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When Are Phylogenetic Analyses Misled by Convergence? A Case Study in Texas Cave Salamanders

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Abstract.— Convergence, i.e., similarity between organisms that is not the direct result of shared phylogenetic history (and that may instead result from independent adaptations to similar environments), is a fundamental issue that lies at the interface of systematics and evolutionary biology. Although convergence is often cited as an important problem in morphological phylogenetics, there have been few well-documented examples of strongly supported and misleading phylogenetic estimates that result from adaptive convergence in morphology. In this article, we propose criteria that can be used to infer whether or not a phylogenetic analysis has been misled by convergence. We then apply these criteria in a study of central Texas cave salamanders (genus *Eurycea*). Morphological characters (apparently related to cave-dwelling habitat use) support a clade uniting the species *E. rathbuni* and *E. tridentifera*, whereas mitochondrial DNA sequences and allozyme data show that these two species are not closely related. We suggest that a likely explanation for the paucity of examples of strongly misleading morphological convergence is that the conditions under which adaptive convergence is most likely to produce strongly misleading results are limited. Specifically, convergence is most likely to be problematic in groups (such as the central Texas *Eurycea*) in which most species are morphologically very similar and some of the species have invaded and adapted to a novel selective environment. [Adaptation; convergence; *Eurycea*; homoplasy; molecular systematics; morphology; phylogeny; salamanders.]

Convergence is one of the oldest and most important issues in phylogeny reconstruction (Darwin, 1859; Hennig, 1966), and one that lies at the intersection of the fields of systematics and evolutionary biology. Although there is considerable debate over the exact definition of convergence (and the related term, parallelism), there seems to be universal agreement that convergence is similarity between organisms that is not due to common ancestry, with the frequent implication that this similarity is caused by adaptive evolution. Convergence is important to evolutionary biology because convergent evolution facilitates testing hypotheses of adaptation using statistical comparative methods (e.g., Harvey and Pagel, 1991; Larson and Losos, 1996; Martins, 1996, 2000). Convergence is a critical issue in systematics because it can potentially mislead phylogeny reconstruction methods, for example, causing analyses to group distantly related organisms that share similar habitats.

Morphological data sometimes are thought to be particularly prone to adaptive convergence, or at least much more so than molecular data (e.g., Sibley and Ahlquist, 1987; Sytsma et al., 1991; Hedges and Sibley, 1994; Hedges and Maxson, 1996; Givnish and Sytsma, 1997). The possibility of convergence in morphological phylogenetic analyses is often invoked to explain conflicts between trees derived from molecular and morphological data (e.g., Hedges and Sibley, 1994; Hollar and Springer, 1997; McCracken and Sheldon, 1998; McCracken et al., 1999; Teeling et al., 2002). More generally, convergence is sometimes used as a reason to reject morphological data in favor of molecular data for reconstructing phylogenies (e.g., Hedges and Maxson, 1996; Givnish and Sytsma, 1997). The idea that morphological data are highly susceptible to convergent evolution makes intuitive sense. Morphological characters may interact with the environment more directly and frequently than most molecular characters, which are often assumed to be selectively neutral or nearly so (e.g., Kimura, 1983; Hedges and Sibley, 1994; but see Gillespie, 1991, and others). Numerous examples exist of natural selection on morphology (e.g., peppered moths, Darwin's finches) and of overall phenotypic similarity between distantly related organisms sharing the same way of life, such as ichthyosaurs and dolphins (Futuyma, 1998). Yet, morphology has been used widely for phylogeny reconstruction for decades, and many molecular studies have merely confirmed groupings already established by morphologists. In fact, much of modern systematic theory was developed based almost exclusively on morphological data (Hennig, 1966; Wiley, 1981). A fundamental assumption of phylogenetic systematics (at least using parsimony) is that convergence is rare enough to be ignored a priori (Hennig, 1966; Wiley, 1981). Is there evidence that convergence can cause morphological analyses to give strongly misleading results? There are studies in which subsets of the total morphological data seem to yield erroneous results caused by convergence (e.g., Trueb and Cloutier, 1991; Quicke and Belshaw, 1999), but much more troubling is the possibility that the entire morphological data set may produce an incorrect tree, as has been suggested by some authors (e.g., Hedges and Sibley, 1994; McCracken et al., 1999). At present, it is unclear how often morphological analyses are misled by convergence (if ever) or even how one determines if this has been the case.

In this article, we (1) briefly review the debate over the definition of convergence and give our working terminology, (2) provide explicit criteria for inferring when convergence has led to an erroneous phylogenetic

conclusion, (3) describe a case study in which a phylogenetic analysis of morphological data appears to have been misled by convergence, and (4) discuss the paucity of well-documented cases of this type in the literature.

Defining Convergence

Despite the importance of convergence to systematics and evolutionary biology, there is surprisingly little consensus in the literature as to what exactly convergence is. Much of the discordance among authors involves attempts to distinguish convergence from a related term, parallelism. Both terms describe the repeated acquisition of similar traits in different lineages. Here, we briefly review the debate to arrive at a working definition.

Patterson (1988) summarized much of the previous controversy over the definitions of convergence and parallelism and provided his own definitions. Patterson (see also Larson and Losos, 1996; Kitching et al., 1998) defined convergence as a type of nonhomologous similarity that involves lack of true similarity of the characters in question (i.e., they fail the similarity test of homology) coupled with incongruence of these characters with others in the analysis (i.e., they fail the congruence test of homology). He defined parallelism as nonhomologous similarity in which the characters pass the similarity test but fail the congruence test. Although Patterson (1988:609) considered both convergence and parallelism to be forms of homoplasy, one implication of his definitions is that convergence should be detectable prior to a phylogenetic analysis, whereas parallelism can only be detected after the analysis. Only the latter definition is consistent with standard notions of homoplasy.

A prevalent and long-standing view in the literature is that convergence involves the acquisition of the same state in separate lineages, where the convergent state arises from different antecedent states (e.g., in lineage A state 2 arises from state 0, whereas in the unrelated lineage D, state 2 arises from state 1). In cases of parallelism, the parallel state evolves from the same primitive state in each instance (i.e., state 2 arises from state 1 in both lineages A and D). This definition is widespread (e.g., Hennig, 1966; Gosliner and Ghiselin, 1984; Wake, 1991; McShea, 1996; Parra-Olea and Wake, 2001) and has been used in major textbooks on phylogenetics (Wiley, 1981), evolutionary biology (Futuyma, 1998), and molecular evolution (Li, 1997). Similarly, some authors (e.g., Simpson, 1961) have also distinguished parallelism and convergence in terms of the genetic or developmental mechanism that gives rise to the similar traits in different lineages—parallelism involves the same mechanism in each lineage, and convergence involves different mechanisms. Another group of authors (e.g., Mayr, 1969; Brooks, 1996) have argued that parallelism and convergence differ in the degree of relatedness of the taxa that acquire the independently evolved states, whether closely related (parallelism) or distantly related (convergence). These definitions are not entirely dissimilar and may even overlap to some extent.

In contrast to these authors, many recent phylogenetic workers have simply treated convergence as a type of homoplasy involving the repeated gain of the same character state(s) in different lineages, with the frequent assumption that these gains are caused by adaptation to a similar selective environment (e.g., Doolittle, 1994; Hedges and Sibley, 1994; Hedges and Maxson, 1996; Bull et al., 1997; McCracken et al., 1999; Quicke and Belshaw, 1999; Hillis and Wiens, 2000). These authors have not been concerned with the antecedent state, the developmental or genetic mechanisms, or the degree of relatedness among lineages, and many have used parallelism as synonymous with convergence (or else have not addressed the differences between these terms).

In summary, a dichotomy has developed between how convergence is usually defined and how the term is used in the recent phylogenetics literature. Researchers in phylogenetics frequently use convergence as a general term for homoplastic gains of a derived state, particularly when these gains are adaptive (e.g., Hedges and Sibley, 1994; Bull et al., 1997; McCracken et al., 1999; Quicke and Belshaw, 1999). In contrast, explicit definitions of the term have emphasized more specific criteria, such as antecedent states, developmental and genetic mechanisms, and degree of relatedness of the taxa that have gained these traits. Here, we are interested in the phenomenon that has been the focus of recent phylogenetics literature on convergence, in which a phylogenetic analysis of a set of morphological characters is thought to be misled by similar adaptations to a similar selective environment in unrelated lineages. One way that these different usages might be reconciled is to treat the traditional definitions as pertaining to an individual character and to consider the recent usage of these terms in the phylogenetics literature as pertaining to overall morphology (or some other aspect of the overall phenotype or genotype). Under the latter usage, convergence would represent cases where similar derived morphologies are produced from different ancestral morphologies, and parallelism would be those cases in which similar derived morphologies are produced from similar ancestral morphologies. Thus, the terms convergence and parallelism would retain similar meanings but could refer to individual characters (traditional) or to sets of characters (phylogenetic). We follow this latter usage of convergence here.

Criteria for Detecting When Phylogenetic Analyses Are Misled by Convergence

We propose that explicit criteria are needed to establish that adaptive convergence has misled a phylogenetic study. Merely showing that trees from morphological and molecular data are incongruent is not enough. In general, there must be evidence that the tree estimated from morphological data is actually wrong and that convergence is the cause of the error. Minimally, three pieces of evidence are needed.

1. Strong morphological support for a clade that unites the taxa that share the similar selective environment.—The clade linked by putatively convergent characters should

be statistically well supported (e.g., from bootstrapping) to rule out the possibility that the association between these similar species is merely due to random homoplasy combined with sampling too few characters.

2. Evidence that the characters that unite the putatively convergent clade are associated with the shared selective environment.—This piece of evidence is important to rule out the possibility that the strongly misleading morphological result is caused by some factor other than adaptive convergence. For example, species may be placed together on a tree based on shared homoplasies caused by long-branch attraction, in which the long-branch attraction is caused by random homoplasy and weak taxon sampling (e.g., Hillis, 1998) or by parallel fixation of polymorphic characters in lineages with small population sizes through genetic drift (e.g., Wiens and Servedio, 1998). Another possibility is that multiple homoplasies are shared between lineages because of character nonindependence, for example caused by small size or paedomorphosis (e.g., Hanken and Wake, 1993; Emerson and Hastings, 1998) or some other mechanism causing developmental, genetic, or functional coupling of traits. We consider adaptive convergence (as a source of error in phylogeny reconstruction) to result from selection acting on independently evolving characters. Violation of the assumption of character independence is a different problem, although convergence may be involved in some cases (e.g., convergent selection for small size leads to a suite of correlated, developmentally coupled homoplasies).

Demonstrating that a given morphological character is an adaptation to a given selective environment is not a trivial task (see Rose and Lauder, 1996), but several approaches can be used. The association between morphological characters and ecological settings (e.g., fins and aquatic habitat) can be evaluated statistically by using phylogenetic comparative methods. These methods are designed for detecting correlations between pairs of characters, including discrete variables (e.g., Maddison, 1990; Pagel, 1994), continuous variables (e.g., Felsenstein, 1985b), or combinations of the two (e.g., Grafen, 1989; McPeek, 1995). These tests should be carried out on the trees based on molecular or other nonmorphological data (or the combined molecular and morphological data) rather than on trees based on morphological data alone. Otherwise, the association between traits and ecological settings is likely to be underestimated using these methods, if the morphology-based phylogeny has been misled by convergence. Another approach is to use experimental evidence that the putative convergent trait actually confers a performance advantage in the shared ecological setting (i.e., is adaptive; Coddington, 1988; Baum and Larson, 1991). Similarly, one might use biomechanical or biophysical modeling to argue that the trait is advantageous in the novel environment. These three lines of evidence might be used interchangeably or (better) in combination.

Many clades may be supported by a mixture of adaptive and nonadaptive characters, including both clades that are correctly inferred and those that are artifacts of adaptive convergence. For example, species that are actually closely related may be united in a phylogenetic analysis by shared adaptive character states, and an incorrect clade might be supported by one or more nonadaptive characters (in addition to characters shared through convergence) by chance. However, if a phylogenetic analysis has been misled by adaptive convergence, we predict that the clade of species that share the same selective environment will no longer be supported in the optimal tree(s) when the putatively adaptive characters are removed and the data are reanalyzed. If the putatively convergent clade is still supported, this result would suggest (among other things) that this clade may have been correctly estimated.

3. Phylogenetic evidence that the species that share the common selective environment are not actually a monophyletic group, preferably consisting of strong support for the contradictory clades from two or more unlinked molecular data sets.—Statistically well-supported clades that contradict the putative convergent clade are important in order to ensure that the conflict between data sets is not merely due to spurious resolution caused by random homoplasy and limited character sampling in the nonconvergent data set. Furthermore, at least two unlinked data sets are desirable, given that there may be a systematic error in one of these data sets. For example, if the data set that rejects the putative convergent clade is from mitochondrial DNA (mtDNA) sequences, supporting evidence from a nuclear data set is important to address the possibility that incongruence between the morphological and mtDNA trees is caused by failure of the mtDNA phylogeny to match the species phylogeny (e.g., through lateral transfer or incomplete lineage sorting; Maddison, 1997). Although it may be difficult to demonstrate that the relevant clade(s) from the morphological tree are likely to be incorrect, many kinds of evidence might be used as support, including characters from behavior and karyology, and the fit between alternative phylogenies and the temporal appearance of taxa in the fossil record (e.g., Brochu, 1997).

A fourth criterion might also be added in order to distinguish cases of strongly misleading convergence from strongly misleading parallelism. To make a case for convergence in overall morphology (according to our definition), it would be necessary to infer differences in overall morphology between the the reconstructed ancestors of the putatively convergent lineages.

Study System

The Edwards Plateau region of central Texas (USA) contains a monophyletic radiation of plethodontid salamanders of the genus *Eurycea* (Chippindale, 2000; Chippindale et al., 2000). This radiation consists of 13 currently described species of aquatic, mostly paedomorphic (nontransforming) salamanders confined to springs and caves in a generally semi-arid landscape (Chippindale, 2000; Chippindale et al., 2000; Hillis et al., 2001). Most surface-dwelling species are extremely similar morphologically, whereas cave-dwelling species

show varying degrees of morphological modification that are seemingly associated with subterranean life (Mitchell and Reddell, 1965; Wake, 1966; Mitchell and Smith, 1972; Potter and Sweet, 1981). Several species have both surface and cave-dwelling populations (i.e., *E. latitans*, *E. naufragia*, *E. pterophila*, *E. tonkawae*, and *E. troglodytes*), and the cave-dwelling populations of these species may show weaker expression of the same traits that characterize exclusively cave-dwelling species (e.g., broader and flatter head, reduced pigmentation and eye size; Chippindale et al., 2000). The most highly modified cave-dwelling species are *E. rathbuni*, *E. robusta*, *E. tridentifera*, and *E. waterlooensis* (Mitchell and Reddell, 1965; Wake, 1966; Mitchell and Smith, 1972; Potter and Sweet, 1981; Hillis et al., 2001). These are the only species that seem to live almost exclusively in caves (although a few individuals have occasionally been found on the surface). Prior to the molecular study of Chippindale et al. (2000), *E. rathbuni* and *E. robusta* were usually recognized as a separate genus (*Typhlomolge*) because of their unusual morphology (*E. waterlooensis* was described very recently but is closely related to *E. rathbuni* based on mtDNA data; Hillis et al., 2001). Previous authors have attributed the similarity between *E. tridentifera* and *Typhlomolge* to either close phylogenetic relatedness (Wake, 1966) or cave-associated convergence (Mitchell and Reddell, 1965; Mitchell and Smith, 1972; Potter and Sweet, 1981), but these morphological studies did not present a data matrix or explicit phylogenetic analysis.

Materials and Methods

General

Taxon sampling focused on those species for which all three data sets (morphology, allozymes, mtDNA) could be obtained. Species were used as terminal taxa for the allozyme and morphological data, and individual haplotypes were used as terminals for the mtDNA data. Although only one or two individuals per species were sequenced for the cytochrome *b* data set, Chippindale et al. (2000) addressed the population status of >30 populations of central Texas *Eurycea* using mtDNA data and >60 populations using allozyme data. Chippindale et al. sampled large numbers of individuals for all allozyme loci used here and for a smaller cytochrome *b* fragment. The more extensive sampling of individuals for the smaller cytochrome *b* fragment confirms the exclusivity (i.e., monophyly at or below the species level) of the mtDNA lineages for most of the species used in this study and justifies use of reduced sample sizes for the longer cytochrome *b* sequences in the present study (i.e., sampling multiple individuals of exclusive species should yield the same species-level phylogeny).

All phylogenetic analyses (morphological and molecular) were carried out using Swofford's (2001) PAUP* program, version 4.0b8. The data matrices used are available on the *Systematic Biology* web site. For the data sets that used time-intensive step matrices (morphology and allozymes), we used the heuristic search option to find the shortest tree, with tree bisection–reconnection branch swapping (TBR) and 50 random-taxon-addition sequence replicates. Branch-and-bound searching was used for the unweighted parsimony analysis of the mtDNA data. Support for individual clades was evaluated using the nonparametric bootstrap (Felsenstein, 1985a), with 500 bootstrap pseudoreplicates (with 5 random-addition sequence replicates for heuristic searches). Bootstrap values >70% were considered to indicate strong support (Hillis and Bull, 1993, but see their caveats).

Statistical support for alternative topologies is difficult to evaluate for the morphological and allozyme data because these data are fundamentally continuous. For the mtDNA data, we compared the fit of the best (unconstrained) likelihood trees to the best likelihood tree in which the extremely cave-modified species (*E. rathbuni* and *E. tridentifera*) were constrained to form a monophyletic group, using the test of Shimodaira and Hasegawa (1999; following Goldman et al., 2000). This test was implemented in PAUP* using 1,000 bootstrap pseudoreplicates. Unfortunately, the Shimodaira–Hasegawa test was not applicable to the allozyme, morphological, or combined data, and use of the Kishino–Hasegawa (Kishino and Hasegawa, 1989) test would be invalid in this case (i.e., alternative topologies not specified a priori; Swofford et al., 1996; Goldman et al., 2000).

Morphological Data and Methods

Morphological data were obtained largely from cleared-and-stained osteological preparations, prepared following Dingerkus and Uhler (1977). Two to nine adult individuals were sampled per species (mean = 5.5, see Appendix 1), depending largely upon availability of specimens for skeletal preparation. For example, only two cleared-and-stained specimens of *E. rathbuni* were used because this taxon is federally listed as an Endangered Species. A paedomorphic (nontransforming) population of the *E. multiplicata* complex was included as an outgroup to root the tree. This taxon is related to but outside of the central Texas clade (Chippindale et al. 2000; Chippindale et al., unpubl.) and is at a comparable ontogenetic stage to the paedomorphic Texas species. A population of the *E. multiplicata* complex was also used as an outgroup in the molecular data sets. *Eurycea robusta*, a taxon known from a single specimen (Potter and Sweet, 1981; Chippindale et al., 2000), was not included in this analysis because no molecular data were available. However, if it is included, it is strongly supported as the sister taxon of *E. rathbuni* in both morphological and combined analyses (when the molecular data are treated as missing; Wiens, unpubl.). *Eurycea waterlooensis* was discovered very recently and was unavailable for inclusion in the allozyme and morphological analyses. This species is a member of the same clade as *E. rathbuni* based on DNA sequence data (Hillis et al., 2001).

The 16 morphological characters used are described in Appendix 2. Characters were selected that were used in previous systematic studies of Texas *Eurycea* (e.g., Wake,

TABLE 1. Summary of character state frequencies (state 1) and mean trait values for each *Eurycea* taxon for the morphological characters described in Appendix 2. Values for all characters are percentages, except for meristic characters 1 and 11, for which mean values are given.

	Characters															
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
multiplicata	19.2	25	100	25	0	0	100	0	0	75	21.5	0	0	0	0	0
chisholmensis	17.5	25	100	12.5	0	25	37.5	75	75	100	18.8	100	50	50	0	0
naufragia	15.3	75	75	0	0	0	75	100	100	100	18	100	25	0	100	0
tonkawae	13.9	0	11.1	16.7	0	0	27.8	55.6	100	100	17.9	100	27.8	0	38.9	0
nana	13	75	100	50	0	0	0	100	100	100	18.2	100	100	100	75	0
pterophila	14.9	25	50	87.5	0	0	0	37.5	100	75	18.1	100	50	100	25	0
neotenes	12.6	25	37.5	81.2	0	0	0	6.2	87.5	25	18.2	87.5	57.1	50	6.2	0
troglodytes	15	33.3	75	75	0	0	75	0	0	100	17.8	75	100	66.7	12.5	0
latitans	13.7	85.7	100	100	0	0	7.1	64.3	100	71.4	18	100	0	100	8.3	0
sosorum	19.2	0	80	90	0	0	0	0	60	80	17	40	40	80	0	0
Comal	12.7	83.3	100	83.3	0	0	16.7	33.3	100	40	18	83.3	60	100	8.3	0
tridentifera	19.5	28.6	71.4	57.1	0	64.3	14.3	0	42.9	85.7	14.7	42.9	85.7	50	64.3	100
rathbuni	43	0	100	0	100	100	100	0	0	100	14	0	100	0	50	100

1966; Potter and Sweet, 1981) or that were found to vary during our own studies (e.g., Chippindale et al., 2000). Characters were not excluded because of polymorphism, continuous variation, or a priori notions about homoplasy, but characters were excluded when they were suspected to be nonindependent of other characters or that were too difficult to measure consistently or describe qualitatively (following Poe and Wiens, 2000). Most characters were described qualitatively (e.g., presence or absence of contact between two elements), although the underlying variation ranged from largely discrete to nearly continuous. Intraspecific variation is abundant in the morphology of Texas *Eurycea* (e.g., Mitchell and Smith, 1972; Sweet, 1978; Potter and Sweet, 1981). Polymorphic characters were included in the phylogenetic analysis and coded using a frequency-parsimony approach because the inclusion of polymorphic characters and frequency information has been shown to generally improve phylogenetic results in statistical analyses (Wiens, 1995), simulations (Wiens and Servedio, 1997, 1998), and congruence studies (Wiens, 1998a). Characters were frequency-coded using the step-matrix method described by Wiens (1995) and Berlocher and Swofford (1997). Thus, for a given character each taxon was given a unique character state, and differences between each pair of character states were weighted based on the difference in frequencies between each pair of taxa (using the Manhattan distance metric).

Two meristic characters were included and coded as continuous variables using step matrix gap-weighting (Wiens, 2001). The best method for scaling meristic characters relative to qualitative characters is an unresolved issue, but in general characters with large ranges of trait values (>10) may best be analyzed using between-character scaling (such that they have weight equivalent to that of a fixed binary character), whereas characters with low ranges may be better analyzed using between-state scaling (equivalent to a fixed multistate character); these issues and methods were described by (Wiens, 2001). In this study, we used between-character scaling for number of premaxillary teeth (range of species means, 12.6–43) and between-state scaling for number of vertebrae (range of species means, 14–21.5). Trait frequencies and means are summarized in Table 1.

The association between habitat (cave usage) and specific morphological characters was tested using Maddison's (1990) concentrated changes test, a standard parsimony-based method for testing the relationships between qualitative (binary) characters in a phylogenetic context. The test was implemented using MacClade 3.0 (Maddison and Maddison, 1992). Habitat use was the independent variable, and the putatively cave-associated morphological characters were the dependent variables. For habitat, species that were exclusively cave-dwelling were coded as "1" and other species (either surface-dwelling or variably surface-dwelling) were coded as "0." Polymorphic morphological characters were made binary using majority coding (which provides the best approximation to frequency methods possible using binary coding; Wiens, 1995), and vertebral number was made discrete and binary using a cutoff value (>15 mean vertebrae = 0; ≤15 vertebrae = 1). Although recoding polymorphic or continuous variables as binary may entail considerable loss of information in phylogeny reconstruction (i.e., Wiens, 1995, 2001), for this comparative analysis we were interested only in the relationship between the most extreme forms of the morphological traits and the habitat (i.e., exclusive cave use) rather than incorporating all possible variation in these characters. The analysis was expanded to include all the outgroup taxa in the analysis of Chippindale et al. (2000) to increase the power of the test by including a taxon (*Haideotriton wallacei*) that is strictly cave dwelling but outside of the central Texas clade. The additional taxa were *E. bislineata, E. longicauda, E. quadridigitata, E. wilderae, H. wallacei,* and *Typhlotriton spelaeus.* Simulations by Lorch and Eadie (1999) suggest that including more taxa should not increase the type I error rate of the test (contra Grafen and Ridley, 1997) and that the type II error rate may be high if too few taxa are included. Data on habitat were obtained from direct observations for Texas species (Chippindale et al., 2000) and from the literature for non-Texas taxa (Petranka, 1998). In no case was habitat inferred from morphology. Morphological data for non-Texas species

were obtained from Wake (1966), Petranka (1998), and a larger survey of plethodontid morphology including all of these taxa (Wiens, unpubl. data). The tree used for the concentrated changes test was based on the combined DNA and allozyme data (Chippindale et al., 2000) which is similar to the trees from DNA and allozyme data from this study (the only differences are within the poorly resolved southeastern Edwards Plateau clade) but includes additional outgroup taxa. After the putatively cave-related characters were identified, the morphological data were reanalyzed with these characters removed to determine if the clade of cave-dwelling species was still supported.

Allozyme Data and Methods

The allozyme data of Chippindale et al. (2000) consist of 25 loci, and 20 of the loci were parsimony informative among the taxa included in this study. The allozyme data were analyzed using the same step matrix frequency-parsimony approach described for the morphological characters. The accuracy of this method specifically for use with allozyme data was demonstrated by Wiens (2000). Data from conspecific populations that were analyzed as separate units by Chippindale et al. (2000) were combined to increase sample sizes in this study, but a few populations that could not be assigned unambiguously were excluded (e.g., certain populations of the *E. troglodytes* complex and in the "northern clade").

Mitochondrial DNA Data and Methods

A fragment of the mitochondrial cytochrome *b* gene was analyzed (including almost the entire gene), consisting of 1,141 bp from each taxon (but only 1,118 bp for *E. naufragia*) with 269 parsimony-informative positions. These data are largely taken from Hillis et al. (2001), and specimen numbers and GenBank accession numbers are presented in Appendix 1 of that paper. Sequence data from an individual representing an undescribed species from Comal Springs (field number PC/DMH 90-171; GenBank AY 260759) and from a second individual of *E. latitans* (field number: PC/DMH 90-128; GenBank AY 260758) were also added and were obtained using methods similar to those described by Hillis et al. (2001).

Data were analyzed using both equally weighted parsimony and maximum likelihood, which gave very similar results. The fit of different likelihood models to the data was evaluated using Modeltest 3.06 (Posada and Crandall, 1998). This program generates an initial distance tree (using neighbor joining and the minimum evolution criterion) assuming the simplest model of sequence evolution (Jukes and Cantor, 1969) and then compares the fit of 56 models to the data using this tree and a likelihood ratio test. Application of Modeltest to our data showed that the HKY (Hasegawa et al., 1985) + Γ (gamma distribution of variable sites) model has the highest likelihood (fit) without adding unnecessary parameters. However, current versions of Modeltest do not include models that use codon-specific rates to incorporate among-site rate heterogeneity in protein-coding genes (r; Swofford et al., 1996). Because the Γ and r parameters are different ways of describing among-site rate variation, models that include these parameters are not nested and therefore cannot be compared using the chi-square test. We compared phylogenetic results using both models and evaluated the fit of both models to the initial minimum evolution tree by comparing likelihoods and using the Akaike (1974) information criterion (following Hasegawa et al., 1991).

The best-fitting model was then used in a heuristic search to find the overall best likelihood topology, using TBR branch swapping and 10 random-taxon-addition sequence replicates. Model parameters were initially generated using the minimum evolution tree. Once the likelihood tree was generated using these parameters, the parameters were estimated on this new tree and the analysis was repeated to determine whether there was any change in topology using these refined parameter estimates (following Wilgenbusch and de Queiroz, 2000). Support for individual branches of the likelihood tree was evaluated using nonparametric bootstrapping, with 200 pseudoreplicates and five random-taxon-addition sequence replicates per bootstrap pseudoreplicate.

Combined Data Analysis

Combination of strongly conflicting data sets is controversial (e.g., de Queiroz et al., 1995; Huelsenbeck et al., 1996) but may improve overall accuracy if conflict is localized on the trees (Wiens, 1998b) and often reveals phylogenetic patterns not seen in analyses of separate data sets alone (e.g., Chippindale and Wiens, 1994). In order to evaluate the relative strength of the hypotheses supported by each data set, we performed a combined analysis of the morphological, allozyme, and mtDNA data sets, scaling all characters to have the same maximum weight (except for the meristic character describing the number of vertebrae, see above). A single (arbitrarily chosen) mtDNA haplotype of *E. latitans*, *E. rathbuni*, and *E. troglodytes* was used to represent each of these species in the combined data matrix.

RESULTS

Parsimony analysis of the morphological data yielded a single shortest tree (Fig. 1; length = 39.6695; consistency index = 0.5430 [informative characters only]; retention index = 0.5317), with strong support for a clade consisting of the two species with the most extreme cave-associated morphologies, *E. rathbuni* and *E. tridentifera* (bootstrap = 79%). Three characters show the strongest evidence (highest weight) for this clade: loss of the orbitosphenoid (character 6), reduction in the number of vertebrae (character 11), and the extreme reduction in eye size (character 16). Bootstrap values elsewhere in the morphological tree are very weak (<50%).

The three characters (6, 11, and 16) that most strongly support the *rathbuni–tridentifera* clade show a significant association with the exclusive use of cave habitat ($P < 0.001$), based on Maddison's concentrated changes test on the tree from combined allozyme and mtDNA

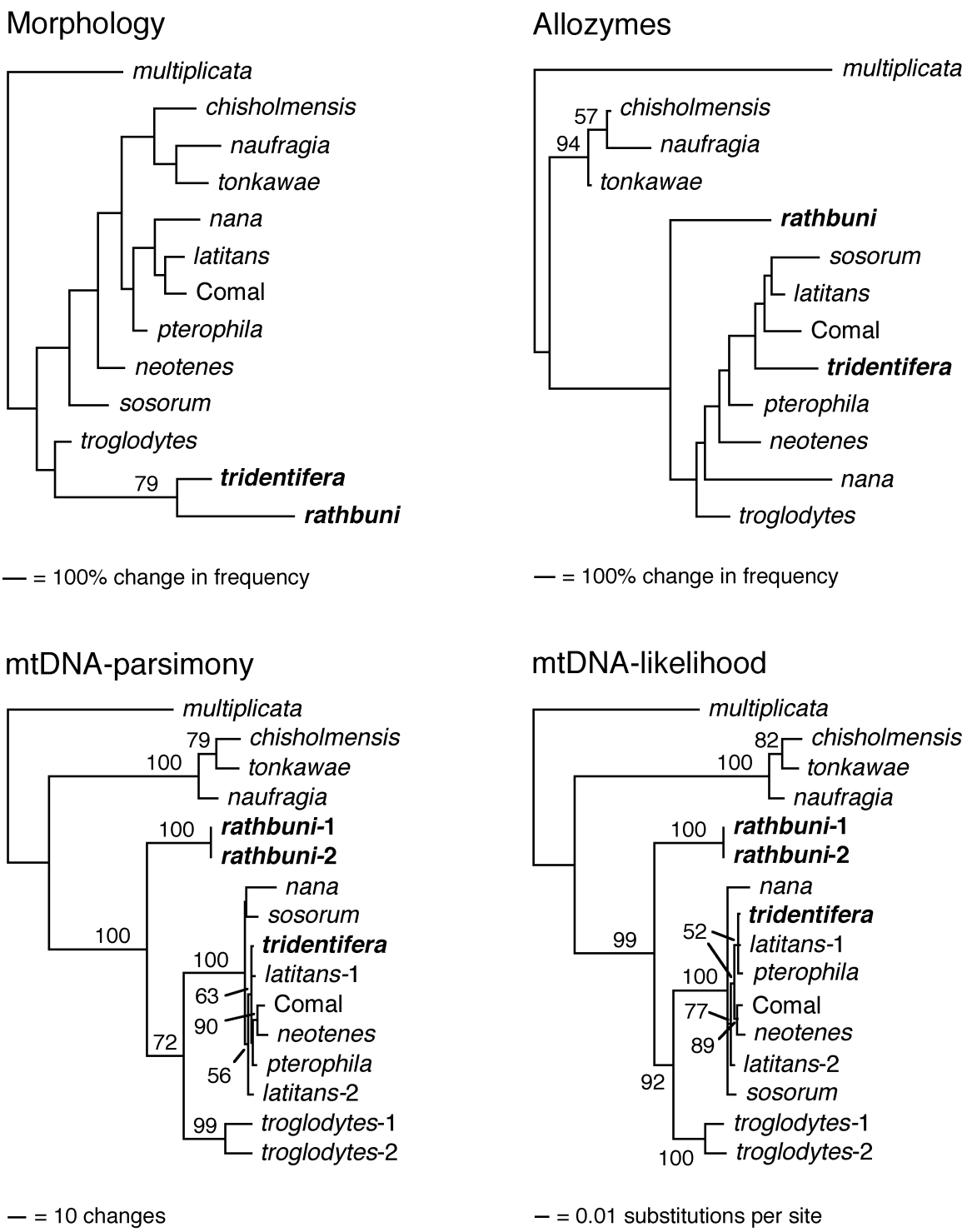

FIGURE 1. Trees from phylogenetic analyses of diverse data sets for central Texas salamanders of the genus *Eurycea*. The species with the most extreme cave-associated morphologies (*E. rathbuni* and *E. tridentifera*) are shown in bold. All trees are shown as phylograms, with the length of each branch proportional to the amount of estimated change for that lineage. Numbers at nodes indicate bootstrap values >50%. For the trees from the morphological and allozyme data, the scale bar indicates the length for a single binary character in which the frequency of the derived state changes from 0 to 100%. The parsimony mtDNA tree is one of a set of eight shortest trees; differences in the topology of these eight trees all involve relationships within the southeastern clade (Comal, *latitans*, *nana*, *neotenes*, *pterophila*, *sosorum*, *tridentifera*). The parsimony tree is based on equal weighting of all characters and substitution types. The maximum likelihood tree is based on the HKY + r model (the HKY + Γ model gives the same topology and almost identical branch lengths and clade support). *latitans* -1 = TNHC 54536 (Pfeiffer's Water Cave); *latitans* -2 = PC/DMH 90-128 (Cibolo Creek); *rathbuni* -1 = TNHC 51174; *rathbuni* -2 = TNHC 60314; *troglodytes* -1 = TNHC 60318 (Trough Spring); *troglodytes* -2 = TNHC 60312 (Sutherland Hollow Spring).

data (results not shown). Outside of the Texas clade, the loss of the orbitosphenoid and the extreme reductions in vertebral number and eye size occur only in *Haideotriton wallacei*, which is the only other species in the group that is exclusively cave dwelling. When these three characters are removed from the phylogenetic analysis, the *rathbuni–tridentifera* clade is not supported in the shortest tree (length = 25.6150). In this tree (not shown), the relationships are similar to those in Figure 1, except that *E. rathbuni* is the sister taxon of all other central Texas species, *E. troglodytes* is the sister taxon of the remaining species, *E. tridentifera* is the sister taxon of the rest, and *E. nana* and *E. latitans* are sister taxa.

Analysis of the allozyme data resulted in a single shortest tree (Fig. 1; length = 58.31; consistency index = 0.8156 [informative characters only]; retention index = 0.7354). The allozyme data place the three species from the northern Edwards Plateau in a single clade (*E. chisholmensis, E. naufragia, E. tonkawae*), which is the sister taxon to all other Central Texas *Eurycea*. *Eurycea rathbuni* is the sister taxon of all other southern species, and *E. troglodytes* is the sister taxon of a clade of seven species from the southeastern Edwards Plateau (*E. latitans, E. nana, E. neotenes, E. pterophila, E. sosorum, E. tridentifera, Eurycea* sp. Comal Springs). The allozyme data do not place *E. rathbuni* with *E. tridentifera*. This tree is not generally well supported (most bootstrap values <50%), but the monophyly of a northern clade has high bootstrap support.

Equally weighted parsimony analysis of the cytochrome *b* data yielded eight shortest trees (Fig. 1; length = 545) that are largely congruent with the tree from the allozyme data. Bootstrap analysis shows strong support for division of the Texas species into a northern clade (*E. chisholmensis, E. naufragia, E. tonkawae*) and a southern clade (all other species), as found in the allozyme data. Also concordant with the allozyme data (but more strongly supported), the mtDNA data show that *E. rathbuni* is the sister taxon of the rest of the southern clade and *E. troglodytes* is the sister taxon of the remaining members of this clade, and there is a well-supported clade of seven species from the southeastern region of the Edwards Plateau (*E. latitans, E. nana, E. neotenes, E. pterophila, E. sosorum, E. tridentifera, Eurycea* sp. Comal Springs). Relationships within this southeastern clade are generally poorly resolved and weakly supported, and branch lengths are extremely short, suggesting that these taxa have speciated only recently. Relationships within this clade are largely incongruent with those from allozyme data, and all conflicting clades are weakly supported by one or both data sets. Furthermore, the two individuals of *E. latitans* sequenced for cytochrome *b* do not form an exclusive group, which is the expected pattern when species have diverged very recently (Neigel and Avise, 1986). Parsimony analyses of the mtDNA strongly suggest that *E. rathbuni* and *E. tridentifera* are not sister taxa.

The maximum likelihood model-fitting analysis using Modeltest showed that the HKY + Γ model provides the best fit to the data without adding unnecessary parameters. Maximum likelihood analysis using the parameters estimated from the minimum evolution tree yielded a single tree (Fig. 1; −ln likelihood = 4069.27178). This tree is very similar to the tree based on parsimony analysis in terms of topology, bootstrap support, and branch lengths, differing primarily in some of the relationships among species in the southeastern clade. Optimizing model parameters on this tree gives nearly identical parameter estimates, and analyses using these reoptimized parameters gives basically the same topology (−ln likelihood = 4069.27174), branch lengths, and bootstrap support. Using these optimized parameters, searching for an optimal topology in which the highly modified cave species are monophyletic yields a −ln likelihood of 4120.78977. The Shimodaira–Hasegawa test with 1,000 bootstrap pseudoreplicates shows this difference to be highly significant ($P = 0.001$).

Maximum likelihood analysis using the HKY + *r* model yields topology, branch lengths, and levels of bootstrap support (Fig. 1) that are very similar or identical to those based on the HKY + Γ model, although the fit of the model to the data is considerably higher (−ln likelihood = 3892.18548). The Akaike information criterion suggests that the HKY + *r* model offers a better fit to the data than the HKY + Γ model. Results using the HKY + *r* model are identical before and after optimization of the parameters of this model on the initial neighbor-joining tree. The Shimodaira–Hasegawa test using this model also significantly rejects the best likelihood tree (−ln likelihood = 3969.3204) in which the cave species are constrained to be monophyletic ($P < 0.001$).

Combined analysis of the morphology, allozyme, and mtDNA data sets supports the same basic relationships estimated from analysis of the allozyme and mtDNA data alone (Fig. 2; length = 583.7900; consistency index = 0.7490 [informative characters only]; retention index = 0.8289) and does not support monophyly of the *rathbuni–tridentifera* clade.

DISCUSSION

Eurycea *as a Case Study for Detecting Misleading Results Caused by Convergence*

Our study suggests that central Texas *Eurycea* meet the three criteria necessary to claim that adaptive convergence has led to a strongly misleading phylogenetic result in the morphological data (i.e., the *rathbuni–tridentifera* clade).

1. Support for convergent clade.—The clade uniting *E. rathbuni* and *E. tridentifera* in the morphological analysis is well supported, with a bootstrap value of 79%. Although a value ≥95% would be more compelling, bootstrap values are conservative indicators of phylogenetic accuracy, and values as low as 70% may indicate a 95% probability of estimating the clade correctly (Hillis and Bull, 1993). We therefore conclude that the *rathbuni–tridentifera* clade is not simply a spurious resolution resulting from the stochastic effects of random homoplasy and undersampling of characters.

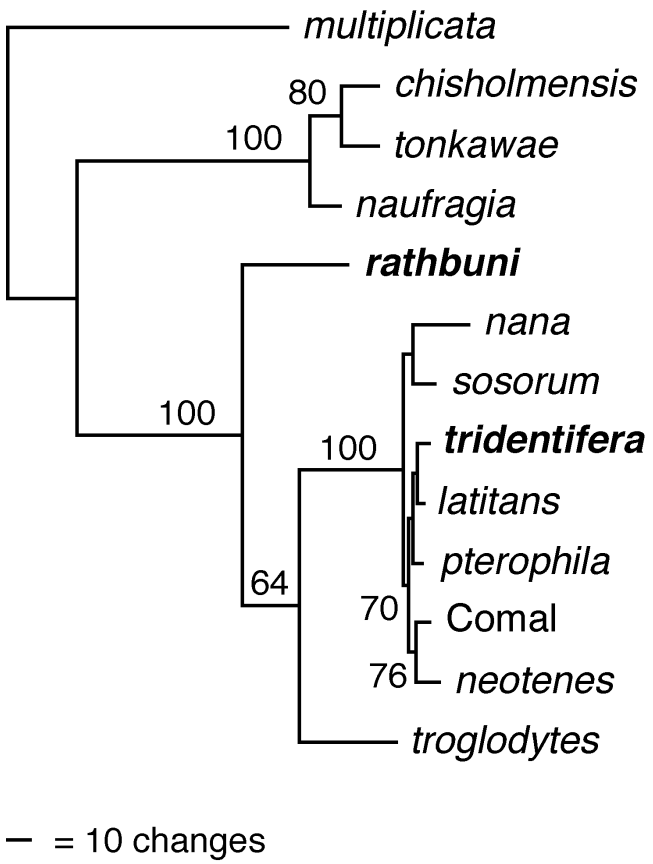

FIGURE 2. Shortest tree from a combined parsimony analysis of the allozyme, morphological, and mtDNA data for central Texas *Eurycea*. Branch lengths are drawn proportional to the amount of estimated change for each lineage (the scale bar indicates 10 changes, where each change is a change in estimated character state frequencies from 0 to 100%), and numbers at nodes indicate bootstrap values >50%. The species with the most extreme cave-associated morphologies (*E. rathbuni* and *E. tridentifera*) are shown in bold. For this analysis, we included mtDNA data from only a single individual of *E. latitans* (TNHC 54536), *E. rathbuni* (TNHC 51174), and *E. troglodytes* (TNHC 60318).

2. Association between putative convergent characters and ecological setting of species.—Three characters provide the strongest support for the *rathbuni–tridentifera* clade: reduced eye size, loss of the orbitosphenoid bone, and reduced number of vertebrae. These characters show a significant association with the exclusive use of caves, and the *rathbuni–tridentifera* clade is no longer supported when these characters are removed. Reduction in eye size is a common phenomenon in cave animals (Culver, 1982), including both vertebrates and invertebrates. Among hemidactyliine salamanders, the loss of the orbitosphenoid and extreme reduction in number of vertebrae are shared only by *E. tridentifera, E. rathbuni, E. robusta*, *E. waterlooensis*, and *H. wallacei* (Wake, 1966; Potter and Sweet, 1981; Hillis, pers. obs.). *Haideotriton* is a monotypic, highly cave-modified genus from Florida and Georgia, which is relatively closely related to the central Texas *Eurycea*, but clearly became cave dwelling independently, based on biogeography, allozymes, and mtDNA (Chippindale et al., 2000) and on nuclear DNA sequences (Chippindale, unpubl.).

We have little information on the adaptive significance of the morphological characters uniting the highly cave-modified *Eurycea*. All three characters involve loss or reduction, a pattern of morphological change that is typical for cave organisms (Culver et al., 1995; Fong et al., 1995). It is possible that the evolution of cave-associated reductive characters involves the relaxation of positive selection maintaining these traits and the accumulation of neutral mutations causing their loss (e.g., Wilkens, 1988), and that these traits may not be adaptations in the usual sense. However, Jones et al. (1992) examined selection on morphological traits in the cave-dwelling amphipod *Gammarus minus* and found evidence for direct selection for small eye size (possibly involving a trade-off for increasing size of other sensory structures). A single reductive character may evolve through a combination of both neutral mutation and directional selection (Fong et al., 1995). The loss of the orbitosphenoid may be associated with the highly modified skull shape in these salamanders, although skull shape is somewhat different in the cave-dwelling *Haideotriton*, which also lacks an orbitosphenoid. The reduced number of trunk vertebrae seems to reflect decreased body elongation (meaning that the length of the trunk has decreased relative to the length of the head, limbs, and tail). Both the unusual skull shape and decreased body elongation may be adaptations for increased locomotor performance associated with decreased use of cryptic habitats (e.g., under rocks, in gravel) and increased use of open water in predator-depauperate cave habitats. Although there currently are insufficient data with which to address these hypotheses in salamanders, it is possible that the cave-associated traits seen in *Eurycea* are truly adaptive, and we tentatively consider these traits to be the result of adaptive convergence.

3. Phylogenetic evidence that the convergent clade is wrong.—The DNA sequence data strongly support placement of *E. tridentifera* in a clade with six other species (*E. latitans, E. nana, E. neotenes, E. pterophila, E. sosorum*, and *Eurycea* sp. Comal Springs) from the southeastern Edwards Plateau and demonstrate that *E. rathbuni* is not part of this clade. These results show that incongruence between the morphology and DNA is not merely due to sampling too few DNA characters. However, they leave open the possibility that the data sets are incongruent because of mismatch between the gene (mtDNA) and species phylogeny or some other systematic error. The fact that the allozyme data support placement of *E. tridentifera* in the same geographically coherent clade of seven species as the DNA data (excluding *E. rathbuni*) makes this possibility seem extremely unlikely. Although this clade is admittedly not strongly supported by the allozyme data, the allozyme and mtDNA trees are highly concordant (i.e., both support the northern clade, southern clade, and basal positions of *E. rathbuni* and then *E. troglodytes* in the southern clade).

A final issue is whether the Texas *Eurycea* represent a case of strongly misleading convergence or

parallelism. The surface-dwelling Texas *Eurycea* are generally similar in overall morphology (Chipppindale, 2000; Chippindale et al., 2000). This similarity implies that the two exclusively cave-dwelling lineages had morphologically similar ancestors and that the strongly misleading homoplasy in this system is explained by parallelism rather than convergence. However, studies by Potter and Sweet (1981), comparing the head shape of *E. rathbuni* and *E. tridentifera,* suggest that different evolutionary trajectories gave rise to the superficially similar morphologies of these cave species. Thus, we tentatively consider this a case of misleading convergence rather than parallelism.

Is Strongly Misleading Morphological Convergence Rare?

Our study of *Eurycea* may be one of the first to document strongly misleading phylogenetic results caused by adaptive morphological convergence, at least according to our criteria. Given that there are now many groups for which both molecular and morphological data are available, why are there so few well-supported examples of this phenomenon? Although several explanations are possible, perhaps the simplest explanation is that the scarcity of well-documented cases of convergence in the literature accurately reflects the rarity of this phenomenon. We speculate that the conditions under which strongly misleading adaptive convergence is most likely to occur are limited—namely, in groups in which most species are poorly differentiated morphologically (e.g., recently diverged or highly constrained) but a few are extensively modified in association with a novel ecological setting (Hillis and Wiens, 2000). In these cases, there is relatively little true historical signal in the morphological data, and the true phylogenetic signal is easily overwhelmed by the false signal generated by convergence (analogous to the case of long-branch attraction described by Felsenstein, 1978).

The central Texas species of *Eurycea* clearly illustrate this scenario for strongly misleading convergence. Surface-dwelling populations are very similar morphologically, and many of the species currently recognized using molecular markers were long considered to be conspecific based on morphology (Chippindale, 2000; Chippindale et al., 2000). Except for the clade uniting the species with the most extreme cave-associated morphologies (*E. rathbuni* and *E. tridentifera*), all bootstrap values in the morphological tree are <50% (Fig. 1), and the overall number of informative morphological characters in the analysis is very small (almost equal to the number of taxa). There is also extensive polymorphism in nearly all of the morphological characters used in this study (Table 1), further supporting the weak differentiation of these species in morphology. The morphological similarity among surface-dwelling central Texas *Eurycea* may reflect the widespread morphological stasis found in many plethodontid salamanders (Wake et al., 1983; Larson and Chippindale, 1993) rather than recent diversification, because levels of sequence and allozyme divergence are high for some clades within this group (Chippindale et al., 2000). It is also possible that convergence that is strictly adaptive is unlikely to be rapid and/or anatomically widespread enough to frequently mislead phylogenetic analyses of morphology, and that strongly misleading results in *Eurycea* occur only because of the unusual cave environment and the combination of relaxed selection, neutral mutations, and reductive character evolution.

Another explanation for the rarity of reported cases of morphological convergence is that morphologists may tend to screen from their data sets those characters that they consider prone to convergence. This screening process is difficult to document because morphologists typically do not report the criteria they use for character selection (Poe and Wiens, 2000). Nevertheless, a few morphologists have explicitly reported that they excluded characters based on suspected homoplasy (Poe and Wiens, 2000), even though the first principle of phylogenetic systematics is that convergence should not be assumed a priori (Hennig, 1966; Wiley, 1981). It is unclear to what extent morphological results might be biased in this manner.

A third explanation, which also seems likely, is that many of the cases of convergence reported in the literature that currently fail to meet our three criteria will prove to be real upon further study (e.g., by adding unlinked molecular data sets or by more rigorous analysis of the morphology). For example, McCracken et al. (1999) postulated misleading morphological convergence in a clade of ducks, but the molecular evidence that was used to claim that the morphological tree was incorrect came from a single mitochondrial gene. Moreover, the DNA-based tree was only weakly supported, and the authors did not critically examine the distribution of putative morphological convergences among taxa (e.g., testing for associations between morphological characters and habitat). Although this case clearly fails to meet our three criteria, future studies applying additional molecular data sets and detailed analysis of the morphological data may show that convergence explains the incongruent results in this group. Similarly, Hollar and Springer (1997) and Teeling et al. (2002) found evidence from multiple molecular data sets for nonmonophyly of bat taxa that are supported by morphological data and suggested that convergence might be involved in generating these putatively misleading morphological results. However, these authors did not examine the morphological evidence thoroughly to address the hypothesis that convergent evolution explains the morphological results, for example, by examining levels of support for the purportedly misleading clades, identifying and deleting presumed convergent characters from the morphological analysis, or testing for correlations between these convergent characters and ecological variables. Finally, many recent studies have noted disagreement between molecular and morphological data sets—and have implicitly assumed that the morphological results are incorrect—without specifically implicating convergence as the source of error (e.g., see articles on mammalian phylogeny in *Systematic Biology* 1999, vol. 48,

no. 1). These disagreements might also prove to be cases of strongly misleading convergence upon further study.

Adaptive convergence (and parallelism) is a very general problem in phylogenetic inference. In this article, we have focused on a very specific and extreme aspect of the problem: cases in which an analysis yields a well-supported but incorrect phylogenetic conclusion. The problem of adaptive homoplasy may also be manifested in clades that are incorrect but weakly supported, in polytomies, or in clades that are correctly reconstructed but only weakly supported because of the contradictory evidence caused by convergence. It should also be noted that adaptive characters potentially can be reliable indicators of phylogeny (even if they exhibit some homoplasy), especially if there is strong selection to maintain a given trait in a lineage for millions of years without reversals (Donoghue and Sanderson, 1992).

CONCLUSIONS

In this study we have provided criteria for detecting misleading phylogenetic results caused by adaptive convergence and presented an empirical example of strongly misleading convergence in morphology. Although the idea of frequent convergence in morphological characters has been a rationale for abandoning or ignoring morphological data in phylogeny reconstruction, we reject the use of our results as a justification for molecular chauvinism. We speculate that the conditions under which adaptive convergence is most likely to cause well-supported but misleading results may be limited (i.e., among species that are generally very poorly differentiated morphologically but that show striking differences in ecology). Furthermore, there are several processes that may cause strongly misleading molecular results, such as long-branch attraction and mismatch between gene and species trees. Adaptive convergence may also be problematic for phylogenetic analysis of molecular data, as suggested by a laboratory study of viruses (Bull et al., 1997).

While our results do not justify the exclusion of morphological data from phylogenetic analyses, they do suggest the need for caution in some cases. We have demonstrated that convergence can be problematic in phylogenetic studies and can seemingly overwhelm true phylogenetic signal. A clade that unites species that share a similar, derived ecology should be viewed with some caution, especially if all or most of the characters uniting the clade are potentially adaptations to this shared environment, and if the clade conflicts with trees based on other types of data. A major theme of biology in recent years has been the importance of incorporating phylogeny into studies of evolution and ecology (e.g., Felsenstein, 1985b; Brooks and McLennan, 1991; Harvey and Pagel, 1991; Martins, 1996; Webb et al., 2002). Studies of convergence demonstrate the importance of considering evolution and ecology in analyses of phylogeny.

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APPENDIX 1

SPECIMENS EXAMINED FOR OSTEOLOGICAL CHARACTERS

Eurycea chisholmensis ($n = 4$): Texas: Bell Co.: Salado Springs: TNHC 51141, 51142, 52770, 52771.

Eurycea latitans ($n = 7$): Texas: Kendall Co.: 8.2 mi WNW Boerne, 7-11 Ranch, tributary of Cibolo Creek at Springs: MVZ 121254; 8.3 mi WNW Boerne, 7-11 Ranch, tributary of Bear Creek at Springs: MVZ 121342, 121347, 121354; Pfeiffer's Water Cave, Chester Pfeiffer Ranch on Cascade Caverns Road: TNHC 54356. Kerr Co.: Cherry Creek Ranch, Cloud Hollow Springs: TNHC 59933, 59934.

Eurycea multiplicata ($n = 4$): Missouri: Christian Co.: 3 mi E of Spokane, Busick State Forest, Wood Fork Creek: TNHC 54011, 54013–54015.

Eurycea nana ($n = 4$): Texas: Hays Co.: San Marcos: MVZ 56239A, 56239B, TNHC 52757, 52758.

Eurycea naufragia ($n = 4$): Texas: Williamson Co.: Avent's Spring: TNHC 51026. Williamson Co.: Buford Hollow Springs, just downstream of Lake Georgetown Dam: 51008, 51009, 59691.

Eurycea neotenes ($n = 8$): Texas: Bexar Co.: 5.2 mi WNW Helotes, Helotes Creek, B. Sams Ranch at Springs: MVZ 120084; Helotes Creek Spring: TNHC 52766, 52767; 5.3 mi SE Bulverde, Clear Fork Cibolo Creek at Springs: MVZ 119960, 119961, 119964, 120007, 120009.

Eurycea pterophila ($n = 8$): Texas: Hays Co.: 6.4 mi E Wimberly, Fern Bank Springs: TNHC 52098–52101, 52772, 52773; Blanco Co.: Boardhouse Spring: TNHC 52764, 52765.

Eurycea rathbuni ($n = 2$): Texas: Hays Co.: Ezell's Cave near San Marcos: FMNH 18350; Edwards Aquifer: TNHC 53624.

Eurycea sosorum ($n = 5$): Texas: Travis Co.: Austin, Zilker Park, Eliza Springs (Polio Pit), adjacent to Barton Springs Pool: TNHC 50915, 50921, 50923, 51178, 51179.

Eurycea tonkawae ($n = 9$): Texas: Travis Co.: Stillhouse Hollow: TNHC 50950, 50955, 52759. Bull Creek Spring: TNHC 50963; Canyon Creek, tributary to Bull Creek: TNHC 55144; spring on Wheelis Tract: TNHC 55152; Canyon Vista Spring: TNHC 50961. Williamson Co.: Round Rock, Brushy Creek Spring: TNHC 50991; Round Rock, Krienke Spring: TNHC 53474.

Eurycea tridentifera ($n = 7$): Texas: Comal Co.: Honey Creek Cave: TNHC 31522, 31526, 53856l; 5.5 mi SW Bergheim, Badweather Pit: MVZ 120565, 120566, 120578; 6.0 mi SW Bergheim, Grosser's Sinkhole, MVZ 120583.

Eurycea troglodytes ($n = 4$): Texas: Bandera Co.: Sutherland Hollow: TNHC 52762, 52763. Kerr Co.: Fessenden Spring: TNHC 52760, 52761. These populations were referred to as the Carson Cave group of the *E. troglodytes* complex by Chippindale et al. (2000).

Eurycea sp. ("Comal Springs"; $n = 6$): Texas: Comal Co.: New Braunfels, Landa Park, Comal Springs: MVZ 120417, 120420, 120429, 120447, TNHC 52768, 52769.

APPENDIX 2

CHARACTERS USED IN PHYLOGENETIC ANALYSIS OF CENTRAL TEXAS *Eurycea*

Designation of character states as 0 or 1 does not necessarily indicate ancestral versus derived states.

1. Number of teeth on the fused premaxillae (Potter and Sweet, 1981). Meristic character.

2. Median contact (or fusion) of pars dorsalis of premaxillae: (0) absent, pars dorsalis separate; (1) present.
3. Median contact of frontal bones: (0) absent; (1) present.
4. Posterior border of frontal: (0) rounded; (1) jagged, with irregular projections.
5. Frontal: (0) not expanded laterally at midlength; (1) expanded laterally at midlength (Potter and Sweet, 1981).
6. Orbitosphenoid: (0) present; (1) absent (Wake, 1966).
7. Contact between palatopterygoid and quadrate bones: (0) absent; (1) present (Potter and Sweet, 1981).
8. Mineralization on distal portion of ceratohyal: (0) absent; (1) present.
9. Mineralization on distal portion of ceratobranchial I: (0) absent; (1) present. Mineralization of ceratobranchials II and III has largely the same distribution among taxa as that of ceratobranchial I; thus, only the mineralization of ceratobranchial I was used in the phylogenetic analyses to avoid character nonindependence.
10. Mineralization on posterior portion of second basibranchial: (0) absent; (1) present.
11. Number of vertebrae (Wake, 1966). Meristic character.
12. Distinct basapophyses on centra of some or all presacral vertebra: (0) absent; (1) present (Wake, 1966).
13. Rib of last presacral vertebra: (0) bicapitate; (1) unicapitate, usually through loss of the dorsal process.
14. Coracoids: (0) overlapping medially; (1) separated medially, not overlapping. Some individuals are difficult to score for this character because of damage to the connective tissue surrounding the coracoids.
15. Distal tarsals IV and V: (0) separate; (1) fused (Wake, 1966).
16. Eye size: (0) normal; (1) greatly reduced (Wake, 1966). Although there is some variation in eye size within species, the eyes of *E. tridentifera* and *E. rathbuni* are strikingly reduced. In other ingroup and outgroup taxa, the lenses are invariably present, whereas the lenses are variably absent in *E. tridentifera* and consistently absent in *E. rathbuni* (Wake, 1966). The ratio of eye diameter to head length (tip of snout to center of gular fold) is roughly 0.15-0.26 in most Texas *Eurycea* and roughly 0.09–0.04 in *E. rathbuni* and *E. tridentifera* (Wiens, unpubl. data).