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MOLECULAR SYSTEMATICS OF THE GENUS *CLEMMYS* AND THE INTERGENERIC RELATIONSHIPS OF EMYDID TURTLES

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ABSTRACT: Nucleotide sequence data from a 556 bp segment of the mitochondrial 16S ribosomal RNA gene support the partition of emydid turtles into two distinct subfamilies. Phylogenetic analysis identifies two major clades and corroborates previous morphological assignments of genera to the subfamilies Deirochelyinae and Emydinae. Within the subfamily Deirochelyinae, *Deirochelys* appears to be the sister taxon to all other genera in the subfamily, and support is found for a clade that includes *Trachemys*, *Graptemys*, and *Malaclemys*. However, the other generic relationships within this subfamily are not well resolved. Within the Emydinae, the genus *Terrapene* is monophyletic. Conversely, the genus *Clemmys* is paraphyletic, necessitating a new generic arrangement of the species now considered to be in *Clemmys*, *Emydoidea*, and *Emys*.

Key words: Systematics; Ribosomal DNA; Turtles; Emydidae; Nucleotide sequences

THE status of the genus *Clemmys* and its proposed relationships to other genera within the family Emydidae have had a varied taxonomic history. Prior to 1964, *Clemmys* included four North American and four Eurasian species. McDowell (1964) recognized the polyphyletic nature of this group and partitioned the genus such that New World forms were retained in *Clemmys* and the Old World forms were relegated to *Mauremys* and *Sacalia*. At the time, emydid turtles were considered a subfamily (Emydinae) of the Testudinidae. McDowell (1964) subdivided this subfamily by reassigning the predominantly Old World emydine turtles (including *Mauremys* and *Sacalia*) to a new subfamily, the Batagurinae. The three testudinid subfamilies recognized by McDowell (1964) now represent the three testudinoid families Testudinidae, Emydidae, and Bataguridae (Gaffney and Meylan, 1988).

As currently recognized, the family Emydidae comprises 10 genera and 37 species (Gaffney and Meylan, 1988). It is thought to have originated in North America where nine of the 10 genera currently occur. Two exceptions to this North American distribution include *Emys*, found in Europe, North Africa, and parts of the Middle East, and *Trachemys*, the range of which encompasses portions of both North and South America (Iverson, 1992). The family clearly is monophyletic but has been divided into two subfamilies by Gaffney and Meylan (1988). The Deirochelyinae includes the predominantly aquatic *Chrysemys, Deirochelys, Graptemys, Malaclemys, Pseudemys*, and *Trachemys*, whereas the Emydinae includes both aquatic and terrestrial species in the genera *Emys, Emydoidea, Clemmys*, and *Terrapene*. This arrangement has received support from Seidel and Adkins (1989) based on isoelectric focusing.

Relationships among the four genera of emydine turtles have been the subject of recurring debate. *Clemmys* (McDowell, 1964), *Emys* (Milstead, 1969), and *Emydoidea* (Bramble, 1974; Gaffney and Meylan, 1988) all have been hypothesized to be the sister taxon to *Terrapene*. The position of *Emydoidea* is particularly controversial, as several investigators have inferred a close relationship to *Deirochelys* (Loveridge and Williams, 1957; McDowell, 1964; Tinkle, 1962; Zug, 1966) whereas others have shown a close relationship between *Emydoidea* and the other emydine genera (Bramble, 1974; Frair, 1982; Gaffney and Meylan, 1988; Seidel and Adkins, 1989).

Within the Emydinae, only *Clemmys* and *Terrapene* contain more than one species. Milstead (1969) subdivided *Terrapene* into two species groups (i.e., *T. carolina* and *T. coahuila; T. nelsoni* and *T. ornata*), an interpretation that generally is well accepted. The relationships of the four species of *Clemmys* are not as well established. Some studies have suggested that *C. insculpta* is basal (the sister taxon) to the other three species (Merkle, 1975;) and that *C. muhlenbergii* and *C. guttata* are closest relatives (Parsons, 1962; Zug, 1966). Merkle (1975) hypothesized that *C. guttata* and *C. marmorata* were the most closely related species, with *C. muhlenbergii* being their sister taxon and *C. insculpta* being the most distantly related taxon. However, Lovich et al. (1991) concluded that *C. muhlenbergii* shares fewer similarities with the other three species.

In this study, we examine phylogenetic relationships among emydid turtles using nucleotide sequence from the mitochondrial 16S ribosomal gene. We employ a phylogenetic analysis to address three major issues. (1) We test Gaffney and Meylan's (1988) hypothesis regarding the status and generic composition of the subfamilies Deirochelyinae and Emydinae. (2) We concentrate specifically on relationships within the genus *Clemmys*, and (3) in so doing, we examine the phylogenetic status of *Clemmys* relative to the other emydine genera.

MATERIALS AND METHODS

We obtained blood samples from a single individual for each of 15 emydid species and two batagurid species. Representatives of the Emydinae included all four currently recognized species of *Clemmys* (*C. marmorata, C. muhlenbergii, C. guttata,* and *C. insculpta*), three (of the four) species of *Terrapene* (*T. coahuila, T. ornata,* and *T. carolina*), *Emys orbicularis,* and *Emydoidea blandingii.* Representatives of the Deirochelyinae included *Deirochelys reticularia, Trachemys scripta, Graptemys geographica, Malaclemys terrapin, Pseudemys concinna,* and *Chrysemys picta. Orlitia borneensis* and *Malayemys subtrijuga* were chosen as outgroups because they (along with *Siebenrockiella crassicollis*) are the only batagurids to have a karyotype (2N = 50) like that of emydids (Bickham, 1975; Bickham and Baker, 1976; Bickham and Carr, 1983; Carr and Bickham, 1981) and because the Bataguridae is considered the sister taxon of the Emydidae (Gaffney and Meylan, 1988).

Genomic DNA was extracted from 30–50 μl of blood by incubation in 450 μl of STE buffer (5.0 M NaCl, 2.0 M Tris-HCl, 0.5 M EDTA, pH 7.5), 25 μl of 20% SDS, and 25 μl of proteinase-K at 10 μg/ml for 1 h. We then added 5M NaCl (150 μl) and placed the solution on ice for 1 h, centrifuged it at 9000 RPM for 15 min on a Savant HSC 10K Microcentrifuge, and extracted the supernatant with a phenol-methylene chloride-isoamyl alcohol mix (25:24:1). DNA was precipitated with an equal volume of 100% isopropyl alcohol, centrifuged at 9000 RPM for 1 min, and washed in 70% isopropyl alcohol. Precipitated DNA was resuspended in 200 μl of TE (1 mM Tris, 100 μM EDTA, pH 7.5). We amplified a 598 bp portion of the 16S gene of the mitochondrial DNA by PCR with the primers LGL 381 (5′-ACC CCG CCT GTT TAC CAA AAA CAT-3′) and LGL 286 (5′-AGA TAG AAA CCG ACC TGG AT-3′). These primers correspond to positions 2487–2510 and 3104–3085 of the human mitochondrial genome map, respectively. These primers are "universal" and were constructed based on similarities between published mtDNA sequences of diverse vertebrate taxa. Samples were amplified by 50 μl reactions which consisted of the following: 0.1–0.5 μg genomic DNA; 5 μl 10X buffer (0.1 M Tris-HCl pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl), 5 μl dNTP mix (2 mM dATP, dTTP, dCTP, dGTP, in 0.1 M Tris-HCl, pH 7.9), 5 μl of a 10 μM solution of each primer, 0.025–0.5 μl Taq DNA polymerase, and brought up to volume with deionized water. Amplifications were done by 32 cycles of 95 C for 45 s of denaturing, 50 C for 30 s of annealing, 70 C for 2.5 min of extension, and 4 s of auto-extension. The PCR products were used to sequence a total of 566 bp using an Applied Biosystems 373A Automated DNA sequencer employing dye labelled terminators (Ferl et al., 1991). Sequence alignments were made using the computer software package SeqEd from Applied Biosystems Inc., Foster City, California.

Phylogenetic relationships were reconstructed using Paup 3.1 (Swofford, 1990). Transversions were weighted 3:1 relative to transitions and gaps were treated as missing data. A heuristic analysis using 100 searches with the random addition of taxa was used to search for trees of the shortest length. We estimated the reliability of the branches of the shortest trees by using a bootstrap analysis with 250 replicates. Subsequently, an exhaustive search was performed separately on just the emydine taxa, using *Deirochelys* as the outgroup, and on just the deirochelyine taxa, using *Clemmys guttata* and *Terrapene coahuila* as the outgroup. Again, bootstrapping was performed to test the reliability of specific nodes of the trees.

RESULTS AND DISCUSSION

Unambiguous alignment was achieved for a 556 bp segment of the 16S ribosomal gene for the 17 taxa studied (Fig. 1). We found a total of 140 variable nucleotide positions of which 105 had only two nucleotides present among the taxa, 20 had three or four nucleotides, and 15 were variable by deletion-duplication events (including three with nucleotide variation as well). Of the 105 variable positions possessing only two nucleotides, 56 were C ↔ T transitions and 29 were A ↔ G transitions. There were 20 transversions including six A ↔ C, 13 T ↔ A, and one G ↔ T. Of the 20 positions that had three or four alternative nucleotides, one had A-G-C, two had A-G-T, 14 had A-C-T, one had T-G-C, and two had all four nucleotides. Just considering sites at which only two nucleotides are present (N = 105), the transition : transversion ratio is 4.25:1.

Phylogenetic analysis of all species using a heuristic search (Swofford, 1990) identified four most-parsimonious trees of 323 steps. In each of these trees, the two subfamilies of emydid turtles were readily distinguished (Fig. 2). In the bootstrap analysis, the emydine clade occurred in 94% of the trees and the deirochelyine clade occurred in 99% of the trees (Fig. 3). Thus, monophyly for these two taxa, as suggested by Gaffney and Meylan (1988), is strongly supported in this study and is consistent with another molecular data set obtained by isoelectric focusing (Seidel and Adkins, 1989). Synapomorphies that define the Deirochelyinae were observed at positions 78, 79, 166, 245, 263, 336, 377, 443, and 500. Synapomorphies that defined the Emydinae were found at positions 212, 231, 253, 256, 260, 362, 385, and 435. These were all transitions.

In order to explore more fully the relationships of the emydine genera, we reanalyzed the data for the nine emydine species using only *Deirochelys* as an outgroup. An exhaustive search for all most parsimonious trees was performed which yielded two trees with a length of 166. These trees differed only in the relationships of the three species of *Terrapene*. These were combined in a strict-consensus tree on which the results of bootstrap analysis using 250 replicates are illustrated (Fig. 4). The monophyletic nature of the genus *Terrapene*, the box turtles, is supported in 74% of the bootstrap trees. A clade including *T. carolina* and *T. ornata* was weakly supported (32% of the trees in the bootstrap trees and one of the two most-parsimonious trees), which possibly confirms the arrangement of Milstead (1969). The only other polytypic genus within this group, *Clemmys*, does not appear to be monophyletic. The position of *C. guttata* is problematic in that it falls outside the clade that includes all of the rest of the emydine taxa. However, the latter clade is only supported by 32% of the bootstrap trees. Strong support (93% of the bootstrap and both of the most-parsimonious trees) was found for a close relationship between

	10	20	30	40	50	60	70	80	90	100	110	120	130	140
Clemmys muhlenbertgii	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TAGTCTTTTA	AATAAAGACT	AGAATGATTG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ACAATCAGTG
Clemmys insculpta	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ACAATCAGTG
Clemmys guttata	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTATAG	ATAATCAGTG
Clemmys marmorata	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATTCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTATAG	GTAATCAGTG
Emys orbicularis	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATTCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTATGG	ATAATCAGTG
Emydoidea blandingii	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATTCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTATGG	GTAATCAGTG
Terrapene carolina	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACTG	ATAATCAGTG
Terrepene coahuila	GTAATGNCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTICTACCTG	TCTCTTACTG	ATAATCAGTG
Terrepene ornata	GTAATGTCTG	CCCAGTGACA	CTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCCACCTG	TCTCTTACTG	ATAATCAGTG
Deirochelys reticularia	GTAATGCCTG	CCCAGTGACA	TTGTTT-AAA	CGGCCGCGGT	ATACTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCCA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCIG	TCTCTTACAG	ATAATCAGAG
Trachemys scripta	GTAATGNCTG	CCCNGTGACA	CTGTTTTAAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ATAATCAGTG
Graptemys geographica	GTAATGCCTG	CCCAGTGACA	CTGTTT-AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ATAATCAGTG
Malaclemys terrapin	GTAATGCCTG	CCCAGTGACA	CTGTTTTAAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCCA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ATAATCAGTG
Pseudemys concinna	GTAATGNCTG	CCCAGTGACA	CTGTTTTAAA	CGGCCGCGGT	ATCATAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCTA	AATAAAGACT	AGAATGAACG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ATAATCAGTG
Chrysemys picta	GTAATGCCTG	CCCAGTGACA	CTGTTTT-AA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTCCA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAG	ATAATCAGTG
Orlitia borneensis	GTAATGCCTG	CCCAGTGACA	TTGTT--AAA	CGGCCGCGGT	ATCCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCCAAACGAG	GTTCTACCTG	TCTCTTACAA	ATAATCAGTG
Malayemys subtrijuga	GTAATGCCTG	CCCAGTGACA	CTGTT--AAA	CGGCCGCGGT	ATTCTAACCG	TGCAAAGGTA	GCGTAATCAC	TTGTCTTTTA	AATAAAGACT	AGAATGAATG	GCTAAACGAG	GTTCTACCTG	TCTCTTACAA	ATAATCAGTG

	150	160	170	180	190	200	210	220	230	240	250	260	270	280
Clemmys muhlenbergii	AAATTGATCT	CCCCGTGCAA	AAGCGGGGAT	AACCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TAATCAA-TA	T--CCAACTA	AGGACTTATA	TTCAATTAGT	ATTTGATCCA	TA-TTTTC-G
Clemmys insculpta	AAATTGATCT	CCCCGTGCAA	AAGCGGGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTCAAATAC	AGATCAACTA	TAATCAA-TA	T--CTAACTA	AGGACTTATA	TTAAATTAGT	ATTTGATCCA	TA-TTTTCCG
Clemmys guttata	AAATTGATCT	CCCCGTGCAA	AAGCAGGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TCATTA--CA	C--CCAACTA	AGGATTTATA	CTTAATTAGG	ATTTGATCCA	TA-TTTTC-G
Clemmys marmorata	AAATTGATCT	TCCCGTGCAA	AAGCGAGAAT	AACCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TAATCAA-CA	T--CCTACTA	AGGACTTATA	CTTAATTAGC	ATTTGATCTA	TA-TTTTT-G
Emys orbicularis	AAATTGATCC	CCCCGTGCAA	AAGCGGGGAT	AACCATATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TCATCAA-TA	T--CCAACTA	AGGACTTATA	TTTAATTAGC	ATTTGATCCA	TA-TTTTT-G
Emydoidea blandingii	AAATTGATCT	CCCCGAGCAA	AAGCGGGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TCATCAA-CA	T--CCAACTA	AGGACTTATA	CTTAATTAGC	ATTTGATCCA	TA-TTTTT-G
Terrapene carolina	AAATTGATCT	CCCCGTGCAA	AAGCAGGGAT	AACCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TCATCAA-TA	T--CTAACCA	AGGACTTATA	TTTAATTAGA	ATTTGATCCA	TA-TTTTC-G
Terrapene coahuila	AAATTGATCT	CCCCGTGCAA	AAGCAGGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TCATCAA-TA	T--CTAGCCA	AGGACTTATA	TTCAATTAGC	ATTTGATCCA	TA-TTTTC-G
Terrapene ornata	AAATTGATCT	CCCCGTGCAA	AAGCAGGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AGATCAACTA	TTATCAA-TA	T--CTAACCA	AGGACTTATA	TTTAATTAGC	ACTTGATCCA	TA-TTTTC-G
Deirochelys reticularia	AAATTGGTCT	CCCCGTGCAA	AAGCGAGGAT	ATCCTTATAT	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAA	AAATCAACCA	TCATCATACA	C-CCTAACCA	AGGGTTTATA	TCCAACTAGT	ACATGATCTA	TA-TTTTC-G
Trachemys scripta	AAATTGGTCT	CCCCGTGCAA	AAGCGAGGAT	AATCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAACAC	AAATCAACTA	CCATCAAACA	C--CAAACTA	AGGATTTATG	ACCAACTAGT	ATATGATCCA	CG-TTTTC-G
Graptemys geographica	AAATTGGTCT	CCCCGTGCAA	AAGCGGGAAT	AACCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAACAC	AAATCAACTA	CCATTAA-CA	C--CAAACTA	AGGATTTATA	ATCAACTAGT	ATATGATCCA	CG-TTTTC-G
Malaclemys terrapin	AAATTGGTCT	CCCCGTGCAA	AAGCGAGGAT	AACCCTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAACGC	AAATCAACTA	CCATCAA-CA	C--CAAACTA	AAGATTTATA	ATAAACTAGT	ACATGATCCA	CG-TTTTC-G
Pseudemys concinna	AAATTGATCT	CCCCGTGCAA	AAGCGAGGAT	AACCTTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAACAC	AAATCAACTA	CCAT-AAGCA	C--CTAACTA	AGGACTTATA	ATCAACCAGT	ACATGATCCA	CG-TTTTC-G
Chrysemys picta	AAATTGATCT	CCCCGTGCAA	AAGCGGGGAT	AACCATATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAACAC	AAATCAACTA	CCAT-AA-TA	TACCCAACTA	AGGATTTATA	ATCAACCAGT	GCATGATCTA	CG-TTTTC-G
Orlitia borneensis	AAATTGATCT	CCCTGTGCAA	AAGCAGGAAT	AAAACTATAA	GACGAGAAGA	CCCTGTGGAA	CTTTAAATAC	AAGTCAACTA	TCATCAA-TA	T--TCACCCA	CGGGCCTACA	CCCAACTA-T	ACCTGACTTA	TATTTTTC-G
Malayemys subtrijuga	AAATTGATCT	TCCTGTGCAA	AAGCAGGAAT	AAAATTATAA	GACGAAAAGA	CCCTGTGGAA	CTTCAAATAC	AAGTCAATTA	TCACTAA-CA	C--TAACCTA	CAGGCTCATA	TTCAACTAAT	ATCTGACTTA	TA-TTTTC-G

	290	300	310	320	330	340	350	360	370	380	390	400	410	420
Clemmys muhlenbergii	GTTGGGGGGA	CCTCGGAGTA	AGAGGAAA-C	CTCCGAAAAA	-AGAACACGC	TT---CTAAA	CCTAGACTTA	CAACTCAAAG	TACTTAACAG	TA-AAACGAT	CCAACATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Clemmys insculpta	GTTGGGGTGA	CCTCGGAGTA	AAAGAAAAAC	CTCCGAAAAA	-AGAACACGC	TT---CTAAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TA-AAATGAT	CCAACATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Clemmys guttata	GTTGGGGCGA	CCTCGGAGTA	AAATGAAA-C	CTCCGAAAAA	-AGAACACAC	TT---CTAAA	CCTAGACTTA	CAATTCAAAG	CACTTAACAG	TA-AAACGAT	CCAATATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Clemmys marmorata	GTTGGGGCGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	TTC-TCTAAA	CCTAGACTTA	CAATTCAAAG	TACCTAACAG	TATAAACGAT	CCAATATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Emy orbicularis	GTTGGGGTGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	TT---CTAAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TATAAATGAT	CCAACATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Emydoidea blandingii	GTTGGGGCGA	CCTCGGAGTA	AAACGAAA-C	CTCCGAAAAA	-AGAACATAC	TT---CTTAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TA-AAACGAT	CCAACATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Terrapene carolina	GTTGGGGCGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	TTC--CCAAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TA-AAACGAT	CCAACATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Terrapene coahvuila	GTTGGGGCGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	CTC--CTAAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TA-AAACGAT	CCAACATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Terrapene ornata	GTTGGGGCGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	TTC--CTTAA	CCTAGACTTA	CAATTCAAAG	TACTTAACAG	TA-AAACGAT	CCAATATATT	TTATCAACGG	ACCAAGCTAC	CCCAGGGATA
Deirochelys reticularia	GTTGGGGCGA	CCTCGGAGTA	GAACAAAA-C	CTCCGAAAAA	-AGAACACTT	ATC-TTTAAA	CCTAGACTTA	CAATTCAAAG	TGC-TAACAG	TA-AAAAGAT	CCAATATATT	TGATCAACGG	ACCAAGCTAC	CCCAGGGATA
Trachemys scripta	GTTGGGGCGA	CCTCGGAGTA	AAGCACAA-C	CTCCGAAAAA	-AGAGCATAC	TTC-TCTAAA	CCTAGACTTA	CAACTCAAAG	TGC-TAACAG	CA-AAAAGAT	CCAATATATT	TGATCAACGA	ACTAAGCTAC	CCCAGGGATA
Graptemys geographica	GTTGGGGCGA	CCTCGGAGTA	AAGCAAAA-C	CTCCGAAAAA	-AGAGCATAC	TTC-TTTAAA	CCTAGACTTA	CAACTCAAAG	TGC-TAACAG	TA-AAAAGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Malaclemys terrapin	GTTGGGGCGA	CCTCGGAGTA	AAGCAAAA-C	CTCCGAAAAA	-AGAGCATAC	TTC-TTTAAA	CCTAGACTTA	CAACTCAAAG	TGC-TAACAG	TA-AAAAGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Pseudemys concinna	GTTGGGGCGA	CCTCGGAGTA	AAACAAAA-C	CTCCGAAAAA	-AGAACATTC	TTCTTTTTAA	CCTAGACCCA	CAATTCAAAG	TGC-TAACAG	TA-AAAAGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Chrysemys picta	GTTGGGGCGA	CCTCGGAGCA	AAACAAAA-C	CTCCGAAAAA	-AGAACATAC	TTCTTTTAAA	CCTAGACTTA	CAATTCAAAG	TGC-TAACAG	CA-AAAAGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Orlitia borneensis	GTTGGGGCGA	CCTCGGAGCA	AAACAAAA-C	CTCCGAAAAA	TAGAATTTAA	C----CTATA	CCTAGACACA	CAACCCAAAG	TGCTTCCGGC	TA-AAATGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA
Malayemys subtrijuga	GTTGGGGCGA	CCTCGGAGCA	AAATAAAA C	CTCCCAAAAC	-AGAACTCTA	T----CTAAA	CCTAGACCCA	CAATCCAAAG	TGCTTCCGGC	TA-AAACGAT	CCAATATATT	TGATCAACGA	ACCAAGCTAC	CCCAGGGATA

	430	440	450	460	470	480	490	500	510	520	530	540	550	556
Clemmys muhlenbergii	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Clemmys insculpta	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Clemmys guttata	ACAGCGCAAT	CCCACCCTAG	AGTTCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Clemmys marmorata	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Emys orbicularis	ACAGCGCAAT	CCCATCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Emydoidea blandingii	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTCATC	TGAGTT
Terrapene carolina	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Terrapene coahuila	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Terrapene ornata	ACAGCGCAAT	CCCACCCTAG	AGTCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGT	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Deirochelys reticularia	ACAGCGCAAT	CCCATCCAAG	AGCCCCTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATT	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Trachemys scripta	ACAGCGCAAT	CCCATCCTAG	AGCCCTTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Graptemys geographica	ACAGCGCAAT	CCCATCCTAG	AGCCCTTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Malaclemys terrapin	ACAGCGCAAT	CCCATCCTAG	AGCCCTTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Pseudemys concinna	ACAGCGCAAT	CCCATCCCAG	AGCCCTTATC	GACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Chrysemys picta	ACAGCGCAAT	CCCATCCTAG	AGCCCTTATC	AACGATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGA	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT
Orlitia borneensis	ACAGCGCAAT	CCCATCCTAG	AGTTCCTATC	GACAATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGC	AGCCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAACAGTC	CTACGTGATC	TGAGTT
Malayemys subtrijuga	ACAGCGCAAT	CCCATCCTAG	AGTCCCTATC	GACAATGGGG	TTTACGACCT	CGATGTTGGA	TCAGGACATC	CTGATGGTGC	AACCGCTATC	AAGGGTTCGT	TTGTTCAACG	ATTAATAGTC	CTACGTGATC	TGAGTT

FIG. 1.—Aligned sequences for a 556-bp region of the mitochondrial 16S ribosomal gene for 15 species of emydid and two species of batagurid turtles. N refers to an undetermined nucleotide and a hyphen indicates the absence of a nucleotide resulting from a deletion or an addition.

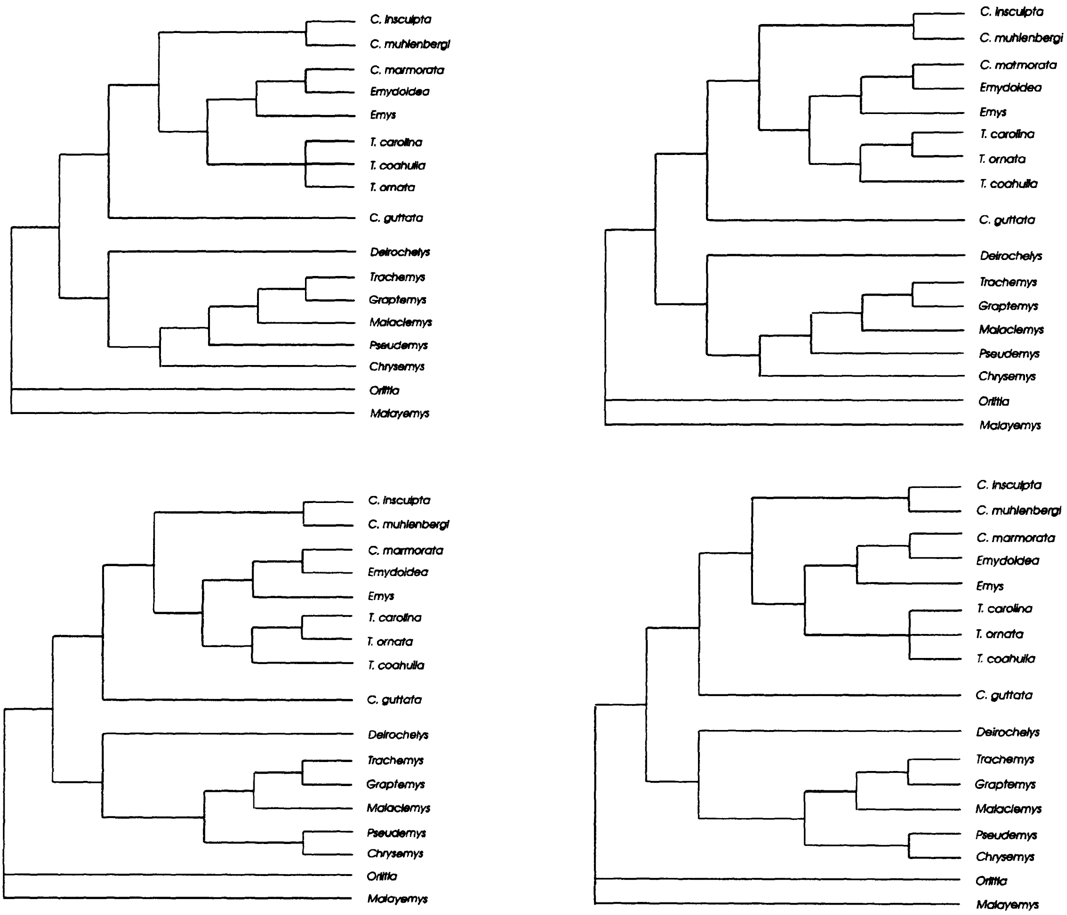

FIG. 2.—Four most-parsimonious trees found with a heuristic search of the 556 bp sequence of the mitochondrial 16S gene from 15 taxa of emydid turtles and using two batagurid species as outgroups. Note that in all trees the emydid taxa are separated into two clades that correspond to the Emydinae and Deirochelyinae of Gaffney and Meylan (1988) and that the genus *Clemmys* is paraphyletic.

Clemmys muhlenbergii and *C. insculpta*, two species found together in the eastern United States (Iverson, 1992). These two form a clade that is the sister taxon to the remaining six emydine taxa (Fig. 4). A clade including (*Emys orbicularis* (*Emydoidea blandingii*, *C. marmorata*)) is supported by 54% of the bootstrap trees and is the sister taxon to the genus *Terrapene*. The arrangement of the emydine taxa in the two trees produced in the exhaustive search was identical to the two arrangements for the emydine clade observed in the heuristic search conducted using the entire data set (Fig. 2). That significant phylogenetic signal was present in the data set was indicated by the distribution of the trees being significantly skewed ($g1 = -0.843$).

An exhaustive search for all most parsimonious trees was conducted for the six deirochelyine taxa using *Clemmys guttata* and *Terrapene coahuila* as the outgroup (Fig. 4). This search yielded a single most-parsimonious tree of 149 steps (Fig. 4). This tree is identical to one of the two arrangements of the deirochelyine taxa found in the heuristic search using the entire data set (Fig. 2). *Deirochelys* is well supported as the sister taxon to the rest of

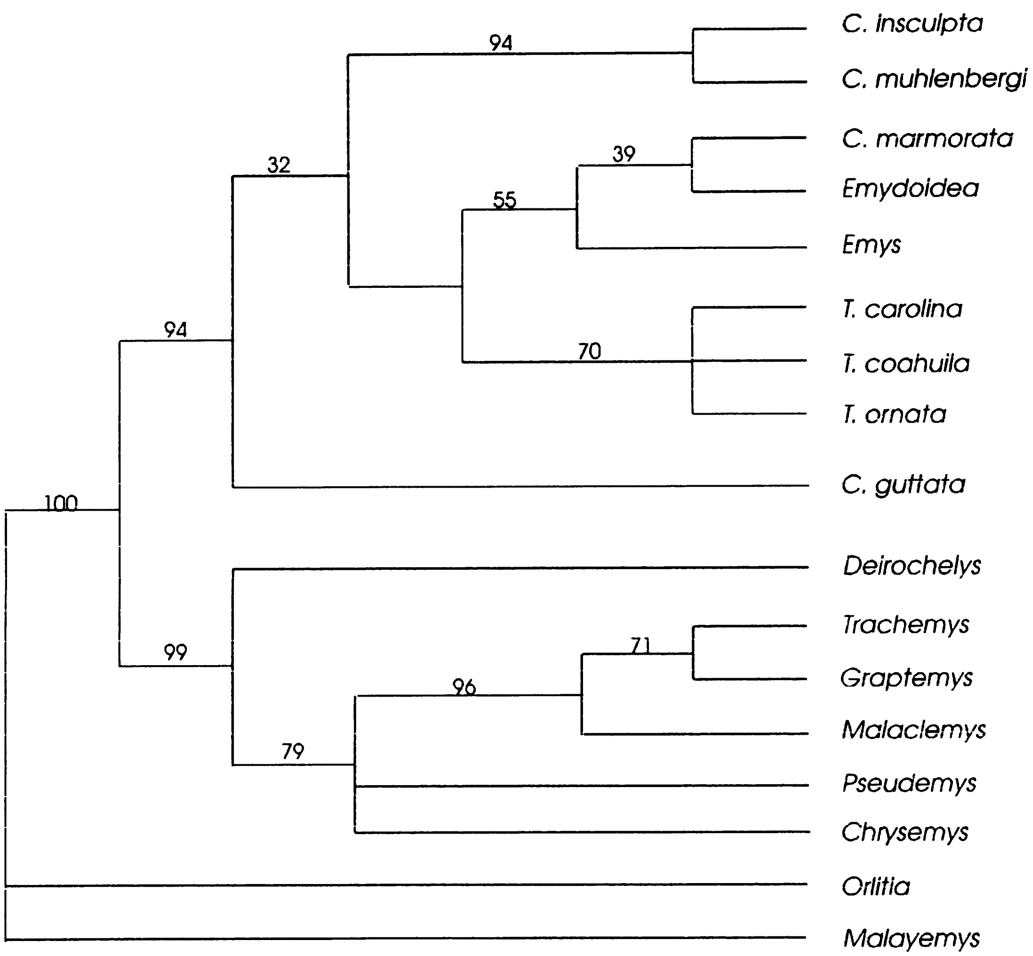

FIG. 3.—A strict-consensus tree was constructed from the four most-parsimonious trees in Fig. 2. The tree has a consistency index of 0.929. The numbers above certain branches are the percentage of trees generated by a bootstrap analysis that supported that particular branch.

the subfamily (85% of the bootstrap trees). Within the rest of the Deirochelyinae, a clade comprised of *Trachemys*, *Graptemys*, and *Malaclemys* occurred in 96% of the bootstrap trees (Fig. 4). A sister-taxon relationship between *Graptemys* and *Trachemys* was weakly supported (66% of the bootstrap trees) as was a sister-taxon relationship between *Pseudemys* and *Chrysemys* (63% of the bootstrap trees). Tree distributions were significantly skewed (g1 = −1.4) indicating phylogenetic signal was present in the data set.

Although monophyly for the two subfamilies is strongly supported by our nucleotide sequence data for the 16S gene and is consistent with recent morphological analysis (Gaffney and Meylan, 1988), the relationships among the genera within each subfamily do not closely correspond to those found in any previous analysis. Within the Emydinae, the traditional congeneric relationships of the three species of *Terrapene* are confirmed, but the same cannot be said for *Clemmys*. Our data suggest that this genus, as currently recognized, is paraphyletic. This could result in taxonomic name changes with *Clemmys*

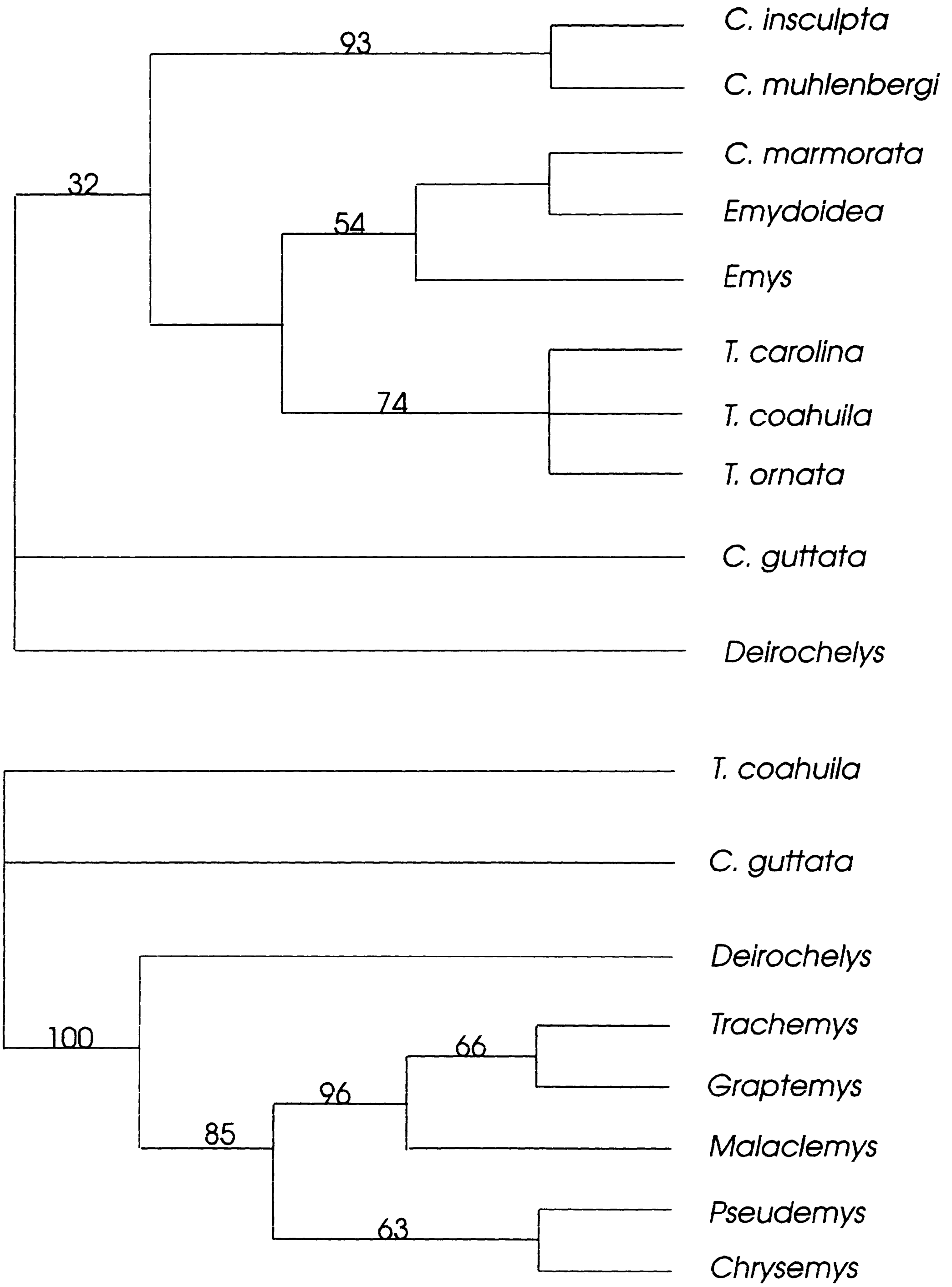

FIG. 4.—A strict-consensus of two most-parsimonious trees of length 166 was generated from the results of an exhaustive search of (top) nine emydine taxa using *Deirochelys* as the outgroup. A single tree with a length of 149 (bottom) was produced by an exhaustive search of six deirochelyine taxa using *Terrapene coahuila* and *Clemmys guttata* as outgroups. Numbers refer to the percent of trees in a bootstrap analysis that supported that branch.

marmorata and *Emydoidea* (Holbrook, 1838) being synonymized with *Emys* (Linnaeus, 1758). However, our data do not fully resolve this question. What is clear, however, is that the generic relationships of the taxa assigned to *Clemmys*, *Emys*, and *Emydoidea* appear to be in need of reconsideration. In order to test this theory of paraphyly for *Clemmys*, we reanalyzed the data using all 17 taxa, but we constrained the analysis to include only trees with a monophyletic *Clemmys*. A heuristic search using 100 replicates with random addition of taxa found 14 most-parsimonious trees of length 331. This compares to the four most-parsimonious trees found in the unconstrained heuristic search (Fig. 2) with lengths of 323 steps. Therefore, eight additional steps are required in the analysis in order to support the traditional generic arrangement of the four species presently included in the genus *Clemmys*.

Within the subfamily Deirochelyinae, Gaffney and Meylan (1988) considered *Graptemys*, and possibly *Malaclemys*, to be the most basal branch of a mostly pectinate phylogeny with the following branching sequence: ((*Graptemys Malaclemys*) (*Chrysemys* (*Deirochelys* (*Trachemys Pseudemys*)))). As in Gaffney and Meylan (1988), a close relationship between *Malaclemys* and *Graptemys* is supported by the molecular data, although they were not found to be sister taxa. However, our demonstration of a relationship between *Trachemys* and the *Graptemys*-*Malaclemys* clade is inconsistent with the morphological analysis of Gaffney and Meylan (1988). Moreover, the status of *Deirochelys* as the sister taxon to all other deirochelyine genera is well supported by the molecular data (Fig. 4) and in conflict with the relationships proposed by Gaffney and Meylan (1988). The relationships of the deirochelyine genera presented here must be considered as preliminary, because only a single species from each genus was included and because the phylogeny is based on nucleotide sequence data from a single mitochondrial gene. In particular, additional species of the polytypic genera *Pseudemys*, *Trachemys*, and *Graptemys* should be included in future analyses, and additional genes, both mitochondrial and nuclear, should be analyzed.

In summary, we have presented evidence, based upon nucleotide sequence analysis of the mitochondrial 16S gene, that the emydid turtles should be divided into two monophyletic subfamilies corresponding to the Emydinae and Deirochelyinae of Gaffney and Meylan (1988). Except for the placement of *Deirochelys* as the sister-taxon to the rest of the genera, the arrangement of the genera within the Deirochelyinae was not well resolved, but within the emydinae, strong evidence was obtained for a paraphyletic genus *Clemmys*. We have demonstrated that the 16S gene appears to have good resolving power for relatively ancient divergences, such as that between the two subfamilies, but intergeneric affinities are less well resolved.

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