sequence divergence between *M. iversoni*/*O. philippeni* and this clade of *Cuora* is relatively large (2.5–4.0%), suggesting that either the hybridization event(s) that we are identifying are ancient (Wink et al., 2001), or that there is additional sequence variation within *Cuora* that we have not sampled.

4.6. Mauremys pritchardi

Mauremys pritchardi was described from 20 specimens reportedly from Myanmar and Yunnan Province, China. The holotype and two paratypes were reportedly collected in Myanmar while the remaining 17 specimens were reportedly purchased from local markets in both Myanmar and China (McCord, 1997). Subsequent work indicated that some specimens of *M. pritchardi* are hybrids between *M. mutica* and *Chinemys reevesii* (Wink et al., 2001) and in our reconstructions, *M. pritchardi* is grouped with strong support with some *M. mutica* haplotypes to the exclusions of others (BP = 100%, DI = 12, PP = 100%, Fig. 3), thereby indicating paraphyly for *M. mutica*. Thus, our data are consistent with the hypothesis that our specimen of *M. pritchardi* is a hybrid between a female *M. mutica* and another species. However, our data are also consistent with the interpretation that *M. pritchardi* is a recently derived member of the genus *Mauremys*, and that *M. mutica* is composed of more than one species.

4.7. Cuora serrata

Cuora serrata was first described as a subspecies (*C. galbinifrons serrata*) from 29 specimens purchased from Hainan Island, China (Iverson and McCord, 1992) and was later elevated to species status (Fritz and Obst, 1997).

Based on allozyme and mtDNA data (from two and three individuals, respectively), Parham et al. (2001) proposed that *C. serrata* is a hybrid between *C. galbinifrons* and *C.* (previously *Pyxidea*) *mouhotii*. In a relatively large mtDNA (COI and ND4) analysis of the genus *Cuora* (including five *C. serrata*) Stuart and Parham (in press) found strong support for a hybrid origin for *C. serrata*. In their analyses, *C. serrata* was paraphyletic; two *C. serrata*, grouped within *Cuora galbinifrons* and the remaining three grouped within *Cuora boureti*. In our MP and ML results *C. serrata* is the sister taxon to *C. mouhotii*, a result consistent with hybrid origin. However, the cyt*b* sequence divergence between *C. serrata* and *C. mouhotii* is reasonably large (~2.7%), which is also consistent with many species-level divergences.

4.8. Taxonomic issues

Aside from potential hybrid species, our analyses highlight taxonomic issues with respect to well-established geoemydid genera. In making taxonomic recommendations, we share the view of most current systematists that named taxa above the species level should be monophyletic, and demonstrably non-monophyletic taxa should be revised with the goal of achieving monophyly. While most would agree that paraphyletic groups should be revised, practical solutions are often not straightforward. Our position is that taxonomy should be revised to convey the greatest genealogical information, while promoting taxonomic stability. In our view, taxonomic stability dictates that changes at the generic or higher levels only be made when non-monophyletic groups are discovered and need to be replaced by monophyletic ones. Maximal genealogical information implies that, when changes are proposed, monotypic genera be avoided if possible, since they fail to convey meaningful phylogenetic information concerning group membership (Hennig, 1966; Maddison, 1996; Mayr, 1943; Miyamoto and Cracraft, 1991; Stanley, 1979).

For turtles in general, the solution to previous examples of non-monophyly has been a proliferation of monotypic genera. Ostensibly, monotypic genera are often erected in an attempt to indicate large levels of interspecific differentiation, and they are frequently interpreted as species that are so different from all others that they "demand" classification in their own genus. However, we agree with Mayr (1943) in his assessment that the function of the genus name is to identify group membership, while the function of the species is to identify distinctiveness. Within the Geoemydidae, about half (11/23) of the currently recognized genera are monotypic, a condition that seems at odds with the concept of the genus as a mechanism whose primary function is to indicate inclusion within monophyletic groups.

4.9. The Mauremys/Ocadia/Chinemys clade

Although based on DNA alone, and primarily on mtDNA, our analyses strongly support the paraphyly of *Mauremys* as currently recognized (Honda et al., 2002a) (Fig. 3). *Mauremys* is demonstrably paraphyletic with respect to *Chinemys* (*reevesii* and *megalocephala*) and *Ocadia sinensis* (the remaining species of *Ocadia* are probably of hybrid origin). The demonstrable paraphyly of *Mauremys* is based on the position of *M. japonica* within a well-supported clade (BP = 100, DI = 17, PP = 100, Fig. 3) including both *Chinemys* and *Ocadia* to the exclusion of all other *Mauremys* (Fig. 3). Clearly, taxonomic revision within the *Mauremys/Chinemys/Ocadia* clade is required. However, the resolution of this issue is somewhat complicated by the unresolved phylogenetic position of *M. leprosa*. In most analyses, *leprosa* is the sister group to the remainder of the *Mauremys/Chinemys/Ocadia* clade but without strong statistical support, whereas there is strong statistical support for the *Mauremys/Chinemys/Ocadia* clade (BP = 100, DI = 17, PP = 100, Fig. 3). There are two reasonable strategies to replace this demonstrably non-monophyletic group. Either new genera can be erected for *japonica* and potentially *leprosa* (depending on its ultimate phylogenetic position), or both *Chinemys* and *Ocadia* could be synonymized with *Mauremys* sensu latu. We prefer the latter choice for the following reasons: (1) *Mauremys* (exclusive of *M. iversoni*) is a well-supported clade (BP = 98%, DI = 13, PP = 100%, Fig. 3) of relatively closely-related species that contains roughly as much cyt*b* genetic divergence as other non-monotypic geoemydid genera (Fig. 3, S1) [while we do not propose defining clades based on sequence divergence alone, it can be a useful heuristic tool to compare relative divergences within clades], (2) our unpublished data sets with increased taxon sampling (Spinks and Shaffer, unpublished) support the position of *leprosa* as the sister group to the remaining members of the *Mauremys/Ocadia/Chinemys* clade, and (3) recognizing a monophyletic *Mauremys* that includes *C. nigricans*, *C. reevesii*, *C. megalocephala*, and *O. sinensis* promotes long-term taxonomic stability in that a single, well-supported clade receives recognition at the generic level.

Our proposed action requires shifting generic names of four species, two of which (*C. reevesii* and *O. sinensis*) are among the best-known geoemydid taxa. However, it also eliminates the monotypic, and therefore phylogenetically uninformative genus *Ocadia*, and maintains the use of the name *Mauremys* in the spirit that it has been used for over a century, to represent a monophyletic group of generalized pond turtles that spans most of Europe, the Middle East, and Asia. As future phylogenetic studies on this group of turtles resolve relationships within *Mauremys*, there is room for the recognition of subgenera for clades of particular mor-

phological or biogeographic interest. Regardless, the phylogeny and resulting biogeographic interpretations of *Mauremys* species across much of Eurasia is a fascinating area for future research.

4.10. The Rhinoclemmys/Chelopus clade

Yasukawa et al. (2001) found *Rhinoclemmys* to be paraphyletic and partitioned this genus into *Rhinoclemmys* (with seven species) and *Chelopus* (including *annulata* and *rubida*). Based on our larger dataset, *Rhinoclemmys* is monophyletic and the species *annulata* and *rubida* do not form a monophyletic group. Thus, we do not follow Yasukawa et al. (2001) in recognizing *Chelopus*. Rather, we retain the name *Rhinoclemmys* for the entire clade of genetically, morphologically, and biogeographically distinct New World geoemydids.

4.11. The Hieremys/Heosemys clade

Hieremys annandalii is another phylogenetically problematic, monotypic genus. The genera *Heosemys* and *Hieremys* form a well-supported clade (BP = 97%, DI = 13, PP = 100%, Fig. 3) and, in our combined data MP analysis (not shown), *H. annandalii* is the sister group to *Heosemys*. However, in the combined data ML analysis *Heosemys* is paraphyletic with respect to *Hieremys annandalii* (Fig. 3), rendering *Heosemys* potentially, but not demonstrably, paraphyletic. Interestingly the cyt*b* only tree (Fig. 2) provides strong Bayesian support for the position of *Hieremys* nested within *Heosemys*, but this support disappears in the larger dataset (Fig. 3). Thus, we refrain from recommending taxonomic revisions for this clade, and await further data to resolve the potential for non-monophyly of *Heosemys*.

5. Conclusions

With the large number of molecular data sets and analyses now available, researchers can tailor genes to particular phylogenetic questions. In our analyses, cyt*b* as well as 12S and R35 sequence data analyzed separately and in combination provided relatively clear resolution for the intrafamilial phylogenetics of the Geoemydidae. Both MP and ML analyses recovered similar topologies with many well-supported clades, although resolution for some of the deeper nodes in our analyses remain elusive or are only recovered with Bayesian analysis. Our data sets allowed us to test, and statistically to reject previous hypotheses of geoemydid relationships, and to provide a new phylogeny for the group. Our results strongly suggest that the genus *Mauremys* as previously defined is paraphyletic with respect to both *Ocadia* and *Chinemys*, and we suggest that *Mauremys* should be redefined to include the spe-

cies *Mauremys megalocephala*, *Mauremys nigricans*, *Mauremys reevesii*, and *Mauremys sinensis*. We also tentatively support the hypotheses that *Mauremys iversoni*, *Ocadia glyphistoma*, and *O. philippeni* are hybrid taxa due to their well-supported phylogenetic positions within genera to which they are not currently classified, combined with the relatively small levels of genetic divergence between them and their non-congeneric sister taxa. Knowledge of the extent of genetic variation within *Mauremys* and *Cuora*, combined with more extensive sampling of the purported hybrid taxa, should bring final resolution to the hybrid issue, and we are pursuing this additional work. As a group, the Geoemydidae contains a disproportionately high number of endangered and threatened turtles. According to the IUCN, 12 of the 16 most critically endangered turtle species are geoemydids, as are 14 of 30 endangered species and 9 of 37 vulnerable species; one geoemydid turtle—*Cuora yunnanensis*—is considered to be extinct (IUCN, 2002). Thus, a critical problem for the immediate future is to elucidate patterns of genetic variation within clades and species, and use this information to further determine the validity of species recently described from China.

Appendix A

List of species, UC Davis tissue number (preceeded by HBS), and GenBank Accession numbers for DNA sequence data. UF numbers refer to type specimens of potential hybrid taxa deposited in the Florida Museum of Natural History (FLMNH).

Batagur baska baska (HBS38418, AY434600, AY434638, AY434658), *Callagur borneoensis* (HBS38419, AY434601, AB090044, AY434659), *Chelus fimbriata* (U81343, U40636), *Chrysemys picta* (5902189, 5902189), *Clemmys guttata* (AF258870), *Cuora amboinensis amboinensis* (HBS38398, AY434580, AB090029), *Cuora amboinensis couro* (HBS38399, AY434581), *Cuora amboinensis kamaroma* (HBS38394, AY434575), *Cuora amboinensis lineata* (HBS38438, AY434620), *Cuora aurocapitata* (HBS38444, AY434626, AB090030, AY434671), *Cuora flavomarginata evelynae* (HBS38424, AY434606, AB090034, AY434663), *Cuora flavomarginata sinensis* (HBS38389, AY434570), *Cuora mccordi* (HBS38387, AY434568), *Cuora mouhotii* (HBS38422, AY434604, AB090036, AY434661), *Cuora pani* (HBS38393, AY434574, AB090031), *Cuora picturata* (UC Davis Museum of Vertebrate Zoology voucher number = HBS38448, AY434576, AB090028), *Cuora serrata* (UF 81791-98, HBS38442, AY434624, AY434670), *Cuora trifasciata* (Museum of Vertebrate Zoology (MVZ) UC Berkeley voucher number = MVZ 230636, AY434627, AB090032), *Cuora zhoui* (HBS38402, AY434584, AB090033), *Cyclemys atripons* (HBS38435, AY434617), *Cyclemys dentata* (HBS38397, AY434579, AF043402, AY434653), *Cyclemys tcheponensis* (HBS38395, AY434577), *Emys blandingii* (AF258869), *Emys marmorata* (AF258867, U81321), *Emys orbicularis* (AF258868, AB090021), *Geoclemys hamiltoni* (HBS38392, AY434573, AY434632, AY434651), *Geoemyda japonica* (HBS38420, AY434602, AB090038), *Geoemyda spengleri* (HBS38404, AY434586, AY434634, AY434655), *Glyptemys insculpta* (AF258876), *Glyptemys muhlenbergii* (AF258875), *Gopherus agassizii* (HBS38449, AY434562, AY434630, AY434646), *Hardella thurjii thurjii* (HBS38421, AY434603, AB090025, AY434660), *Heosemys depressa* (HBS38425, AY434607), *Heosemys grandis* (HBS38385, AY434566, AB090039), *Heosemys spinosa* (HBS38396, AY434578, U81339, AY434652), *Hieremys annandalii* (HBS38416, AY434598, AF043408, AY434657), *Indotestudo elongata* (HBS38450, AY434643, AF175338), *Indotestudo forsteni* (HBS38451, AY434561, AY434645), *Indotestudo travancorica* (Tissue donated by I. Das, HBS38452, AY434644), *Kachuga dhongoka* (HBS38388, AY434569, AY434631, AY434649), *Leucocephalon yuwonoi* (HBS38426, AY434608, AY434664), *Malayemys subtrijuga* (HBS38409, AY434591, AF043398, AY434656), *Manouria emys* (HBS38453, AY434563, AY434647), *Mauremys annamensis* (HBS38383, AY434564, AB090041), *Mauremys caspica* (HBS38412, AY434594, AB090043), *Mauremys iversoni* 1 (Florida State Museum 71865, UF 71866, HBS38384, AY434565), *Mauremys iversoni* 2 (HBS38427, AY434609), *Mauremys japonica* (HBS38405, AY434587, AB090042), *Mauremys leprosa* (HBS38410, AY434592, AY434635), *Mauremys megalocephala* (HBS38411, AY434593, AY434636), *Mauremys mutica* (HBS38446, AY434628, AB090040, AY434672), *Mauremys mutica kami* (HBS38440, AY434622), *Mauremys pritchardi* (UF

105422-24, HBS38391, AY434572), *Mauremys reevesi* (HBS38386, AY434567, AB090026, AY434648), *Mauremys rivulata* (Tissue donated by P. Vander Schouw, HBS38441, AY434623, AY434641), *Mauremys sinensis* (HBS38433, AY434615, AF043391, AY434666), *Melanochelys trijuga edeniana* (HBS38447, AY434629, AY434642, AY434673), *Melanochelys trijuga trijuga* (HBS38406, AY434588, AF043405), *Morenia ocellata* (HBS38423, AY434605, AF043409, AY434662), *Notochelys platynota* (HBS38431, AY434613, AB090037, AY434665), *Ocadia glyphistoma* (UF 84818, HBS38414, AY434596), *Ocadia philippeni* (UF 80765-66, HBS38400, AY434582), *Orlitia borneensis* (HBS38437, AY434619, AB090024, AY434669), *Pangshura smithii smithii* (HBS38407, AY434589), *Pangshura tecta* (HBS38401, AY434583, AY434633, AY434654), *Pangshura tentoria circumdata* (HBS38428, AY434610), *Pangshura tentoria flaviventer* (HBS38430, AY434612, AY434639), *Pelodiscus sinensis* (AY259553, AB090045), *Rhinoclemmys annulata* (HBS38439, AY434621), *Rhinoclemmys areolata* (HBS38429, AY434611), *Rhinoclemmys diademata* (HBS38434, AY434616, AY434640, AY434667), *Rhinoclemmys funerea* (HBS38417, AY434599), *Rhinoclemmys melanosterna* (HBS38408, AY434590), *Rhinoclemmys pulcherrima incisa* (HBS38415, AY434597), *Rhinoclemmys punctularia punctularia* (HBS38413, AY434595, AY434637), *Rhinoclemmys rubida* (Tissue donated by R. Wicker, Frankfurt Zoo, HBS38443, AY434625), *Sacalia bealei* (HBS38403, AY434585, AB090023), *Sacalia quadriocellata* (HBS38436, AY434618, AF043392, AY434668), *Sacalia pseudocellata* (HBS38432, AY434614), *Siebenrockiella crassicollis* (HBS38390, AY434571, AF043406, AY434650), *Staurotypus triporcatus* (U81349, AB090018), *Terrapene ornata* (AF258874), *Trachemys scripta* (AF207750, AB090022).

References

Alfaro, M.E., Arnold, S.J., 2001. Molecular systematics and evolution of *Regina* and the Thamnophiine snakes. Mol. Phylogenet. Evol. 21, 408–423.

Barth, D., Bernhard, D., Guicking, D., Stock, M., Fritz, U., 2003. Is *Chinemys megalocephala* Fang, 1934 a valid species? New insights based on mitochondrial DNA sequence data. Salamandra 38, 233–244.

Bickham, J.W., 1975. A cytosystematic study of turtles in the genera *Clemmys*, *Mauremys* and *Sacalia*. Herpetologica 31, 198–201.

Bour, R., Dubois, A., 1986. Nomenclature ordinale et familiale des tortues (Reptilia). Note complimentaire. Bull. Mensuel de la Société Linnéenne de Lyon 55, 87–90.

Bowen, B.W., Nelson, W.S., Avise, J.C., 1993. A molecular phylogeny for marine turtles: trait mapping, rate assessment and conservation relevance. Proc. Natl. Acad. Sci. USA 90, 5574–5577.

Bramble, D.M., 1974. Emydid shell kinesis: Biomechanics and evolution. Copeia 1974, 707–727.

Broughton, R.E., Stanley, S.E., Durrett, R.T., 2000. Quantification of homoplasy for nucleotide transitions and transversions and a

reexamination of assumptons in weighted phylogenetic analysis. Syst. Biol. 49, 617–627.

Caccone, A., Gibbs, J.P., Ketmaier, V., Suatoni, E., Powell, J.R., 1999. Origin and evolutionary relationships of giant Galápagos tortoises. Proc. Natl. Acad. Sci. USA 96, 13223–13228.

Carr, J.L., Bickham, J.W., 1986. Phylogenetic implications of karyotype variation in the Batagurinae (Testudines: Emydidae). Genetica 70, 89–106.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

Dolphin, K., Belshaw, R., Orme, C.D.L., Quicke, D.L.J., 2000. Noise and incongruence: interpreting results of the incongruence length difference test. Mol. Phylogenet. Evol. 17, 401–406.

Dowton, M., Austin, A.D., 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behavior of the Incongruence Length Difference test in mixed-model analyses. Syst. Biol. 51, 19–31.

Engstrom, T.N., Shaffer, H.B., McCord, W.P., 2002. Phylogenetic diversity of endangered and critically endangered southeast Asian softshell turtles (Trionychidae: *Chitra*). Biol. Cons. 104, 173–179.

Ernst, C.H., Barbour, R.W., 1989. Turtles of the World. Smithsonian Institution Press, Washington, DC.

Eriksson, T., 1998. AutoDecay ver. 4.0 (program distributed by author). Department of Botany. Stockholm University, Stockholm. (Available from http://www.bergianska.se/index_forsk-ning_soft.html).

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–320.

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

Friedel, R.H., Stubbusch, J., Barde, Y., Schnuerch, H., 2001. A novel 7-transmembrane receptor expressed in nerve growth factor-dependent sensory neurons. Mol. Cell. Neurosci. 17, 31–40.

Fritz, U., Obst, F.J., 1997. Zum taxonomischen status von *Cuora galbinifrons serrata* (Iverson and McCord, 1992) und *Pyxidea mouhotii* (Gray, 1862) (Reptilia:Testudines: Bataguridae). Zool. Abh. Staatl. Mus. Tierk. Dresden 49, 261–279.

Fujita, M.K., Engstrom, T.N., Starkey, D.E., Shaffer, H.B., Turtle Phylogeny: insights from a novel nuclear intron. Mol. Phylogenet. Evol., in press.

Gaffney, E.S., Meylan, P.A., 1988. A phylogeny of turtles. In: Benton, M.J. (Ed.), The Phylogeny and Classification of Tetrapods. Clarendon Press, Oxford, England, pp. 157–219.

Gerlach, J., 2001. Tortoise phylogeny and the 'Geochelone' problem. Phelsuma 9, 1–24.

Georges, A., Birrel, J., Saint, K.M., McCord, W., Donnellan, S.C., 1998. A phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation. Biol. J. Linn. Soc. 67, 213–246.

Giannasi, N., Malhotra, A., Thorpe, R.S., 2001. Nuclear and mtDNA phylogenies of the *Trimeresurus* complex: implications for the gene versus species tree debate. Mol. Phylogenet. Evol. 19, 57–66.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T., 2000. The global decline of reptiles, DéjàVu Amphibians. Bioscience 50, 653–666.

Gray, J.E., 1870. Supplement to the Catalogue of Shield Reptiles in the Collection of the British Museum. Part 1. Testudinata (Tortoises). Taylor and Francis, London.

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 (Reptilia: Testudines: Geoemydidae). Zool. Abh. Staatl. Mus. Tierk. Dresden 23, 75–86.

Hennig, W., 1966. Phylogenetic Systematics. University of Illinois Press, Urbana, IL.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.

Hirayama, R., 1984. Cladistic analysis of batagurine turtles (Batagurinae: Emydinae: Testudinoidea): A preliminary result. Studia Geologica Salmanticensia, vol Especial 1. Studia Palaeocheloniol. 1, 141–157.

Honda, M., Yasukawa, Y., Hirayama, R., Ota, H., 2002a. Phylogenetic relationships of the Asian box turtles of the genus *Cuora* sensu lato (Reptilia: Bataguridae) inferred from mitochondrial DNA sequences. Zool. Sci. 17, 1305–1312.

Honda, M., Yasukawa, Y., Ota, H., 2002b. Phylogeny of the Eurasian freshwater turtles of the genus *Mauremys* Gray 1869 (Testudines), with special reference to a close affinity of *Mauremys japonica* with *Chinemys reevesii*. J. Zool. Syst. Evol. Res. 40, 195–200.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

IUCN Asian Turtle Workshop: developing strategies through captive management (Briefing Book). 2001. Fort Worth Zoo, Fort Worth Texas, 26–28 January.

IUCN, 2002. 2002 IUCN Red List of Threatened Species. Iverson, J.B., 1992. A revised checklist with distribution maps of the turtles of the world. Privately printed. Richmond, IN.

Iverson, J.B., 1992. A revised checklist with distribution maps of the turtles of the world. Privately printed, Richmond, IN.

Iverson, J.B., Gotte, S., Lovich, J., Ernst, C.H., 1989. The validity of *Chinemys megalocephala* (Testudines: Batagurinae). Copeia 1989, 494–498.

Iverson, J.B., McCord, W.P., 1992. A new subspecies of *Cuora galbinifrons* (Testudines: Batagurinae) from Hainan Island, China. Proc. Biol. Soc. Wash. 105, 433–439.

Källersjö, M., Albert, V.A., Farris, J.S., 1999. Homoplasy *increases* phylogenetic structure. Cladistics 15, 91–93.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kou, Z., 1989. *Cyclemys* from Yunnan, a description of a new species and a new record to China (Testudinata: Emydidae). Curr. Herpetol. East Asia, 193–197.

Lamb, T., Lydeard, C., 1994. A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the Testudinoidea. Mol. Phylogenet. Evol. 3, 283–291.

Lau, M., Chan, B., Crow, P., Ades, G., 2000. Trade and conservation of turtles and tortoises in the Hong Kong Special Administrative Region, People's Republic of China. In: van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J. (Eds.), Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia. Chelonian Research Foundation, Lunenburg, MA, pp. 39–44.

Lau, M., Shi, H., 2000. Conservation and trade of terrestrial and freshwater turtles and tortoises in the People's Republic of China. In: van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J. (Eds.), Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia. Chelonian Research Foundation, Lunenburg, MA, pp. 30–38.

Maddison, D.R., 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. 40, 315–328.

Maddison, W.P., 1996. Molecular approaches and the growth of phylogenetic biology. In: Ferraris, J.D., Palumbi, S.R. (Eds.), Molecular Zoology Advances, Strategies, and Protocols. Wiley, New York, pp. 47–63.

Mayr, E., 1943. Criteria of subspecies, species and genera in ornithology. Ann. N. Y. Acad. Sci. 43, 133–139.

McCord, W.P., 1997. *Mauremys pritchardi*, a new batagurid turtle from Myanmar and Yunnan, China. Chelonian Conserv. Biol. 2, 555–562.

McCord, W.P., Iverson, J.B., 1992. A new species of *Ocadia* (Testudines:Bataguridae) from Hainan Island, China. Proc. Biol. Soc. Wash. 105, 13–18.

McCord, W.P., Iverson, J.B., 1994. A new species of *Ocadia* (Testudines:Batagurinae) from Southwestern China. Proc. Biol. Soc. Wash. 107, 52–59.

McCord, W.P., Iverson, J.B., Spinks, P.Q., Shaffer, H.B., 2000. A new genus of geoemydid turtle from Asia. Hamadryad 25, 20–24.

McDowell, S.B., 1964. Partition of the genus *Clemmys* and related problems in the taxonomy of aquatic Testudinidae. Proc. Zool. Soc. Lond. 143, 239–279.

Mindell, D.P., Sorenson, M.D., Dimcheff, D.E., Hasegawa, M., Ast, J.C., Yuri, T., 1999. Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. Syst. Biol. 48, 138–152.

Miyamoto, M.M., Cracraft, J., 1991. Phylogenetic inference, DNA sequence analysis, and the future of molecular systematics. In: Miyamoto, M.M., Cracraft, J. (Eds.), Phylogenetic Analysis of DNA Sequence Data. Oxford University Press, New York, pp. 3–17.

Page, R.D.M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12, 357–358. Available from <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>.

Palkovacs, E.P., Gerlach, J., Caccone, A., 2002. The evolutionary origin of Indian Ocean tortoises (*Dipsochelys*). Mol. Phylogenet. Evol. 24, 216–227.

Palumbi, S.R., Martin, A.P., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR. Special Publ. Dept. Zool. University of Hawaii, Honolulu.

Parham, J.F., Simison, B.W., Kozak, K.H., Feldman, C.R., Shi, H., 2001. New Chinese turtles: endangered or invalid. A reassessment of two species using mitochondrial DNA, allozyme electrophoresis and known-locality specimens. Anim. Conserv. 4, 357–367.

Perry, W.L., Lodge, D.L., Feder, J.L., 2002. Importance of hybridization between indigenous and nonindigenous freshwater species: an overlooked threat to North American biodiversity. Syst. Biol. 51, 255–275.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Pritchard, P.C.H., McCord, W.P., 1991. A new emydid turtle from China. Herpetologica 47, 139–147.

Prychitko, T.M., Moore, W.S., 2000. Comparative evolution of the mitochondrial cytochrome *b* gene and nuclear beta-fibrinogen intron 7 in woodpeckers. Mol. Biol. Evol. 17, 1101–1111.

Ruvolo, M., 1997. Molecular phylogeny of the hominoids: inferences from multiple independent DNA sequence data sets. Mol. Biol. Evol. 14, 248–265.

Sanderson, M.J., Shaffer, H.B., 2002. Troubleshooting molecular phylogenetic analyses. Ann. Rev. Ecol. Syst. 33, 49–72.

Shaffer, H.B., Meylan, P., McKnight, M.L., 1997. Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. Syst. Biol. 46, 235–268.

Shi, H., Parham, J.F., 2001. Preliminary observations of a large turtle farm in Hainan Province, People's Republic of China. Turtle Tortoise Newslett. 3, 4–6.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inferences. Mol. Biol. Evol. 16, 1114–1116.

Sites, J.W., Greenbaum, I.F., Bickham, J., 1981. Biochemical systematics of neotropical turtles of the genus *Rhinoclemmys* (Emydidae: Batagurinae). Herpetologica 37, 256–264.

Sites, J.W., Bickham, J.W., Pytel, B.A., Greenbaum, I.F., Bates, B.B., 1984. Biochemical characters and the reconstruction of turtle phylogenies: relationships among batagurine genera. Syst. Zool. 33, 137–158.

Stanley, S., 1979. Macroevolution Pattern and Process. W.H. Freeman, San Francisco.

Stuart, B.L., Parham, J.F., Molecular phylogeny of the critically endangered Indochinese box turtle (*Cuora galbinifrons*). Mol. Phylogenet. Evol., in press.

Swofford, D.L., 2001. PAUP* Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA.

Thirakhupt, K., van Dijk, P.P., 1994. Species diversity and conservation of turtles of western Thailand. Nat. Hist. Bull. Siam. Soc. 42, 207–259.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

van Dijk, P.P., 2000. The status of turtles in Asian. In: van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J. (Eds.), Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia. Chelonian Research Foundation, Lunenburg, MA, pp. 15–23.

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. Chelonian Research Foundation. MTC Printing, Lunenburg, MA.

Weisrock, D.W., Janzen, F.J., 2000. Comparative molecular phylogeography of North American softshell turtles (*Apalone*): implications for regional and wide-scale historical evolutionary forces. Mol. Phylogenet. Evol. 14, 152–164.

Wink, M., Guiking, D., Fritz, U., 2001. Molecular evidence for hybrid origins of *Mauremys iversoni* Pritchard and McCord, 1991, and *Mauremys pritchardi* McCord, 1997 (Reptilia: Testudines: Bataguridae). Zool. Abh. Staatl. Mus. Tierk. Dresden 52, 41–49.

Wu, P., Zhou, K., Yang, Q., 1998. Evolution of hinged turtles in Bataguridae inferred from sequences of 12S rRNA gene. Chin. J. Appl. Environ. Biol. 4, 374–378.

Yasukawa, Y., Hirayama, R., Hikida, T., 2001. Phylogenetic relationships of geoemydine turtles (Reptilia: Bataguridae). Curr. Herpetol. 20, 105–133.

Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine combinability for Slow Loris phylogeny. Syst. Biol. 50, 408–424.