Molecular Phylogenetics and Conservation of *Tupinambis* (Sauria: Teiidae)

LEE A. FITZGERALD, JOSEPH A. COOK, AND A. LUZ AQUINO

At least five species of *Tupinambis* lizards (Sauria: Teiidae) occur in South America east of the Andes. In Argentina and Paraguay, two species of *Tupinambis* have been commercially exploited for the leather trade since the 1970s. Current quotas total 1.35 million skins/yr. Despite this large and sustained harvest, few studies have examined genetic or morphological variation in these lizards. We sequenced parts of the mitochondrial cytochrome b (300bp) and ND4 (375bp) genes and examined this variation in light of morphological characters traditionally used to identify these species. DNA sequences provided a preliminary view of intraspecific and interspecific variation and were used to explore evolutionary relationships among 17 individuals representing *T. merianae*, *T. rufescens*, and *T. duseni* from Paraguay, and *T. longilineus* and *T. teguixin* from Roraima, Brazil, and Cuyabeno, Ecuador. *Kentropyx viridistriata*, *Ameiva ameiva*, and *Cnemidophorus ocellifer* were included as outgroups. Maximum-parsimony and neighbor-joining analyses revealed two distinctive groups within *Tupinambis*: a northern South American and Amazonian clade (*T. teguixin* and *T. longilineus*) and a clade (*T. duseni*, *T. rufescens*, and *T. merianae*) that is distributed primarily south of Amazonia. Although genetically similar and previously considered synonymous with *T. rufescens*, *T. duseni* is morphologically distinct based on squamation, coloration, and morphometrics. This incongruence between the molecular data and morphology suggests that *T. duseni* and *T. rufescens* may have undergone extensive and recent morphological evolution or there has been introgression of mitochondrial DNA between these species. Sequence divergence between *T. teguixin* from Brazil and Ecuador was similar to that found between *T. rufescens* and *T. merianae* and may indicate these *T. teguixin* populations are not conspecific. The *T. teguixin* clade was sister to *T. longilineus*. These findings, combined with the large-scale commercial exploitation of the genus, suggest an urgent need to address geographic variation and the systematics of species of *Tupinambis.*
1995), for example, and the lack of consistent taxonomy and genetic and morphological markers useful for forensics pose impediments to management and long-term conservation. Characterization of geographic variation and species limits is necessary to quantify the trade effectively, enforce harvest quotas, and monitor trends in the take by geographic area.

The taxonomic history of the genus *Tupinambis* Daudin 1802, and particularly that of *T. teguixin* (Linnaeus 1758) and *T. duseni* Lönnberg in Lönnberg and Andersson 1910, is tortuous. Four species were recognized by Peters and Donoso-Barros (1970): *T. teguixin*, *T. nigropunctatus* Spix 1825, *T. rufescens* Günther 1871, and *T. duseni*. Presch (1973) reviewed the genus and recognized only two species: *T. rufescens*, the red tegu, which included the single known specimen of *T. duseni* (following Burt and Burt, 1931), and *T. teguixin*, comprised of all other tegus, including *T. nigropunctatus* from northern populations and *T. teguixin* (sensu Boulenger, 1885) from the Southern Cone region of South America. Presch’s (1973) arrangement for *T. teguixin*, however, was not followed by subsequent authors (e.g., Hoogmoed, 1973; CeI, 1986, 1993). In her recent review of available names and type material for “teguixin” forms, Avila-Pires (1995) clarified the situation by renaming *T. nigropunctatus* as *T. teguixin* and renaming *T. teguixin* (sensu Boulenger, 1885) as *T. merianae*. Hence, northern South American and Amazonian populations are *T. teguixin*, whereas the larger southern form that is exploited for skins is *T. merianae*.

Two additional species of *Tupinambis* have recently been described. Avila-Pires (1995) described *T. longilineus* from Rondônia, Brazil, and Manzani and Abe (1997) described *T. quadrilineatus* from central Brazil. Colli et al. (1998) provided a more comprehensive description of the same taxon, with additional data. Although Colli et al. (1998) compared five species of *Tupinambis* using morphological and allozyme electrophoretic data, reconstruction of evolutionary relationships among *Tupinambis* species using explicit phylogenetic methods has not been completed. Herein, we provide an independent view of phylogenetic relations and species limits among five nominal species of *Tupinambis* based on sequences of the mitochondrial cytochrome *b* (cyt *b*) and ND4 genes. We relate the genetic data to morphological features previously used to identify these taxa.

**MATERIALS AND METHODS**

*Specimens examined.*—Partial cyt *b* (300 bp, corresponding to positions 14618–14917 of the bovine mitochondrial genome; Anderson et al., 1982) and ND4 (375 bp, positions 11204–11578 of the bovine mitochondrial genome; Anderson et al., 1982) sequences were obtained from the mitochondria of 17 individuals representing *T. merianae*, *T. rufescens*, *T. duseni*, *T. longilineus*, and *T. teguixin* and from one individual each of *Kanjops viridistriga*, *Ameiva ameria*, and *Cnemidophorus ocellifer*. The *T. longilineus* and *T. teguixin* were from Brazil and Ecuador and the remainder of the material was from Paraguay (Fig. 1).

DNA was extracted from dried skin samples or from tissues frozen or preserved in alcohol. The sequence data for these individuals have been submitted to the GenBank Data Libraries (accessions AF151174–AF151213).

**DNA extraction, amplification, and sequencing.—** Tissues were subjected to proteinase K digestion, NaCl precipitation of proteins, and DNA precipitation with ethanol, following a protocol modified from Miller et al. (1988). Partial segments of the cyt *b* and ND4 genes were amplified by the polymerase chain reaction (PCR, usually 30 cycles alternating denaturation at 95
C for 1 min, annealing at 45 °C for 1 min, extension at 72 °C for 1.5 min) using the following primer combinations: for cyt b, LIZ1 (5'-3', AGC CCC ATC CAA CAT CTC TGC ATG AAA)–LIZ2 (5'-3', TGA CTC TGG CAC CTC AGA ATG ATA TTA GGG CTC A) and for ND4, ND4-LEU (Arévalo et al., 1994). LIZ1–LIZ2 were modified from the universal cyt b primers of Kocher et al. (1989) based on the chicken sequence (Desjardins and Morais 1990). All PCR experiments included negative controls.

Aliquots (5 µl) of the PCR products and negative controls were visualized in agarose mini-gels. Each remaining product was precipitated with polyethylene glycol, recovered by vacuum centrifugation, and resuspended in 1X TE buffer. The clean products were used as templates in cycle sequencing utilizing a Perkin-Elmer kit (Fst-RR, 402119). Cycle sequencing products were purified with Sephadex G-50 (Sigma) in reusable columns (Princeton Separations) and run on 2% polyacrylamide gels using an automated sequencer (Applied Biosystems, Inc., 375). Both heavy and light strands were sequenced in all cases.

Sequence and phylogenetic analysis.—Partial sequences were examined, assembled based on overlapping regions and aligned by eye using the Sequence Navigator program (Applied Biosystems, Inc., vers. 1.01). Kimura 2-parameter distance values, nucleotide composition and numbers of variable and phylogenetically informative sites for nucleotides were obtained using PAUP* (vers. 4.0b1, D. Swofford, 1998, unpubl.).

Phylogenetic analyses used two primary methods: maximum parsimony (MP) and neighbor joining (NJ). Weighted and unweighted MP trees were generated using PAUP*. Weighting schemes comprised equal weights or differential weights for first, second, and third codon positions (weighted 2, 5, and 1, respectively) in combination with a stepmatrix awarding greater weight to transversions than transitions (10:1). The latter scheme attempts to accommodate known biases in the evolution of cyt b and ND4 genes (Irwin et al., 1991; Arévalo et al., 1994). We used the branch-and-bound search algorithm for the weighting scheme (and equal weights) in PAUP*. Because the topologies of these trees were identical, we used only the equal-weight dataset in subsequent analyses. Values of skewness were computed from 10,000 random trees and used to assess overall phylogenetic signal in the dataset (Hillis and Huelsenbeck, 1992). Trees were rooted using the outgroup criterion, with Ameiva, Cnemidophorus, and Kentropyx defined as outgroups, collectively. To test for the monophyly of the genus Tupinambis with respect to these three genera, separate MP analyses (equal weights) were conducted with each genus defined as the sole outgroup.

Distance-based NJ trees using Kimura 2-parameter distances were computed and assessed using PAUP*. MP and NJ trees were bootstrapped (500 replicates) to assess the strength of associations and all trees were rooted for presentation. Decay indices (Bremer, 1988) were computed for the MP tree using TreeRot (Sorenson, 1996).

Morphological analyses.—We measured specimens of T. merianae, T. rufescens, and T. duseni from Paraguay. We determined the sex of each specimen and measured snout-vent length (SVL), head length, head width, tip of snout to eye, length of the pes, and length of the fourth toe. We counted the number of ventral scale rows, subdigital lamellae on the fourth toe, femoral and preanal pores, and labials. Descriptive statistics of scale counts were compared to values reported in the literature for species of Tupinambis (Presch, 1973; Avila-Pires, 1995). Ten T. duseni were hatchling clutch mates. We tabulated the scale counts for these individuals but excluded them from statistical analyses.

The individuals used in the study varied substantially in body size (85–423 mm SVL). Prior to analysis, individuals < 200 mm SVL were deleted from the dataset to reduce bias in statistical analyses. Analyses of covariance, using SVL as the covariate, were performed on five measurement variables (log10-transformed) to test for differences in the relative size of traits among the samples from Paraguay. In cases of overall significance, contrasts were computed to test for differences in least-square means between species.

To remove the confounding effects of body size for multivariate analyses, log10-transformed observations were pooled, and each morphometric character was regressed against SVL. Residuals resulting from the regressions were retained as size-adjusted variables and allowed statistical comparisons of morphological shape variation among individuals of different size (Miles, 1994; Losos et al., 1998). A canonical discriminant analysis (PROC Candisc, SAS Institute, Cary, NC, 1989, unpubl.) was performed on the five measurement variables to evaluate morphological differences among species. Correlations between canonical variates axis scores
and original variables were used to assess the relative importance of each trait. Mahalanobis' distances \(D^2\) were computed, and resulting \(P\) values allowed us to accept or reject the null hypothesis of no significant morphometric variation among the three Paraguayan taxa (Miles, 1994).

The five size-adjusted body measurements and the five scale characters were used in a cluster analysis to assess morphological similarity among the species. Gower's median method for computing distances (a weighted pair-group method using centroids) was used to minimize effects of combining measurement and meristic data (SAS Institute, Cary, NC, 1989, unpubl.; D. B. Miles, pers comm).

**RESULTS**

**Compositional biases, and patterns and levels of variation.**—All sequences obtained follow the patterns of compositional bias common among vertebrate mitochondrial cyt \(b\) (e.g., Irwin et al., 1991; Petren and Case, 1997) and ND4 (e.g., Benabib et al., 1997) genes. Average base frequencies for the coding strand were: \(A\) (28.8% overall, 27.5% cyt \(b\), 29.9% ND4); \(C\) (28.1%, 27.8%, 28.3%); \(G\) (15.3%, 15.0%, 15.5%); and \(T\) (27.8%, 29.7%, 26.3%). There is a deficiency in guanines in the light strand of all individuals (14.5-16.3% overall, 13.7-16.7% cyt \(b\), 14.4-16.5% ND4). Observed substitutions were most abundant in third positions and least common in second positions. Observed, uncorrected transitions outnumber transversions in pairwise comparisons of haplotypes that exhibit relatively little divergence (e.g., 9:1 in cyt \(b\) and 13:1 in ND4 for the comparison of two samples of \(T. \) duseni). These substitutions and biases are expected for functional mitochondrial cyt \(b\) and ND4 genes. We subsequently combined these data for all phylogenetic analyses.

Distance values for pairwise comparisons ranged from 0.0 for two specimens of \(T. \) duseni (issue 766 and issue 777) and two \(T. \) teguixin (LSUMNS 12431 and LSUMNS 12450) to 0.424 for comparisons between \(T. \) merianae and \(T. \) rufescens (issue 740). Samples 777 and LSUMNS 12450 subsequently were excluded from further phylogenetic analyses of the sequence data. Largest intraspecific distances (0.099) were found within \(T. \) teguixin (LSUMNS 12405 and LSUMNS 12715) and were nearly as large as the distance (0.109) between comparisons of \(T. \) merianae (issue 649) and \(T. \) rufescens (issue 740).

**Relationships among species of Tupinambis.**—Strong phylogenetic signal in the sequence data was indicated by the highly significant G1 value (4.8580) obtained from 10,000 random trees \((P < 0.01\), table 2 of Hillis and Huelsenbeck, 1992). Of the 675 characters, 205 were parsimoniously informative. Maximum-parsimony analyses recovered a single most-parsimonious tree (Fig. 2A) when characters were unweighted (541 steps in length). Under several weighting permutations, the same most-parsimonious tree was consistently recovered. One of the weighting schemes (transversion/transition weighted 10/1 and first, second, and third position codon sites weighted 2/5/1, respectively) produced an additional shortest tree, but it differed only with respect to the relationships among the outgroup taxa. The neighbor-joining analysis produced a tree with identical topology (Fig. 2B).

These analyses suggest the following relationships: (1) species of Tupinambis form a monophyletic group with respect to the representatives of Ameiva, Cnemidophorus, and Kentropyx used in this study; (2) there are two primary and deeply divergent clades in Tupinambis (a southern group consisting of \(T. \) merianae and \(T. \) rufescens, and \(T. \) duseni and a northern/Amazonian clade comprised of \(T. \) teguixin and \(T. \) longipes); (3) within the southern clade, \(T. \) merianae is sister to a clade composed of \(T. \) rufescens and \(T. \) duseni (which show very low levels of divergence); (4) within the northern clade, the level of divergence between \(T. \) teguixin samples from Roraima, Brazil, and Cuyabeno, Ecuador, is comparable to levels of divergence found among other interspecific comparisons.

**Morphology.**—Morphological analyses quantified several consistent differences among the three species of Tupinambis in Paraguay. There was significant variation in snout–eye length \((n = 48, F_{2,45} = 5.224, P < 0.008)\), and contrasts showed \(T. \) duseni had a relatively longer snout–eye length than the other two species \((P < 0.006)\). As noted in Lönngberg and Andersson (1910), length of the fourth toe also varied statistically among the species \((n = 48, F_{2,45} = 5.224, P < 0.008)\). Tupinambis duseni had a relatively shorter fourth toe than the other species \((P < 0.005)\), whereas the fourth toe in \(T. \) rufescens was shorter than in \(T. \) merianae \((P < 0.02)\). Adjusted mean head length, head width, and hind foot length did not differ among the species \((n = 48; df = 2,43; P > 0.13, P > 0.37, P > 0.95, respectively)\).

The canonical discriminant analysis corroborated the univariate comparisons. Canonical variate axis 1 was statistically significant \(F_{2,48} = 5.057; P < 0.001\) and explained 92.1% of the variance in the dataset. The relative length of
Fig. 2. (A) Results of unweighted maximum-parsimony analysis of tegu relationships with Kentropyx, Ameiva, and Cnemidophorus designated as outgroup taxa. This branch-and-bound analysis produced a single tree (length 541 steps, CI = 0.691, RI = 0.824). Percentage of 500 bootstrap pseudoreplicates are reported near each node followed by decay indices. (B) Neighbor-joining tree of relationships between species of Tupinambis and three other genera of macroteiids using pairwise Kimura two-parameter distances and associated gamma distributions. Numbers above branches represent percentage values from 500 bootstrap replicates. Numbers following the names identify tissue specimen vouchers and are listed in Materials Examined.

the fourth toe and snout-eye length were strongly correlated with CV axis 1 (Table 1), and T. duseni formed a distinct group on the low end of this axis (Fig. 3). Although CV axis 2 explained an additional 7.9% of the variance, it was not statistically significant ($F_{4,42} = 1.217; P < 0.317$). Canonical variate axis 2 represented a morphological gradient based on relative head length and snout-eye length, but it did not separate the species well (Table 1). Mahalanobis' distances with corresponding $P$-values showed T. duseni occupied distinct multivariate space from T. rufescens and T. merianae ($P < 0.002$ for both comparisons; Fig. 3). Tupinambis rufescens and T. merianae did not occupy statistically distinct morphological space in this analysis.

Scale characters were also informative for distinguishing the species. Tupinambis merianae had a high number of femoral + precloacal pores (20–25), a characteristic not shared with its congeners (Table 2). The number of ventral scales in a single row across the body correlated well with the northern and southern clades identified in the phylogenetic analyses. Tupinambis merianae, T. rufescens, and T. duseni all shared a high number of ventrals across midbody (minimum ventrals in a row for these species = 34),

### Table 1. Summary of a Canonical Discriminant Analysis Based on Five Size-Adjusted Morphological Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>CV axis 1</th>
<th>CV axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head length</td>
<td>-0.077</td>
<td>0.840</td>
</tr>
<tr>
<td>Snout-eye length</td>
<td>-0.519</td>
<td>0.614</td>
</tr>
<tr>
<td>Head width</td>
<td>-0.295</td>
<td>0.116</td>
</tr>
<tr>
<td>Hind foot</td>
<td>-0.035</td>
<td>0.194</td>
</tr>
<tr>
<td>Fourth toe</td>
<td>0.939</td>
<td>0.234</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>1.942</td>
<td>0.116</td>
</tr>
<tr>
<td>Percent variance explained</td>
<td>92.1%</td>
<td>7.9%</td>
</tr>
</tbody>
</table>
The generality of this deep north-south biogeographic split in other South American taxa should be investigated further. Given the early cladogenesis of these primary clades, the inclusion of other macroteiid genera, especially *Callopistes*, *Teius*, *Crocodilurus*, and *Dracoena*, is crucial to addressing the issue of monophyly. These genera are thought to be close relatives of *Tupinambis* (Fresch, 1974; Sullivan and Estes, 1997). The genetic data agree with previous hypotheses based on morphology. *Tupinambis longilineatus* was the sister taxon to *T. teguixin* from Roraima, Brazil, a finding consistent with the conclusions of Avila-Pires (1995) and Colli et al. (1998). Interestingly, the split among *T. teguixin* and *T. longilineatus* remained as that between *T. rufescens* and *T. merianae*. An expanded analysis, including samples of *Tupinambis* from throughout Amazonia and northern South America, might reveal significant variation within the currently defined taxon, *T. teguixin*.

In spite of morphological similarities between *T. teguixin* and *T. merianae*, the latter taxon is apparently the sister group to *T. rufescens* and *T. duseni*, and part of the distinctive clade in southern South America. We hypothesize that, during or after the north-south split of the *Tupinambis* clade, *T. rufescens* derived from a *T. merianae*-like ancestor. *Tupinambis rufescens* is restricted to the xeric chaco environment appearing to be a derived condition in this genus.

Further work is needed to resolve the relationship between *T. rufescens* and *T. duseni*. Burt and Burt (1931) considered the single specimen of *T. duseni* a geographical variant of *T. rufescens*, distinguishable only by relative sizes of limb elements. They apparently did not place importance on the striking differences in coloration and scalation of the type specimen of *T. duseni*. Peters and Donoso-Barros (1970) did recognize *T. duseni* as distinct, but Fresch (1973) followed Burt and Burt (1931), retaining *T. duseni* within *T. rufescens*. He did not examine the type specimen of *T. duseni*, however. Although Avila-Pires (1995) also recognized *T. duseni*, Colli et al. (1998) preferred to retain *T. duseni* as a junior synonym of *T. rufescens* until further information is available.

DISCUSSION

These mitochondrial data support some previous hypotheses of evolutionary relationships, provide evidence for unsuspected relationships and suggest substantial and previously undetected geographic variation in these large lizards. Deep divergence between the southern

Fig. 3. A plot of the positions of individual *Tupinambis* on CV axis 2 versus CV axis 1. The analysis was conducted using five size-adjusted morphological variables on individuals > 200 mm SVL. Canonical variate axes represented a morphological gradient with individuals having long fourth toe and short snout-eye length on the high end, and short fourth toe and long snout-eye length on the low end. Canonical variate axis 2 represented a gradient of head length but did not separate the species well. Closed circles = *T. merianae*; closed triangles = *T. rufescens*; closed squares = *T. duseni*. Mahalanobis' distances with corresponding P-values are shown in the inset.

whereas *T. teguixin*, *T. longilineatus*, and *T. quadri-lineatus* have a much smaller number (maximum ventrals in a row for these species = 28).

The cluster analysis using five size-adjusted morphological variables and the five scale counts from Paraguayan material produced a dendrogram that described well the southern clade of *Tupinambis* (Fig. 4). Morphological distances among the *T. merianae* were generally mostly small. One individual each of *T. rufescens* and *T. duseni* clustered with five *T. merianae* that were relatively distant from their conspecifics. These individuals clustered together because the five *T. merianae* possessed a relatively high number of ventral scales across midbody, and the *T. rufescens* and *T. duseni* each had a relatively high number of femoral pores. The remaining three *T. duseni* clustered together, and this group was paired with the remaining seven *T. rufescens*.

clade (*T. merianae* and *T. rufescens*) and the Amazonian forms is indicative of an old split within *Tupinambis*. The generality of this deep north-south biogeographic split in other South American taxa should be investigated further. Given the early cladogenesis of these primary clades, the inclusion of other macroteiid genera, especially *Callopistes*, *Teius*, *Crocodilurus*, and *Dracoena*, is crucial to addressing the issue of monophyly. These genera are thought to be close relatives of *Tupinambis* (Fresch, 1974; Sullivan and Estes, 1997). The genetic data agree with previous hypotheses based on morphology. *Tupinambis longilineatus* was the sister taxon to *T. teguixin* from Roraima, Brazil, a finding consistent with the conclusions of Avila-Pires (1995) and Colli et al. (1998). Interestingly, the split among *T. teguixin* and *T. longilineatus* and even to that between *T. rufescens* and *T. merianae*. An expanded analysis, including samples of *Tupinambis* from throughout Amazonia and northern South America, might reveal significant variation within the currently defined taxon, *T. teguixin*.

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*Tupinambis duseni* is yellowish in color and, in addition to quantified differences in head shape and fourth toe, it is also distinct from *T. rufescens*.
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source</th>
<th>Ventral scale rows</th>
<th>Ventrals across midbody</th>
<th>Subdigital lamellae, 4th toe</th>
<th>Femoral + precloacal pores</th>
<th>Labials (superior + inferior)</th>
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<tr>
<td><em>Tupinambis merianae</em></td>
<td>this study</td>
<td>40; 51–(35.2)–36</td>
<td>41; 37–(42.7)–49</td>
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<td>10; 42–(46.1)–55</td>
<td>10; 25–(27.1)–29</td>
<td>10; 12–(18.2)–17</td>
<td>10; 14–(16.7)–18</td>
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<td>18; 34–(40.1)–46</td>
<td>16; 21–(23.7)–28</td>
<td>16; 14–(15.4)–19</td>
<td>18; 15–(15.0)–20</td>
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<td>Manzani and Abe (1997)</td>
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</tbody>
</table>

* Sample sizes were not given in Avila-Pires (1995).

* Femoral pores and precloacal pores were reported separately.

* Supralabials and infralabials were reported separately and counted on both sides of the body (Colli et al., 1998).

* Means were not reported in Manzani and Abe (1997).
and *T. merianae* in having large, convex scales on the anterior part of the dorsum. The description of *T. duseni* (Lönnberg and Andersson, 1910) points out these features and is entirely consistent with the specimens collected in Paraguay. One of us (ALA) examined the holotype (NRM 2886) and found no differences between it and Paraguayan material.

*Tupinambis duseni* was previously known only from the type locality, Parana, Brazil. Our discovery of *T. duseni* at three localities in Paraguay since 1993 provided additional material that gave insight into, but did not resolve, the question of the specific status of *T. duseni*. The minor genetic divergence between *T. rufescens* and *T. duseni* suggests the two forms are very closely related. Despite this close genetic association, *Tupinambis duseni* exhibits consistent and striking differences from *T. rufescens* in coloration, shape of the head, and limb morphology. Additionally, *T. duseni* has been found only in isolated cerrado habitats in eastern Paraguay, whereas all Paraguayan records of *T. rufescens* are far west of the Paraguay River (Fig. 1). Summarizing, *T. duseni* differs from *T. rufescens* in many morphological and ecological characters, and there is presumably some heritable genetic component to this variation. *Tupinambis duseni* may have split recently from a *T. rufescens* ancestor and is experiencing rapid morphological divergence in the isolated cerrado habitats. The paraphyletic relationship indicated by our limited sampling scheme suggests the possibility of incomplete lineage sorting (Avise, 1994; Patton and Smith, 1994). Thus, these taxa do not appear to be reciprocally monophyletic because of the retention of ancestral alleles. Alternatively, the similarity among the mitochondrial gene sequences examined could have resulted from introgression of mitochondrial genes between the two taxa. This explanation appears less likely as these species are not sympatric in Paraguay. Finally, we know very little about the occurrence of *T. rufescens* in Brazil, and how Brazilian *T. rufescens* compare to *T. rufescens* from the Chaco. The records in Fresno (1975) are unreliable because of the general confusion of northern and southern forms of *Tupinambis*, the previous identification of the specimens as *T. teguixin*, and his misidentification of *Seis marmoratus* Laurenti 1768 as *T. rufescens* (a juvenile *T. teguixin* according to Avila-Pires, 1995).

Although *T. merianae* is sympatric with *T. rufescens* in the transition zone of the Paraguayan Chaco, *T. duseni* is allopatric to populations of *T. rufescens*. Abe et al. (1992) reported sympatry among *T. duseni*, *T. merianae*, and *T. teguixin* at Baliza, Goiás, Brazil. We suggest that *T. duseni* is a valid taxon exhibiting rapid morphological divergence from a *T. rufescens*-like ancestor. Larger samples of *T. rufescens* from throughout its range, especially from Brazil, should clarify whether *T. duseni* is distinctive, and define the extent of morphological and genetic variation within *T. rufescens*.

**Implications for conservation, management, and trade**—This phylogeny, along with the taxonomic clarifications by Avila-Pires (1995), carries serious implications for conservation of these exploited lizards. First, all species of *Tupinambis* are managed as a single entity at this time, with quotas allocated for the entire genus within a country. Though harvest monitoring programs identify skins according to species and sex (Fitzgerald et al., 1991, 1994), skins are mixed during the tanning process making it difficult to verify quantities of each species that are exported. Although species of *Tupinambis* are morphologically similar, our results indicate substantial
genetic variation exists within them, and this variation is likely to be associated with geographically structured populations. The availability of phylogenetic information should make it easier to move toward management of these lizards at the level of individual species and identify species and populations that may need distinct management practices.

A striking example of the relevance of systematics to the conservation of *Tupinambis* is the situation of *T. duseni*. This taxon was recently discovered in Paraguay where it is apparently confined to patchy cerrado habitats. A substantial portion of cerrado vegetation has been converted to pasture for cattle grazing, yet the impact of this habitat modification on populations of *T. duseni* is unknown. Meanwhile, the species has been harvested for the skin trade since before 1980 along with *T. meriana* and *T. rufescens*. Skins of *T. duseni* were identified in samples of the yearly harvest used for monitoring the lizards in Paraguay. An estimated 5000 *T. duseni* entered the trade in 1996 (M. A. Mieres and A. L. Aquino, Report on the *Tupinambis* harvest to CITES-PY, Ministerio de Agricultura y Ganadería, Paraguay, 1997, unpubl.).

Second, a taxonomy based on the partitioning of geographic and genetic variation and the delineation of species limits will facilitate international monitoring (e.g., CITES) of the skin trade. *Tupinambis teguixin* does not occur in Argentina and Paraguay, for example, so any *T. teguixin* in the lizard trade must originate from elsewhere. Our preliminary data provide a framework for forensic identification of species and the geographic origins of skins. The morphological analyses indicated, for example, that any skins with fewer than 28 ventral scales in a row belong to a species from the northern/Amazonian clade, and could not have originated from Paraguay or Argentina. Genetic markers identified in this study may identify taxa with a high degree of confidence. The substantial intraspecific genetic variation found in this study indicates, however, that more complete geographic sampling of genetic and morphological variability is necessary to manage and monitor effectively such a heavily harvested group of species.

Finally, a phylogeny of *Tupinambis* allows predictions to be made about the potential for sustainability of these little-studied lizards. The clade containing *T. rufescens* and *T. meriana* is characterized by large body size, relatively large clutch size, nesting in burrows, nest attendance, and a seasonal activity pattern (Fitzgerald et al., 1991, 1993). Based on our results, this group is distant from the northern/Amazonian clade, and comparisons between *T. meriana* and *T. teguixin* should be interpreted with caution (Harvey and Pagel, 1991). The northern/Amazonian clade, though less studied, contains smaller species with a correspondingly smaller clutch size (Reese, 1922). In Venezuela and Ecuador, *T. teguixin* nests in termite mounds (Reese, 1922; Beebe, 1945; Dixon and Soini, 1975). Hence, management should account for phylogenetic similarity, particularly when attempting to predict species specific responses to exploitation and habitat change when faced with limited data on population ecology.

**Materials Examined**

Corresponding voucher specimens are listed by species, catalog number, and locality. Institutional abbreviations are as listed in Leviton et al. (1985).

*Tupinambis teguixin* (LSUMNS H12405, H12450, H12431): BRAZIL; Roraima; Fazenda Nova Esperança, 44 km W BR-174 on BR-210; *T. teguixin* (LSUMNS H12678, LSUMNS H12703, LSUMNS H12715): ECUADOR; Sumbios Province; Reserva Faunística Cuyabeno; Estación Biológica da Universidad Católica.

*Tupinambis longilineus* (LSUMNS H11415, LSUMNS H11416): BRAZIL; Amazonas; Rio Ituxi at the Madeireira Schaffer; S 8°20'47.0" W 65°42'57.9".

*Tupinambis duseni* (USNMField 166766 (tissue 766), MNHN 5074-5): PARAGUAY; Concepción; 17 km S 20 km E Yby Yau; Estancia Siete Lagunas; S 25°06.586' W 56°20.001'; *T. duseni* (MNHN 6181-94): Concepción; Paso Barreto; *T. duseni* (USNMField 166778 (tissue 778), MNHN 4342-3, 5076): Amambay; Colonia Ybycuí; S 23°38' W 55°82'; *T. duseni* (NRM 2886 holotype): BRAZIL; Paraná.

*Tupinambis rufescens* [MNHN 5068, USNMField 166740 (tissue 740)]: PARAGUAY; Presidente Hayes; Estancia Santa Elisa; S 22°24'33" W 56°58'53"; *T. rufescens* [MNHN 4141, 5073, USNMField 166743 (tissue 743)]: Presidente Hayes; Juan de Zalazar; S 23°04'44" W 59°14'12"; *T. rufescens* (MNHN 4145): Reserva Indigena Casanillo; S 22°11'02" W 59°19'07"; *T. rufescens* (MNHN 4340-1): Boquerón; 7 ma. División de Infantaria Tte 1º Alfredo Stroessner; S 22°39.5' S 61°30.5'; *T. rufescens* (MNHN 4359): Boquerón; Neuland; *T. rufescens* (MNHN 4358): Boquerón; 5 km W Filadelfia; *T. rufescens* (MNHN 4146-7): Boquerón; 60 km ENF Filadelfia; *T. rufescens* (MNHN 4337): Boquerón; Colonia Campo Alegre; 25 km W de Neuland; *T. rufescens* (MNHN 3044): Boquerón; Filadelfia; T.
rufescens (MNHNP 3036, 3040): Chaco; 70 km NW Filadelfia on road to Madrejón.

*Tupinambis* merianae (MNHNP 3984–4000, 4345): PARAGUAY; Alto Paraguay; 30 km SW Bahia Negra; Colonia Poterrito; *T. merianae* (MNHNP 3043): Alto Paraguay; Bahia Negra; *T. merianae* (MNHNP 4326): Puerto Casado; *T. merianae* (MNHNP 3042): Canindeyu; Reserva Mbaracayu; 10 km W Cerro del Norte; 7: merianae (MNHNP 3041): Itapúa; 10 km W Cerro del Norte; *T. merianae* (MNHNP 4365): Paraguari; Lago Ypoa; 8 km W Valle Apua; *T. merianae* (MNHNP 3039): Amamary; Reserva Indigena Casanillo; 7: merianae (MNHNP 4426): Alto Parana; 15 km SW Santa Fe; *T. merianae* (MNHNP 4011): Canindeyu; Reserva Mbaracayu; 7: merianae (MNHNP 4336): Presidente Hayes; 10 km N of Ruta Trans-Chaco; 7: merianae co; S 25°20'6" W 57°31'05"; *T. merianae* [USNMField 166779 (tissue 780), MNHNP 6234-5]: Caazapa; Parque Nacional Caaguazu; Administración S 26°05'59.0" W 55°31'31.3"; *T. merianae* (MNHNP 4008): Canindeyu; Reserva Mbaracayu; Puesto Carapa; *T. merianae* (USNMField 167299): Canindeyu; Reserva Mbaracayu; 8 km S Puesto Laguneta; 7: merianae (MNHNP 3043): Itapua; 10 km W Centro de Estudios, 7: merianae (MNHNP 4344, 6176): Cordillera; Estancia Sombrero; 25°04' S 56°36' W; *T. merianae* (MNHNP 4364): Cordillera; Estancia Ypoa; S 25°55'5 W 59°25'9; *T. merianae* (MNHNP 4344, 6176): Cordillera; Estancia Sombrero; 8 km NW Cleto Romero; S 25°04'26" W 56°36'08"; *T. merianae* (MNHNP 3041): Itapua; 10 km W Centro de Desarrollo Forestal; *T. merianae* (MNHNP 3977): Misiones; 15 km SSW Santiago; S27°15'8" W 56°49'8; *T. merianae* (MNHNP 3815): Misiones; Yabebyr; *T. merianae* (MNHNP 3052): Misiones; San Ignacio; S 25°53'01" W 57°01'06"; *T. merianae* (MNHNP 6175): Paraguari; Lago Ypoa; S 25°56'71" W 57°26'80"; *T. merianae* (MNHNP 4365): Paraguari; Lago Ypoa; Estancia Ypoa; 32 km W Valle Apua; *T. merianae* (MNHNP 2838–9, 3037, 4327): Paraguari; Parque Nacional Yby-cui; S 26°56'5" W 56°50'2; *T. merianae* (MNHNP 6195): Presidente Hayes; Estancia Villa Rey, Casco Principal; *T. merianae* (MNHNP 6237): Presidente Hayes; Juan de Zalazar; *T. merianae* (MNHNP 4142–4): Presidente Hayes; Juan de Zalazar; S 23°04'36" W 59°14'07"; *T. merianae* (MNHNP 5070): Presidente Hayes; Juan de Zalazar; 2 km E administration; S 22°26'40" W 58°42'40"; *T. merianae* (MNHNP 4328): Presidente Hayes; km 75.5 Ruta Trans-Chaco; *T. merianae* (MNHNP 6218, 6228): Presidente Hayes; Reserva Indigena Casanillo; S 22°11'02" W 59°19'07"; *T. merianae* [USNMField 166649 (tissue 649)]: PARAGUAY; Presidente Hayes; Ruta Pozo Colorado to Concepcion; S 23°31'07.0" W 57°50'56.7"; *T. merianae* (MNHNP 4346): Central; San Lorenzo; S 25°20'6" W 57°31'05"; *T. merianae* [USNMField 166779 (tissue 780), MNHNP 6234-5]: Caazapa; Parque Nacional Caaguazu; Administración S 26°05'59.0" W 55°31'31.3"; *T. merianae* (MNHNP 6226): Concepción; 17 km S 20 km E Yby Yau; near Estancia 7 Lagunas; S 23°10.131' W 56°21.568'. *Kentropyx viridistriga* [USNMField 166784 (tissue 784)]: PARAGUAY; Amambay; Parque Nacional Cerro Cora.

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