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Molecular Phylogenetics of Emydine Turtles: Taxonomic Revision and the Evolution of Shell Kinesis

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The 10 extant species of emydine turtles represent an array of morphological and ecological forms recognizable and popular among scientists and hobbyists. Nevertheless, the phylogenetic affinities of most emydines remain contentious. Here, we examine the evolutionary relationships of emydine turtles using 2092 bp of DNA encoding the mitochondrial genes cyt *b*, ND4, and adjacent tRNAs. These data contain 339 parsimony informative characters that we use to erect hypotheses of relationships for the Emydinae. Both maximum parsimony and maximum likelihood methods yield a monophyletic Emydinae in which all but three nodes are well resolved. *Emys orbicularis, Emydoidea blandingii,* and *Clemmys marmorata* form a monophyletic clade, as do the species of *Terrapene*. *Clemmys muhlenbergii* and *Clemmys insculpta* form a third monophyletic group that may be sister to all other emydines. *Clemmys guttata* is problematic and probably related to *Terrapene*. Based on this phylogeny, and previous molecular work on the group, we suggest the following taxonomic revisions: (1) *Clemmys* should be restricted to a single species, *C. guttata*. (2) *Calemys* should be resurrected for *C. muhlenbergii* and *C. insculpta*. (3) *Emys* should be expanded to include three species: *E. orbicularis, E. blandingii,* and *E. marmorata*. Furthermore, our analyses show that neither kinetic-shelled nor akinetic-shelled emydines form monophyletic groups. Therefore, shell kinesis was either independently gained in *Emys* and *Terrapene* or secondarily lost in *E. marmorata* and *C. guttata*. Parsimony, paleontological evidence, and the multiple origins of shell kinesis in related turtle lineages (especially geoemydines) support the independent origin of plastral kinesis. © 2002 Elsevier Science (USA)

Key Words: **Emydidae; molecular phylogenetics; mitochondrial DNA; turtles; taxonomy; shell kinesis.**

INTRODUCTION

The Emydinae (*sensu* Gaffney and Meylan, 1988) is a small turtle subfamily represented by 10 extant species (9 North American, 1 European) currently assigned to four morphologically and ecologically diverse genera: *Clemmys, Emydoidea, Emys,* and *Terrapene.* This group contains some of the most well studied, popular, and recognizable turtles in the world. For example, the European pond turtle, *Emys orbicularis,* is one of the oldest named chelonians (Linnaeus, 1758) and box turtles of the genus *Terrapene* are among the most popular reptile pets in North America and Europe. Other members of this group are well known to scientists and laymen alike because of their declining numbers (e.g., *Clemmys muhlenbergii;* Bury, 1979; Collins, 1990).

This small clade of turtles exhibits greater ecological and morphological diversity than its more speciose sister group, the deirochelyines (*sensu* Gaffney and Meylan, 1988). Some species, such as *Clemmys marmorata,* have webbed feet, a hydrodynamic shell, and an aquatic life style. Others, such as *Terrapene ornata,* have robust terrestrial limbs, have a high-domed shell, and inhabit arid landscapes (Legler, 1960). Still other species, such as *Clemmys insculpta,* are morphologically and ecologically intermediate.

The emydine genera *Emys, Emydoidea,* and *Terrapene* have moveable hinges on the ventral part of their shells (plastron). This trait, known as plastral kinesis, is relatively rare among living chelonians. In most turtles, including all other emydids (*sensu* Gaffney and Meylan, 1988), the plastron is rigid and immovable (Fig. 1A). Turtles with advanced plastral kinesis, however, can pull the bottom shell up toward the top shell (carapace) and more securely protect the limbs and head (Fig. 1B). Hence, this form of plastral kinesis might be viewed as an antipreditor adaptation. Emydine plastral kinesis is unique and is thought to have originated only once within this turtle subfamily (Gaffney and Meylan, 1988; Bramble, 1974). Emydine shell kinesis involves several morphological specializa-

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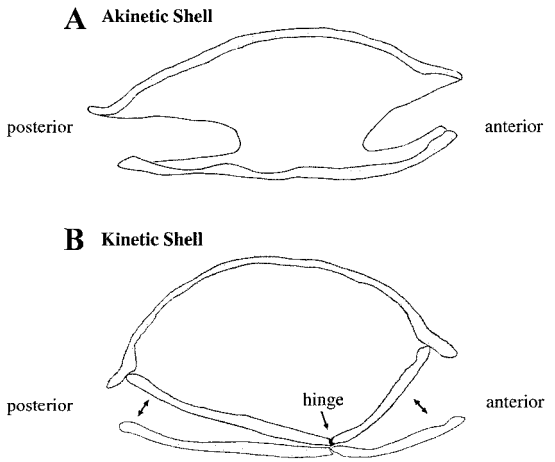

FIG. 1. Sagittal view of turtle shells. (A) Typical turtle shell lacking plastral kinesis; the rigid plastron is firmly attached to the carapace by a solid bony bridge (modified from Meylan and Gaffney, 1989). (B) Turtle shell exhibiting advanced plastral kinesis; the hinged plastron, shown in both open and closed positions, is loosely attached to the carapace (modified from Bramble, 1974).

tions: (1) an alignment of scales with plastral sutures and a reduction of sutural connections to form a hinge, (2) segmented scapulae that facilitate head and limb retraction, and (3) a closing mechanism exapted from cervical musculature (Bramble, 1974). These specializations are most pronounced in the genus *Terrapene,* which can completely conceal its head and limbs within a closable shell (hence the common name, box turtle).

Although emydine turtles are extensively studied and popular, the phylogenetic relationships among lineages remain contentious. Morphological treatments of the Emydinae (Bramble, 1974; Gaffney and Meylan, 1988) have suggested that the box turtles and other hinged genera form a monophyletic group (Fig. 2A). By default, the species without plastral kinesis were lumped into the genus *Clemmys.* Molecular evidence from the 16S ribosomal gene (Bickham *et al.,* 1996), on the other hand, indicated that the genus *Clemmys* is not monophyletic (Fig. 2B). The ribosomal DNA data further suggested that the six hinged emydines might not all be one another's closest relatives. An attempt to combine these morphological and molecular data (Burke *et al.,* 1996) in a total evidence analysis (Eernisse and Kluge, 1993) resolved a monophyletic hinged clade but also suggested a paraphyletic *Clemmys* (Fig. 2C). While some consensus has emerged for a few nodes (e.g., monophyly of *Terrapene*), the weak support for most hypothesized arrangements may be improved with additional molecular data.

Our objective is to elucidate the evolutionary history of the Emydinae using rapidly evolving molecular markers and samples of all extant species. Then, based on our proposed phylogeny, we revise the taxonomy of the Emydinae and discuss the evolution of plastral kinesis.

MATERIALS AND METHODS

Genes Chosen

The rate of mitochondrial DNA evolution has been shown to be up to eight times slower in turtles than in other vertebrate groups (Avise *et al.,* 1992; Lamb *et al.,* 1994). Accordingly, many systematists have demonstrated the utility of quickly evolving molecular markers for higher level chelonian questions (e.g., Dutton *et al.,* 1996; Shaffer *et al.,* 1997; Lamb and Osentoski, 1997; Caccone *et al.,* 1999). In emydid turtles in particular, Bickham *et al.* (1996) showed that the 16S ribosomal gene is helpful for resolving relationships at or above the family level, while Lenk *et al.* (1999) demonstrated that cyt *b* is a practical marker for intraspecific questions. Thus, we chose cyt *b* and the rapidly evolving ND4 gene. Both of these protein-coding genes, which have been valuable in lower level questions in other reptiles (e.g., Zamudio and Greene, 1997; Feldman, 2000; Rodriguez-Robles *et al.,* 2001), have also been useful in higher level turtle studies (e.g., Dutton *et al.,* 1996; Shaffer *et al.,* 1997).

Taxon Sampling and Laboratory Protocols

We obtained liver tissue from museum specimens and blood samples from living zoo specimens for all 10 extant emydine species and two deirochelyine outgroup species (Appendix 1). We isolated genomic DNA from tissue and blood samples by standard proteinase K digestion and phenol/chloroform purification (Maniatis *et al.,* 1982). We amplified a 1200-bp region of mtDNA encoding the entire cyt *b* gene and part of the adjacent tRNA threonine ($tRNA^{thr}$) via PCR (Saiki *et al.,* 1988) using primers GLUDG-L (Palumbi *et al.,* 1991) and M (Shaffer *et al.,* 1997) (Table 1). We amplified an additional 900 bp of mtDNA encoding a portion of ND4 and flanking $tRNA^{his}$, $tRNA^{ser}$, and a portion of $tRNA^{leu}$ using primers ND4 and Leu (Arevalo *et al.,* 1994) (Table 1). We used the following thermal cycle parameters for 50-μl amplification reactions: 35 cycles of 1 min denaturing at 94°C, 1 min annealing at 50–52°C, and 2 min extension at 72°C. We purified PCR products using the Wizard Prep Mini Column Purification Kit (Promega, Inc.) and used purified template in 10-μl dideoxy chain-termination reactions (Sanger *et al.,* 1977) using ABI Big Dye chemistry (Perkin–Elmer Applied Biosystems, Inc.) and the primers listed in Table 1. We ran cycle-sequenced products on a 4.8% Page Plus (Ameresco) acrylamide gel using an ABI 377 automated sequencer (Perkin–Elmer Applied Biosystems, Inc.). We sequenced all samples in both directions.

Sequence Analyses

We aligned DNA sequences with the program Sequencher 3.0 (Gene Codes Corp.). We translated protein-coding nucleotide sequences into amino acid sequences using MacClade 3.06 (Maddison and

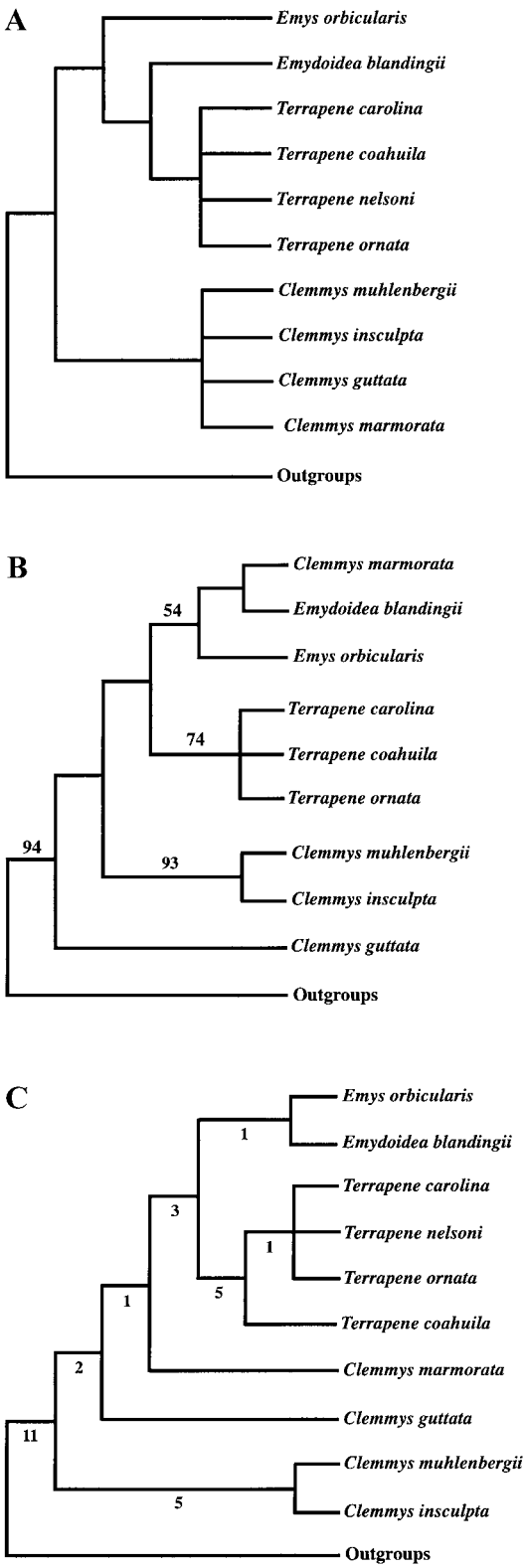

FIG. 2. Previous phylogenetic hypotheses for the Emydinae. (A) Phylogeny based on five osteological characters (Gaffney and Meylan, 1988). (B) Phylogeny based on mitochondrial rRNA sequence data (Bickham *et al.*, 1996) shown with bootstrap support. (C) Phylogeny based on the Bickham *et al.* (1996) sequence data as well as additional morphometric, ecological, behavioral, and biochemical information (Burke *et al.*, 1996) shown with decay indices.

Maddison, 1992). We identified tRNA genes by manually reconstructing their secondary structures using the criteria of Kumazawa and Nishida (1993). We deposited all mitochondrial DNA sequences in GenBank (Appendix 1).

We performed a partition homogeneity test (PH), similar to the incongruence length differences test (Farris *et al.*, 1994), to determine whether the ND4 and cyt *b* data could be combined. We used PAUP* 4.0b3a (Swofford, 1998) to generate a null distribution of length differences using 1000 same-sized, randomly generated partitions from the original data with replacement.

To judge base substitution saturation at first, second, and third codon positions, we plotted the uncorrected percentage sequence divergence of transitions and transversions versus the corrected Kimura two-parameter (Kimura, 1980) estimates of divergence for each codon position.

Phylogenetic Analyses

We used maximum parsimony (MP; Swofford *et al.*, 1996) and maximum likelihood (ML; Felsenstein, 1981) phylogenetic methods to infer evolutionary relationships of emydine species. We conducted all phylogenetic analyses in PAUP*. We polarized the phylogeny via outgroup comparison (Maddison *et al.*, 1984) using the chicken turtle, *Deirochelys reticularia*, and the painted turtle, *Chrysemys picta*. Previous morphological and molecular phylogenetic studies suggest that these deirochelyine turtles are appropriate outgroup taxa (Gaffney and Meylan, 1988; Bickham *et al.*, 1996).

We executed MP analyses with the branch-and-bound search algorithm (Hendy and Penny, 1982) using unordered characters. To assess the robustness of individual nodes, we used the bootstrap resampling method (Felsenstein, 1985) employing 1000 replicates of branch-and-bound searches in PAUP*. Additionally, we calculated branch support (Bremer, 1994) for all nodes using the program TreeRot 2 (Sorenson, 1999).

We performed ML analyses to estimate branch lengths and search for additional tree topologies. To determine the most appropriate model of DNA substitution for reconstructing emydine relationships under ML, we executed a hierarchical likelihood ratio test (Felsenstein, 1993; Goldman, 1993; Yang, 1996) in the program Modeltest 3.0 (Posada and Crandall, 1998). The model of DNA evolution that best fit these sequence data was the general time reversible model (GTR; Rodriguez *et al.*, 1990) of nucleotide substitution in conjunction with gamma (Γ; Yang, 1994a,b). The GTR + Γ model accommodates unequal base composition by using the empirical base frequencies, estimates the uneven ratio of each type of nucleotide substitution, and accounts for the heterogeneous rates of nucleotide substitutions across all sites.

TABLE 1

Oligonucleotide Primers Used to Amplify and Sequence Turtle mtDNA in This Study

Primer	Gene	Sequence	Position	Reference
(L) ND4	ND4	5′-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3′	10,919	Arevalo *et al.*, 1994
(H) Leu	$tRNA^{leu}$	5′-AC CAC GTT TAG GTT CAT TTT CAT TAC-3′	11,837	Arevalo *et al.*, 1994
(L) GLUDG	$tRNA^{Glu}$	5′-TGA CTT GAA RAA CCA YCG TTG-3′	14,378	Palumbi *et al.*, 1991
(H) Primus-rev	cyt *b*	5′-CGG TTG CAC CTC AGA AGG ATA TTT GGC CTC A-3′	14,804	This study
(L) Primus	cyt *b*	5′-TGA GGC CAA ATA TCC TTC TGA GGT GCA ACC G-3′	14,834	This study
(H) Rush-rev	cyt *b*	5′-GTT GGG TTG TTT GAT CCG GTT TCA TGT AGA AA-3′	14,996	This study
(L) Rush	cyt *b*	5′-TTC CTA CAT GAA ACC GGA TCA AAC AAC CCA AA-3′	15,027	This study
(H) M	$tRNA^{thr}$	5′-TCA TCT TCG GTT TAC AAG AC-3′	15,574	Shaffer *et al.*, 1997

Note. The 3′ ends of the primers match nucleotide positions of the heavy strand of the mitochondrial genome of the deirochelyine turtle *Chrysemys picta* (Mindell *et al.*, 1999). Ambiguity codes: R = A or G; Y = C or T.

RESULTS

Genetic Variation

Sequences from the protein-coding regions appear functional and there are no gene rearrangements in the data (Kumazawa and Nishida, 1995; Kumazawa *et al.*, 1996; Macey and Verma, 1997; Macey *et al.*, 1997). However, cyt *b* positions 1108–1110 of *D. reticularia* possess a codon deletion (all other specimens have alanine at this codon site). Additionally, $tRNA^{ser}$ is unusual in both emydines and deirochelyines because it has a short D-stem instead of a D-arm replacement loop like that of most metazoan taxa (Kumazawa and Nishida, 1993).

The PH test shows that length difference between the sum of the ND4 and cyt *b* trees and the combined ND4 and cyt *b* trees is not significantly different from the randomly generated test statistic ($P > 0.05$). Therefore, the aligned DNA sequences are sufficiently homogeneous (Bull *et al.*, 1993) and ND4 and cyt *b* data can be combined and examined using MP and ML methods.

Of the 2092 aligned nucleotides, 609 are variable and 339 are parsimony informative. Among ingroup taxa, 461 bp are variable and 251 parsimony informative. Of the 609 variable characters, 139 occur at first codon positions, 61 at second positions, 363 at third positions, and 46 in tRNAs. The scatter diagrams are linear and show no evidence of multiple hit problems for transversions and transitions within the ingroup (Fig. 3). Comparisons between ingroup and outgroup taxa, however, show that third-codon-position transitions may be saturated.

Phylogenetic Relationships

The branch-and-bound equally weighted MP analysis produces a single most parsimonious tree (L = 1088; CI = 0.642; RI = 0.466) and the ML GTR + Γ reconstruction also yields one tree (−lnl = 7798.4184; $\alpha = 0.2766$). The two trees differ only in the placement of a single taxon (Fig. 4). In all analyses emydine turtles unambiguously group to the exclusion of the two deirochelyines (100% bootstrap; 37 decay index) and phylogenetic relationships are well resolved and well supported for all but three nodes of the tree.

The hinged genus, *Terrapene*, forms a monophyletic assemblage (100% bootstrap; 16 decay index) in which *T. ornata* and *T. nelsoni* form one clade (99% bootstrap; 10 decay index) and *T. coahuila* and *T. carolina* form another (94% bootstrap; 5 decay index). The genus *Clemmys* is not monophyletic. Instead, *C. marmorata* is a member of a clade containing *Emydoidea blandingii* and the Old World turtle *Emys orbicularis* (98% bootstrap; 10 decay index). Relationships among these three taxa are not well resolved, as indicated by the conflict between the MP and the ML reconstructions; the MP tree connects *Emys orbicularis* to *C. marmorata* (56% bootstrap; 1 decay index) while the ML tree links *Emys orbicularis* to *Emyd. blandingii*. Additionally, the turtle *C. guttata* does not group with the other eastern U.S. *Clemmys*, *C. insculpta* and *C. muhlenbergii*. Instead, *C. guttata* garners meager support as the sister taxon to the *Terrapene* clade (70% bootstrap; 3 decay index) in both the MP and the ML reconstructions. Finally, *C. insculpta* and *C. muhlenbergii* form a strong monophyletic group (100% bootstrap; 10 decay index). Both MP and ML analyses suggest that *C. insculpta* and *C. muhlenbergii* are sister to a monophyletic clade containing the rest of the Emydinae. This phylogenetic position, however, is not well supported (63% bootstrap; 2 decay index).

DISCUSSION

Our analyses of the cyt *b*, ND4, $tRNA^{thr}$, $tRNA^{his}$, $tRNA^{ser}$, and partial $tRNA^{leu}$ mitochondrial genes show that these markers are evolving at a rate appropriate for the study of emydine systematics. Because of the large number of informative nucleotides most nodes are well resolved and well supported in both MP and ML analyses. Therefore, we address several hypotheses of emydine phylogeny, taxonomy and evolution using our molecular phylogeny.

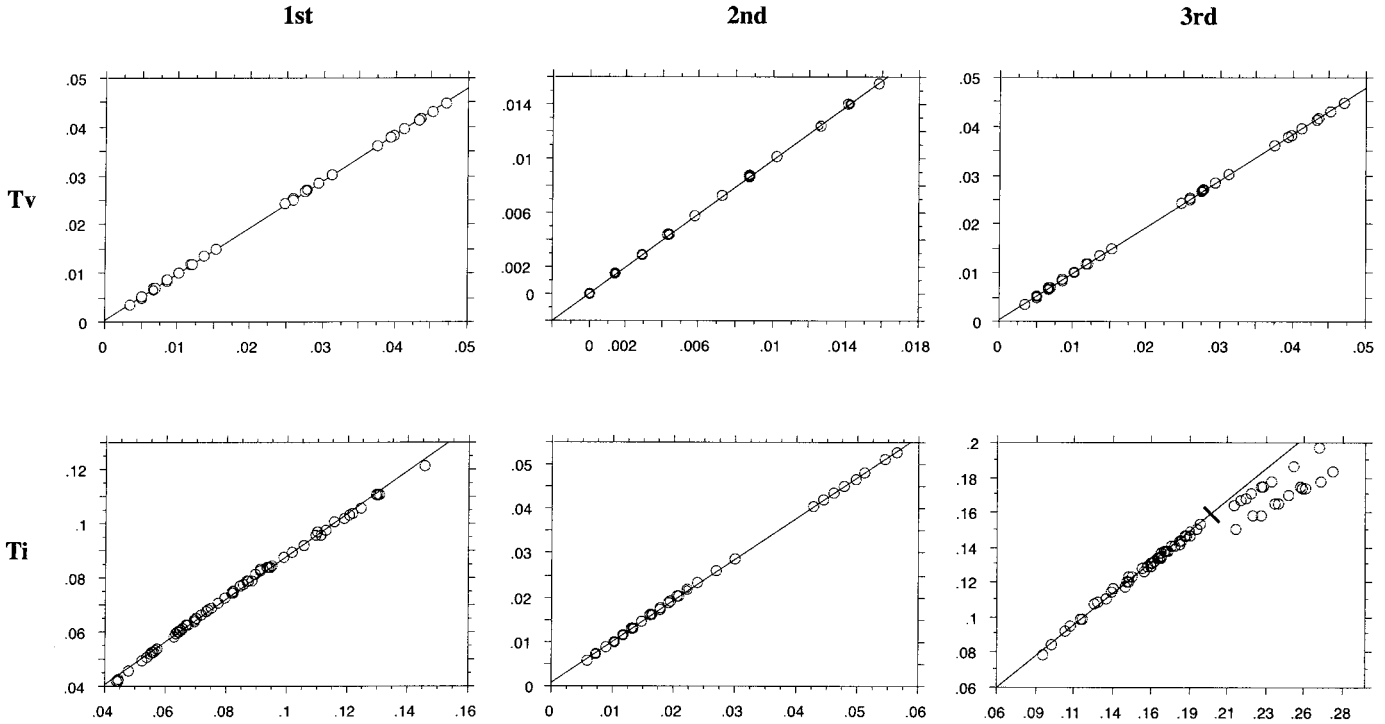

FIG. 3. Saturation plots of uncorrected pairwise sequence differences versus Kimura two-parameter estimates of pairwise distances for transversions and transitions at each codon position.

Phylogenetic Relationships

The phylogenetic relationships of emydine turtles have been the subject of recent studies using morphological evidence (Gaffney and Meylan, 1988), molecular evidence (Bickham *et al.*, 1996), and both character types (Burke *et al.*, 1996). Our mtDNA phylogeny is both concordant and discordant with these previous phylogenies in several noteworthy ways. Here, we emphasize the most important points of our proposed phylogeny: relationships within *Terrapene*, paraphyly of *Clemmys*, and the multiple origins of emydine turtles with plastral kinesis.

While the monophyly of the North American box turtles has never been questioned, the evolutionary relationships among species have been frequently debated. Several authors have hypothesized that the genus *Terrapene* forms a bipartite monophyletic group (Milstead, 1960, 1967, 1969; Milstead and Tinkle, 1967; Minx, 1992, 1996). They proposed that box turtles form two clades: a *T. ornata* group comprising the western box turtle, *T. ornata*, and the Sonoran box turtle, *T. nelsoni*, and a *T. carolina* group composed of the Mexican *T. coahuila* and eastern box turtle, *T. carolina*. An alternative hypothesis, largely based on the less developed plastral kinesis and aquatic ecology of *T. coahuila*, suggested that the Coahuilan box turtle is sister to a group containing the remaining box turtle species (Auffenberg, 1958; Legler, 1960; Williams *et al.*, 1960; Bramble, 1974; Burke *et al.*, 1996). Our data support the former hypothesis, separating *Terrapene* into western and eastern clades (Fig. 4). The phylogenetic position of the aquatic *T. coahuila*, sister to the semiterrestrial *T. carolina*, could indicate that some degree of terrestriality evolved more than once within *Terrapene* (once within *T. carolina* and once within the *T. ornata* group). Alternatively, the Coahuilan box turtle might have became secondarily aquatic to suit the spring habitats of the Cuatro Cieneagas region of Mexico (Milstead, 1969; Minx, 1996). A comprehensive review of the *T. carolina* complex using molecular techniques is long overdue. If *T. coahuila* is nested within the semiterrestrial *T. carolina*, as hypothesized by Milstead (1960, 1967, 1969) and Minx (1992), it would imply a secondarily aquatic ecology.

Our molecular data unambiguously demonstrate the paraphyly of the genus *Clemmys* (Fig. 4). A paraphyletic *Clemmys* stands in contrast to the purely morphological analysis of the Emydinae (Gaffney and Meylan, 1988; Fig. 2A), but has been suggested by molecular (Bickham *et al.*, 1996; Fig. 2B) and combined analyses (Burke *et al.*, 1996; Fig. 2C). As a second test of the paraphyly of *Clemmys*, we constrained the equally weighted, branch-and-bound MP searches to recover only those trees that produce a monophyletic *Clemmys*. The shortest tree generated by the constraint search is 1114 steps long (CI = 0.624; RI = 0.428), 26 steps longer than the most parsimonious unconstrained estimate of emydine phylogeny. A comparison of the con-

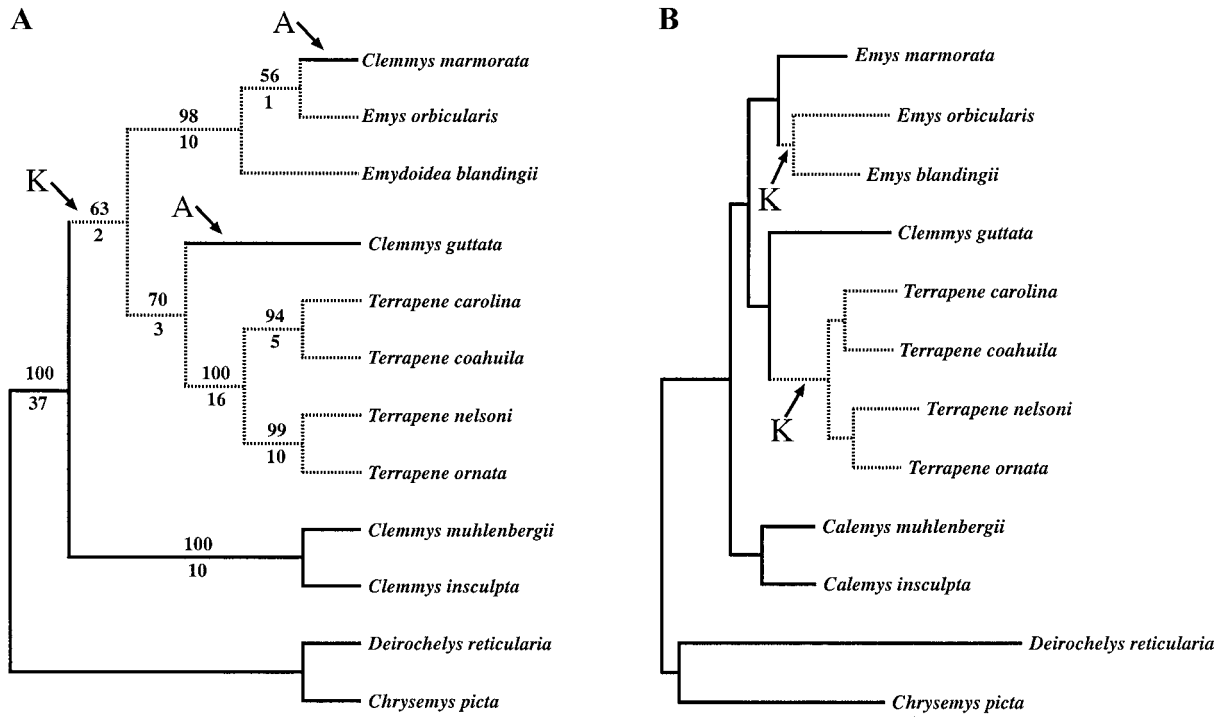

FIG. 4. Phylogenetic trees for emydine mtDNA lineages. Also shown are two alternative hypotheses for the evolution of plastral kinesis. Lineages reconstructed as exhibiting plastral kinesis illustrated with dashed lines: K, kinetic plastron; A, akinetic plastron. (A) Single most parsimonious tree (L = 1088; CI = 0.642; RI = 0.466). Numbers above nodes indicate bootstrap support, those below nodes represent decay indices. MP tree indicating a single origin of plastral kinesis followed by two losses. (B) Maximum likelihood estimate of emydine phylogeny ($-\ln l = 7798.4184$; $\alpha = 0.2766$). Branch lengths drawn proportional to maximum likelihood estimates of genetic divergence. ML tree showing two independent evolutionary gains of shell kinesis. Tree also drawn with proposed taxonomic revision of the Emydinae.

strained and unconstrained phylogenies in PAUP* using a two-tailed Wilcoxon signed-ranks test (Templeton, 1983) suggests the two hypotheses are incompatible ($P = 0.0032$) and a monophyletic *Clemmys* is unsupported.

Our phylogenetic hypothesis suggests that the spotted turtle, *C. guttata* (the type species of the genus *Clemmys* Ritgen 1828), is the closest living relative to the North American box turtles. This hypothesis is novel; previous treatments of the Emydinae have implicated *C. guttata* as either the sister taxon to all other emydines (Bickham *et al.*, 1996) or the second most basal emydine lineage (Burke *et al.*, 1996). Unfortunately, all three proposed arrangements lack good statistical support. In addition, our mtDNA data show *C. guttata* to be equally distant and highly divergent from all other emydine turtles (Table 2). Most emydine species pairs differ by only 4–6% uncorrected sequence divergence (e.g., "*C.*" *insculpta* and "*C.*" *muhlenbergii*). The spotted turtle, on the other hand, shows uncorrected nucleotide differences of 8–9% from any another emydine. In short, the conflicting phylogenetic hypotheses and sizeable molecular differences exhibited by *C. guttata* indicate that this taxon cannot be easily allied to the other emydines, including other "*Clemmys*."

Bickham *et al.* (1996) and Burke *et al.* (1996) have demonstrated a close kinship between "*C.*" *muhlenbergii* and "*C.*" *insculpta*. Our mtDNA phylogeny confirms this relationship and corroborates the hypothesis that the deepest split in the Emydinae occurs between all other emydines and a "*C.*" *muhlenbergii* + "*C.*" *insculpta* clade (Burke *et al.*, 1996).

Finally, our data also suggest that "*C.*" *marmorata* is not closely related to other "*Clemmys*," but shares a more recent common ancestor with *Emys orbicularis* and *Emyd. blandingii*. These data, and the putative relationship between "*C.*" *guttata* and *Terrapene*, indicate that the hinged emydines (i.e., *Emys orbicularis*, *Emyd. blandingii*, and *Terrapene*) represent a paraphyletic assemblage. As with "*Clemmys*," we tested the paraphyly of the hinged emydines by constraining the equally weighted, branch-and-bound MP searches to recover only those trees that produce a monophyletic hinged clade. The shortest tree generated by the constraint search is 1115 steps long (CI = 0.623; RI = 0.426), 27 steps longer than the unconstrained MP estimate of emydine phylogeny. The two-tailed Wilcoxon signed-ranks test fails to support ($P = 0.0018$) the monophyly of the hinged emydines. This result is in sharp contrast to the phylogenetic hypothesis based on osteological characters associated with plastral hinging (Gaffney and Meylan, 1988) and total evidence (Burke *et al.*, 1996), but is in rough agreement

TABLE 2

Pairwise Comparisons of mtDNA Sequences among Emydines and Outgroup Taxa

	1	2	3	4	5	6	7	8	9	10	11	12
1 *Clemmys marmorata*	—	8.11	7.36	12.58	11.90	12.16	13.03	13.43	9.89	9.83	27.09	21.88
2 *Emys orbicularis*	6.14	—	8.40	13.15	13.93	13.13	14.43	13.57	11.00	11.56	28.35	21.89
3 *Emydoidea blandingii*	5.66	6.33	—	12.11	12.80	12.69	13.93	13.60	10.04	10.50	27.80	21.12
4 *Clemmys guttata*	8.44	8.73	8.20	—	12.61	12.30	13.47	13.05	11.37	12.91	28.73	22.42
5 *Terrapene carolina*	8.01	9.07	8.39	8.30	—	5.15	8.05	7.01	13.06	12.99	28.80	19.24
6 *Terrapene coahuila*	8.20	8.78	8.44	8.25	4.22	—	7.89	6.52	11.77	11.73	25.55	19.95
7 *Terrapene nelsoni*	8.59	9.26	8.97	8.73	6.09	6.00	—	5.79	13.26	13.07	29.60	21.14
8 *Terrapene ornata*	8.78	8.87	8.78	8.54	5.37	5.13	4.70	—	13.24	12.74	29.99	21.85
9 *Clemmys muhlenbergii*	7.01	7.64	7.06	7.73	8.50	7.98	8.70	8.70	—	5.46	25.23	19.72
10 *Clemmys insculpta*	7.05	8.01	7.43	8.59	8.54	8.01	8.59	8.44	4.42	—	24.83	18.42
11 *Deirochelys reticularia*	13.36	13.75	13.60	13.75	13.75	12.74	14.08	14.18	12.61	12.69	—	26.92
12 *Chrysemys picta*	11.85	11.80	11.56	12.09	10.74	11.18	11.56	11.80	11.10	10.70	13.45	—

Note. Figures above the diagonal denote ML GTR + Γ sequence divergences (%) while those below the diagonal indicate uncorrected pairwise differences (%).

with the previous molecular treatment of the Emydinae (Bickham *et al.*, 1996).

In summary, the Emydinae can be divided into four well-supported clades: (1) *Terrapene,* (2) *C. guttata,* (3) "*C.*" *insculpta* and "*C.*" *muhlenbergii,* and (4) "*C.*" *marmorata, Emys orbicularis,* and *Emyd. blandingii.* Unfortunately, relationships between the emydine clades are not completely clear. Both MP and ML phylogenetic analyses yield the same topology, placing "*C.*" *insculpta* and "*C.*" *muhlenbergii* as the sister group to a monophyletic clade containing the rest of the emydine turtles. This arrangement is consistent with the total evidence analysis presented by Burke *et al.* (1996), but receives little statistical support in their study and ours.

Taxonomy

The paraphyly of the genus *Clemmys* Ritgen 1828 requires a taxonomic revision of the nonhinged emydines. Our phylogenetic hypothesis suggests that the type species, *C. guttata,* is not closely related to other "*Clemmys,*" but may share a more recent common ancestor with *Terrapene.* Although this molecular grouping is weak, it is supported by a morphological character (Burke *et al.*, 1996). Regardless of its true affinities, *C. guttata* does not appear closely related to any other emydine, so we suggest the name *Clemmys* be reserved for *C. guttata* alone.

The species "*C.*" *muhlenbergii* and "*C.*" *insculpta* form a robust monophyletic group exclusive of, and sister to, all other emydine turtles. In 1857, Agassiz gave these species their own generic names, *Calemys muhlenbergii* and *Glyptemys insculpta.* We recommend that "*Clemmys*" *muhlenbergii* and "*Clemmys*" *insculpta* be referred to the genus *Calemys,* the former representing the type species.

Our mtDNA phylogeny also shows that "*C.*" *marmorata* is not closely related to *Calemys* or *Clemmys,* but shares a more recent common ancestor with *Emys orbicularis* and *Emyd. blandingii.* The generic name *Actinemys* Agassiz 1857 is available for "*C.*" *marmorata,* but use of this name would obscure the phylogenetic affinities of "*C.*" *marmorata* and its relatives. Excluding fossil taxa, the resurrection of *Actinemys* results in a well-supported clade composed of three closely related yet monotypic genera (*Actinemys, Emys,* and *Emydoidea*). Instead, the oldest generic name applied to this clade is *Emys* Dumeril 1806, and we recommend this name be applied to all three species. An expanded *Emys* more accurately demonstrates our knowledge of evolutionary descent. Before Loveridge and Williams (1957), most authors recognized the affinities of *Emys orbicularis* and *Emyd. blandingii* by placing both in the genus *Emys.* Similarly, "*Clemmys*" *marmorata* was originally described as *Emys marmorata* Baird and Girard, 1852. Although *Emys marmorata* lacks plastral kinesis, it does have reduced plastral buttresses. Furthermore, *Emys* species share several morphological similarities; all are medium-sized turtles that possess nonkeeled shells with patterns of radiating spots or lines on the carapace. *Emys* species are typically olive, brown, or black with some yellow. Unlike other emydines, they lack red scales. And with the exception of *Calemys insculpta,* the only emydines to range higher than 45° latitude are the species of *Emys.* Finally, *Emys* are the only emydines that retain fully webbed feet.

Nevertheless, each species of *Emys* represents a lineage extending 12–14.5 million years into the past (Hutchison, 1981; Holman, 1995). Their independent history is demonstrated by the distinctive morphology of the different species. The type species, *Emys orbicularis,* is an aquatic turtle with a hinged plastron; *Emys marmorata* has an akinetic plastron; and *Emyd. blandingii* has a specialized feeding mechanism that involves an elongated cervical series and a highly modified skull (Loveridge and Williams, 1957). For these

reasons, we suggest the names *Emydoidea* and *Actinemys* be used as subgeneric taxa for fossil lineages within *Emys*. This taxonomy is consistent with the Linnaean system of ranks as well as the highly informative system of phylogenetic taxonomy (de Queiroz and Gauthier, 1992).

Plastral Kinesis

The most striking result of our proposed phylogeny is the paraphyly of the hinged emydines. The absence of a hinged plastron or segmented scapulae in *Emys marmorata* makes it impossible to optimize these characters without homoplasy. Our ML tree suggests that shell kinesis evolved twice (Fig. 4B), once in *Terrapene* and once in *Emys*. Alternatively, shell kinesis evolved once (Fig. 4A) and was lost twice (in *Emys marmorata* and *C. guttata*). The single-origin hypothesis is only slightly less parsimonious (by one step). But because the intergeneric relationships of emydines are not well supported we are reluctant to accept the multiple origins of plastral kinesis based on parsimony alone. However, independent lines of evidence also suggest a multiple origin of the hinged emydines.

First, a survey of other living turtles reveals that plastral kinesis has evolved repeatedly: twice within kinosternids (*Kinosternon, Staurotypus;* Bramble *et al.*, 1984), once within pleurodires (*Pelusios;* Bramble and Hutchison, 1981), and as many as seven times in the close relatives of emydines ("Bataguridae" and Testudinidae). Testudinids evolved plastral kinesis at least twice (*Testudo, Pyxis;* see Ernst and Barbour, 1989) and the geoemydine "batagurids" may have evolved plastral kinesis five times (*Notochelys, Cyclemys, Cuora, Cistoclemmys,* and *Pyxidea;* Bramble, 1974; Hirayama, 1985). These geoemydines are the Old World ecological analogs of emydines, occupying a variety of aquatic, semiterrestrial, and terrestrial environments. Although they lack segmented scapulae, geoemydines demonstrate the likelihood for parallel evolution of plastral kinesis in testudinoids.

Second, within emydines, the fossil record reveals a trend of increasing specialization of plastral kinesis through time. *Emys* (*Emydoidea*) *hutchisoni,* from the middle Miocene of Nebraska (Late Barstovian, 12 mya), has less developed shell kinesis than extant *Emyd. blandingii* (Hutchison, 1981; Holman, 1995). The oldest *Terrapene,* also from the middle Miocene of Nebraska (Holman, 1987), resembles *T. coahuila,* the extant *Terrapene* that has the least derived plastral kinesis. So, whereas decreasing specialization of plastral kinesis may have occurred, the available fossil evidence indicates just the opposite.

Given the molecular phylogeny, the many independent derivations of shell kinesis in other testudinoids, and the apparent trend toward increasing specialization of plastron kinesis among fossil emydines, the multiple origins of plastral kinesis appear to be the best explanation for the observed homoplasy in emydines. If correct, this means that the segmented scapulae and cervical musculature modifications that accompany plastron kinesis also evolved in parallel in species of *Emys* and *Terrapene.* These modifications are unknown in other kinetic-shelled clades. Bramble (1974) was so impressed with these characters that he wrote, "A multiple origin for the complex closing mechanism held in common by these box turtles appears extremely remote" (p. 724). Similarly, Gaffney and Meylan (1988) relied entirely on the specializations of the scapulae to resolve their phylogeny of emydine genera. We submit that the independent evolution of these characters in *Emys* and *Terrapene* may be the result of structural constraints particular to emydine turtles. In other words, the parallel evolution of suprascapular processes indicates that the ancestors of *Emys* and *Terrapene* shared a similar morphology and so evolved plastral kinesis in a similar way.

We can test this hypothesis with additional fossil evidence. Unfortunately, the Tertiary sediments of North America, though bearing the remains of numerous deirochelyines, "batagurids," and testudinids, have not yielded a large number of emydine fossils (Hutchison, 1996). The oldest known emydines are *Terrapene* and "*Clemmys*" from the Miocene of Nebraska (middle Barstovian, 13–14.5 mya; Holman, 1987) while the oldest deirochelyines, *Chrysemys antiqua* (Clark, 1937; Hutchison, 1996), are at least 35 million years old (late Eocene, Chadronian). Extending the lineage leading to emydine turtles to that time reveals that the first 20 million years of emydine history are completely unknown, and by the time they appear in the fossil record, the modern lineages of emydines are already established. In short, the most significant evolutionary events in the history of emydines remain undocumented.

CONCLUSIONS

Our proposed phylogeny offers additional insights into the relationships of emydine turtles and the evolution of complex morphological characters. Our taxonomic revision of the Emydinae is consistent with these relationships. We suggest that (1) the genus *Clemmys* should be restricted to a single species, *C. guttata.* (2) The genus *Calemys* should be resurrected for *C. muhlenbergii* and *C. insculpta.* (3) The genus *Emys* should be expanded to include three species: *E. orbicularis, E. blandingii,* and *E. marmorata.* (4) The names *Actinemys* and *Emydoidea* should be preserved as subgeneric taxa for lineages within *Emys.*

Neither kinetic-shelled nor akinetic-shelled emydines form a monophyletic group. Thus, the evolution of plastral kinesis is more complex than previously supposed. Shell kinesis was either independently gained in *Emys* and *Terrapene* or secondarily lost in *Emys marmorata* and *C. guttata.* Parsimony, paleontological evidence, and the multiple origins of kinesis

in related turtle lineages (especially geoemydines) lend credibility to the multiple origin explanation. We suggest that the plastron-closing mechanism shared by *Emys* and *Terrapene* demonstrates the ability of closely related turtle lineages to evolve complex characters in parallel. Although the morphology of turtles is notoriously homoplastic, the actual amount and extent of homoplasy may be greater than previously thought.

APPENDIX

Specimens Used and GenBank Accession Numbers for DNA Sequence Data

Acronyms are MVZ, Museum of Vertebrate Zoology, Berkeley, California; CAS, California Academy of Sciences, San Francisco, California; ROM, Royal Ontario Museum, Toronto, Ontario; and AF, GenBank (http://www.ncbi.nlm.nih.gov).

Clemmys guttata—Dulpin Co., North Carolina, MVZ 175961; AF258858, AF258870.

Calemys (=*Clemmys*) *insculpta*—Ontario, Canada, ROM 1523; AF258864, AF258876.

Calemys (=*Clemmys*) *muhlenbergii*—zoo specimen, Wildlife Conservation Society, Bronx Zoo, New York, New York; AF258863, AF258875.

Emys (=*Emydoidea*) *blandingii*—Ontario, Canada, ROM 20922; AF258857, AF258869.

Emys (=*Clemmys*) *marmorata*—Lake Co., California, MVZ 164994; AF258855, AF258867.

Emys orbicularis—Schelkovskya Dist., Russia, CAS 182905; AF258856, AF258868.

Terrapene carolina—Jackson Co., North Carolina, MVZ 137441; AF258859, AF258871.

Terrapene coahuila—zoo specimen (T00228), Gladys Porter Zoo, Brownsville, Texas; AF258860, AF258872.

Terrapene nelsoni—zoo specimen, Arizona Sonoran Desert Museum, Tucson, Arizona; AF258861, AF258873.

Terrapene ornate—Cochise Co., Arizona, MVZ 137743; AF258862, AF258874.

Chrysemys picta—no locality, MVZ 230532; AF258866, AF258878.

Deirochelys reticularia—no locality, MVZ 230923; AF258865, AF258877.

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