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Ecological diversification and phylogeny of emydid turtles

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Ecological diversification is a central topic in ecology and evolutionary biology. We undertook the first comprehensive species-level phylogenetic analysis of Emydidae (an ecologically diverse group of turtles), and used the resulting phylogeny to test four general hypotheses about ecological diversification. Phylogenetic analyses were based on data from morphology (237 parsimony-informative characters) and mitochondrial DNA sequences (547 parsimony-informative characters) and included 39 of the 40 currently recognized emydid species. Combined analyses of all data provide a well-supported hypothesis for intergeneric relationships, and support monophyly of the two subfamilies (Emydinae and Deirochelyinae) and most genera (with the notable exception of *Clemmys* and *Trachemys*). Habitat and diet were mapped onto the combined-data tree to test fundamental hypotheses about ecological diversification. Using continuous coding of ecological characters showed that lineages changed in habitat before diet, ecological change was most frequently from generalist to specialist, and habitat and diet rarely changed on the same branch of the phylogeny. However, we also demonstrate that the results of ancestral trait reconstructions can be highly sensitive to character coding method (i.e. continuous vs. discrete). Finally, we propose a simple model to describe the pattern of ecological diversification in emydid turtles and other lineages, which may reconcile the (seemingly) conflicting conclusions of our study and two recent reviews of ecological diversification. *Biological Journal of the Linnean Society*, 2003, **79**, 577–610.

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INTRODUCTION

Ecological diversification, the evolution of divergent ecological characteristics within a lineage, is a topic central to the study of adaptation, macroevolution and community ecology (Schluter, 2000). Many authors have proposed that there are general rules of ecological diversification which explain ecological and evolutionary patterns across diverse groups of organisms. For example, it is often hypothesized that during ecological diversification the direction of change in ecological characteristics will generally be from generalized to specialized and not vice versa (Cope, 1896; MacArthur & Pianka, 1966; Futuyma & Moreno, 1988; Thompson, 1994; Schluter, 2000; Nosil, 2002). Even though phylogenetic methods have become indispensable to testing evolutionary hypotheses (Donoghue, 1989; Brooks & McLennan, 1991; Harvey & Pagel, 1991; Wainwright & Reilly, 1994; Martins, 1999; Pianka, 2000; Schluter, 2000), the extent to which hypotheses about ecological diversification have been tested in a phylogenetic context varies widely, depending on the hypothesis.

There are two fundamental and opposing hypotheses about the general sequence of ecological diversification within lineages, which have only rarely been tested. The character displacement hypothesis (Williams, 1972; Losos, 1992) predicts that ecological diversification will occur first in diet and that organisms

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will evolve to occupy new habitats only after the previous one has become 'saturated' with dietary specialists. Conversely, the niche compression hypothesis (MacArthur & Wilson, 1967; Schoener, 1974; Losos, 1992) predicts that species in an evolving lineage will first change in habitat use to completely avoid competition for resources within a habitat (rather than merely reduce competition with the initial phases of dietary specialization). We refer to these as the 'habitat-first' and 'diet-first' hypotheses hereafter to avoid confusion, because the terms 'character displacement' and 'niche' are generally used more broadly in the ecological literature (e.g. Diamond & Case, 1986; Brown & Lomolino, 1998; Morin, 1999). The habitat-first and diet-first hypotheses have only been tested in a handful of studies (e.g. Losos, 1992; six studies reviewed in Schluter, 2000), and these studies have not consistently supported either hypotheses.

Implicit in both of these hypotheses is the assumption that simultaneous changes on one or more ecological axes (e.g. in both habitat and diet) are unlikely, an assumption that we refer to as the 'limited-diversification' hypothesis. So far as we know, the limited-diversification hypothesis has never been explicitly tested in a phylogenetic study.

In contrast to the previous hypotheses, the generalist-to-specialist hypothesis has been relatively well studied using phylogenetic methods (reviewed in Futuyma & Moreno, 1988; Thompson, 1994; Schluter, 2000). It is commonly hypothesized that ecological diversification proceeds from generalized ancestors to more specialized descendants, and that ecological specialization will be largely irreversible once achieved (Futuyma & Moreno, 1988; Thompson, 1994; Schluter, 2000). Schluter (2000) lists five different theoretical reasons to expect a generalist to give rise to specialists and not vice versa. This hypothesis has even been put forth as a general 'rule' of ecological diversification (e.g. the law of the unspecialized: Cope, 1896; the compression hypothesis: MacArthur & Pianka, 1966). However, despite its wide acceptance and strong theoretical basis, many exceptions to this hypothesis have been found (reviewed in Futuyma & Moreno, 1988; Thompson, 1994; Schluter, 2000; but see Nosil, 2002).

The general phylogenetic approach most commonly used in studies of ecological diversification is ancestral trait reconstruction (Schluter, 2000). It has been shown that how characters are defined and coded for in ancestral reconstructions can strongly affect the results (Wiens, 1999), in some cases even reversing the conclusion with respect to the hypothesis being tested (Wiens & Morris, 1996). Very few phylogenetic tests of ecological diversification hypotheses have considered the potential impact of their choice of coding method (i.e. continuous vs. discrete). In fact, most previous studies of ecological diversification have coded characters as discrete during ancestral reconstruction, even though ecological traits typically vary continuously within and between species (Wainwright & Reilly, 1994; Pianka, 2000). The impact of reconstructing traits as continuous vs. discrete on comparative studies has not previously been investigated.

The generalist-to-specialist, habitat-first, diet-first and limited-diversification hypotheses are all essentially phylogenetic hypotheses about patterns of ecological character-state change during the diversification of a lineage. To test these hypotheses in a phylogenetic context, the ideal group would be one that is speciose, whose species have been the subjects of detailed descriptive ecological studies, and which includes generalists and specialists in both habitat and diet. The turtle family Emydidae satisfies all of these criteria, but their phylogenetic relationships remain uncertain.

The Emydidae

Emydidae contains 40 currently recognized species in ten genera (Ernst & Barbour, 1989; Ernst, Lovich & Barbour, 1994; Vanzolini, 1995; but see Feldman & Parham, 2002 and Discussion below). The family includes many common North American turtles such as the box turtles (*Terrapene*), painted turtles (*Chrysemys picta*), cooters (*Pseudemys*) and sliders (*Trachemys*). Within North America, emydids are by far the most abundant, speciose, and ecologically diverse group of turtles (Ernst *et al*., 1994). Emydids also occur in Central America, the West Indies, South America, Europe and northern Africa (Iverson, 1992). Emydid lineages have specialized along two different ecological axes (habitat and diet) and in different directions on each axis (i.e. carnivore vs. herbivore and aquatic vs. terrestrial; Ernst & Barbour, 1989; Ernst *et al*., 1994). In addition to specialists, the family also includes generalists in both diet and habitat. This ecological diversity makes Emydidae an ideal group in which to test the generalist-to-specialist, habitat-first, diet-first and limited-diversification hypotheses. The most direct test of these hypotheses would involve reconstructing changes in emydid ecological characters on a well-supported phylogeny to determine if the direction and sequence of state changes are consistent with each hypothesis. Unfortunately, many ambiguities remain in our knowledge of emydid phylogenetic relationships.

Only two recent studies have examined the phylogenetic relationships of all emydid genera: one based on morphological data (Gaffney & Meylan, 1988) and one on mitochondrial ribosomal DNA sequence data (Bickham *et al*., 1996). These studies agree on the placement of the genera into two subfamilies, the Emydinae (*Clemmys*, *Emydoidea*, *Emys* and *Terrap-*

ene) and Deirochelyinae (*Chrysemys*, *Deirochelys*, *Graptemys*, *Malaclemys*, *Pseudemys* and *Trachemys*). However, there are also numerous conflicts in the findings of these studies. For example, the studies disagree on the monophyly of *Clemmys*, the most basal member of the Deirochelyinae, and the sister-group of *Trachemys*. These studies were limited in several respects: none attempted to include all the species within the family, relatively few morphological characters have been used ($N = 25$ for Gaffney & Meylan, 1988), and there was no attempt to combine the available morphological and molecular data for all genera. Other recent studies of emydid phylogeny have been limited to the subfamily Emydinae (Burke, Leuteritz & Wolf, 1996; Feldman & Parham, 2002) or to single genera (e.g. Seidel, 1988, 1994, 2002; Lamb & Avise, 1992; Lamb *et al*., 1994; Minx, 1996; Lenk *et al*., 1999; Ultsch *et al*., 2001).

Goals of the study

Herein we undertake the first comprehensive species-level analysis of the Emydidae, by combining molecular data from the literature (Lamb *et al*., 1994; Bickham *et al*., 1996; Shaffer, Meylan & McKnight, 1997; Lenk *et al*., 1999; Feldman & Parham, 2002) and morphological data from our own observations. Although some might question the use of morphological data in groups that have undergone extensive ecological diversification (e.g. Givnish & Sytsma, 1997), we show that our phylogenetic results are not biased by adaptive convergence.

Next, we examine emydid ecological diversity in a phylogenetic context to test four general hypotheses about patterns and processes of ecological diversification. The generalist-to-specialist hypothesis is tested by performing ancestral character state reconstructions of habitat and diet, and examining the direction of change in both ecological characteristics. The habitat-first, diet-first and limited-diversification hypotheses are tested by examining the sequence of evolutionary changes in habitat and diet. We also compare (possibly for the first time) the consequences of coding ecological variables as either discrete or continuous in ancestral reconstructions.

MATERIAL AND METHODS

Phylogenetic analyses

Morphological taxon sampling

Specimens representing all currently recognized emydid species (Ernst & Barbour, 1989; Ernst *et al*., 1994; Seidel, 1994; Vanzolini, 1995) were included, except the recently described species *Trachemys adiutrix* (Vanzolini, 1995) for which no specimens were available at the time of this study. Ten additional species were included for use as outgroups, from the families Bataguridae and Kinosternidae. Bataguridae (which includes Testudinidae; Gaffney & Meylan, 1988) is the sister-group of Emydidae, and Kinosternidae belongs to the sister-group of Emydidae + Bataguridae (Gaffney & Meylan, 1988; Shaffer *et al*., 1997). Because many emydid taxa currently recognized as subspecies may represent distinct species (Iverson, 1992), subspecies were generally used as terminal units. For monotypic species or those with poorly differentiated subspecies (e.g. *Graptemys nigrinoda*, *Deirochelys reticularia*), species were used as the terminal taxa. Recent workers disagree on the alpha taxonomy of the *Pseudemys concinna–floridana* complex (*sensu* Ernst *et al*., 1994; compare Ward, 1984; Ernst *et al*., 1994; Seidel, 1994). However, all agree that *P. concinna* and *P. floridana peninsularis* (*sensu* Ernst *et al*., 1994) are distinct species, and these taxa were used as terminals in our analyses. *Malaclemys terrapin* was treated as two terminal taxa (Atlantic and Gulf coast) based on molecular phylogeographical evidence that these geographical units are distinct monophyletic groups and that presently recognized subspecies within these two taxa are merely clinal variants (Lamb & Avise, 1992). Very few specimens of *Emys orbicularis* were available, and these were pooled to form a single terminal taxon, although *E. orbicularis* may consist of more than one species (Lenk *et al*., 1999). For the majority of terminal taxa, six osteological specimens and ten fluid-preserved specimens were examined. In some cases (13 of the 64 ingroup taxa), osteological specimens were not available for taxa for which fluid preserved specimens were available. These taxa were included in some analyses, despite the large amount of missing data, because previous studies suggest that limited taxon sampling may be more problematic than the inclusion of missing data cells in incomplete taxa (Wiens & Reeder, 1995; Wiens, 1998a, 2003a,b). A complete list of specimens examined is available upon request from the authors.

Morphological character sampling

Over 300 morphological characters drawn from the literature and personal observations were evaluated, including nearly all osteological and external morphological characters described in published systematic studies of the Emydidae during the last 40 years. All seemingly independent characters that could be described unambiguously were included, and characters were not excluded because of overlap in trait values between species, intraspecific variation, missing data, or a priori notions of homoplasy (following Poe & Wiens, 2000). In total, 116 osteological and 109 external morphological parsimony-informative characters

were included (see Appendix for description of characters and citations). Character states were scored by direct observation of specimens. Additional characters that could not be observed in preserved or skeletal specimens (or not without elaborate dissections) were taken from the literature, including characters of colouration-in-life ($N = 1$), penial morphology ($N = 4$), egg-shell morphology ($N = 1$), behaviour ($N = 2$), development ($N = 1$) and allozymes ($N = 3$). We refer to this set of 237 non-nucleotide characters as the morphological data set hereafter.

Coding and weighting of morphological characters

Morphological data were analysed using PAUP* v.4.0b1 (Swofford, 2001). Qualitative binary characters that exhibited within-species variation (polymorphism) were coded using the frequency-bins method (Wiens, 1995). Quantitative characters (i.e. morphometric and meristic characters) were coded using gap-weighting (Thiele, 1993). Both the frequency-bins and gap-weighting methods were implemented by dividing up the range of variation into 25 bins (coded as ordered character states 'a' to 'y'). Evidence from simulations (Wiens & Servedio, 1997, 1998), congruence testing (Wiens, 1998b), and statistical analyses (Wiens, 1995) suggest that frequency methods (e.g. frequency-bins and gap-weighting) are generally as accurate or more accurate than discrete methods for coding polymorphic data. Because of the large number of taxa, frequency-bins and gap-weighting methods were used as opposed to more precise step-matrix approaches (e.g. Wiens, 1995, 2001; Berlocher & Swofford, 1997). For step-matrix methods, the number of taxa that can be effectively given unique states and weights is limited by the number of states allowed by PAUP* (i.e. 32).

Morphometric variables were size-corrected by regression with a measure of overall size appropriate to each variable (e.g. carapace width was size-corrected by regression with carapace length, length of supraoccipital crest was size-corrected by regression with skull length; see Appendix). These residuals were then regressed against these size variables to verify that the residuals were adequately size-corrected. All analyses showed no significant relationship ($P > 0.05$), and no further transformations were deemed necessary.

Multistate qualitative characters were coded as integers from 0 to $N - 1$, where N is the numbers of states observed across all taxa, and were generally treated as unordered. Intraspecific variation in multistate characters was coded using the majority approach (*sensu* Wiens, 1995, 1999), in which terminal taxa are coded as having the state with the highest frequency (since frequency-based step matrix methods were impractical). Taxa which had two conditions at equal frequencies were coded using the polymorphic method (*sensu* Wiens, 1999). All characters that were not coded using frequency-bins or gap-weighting (i.e. discrete, non-polymorphic characters) were weighted by 24, so that a change in character state frequencies from 0 to 100% had equal weight using both frequency and non-frequency coding.

Several characters exhibited sexual dimorphism within a taxon, but also showed considerable variation among taxa that was neither strictly correlated between sexes nor strictly sex-independent. To incorporate this variation but reduce potential problems of character non-independence, we coded each sex separately for these characters (e.g. male carapace length, female carapace length) and then weighted each character by 0.5. Although this weighting scheme is somewhat arbitrary, it includes all of the variation in each sex, but does not give the two characters any more weight than if they were treated as a single character.

All analyses that included morphological characters were conducted using both between-character scaling and between-state scaling (*sensu* Wiens, 2001) to weight meristic characters. A disadvantage of between-state scaling is that meristic characters with very large ranges of mean trait values between species may have undue influence on the analysis. Although the best cut-off value is unclear, we only coded meristic characters with a range of mean species values less than 10 using between-state scaling. Because of small sample sizes for many taxa for osteological characters, statistical scaling (Wiens, 2001) was not attempted.

Tree searching

Each parsimony analysis consisted of two parts. First, a heuristic search (using tree-bisection-reconnection (TBR) branch-swapping) was performed to find the shortest tree, using 1000 random taxon-addition-sequence replicates. Second, a non-parametric bootstrap analysis (Felsenstein, 1985) was performed to assess support for individual branches using 200 pseudoreplicates and heuristic searches with TBR branch-swapping and five random-taxon-addition-sequence replicates per bootstrap pseudoreplicate. Topological constraints for outgroup species, based on the phylogenies reconstructed by Gaffney & Meylan (1988) and Shaffer *et al*. (1997), were enforced in all analyses.

Molecular data and analysis

Cytochrome *b* and control region mitochondrial DNA (mtDNA) sequence data were available for *Malaclemys* and the species of *Graptemys* (Lamb *et al*., 1994). Homologous sequences from the complete mitochondrial genome of *Chrysemys picta* (Mindell *et al*., 1999) were added from GENBANK. Homologous cytochrome *b* sequences from all emydine species, *Deirochelys reticularia*, *Trachemys scripta* and three out-

group taxa (*Chinemys reevesii*, *Heosemys spinosa* and *Sternotherus odouratus*) were also added from GENBANK (Shaffer *et al*., 1997; Lenk *et al*., 1999; Feldman & Parham, 2002). Of 1526 positions (345 control region, 1181 cytochrome *b*), 292 positions (36 control region, 256 cytochrome *b*) were parsimony-informative after realignment (see below). In addition, 16S (= large subunit) mitochondrial ribosomal DNA sequence data were available for at least one species of each emydid genus (Bickham *et al*., 1996). Out of 558 positions, 68 were parsimony-informative after realignment. Finally, ND4 (NADH dehydrogenase 4) sequences were available for every currently recognized species of emydine as well as the deirochelyines *Chrysemys picta* and *Deirochelys reticularia* (Feldman & Parham, 2002). Of 895 positions, 136 were parsimony-informative after realignment. At least some molecular data were available for 28 of the 39 emydid species represented in this study. Although many of the molecular data sets used in the combined analysis were missing data for some taxa, simulations show that adding sets of incomplete characters can improve phylogenetic accuracy relative to excluding these characters (Wiens, 1998a; Wiens, 2001).

Because some previous authors did not describe their methods of sequence alignment (and did not incorporate secondary structure for the ribosomal sequences), sequences were realigned following Wiens, Reeder & Nieto Montes de Oca (1999). Control region and 16S sequences were aligned using Clustal X v.1.4b (Thompson, Higgins & Gibson, 1994) with three different gap opening costs (gap opening cost = 5, 10 and 15). Other parameters were held constant at the default values (gap extension cost = 0.05; transition weight = 0.50). After initial alignment, 16S sequences were constrained to favour the placement of gaps in hypothesized loop regions rather than stems (following Wiens & Reeder, 1997). Stems and loops were determined by comparing sequences to secondary structure models published for *Xenopus* and *Bovis* (Gutell & Fox, 1988). Only regions that were invariant in alignment across all three gap opening costs (unambiguously aligned) were used in the phylogenetic analyses. A total of 51 positions (32 for 16S and 19 for control region) were considered ambiguously aligned. No contiguous gaps (e.g. AG---CCT) appeared in unambiguously aligned regions of the 16S gene or control region. Gaps were therefore treated as an informative fifth character state during parsimony analyses, assuming that all insertion and deletion events were independent of each other. Cytochrome *b* and ND4 sequences, which are protein-coding and contained no gaps, were aligned by eye. Cytochrome *b* and control region sequences were combined during all analyses (hereafter cytochrome *b* is used synonymously with cytochrome *b* + control region), because of the overlapping taxon sampling and because preliminary analyses showed no strongly supported conflicts between trees based on separate analyses of these data sets (bootstrap values for conflicting clades were <50%). We were unable to extract data on the presence or absence of restriction sites from the information reported by Lamb *et al*. (1994), and this data set was therefore not included.

Each set of aligned sequences was tested for the presence of statistically significant phylogenetic signal using the g_1 method of Hillis & Huelsenbeck (1992). The g_1 test statistic was calculated for each set of sequences from the lengths of 100 000 randomly generated trees. These tests showed that all three sequence data sets (cytochrome *b*, 16S, ND4) are significantly more structured than expected for random data ($P < 0.01$ for each).

We used both maximum likelihood and equally weighted parsimony to estimate phylogenies for the three mtDNA data sets using PAUP* v.4.0b1 (Swofford, 2001). Equally weighted parsimony was used to assess the results given a simple model of sequence evolution. These results were contrasted with the results obtained from a more complex model using maximum likelihood. Prior to our maximum likelihood analysis, we used Modeltest (Posada & Crandall, 1998) to choose the model that best fitted the data via a hierarchical likelihood-ratio test. The starting model and parameter-addition hierarchy used by Modeltest corresponds to ηLRT_1 of Posada & Crandall, 2001), which was shown in their simulation study to have accuracy similar or superior to that of the other model selection procedures evaluated. The best-fitting model was GTR + Γ + I (Yang, 1994; Gu, Fu & Li, 1995) for the cytochrome *b* and 16S data sets and HKY85 + G (Hasegawa, Kishino & Yano, 1985) for the ND4 data set. The best-fitting model for each data set was then used in a heuristic search to find the best overall likelihood topology (TBR branch-swapping, 20 random taxon-addition-sequence replicates). Because estimating model parameters during the tree search was extremely time intensive for these data, model parameters were initially estimated using the best fitting-model and the shortest parsimony tree. Following the iterative procedure outlined in Wilgenbusch & de Queiroz (2000), likelihood parameters were then re-estimated on the tree with the highest likelihood score and these new parameters were used for another heuristic search. In all cases, the tree with the highest likelihood score found during the second heuristic search had the same topology as the tree found during the first heuristic search, and no additional searches were performed. The likelihood parameters estimated on the tree with the highest likelihood score were then used to perform a bootstrapping analysis to assess support for individual branches (100 pseudoreplicates,

TBR branch-swapping, two random taxon-addition-sequence replicates per bootstrap pseudoreplicate). Combined analysis (see below) of the molecular data sets followed the same procedure. The best-fitting likelihood model chosen by Modeltest for the combined molecular data was the same as that chosen for the cytochrome *b* and 16S molecular sequence data sets (GTR + Γ + I), and the likelihood parameters chosen were generally within the range of those chosen for these two data sets. Maximum likelihood analysis of combined data sets with somewhat different model parameters is an unresolved issue (e.g. Wilgenbusch & de Queiroz, 2000). We speculate that the advantages of the large increase in the number of characters (relative to separate analysis of the data sets) should outweigh the disadvantages of the slight mismatch in model parameters between data sets for the combined analyses.

Combining data sets

Methods for integrating data sets are controversial (for reviews see de Queiroz, Donoghue & Kim, 1995; Hillis & Wiens, 2000). We used the approach outlined by Wiens (1998c), in which data sets are analysed separately and then combined, but only those parts of the combined-data tree that are not in strongly supported conflict in the separate analyses are considered to be adequately resolved. We consider a strongly supported conflict to be clades that are in disagreement between data sets with bootstrap values in each ≥70% (after Hillis & Bull, 1993; but see their caveats). This procedure was first used to look for strongly supported conflicts between trees from the three mitochondrial sequence data sets (16S, cytochrome *b* and ND4). Combined analyses of all molecular data was then performed (using both parsimony and likelihood) and the results of these analyses were compared to trees from separate analyses of the morphological data. A combined analysis using all data and all taxa was then performed. Because likelihood and equally weighted parsimony analyses gave similar results (and because choice of DNA weighting schemes is problematic when DNA and morphological characters are combined), DNA sequence characters were equally weighted in all analyses that combined molecular and morphological data. Morphological and DNA sequence characters were given equal base weight, assuming that a nucleotide substitution or indel is equivalent to a change in a fixed morphological character.

Combining data sets with incompletely overlapping sets of taxa can be problematic because of the inclusion of taxa with many missing data cells. However, analyses of natural, experimental and simulated phylogenies suggest that the inclusion of these incomplete taxa should not greatly decrease accuracy (Wiens & Reeder, 1995; Wiens, 1998a, 2003a) particularly when large numbers of characters have been scored in the incomplete taxa (regardless of how much missing data they bear; Wiens, 2003a). The preferred estimate of emydid relationships was based on the combined analysis including all taxa and characters, but taking into account areas of strongly supported incongruence. This was the tree used for interpreting patterns of ecological diversification.

Analyses of ecological diversification

Direction of change

The generalist-to-specialist hypothesis was tested for both habitat and diet by determining the number of changes from generalized to specialized character states, from specialized to generalized, and between specialized states (e.g. aquatic to terrestrial). Ecological characters (diet and habitat) were mapped onto the phylogeny and ancestral states were reconstructed both as discrete characters (using parsimony) and as continuous characters (using both parsimony and maximum likelihood). Maximum likelihood reconstruction of discrete characters was not attempted because current implementation of the method (i.e. Pagel's (1994, 1999) program Multistate) do not allow for the ordering of multistate characters. The states of the two ecological characters considered in the study should clearly be ordered (see below). Diet and habitat were chosen because these are the two primary axes along which turtle species divide ecospace (Bury, 1979; Vogt & Guzman, 1988; Lindeman, 2000a).

Parsimony reconstructions of discrete character states were performed using MacClade, version 3.04 (Maddison & Maddison, 1992). The character states for diet were carnivorous (0; specialist), omnivorous (1; generalist), and herbivorous (2; specialist). The character states for habitat were aquatic (0; specialist), semi-terrestrial (1; generalist), and terrestrial (2; specialist). Characters were scored for each species based upon literature surveys (Smith & Smith, 1979; Seidel, 1988; Ernst & Barbour, 1989; Ernst *et al*., 1994). A species was scored as an omnivore unless its diet was reported to consist entirely of plant matter or animal matter or more than 90% by volume in literature reports of stomach contents. We assume that volumes >10% do not represent accidental ingestion. For habitat use, those species scored as aquatic were those reported to spend their active season primarily in aquatic habitats, generally leaving the water only to bask, migrate to a new aquatic habitat, or nest. Species scored as terrestrial were those reported to spend the active season primarily in terrestrial environments, and that generally enter water only to thermoregulate or to cross to another terrestrial site. Species that fell between these two extremes were

scored as semi-terrestrial. In the majority of species, little variation in ecological characters has been reported between subspecies, and so subspecies were scored identically. However, Mesoamerican subspecies of *Trachemys scripta* were scored based strictly upon reports for individual subspecies because of evidence that these subspecies vary extensively in diet (Legler, 1960, 1990). Both diet and habitat use were treated as ordered in ancestral reconstructions, based on the obvious intermediacy of omnivory and semi-terrestriality.

For a given character, all equally parsimonious character-state reconstructions were identified using the equivocal cycling feature of MacClade. To ensure that the ancestral state for the entire family was not biased by the choice of representative outgroup species (which varied between data sets), an outgroup consisting of all currently recognized batagurid genera was used during all ancestral character state reconstructions using parsimony. The topology of the outgroup conformed to the phylogenetic arrangement reported in Gaffney & Meylan (1988).

To investigate the robustness of results obtained using the preferred tree to alternative hypotheses of emydid phylogenetic relationships, parsimony reconstructions of ecological characters were repeated using every tree topology reported in this study (see Figs 1–8). The total number of reconstructed state changes varied with the number of included taxa. However, the results were qualitatively the same with respect to the ecological hypotheses tested, regardless of the tree used (i.e. a hypothesis supported by mapping characters onto the preferred tree was also supported by the alternative topologies). Only results using the preferred tree are reported (the tree based on the combined morphological and molecular data; Fig. 7), as we consider this to be the best overall estimate of emydid phylogenetic relationships.

Different methods for coding traits can have a significant impact on reconstructing the evolutionary history of that trait (e.g. fig. 7 in Wiens, 1999), although the effects of different coding methods are rarely considered or compared in evolutionary studies. Coding continuous morphological variables directly as continuous (rather than qualitative or discrete) appears to have many advantages in phylogeny reconstruction (Wiens, 2001). Therefore, reconstructions were repeated after scoring the ecological character states of terminal taxa as continuous variables. In the case of diet, terminal taxa were assigned a score between 0 and 1 according to the percentage (by volume) of animal matter reported in quantitative studies of stomach contents (reviewed in Ernst & Barbour, 1989; Ernst *et al*., 1994). Taxa for which quantitative dietary data were unavailable were assigned a score using the qualitative states described above: 0 for herbivores, 0.5 for omnivores, and 1 for carnivores. Taxa for which no dietary data (quantitative or qualitative) were available were excluded from this analysis. Similarly, terminal taxa were also assigned a score between 0 and 1 to represent degree of terrestriality (based on the discrete states described above): 0 for aquatic species, 0.5 for semi-terrestrial species, and 1 for terrestrial species.

Maximum likelihood (Schluter *et al*., 1997) and parsimony (Maddison, 1991) reconstructions of quantitative trait values were performed using COMPARE v.4.4 (Martins, 2001). For maximum likelihood reconstructions, within-species variation was set to zero and a linear relationship between branch lengths and phylogenetic divergence was assumed (linear generalized least-squares method). This method provides maximum likelihood estimates of ancestral states for continuous characters (Schluter *et al*., 1997; Garland, Midford & Ives, 1999; Martins, 1999). Branch lengths were derived from external morphological data (109 characters, available for all but two of the 64 ingroup taxa), assuming that overall levels of change in external morphology reflects divergence times. Both ACCTRAN and DELTRAN optimization were used to estimate branch lengths for the morphological data; results were similar using both methods and only results using ACCTRAN are reported. For parsimony reconstructions, within-species variation was set to zero and all branch lengths were set to one (equivalent to least-squares parsimony; Maddison, 1991).

To test the generalist-to-specialist hypothesis (and the other hypotheses discussed below), we interpreted the reconstructed ancestral trait values as discrete characters using the same cut-offs applied to quantitative literature accounts of diet during reconstruction of discrete ancestral states. Reconstructed values for degree of carnivory were considered specialist (herbivore) if ≤10%, specialist (carnivore) if ≥90%, and generalist (omnivore) if between 10–90%. Reconstructed values for degree of terrestriality were considered specialist (aquatic) if ≤10%, specialist (terrestrial) if ≥90%, and generalist (semi-terrestrial) if between 10–90%. Parsimony and maximum likelihood reconstructions of ancestral values gave similar results. Therefore, only results using maximum likelihood are reported, and hereafter 'continuous coding' refers to results from maximum likelihood reconstruction of continuous ancestral trait values, and 'discrete coding' refers to results from parsimony reconstruction of discrete ancestral states.

Sequence of ecological diversification

We tested the habitat-first, diet-first and limited-diversification hypotheses by examining the order of ecological character state changes reconstructed on the preferred tree. The diet-first and habitat-first

hypotheses predict that, for a given clade, the first ecological character to change will be diet or habitat, respectively. The limited-diversification hypothesis predicts that there will be no changes in both characters on the same branch of the phylogeny.

RESULTS

Phylogenetic analysis

In the following section, we focus on areas of agreement and disagreement between data sets and methods, and allow the figures to summarize the hypothesized relationships. All analyses agreed on the monophyly of the subfamily Emydinae and of most genera (Figs 1–8). Analyses also agreed on the monophyly of Deirochelyinae, with the exception of a conflict as to the position of *Deirochelys* (see below). Disagreements between data and methods centred around intergeneric relationships within the subfamilies, a few intrageneric relationships, and the monophyly of *Clemmys*. All data matrices will be made available on the personal website of J.J.W. in late 2003 (http://life.bio.sunysb.edu/ee/people/wiensindex.html).

Molecular data

There was considerable congruence between trees based on cytochrome *b* sequences (Fig. 1a,b), 16S sequences (Fig. 1c,d), and ND4 sequences (Fig. 2). Both cytochrome *b* and 16S data sets agreed on the monophyly of the two subfamilies (outside of the placement of *Deirochelys*) and a *Graptemys* + *Malaclemys* + *Trachemys* clade. All three data sets agree on a *Clemmys insculpta* + *C. muhlenbergii* clade and a *C. marmorata* + *Emys* + *Emydoidea* clade. Conflicts between these trees were generally weakly supported. A conflict over the sister-taxon of *Graptemys* (either *Trachemys* or *Malaclemys*) was strongly supported by bootstrapping in the cytochrome *b* and 16S data sets (Fig. 1a vs. 1c), but only when analysed using parsimony (Fig. 1c vs. 1d). Maximum likelihood trees were similar to trees based on parsimony (Figs 1–3) and the few conflicting clades were weakly supported by bootstrapping in all data sets.

There was some conflict among the molecular data sets regarding the position of *Deirochelys*. In the trees based on 16S sequences, *Deirochelys* is part of a strongly supported monophyletic Deirochelyinae (Fig. 1c,d). In trees based on parsimony analysis of cytochrome *b*, *Deirochelys* is weakly supported as the sister-taxon of the Emydidae (Fig. 1a). In trees based on maximum likelihood analysis of cytochrome *b, Deirochelys* appears as the sister-taxon of the Emydinae (Fig. 1b), and this Emydinae + *Deirochelys* clade is strongly supported by bootstrapping (occurring in 71% of pseudoreplicates). In the tree based on parsimony analysis of all molecular data, *Deirochelys* is weakly supported as the sister-taxon of the Emydidae (Fig. 3a). However, when the same data are analysed using maximum likelihood, *Deirochelys* is part of a strongly supported monophyletic Deirochelyinae (Fig. 3b).

Morphological data

Analyses of morphological data using between-character (Fig. 4) and between-state scaling (Fig. 5) of meristic characters yielded similar trees that agreed on the monophyly of the subfamilies, the monophyly of most genera, relationships within the Emydinae (both within and between genera), and many intrageneric relationships. Trees based on the two scaling methods disagreed on dierochelyine intergeneric relationships, monophyly of *Graptemys*, and placement of the species and subspecies of *Trachemys*. None of the conflicting results were strongly supported by bootstrapping (Figs 4,5) and the trees based on between-state and between-character scaling were largely congruent when many taxa that lack complete morphological data are excluded (Fig. 6).

Conflicts between morphological and molecular data

Trees from the morphological and combined molecular data agree on the monophyly of the two subfamilies, the monophyly of *Graptemys* and *Terrapene*, and the sister relationship of *Clemmys insculpta* and *C. muhlenbergii* (Figs 3–5). Most disagreements were only weakly supported, but there were two localized but well-supported points of contention between the two data sets. The monophyly of *Clemmys* was close to our criterion for strong support in the trees based upon morphological data (Figs 4,5), appearing in 64 and 67% of bootstrap pseudoreplicates in analyses based on between-character and between-state scaling of meristic characters, respectively, whereas a clade consisting of *Emys*, *Emydoidea* and *Clemmys marmorata* had strong bootstrap support in all analyses that included cytochrome *b* sequences (Figs 1,3,7,8). Furthermore, when the morphological data are reduced to include only the 28 ingroup taxa represented in the combined molecular data, a clade consisting of *Pseudemys*, *Trachemys* and *Chrysemys* was strongly supported (Fig. 6), whereas a clade consisting of *Trachemys*, *Malaclemys* and *Graptemys* was strongly supported by all molecular data sets (Figs 1,3).

Combined analysis

Combined analysis of all taxa and characters using parsimony produced a tree that was well resolved, but weakly supported by bootstrapping in some areas (Fig. 7). Between-character and between-state scaling of meristic characters gave identical results for the combined-data analyses. The combined data

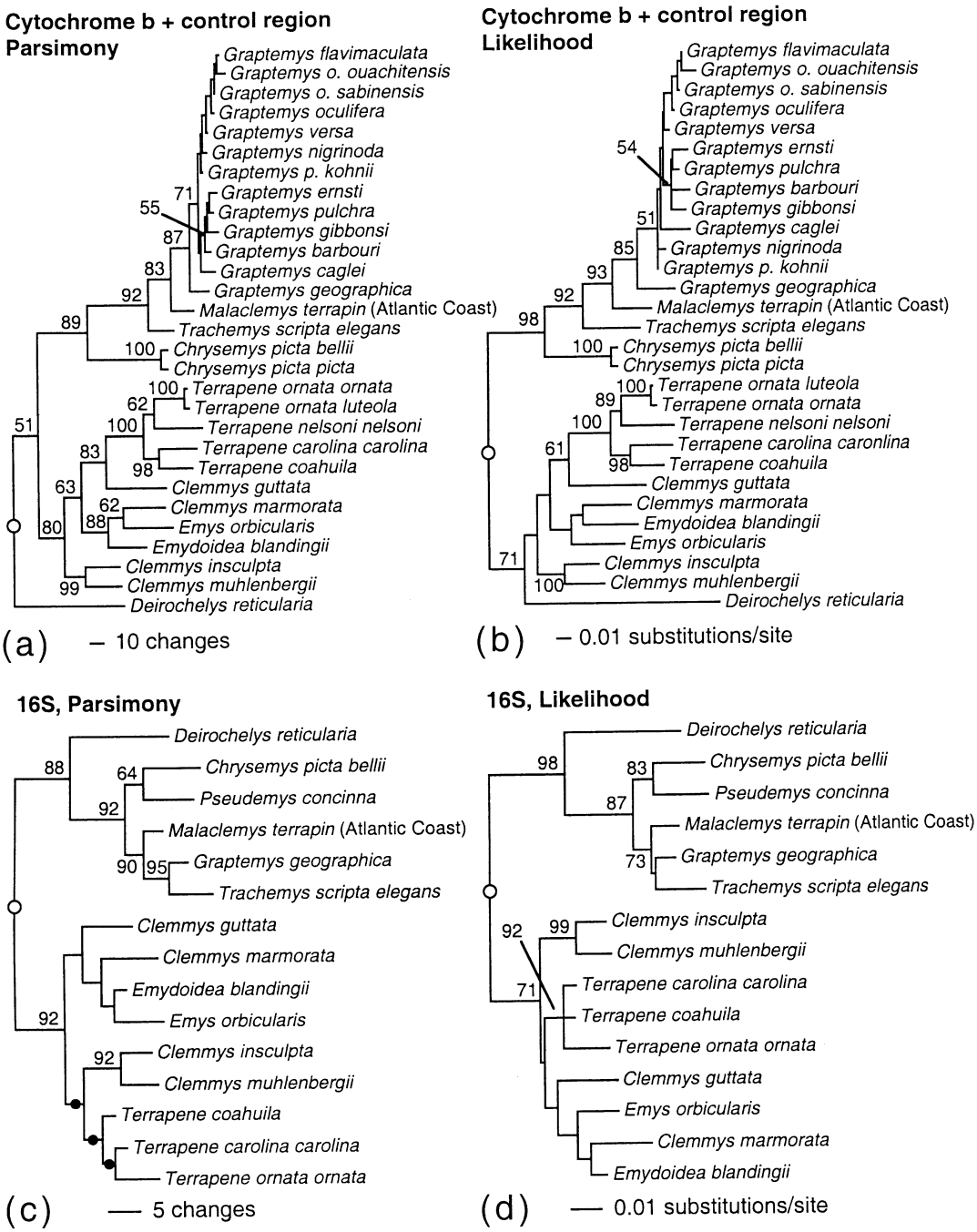

Figure 1. Trees from analyses of separate molecular data sets. Numbers associated with each branch are bootstrap proportions (values below 50% not shown). Open circles indicate the position of the root. Filled circles indicate branches that are collapsed in a strict consensus of multiple equally parsimonious trees. (a) One of two equally parsimonious trees from parsimony analysis of all mitochondrial cytochrome *b* + control region sequences (TL = 1222, CI = 0.576, RI = 0.607). *Chinemys reevesi* and *Heosemys spinosa* are used as the first outgroup whereas *Sternotherus odouratus* is used as the second outgroup. Neither outgroup is shown. (b) Maximum likelihood tree (– ln likelihood = 7563.1). (c) One of three equally parsimonious trees from parsimony analysis of 16S mitochondrial ribosomal sequences (TL = 219, CI = 0.676, RI = 0.703). The outgroup taxa, *Orlitia borneensis* and *Malayemys subtrijuga*, are not shown. (d) Maximum likelihood tree (–ln likelihood = 1701.3). Abbreviations: *p.* = *pseudogeographica*, *o.* = *ouachitensis*, TL = tree length, CI = consistency index excluding uninformative characters, and RI = retention index.

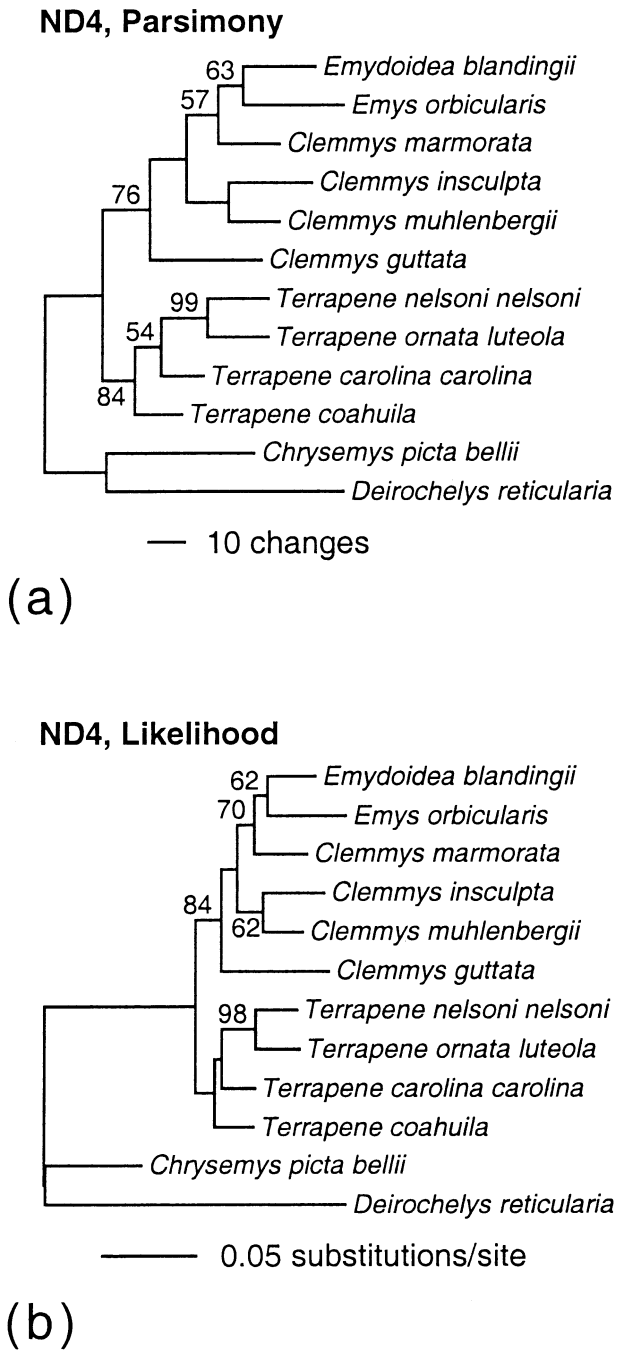

Figure 2. Shortest trees from analysis of ND4 sequence data using (a) equally weighted parsimony (TL = 413, CI = 0.656, RI = 0.486) and (b) maximum likelihood (–ln likelihood = 3117.1). Numbers associated with each branch are bootstrap proportions (values below 50% not shown). The outgroup taxa, *Deirochelys reticularia* and *Chrysemys picta*, are shown for comparative purposes.

support the monophyly of the two subfamilies and of most genera, with the exception of *Trachemys* and *Clemmys*. To investigate the effects of including taxa with incomplete data on the overall tree topology, combined analyses were also performed including only taxa for which both morphological and molecular data were available. For these analyses, the topology of trees based upon between-character and within-character scaling of meristic characters were identical, and bootstrap support was relatively high (Fig. 8). The topology of trees based upon all taxa and characters (Fig. 7) was consistent with the well-supported topology based on the complete taxa alone (Fig. 8).

EMYDID ECOLOGICAL DIVERSIFICATION

Direction of change

When habitat and diet are mapped onto the preferred tree (Fig. 9), the common ancestor of the Emydidae is reconstructed as an aquatic omnivore (habitat specialist, dietary generalist) using discrete coding and a semi-terrestrial omnivore (habitat and dietary generalist) using continuous coding. Reconstruction of discrete states for habitat shows two or three changes from generalist (semi-terrestrial) to specialist (terrestrial or aquatic) and one or two changes from specialist to generalist, depending upon the equally parsimonious reconstruction. Reconstruction of continuous trait values shows 12 changes from generalist to specialist and none from specialist to generalist (after ancestral trait values are scored as specialist or generalist). The results also suggest the possibility of a change between extreme forms of ecological specialization. The seemingly specialized Mexican aquatic box turtle (*Terrapene coahuila*) is descended from an ancestor that was either a terrestrial specialist (using discrete coding) or semi-terrestrial generalist (using continuous coding).

In summary, reconstruction of discrete states for diet shows that there have been between seven and eight changes from generalist to specialist and two or three changes from specialist to generalist, depending upon the specific parsimony reconstruction (changes from herbivore to carnivore or vice versa do not appear in any reconstruction). Using continuous coding there have been 16 changes from generalist to specialist in diet and none from specialist to generalist.

Sequence of ecological diversification

When diet and habitat are mapped onto the preferred tree using discrete coding, the earliest major ecological change occurred in habitat, with a shift from aquatic to semi-terrestrial habitat use in the ancestor of the

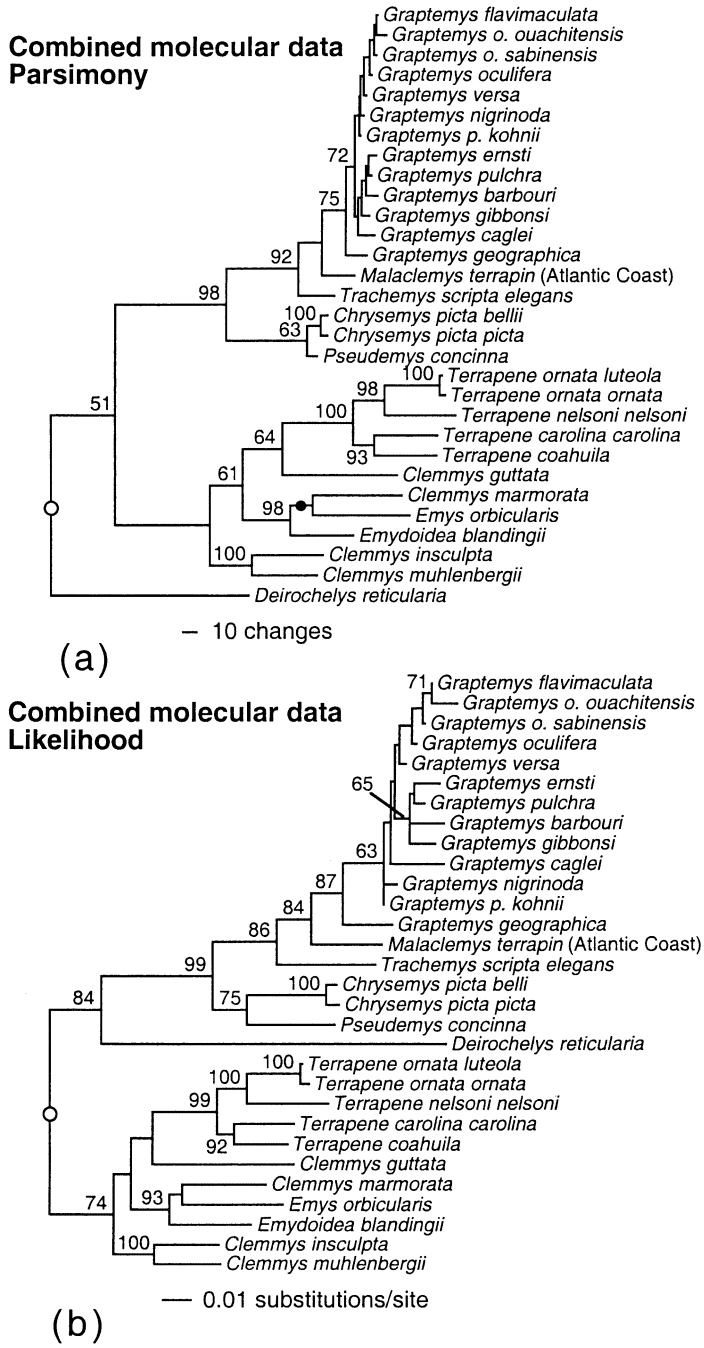

Figure 3. Trees from analysis of all molecular sequence data combined. Numbers associated with each branch are bootstrap proportions (values below 50% not shown). Open circles indicate the position of the root. Filled circles indicate branches that are collapsed in a strict consensus of multiple equally parsimonious trees. The outgroup taxa, *Chinemys reevesi*, *Heosemys spinosa*, *Orlitia borneensis* and *Malayemys subtrijuga* are used as the first outgroup whereas *Sternotherus odouratus* is used as the second outgroup. Neither outgroup is shown. The subspecies of *Chrysemys* were constrained to be monophyletic in both analyses because non-overlapping sequences were available for different subspecies and *Chrysemys* consists of a single species. (a) One of three equally parsimonious trees from parsimony analysis of combined sequence data (TL = 39716, CI = 0.981, RI = 0.592). (b) Maximum likelihood tree (–ln likelihood = 12501.9).

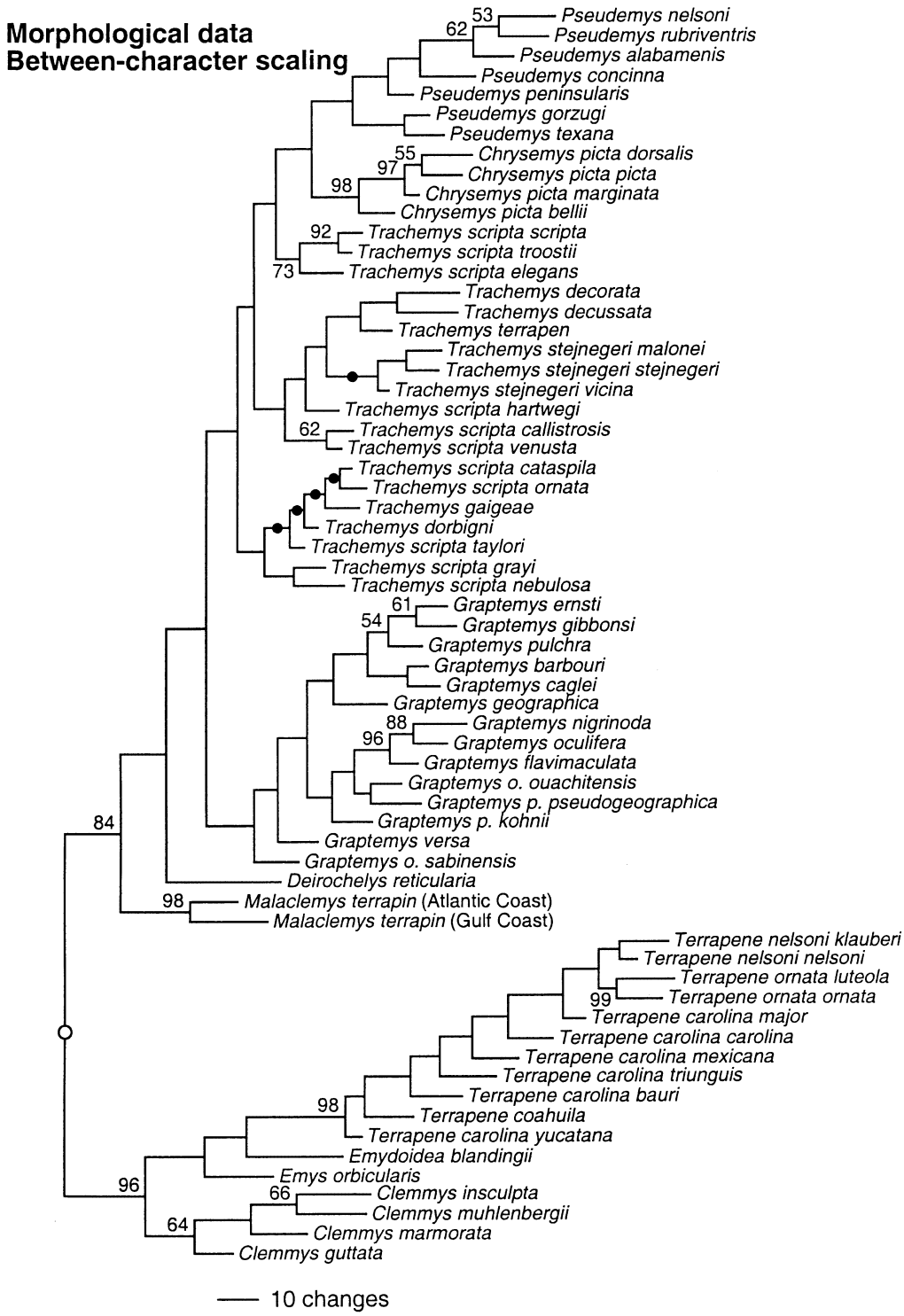

Figure 4. One of 24 shortest trees from parsimony analysis of all morphological data using between-character scaling of meristic data (TL = 1503, CI = 0.248, RI = 0.554). Numbers associated with each branch are bootstrap proportions (values below 50% not shown). The open circle indicates the position of the root. Filled circles indicate branches that are collapsed in a strict consensus of multiple equally parsimonious trees. The outgroup taxa (*Kinosternon subrubrum*, *Stenotherus odouratus*, *Mauremys caspica*, *Morenia petersi*, *Notochelys platynota* and *Rhinoclemmys areolata*) are not shown.

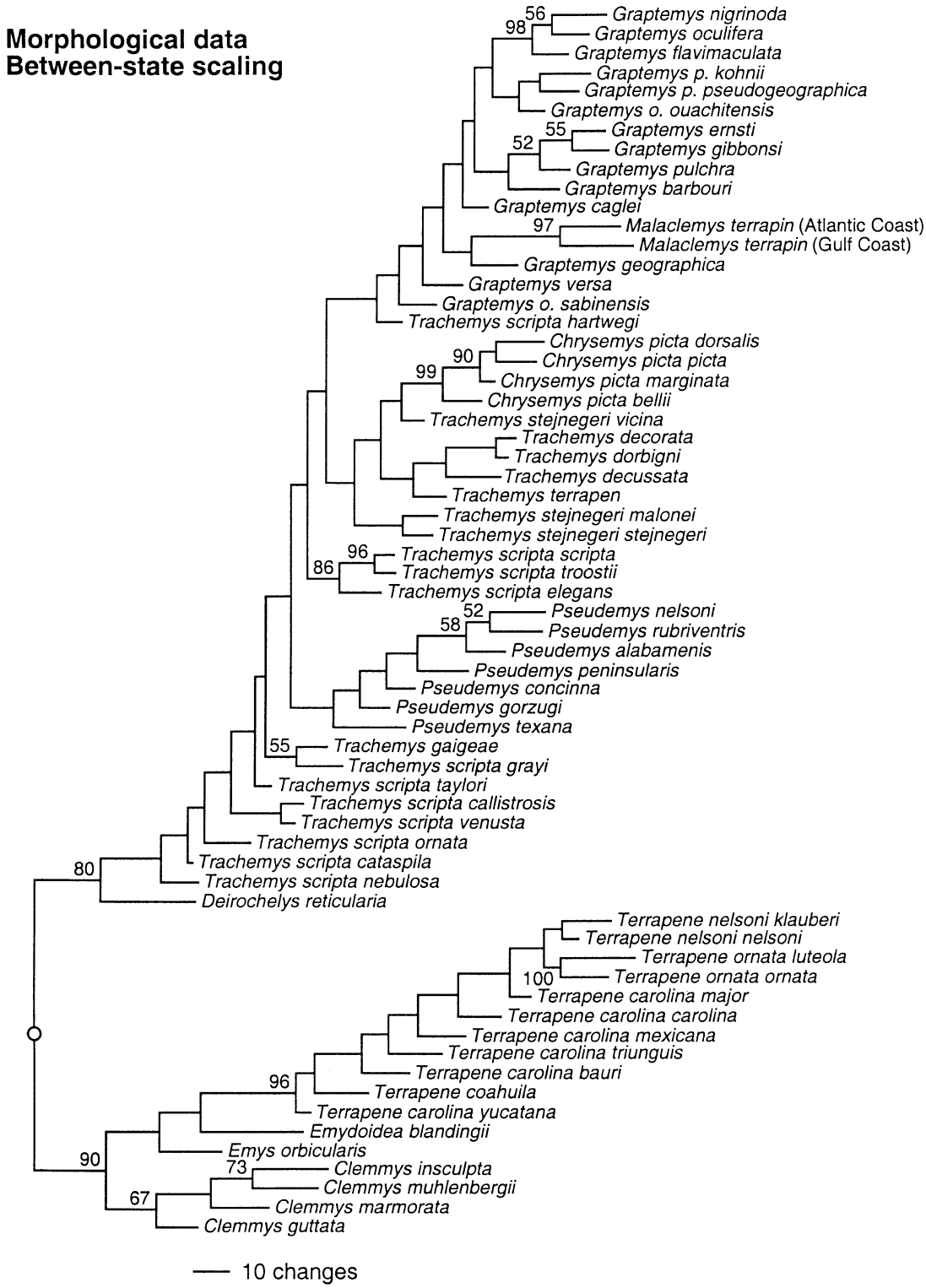

Figure 5. Shortest tree from parsimony analysis of all morphological data using between-state scaling of meristic data (TL = 1649, CI = 0.248, RI = 0.543). Numbers associated with each branch are bootstrap proportions (values below 50% not shown). The open circle indicates the position of the root. The outgroup taxa (not depicted) are the same as those listed in Fig. 4.

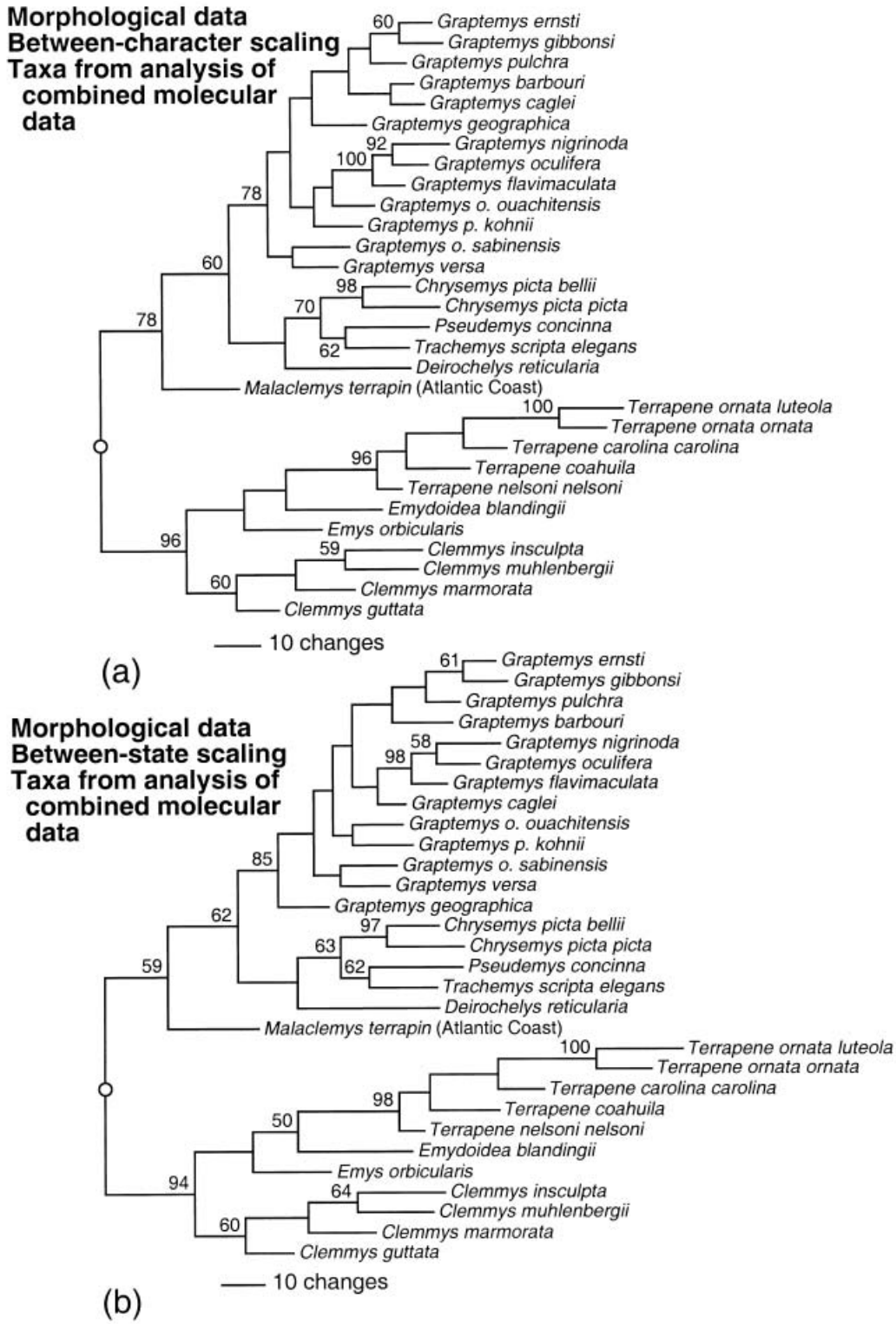

Figure 6. Shortest trees from parsimony analysis of the morphological data, with ingroup taxa that are not present in the combined molecular data set deleted (Fig. 2). The outgroup taxa (not depicted) are the same as those listed in Fig. 3. Numbers associated with each branch are bootstrap proportions (values below 50% not shown). Open circles indicate the position of the root. (a) Single shortest tree based on between-character scaling of meristic data (TL = 926, CI = 0.325, RI = 0.541). (b) Single shortest tree based on between-state scaling (TL = 1025, CI = 0.323, RI = 0.530).

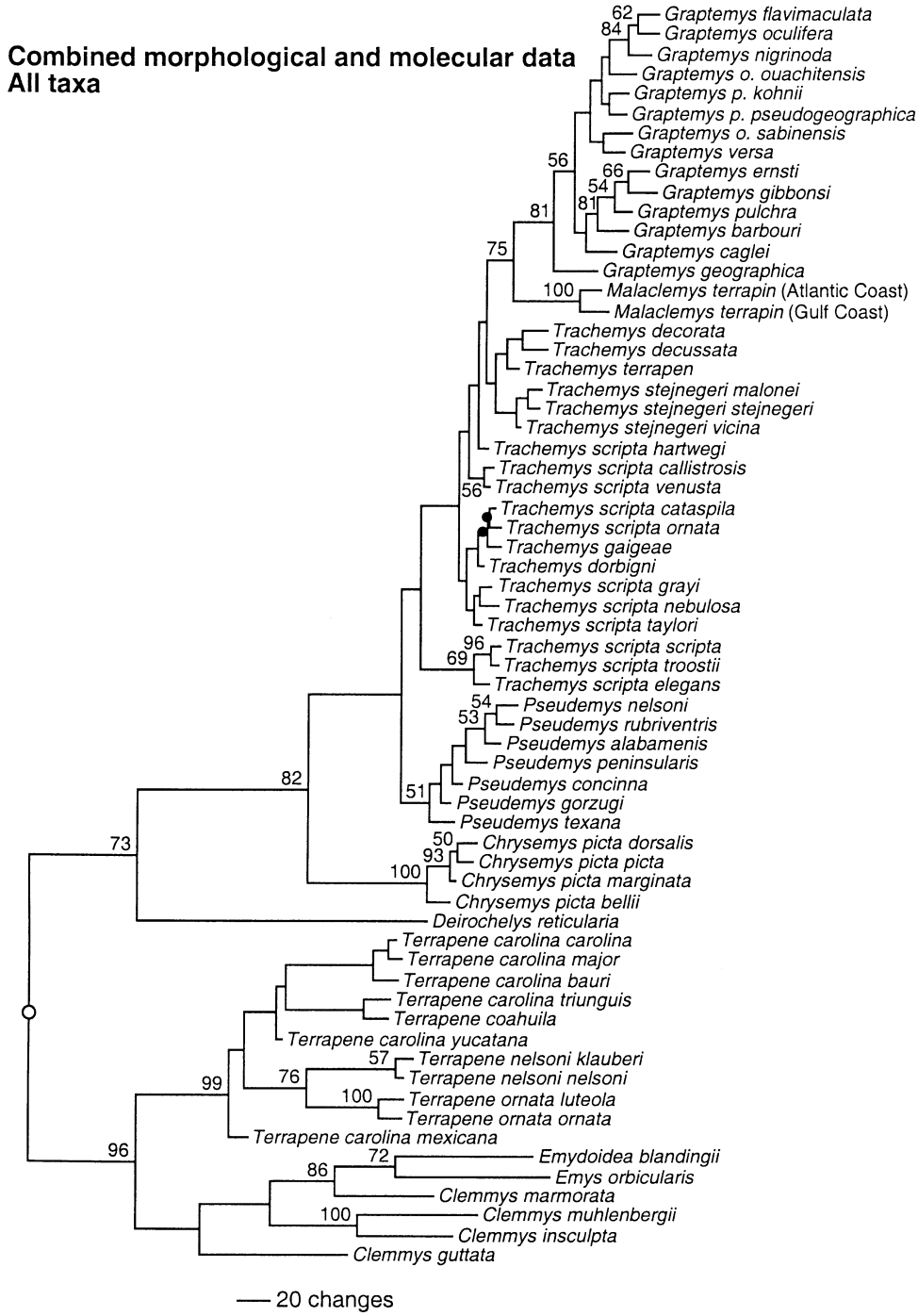

Figure 7. One of five shortest trees from parsimony analysis of combined morphological and molecular data for all taxa based on between-character scaling of meristic characters (TL = 41255, CI = 0.954, RI = 0.562). Numbers associated with each branch are bootstrap proportions (values below 50% not shown). The open circle indicates the position of the root. Filled circles indicate branches that are collapsed in a strict consensus of multiple equally parsimonious trees. Between-state scaling of meristic characters generated five trees with the same topology (TL = 41278, CI = 0.951, RI = 0.554). The outgroup taxa are not shown (*Kinosternon subrubrum*, *Stenotherus odouratus*, *Chinemys reevesi*, *Heosemys spinosa*, *Malayemys subtrijuga*, *Mauremys caspica*, *Morenia petersi*, *Notochelys platynota*, *Orlitia borneensis* and *Rhinoclemmys areolata*).

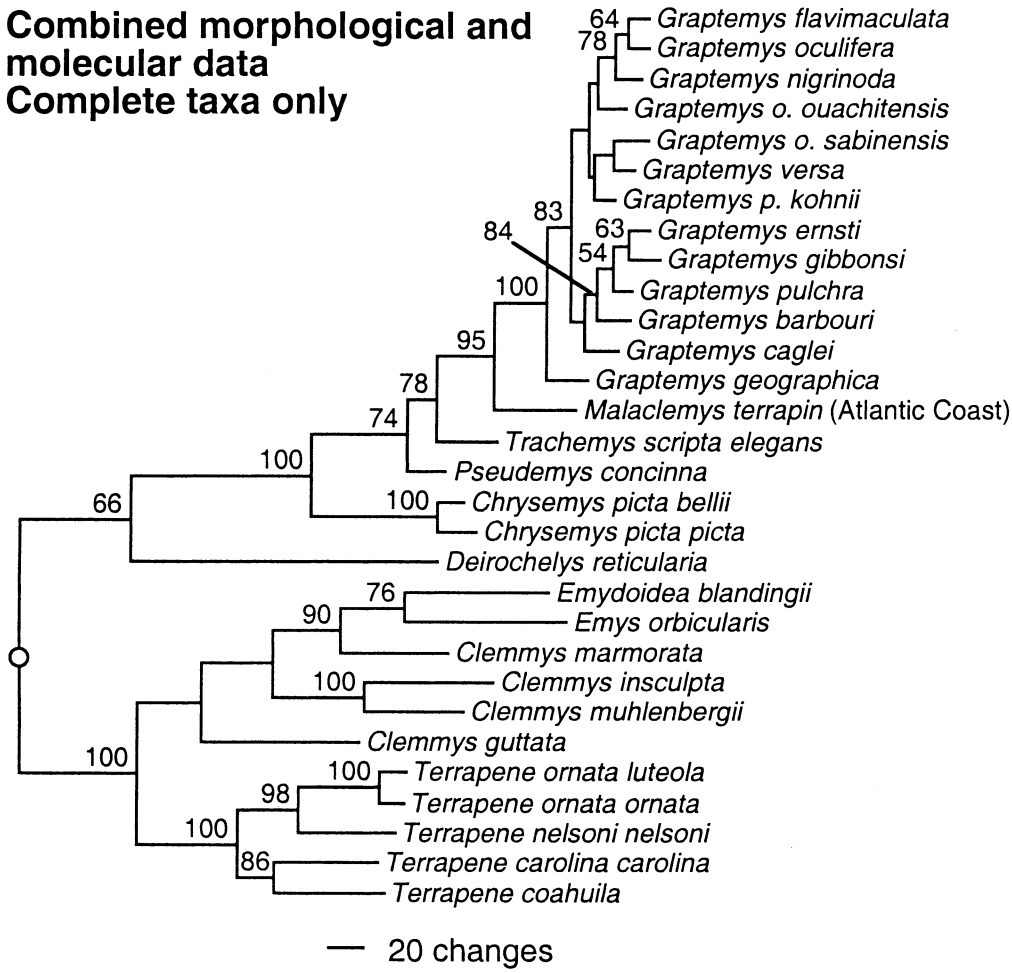

Figure 8. Single shortest tree from parsimony analysis of combined morphological and molecular data for taxa with complete data based on between-character scaling of meristic characters (TL = 40681, CI = 0.965, RI = 0.558). Numbers associated with each branch are bootstrap proportions (values below 50% not shown). The open circle indicates the position of the root. An analysis using between-state scaling generated a single tree with the same topology, save that the positions of *Terrapene ornata* and *T. coahuila* are reversed (TL = 40659, CI = 0.964, RI = 0.552). Outgroup taxa (not depicted) are the same as those listed in Fig. 7.

emydines (Fig. 9b). Very different patterns of diversification occur subsequently in the two subfamilies (Fig. 9). Within Emydinae there have been four major habitat changes and two major changes in diet. All changes in diet occur on the same branch or after changes in habitat (there is a simultaneous change in diet and habitat in *Emys orbicularis*). Overall, the pattern in emydines supports the habitat-first hypothesis. In contrast, all deirochelyines exhibit the plesiomorphic habitat character state (aquatic) and there have been seven major changes in diet. This pattern supports the diet-first hypothesis. Using continuous coding, the broad result that deirochelyines have undergone more changes in diet and fewer changes in habitat compared to emydines is supported. However, based upon continuous coding, habitat always changes before diet in both the deirochelyines and emydines (with the exception of a simultaneous change in *Emys orbicularis*).

Whereas support for the habitat-first and diet-first hypotheses is clade- and method-specific, the results overall strongly support the limited-diversification hypothesis. There is only one case in which habitat and diet changed on the same branch, whereas there have been at least nine changes in diet (16 using continuous coding) and at least four changes in habitat (12 using continuous coding) on different branches.

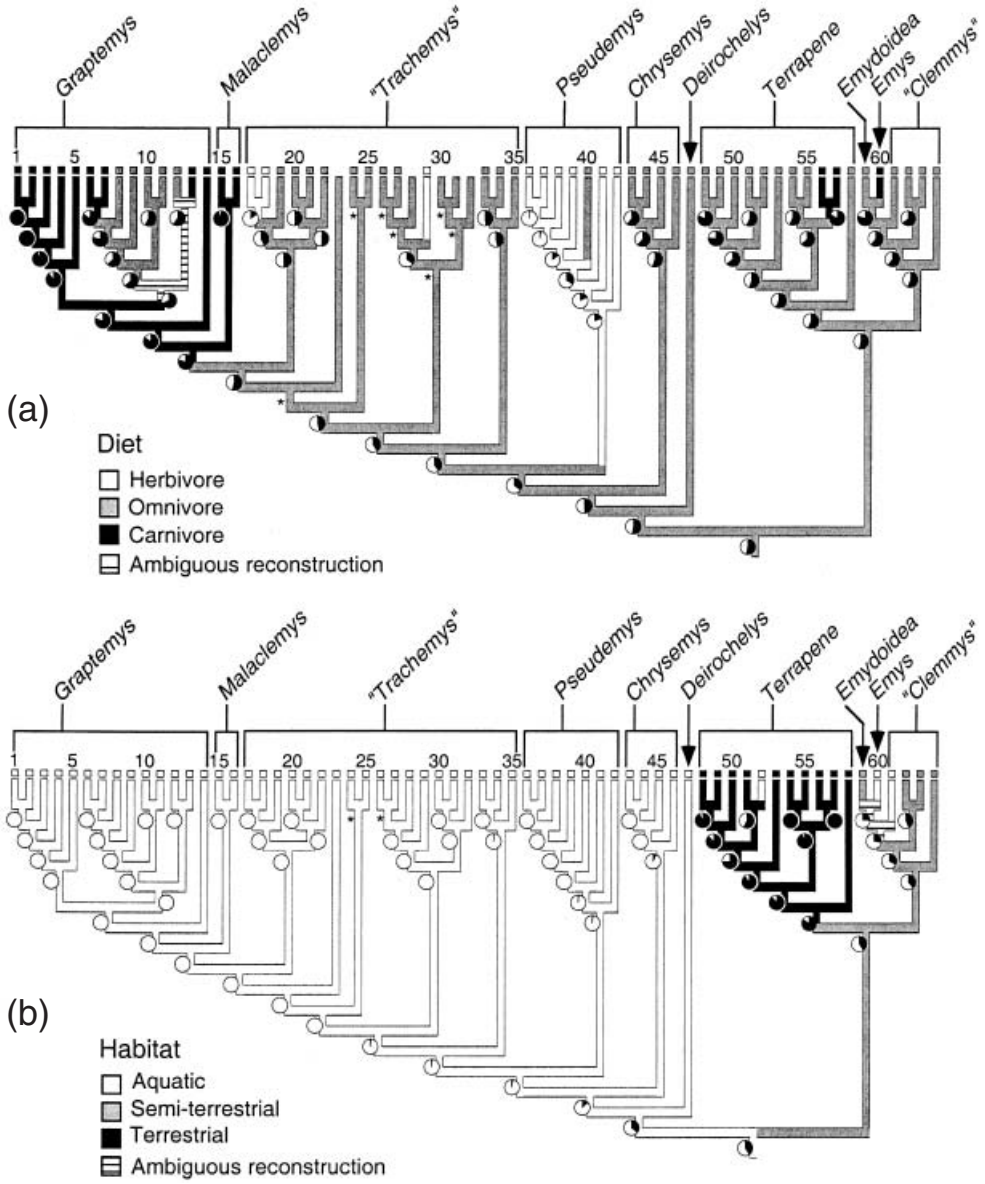

Figure 9. Ancestral character state reconstructions for (A) diet and (B) habitat use. Squares above each branch indicate character states for terminal taxa, missing squares indicate terminal taxa for which no ecological data were available. The black zone in the pie charts indicate the reconstructed proportion of animal matter in the diet in (A), and the reconstructed proportion of terrestrial habitat use in (B). The asterisk (*) indicates taxa that were excluded from maximum likelihood analyses due to missing data. Terminal taxa are as follows: (1) *Graptemys ernsti*; (2) *G. gibbonsi*; (3) *G. pulchra*; (4) *G. barbouri*; (5) *G. caglei*; (6) *G. flavimaculata*; (7) *G. oculifera*; (8) *G. nigrinoda*; (9) *G. ouachitensis ouachitensis*; (10) *G. pseudogeographica kohnii*; (11) *G. p. pseudogeographica*; (12) *G. o. sabinensis*; (13) *G. versa*; (14) *G. geographica*; (15) *Malaclemys terrapene* (Atlantic Coast subspecies); (16) *M. terrapene* (Gulf Coast subspecies); (17) *Trachemys decorata*; (18) *T. decussata*; (19) *T. terrapen*; (20) *T. stejnegeri malonei*; (21) *T. s. stejnegeri*; (22) *T. s. vicina*; (23) *T. scripta hartwegi*; (24) *T. s. callistrosis*; (25) *T. s. venusta*; (26) *T. dorbigni*; (27) *T. gaigeae*; (28) *T. s. cataspila*; (29) *T. s. ornata*; (30) *T. s. grayii*; (31) *T. s. nebulosa*; (32) *T. s. taylori*; (33) *T. s. scripta*; (34) *T. s. elegans*; (35) *T. s. troosti*; (36) *Pseudemys nelsoni*; (37) *P. rubriventris*; (38) *P. alabamensis*; (39) *P. peninsularis*; (40) *P. concinna*; (41) *P. gorguzi*; (42) *P. texana*; (43) *Chrysemys picta marginata*; (44) *C. p. picta*; (45) *C. p. marginata*; (46) *C. p. bellii*; (47) *Deirochelys reticularia*; (48) *Terrapene carolina carolina*; (49) *T. c. major*; (50) *T. c. bauri*; (51) *T. c. triunguis*; (52) *T. coahuila*; (53) *T. carolina yucatana*; (54) *T. nelsoni klauberi*; (55) *T. n. nelsoni*; (56) *T. ornata luteola*; (57) *T. o. ornata*; (58) *T. c. mexicana*; (59) *Emydoidea blandingii*; (60) *Emys orbicularis*; (61) *Clemmys marmorata*; (62) *C. insculpta*; (63) *C. muhlenbergii*; (64) *C. guttata*.

DISCUSSION

EMYDID SYSTEMATICS

Our preferred tree, based on the combined analysis of all taxa and characters (Fig. 7), supports the monophyly of the two subfamilies and of all the genera, with the exception of *Trachemys* and *Clemmys*. Although the combined-data estimate is well-resolved, levels of bootstrap support are weak in many parts of this tree. When highly incomplete taxa are removed, however, the result is a 'backbone' tree with generally high levels of bootstrap support (Fig. 8) that is consistent with the topology of the tree with all taxa. This finding suggests that most of the low support in the comprehensive phylogeny comes from the weakly supported placement of the incomplete taxa (see Wiens, 2003a,b)

We consider the tree based on the combined data to be our preferred estimate of emydid phylogeny. This phylogenetic hypothesis represents the first comprehensive species-level phylogeny of Emydidae, and almost all characters used in previous studies of emydid phylogeny have been incorporated into this analysis. The combined morphological and molecular data set includes 733 parsimony-informative characters, more than twice the number of any previous study of emydid phylogeny. However, additional sampling of taxa and characters (particularly molecular data sets with complete taxon sampling) are needed to provide a more strongly supported hypothesis for all taxa. Below we discuss in more detail the major conflicts between data sets, as well as taxonomic implications of the results.

Previous studies

Only two recent studies have considered the phylogenetic relationships of all emydid genera (Fig. 10a,b): Gaffney & Meylan (1988; based on morphological data) and Bickham *et al*. (1996; based on 16S sequence data). Overall, our combined-data tree (Fig. 7) shows little concordance with the results of Gaffney & Meylan (1988) (Fig. 10a). Major differences include generic-level relationships in the two subfamilies and the monophyly of *Clemmys* and *Trachemys*. When morphological characters are considered alone, generic-level relationships are similar (Figs 4,5) to those reported by Gaffney & Meylan (1988) (Fig. 10a) within the Emydinae, but not the Deirochelyinae. However, Gaffney & Meylan (1988) noted that their hypothesis, based upon only 25 characters, was preliminary. Our results are more concordant with those of Bickham *et al*. (1996) (Fig. 10b), showing similar generic-level arrangements within the Deirochelyinae (differing only in the placements of *Trachemys*) and many similarities within the Emydinae (including a paraphyletic *Clemmys*, a monophyletic *Terrapene*, and a clade consisting of *Emydoidea*, *Emys* and *Clemmys marmorata*).

Two recent studies (Burke *et al*., 1996, based on 16S sequences and morphology, and Feldman & Parham, 2002, based on cytochrome *b* and ND4 sequences) have addressed phylogenetic relationships within the subfamily Emydinae (Fig. 10c,d). Our results within Emydinae (Figs 7,8) show both agreement and disagreement with those of Burke *et al*. (1996; Fig. 10c). Our study agrees on the monophyly of *Terrapene* and of the *Clemmys insculpta* + *C. muhlenbergii* clade and disagrees over the position of *C. marmorata* and *C. guttata*. We show the same strongly supported emydine relationships as Feldman & Parham (2002) (Fig. 10d) in our combined-data trees (Figs 7,8).

Monophyly of Clemmys

Our results show a conflict regarding the monophyly of *Clemmys* between the morphological data (supporting monophyly; Figs 4,5) and molecular data (rejecting monophyly; Fig. 3a,b). A similar conflict has been found in previous studies (Burke *et al*., 1996; Feldman & Parham, 2002). Monophyly of *Clemmys* is supported by six morphological characters that show extensive change on this branch (i.e. a change in frequency of 50% or more). These characters share no obvious functional or developmental relationship and seem to be evolving independently (the characters include variation in cranial ($N = 3$), plastral ($N = 2$) and penial ($N = 1$) morphology; characters 36, 68, 76, 98, 100 and 240 in the Appendix). Thus their concordance is difficult to explain. Nevertheless, morphological data do not strongly support monophyly of *Clemmys* using our threshold for bootstrap support, and we therefore consider *Clemmys* to be non-monophyletic.

Our phylogenetic conclusions based on the combined molecular data and the combined molecular and morphological data agree with those of Feldman & Parham (2002; based on ND4 and cytochrome *b*), but our preferred classification differs somewhat. Because of the seeming paraphyly of *Clemmys*, Feldman & Parham (2002) proposed three taxonomic changes within emydines. First, they proposed that the generic name *Clemmys* be restricted to the type species of the genus (*Clemmys guttata*). Second, they proposed that the generic name *Calemys* (Agassiz, 1857) be applied to *Clemmys insculpta* and *Clemmys muhlenbergii*, as these species together form a strongly supported monophyletic group. Finally, they recommended that *Emydoidea blandingii* and *Clemmys marmorata* be assigned to *Emys*, given that these two species form a well-supported clade with *Emys orbicularis*. We agree with their first two recommendations, but disagree with the third. Although we wish to avoid recognizing monotypic genera, it is more conservative (i.e. one taxonomic change vs. two) to recognize *Clemmys marm-*

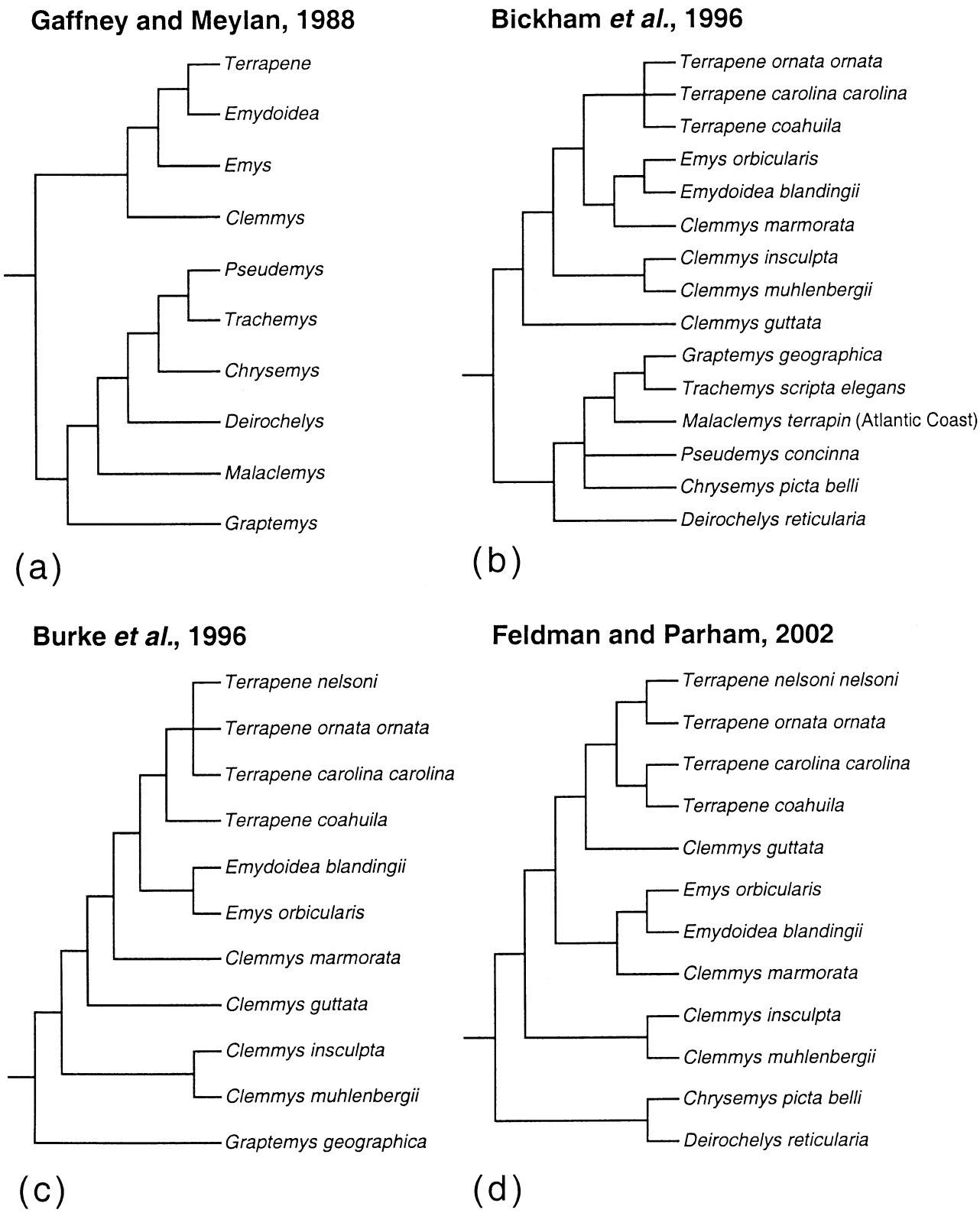

Figure 10. Past phylogenetic hypotheses for the Emydidae and Emydinae from (a) Gaffney & Meylan (1988), (b) Bickham *et al*. (1996), (c) Burke *et al*. (1996), and (d) Feldman & Parham (2002).

orata as a separate genus than to expand *Emys*. Furthermore, *Emys orbicularis* contains at least four geographically distinct haplotype clades that are also morphologically diagnosable: these will most likely be recognized as distinct species in the near future (Lenk *et al*., 1999). Thus, *Emys* may not remain a monotypic genus for long. In the interest of stability, we recommend applying the generic name *Actinemys* (Agassiz (1857) to *Clemmys marmorata* and leaving the genera *Emydoidea* and *Emys* unchanged.

Position of Deirochelys

There is a conflict between trees based on cytochrome *b* and 16S sequences regarding the position of *Deirochelys*. In trees based on 16S sequences *Deirochelys* appears as part of a well-supported monophyletic Deirochelyinae (Fig. 1c,d), whereas in those based on cytochrome *b Deirochelys* appears as the sister to all other emydids (Fig. 1a) or to emydines (Fig. 1b). This conflict was only strongly supported when these sequences were analysed using maximum likelihood (Fig. 1b,d), yet when the combined molecular data are analysed using likelihood *Deirochelys* is part of a strongly supported monophyletic Deirochelyinae (Fig. 3b). Trees based on morphological data agree with those based on 16S sequences and maximum likelihood analysis of the combined molecular data in placing *Deirochelys* within a monophyletic and strongly supported Deirochelyinae (Figs 4–6). All analyses based on sequence data agree that *Deirochelys* occurs near the base of the Emydidae at the end of a relatively long branch (Figs 1–3). We therefore suggest that the conflict over the placement of this taxon represents long-branch attraction (Felsenstein, 1978; Huelsenbeck, 1995) in the cytochrome *b* sequences, and that the Deirochelyinae is monophyletic (see Wiens & Hollingsworth (2000) for another example in which cytochrome *b* seems to give misleading intergeneric results in reptiles due to long-branch attraction).

Sister-taxon of Graptemys

There is a strongly supported conflict between the molecular data sets concerning the sister-taxon of *Graptemys*. In the tree based on parsimony analysis of 16S sequences, *Trachemys* is strongly supported as the sister-taxon of *Graptemys* (Fig. 1c), whereas in the parsimony trees based on cytochrome *b* sequences, *Malaclemys* and *Graptemys* are sister-taxa (Fig. 1a). When the 16S sequences are analysed using maximum likelihood, the *Graptemys* + *Trachemys* clade is no longer strongly supported by bootstrapping, and the branch length of the ancestor of this clade is dramatically reduced (Fig. 1b). These changes in bootstrap values and branch lengths suggest that many changes interpreted by parsimony as synapomorphies of *Graptemys* + *Trachemys* are interpreted as homoplastic autapomorphies of these genera by maximum likelihood. Although maximum likelihood may be less sensitive to long-branch attraction than parsimony, both methods may be misled in the same way when relatively few characters are sampled (Huelsenbeck, 1995). We speculate that the placement of *Trachemys* as the sister-taxon *Graptemys* is an artefact of long-branch attraction in the 16S sequences. In the trees based on combined molecular data (Fig. 3) and in the trees based on combined morphological and molecular data (Figs 7,8) *Graptemys* and *Malaclemys* are strongly supported as sister-taxa.

Position of Trachemys

When the morphological data set is reduced to include only the taxa for which molecular data are available, the resulting tree strongly supports a clade consisting of *Chrysemys*, *Pseudemys* and *Trachemys* (Fig. 6). In contrast, both molecular data sets strongly support a clade consisting of *Graptemys*, *Malaclemys* and *Trachemys* (Figs 1,3). Because the *Pseudemys* + *Trachemys* + *Chrysemys* clade does not appear in any morphological analysis that includes more taxa (Figs 4,5) and is inconsistent with the molecular data, it seems most likely that this clade is an artefact of limited taxon sampling in the morphological data.

Intrageneric results with taxonomic implications

Four sets of intrageneric relationships in the preferred tree have implications for emydid taxonomy. First, the species and subspecies of *Trachemys* form a paraphyletic series of five lineages leading up to the *Graptemys* + *Malaclemys* clade. These five lineages include a clade of West Indian taxa, a clade of primarily Mexican taxa, and a clade of North American taxa. The subspecies of the species *T. scripta* are grossly paraphyletic, appearing in four of the five clades, strongly suggesting that *T. scripta* may represent multiple species. No intergradation between subspecies has been reported between Neotropical subspecies, even in a case where many specimens of geographically adjacent subspecies were kept in the same pond (see account of *T. s. grayi* in Smith & Smith, 1979). Seidel (2002) also concluded, based on morphological data, that *T. scripta* consists of multiple species. However, hypothesized relationships for these subspecies are not well-supported in this study or that of Seidel (2002). We suggest that it is premature to define species boundaries within the *Trachemys scripta* complex based on current evidence, and that this is a problem in need of additional study.

Second, *Graptemys ouachitensis* may also consist of multiple species. The two subspecies of *G. ouachitensis* were not closely related in our preferred tree (Fig. 7), and did not appear as sister-taxa in any analysis.

Ward (1980) reported features of cranial osteology that reliably diagnose *G. o. ouachitensis* from *G. o. sabinensis* and suggested that the latter be elevated to a full species. Furthermore, no hybrids or areas of natural sympatry between *G. o. ouachitensis* and *G. o. sabinensis* have been reported.

Third, within *Pseudemys*, the monophyly of the subgenus *Pytchemys* (*sensu* Seidel, 1994; *P. alabamensis*, *P. nelsoni* and *P. rubriventris*) was supported, but not that of the subgenus *Pseudemys* (*sensu* Seidel, 1994; all remaining species).

Fourth, within *Terrapene*, the monophyly of the *ornata* species group (*sensu* Minx, 1996; *T. ornata* and *T. nelsoni*) was strongly supported but the *carolina* group (*sensu* Minx, 1996; *T. coahuila* and *T. carolina*) was not. Instead, *Terrapene carolina mexicana* was weakly supported as the sister to all other *Terrapene* (Fig. 7). Our results suggest that *T. carolina* is paraphyletic and might also consist of multiple species. *Terrapene carolina mexicana* and *T. c. yucatana* seem likely to represent full species based on their geographical isolation from other *T. carolina* subspecies. *Terrapene carolina yucatana*, in particular, is morphologically and biogeographically distinct from the other subspecies of *T. carolina* (Smith & Smith, 1979).

Convergence, ecological diversification and morphological phylogenetics

The impressive ecological diversification of emydids suggests the possibility that our morphology-based phylogenies might be misled by adaptive convergence (e.g. Hedges & Maxson, 1996; Givnish & Sytsma, 1997). This appears not to be the case. Molecular data were available for those taxa that show greatest diversification in ecological characters, and adaptation to habitat and diet cannot explain any of the conflicts between the morphological and molecular data. Habitat use is highly variable among emydines and molecular data were available for every species of emydine turtle. Only one point of (marginally) well-supported incongruence was found between trees from molecular and morphological data in emydines: the monophyly of *Clemmys* was moderately well-supported by morphological data, and strongly rejected by molecular data. Three of the species of *Clemmys* exhibit ecological character states that are plesiomorphic for emydines (Fig. 9). Only one species of *Clemmys* (*C. marmorata*) exhibits an apomorphic ecological characteristic (aquatic habitat), which it shares with *Emys orbicularis*. Surprisingly, a *C. marmorata* + *E. orbicularis* clade is supported only by the molecular data (Fig. 3 vs. Figs 4,5). Although molecular data are not available for all deirochelyine species, habitat use varies little in this clade (all are aquatic).

Diet also does not appear to have caused phylogenetically misleading convergence. The majority of emydines are omnivores, and the two carnivorous species (*Terrapene ornata* and *Emys orbicularis*) do not appear as close relatives in any analysis. Among deirochelyines, the species that show the most extreme morphological adaptation to diet are the 'broad-headed' species of *Graptemys* (*G. barbouri*, *G. ernsti*, *G. gibbonsi* and *G. pulchra*), the females of which display extreme megacephaly as an adaptation to molluscivory (Lindeman, 2000b). The monophyly of the broad-headed *Graptemys* is strongly supported by morphological data (Figs 4,5), but is also supported by molecular data (Fig. 1a,b). *Emydoidea blandingii* and *Deirochelys reticularia* show an amazing degree of morphological convergence (e.g. both have relatively flattened and narrow skulls, narrow triturating surfaces, elongate cervical vertebrae and elongate thoracic rib heads), apparently related to similarly specialized feeding strategies (Loveridge & Williams, 1957; Bramble, 1974). However, these genera did not appear as close relatives in any analysis, even in those based upon morphology alone. The placement of *Deirochelys* within the Deirochelyinae and *Emydoidea* within the Emydinae was strongly supported in trees based upon both morphological and molecular data (Figs 1c,d, 3b, 4, 5–8).

Overall, our results demonstrate that morphological data can be used to reconstruct phylogenies in groups that have undergone ecological diversification, without being misled by convergence. Convergence may be most likely to mislead in cases where species that retain the plesiomorphic selective regime are extremely similar morphologically (Hillis & Wiens, 2000), and this does not appear to be the case in emydids.

Emydid ecological diversification

Comparison of coding methods

This study may be the first to compare the results of ancestral reconstructions based on continuous and discrete coding of the same characters. Overall, our observations suggest that the choice of coding method can have a significant impact on the conclusions of a comparative ecological or evolutionary study. In our study, the choice of coding method changed our conclusions with respect to two of the four hypotheses tested. When ecological characters were reconstructed as continuous characters three hypotheses were unambiguously supported (i.e. there were no exceptions to the patterns predicted by the generalist-to-specialist, habitat-first and limited-diversification hypotheses). However, when characters were scored discretely we found excep-

tions to the generalist-to-specialist and habitat-first hypotheses.

There were two general differences between our results using discrete and continuous coding. First, analyses using continuous coding were more likely to reconstruct ancestors as generalists (50 ancestors were reconstructed as generalist for diet, 15 were reconstructed as generalists for habitat) than analyses using discrete coding (43 ancestors were resolved as generalist for diet, four were resolved as generalists for habitat). Second, changes in character states were much more common using continuous coding. When using continuous coding, the criterion we used to determine number of ecological shifts was highly conservative (i.e. any value from 10–90% would be scored as a generalist, and thus many changes within this wide range were not treated as ecological shifts). However, we found that nearly twice the overall number of changes were observed using continuous coding (27 changes) vs. discrete coding (14 changes).

Both of these differences may have a similar underlying explanation. The continuous coding methods that we used (squared-change parsimony and maximum likelihood) assume a Brownian motion model which reconstructs ancestral nodes by effectively 'averaging' across nodes (e.g. Maddison, 1991). This property may make continuous coding methods more likely to reconstruct 'intermediate' values at ancestral nodes (i.e. generalist), and may lead to many small changes in ancestral values being reconstructed across the tree (leading to more changes being inferred), relative to coding with a small number of discrete states. Although this property of continuous coding methods may seem like a source of bias, it may also more accurately reflect biological reality than discrete coding (given that the underlying variables are continuous). The sensitivity of evolutionary conclusions to different coding methods suggests that the relative accuracy of different coding methods is a topic in urgent need of study. It should also be pointed out that in our study, continuously coded characters were reinterpreted as discrete states after the reconstructions in order to test the hypotheses of interest, and this practice may also have influenced our results.

Direction of change

Three recent reviews examined the evidence for the generalist-to-specialist hypothesis from natural systems (Futuyma & Moreno, 1988; Thompson, 1994; Schluter, 2000) and all reached the same conclusion: exceptions are common in nature. However, the great majority of studies considered in these reviews used parsimony reconstruction of discrete states. When we reconstructed ancestral states as discrete characters using parsimony we also found exceptions to the generalist-to-specialist hypothesis. However, when we reconstructed ancestral states as continuous characters (using parsimony or likelihood) we found no exceptions to the generalist-to-specialist hypothesis. This result suggests the possibility that other studies showing exceptions to the generalist-to-specialist hypothesis using discrete coding might have supported it using continuous coding (although it remains unclear which method is more likely to give correct inferences).

Sequence of ecological diversification

Our results using discrete coding suggest that there is no general sequence of ecological diversification, and that the habitat-first and diet-first hypotheses are clade-specific. For example, results in Deirochelyinae supported the diet-first hypothesis whereas those in Emydinae supported the habitat-first hypothesis. Several recent studies have tested these hypotheses, and collectively these studies have also found that support for these hypotheses varies from clade to clade. For example, Losos (1992) showed divergence in habitat prior to divergence in diet in *Anolis* lizards. Conversely, Richman & Price (1992) showed that divergence in diet occurred first in birds of the genus *Phylloscopus*. Schluter (2000) considered evidence for the habitat-first hypothesis in birds and found that it was supported in five of eight studies. Taken together, there does not seem to be convincing evidence to favour the greater generality of the habitat-first or the diet-first hypothesis. However, ours is the first study to test these hypotheses by reconstructing ecological characters as continuous variables. We found that use of continuous coding supported the habitat-first hypothesis both in the family overall and in the two subfamilies.

This study, and all of the studies mentioned above, found that simultaneous change on two or more ecological axes on the same branch of the phylogeny are extremely rare, lending support to the limited-diversification hypothesis. A change in any ecological characteristic (particularly broadening a niche or moving the 'position' of a niche on an axis) may require correlated change in morphology, behaviour and/or physiology (Wainwright & Reilly, 1994). There may be clade-specific trends in variability for the traits that allow ecological changes to occur, and these trends may determine which axis diversification will occur on first (Brooks & McLennan, 1991; Schluter, 1996). A simultaneous change on two ecological axes might be a more unlikely evolutionary change than a change on one axis due to the additional correlated phenotypic modification that it entails or, in some cases, because the phenotypic modification needed for a change on one axis is in opposition to that needed to change others (Wainwright & Reilly, 1994; Schlichting & Pigliucci, 1998). Furthermore, a change along only one

ecological axis may be all that is required to reduce competition (Tilman, 1997), if competition is driving ecological diversification of sympatric species. For example, species that exhibit the same diet but live in different habitats would not be expected to compete directly. Note also that the ecological character states that we used were quite broad, and could potentially have masked significant ecological differences between species that would also be expected to reduce competition.

General patterns of ecological diversification

Schluter (2000) and Streelman *et al*. (2002) recently reviewed phylogenetic studies of ecological diversification, and came to surprisingly different conclusions. Schluter (2000) found that in many groups the majority of speciation events are associated with ecological changes, and suggested that most speciation may be driven by adaptive evolution (e.g. Hawaiian silverswords: Baldwin & Sanderson, 1998; *Anolis* lizards: Losos *et al*., 1998; Galapagos finches: Grant, 1986, Petren, Grant & Grant, 1999). Conversely, Streelman *et al*. (2002) showed that in other groups ecological shifts between habitats are associated with basal splits, but that higher in the tree sexual selection, and not ecological changes, are associated with speciation events (e.g. cichlid fishes: Albertson *et al*., 1999; and parrotfishes: Streelman *et al*., 2002). In emydids, the basal split in the family is marked by a shift between aquatic and semi-terrestrial habitats (as predicted by Streelman *et al*., 2002), there are few changes in habitat higher in the tree, and most changes are associated with a few taxa that are geographically distant from other emydids (i.e. *Clemmys marmorata*, *Emys orbicularis*). In contrast to the taxonomic groups reviewed by Schluter (2000) and Streelman *et al*. (2002), recent speciation events in emydids do not appear to have been driven by ecological changes or sexual selection. Instead recent speciation in emydids appears to have involved allopatric speciation between ecologically similar taxa (Stephens & Wiens, 2003).

We suggest a simple model of ecological diversification that might reconcile the results of our study and those reviewed by Schluter (2000) and Streelman *et al*. (2002) (Fig. 11). Early in the history of the group, speciation is driven by ecological divergence in response to competition. This would correspond to the pattern in studies reviewed by Schluter (2000), where the majority of speciation events seem to be associated with ecological changes. Many of these groups appear to be relatively 'young' with recent origins and few species (e.g. Hawaiian silverswords: 28 species, *c*. 5.2 million years old (myo), Baldwin & Sanderson, 1998; and Galapagos finches: 14 species, *c*. 3 myo; Grant & Grant, 1996). Later, as niche space becomes increasingly filled, adaptive evolution will slow down and non-ecological mechanisms will become more important in driving speciation (e.g. allopatry, sexual selection). Thus, for older and more speciose groups, we expect major ecological differences between the oldest clades but relatively few within these clades. This pattern corresponds to that in parrotfishes (90 species, *c*. 42 myo; Streelman *et al*., 2002) and emydids (41 species, > 58 myo; Holman, 1995). We also speculate that non-adaptive speciation mechanisms will come to predominate even in relatively young groups once the available niche space is filled. For example, the cichlid fishes of Lake Malawi consist of

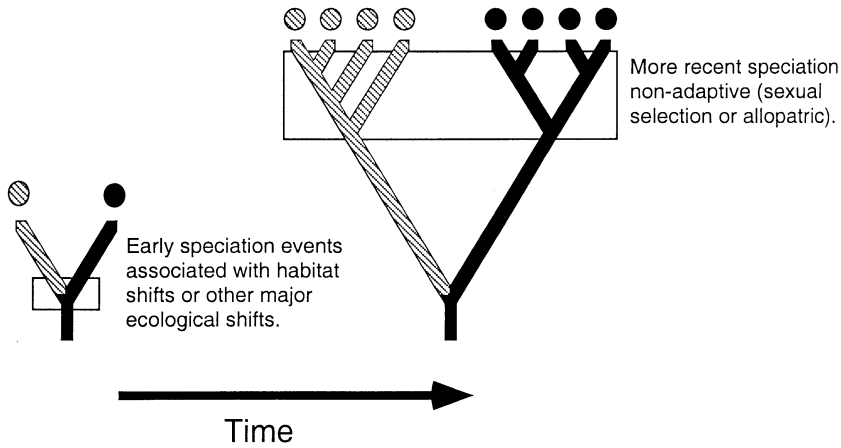

Figure 11. Hypothetical example illustrating a simple model of ecological diversification and speciation over time. This model is compatible with our results and the conclusions of Schluter (2000) and Streelman *et al*. (2002). Early in the history of a group (tree on left) speciation events are associated with major ecological changes (i.e. change from black to striped shading), whereas later speciation events (tree on right) are associated with non-adaptive mechanisms.

>500 species which diverged less than 1 myo (Albertson *et al*., 1999). Despite their recent origin, these cichlids show a pattern similar to that in emydids and parrotfishes, with ecological differences between basal groups but more recent speciation events being driven by sexual selection (Albertson *et al*., 1999).

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APPENDIX

CHARACTERS USED IN STUDY AND MORPHOLOGICAL CHARACTER STATES

Terminology follows Zangerl (1969; for shell and scute morphology), Bramble (1974; for hinge and limb girdle morphology), Gaffney (1979; for cranial osteology), or Ernst *et al*. (1994; for most external morphology). Only the earliest relevant study is cited for characters derived from literature sources. The definitions of many characters originally taken from the literature were modified to reduce ambiguity between character states. The numbering of character states is unrelated to polarity. Unless stated otherwise, all multistate characters were treated as unordered. The presence of a structure was treated as a separate character from variation in a structure, and variation in a structure was coded as unknown ("?") for those taxa in which the structure was missing (for example the position of the middorsal keel would be scored as unknown in a specimen that did not have a keel; see Maddison, 1993; Wilkinson, 1995). All colouration characters were scored based on preserved specimens. These characters seem to be unaffected by how long specimens have been preserved – even in very old specimens the characters could still be scored reliably. The numbers used for each character correspond to the order in which they appear in the data matrix.

MORPHOMETRIC OSTEOLOGICAL CHARACTERS

Cranial

1. Female maximum skull width. Size corrected by regression with skull length.
2. Width of female maxillary triturating surface (McDowell, 1964). Size corrected by regression with skull length.
3. Width of female postorbital arch (Minx, 1996), measured midway between dorsal origin of postorbital arch and contact with zygomatic arch (or 1/4 of the way from dorsal origin to ventral origin if zygomatic arch absent). Size corrected by regression with skull length.
4. Length of female supraoccipital crest, measured from posterior margin of occipital condyle to posterior tip of supraoccipital crest. Size corrected by regression with skull length.
5. Width of female zygomatic arch, measured at narrowest point. Size corrected by regression with skull length.
6. Female skull length, measured between anterior tip of skull and posterior margin of occipital condyle. Size corrected by regression with carapace length
7. Male maximum skull width. Size corrected by regression with skull length.
8. Width of male maxillary triturating surface (McDowell, 1964). Size corrected by regression with skull length.
9. Width of male postorbital arch (Minx, 1996), measured midway between dorsal origin and contact with zygomatic arch (or 1/4 of the way from dorsal origin to ventral origin if zygomatic arch absent). Size corrected by regression with skull length.
10. Length of male supraoccipital crest, measured from posterior margin of occipital condyle to posterior

tip of supraoccipital crest. Size corrected by regression with skull length.
11. Width of male zygomatic arch, measured at narrowest point. Size corrected by regression with skull length.
12. Male skull length, measured between anterior tip of skull and posterior margin of occipital condyle. Size corrected by regression with carapace length.

Postcranial
13. Length of acromion process of scapula divided by length of scapular blade (Minx, 1996). Length of each process was measured from medial surface of glenoid fossa to distal end of process.

Meristic and qualitative osteological characters

14. Ventromedial surface of palate: flat (0), or depressed (1).
15. Contact of vomer with pterygoids: vomer does not come to a distinct point at contact with pterygoids on ventral surface of palate, suture is broadly rounded at contact or forms jagged horizontal line (0), vomer tapers to a single point at contact with pterygoids, and often flared just anterior to contact (1), vomer bifurcate at contact with pterygoids (2), vomer trifurcate (3), vomer with four distinct points (4), vomer with five distinct points (5).
16. Vomer–pterygoid contact occurs: at (0), or anterior to posterior border of palate (1).
17. Foramen palatinum posterius (Burke *et al*., 1996): narrower than or equal to (0), or wider than palatine process of vomer (1). In the latter case the foramen usually consists of a large opening defined by a thin membranous palatine.
18. Foramen palatinum posterius: occurs at bottom of deep furrow formed by posteroventral projections of palatine and maxilla (0), or is clearly visible (1). In the latter case the foramen occurs in a flat surface of bone or in a shallow depression.
19. Foramen orbito-nasal: equal in size to or smaller than palatine process of vomer (0), or large opening with width greater than or equal to that of palatine process of vomer (1).
20. Foramen orbito-nasal: not bisected, only one opening present (0), or bisected partially or completely by thin process of palatine (1).
21. Shape of foramen orbito-nasal: elongate anteriorly and posteriorly, diameter of longitudinal axis two or more times diameter of perpendicular axis (0), or rounded with length and width subequal (1).
22. Foramen palatinum posterius (Gaffney & Meylan, 1988): larger than (0), or smaller or same size as foramen orbito-nasal (1).
23. Pterygoid (Gaffney & Meylan, 1988): does not contact foramen palatinum posterius (0), or contacts foramen palatinum posterius and forms part of posterior border (1).
24. Palatine (Gaffney & Meylan, 1988): excluded entirely from triturating surface of skull (0), or not excluded from triturating surface (1).
25. Vomer (McDowell, 1964): does not contribute to (0), or contributes to triturating surface (1).
26. Median maxillary ridge (Gaffney & Meylan, 1988): absent (0), or present (1).
27. Concavities medial to maxillary ridge (Gaffney & Meylan, 1988): absent (0), or present (1).
28. Number of concavities medial to maxillary ridge (Gaffney & Meylan, 1988): one (0), or two (1).
29. Anteroventral border of premaxilla: smooth (0), smooth but with notch (1), notched, notch defined by two cusps (2), or hooked beak (3).
30. Foramina praepalatinum (McDowell, 1964): exposed ventrally (0), or not visible ventrally (1).
31. Posteroventral surface of upper jaw: not serrated (0), or serrated (1).
32. Foramen carotico-pharyngeale (Gaffney & Meylan, 1988), foramen located on the ventromedial pterygoid surface: absent (0), or present (1).
33. Foramen carotico-pharyngeale: large, easily seen with naked eye, subdivisions of foramen visible just below surface (0), or small, barely visible to naked eye, subdivisions not visible (1).
34. Foramen carotico-pharyngeale (McDowell, 1964): contacts (0), or does not contact pterygoid-basisphenoid suture (1).
35. Foramen carotico-pharyngeale, when not contacting pterygoid-basisphenoid suture: is not (0), or is connected to basisphenoid-pterygoid suture by short (pterygoid-pterygoid) suture (1).
36. Depression in pterygoid just lateral to basisphenoid: absent (0), or present (1). When such a depression is present the foramen carotico-pharyngeale usually occurs in the wall of the depression.
37. Lateral edges of rostral projection of basisphenoid (Killebrew, 1979), in ventral view: convex (0), concave (1), convex posteriorly, concave anteriorly (2), or straight (3).
38. 'Wings' on rostral projection of basisphenoid, anterolateral processes of basisphenoid that often contact foramen carotico-pharyngeale: absent (0), or present (1).
39. Anterior tip of basisphenoid: acute (0), or rounded (1).
40. Foramen at anterior tip of basisphenoid: absent (0), or present (1). When such a foramen is present, the previous character (i.e. 39) usually cannot be scored because the tip of the basisphenoid is not fully ossified.
41. Basisphenoid-basioccipital suture (Bertl & Killebrew, 1983): straight (0), curved anteriorly (1), or

straight medially, but lateral edges sloped posteriorly (2).
42. Basisphenoid-basioccipital suture: not notched (0), or with medial notch (1).
43. Basioccipital process of basisphenoid, small posteromedial projection of basisphenoid: absent (0), or present (1).
44. Lateral edge of basisphenoid: forms simple two-sided corner with posterior edge of basisphenoid (0), or three-sided corner with posterior edge of basisphenoid (1).
45. Tuberculum basioccipitale: absent (0), or present (1).
46. Tuberculum basioccipitale: directed posteriorly (0), or ventrally (1).
47. Contact of pterygoid with basioccipital (Gaffney & Meylan, 1988): absent (0), or present (1).
48. Contact of pterygoid with exoccipital (McDowell, 1964): absent (0), or present (1).
49. Posteriorly directed process of premaxilla in nasal chamber (Killebrew, 1979): absent (0), or present (1). When present, the process is visible through the fossa nasalis.
50. Lateral edges of prefrontal (Burke *et al*., 1996), in dorsal view: taper anteriorly (0), or not tapered, lateral edges parallel or form hourglass shape (1).
51. Minimum interorbital distance (McDowell, 1964): wider than nasal chamber (0), or narrower than nasal chamber (1).
52. Prefrontal process of frontal (Bertl & Killebrew, 1983): absent (0), or present (1).
53. Anterior termination of prefrontal process of frontal, when prefrontal process present: blunt or rounded (0), or acute (1).
54. Frontal (McDowell, 1964), in dorsal view: reaches orbital margin (0), or does not contact orbital margin (1).
55. Jugal (Bertl & Killebrew, 1983), in lateral view: does not reach orbit (0), or contributes to orbit (1).
56. Dorsal surface of supraoccipital crest in lateral view: rounded (0), straight (forming continuous line) along more than 3/4 of length (1), or peaked such that both anterior and posterior halves of the supraoccipital crest are straight, but the posterior half slopes ventrally at an angle from anterior half (2).
57. Ventral slope of supraoccipital crest, when crest is sloped: begins anterior to supraoccipital-parietal suture (0), begins at supraoccipital-parietal suture (1), or begins posterior to supraoccipital-parietal suture (2).
58. Posterior termination of supraoccipital crest: rounded (0), or acute to subacute (1).
59. Zygomatic arch (Minx, 1996): absent (0), or present (1).
60. Quadratojugal: absent (0), or present (1).
61. Quadratojugal, when present: reduced, contacting quadrate but not to jugal (0), or contacts both jugal and quadrate (1).
62. Crista praetemporalis, dorso-ventral ridge along the posterior margin of the fossa temporalis, near parietal-prootic suture (McDowell, 1964): small to absent (0), or present and consisting of heavy, enlarged area of spongy bone, with width 1/2 or more that of the posterior margin of fossa temporalis (1). After preliminary analysis the character 'presence or absence of the crista praetemporalis' was discarded because it showed approximately the same pattern of inter-taxon variation but was more difficult to define unambiguously.
63. Anterior border of processus inferior parietalis (McDowell, 1964): thin (0), or thick, width at least one quarter the distance between posterior margins of right and left interorbital foramina (1).
64. Parietal-palatine contact (McDowell, 1964): absent, elements separated by pterygoid (0), or contact occurs (1).
65. Maxilla (McDowell, 1964): separated from quadratojugal by jugal (0), or has posterior process that contacts quadratojugal (1). Our description of this character follows McDowell (1964) except that the element we refer to as the quadratojugal (following Gaffney, 1979) was described and figured as the squamosal by McDowell (1964).
66. Jugal (Gaffney & Meylan, 1988): does not contact palatine, jugal cut off from palatine by maxilla (0), or contacts palatine (1).
67. Epipterygoid (Gaffney & Meylan, 1988): does not contact (0), or contacts jugal at posterior of palate (1).
68. Jugal (McDowell, 1964): does not contact pterygoid (0), or contacts pterygoid at posterior of palate in area of fossa temporalis (1).
69. Pterygoid (Bertl & Killebrew, 1983): does not contribute to ventral border of foramen nervi trigemini (f.n.t.), dorsal projection of pterygoid separated from f.n.t. by anterior projection of quadrate (processus epipterygoideus) and posterior projection of epipterygoid and/or parietal (0), or contributes to ventral border of f.n.t. (1).
70. Apex of lower jaw (Seidel & Palmer, 1991): angled (0), or rounded (1).
71. Anterior margin of dentary (McDowell, 1964), in lateral view: rounded (0), or forms 90° angle ventrally with ventral margin of dentary (1).
72. Ventromedial surface of dentary in anterior view (McDowell, 1964): rounded (0), or flattened (1).
73. Lower jaw: not serrated (0), or serrated (1).
74. Lower jaw: not hooked (0), or hooked (1).
75. Triturating surface of dentary: not spatulate (0), or broad and spatulate (1).
76. Edge of triturating surface of dentary (McDowell, 1964): with distinct lingual border, a sharp

angularity setting off the horizontal triturating surface from the medial surface of the dentary (0), or lacks distinct lingual border, and slopes gradually towards the vertical medial face of the dentary (1).
77. Lower triturating surface of dentary (McDowell, 1964): in dorsal view not sharply defined anteromedially, width of anteromedial and lateral triturating surfaces roughly equal (0), or sharply defined anteromedially, width of anteromedial triturating surface at least twice width of lateral triturating surface (1).
78. Ridge of median lower triturating surface of dentary (McDowell, 1964): absent (0), or present and separate from and lateral to lingual ridge (1).
79. Dorsal projection of angular (Gaffney & Meylan, 1988): contacts (0), or does not contact Meckel's cartilage (1).
80. Processus coronoideus (Bertl & Killebrew, 1983): not hooked (0), or hooked (1).

Postcranial
81. Carapace-plastral connection (Burke *et al*., 1996): ligamentous (0), or bony (1).
82. Plastral buttresses (Burke *et al*., 1996): absent (0), or present, with axillary and inguinal buttresses (dorsal processes of plastron) contacting carapace (1).
83. Peripherals (Minx, 1996): not thickened (0), or with lateral edges swollen to form lip (1).
84. Number of sides of neural I (Minx, 1996).
85. Number of sides of neural II (Minx, 1996).
86. Number of sides of neural III (Minx, 1996).
87. Number of sides of neural IV (Minx, 1996).
88. Number of sides of neural V (Minx, 1996).
89. Number of sides of neural VI (Minx, 1996).
90. Neural VII absent (0) or present (1).
91. Number of sides of neural VII (Minx, 1996).
92. Neural VIII absent (0) or present (1).
93. Number of sides of neural VIII (Minx, 1996).
94. Inward depression in the posterior half of the fourth costal (Minx, 1996): absent (0), or present (1).
95. Number of sides of posterior suprapygal (Minx, 1996).
96. Suprapygals: separated from neurals by last pair of costals (0), or contacting neurals (1).
97. Number of suprapygals.
98. Anterior epiplastral margin (Seidel, 1994) underlying gular scutes: not swollen (0), or swollen to form lip (1).
99. Epiplastra: with smooth lateral margins (0), or each bearing a tooth-like swelling on dorsal surface at margin of gular and humeral scutes (1).
100. Anterior epiplastral margin (Seidel, 1994), in ventral view: straight (0), curved anteromedially and usually forming smooth line with rest of epiplastral margin (1), or curved and bearing shallow medial cleft, having an appearance similar to the top of a 'heart' symbol (2).
101. Anterior epiplastral margin underlying gular scutes, in anterior view: straight and flat (0), or curved dorsally at margins (1).
102. Entoplastron: absent (0), or present (1).
103. Number of sides of entoplastron (Seidel & Miranda, 1984), in ventral view.
104. Entoplastron: extended anteriorly, majority of element is anterior to point of greatest width (0), anterior and posterior halves of entoplastron equal (1), or entoplastron extended posteriorly, majority of element posterior to point of greatest width (2).
105. Number of phalanges of manal digit I.
106. Number of phalanges of manal digit II.
107. Number of phalanges of manal digit III.
108. Number of phalanges of manal digit IV.
109. Number of phalanges of manal digit V.
110. Number of phalanges of pedal digit I.
111. Number of phalanges of pedal digit II.
112. Number of phalanges of pedal digit III.
113. Number of phalanges of pedal digit IV.
114. Number of phalanges of pedal digit V.
115. Suprascapula (Burke *et al*., 1996): absent (0), or present (1).
116. Episcapula (Burke *et al*., 1996): absent (0), or present (1).
117. Cervical vertebrae (McDowell, 1964): not elongate (0), or elongate (1).
118. Vertebra VIII (McDowell, 1964): shorter than vertebrae II–VII (0), or vertebrae all equal in length (1).
119. Articulation of cervical V and VI (Gaffney & Meylan, 1988): double (0), or single (1).
120. Thoracic rib heads (McDowell, 1964): straight, relatively short and thick (0), long, slender, and bowed ventrally (1), or long and slender but not bowed ventrally (2).
121. Epipubes (Gaffney & Meylan, 1988): not ossified, cartilaginous (0), or at least partially ossified (1).
122. Opening in pelvis: single (0), or two openings present, anterior and posterior halves of pelvis contact medially (1).
123. Seams between right and left halves of pelvis: visible (0), or right and left half of pelvis completely fused, seams no longer visible ventrally (1).

Morphometric characters of external morphology

124. Maximum female plastron length. Size corrected by regression with carapace length.
125. Width of female carapace along midline. Size corrected by regression with carapace length.
126. Maximum male plastron length. Size corrected by regression with carapace length.

127. Width of male carapace along midline. Size corrected by regression with carapace length.
128. Width of cervical scute. Size corrected by regression with cervical scute length.
129. Length of intergular seam. Size corrected by regression with plastron length.
130. Length of interhumeral seam. Size corrected by regression with plastron length.
131. Length of interpectoral seam. Size corrected by regression with plastron length.
132. Length of interabdominal seam. Size corrected by regression with plastron length.
133. Length of interfemoral seam (Seidel, 1994). Size corrected by regression with plastron length.
134. Length of interanal seam. Size corrected by regression with plastron length.

Meristic and qualitative characters of external morphology

Head morphology

135. Snout, in lateral view: does not extend anterior to rest of head (0), or extends anterior to rest of head (1).
136. Snout, in dorsal view (Minx, 1996): rounded (0), or squared, with three distinct sides (1).
137. Nostrils: round, length and width equal (0), or oval (1).
138. Orientation of nostrils, if oval, in anterior view: horizontal (0), vertical (1), or diagonal (2). This character was scored as unknown for specimens with round nostrils.

Head colouration

139. Background colour of head and neck: monotone (0), or two tone, one colour dorsally, another ventrally (1).
140. Markings on head and neck: absent, although head may be different colour from neck (0), or present, including stripes, spots, specks, or blotches present on head and or neck (1).
141. Markings on head and neck (Seidel, 1981), if present: consist of stripes or curved lines, rarely in combination with specks or spots (0), or spots or speckles, with no lines present (1).
142. Number of stripes contacting orbit. Scored as unknown for specimens lacking head and neck stripes.
143. Number of stripes (Seidel & Palmer, 1991) between eyes on dorsal surface of head. This character was scored as unknown for specimens lacking head and neck stripes.
144. Light coloured markings resembling hairpins: absent (0), or present on dorsal surface of head (1). For illustration see Conant & Collins (1998 : 52, fig. 8). This character was scored as unknown for specimens lacking head and neck stripes.
145. Arrow shaped mark on dorsal surface of snout: absent (0), or present (1). This character was scored as unknown for specimens lacking head and neck stripes.
146. Postorbital mark, a large distinct mark posterior to orbit on lateral surface of head or neck, often bordering orbit: absent (0), or present (1). In the case of turtles with prominent head stripes such a mark will be at least twice the thickness of other head and neck stripes and/or of a different colour.
147. Orientation of postorbital mark, if present: horizontal (0), or vertical (1).
148. Number of postorbital markings, if present.
149. Postorbital mark, if present: does not contact (0), or contacts orbit (1).
150. Postorbital mark: isolated from neck stripes (0), or contacts neck stripes (1). Scored as unknown in specimens that did not have both neck stripes and at least one postorbital mark.
151. Middorsal mark, large triangular mark covering dorsal surface of skull from interorbital region to tip of snout: absent (0), or present (1).
152. Middorsal mark: does not contact (0), or contacts postorbital mark (1). Scored as unknown in specimens that did not have both a middorsal mark and at least one postorbital mark.
153. Mandibular stripe, a large distinct stripe running along lower jaw distinct from and at least twice the thickness of other stripes on lower jaw: absent (0), or present (1).
154. Mandibular stripe, when present: not forked (0), or forked anteriorly (1).
155. Mandibular stripe (Legler, 1990), when present: does not contact (0), or contacts one or more neck stripes (1).

Shell morphology

156. Overall shape of carapace (Minx, 1996), in dorsal external view: circular (0), oval, sometimes expanded posteriorly (1), or with slightly concave lateral edges (2).
157. Carapace shape in lateral view (Seidel & Miranda, 1984): domed, having distinct 'highest point' (0), or flattened, no distinct highest point (1).
158. Location of highest point of carapace, when present: anterior to midline (0), at midline (1), or posterior to midline (2).
159. Growth annuli of scutes of carapace (Minx, 1996): distinct (0), or not visible (1).
160. Growth point of scutes of carapace, centre of growth annuli: in middle of each scute (0), or along posterior margin of each scute (1). This character was scored as unknown when growth annuli were not visible on scutes of carapace.

161. Pleural scutes: thick, each with raised point (0), or thin, lacking raised points (1).
162. Longitudinal ridges (Burke *et al*., 1996): present on scutes of carapace (0), or scutes smooth, not bearing ridges apart from growth rings (1). If such ridges are present in combination with distinct growth rings, the ridges may be vermiculate rather than continuous.
163. Outline of neurals: not visible (0), or visible through dorsal scutes of carapace (1).
164. Anterior marginals of carapace: not serrate (0), or serrate (1).
165. Borders between all marginals posterior of contact of the bridge (connection between carapace and plastron): smooth (0), or some or all notched (1).
166. Borders between marginals anterior to bridge: smooth (0), or some or all notched (1).
167. Number of most posterior marginal bearing a notched posterior border among marginals anterior of bridge. In specimens where the border between all marginals anterior of the bridge was smooth, this character was scored as unknown. Marginals were numbered from anterior to posterior, following Zangerl (1969).
168. Posterior marginals, median notch: absent (0), or present (1). Median notches generally occur in addition to notches at the borders of posterior marginals.
169. Markings on dorsal surface of marginals: absent and marginals monotone (0), or some marking (spots, blotches, or lines) found on marginals (1). These markings can be darker or lighter than the background colour, depending upon the species.
170. Markings on dorsal surface of marginals, if present, consist of: single dark spot at posterolateral margin of each marginal (0), dorsally facing C-shaped markings (1), vertical lines, sometimes bifurcated dorsally to form Y- or T-shape (2), irregular dark blotches (3), ventrally facing C-shaped markings (3), posterolateral facing C-shaped markings (4), posterolateral facing C-shaped mark with dark blotch at posterolateral corner of each marginal (6), single light spot in the centre of each marginal (7), pattern of spots and lines radiating from one point on each marginal (8), or many small spots or specks found on each scute (9). Emydids display a bewildering diversity of marginal markings as a group. In order to deal with this variation in a conservative manner assumptions about the independence and homology of the various types of markings were minimized (i.e. type of marginal markings was treated as a single unordered character) and the states of this character were chosen such that only specimens with very similar marginal markings would receive the same score.
171. Pleural scutes: bearing no distinct markings (0), or marked (1).
172. Markings on second pleural scute (Seidel & Palmer, 1991), if present, consist of: multiple spots or speckles only (0), pattern of curving lines, reticulate pattern, or multiple ocelli (1), large isolated ring shaped mark or large isolated blotch that does not contact edges of scute (2), spots or blotches dorsally, vertical lines ventrally, often forming radiating pattern (3), concentric series of ring shaped markings (4), many equally distinct vertically orientated lines, sometimes bifurcated dorsally (5), one pronounced vertical line, sometimes with a few smaller vertical lines, usually bifurcated ventrally and forming upside-down 'Y' (6), single light spot (7). As with marginals' markings, emydid display considerable diversity in pleural scute markings. This variation was dealt with in a similar manner, by coding pleural scute markings as a single unordered character with states chosen such that only specimens with very similar markings would receive the same score.
173. Bright yellow colouration of seams of carapace: absent (0), or present (1).
174. Median keel along dorsal midline of carapace (Burke *et al*., 1996): absent (0), present (1).
175. Keel, when present: primarily located on anterior half of carapace (0), distributed equally on anterior and posterior halves of carapace (1), or primarily located on posterior half of carapace (2).
176. Keel, when present, consists of: single ridge (0), apically blunt knobs (1), or apically acute serrations (2).
177. Height of keel elements, when present: less than 10% length of scutes on which they occur (0), or more than 17% length of scutes on which they occur (1).
178. Coloration of keel, when keel present: darker than that of surrounding carapace (0), not distinct (1), or lighter than that of surrounding carapace (2).
179. First vertebral scute (Seidel, 1994): not constricted, edges relatively straight (0), constricted anteriorly (1), or constricted at mid-length, forming hour-glass shape (2).
180. First marginal (Minx, 1996): long and narrow, maximum length exceeds maximum width (0), square, width and length roughly equal (1), or short and wide, width exceeds length (2).
181. Posteriormost marginals: form smooth horizontal line with marginals lateral to them (0), or higher than marginals just lateral to them (1).
182. Orientation of edge of posterior row of marginals: posteroventral, not flared (0), or flared posteriorly or posterodorsally out and up to form lip (1).
183. Marginal or marginals contacted by seam A (Tinkle, 1962), seam between vertebral I and pleural scute I. The score for this character indicates which marginal is contacted by seam A and whether the contact is in the anterior half, posterior half, or middle of the marginal or, if the contact happens to occur at the border between two marginals, between which marginals the contact occurs.

184. Marginal or marginals contacted by seam B (Tinkle, 1962), seam between pleural scutes I and II.
185. Marginal or marginals contacted by seam C (Tinkle, 1962), seam between pleural scutes II and III.
186. Marginal or marginals contacted by seam D (Tinkle, 1962), seam between pleural scutes III and IV.
187. Marginal or marginals contacted by seam E (Tinkle, 1962), seam between pleural scutes V and VI.
188. Number of marginals (Tinkle, 1962) on each side of carapace.
189. Number of plastral hinges: none (0), one (1), or two (2). Note that in all specimens that bear a single plastral hinge, the hinge is present in the anterior half of the carapace.
190. Posterior plastron (Burke *et al*., 1996): not closeable (0), or closeable (1).
191. Anterior plastron (Burke *et al*., 1996): not closeable (0), or closeable (1).
192. Seams of plastral bones: not visible (0), or visible through plastral scutes (1).
193. Seams of plastral scutes (Minx, 1996): not darker than scutes (0), or darker than scutes (1).
194. Markings on plastron: absent, scutes of plastron monotone (0), or present (1).
195. Markings on plastron, when present: consist of dark markings on a light background (0), or light markings on a dark background (1).
196. Markings of plastron, when present: located on lateral edges of plastron (0), in the middle of individual plastral scutes, not contacting edges (1), along central axis of plastron, spreading along seams of plastron (2), or occur in at least two of the above locations (3). Within the species scored as '3', some individuals would exhibit a combination of 1 and 2 while other individuals of the same species would exhibit a combination of 0, 1, and 2. Thus, the various specimens scored as 3 seemed to be exhibiting homologous states of this character.
197. Plastral markings, when present (Seidel & Palmer, 1991): consist of a single dark figure (0), or isolated markings that are not interconnected (1).
198. Male plastron (Minx, 1996): flat (0), or bearing concavity (1).
199. Concavity of male plastron, when present: restricted to posterior half of plastron (0), or extends along entire length of plastron (1).
200. Gulars, in ventral view: flush with anterior margin of humerals (0), or extend anteriorly to margin of humerals (1).
201. Humoral-pectoral seam (Gaffney & Meylan, 1988): does not contact or is posterior to (0), or contacts entoplastron (1).
202. Contour of pectoral-abdominal seam (Gaffney & Meylan, 1988): horizontal (0), or sloped posteromedially to approach abdomino-femoral seam (1).
203. Contour of anterior apex of femoral-anal seam: acute (0), or smooth curving line (1).
204. Notch at lateral edge of femoral-anal seams: absent (0), or present (1).
205. Posteromedial margin of plastron (Seidel, 1994): curved anteromedially (0), consists of deep V shaped indentation between posterior anals (1), forms a horizontal line (2), or rounded posteriorly (3).
206. Anterior margin of plastron: not serrate (0), or serrate (1).
207. Posterior margin of plastron: not serrate (0), or serrate (1).
208. Inguinal scute (Minx, 1996): absent (0), or present (1).
209. Number of posteriormost marginal (Seidel, 1994) that contacts inguinal scute. Marginals were numbered from anterior to posterior.
210. Inguinal scute (Minx, 1996): with smooth surface, growth rings not visible (0), or with visible growth rings (1).
211. Inguinal scute (Seidel, 1994): unmarked (0), or bearing black markings (1).
212. Apical scale (Minx, 1996): absent (0), or present (1).
213. Apical scale (Minx, 1996), when present: cornified with visible growth rings (0), or not cornified and with no visible growth rings (1). In the latter case the apical scale is small and elongate.
214. Axillary scute (Minx, 1996): absent (0), or present (1).
215. Number of anteriormost marginal scute (Minx, 1996) that contacts the posterior margin of the axillary scute. Marginals were numbered from anterior to posterior.
216. Bridge of plastron, a dorsal extension of the plastron that is visible externally and contacts the carapace: absent (0), or present (1). Note that when the plastral bridge is absent, reduced plastral buttresses may or may not be present internally.
217. Markings on bridge: absent (0), or present, consisting of an elongate black blotch or thick black line (1).
218. Dark markings on underside of some or all marginals: absent (0), or present (1).
219. Dark markings under marginals, if present: occur on all marginals (0), on marginals near bridge only (1), or on all marginals near bridge and anterior to bridge (2).
220. Dark markings under marginals, when present, consist of: irregular blotches (0), solid dark circles (1), dark circles with light area inside them or a dark ring (2), dorsally orientated C-shaped mark (3), dark spots and irregular reticulate lines (4), posteriorly orien-

tated C-shaped mark (5), light centred circles in the middle of each scute in addition to dark scute borders (6), or single dark lines at the posterior and ventral margin of each scute (7).

Limb morphology

221. Claws on manus of adult male (Seidel & Miranda, 1984): all same size as or only slightly longer than adjacent claws (0), or elongate, some more than twice as long as some adjacent claws (1). In the latter case digits II and III or II, III and IV will bear elongate claws.
222. Claws on manus of males (Seidel & Miranda, 1984): curved (0), or straight (1).
223. Digits of male manus bearing elongate claws: II and III (0), or II, III, and IV (1).
224. Number of emergent hind claws (Minx, 1996): four (0), or three (1).
225. Hind foot webbing (Burke *et al*., 1996): absent or extending only to proximal margin of claws (0), or extending nearly to distal end of claws (1).
226. Clasping claws (Minx, 1996), thick recurved claws on pes of males that can be flexed independently of the other pedal digits: absent (0), or present (1).
227. Scales on the forelimbs (Minx, 1996): relatively flat (0), or convex, protruding outward and appearing bulbous or rugose (1).
228. Color of scales on forelimbs: same as that of skin on forelegs (0), or different from colour of skin on forelegs (1).
229. Scales on outer edge of forelimbs: serrate (0), or not serrate (1).
230. Stripes on forelimbs: absent (0), or present (1).
231. Number of stripes on forelimb, counted at wrist.
232. Posterior surface of hindlimb: unmarked (0), or marked with stripes or spots (1).
233. Markings on posterior surface of hindlimbs, when present: consist of spots (0), horizontal stripes (1), or vertical stripes (2).

Non-sequence characters scored from literature reports

234. Myoglobin electromorph (Seidel & Adkins, 1987): with isoelectric point, pI, 6.8 (0), or pI 6.9 (1).
235. Liver protein electromorph (Seidel, 1994): pI 8.2 (0), or pI 8.4 (1).
236. Fast anodal electromorph for glycerol-3-phosphate dehydrogenase (Seidel, 1988): absent (0), or present (1).
237. Dark-coloured iris stripe: absent (0), or present (1). Scored based upon photographs of living individuals in Ernst & Barbour (1989) and Ernst *et al*. (1994).
238. Egg shell (Ewert, 1979): hard (0), or soft (1).
239. Sex determination (Ewert & Nelson, 1991): temperature dependent (0), or chromosomal (1).
240. Sulcus that divides pleca media of penis (Zug, 1966) into lateral and medial fold: absent (0), or present (1).
241. Pleca media (Zug, 1966): round (0), or spade or diamond shaped (1).
242. Plica externa (Zug, 1966): not reduced (0), or reduced (1).
243. 'Circular elevation' on penis (Zug, 1966): absent (0), or present, occurring distally on the enlarged ends of the plica media (1).

Molecular sequence data

244–802. Mitochondrial ribosomal (large subunit), 16S ribosomal DNA sequences (Bickham *et al*., 1996).
803–1147. Mitochondrial control region DNA sequences (Lamb *et al*., 1994 and Mindell *et al*., 1999).
1148–2345. Mitochondrial cytochrome *b* DNA sequences (Lamb *et al*., 1994; Shaffer *et al*., 1997; Lenk *et al*., 1999; Mindell *et al*., 1999; Feldman & Parham, 2002).
2346–3240. Mitochondrial ND4 DNA sequences (Feldman & Parham, 2002).

Behavioural characters

3241. Forelimb titillation during mating (Seidel & Fritz, 1997): absent (0), or present (1).
3242. Position of male during forelimb titillation (Seidel & Fritz, 1997), when titilation present: below female (0), or above female (1).