

## EVIDENCE FOR PARALLEL ECOLOGICAL SPECIATION IN SCINCID LIZARDS OF THE *EUMECES SKILTONIANUS* SPECIES GROUP (SQUAMATA: SCINCIDAE)

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**Abstract.**—We identify instances of parallel morphological evolution in North American scincid lizards of the *Eumeces skiltonianus* species group and provide evidence that this system is consistent with a model of ecological speciation. The group consists of three putative species divided among two morphotypes, the small-bodied and striped *E. skiltonianus* and *E. lagunensis* versus the large-bodied and typically uniform-colored *E. gilberti*. Members of the group pass through markedly similar phenotypic stages during early development, but differ with respect to where terminal morphology occurs along the developmental sequence. The morphotypes also differ in habitat preference, with the large-bodied *gilberti* form generally inhabiting lower elevations and drier environments than the smaller, striped morphs. We inferred the phylogenetic relationships of 53 *skiltonianus* group populations using mtDNA sequence data from the ND4 protein-coding gene and three flanking tRNAs (900 bp total). Sampling encompassed nearly the entire geographic range of the group, and all currently recognized species and subspecies were included. Our results provide strong evidence for parallel origins of three clades characterized by the *gilberti* morphotype, two of which are nested within the more geographically widespread *E. skiltonianus*. *Eumeces lagunensis* was also nested among populations of *E. skiltonianus*. Comparative analyses using independent contrasts show that evolutionary changes in body size are correlated with differences in adult color pattern. The independently derived association of *gilberti* morphology with warm, arid environments suggests that phenotypic divergence is the result of adaptation to contrasting selection regimes. We provide evidence that body size was likely the target of natural selection, and that divergences in color pattern and mate recognition are probable secondary consequences of evolving large body size.

**Key words.**—Ecological speciation, heterochrony, parallel evolution, skinks, systematics.

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The idea that ecology can play a major role in species formation dates back to the Evolutionary Synthesis (Mayr 1942; Dobzhansky 1946), but until recently there has been little empirical evidence in favor of ecological speciation, and few methods have been developed to distinguish it from other nonecological speciation mechanisms (e.g., genetic drift, founder events; Schluter 2001). In its most general definition, ecological speciation results from the evolution of reproductive incompatibility as a consequence of divergent natural selection on organismal traits (Schluter 2001). Reproductive isolation is thought to arise secondarily as a by-product of adaptation to alternative environments, with genetic differentiation being facilitated by selection on other traits (i.e., the by-product mechanism; Mayr 1942; Dobzhansky 1951; Schluter 2001). Although recent studies have demonstrated several ways in which divergent selection might promote this scenario (Feder 1998; Nagel and Schluter 1998; Via et al. 2000; Filchak et al. 2000; Podos 2001), few well-supported examples of ecological speciation exist, and more studies involving natural populations are needed to assess its generality.

We have identified a candidate example of ecological speciation in North American scincid lizards of the *Eumeces skiltonianus* species group. Commonly referred to as skinks, these insectivorous lizards have slender bodies with reduced limbs, and characteristically shiny scales that are reinforced with bone (Stebbins 1985). They are well known for their extreme agility and secretive nature. Populations tend to be localized where there is an abundance of ground cover (e.g.,

dead wood, flat rocks, or leaf litter) and are found in a variety of habitats ranging from grassland, pinyon juniper woodland, and open pine forests to broken chaparral (Stebbins 1985). The *skiltonianus* group is widely distributed in the western United States, extending from southwestern Canada into Baja California, and consists of two to five species depending on the authority. The original classification of Taylor (1935) recognizes three species, *E. skiltonianus*, *E. lagunensis*, and *E. gilberti*, and has been accepted by most subsequent authors. However, the monophyly of the group has never been evaluated using phylogenetic methods, and species limits remain controversial and untested.

The ecology and life history of members of the group have been the subject of numerous studies (e.g., Van Denburgh 1896, 1897, 1922; Grinnell 1908; Rodgers and Fitch 1947; Tanner 1957; Lowe and Shannon 1954; Jones et al. 1985), many of which describe contrasting habitat associations between species with alternative morphologies (Rodgers and Fitch 1947; Macey and Papenfuss 1991; Morrison et al. 1999). Among the three currently recognized species, two morphotypes are distinguishable among the adult forms: the small-bodied and striped *E. skiltonianus* and *E. lagunensis* versus the large-bodied and predominantly uniform-colored *E. gilberti*. In general, the large-bodied *E. gilberti* inhabit lower elevations in warmer and drier environments than *E. skiltonianus* and *E. lagunensis* (Rodgers and Fitch 1947; Banta 1962; Macey and Papenfuss 1991; Morrison et al. 1999). Conversely, the small-bodied forms occupy mesic and cooler conditions, such as higher elevations and coastal regions (Rodgers and Fitch 1947; Tanner 1957; Banta 1962; Macey and Papenfuss 1991). The range of *E. gilberti* is largely contained within the ringlike distribution of *E. skiltonianus* (which occupies the majority of the range for the *skiltonianus*

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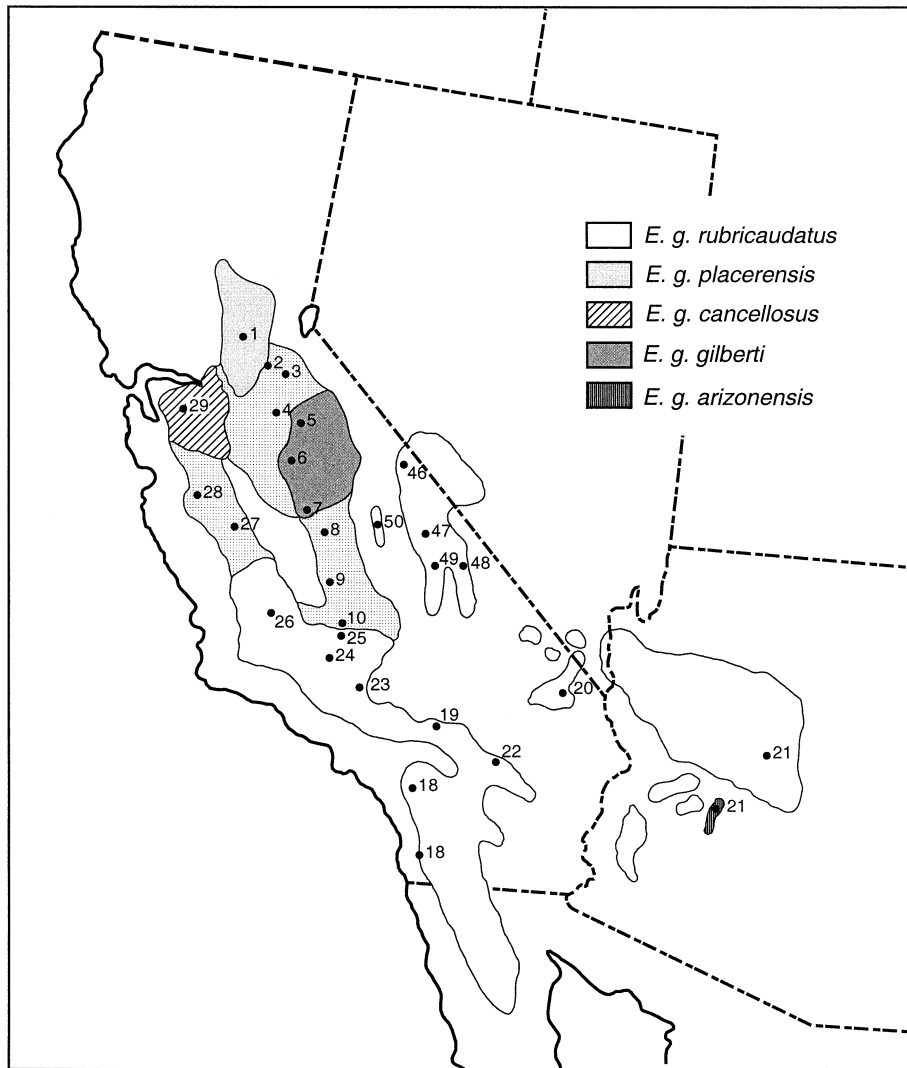


FIG. 1. Distribution of *Eumeces gilberti* (Rodgers and Fitch 1947; Stebbins 1985; Jones 1985). Haplotype localities are indicated by numbered dots, which correspond with localities listed in the Appendix. Stippled areas indicate putative zones of intergradation (Rodgers and Fitch 1947). Note that the distribution of *E. g. arizonensis* is restricted to an 18-km section of riparian woodland along the Hassayampa River near Wickenburg, Arizona (Jones 1985).

group), whereas *E. lagunensis* is allopatrically distributed in the southern portion of the Baja Peninsula (Figs. 1, 2; Stebbins 1985; Grismer 1996). Some areas of sympatry are observed between *E. skiltonianus* and *E. gilberti* in southern California, but the overlap is limited and patchy (J. Q. Richmond, pers. obs.; R. Fisher, pers. comm.).

The morphological diversity in the group has been described in detail, primarily due to the interest of earlier systematists in resolving taxonomic issues (Taylor 1935; Rodgers and Fitch 1947; Tanner 1957; Grismer 1996). Members of the *skiltonianus* group appear to share a common developmental pattern, in which each species passes through markedly similar ontogenetic stages. However, species differ with respect to where their adult morphology occurs on this sequence. In certain areas, neonate morphology is so similar that the different species are indistinguishable (Jones 1985), and some authors have suggested that the three species represent ontogenetic variants of a single polytypic assemblage

(Van Denburgh 1897; Cope 1900; Camp 1916; Grinnell and Camp 1917). All group members share a juvenile color pattern that consists of sharply defined dorsal stripes, a black or dark brown ground color, and bright blue or pink tails (Rodgers and Fitch 1947). As individuals grow, the tail eventually fades to about the same color as the dorsum and the amount of striping retained is related to adult body size. *Eumeces gilberti* gradually loses or greatly reduces the distinctiveness of the striping with maturity, whereas the smaller *E. skiltonianus* and *E. lagunensis* retain the stripes throughout adulthood. With some exceptions, adult *E. gilberti* eventually develop a uniform ground color over the entire body (typically copper brown, drab olive, or bluish gray) and reach body sizes up to 1.5 times larger than *E. skiltonianus* and *E. lagunensis* (Rodgers and Fitch 1947; this study). Because the different terminal morphologies appear to reflect alternative endpoints of a shared ontogeny, diversification has likely been facilitated by heterochronic processes. This hypothesis

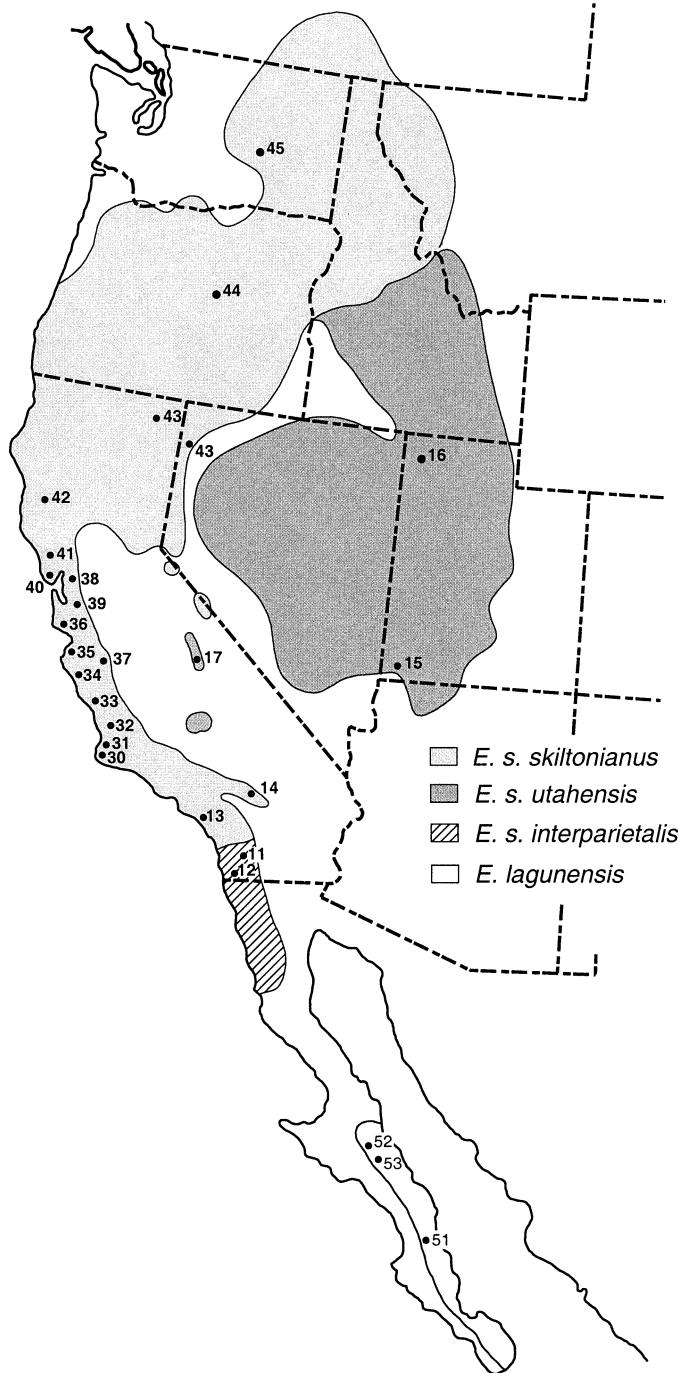


FIG. 2. Distribution of *Eumeces skiltonianus* and *E. lagunensis* (Tanner 1957; Stebbins 1985; Grismer 1996). Haplotype localities are indicated by numbered dots, which correspond with localities listed in the Appendix.

was first alluded to by several early authors (Van Denburgh 1897; Cope 1900; Camp 1916; Grinnell and Camp 1917), but surprisingly little attention has been focused on the role of ontogeny in the phenotypic evolution and speciation of the group.

The association of the large- and small-bodied morphotypes with different preferred environments suggests that natural selection has influenced the phenotypic diversity in the

*skiltonianus* group. This is because body size is often the target of natural selection, and other putative examples of ecological speciation have shown a common tendency for closely related ecomorphs to diverge in size (Schluter and Nagel 1995; Schluter 2001). These differences in body morphology have had important consequences on the speciation process, because mating compatibility is often dictated by body size and in some cases by nuptial coloration (Ratcliffe and Grant 1983; Nagel and Schluter 1998). Previous studies of the *skiltonianus* group provide strong evidence that *E. skiltonianus* and *E. gilberti* do not interbreed in areas of sympatry (Rodgers and Fitch 1947), and females of at least one other North American *Eumeces* species are known to select mates on the basis of body size (Cooper and Vitt 1993). Thus, it seems reasonable to hypothesize that the different adult morphologies serve as a cue for interspecific mate discrimination in the *skiltonianus* group. Whether this qualifies as a case of ecological speciation ultimately rests on the demonstration that contrasting natural selection has promoted the divergence of mate preference (Schluter 2001).

The primary goal of this study was to examine the morphological and phylogenetic diversification in the *skiltonianus* group to evaluate this system as a candidate example of ecological speciation. We inferred the phylogenetic relationships of populations representing all species and subspecies in the group and used these results as a basis for determining the direction of evolutionary change in morphology and for establishing species limits. Our results indicate that independent heterochronic events have led to parallel evolution of three clades characterized by *gilberti* morphology. At least two of these clades are derived from within the more widespread and small-bodied *E. skiltonianus*. The repeated evolution of similar morphology appears to covary with certain environmental conditions (e.g., temperature and moisture), suggesting that natural selection has been the primary cause of phenotypic divergence. Our data also indicate that lineages with different body sizes are reproductively isolated, whereas lineages that have evolved similar body morphology may not be.

MATERIALS AND METHODS

*Taxon Sampling*

Geographic sampling encompassed nearly the entire range of the *skiltonianus* group (sample localities are provided in Appendix). Field collecting emphasis was placed on populations of *E. gilberti* and *E. skiltonianus* in California because most of the sympatry and intraspecific morphological variation is concentrated in this region. Sampling was intentionally broad to identify potential contact zones among genetically divergent populations and to determine if mitochondrial DNA (mtDNA) structuring correlates with patterns of intraspecific variation in morphology. Individuals of *E. gilberti* were sampled from 26 populations in California and Arizona, representing each of the five currently recognized subspecies (Fig. 1). Samples from 25 populations representing the three subspecies of *E. skiltonianus* were obtained from California, Utah, Oregon, and Washington, and the monotypic *E. lagunensis* was sampled from three localities in Baja California (Fig. 2).

TABLE 1. Oligonucleotide primers used in this study.

Primer	Sequence (5'-3')	Position <sup>1</sup>
ND4Gold	CTTTGACTYCCMAARGCCCACGTAGA	10768
ND4B	CTCATTCAAACCCCRGTGAAGCCT	11031
LEU <sup>2</sup>	TACTTTTACTTGGATTTCACCA	11691
tleu2c	TTGACTTTGATCCTTTRAAAGTGAG	11639

<sup>1</sup> 5' nucleotide position in the *Eumeces egregius* mtDNA sequence (Kumazawa and Nishida 1999).

<sup>2</sup> Primer is from Forstner et al. (1995).

The genus *Eumeces* is widespread throughout Southeast Asia, northwest Africa, north America, Mexico, and Central America (Taylor 1935). Selection of an appropriate outgroup for phylogenetic analysis was difficult because hypotheses of species-level relationships within *Eumeces* have never been tested using modern phylogenetic methods. To accommodate this uncertainty, we selected outgroups across a range of different species groups (following Taylor 1935) and included the following Asian and New World species: *E. egregius* (*egregius* group); *E. brevirostris* (*brevirostris* group); *E. fasciatus*, *E. inexpectatus*, *E. laticeps* (*fasciatus* group); *E. obsoletus* (*obsoletus* group); *E. septentrionalis* and *E. tetragrammus* (*anthracinus* group); and *E. quadrilineatus* (*quadrilineatus* group). *Neoseps reynoldsi* was also included because it may represent a lineage derived from within *Eumeces* (Telford 1959). Because we had no a priori evidence as to which species or species group is most closely related to the *skiltonianus* group, we evaluated tree topology on an overall unrooted network resulting from simultaneous analysis of all ingroup (the *skiltonianus* group) and outgroup species. Special consideration was given to the Asian *E. quadrilineatus* because it has been previously considered a close relative (Taylor 1935) or an actual member of the *skiltonianus* group (Lieb 1985).

#### DNA Amplification and Sequencing

Total DNA was extracted from muscle or liver using standard proteinase-K and phenol/chloroform extraction methods (Hillis et al. 1996). The polymerase chain reaction (PCR) was used to amplify a 900-bp fragment of the mitochondrial genome encompassing much of the ND4 protein coding gene and the flanking histine, serine, and leucine tRNA genes. The primers used for amplification are listed in Table 1. Typical PCR parameters were as follows: 94°C for 30 sec, 47–50°C for 30 sec, and 72°C for 30 sec. Sufficient product for direct DNA sequencing was generally obtained after 30 cycles and unincorporated primers and nucleotides were removed by PEG precipitation. DNA templates were sequenced using a dye-labeled dideoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA) on an ABI 373A or 377 automated DNA sequencer. DNA sequences were linked and edited using Sequencher v3.0 (Gene Codes Corp., Ann Arbor, MI) and aligned using Clustal W (Thompson et al. 1994). The tRNA sequences lacked problematic insertions and/or deletions and were easily aligned without using structural models. Sequence data for *E. egregius* was obtained from Genbank (accession number NC 000888; Kumazawa and Nishida 1999).

#### Phylogenetic Analyses

We conducted phylogenetic analyses using both maximum parsimony (MP) and maximum likelihood (ML) to ensure that the different methods were generating similar results. The data were first subjected to heuristic MP searches (100 random taxon-addition-sequence replicates, tree-bisection-reconnection [TBR] branch swapping) with three weighting schemes: uniform weighting, transversion weighting, and successive weighting. The transversion weighting scheme down-weighted transitions according to the substitution bias estimated using ML under the HKY85 + I +  $\Gamma$  model. This model was used to estimate the transition bias because failure to account for among-site rate variation can cause an underestimation of the transition:transversion ratio (ti:tv; Wakeley 1994). Successive character weighting was performed with characters iteratively reweighted based on the maximum re-scaled consistency index among uniformly weighted parsimony trees (base weight = 10; Farris 1969; Swofford et al. 1996). All analyses were conducted using PAUP version 4.0b6 (Swofford 2001).

Models of sequence evolution for the ML analyses were evaluated using the program Modeltest version 3.0 (Posada and Crandall 1998). In all, 56 different models of sequence evolution were tested using the likelihood-ratio test statistic, as described in Huelsenbeck and Crandall (1997). Five topologies were randomly selected from the initial pool of all parsimony trees to determine the preferred model of nucleotide substitution. Our assumption was that convergence on the same model using different parsimony topologies indicates the best fit to the data. Once the appropriate model was selected, the parsimony tree with the best overall likelihood score was used to infer model parameters for the initial likelihood search (Sullivan et al. 1996). The tree(s) generated from the first likelihood search was then used to estimate model parameters again, and this iterative process was repeated until the same topology and parameter estimates were obtained in successive searches (Wilgenbusch and de Queiroz 2000).

Support for inferred clades was assessed by nonparametric bootstrapping (Felsenstein 1985a; Hillis and Bull 1993). For both the equally and differentially weighted MP analyses, 500 bootstrap replicates were performed using heuristic searches (three random-taxon-addition-sequence replicates; TBR branch swapping). Because of the large number of taxa, bootstrapping using ML was prohibitively slow, so we decreased computation time by using the nearest-neighbor-interchange option (NNI) for branch swapping and by performing 100 bootstrap pseudoreplicates with a maximum of one tree saved in each replicate. Reducing the search effort is not expected to significantly affect the bootstrap values of nodes that are well supported in the initial parsimony analyses (DeBry and Olmstead 2000). Clades with bootstrap values  $\geq 70\%$  were considered strongly supported (Hillis and Bull 1993, but see their caveats).

#### Morphology

Evolutionary changes in adult body size and color pattern were evaluated by mapping these characters onto the ML phylogeny (see justification in the Results section). A total

of 564 museum specimens were measured in this study. Some data were culled from the literature (Taylor 1935; Rodgers and Fitch 1947; Tanner 1957; Grismer 1996) and from an undergraduate senior thesis by Dustin A. Wood (unpubl., San Diego State University). Ancestral character state reconstructions were performed in MacClade version 3.08 (Maddison and Maddison 1999).

Snout-vent-lengths (SVL) were used as a standard for body size comparisons and were treated as ordered character states. This measurement represents the distance from the tip of the snout to the posterior edge of the preanal scales that lie anterior to the vent and is a common proxy for body size in herpetological studies. SVLs were obtained from a series of adult individuals collected at or near the same localities as those sampled for mtDNA and were treated as continuous character states. The maximum SVL of a given series was used for comparative analysis and was assumed to represent a genetically determined maximum body size for individuals of a specific geographic area. As such, each terminal taxon in the mtDNA phylogeny was assigned a SVL that corresponded to the largest individual of the appropriate geographic series. Body size evolution was reconstructed using the method of squared-change parsimony (Maddison 1991). Color pattern was scored as a categorical character with three states, one corresponding to the retention of stripes in adults, a second corresponding to the partial retention of the striped pattern in adults, and a third corresponding to the complete loss of stripes. Transformations between states were ordered, with the partial retention of stripes being the intermediate condition. The use of three character states accounted for the incomplete loss of stripes in some *E. gilberti* populations.

Because of the apparent association between body size and the loss or retention of the striped color pattern, we tested for a correlation between these two characters using the method of phylogenetically independent contrasts (Felsenstein 1985b). The data were analyzed using the BRUNCH algorithm in the software package Comparative Analysis by Independent Contrasts (CAIC; Purvis and Rambaut 1995). Under the null hypothesis that evolutionary changes in body size (dependent variable) are independent of developmental shifts in color pattern, it is expected that half the contrasts in body size will be positive and half will be negative, with a mean contrast value of zero. We evaluated the null hypothesis using a two-tailed sign test to determine whether there was a significant bias toward positive scores. A significant result indicates that evolutionary change in adult color pattern is phylogenetically correlated with the evolution of increased body size.

Analyses of character mapping and independent contrasts were limited to the ingroup only because of the lack of strong support regarding which species or species group is most closely related to the *skiltonianus* group (and weak bootstrap support for outgroup relationships in general). As such, outgroup taxa were pruned from the analysis to minimize any inconsistencies associated with the placement of outgroups. This approach had little effect on character state reconstruction and independent contrasts, except at the basal split of the ingroup topology (see Results section).

## RESULTS

### *Haplotype Diversity and Optimal Trees*

Fifty unique haplotypes were discovered across 53 different populations (1–4 individuals/population), with sequence divergences reaching greater than 20% among some ingroup lineages. A total of 900 nucleotide positions were aligned, 400 of which were variable (289 within the ingroup) and 313 of which were parsimony informative (233 within the ingroup). Of the 330 variable sites within the ND4 protein-coding region, 25% were in the first codon position, 7% in the second position, and 68% in the third position. The remaining 70 variable sites (49 parsimony informative) were dispersed among the tRNA gene sequences.

An initial heuristic search using uniformly weighted characters resulted in 24 shortest trees (length [L] = 1402). Successive weighting on these trees resulted in two shortest trees (L = 1403) that were similar to the original 24, but not identical. Estimates of the ti:tv ratio were close to 6:1 across the initial topologies, and transversion weighted analysis implementing a 6:1 step matrix resulted in 12 shortest trees (L = 1408). These trees were also similar to those obtained from the previous weighting schemes. The few minor differences in topology among weighting schemes primarily involved the placement of *E. brevirostris* and the relationships within clusters of similar haplotypes.

Likelihood-ratio tests revealed that the GTR + I +  $\Gamma$  substitution model was consistently preferred over the remaining 55 less general models of sequence evolution, and it was a significant improvement over the next most restrictive model (GTR +  $\Gamma$ ;  $P < 0.001$ ). A ML search using substitution model parameters estimated from one of the two MP trees with the highest likelihood score resulted in a single optimal tree ( $-\ln$  likelihood = 7879.80; not shown). Two searches using re-estimated parameters from successive ML trees resulted in the same topology, but with slightly better likelihood scores (Fig. 3; final tree,  $-\ln$  likelihood = 7866.04). The substitution model parameters for the optimal maximum likelihood tree are shown in Table 2.

Although we explored different methods of phylogenetic analysis, we favor the ML tree because ML incorporates important features of molecular evolution that are pertinent to our mtDNA dataset (e.g., among-site rate variation and unequal base frequencies), provides an objective method for character weighting (Felsenstein 1981), and has been shown to be a reliable estimator of phylogeny under simulated conditions (Huelsenbeck 1995). Thus, the remaining sections of the paper refer to the ML topology unless otherwise stated. It should be noted, however, that the optimal ML tree was similar to the trees generated in the MP searches.

### *Phylogeographic Patterns*

The monophyly of the *skiltonianus* group is supported by high bootstrap values under MP and ML (Fig. 3). Nine major clades are consistently recovered in all analyses, each of which corresponds to distinct, well-supported geographic units (bootstrap values  $\geq 70\%$ ; Fig. 3). These clades are exclusive with respect to either the *skiltonianus* or *gilberti* morphotypes and contain representatives of only one of the cur-

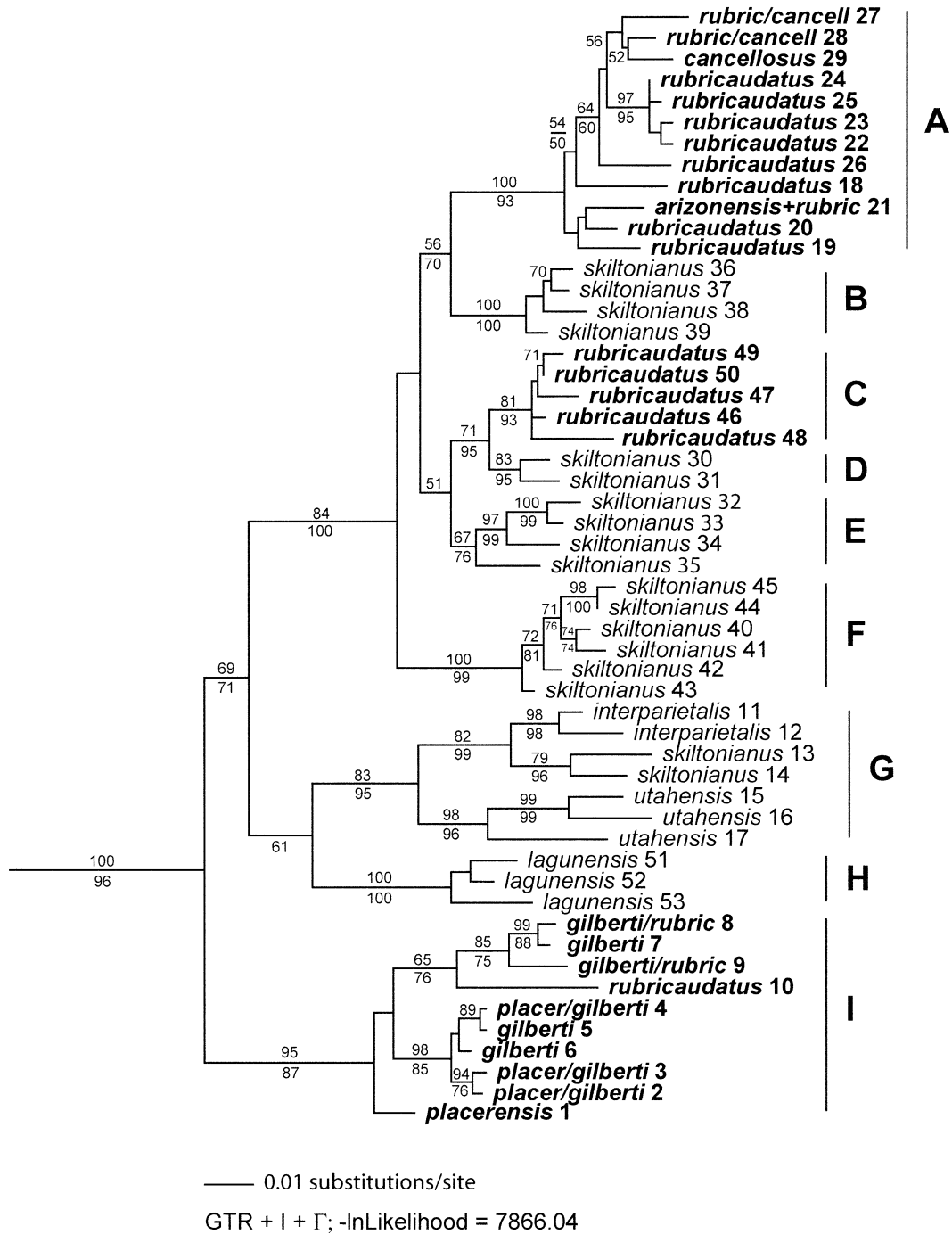


FIG. 3. Phylogeny of the *Eumeces skiltonianus* group inferred from the maximum-likelihood (ML) analysis. Taxa are labeled according to the subspecies that corresponds with the locality of the sampled individual, and names separated by a forward slash indicate intermediate forms (following Rodgers and Fitch 1947). Clade labels are as follows: (A) southwestern *E. gilberti*; (B) San Francisco Bay *E. skiltonianus*; (C) Inyo County *E. gilberti*; (D) Santa Barbara County *E. skiltonianus*; (E) central coastal *E. skiltonianus*; (F) Pacific Northwest *E. skiltonianus*; (G) southern *E. skiltonianus*; (H) *E. lagunensis*; and (I) Sierran *E. gilberti*. Parsimony bootstrap values for the equally weighted analyses are shown above the branches and ML bootstrap values are shown below the branches. Bootstrap values < 50% are not shown.

rently recognized species. However, with the exception of *E. lagunensis*, they do not correspond to traditionally named taxonomic units. Patterns of variation indicate that *E. gilberti* and *E. skiltonianus* are poly- and paraphyletic, respectively. Lineages currently recognized as *E. gilberti* form three dis-

tantly related clades, two of which are nested within *E. skiltonianus* (clades A and C) and a third (I) that comprises the sister group to all remaining ingroup lineages (Fig. 3). *Eumeces skiltonianus* is divided into five major clades (B, D, E, F, and G), with B and D each sharing a most recent com-

TABLE 2. The GTR + I +  $\Gamma$  substitution model parameters for the optimal maximum-likelihood tree. (A) Nucleotide substitution parameters (transition headers are in bold). (B) Base frequency parameters.

A.						
<b>C ↔ T</b>	<b>A ↔ G</b>	A ↔ C	A ↔ T	C ↔ G	I	$\alpha$
6.78	15.81	0.64	0.56	1.00	0.47	1.16
B.						
Nucleotide Frequency	$\Pi_A$	$\Pi_G$	$\Pi_C$	$\Pi_T$		
	0.34	0.32	0.11	0.23		

mon ancestor with the independently evolved *E. gilberti* clades A and C, respectively. The Baja Peninsular species, *E. lagunensis* (H), is also nested within the morphologically similar *E. skiltonianus*.

A phylogeographic overview of the ingroup is shown in Figure 4. Although the nine major clades show a high degree of regional integrity, nearest neighboring clades are not always closely related, nor are the lowest sequence divergences always between clades of the same morphology. One of the most striking results is the distant relationship between geo-

graphically adjoining *E. gilberti* clades at the juncture of the southern Sierra Nevada and Tehachapi Mountains (A vs. I; Fig 4.). North of this mtDNA contact zone, an exclusively Sierran *E. gilberti* clade (I) forms the sister taxon to all remaining lineages of the *skiltonianus* group (A–H; Fig. 3). This clade includes two *E. gilberti* subspecies that are diagnosed primarily by their bright blue juvenile tails, which strongly resemble those in *E. skiltonianus*. The more northern *E. g. placerensis* extends south of El Dorado County (and probably includes unsampled populations up to the Yuba River in Placer Co.) and gradually intergrades with *E. g. gilberti* at approximately the same latitude as the San Francisco Bay (Rodgers and Fitch 1947). The subspecies distributions roughly correspond with the mtDNA phylogeographic structure. However, the precise borders between morphologically pure forms are nebulous, and much of our sampling includes populations that are currently recognized as intermediate.

The *gilberti* populations comprising the southern Sierran/Tehachapi boundary for clades A and I (populations 25 and 10, respectively) have high sequence divergence (see below) and are widely separated on the tree. Specimens collected during this study show that intergrades between the blue-

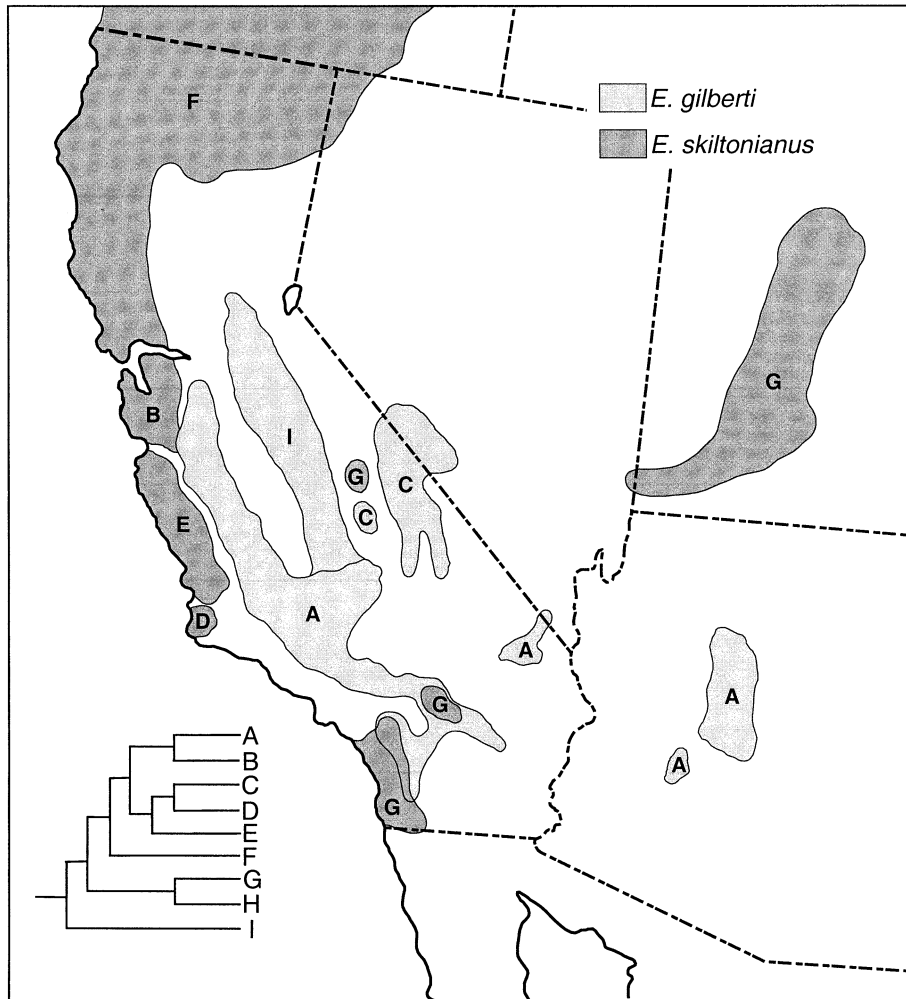


FIG. 4. Map showing the major phylogeographic units within *Eumeces gilberti* and *E. skiltonianus*. Letters refer to clade labels in Figure 3 (Clade H not shown).

tailed *E. g. gilberti* and the pink-tailed *E. g. rubricaudatus* exist in this same general area, with a relatively abrupt transition to pink-tailed populations south of the contact zone (Fig. 1). These pink-tailed populations fall within a second and more recently derived *gilberti* assemblage (A), which is the most broadly distributed of the three *E. gilberti* clades. The majority of the range is recognized as the largest of the five *gilberti* subspecies, *E. g. rubricaudatus* (Rodgers and Fitch 1947). It extends from southern California (Fig. 3; 18) along the western periphery of the San Joaquin Valley up to about the same latitude as the San Francisco Bay (Fig. 3; 26–29). Haplotype 29 represents pure *E. g. cancellosus*, whereas 27 and 28 represent putative intermediates between *E. g. cancellosus* and *E. g. rubricaudatus* (Rodgers and Fitch 1947). These intermediates span much of the inner Coast Ranges, and blend imperceptibly into *E. g. rubricaudatus* in Kern County (Fig. 1). The clade also extends south of the Tehachapi Mountains along the eastern slopes of the Transverse Ranges, and well into the desert regions of southern San Bernardino County (19 and 22–24). Populations traverse eastward from the mouth of the Mojave River in the northern San Bernardino Mountains (19), and occur discontinuously across the eastern Mojave Desert (20). Individuals sampled from two Arizona localities share the same haplotype (21) and are also included in this Mojave phylogeographic axis. The southernmost locality in Arizona represents *E. g. arizonensis*, whereas the more northern population is considered to be *E. g. rubricaudatus* (Jones 1985).

A central-eastern *E. gilberti* clade (C; hereafter called the Inyo clade due to its regional affinity with Inyo County) includes populations that are restricted to the low and middle elevations of the White, Inyo, Argus, and Panamint Ranges (46–49), as well as a population in the eastern Sierra Nevada (50; Figs. 3, 4). This group represents a third independently derived *gilberti*-like clade that is most closely related to coastal lineages of *E. skiltonianus* (31, 32) and has been recognized as *E. g. rubricaudatus* on the basis of adult color pattern. However, we have found that Inyo *E. gilberti* have smaller body size compared to most other *rubricaudatus*-like populations in southern California, and localities in the Panamint Mountains have blue-tailed juveniles (rather than the reddish pink tails that *rubricaudatus* individuals were named for).

The most basal *E. skiltonianus* clade (G) includes localities from southern California, the eastern Sierra Nevada, and Utah (Fig. 4). Structuring within this group shows two well-supported subclades, one including southern California lineages (11–19) and the other consisting of eastern Sierra Nevada and Utah lineages (15–17). The geography of the two subclades largely corresponds with the previously described distributions of *E. s. interparietalis* and *E. s. utahensis*, respectively. Clade G also represents the sister group to a monophyletic *E. lagunensis* (H), but this more inclusive clade is weakly supported by the mtDNA data. *Eumeces lagunensis* is morphologically similar to *E. skiltonianus* from San Diego County and northern Baja California, and differs in minor yet consistent features of squamation. Clade G + *E. lagunensis* forms the sister group to a large clade comprising the remaining ingroup lineages, and is considered strongly supported (although marginally; bootstrap values = 69% MP and 71% ML).

A widespread Pacific Northwest clade of *E. skiltonianus* (F) represents the sister group to clades A–E, but the placement of this clade is not strongly supported (Fig. 3). The morphology of *E. skiltonianus* occurring throughout the Pacific Northwest is similar to that of contiguous populations along the coast of California, all of which are placed in a single subspecies, *E. s. skiltonianus*. Haplotypes north of San Francisco Bay fall within the Pacific Northwest clade, whereas haplotypes south of the bay are regionally partitioned into three distinct clusters (Fig. 4). The most northerly clade of the central coast (B) is restricted to the eastern side of San Francisco Bay and extends to the mouth of the Salinas Valley at roughly the same latitude as Monterey Bay. Another mtDNA clade (E) extends south of Monterey Bay into southern San Luis Obispo County, where it geographically adjoins the third clade (D) in the vicinity of Point Conception in Santa Barbara County. Clades B and D are most closely related to *E. gilberti* clades A and C, respectively, despite having a continuous distribution among morphologically similar populations along the central coast. The southern and eastern limits of the Santa Barbara clade (D) are obscured because of sampling gaps, and further collecting in Kern County may narrow the break with the Inyo *E. gilberti* clade (C).

Our mtDNA data consistently demonstrate that nearest neighboring clades and populations are not necessarily closely related. In fact, some of the highest sequence divergences are observed among geographically adjacent clades of the same morphology. For example, corrected divergence (GTR + I +  $\Gamma$  model) for *E. gilberti* populations in the Tehachapi contact zone is 19.1% (10 vs. 25; Fig. 3), and *E. skiltonianus* populations in the San Francisco Bay area (e.g., 38 vs. 40) are 8.8% divergent. Sympatric *E. gilberti* and *E. skiltonianus* clades in southern California (clades A and G, respectively) are not sister groups, and populations in nearest proximity (*E. skiltonianus* 13 vs. *E. gilberti* 18) are 14.9% divergent. Likewise, *E. gilberti* are parapatric or narrowly sympatric with *E. skiltonianus* in the Gabilan–San Benito County region (28 vs. 37, respectively), but the two populations are nested within separate clades. The mtDNA topology is also inconsistent with ordered or directional geographic trends in a number of cases. For example, a stepwise, north-to-south invasion of skinks across a particular region should reveal a pectinate topology that shows northern populations at the base of a clade, then gradually more southerly populations with subsequent nested branches. However, subdivisions within the continuously distributed *E. gilberti* populations of the Sierran (I) and southwestern (A) clades indicate that most territories were not colonized in a directional fashion. Similar situations are observed in central coastal *E. skiltonianus* (B, D, E, and lineages therein; Fig. 3). These patterns indicate that many of the current distributions reflect secondary overlap between clades that have diversified in allopatry.

#### Outgroup Relationships

The relationships among outgroup species are incongruent with the current *Eumeces* classification (Fig. 5). For example, members of the *fasciatus* group (*E. fasciatus*, *E. laticeps*, and *E. inexpectatus*) do not form a monophyletic clade in any possible rooting of the overall phylogeny, but are interspersed

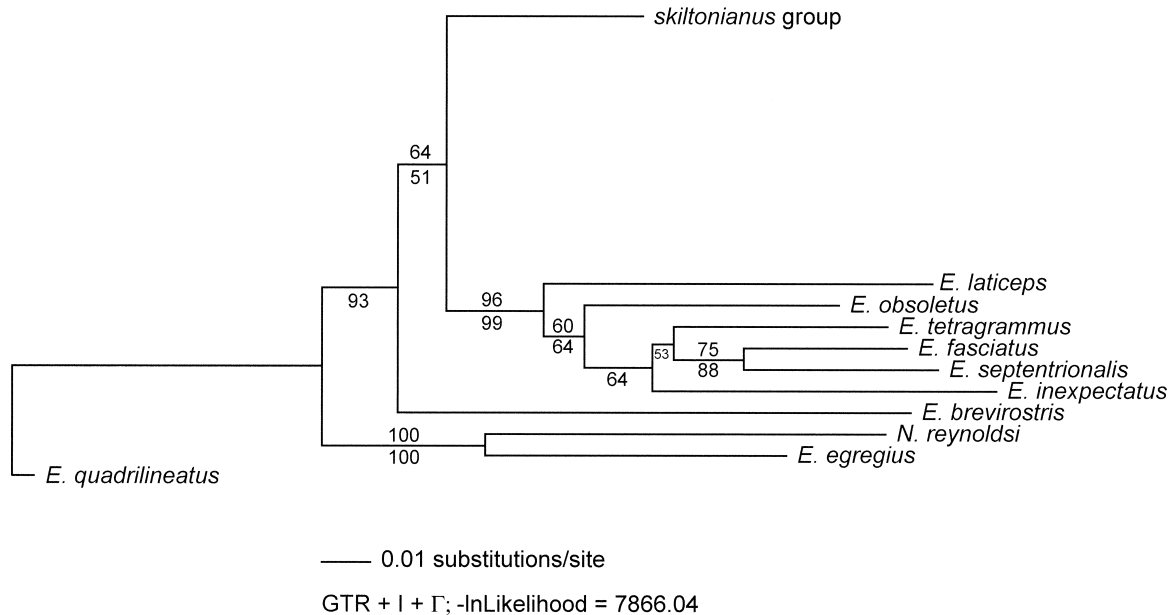


FIG. 5. Outgroup relationships inferred from maximum-likelihood analysis. The topology shown is rooted with *Eumeces quadrilineatus* because of its geographic distribution in Southeast Asia and high sequence divergence from North American *Eumeces*.

among exemplars from other species groups. The mtDNA data show strong support for the sister group relationship between *E. egregius* and *N. reynoldsi* (bootstrap values = 100 for MP and ML), corroborating earlier suggestions that the sand-dwelling *N. reynoldsi* is closely related to members of the *egregius* species group in Florida (Telford 1959) rather than to other geographically disparate scincids with similar morphology (e.g., degenerate limbs, absence of an external ear opening, and modified skulls for burrowing). Our results supported only a distant relationship between the *skiltonianus* group and *E. quadrilineatus* (Fig. 5), indicating that this morphologically similar Asian species represents a separate entity with respect to the North American *skiltonianus* group.

It should be noted that most outgroup relationships were weakly supported under both ML and MP, and it remains unclear which species or species group is the nearest relative of the *skiltonianus* group. Increased sampling of *Eumeces* throughout the range should provide a better understanding of the relationships among these species and provide further insight regarding the taxonomic validity of the currently recognized species and species groups.

#### Reconstruction of Body Size and Adult Color Pattern

Figure 6 shows the reconstruction of body size and color pattern evolution on the ML topology. Body size ranges and the corresponding adult color states are provided in Table 3. Character reconstruction is unambiguous for essentially all portions of the *skiltonianus* group phylogeny. Large body size and ontogenetic color change defines each of the major *E. gilberti* lineages, whereas retention of striping and smaller size characterizes all clades of *E. skiltonianus* and *E. lagunensis*. Six most parsimonious reconstructions are observed for color pattern, but in each case the alternatives do not conflict with the association of synchronous changes in color pattern and body size.

Alternative rootings of the overall phylogeny result in equivocal reconstruction at the basal node of the ingroup. A small-bodied and striped common ancestor would indicate three independent origins of the *gilberti* morphotype (clades A, C, and I). However, if the ancestor of the group was large-bodied, clades A and C would represent independent reversals to large body size and a loss of stripes, with the basal *E. gilberti* clade (I) retaining the plesiomorphic condition. Although the ancestral condition for the *skiltonianus* group is ambiguous, the relationship of body size and color pattern conforms to the expected pattern in all cases. Independent contrast analysis revealed a significant bias toward positive scores (6/6 positive contrasts;  $P = 0.03125$ ; two-tailed sign test), indicating that the loss or reduction of the striped color pattern in adults is correlated with the evolution of increased body size. Comparisons were limited to six branches on which there were ontogenetic changes in color pattern.

Individuals of two populations within the southwestern *E. gilberti* clade A (represented by 20 and 21; Fig. 3) show a reversal toward smaller body size and a tendency to retain faded striping in adults. Haplotype 21 is found in two separate populations in Arizona, only one of which shows the reversal and is recognized as *E. g. arizonensis*. The other consists of individuals with *rubricaudatus* morphology and is located farther to the north along the same river drainage. Specimens collected from the Granite Mountains in the eastern Mojave Desert (20) also have the *arizonensis* phenotype. These reversals appear to occur in isolated populations that persist in the southwestern deserts, where permanent or semipermanent water sources create suitable riparian habitat within warm, arid environments. Similar situations are observed in some Inyo *E. gilberti* populations (C). Although body morphology has reversed to a more *skiltonianus*-like condition, SVL of individuals at these localities is still substantially larger than in *E. skiltonianus*, and the striped pattern is either lost or significantly faded.

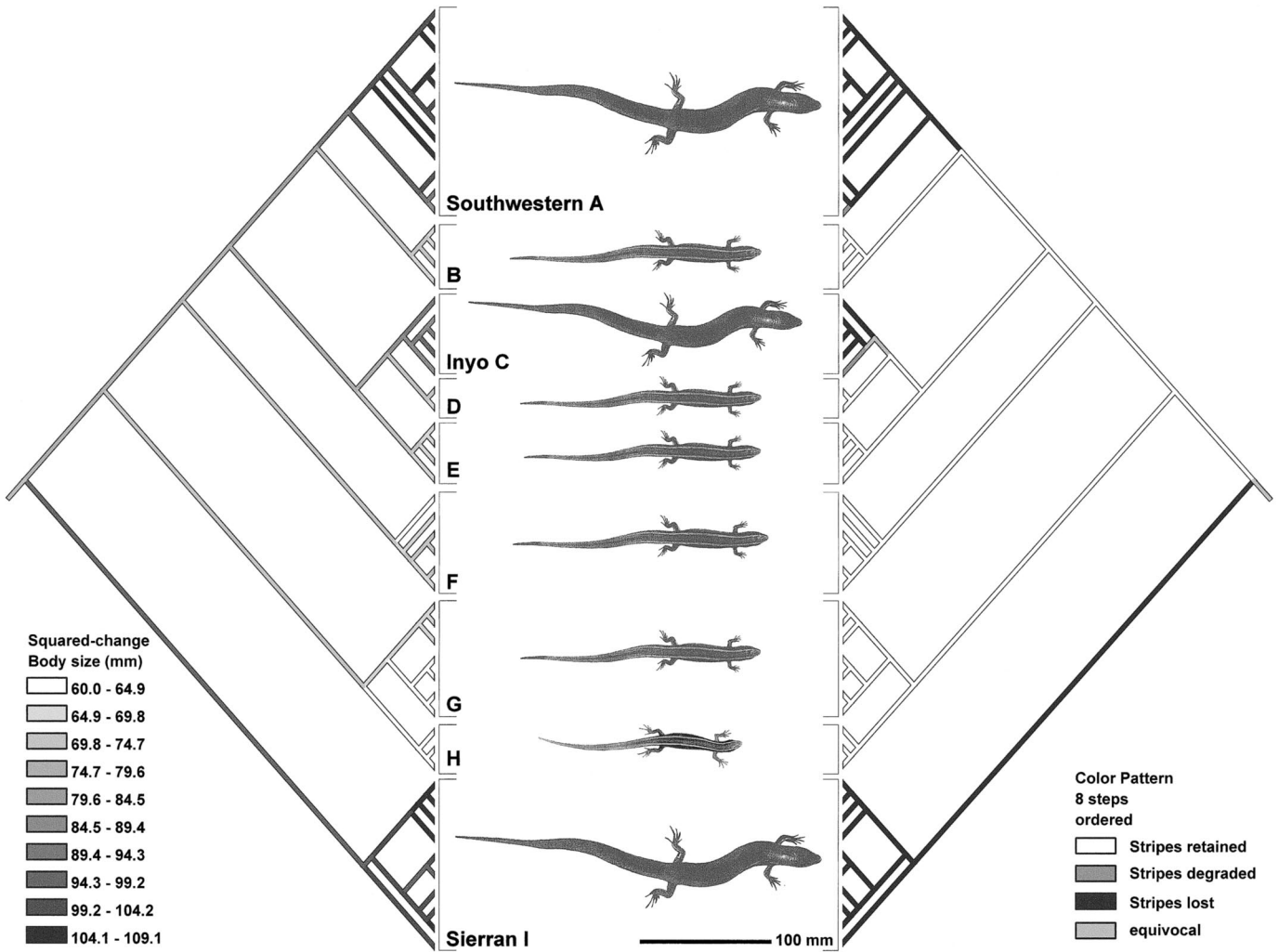


FIG. 6. Phylogenetic reconstruction of body size (left) and color pattern (right) for the *Eumeces skiltonianus* group. Characters are mapped onto the maximum-likelihood topology. Skinks are shown to scale and represent the largest individual for a given geographic clade. Illustrations were redrawn from photographs in Taylor (1935) and Smith (1946) by J. Richmond.

TABLE 3. Body size and color pattern for *Eumeces* mitochondrial DNA clades. Values for snout-vent length (SVL) represent the average  $\pm$  standard deviation and the range of SVLs obtained for each major clade (following Fig. 3). Note that clades A and C contain lineages that are characterized by reversals to smaller body size and partial retention of stripes.

Clade	Morph	n	SVL (mm)	Color pattern
Southwestern (A)	<i>gilberti</i>	134	85.2 $\pm$ 10.7 (55.2–109.1) <sup>1</sup>	stripes lost/reduced
San Francisco Bay (B)	<i>skiltonianus</i>	61	65.0 $\pm$ 5.1 (48.0–73.9)	stripes retained
Inyo Co. (C)	<i>gilberti</i>	25	80.2 $\pm$ 6.9 (66.8–97.9)	stripes lost/reduced
Santa Barbara Co. (D)	<i>skiltonianus</i>	14	62.3 $\pm$ 7.3 (47.0–70.7)	stripes retained
Central Coastal (E)	<i>skiltonianus</i>	24	61.6 $\pm$ 5.7 (49.7–69.5)	stripes retained
Pacific Northwest (F)	<i>skiltonianus</i>	135	63.8 $\pm$ 5.1 (49.0–75.0)	stripes retained
Southern + Utah (G)	<i>skiltonianus</i>	77	62.0 $\pm$ 4.4 (52.1–70.5) <sup>2</sup>	stripes retained
Baja California (H)	<i>lagunensis</i>	24	57.2 $\pm$ 2.0 (54.8–60.0) <sup>3</sup>	stripes retained
Sierran (I)	<i>gilberti</i>	194	88.1 $\pm$ 10.5 (51.0–108.9)	stripes lost

<sup>1</sup> Includes SVL data from Jones (unpubl. data).

<sup>2</sup> Includes SVL data from Tanner (1957).

<sup>3</sup> Includes SVL data from Grismer (1996).

## DISCUSSION

A key step in demonstrating ecological speciation is showing that reproductive isolation evolves as a consequence of divergent natural selection on specific traits (Schluter 2001). Evidence for natural selection is obtained when parallel morphology occurs in separate lineages experiencing similar environments (Schluter and Nagel 1995; Schluter 2001). We consider parallelism to be the result of the same character transformations occurring independently in closely related lineages via modifications of a shared developmental pathway (Futuyama 1997). The similarity in early ontogenetic trajectories and the close association between differences in body size and color pattern provide strong support for parallel evolution of the *gilberti* morphotype. The relationship of divergent morphology with different environments, combined with evidence of reproductive isolation between lineages with alternative morphologies, supports a model of ecological speciation in the *skiltonianus* group.

The following section begins with a discussion of the developmental mechanisms responsible for parallel morphological evolution, as well as the ecological factors that might be associated with the repeated shifts to *gilberti* morphology. Given evidence that body morphology may play a role in assortative mating, we then discuss several lines of evidence supporting this system as an example of parallel speciation, a special case of ecological speciation in which the same (or compatible) mate recognition system evolves independently in clades experiencing similar environments (Schluter and Nagel 1995; Schluter 1998, 2001). A precursor for understanding the speciation process is the determination of which ingroup lineages constitute species. Therefore, the final section addresses species limits.

*Heterochrony and Parallel Morphological Evolution*

Lineages comprising the three *E. gilberti* clades exhibit growth stages that outwardly recapitulate the adult phase of *E. skiltonianus*, with endpoints reflecting an apparent extrapolation of the *E. skiltonianus* developmental trajectory (Camp 1916). The repeated derivation of *gilberti* morphology within *E. skiltonianus* is consistent with the process of peramorphosis, a heterochrony recognized as a shift in the descendant species ontogeny that results in a terminal morphology that transcends the ancestral condition (Gould 1977; Alberch et al. 1979; Reilly et al. 1997). The phylogeny also indicates that reversals have occurred in certain lineages, in which case the descendant morphology reflects truncated development rather than terminal additions (i.e., paedomorphosis). For example, some *E. gilberti* populations in the Mojave and Sonoran Deserts have smaller adult body sizes and tendency to retain degraded striping (i.e., lineages 20 and 21, Fig. 3). These populations are nested within a clade that consists primarily of the largest *E. gilberti* lineages, which unequivocally lose the stripes with maturity. Similar paedomorphic processes might also have influenced the evolution of *E. lagunensis* (H; Fig. 3), which are smaller compared to their closest *E. skiltonianus* relatives and often maintain the dark juvenile ground color and distinctive striping throughout adulthood (Grismer 1996). Thus, the adult phenotypes within

the *skiltonianus* group might be best described as different endpoints along a shared developmental pathway.

Our study emphasizes the phylogenetic aspects of heterochrony, a necessary first step for determining the polarity and frequency of putative heterochronic transformations. However, the quantification of growth data is also needed for assessing the specific types of heterochrony that fall within the broader context of paedo- and peramorphosis (Alberch et al. 1979; Reilly et al. 1997). This is especially important given that developmental modifications other than rate or timing changes can give rise to phenotypes that appear to be caused by heterochrony (Raff 1996; Rice 1997). Earlier studies have provided some insight on growth trajectories for different *E. skiltonianus* populations (Rodgers and Memmler 1943; Tanner 1957), but similar information is currently unavailable for *E. gilberti*. The lack of such data makes it difficult to render any definitive classification of the specific type(s) of heterochrony. However, our study shows that phyletic shifts in ontogeny have repeatedly occurred in multiple directions in independent lineages, with color pattern and body size evolving in a coordinated and consistent manner.

*Factors Influencing the Evolution of Morphology*

Heterochronic processes have served as the probable mechanism by which terminal morphologies have shifted, and the correlation between the loss of stripes and large body size suggests that selection on one character could, as a side-effect of global heterochrony, also produce a change in the other. We think it is significant that other examples of ecological speciation consistently demonstrate that body size is a predominant factor in driving species formation. Reproductive isolation frequently depends on body morphology, and closely related species commonly diverge in body size (Schluter and Nagel 1995). Furthermore, body size is often targeted by natural selection because of the far-reaching implications on physiology and life history. It is also worth noting that studies of female mate choice in broad-headed skinks, *Eumeces laticeps*, found no evidence that females choose mates on the basis of conspicuous features of color pattern, but rather select mates primarily on the basis of body size (Cooper and Vitt 1993). Given this evidence, we favor the idea that selection has acted directly on body size in the *skiltonianus* group and that color pattern and mate recognition have evolved as incidental by-products.

The repeated evolution of a particular morphology in similar environments implies that natural selection was the cause of these changes because genetic drift is unlikely to result in concordant evolutionary transformations (Schluter and Nagel 1995; Schluter 1998). Different habitat associations have been described for *E. gilberti* and *E. skiltonianus*, and the parallel-evolved clades do show similar environmental affinities. In areas of parapatry, *E. gilberti* are known to inhabit lower elevations in more arid and warmer conditions than *E. skiltonianus* (Rodgers and Fitch 1947; Banta 1962; Macey and Papenfuss 1991; Morrison et al. 1999). Additionally, *E. gilberti* has a more restricted and southerly distribution relative to *E. skiltonianus* and penetrates further into the southwestern deserts (Stebbins 1985). These patterns are consistent with distributional and ecological data for other lizard genera

(i.e., *Sceloporus* and *Cnemidophorus*), where large-bodied species inhabit warm regions at lower elevations compared to smaller species, which occupy cooler regions or higher elevations (Bogert 1949). Historical biogeography studies of the *skiltonianus* group have also addressed the environmental correlates of population densities and distribution (Morafka and Banta 1972). For example, the near absence of *E. skiltonianus* in the Gabilan–San Benito County region in California is considered to be the result of post-Pleistocene warming and drying, with the current *E. skiltonianus* populations representing relicts of a previously more widespread species (Morafka and Banta 1972). Conversely, *E. gilberti* are now common throughout the area, presumably due to glacial recession and the persistence of suitable habitats for the larger, xeric species.

One explanation of the independent transitions to large body size is that physiology has served as a driving factor. Physiological processes can lead to selection for larger body size in warm, arid habitats because of its effects on the surface area:volume ratio (Cowles 1945; Bogert 1949; Futuyma 1997). A large skink with low surface area:volume would benefit from higher thermal inertia and lower flux of water across the skin relative to a small skink of similar shape. Conversely, smaller body size might be selected in *E. skiltonianus* habitat because it allows for more rapid heating and cooling in environments with fewer opportunities for achieving preferred body temperature (e.g., due to cloud cover or consistent winds) and for which water loss is not as eminent a threat to survival (Cowles 1945; Bogert 1949; Stevenson 1985). These conditions typically characterize high altitudes, coastlines, and northern latitudes, which comprise the majority of the range for *E. skiltonianus*. Large *E. gilberti* individuals also weigh more than twice the amount of the largest *E. skiltonianus* (~32.0 g vs. ~13.0 g; J. Q. Richmond, unpubl. data) and have substantially higher minimum activity temperatures than *E. skiltonianus* (21.5°C vs. 13.2°C; Brattstrom 1965). The general implication of these observations is that physiology has mediated the directionality of body size divergences in different environments.

Selective advantages for ontogenetic shifts in *gilberti* color expression are more difficult to address. The striped pattern likely serves to confuse predators during rapid movements (Jackson et al. 1976; Brodie 1992), and brightly colored tails are known to attract attention away from the body (Vitt and Cooper 1986). However, reasons for why the *gilberti* coloration changes during ontogeny are less clear. The shift may enhance crypsis as body size increases or it might be the result of selection relating to mate preference. If sexual selection has played some role in the evolution of color expression, the question remains as to how the divergent mate preference became established in the first place. We propose that the *gilberti* color transformation is a consequence of selection on body size given that the persistence, reduction, or complete loss of striping is dependent on where terminal body size falls within the developmental sequence. Once certain size thresholds are reached during growth, the loss of stripes might occur in the absence of any direct selection on color pattern. One way to test this idea is to investigate the possible disadvantages of being a large skink (e.g., *gilberti*-sized) and having stripes, because this phenotype is never

observed in natural *Eumeces* populations. Any disadvantages could be perceived as support for some form of selection on being stripeless above certain body sizes. Approaches might include mate choice or predation experiments using painted live specimens or clay models to determine how mate recognition and mortality are affected by this apparently prohibited phenotype.

The repeated evolution of *gilberti* morphology also draws attention to possible biases in the types of evolutionary change that are permissible. Hypotheses regarding such constraints suggest that intrinsic developmental biases more readily produce changes in certain directions than others, including extreme situations in which certain phenotypes may be inaccessible (Smith et al. 1985; Wake 1991). The morphology of *Eumeces* in general is highly conserved, and some Asian and North American forms have been previously recognized as the same species or closely related members of species groups (Taylor 1935; Lieb 1985). Thus, the *gilberti* phenotype might represent a pathway of least evolutionary resistance, with fundamentally similar morphogenetic mechanisms giving rise to repeated trends. Heterochrony-like diversification is also not unusual in *Eumeces*, with many species appearing to represent variants of shared developmental trajectories (Camp 1916). Eastern North American skinks of the *fasciatus* species group are particularly similar to the *skiltonianus* group in terms of these patterns. Members of the *fasciatus* group share a distinctive striped juvenile phase with bright blue tails, and at least one species (*E. laticeps*) grows substantially larger than its cohorts and loses the striped color pattern in adulthood (Taylor 1935). The morphology of *E. laticeps* is surprisingly *gilberti*-like, but the two species are not closest relatives nor does there appear to be any common ecological factors promoting this particular morphology. These patterns are consistent with the concept of a bauplan, where phylogenetic persistence of an organismal archetype is known to impose limits on variation and the direction of evolutionary change (Wagner 1989; Wake 1991). Parallel evolution of *gilberti* morphology may therefore reflect systematically biased properties of *Eumeces* development, rather than being an immediate artifact of *skiltonianus* group history.

#### Links to Parallel Ecological Speciation

An important distinction between parallel speciation and the more general concept of parallel evolution is that parallel speciation requires independent and repeated evolution of the same mate recognition system (Schluter and Nagel 1995; Schluter 1998). The result of parallel speciation is a lack of reproductive isolation between independently evolved clades experiencing similar environments. This implies that the same isolation mechanism is responsible for each speciation event, and that natural selection has caused the divergence (Schluter and Nagel 1995). Heterochronies might provide a viable pathway through which independent lineages arrive at the same morphological endpoints. If shifts in ontogeny are a consequence of divergent selection on traits that are relevant to mate choice, the dynamics of the system might eventually lead to parallel speciation.

Changes in body size are an important factor relating to mate recognition in sympatric stickleback species (Nagel and Schluter 1998), sockeye and kokanee salmon (Foote and Lar-

kin 1988), and Galapagos finches (Ratcliffe and Grant 1983). In these examples, ecomorphs with the greatest size disparities tend to be the most reproductively isolated. A similar situation occurs between *E. skiltonianus* and *E. gilberti* lineages in areas of parapatry and sympatry. Where the two forms overlap most broadly in southern California, they are easily distinguished by morphology at all stages of the life cycle. Also, the largest *E. gilberti* are found in San Diego County, whereas the smallest *E. skiltonianus* occur in this same region. From a genetic perspective, no mtDNA lineages recognized as *E. skiltonianus* morphologically were ever discovered in any of the major *E. gilberti* clades or vice versa. Thus, the two forms apparently do not interbreed in areas of sympatry.

Analysis of reproductive compatibility in the contact zones between independently evolved *E. gilberti* lineages is necessary to determine whether this system represents a case of parallel speciation. This is important for two reasons: (1) hybridization among descendant clades experiencing similar environments provides a reliable measure for determining whether the traits of interest are responsible for reproductive isolation; and (2) repeated trends are necessary to establish whether traits conferring mate compatibility are correlated with environment (Schluter and Nagel 1995). At present, we have located a secondary contact zone between the Sierran and southwestern *E. gilberti* mtDNA clades (A and I, respectively; Fig. 4) at the juncture of the southern Sierra Nevada and Tehachapi Mountains. This is an unusual situation in that most other putative cases of parallel speciation do not involve sympatry of independently evolved ecotypes. The boundaries of mtDNA clades A and I correspond with an intergradation zone between distinct subspecies (the Sierran *E. g. gilberti* and *E. g. rubricaudatus*). The most conspicuous intermediate character is juvenile tail color, which is a purplish blend of the pink-tailed *E. g. rubricaudatus* and the bright blue tails of *E. g. gilberti*. Other intermediate characters include the size at the onset of shifts in color pattern and other aspects of subadult coloration (Rodgers and Fitch 1947). If the morphology serves as a reliable proxy for gene flow, the implication is that members of the independently derived *E. gilberti* clades are interbreeding in the contact zone. This supports the scenario for parallel ecological speciation, but ultimately requires confirmation from nuclear genetic makers. More information regarding zones of overlap between the Sierran and southern *E. gilberti* lineages with Inyo *E. gilberti* is also needed to address the reproductive capacity of the three clades simultaneously. Museum records indicate that members of the Inyo clade may contact the Sierran or southwestern *E. gilberti* lineages on the eastern slopes of the Sierras in the vicinity of Tehachapi Pass, but little is known of the interactions (if any).

#### *Lineage History and Taxonomic Implications*

de Queiroz (1998) proposed that contemporary species concepts differ only in terms of the specific criteria used to delimit species within the time extended processes of speciation (e.g., coalescence, reproductive isolation). For this very reason, the existence of polyphyletic species presents an interesting dilemma. Parallel speciation studies have pre-

viously focused on the biological species concept (Schluter 1998), where populations belong to the same species only if they are not reproductively isolated (Mayr 1963). Under this definition, parallel-evolved *E. gilberti* clades would be considered a single species if sufficient evidence of gene flow existed among sympatric clades, despite their independent evolutionary origins. However, most contemporary evolutionary biologists, regardless of their preferred species concept, would probably agree that reproductive isolation between good species may be imperfect. Our current knowledge indicates that although some gene flow may be occurring among these *E. gilberti* clades, each maintains a unique identity with respect to mtDNA, some morphological features, and geographic structure. Failure to recognize these clades as distinct species (at least tentatively) would be ignoring important aspects of phylogenetic history and would generate a taxonomy that is inconsistent with evolutionary processes. Furthermore, it would imply some concern about the unknowable, futuristic settings of these clades, rather than emphasizing the current and historical patterns for which we have evidence. Given these circumstances, we consider the evolutionary species definition (e.g., Wiley 1978; Frost and Kluge 1994) to be the most appropriate for delimiting species in the *skiltonianus* group.

We suggest that a minimum of three evolutionary species exist within *E. gilberti*, each representing one of the three independently evolved phylogeographic groups (clades A, C, and I; Fig. 3). Currently, we are unaware of any diagnostic morphological characters that would serve as unambiguous synapomorphies for each clade. Ongoing morphometric studies are investigating possible differences in shape and size among the three clades and will offer an additional and previously unexplored perspective for assessing species limits. Up to five evolutionary species could be recognized within *E. skiltonianus* based on the existence of regionally distinct mtDNA clades (B, D–F, and G; Fig. 3), and strongly supported partitions within clade G suggest the presence of possibly more. However, the majority of the *E. skiltonianus* mtDNA clades correspond to geographically continuous populations that are morphologically similar, with the common ancestor of some clades giving rise to independently derived *E. gilberti* lineages. The existence of polyphyletic lineages within a more widespread parent species may be common in the formation of new species, and widely accepted speciation mechanisms can result in nonexclusive ancestors (e.g., Hedin 1997). Thus, we consider the evolutionary units that merit species recognition to be limited to the *E. gilberti* clades, with *E. skiltonianus* representing a paraphyletic or nonexclusive ancestral species. We also support the continued recognition of *E. lagunensis* as a distinct species based on consistent differences in external characters and exclusive mtDNA haplotypes. The close relationship and morphological similarity to southern *E. skiltonianus* supports the previous inference that this species is an allopatric derivative of a *skiltonianus*-like ancestor (Taylor 1935; Murphy 1983).

We appreciate that mtDNA represents a single line of evidence for species recognition and that its utility is potentially limited due to problems associated with introgression, repeated opportunities for isolation and secondary contact among populations, male biased dispersal, and incomplete lineage sorting

(Avice 1994; Wake and Jockusch 2000). Nonetheless, mtDNA perspectives are an important component in the identification of evolutionary lineages and are especially useful for defining species under certain circumstances and when used in conjunction with other sources of data (Wiens and Penkrot 2001). We await additional evidence from ongoing studies using nuclear markers, increased mtDNA sampling, and morphometric analyses to establish more formal and definitive conclusions regarding species limits. Until such time, we feel that our recommendations best reflect the current knowledge of the diversity in the *skiltonianus* group and capture pertinent attributes of phyletic history that are instructive for understanding the processes of speciation.

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#### APPENDIX

Haplotype number (bold face), voucher specimen, species/subspecies name, and locality data (in this order). Institutional abbreviations follow Leviton et al. (1985) except for KWS (Kirk W. Setser), DGM (Dan G. Mulcahy), TAT (Tom A. Titus), JJW (John J. Wiens), JQR (Jonathan Q. Richmond), LV (Laurie Vitt), TWR (Tod W. Reeder) and RNF (Robert N. Fisher) field series. Abbreviations following RNF samples (tail tips only) correspond to pitfall array localities: (LCR) Little Cedar Ridge, (BBL) Big Bear Lake, and (BPJR) the Burns Pinion Juniper Reserve. RNF samples were obtained from an ongoing study conducted by the U.S. Geological Survey/Western Ecological Research Center in San Diego (principle investigator, Robert N. Fisher).

Clade A. **18** (SDSU 3855) *rubricaudatus* CA, San Diego Co., Escondido, San Diego Wild Animal Park. 33 60.0N, 116 58.8W; (SDSU 3857) *rubricaudatus* CA, Orange Co., Santa Ana Mountains, Limestone Canyon. 33 45.2N, 117 41.4W: **19** (SDSU 4008) *rubricaudatus* CA, San Bernardino Co., Mojave River along Hwy 173, 9.3 km E of Hwy 138/173 jct. 34 35.0N, 118 17.1W: **20** (SDSU 4074) *rubricaudatus* CA, San Bernardino Co., Granite Mountain Reserve (University of California, Riverside), Granite Plateau. 34 19.8N, 117 15.9W: **21** (SDSU 3824) *arizonensis* AZ, Maricopa Co., Hassayampa River ca. 4.0 km S of Wickenburg. 33 56.3N, 112 41.9W: (SDSU 3853) *rubricaudatus* AZ, Yavapai Co., Cherry Rd., 0.4 km from Cherry Rd./I-17 intersection: **22** (RNF-BPJR 0001) *rubricaudatus* CA, San Bernardino Co., Burns Pinion Juniper Reserve (University of California, Irvine). 34 08.9N, 116 26.8W: **23** (SDSU 4139) *rubricaudatus* CA, Los Angeles Co., Leona Valley at Elizabeth Lake. 34 35.0N, 118 17.1W: **24** (SDSU 3812) *rubricaudatus* CA, Los Angeles Co; Hungry Valley State Vehicular Recreation Area, Gold Hill Rd., 2.0 km SW of Peace Valley Rd./Gold Hill Rd. jct. 34 46.8N, 118 51.4W: **25** (SDSU 4138) *rubricaudatus* CA, Kern Co., Brite Valley near the intersection of Brite Creek/Banducci Rd. 34 29.8N, 116 57.0W: **26** (MVZ 147888) *rubricaudatus* CA, Kern Co., E slope Temblor Range, Hwy 58, 17.7 km NW Hwy 33. 35 23.0N, 119 33.2W: **27** (MVZ 147892) *rubricaudatus* CA, Monterey Co., Hwy 198, near Coalginga Mineral Spring Rd. jct. 36 09.0N, 120 52.0W: **28** (SDSU 3786) *rubricaudatus* CA, San Benito Co., 0.5 km N of Pinnacles National Monument Park boundary, 0.1 km W of Hwy 146. 36 28.5N, 121 13.8W: **29** (SDSU 3842) *cancellosus* CA, Alameda Co., Lake Del Valle. 37 36.5N, 121 43.1W.

Clade B. **36** (CAS 203542) *skiltonianus* CA, Santa Cruz Co., China Grade Rd., 3.2 km N of the China Grade Rd./State Route 236 jct. 37 9.9N, 122 12.1W: **37** (SDSU 4099) *skiltonianus* CA, Santa Clara Co., 8.0 km W of Lick Observatory via Mt. Hamilton Rd. 37 20.2N, 121 40.0W: **38** (LSUMZ H-14929) *skiltonianus* CA, Contra Costa Co., Briones Regional Park. 37 56.3N, 122 08.3W: **39** (SDSU 4094) *skiltonianus* CA, San Benito Co., Bickmore Canyon, ca. 4.8 km W of Gloria Rd./Hwy 25 jct. 36 33.6N, 121 13.6W.

Clade C. **46** (SDSU 3811) *rubricaudatus* CA, Inyo Co., adjacent to Hwy 168, ca. 12.9 km E of Hwy 395/Hwy 168 jct. 37 15.0N, 118 10.1W: **47** (SDSU3802) *rubricaudatus* CA, Inyo Co., Nelson Range in Grapevine Canyon. 37 1.8N, 117 20.4W: **48** (LSUMZ H-14874) *rubricaudatus* CA, Inyo Co., Panamint Mountains, Jail Canyon at Hall Mine. 36 11.6N, 117 10.6W: **49** (LSUMZ H-14796) *rubricaudatus* CA, Inyo Co., Argus Mountains, China Lake Naval Weapons Testing, vicinity Betram Spring. 35 93.0N, 117 47.0W: **50** (SDSU 4140) *rubricaudatus* CA, Inyo Co., 6.4 km SW of Olancha at Walker Creek. 36 14.8N, 118 03.2W.

Clade D. **30** (SDSU 3841) *skiltonianus* CA, Santa Barbara Co., Vandenberg Air Force Base, 0.1 km N of water plant on Terra Rd., east of the 13th St. jct. 34 41.2N, 120 31.4W: **31** (SDSU 3833)

*skiltonianus* CA, Santa Barbara Co., Quiota Creek, 3.1 air km NE of Refugio Pass. 34 33.4N, 120 2.5W.

Clade E. **32** (SDSU 4142) *skiltonianus* CA, San Luis Obispo Co., 4.0 km E of Pozo via Pozo Rd., ca. 1.6 km N of Los Padres National Forest boundary between the Garcia Mountains and the La Panza Range. 35 18.6N, 120 20.2W: **33** (SDSU 4089) *skiltonianus* CA, San Luis Obispo Co., Hwy 41 ca. 6.4 km NW of Hwy 41/Hwy 1 jct. 35 25.4N, 120 47.0W: **34** (SDSU 4092) *skiltonianus* CA, Monterey Co., 8.7 km W of the west entrance to the Fort Hunter Liggett Military Reservation along Nacimiento Fergusson Rd. 36 0.9N, 121 25.1W: **35** (SDSU 4093) *skiltonianus* CA, Monterey Co., Carmel Valley at Garland Ranch Regional Park. 36 17.2N, 121 27.7W.

Clade F. **40** (LSUMZ H-14523) *skiltonianus* CA, Marin Co., 9.7 km W of Navato. 38 7.4N, 122 40.1W: **41** (LSUMZ H-14933) *skiltonianus* CA, Napa Co., Lake Berryessa, Hwy 128 at Smittle Creek. 38 34.7N, 122 15.3W: **42** (CAS 202568) *skiltonianus* CA, Tehama Co., Mendocino National Forest Rd. M1, 11.7 km NE of Mendocino Pass Rd. at the Eel River Work Center, 1.4 km W of Boardman Camp. 39 51.3N, 123 0.5W: **43** (CAS 202952) *skiltonianus* NV, Washoe Co., north slope of Steven's Camp. 41 29.3N, 119 29.4W: (CAS 203110) *skiltonianus* CA, Modoc Co., Modoc National Forest, Warner Mountains, ca. 300 m ESE Plum Valley Campground. 41 42.3N, 120 19.2W: **44** (TAT 1295) *skiltonianus* OR, Lane Co., 2 km E of confluence of Wildcat Creek and Siuslaw River. 44 0.1N, 123 37.3W: **45** (LSUMZ H-14916) *skiltonianus* WA, Grant Co., ca. 3.2 km NNE of Vantage. 46 58.1N, 119 58.1W.

Clade G. **11** (SDSU 3831) *interparietalis* CA, San Diego Co., Laguna Mountains, along Sunrise Highway (S1) 21.2 km N of I-8/S1 jct. 32 54.7N, 116 27.8W: **12** (RNF-LCR001) *interparietalis* CA, San Diego Co., San Ysidro Mountains, Otay National Cooperative Land and Wildlife Management Area, Little Cedar Ridge. 32 37.2N, 116 51.7W: **13** (SDSU 3809) *interparietalis* CA, Orange Co., Santa Ana Mountains, Starr Ranch Reserve. 33 36.7N, 117 32.8W: **14** (RNF-BBL155) *skiltonianus* CA, San Bernardino Mountains, Big Bear Lake. 34 15.9N, 116 53.9W: **15** (DGM 049) *utahensis* UT, Box Elder Co., Devil's Playground, NE side of the Bovine Mountains. 41 30.6N, 113 39.8W: **16** (KWS 003), *utahensis* UT, Washington Co., along FR006, ca. 3.2 km S of jct. with FR003. 37 29.0N, 113 50.0W: **17** (SDSU 3816) *utahensis* CA, Inyo Co., Independence Creek at Gray's Meadow campsite. 36 47.2N, 118 15.2W.

Clade H. **51** ROM *E. lagunensis* Mexico, Baja California Sur, San Jose de Comondu: **52** (SDNMH 68703) *lagunensis* Mexico, Baja California Sur, Sierra Guadalupe, Canada Guano, near Rancho Sebastian: **53** (SDNMH 68705) *lagunensis* Mexico, Baja California Sur, Sierra Guadalupe, La Cumbre de San Pedro.

Clade I. **1** (SDSU 3843) *placereensis* CA, El Dorado Co., SE side of Folsom Lake at the Green Valley Rd./Miller Rd. entrance to Folsom Lake. 38 42.7N, 121 5.7W: **2** (SDSU 3834) *placereensis/gilberti* CA, Calaveras Co., Cedar Rd., 0.6 km from Cedar Rd./Hwy 49 jct., 2.4 km N of Mokelumne Hill. 38 18.0N, 120 42.8W: **3** (MVZ 162079) *placereensis/gilberti* CA, Calaveras Co., 2.4 km WNW Hwy 4 at Avery on Avery-Sheep Ranch Rd. 39 12.0N, 120 23.4W: **4** (SDSU 3793) *gilberti* CA, Stanislaus Co., Hwy 132, 7.9 km SE of Hwy 132/J59 jct. near La Grange. 37 38.0N, 120 30.8W: **5** (MVZ 137831) *gilberti* CA, Mariposa Co., 3.7 km S Mormon Bar, intersection of Ben-Hur Rd. and Hwy 49. 37 26.4N, 119 56.4W: **6** (SDSU 3784) *gilberti* CA, Madera Co., North Fork Rd. ca. 11.3 km W of North Fork Rd./Auberry Rd. jct. between North Fork and O'Neals. 37 11.7N, 119 36.5W: **7** (MVZ 500014) *gilberti* CA, Tulare Co. Drum Valley Rd., 4.3 km NW jct. with California Hwy 245. 36 38.4N, 119 6.6W: **8** (SDSU 3787) *gilberti/rubricaudatus* CA, Tulare Co., north side of Hwy 190, 4.3 km E J29/Hwy. 190 jct. 36 2.7N, 118 57.0W: **9** (SDSU 3804) *gilberti/rubricaudatus* CA, Kern Co., 4.8 km N of the Woody Rd./Hwy 155 jct. off Hwy 155 in Donery Gulch. 35 43.3N, 118 48.1W: **10** (SDSU 3852) *rubricaudatus* CA, Kern Co., Tehachapi Pass, 4.5 km SE of Keene, S side of Hwy 58 at the Broome Rd. exit. 35 12.0N, 118 31.3W.

Outgroup taxa. (LACMFS188) *E. quadrilineatus*; (JJW602) *E. brevirostris*; (MVZ11013) *E. egregius*; (USNM541471) *Neoseps reynoldsi*; (SDSU 4115) *E. laticeps*; (JQR 169) *E. obsoletus*; (LV 41032) *E. tetragrammus*; (TWR 316) *E. inexpectatus*; (TWR 438) *E. fasciatus*; (TWR 059) *E. septentrionalis*. Localities available upon request.