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Phylogenetic Relationships of the Endangered Shenandoah Salamander (*Plethodon shenandoah*) and Other Salamanders of the *Plethodon cinereus* Group (Caudata: Plethodontidae)

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ABSTRACT.—**The Shenandoah Salamander (*Plethodon shenandoah*), known from isolated talus slopes on three of the highest mountains in Shenandoah National Park, is listed as state-endangered in Virginia and federally endangered under the U.S. Endangered Species Act. A 1999 paper by G. R. Thurow described *P. shenandoah*-like salamanders from three localities further south in the Blue Ridge Physiographic Province, which, if confirmed, would represent a range extension for *P. shenandoah* of approximately 90 km from its nearest known locality. Samples collected from two of these three localities were included in a molecular phylogenetic study of the known populations of *P. shenandoah*, and all other recognized species in the *Plethodon cinereus* group, using a 792 bp region of the mitochondrial cytochrome-*b* gene. Phylogenetic estimates were based on Bayesian, maximum likelihood, and maximum parsimony methods and topologies examined for placement of the new *P. shenandoah*-like samples relative to all others. All topologies recovered all haplotypes of the *P. shenandoah*-like animals nested within *P. cinereus*, and a statistical comparison of the best likelihood tree topology with one with an enforced (Thurow + Shenandoah *P. shenandoah*) clade revealed that the unconstrained tree had a significantly lower –ln L score ($P < 0.05$, using the Shimodaira-Hasegawa test) than the constraint tree. This result and other anecdotal information give us no solid reason to consider the Thurow report valid. The current recovery program for *P. shenandoah* should remain focused on populations in Shenandoah National Park.**

The Shenandoah Salamander (*Plethodon shenandoah*) was described by Highton and Worthington (1967) from northwest-facing talus slopes on three of the highest mountains in Shenandoah National Park (SNP) in the Blue Ridge Physiographic Province of Virginia. Until recently this was believed to be the entire known range of *P. shenandoah* (Highton, 1988). Because of its very restricted geographic distribution (~ 6 km^2), the species was listed as endangered by the Commonwealth of Virginia in 1987 (Wynn, 1991), and as federally endangered under the U.S. Endangered Species Act in 1989. The Shenandoah Salamander Recovery Plan (United States Fish and Wildlife Service [USFWS], 1994) outlined a number of tasks aimed at further understanding and mitigating any human-caused threats to *P. shenandoah*. One task emphasized the need to "Continue searches of appropriate habitat to define boundaries of existing populations and to determine whether additional populations exist" (task 1.0; p. 13). Thurow (1999) reported *P. shenandoah*-like salamanders from the Blue Ridge Physiographic Province southwest of the known sites in SNP, which would represent a range extension for *P. shenandoah* of approximately 90 km from its nearest known locality. Thurow also hypothesized that hybridization is occurring between *P. shenandoah* and *Plethodon cinereus* in this area and that a threat may exist to the genome of *P. shenandoah* caused by hybridization. Thurow's evidence for recognizing these populations as *P. shenandoah* was morphological, and he supported his findings and interpretations with photographs (figs. 2 and 3; p. 271). However, others have searched these sites as recently as 2000 and have seen only *P. cinereus*-like animals (R. Highton, pers. obs.). *Plethodon shenandoah* has not been documented outside of SNP by other sources (Mitchell and Reay, 1999).

Highton (1999) showed that *P. shenandoah* and *P. cinereus* are genetically divergent, based on 24 allozyme loci (mean Nei $D = 0.22$ between the species, and $D = 0.18$ between a pair of sympatric populations of the two species in SNP). Carpenter et al. (2001) used mtDNA sequence data and

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showed that these two species were reciprocally monophyletic at this locus, thus providing a baseline for a molecular diagnostics approach (Birstein et al., 1998; Heist and Gold, 1999; Shivji et al., 2002) to test Thurow's claim that his specimens represent previously unknown populations of *P. shenandoah*. Our approach here is to compare specimens collected from the ''Thurow localities'' to (1) unambiguously pure individuals of *P. shenandoah* from SNP (many identified on the basis of morphology and mtDNA by Carpenter et al., 2001); (2) all four genetically distinct groups of *P. cinereus* identified by Hass (1985) and discussed by Highton (1999, 2000); and (3) all other recognized species of the *P. cinereus* group, including two species described by Highton (1999) on the basis of allozyme data. Should the identification of Thurow's material as *P. shenandoah* be verified, then the Shenandoah Salamander Recovery Plan would need to be modified to include these populations. Should results show that these populations represent another species, then additional conservation efforts might be needed.

Although assessment of species status might seem easy in principle, there are controversies as to what definitions of species should be applied. One is the distinction among ''evolutionary significant units'' (ESUs, which qualify as species by some criteria), and demographically independent ''management units'' (Avise, 2000; de Queiroz, 1998; Crandall et al. 2001). Further, operational criteria for testing and recognizing these entities are often not clearly defined in empirical conservation genetics studies (Sites and Crandall, 1997), and these issues are compounded in salamanders because rates of morphological evolution are notoriously slow in many groups, and traditional morphologically based species often drastically underrepresent the true number present (Chippindale, 2000; Tilley, 2000; Wake and Jockusch, 2000). This phenomenon is especially evident in eastern North American species of *Plethodon*, where molecular studies often reveal the presence of cryptic species (Highton, 1995, 1997, 1998, 1999, 2000; Highton and Peabody, 2000). However, given clearly stated operational criteria for defining species and ESUs, and a sampling design appropriate to the criterion chosen, empirical results supporting any of the above options should be of value to conservation efforts, simply by defining the number of distinct lineages within and among the named entities, and suggesting the approximate geographic limits of each.

In this study, we sample two of the three localities claimed by Thurow (1999) to harbor populations of *P. shenandoah*-like salamanders, for genetic comparisons to all other recognized species in the *P. cinereus* group (Highton, 1999). Specifically, mitochondrial cytochrome-*b* sequences were obtained from salamanders from multiple localities in the ranges of all but the species with restricted distributions. Hass (1985) showed that there is a considerable amount of geographic protein variation in the southern part of the range of *P. cinereus*. She found four genetically well-differentiated groups within the species but did not recognize them as different species because there is considerable hybridization between the groups at parapatric contacts. The populations of *P. cinereus* in SNP are members of her Group III, whereas those in the area from which Thurow obtained samples are members of Group II. Phylogenetic history is estimated under both maximum likelihood (ML) and maximum parsimony (MP) optimality criteria and tree topologies examined for placement of the new *P. shenandoah*-like samples relative to all others. Conspecificity with *P. shenandoah* from SNP would be confirmed if the Thurow samples are recovered with strong support as part of a monophyletic group with the SNP samples of *P. shenandoah*. A result showing strong support for phylogenetic affinities of the Thurow samples with some other taxon, or support for distinctness of these samples relative to all currently recognized taxa in the *P. cinereus* group, would falsify the hypothesis of conspecificity with the SNP *P. shenandoah*.

Materials and Methods

Taxon Sampling and Outgroup Choice.—Mitochondrial DNA sequences were obtained from a total of 170 salamanders, of which 163 samples from 39 localities represented all recognized species of the *P. cinereus* group (Highton, 1999), including samples of Hass' (1985) four groups of *P. cinereus*. Five species were used as outgroups, representing three other eastern species groups, and although different combinations of these outgroups were used in this study (including combining them as a single clade; Mahoney, 2001), a strongly supported monophyletic *P. cinereus* group with identical internal topologies was always recovered. For simplicity we used *P. punctatus* and *P. wehrlei* to root trees in this study, based on the fact that they had the longest branches in our unrooted trees. Table 1 summarizes the number of individuals sequenced and localities for all taxa used in this study. We sequenced at least two individuals from most localities but included more individuals when possible, particularly for key populations of *P. cinereus* and *P. shenandoah*, as well as salamanders from the Thurow localities (Fig. 1). In most cases, tail tips were aseptically collected from adult salamanders, stored in the field in 99% ethanol, and the animals released at their capture points.

Some tissue specimens were collected by one of us (RH) in connection with systematic studies of eastern *Plethodon*, and in most cases, voucher specimens are deposited in the National Museum of Natural History (NMNH).

Laboratory Procedures.—Total genomic DNA was extracted from liver/muscle tissues and preserved in 96% ethanol, following the protocol developed by Fetzner (1999). Three microliters of extraction product were electrophoresed on 1% agarose gel to estimate the quality and amount of genomic DNA, and sample dilutions were performed where necessary prior to polymerase chain reaction (PCR) amplification. The cytochrome-*b* (cyt-*b*) mitochondrial gene region was amplified via PCR in a cocktail containing 2.0 μl of template DNA (approximate concentration estimated on a 2% agarose gel), 8 μl of dNTPs (1.25 mM), 4 μl of 10x Taq buffer, 4 μl of each primer (10 μM), 4 μl of MgCl (25 mM), 24 μl of distilled water and 0.25 μl of Taq DNA polymerase (5U/μ) from Promega Corp., Madison, Wisconsin. A fragment of approximately 792bp from the cyt-*b* gene was amplified using primers GluDGL (3′-TGACTTGAARAACCAYCGTTG-3′; Palumbi, 1996) and cyt-*b*-3 (5′-GGCAAATAG-GAARTATCATTC-3′; Palumbi, 1996), and the internal primers cyt-*b*-2 (5′-CCCTCAGAATGA-TATTTGTCCTCA-3′; Palumbi, 1996) and F1 (TGAGGACARATATCHTTYTGRGG; A. Whiting, unpubl.; available from JWS upon request) were used for sequencing reactions.

Double-stranded PCR amplified products were checked by electrophoresis on a 1% agarose gel (size of the target region estimated using a molecular weight marker), purified using a GeneClean III kit (BIO101, Inc., Vista, CA), and directly sequenced using the Perking Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA). Excess of Dye Terminator was removed with CentriSep spin columns (Princeton Separations, Inc.), and sequences were fractionated by polyacrylamide gel electrophoresis on a ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA) at the DNA Sequencing Center at Brigham Young University. Sequences were deposited in GenBank under accession numbers AY378025–AY378079.

Phylogenetic Analyses.—Sequences were edited and aligned using the program Sequencher 3.1.1 (Gene Codes Corp., Inc., 1995), and translated into amino acids for confirmation of alignment. Missing bases (in two terminals only) were coded as "?". A matrix with 173 sequences was input in the program Collapse v1.1 (available from http://bioag.byu.edu/zoology/crandall_lab/programs.htm), to obtain a matrix with nonredundant haplotypes ($N = 55$). For MP analysis all characters were equally weighted, and we conducted a heuristic search with 1000 replicates of random taxon addition with tree-bisection-reconnection (TBR) branch-swapping using PAUP* (vers. 4.0b4b; D. L. Swofford, Sunderland, MA, 2001). Nodal support was assessed by 10,000 pseudoreplicates of nonparametric bootstrap analysis (BS; Felsenstein, 1985), with strong support being inferred if BS $\geq$ 70 (Hillis and Bull, 1993; with caveats).

For ML analysis, we used the program Modeltest (vers. 3.04; Posada and Crandall, 1998; available from the same website) to select the model of molecular evolution that best fit the cyt-*b* sequences, and on the basis of likelihood ratio tests, selected the HKY + I + Γ model (Hasegawa et al., 1985). A heuristic search with 10 random addition replicates using the TBR branch-swapping algorithm was performed to obtain the ML tree. Because of computational limitations imposed by ML estimation, we used PAUP* to perform two separate searches with 50 random addition replicates each, in a bootstrap analysis, and then combined the total 100 pseudoreplicates to obtain the bootstrap proportions. All ML analyses were performed on an IBM Sp2 supercomputer in the BYU supercomputing facility.

We implemented two independent Bayesian analyses using MrBayes 2.0 (Huelsenbeck and Ronquist, 2001), based on the GTR + I + Γ model of evolution (Yang, 1994, Gu et al., 1995). A priori the specific parameter values were uniform and were estimated as part of the analysis. To more thoroughly explore the parameter space we ran Metropolis-Coupled Markov Chain Monte Carlo simulations (MCMCMC) with four incrementally heated chains, using the default values. From a random starting tree we ran 2.0×10^6 generations and sampled the Markov chains at intervals of 100 generations to obtain 10,000 sample points. We determined when stationarity was reached (to discard the "burn-in" samples) by plotting the log likelihood scores of sample points against generation time; when the values reached a stable equilibrium, before 40,000 generations, stationarity was assumed. The equilibrium samples (the 19,600 trees retained after burn-in) were used to generate a 50% majority rule consensus tree. The percentage of samples that recover any particular clade on this tree represents that clade's posterior probability (PP); these are the *P*-values, and we consider PP $\geq$ 95% as evidence of significant support for a clade (Huelsenbeck and Ronquist, 2001).

Results

The MP search recovered 80 equally parsimonious trees (L = 883, CI = 0.493, RI = 0.842), from which a strict consensus tree was generated to compare with the ML and Bayesian trees. In

TABLE 1. Number of individuals and museum voucher numbers of all ingroup and outgroup taxa, by locality (presented in order within species by north to south gradient), used in this study for the cytochrome-*b* gene region; locality numbers match those in Figure 1. Museum numbers represent USNM vouchers.

State County	(Loc. #) Locality Description	# Individuals (Museum #s)	Coordinates	
			Latitude N	Longitude W
Ingroup taxa				
		Plethodon cinereus		
West Virginia				
Preston	(1) Near Lake Terra Alta	3 (75177–79)	39°27′24″	79°31′08″
Kentucky				
Owen	(2) Near Glencoe	4 (59864–67)	38°42′00″	84°49′01″
Virginia				
Rappahannock	(3) Pinnacles, Shenandoah National Park	10 (PCP1–P10)	38°37′35″	78°20′09″
Page	(4) Stony Man, Shenandoah National Park	10 (PCS1–S10)	38°35′48″	78°22′45″
Madison	(5) Hawksbill Mountain, Shenandoah National Park	10 (PCH1–H10)	38°33′20″	78°23′11″
Bedford	(7) Thunder Ridge	3 (TR-1–TR-3)	37°32′22″	79°29′24″
Southampton	(8) Near Berlin	3 (75346–47,49)	36°52′26″	76°57′28″
Washington	(9) Low Gap, Brumley Mt.	3 (57215–17)	36°50′13″	82°05′15″
Maryland				
St. Mary's	(6) Near Park Hall	3 (67202–04)	38°13′23″	76°27′12″
North Carolina				
Mitchell	(10) Indian Grave Gap	4 (64109–12)	36°06′36″	82°21′40″
		P. electromorphus		
Pennsylvania				
Beaver	(12) Near Frankfort Springs	3 (54087–89)	40°29′50″	80°25′52″
West Virginia				
Mason	(13) Near New Haven	5 (65644, 68437–40)	38°59′18″	81°58′47″
Gilmer	(15) Cedar Creek State Park	4 (75911–14)	38°52′34″	80°51′03″
Kentucky				
Boone	(14) Near Big Bone Lick	4 (56969–72)	38°52′56″	84°44′53″
		P. hoffmani		
Pennsylvania				
Indiana	(16) Near Kintersburg	4 (54050–53)	40°42′47″	79°05′00″
West Virginia				
Pendleton	(17) Near Circleville	3 (72894–96)	38°39′16″	79°30′55″
Summers	(18) Near Brooks	4 (56950–53)	37°42′49″	80°53′12″
		P. hubrichti		
Virginia				
Botetourt	(19) Bryant Ridge	1 (BR-1)	37°31′00″	79°34′08″
Bedford	(20) Near Sunset Field	4 (67008–11)	37°30′45″	79°30′56″
Bedford	(21) Blue Ridge Skyline	4 (PH-1–PH-4)	37°29′34″	79°32′39″
Bedford	(22) Flyod Field	2 (FF-1–FF-2)	37°29′26″	79°32′54″
Bedford	(23) Overstreet Creek	2 (OC-1, OC-3)	37°29′23″	79°31′55″
Bedford	(24) Falling Water Cascades	2 (FWC-1–FWC-2)	37°28′22″	79°34′38″
Botetourt	(25) Flat Top Trail	2 (FTT-1–FTT-2)	37°27′35″	79°35′11″
		P. nettingi		
West Virginia				
Pocohontas	(26) Gaudineer Knob	4 (66736–39)	38°36′54″	79°50′38″
		P. richmondi		
West Virginia				
Cabell	(28) Ritter Park, Huntington	2 (52930–31)	38°24′13″	82°26′03″
Raleigh	(30) Near Hinton	3 (51447, 51449–50)	37°42′51″	80°53′42″
Kentucky				
Woodford	(29) Near Clear Creek	4 (53584–87)	37°55′31″	84°42′07″

TABLE 1. Continued.

State County	(Loc. #) Locality Description	# Individuals (Museum #s)	Coordinates Latitude N	 Longitude W
North Carolina				
Watauga	(31) Near Deep Gap	4 (58108–11)	36°14′05″	81°30′03″
		P. serratus		
Oklahoma				
McCurtain	(32) Beavers Bend State Park	4 (74643–46)	34°07′29″	94°40′15″
Georgia				
Henry	(33) Near McDonough	4 (77366–67, 70, 72)	33°29′53″	84°11′03″
		P. shenandoah		
Virginia				
Page	(34) Pinnacles, Shenandoah National Park	7 (PSP2–P7, PSP10)	38°37′50″	78°20′02″
Page	(35) Stony Man, Shenandoah National Park	8 (PSS2–S9)	38°35′55″	78°22′41″
Page	(36) Hawksbill Mountain, Shenandoah National Park	10 (PSH1–H10)	38°33′24″	78°23′42″
Nelson	(37) The Priest "Thurow *P. shenandoah*"	4 (P1–P4)	37°49′13″	79°03′50″
Amherst	(38) Pompey Mountain "Thurow *P. shenandoah*"	5 (PO1–3, 5, 6)	37°45′22″	79°10′03″
		P. virginia		
West Virginia				
Hampshire	(39) Nathaniel Mountain	4 (74888–91)	39°11′48″	78°47′30″
Pendleton	(40) Near Cow Knob	4 (75898–901)	38°41′36″	79°05′44″
Outgroup Taxa				
		P. dorsalis		
Kentucky				
Jefferson	(11) Near Caperton Swamp	1 (59859)	38°17′09″	85°40′39″
		P. punctatus		
West Virginia				
Pendleton	(27) Near Cow Knob	2 (67637–38)	38°41′36″	79°05′44″
		P. websteri		
Alabama				
Lee	(41) Near Opelika	1 (77343)	32°36′17″	85°17′57″
		P. wehrlei		
Pennsylvania				
Cambria	(42) Near Emeigh	2 (69395, 69398)	40°42′07″	78°48′08″
		P. yonahlossee		
North Carolina				
Mitchell	(43) Indian Grave Gap	1 (77653)	36°06′36″	82°21′40″

both cases, the two independent Bayesian analyses yielded almost identical consensus topologies, and this topology was very similar to the strict consensus MP tree. The ML search recovered one tree (ln L = –5038.34542) under the HKY parameters, and because it was also was very similar to the Bayesian and MP topologies, it is the only tree presented here (Fig. 2).

There was strong support in all tree reconstruction methods for monophyly of the *P. cinereus* group (Fig. 2, MP and ML Bootstrap Support [BS] = 100; Bayesian Posterior Probabilities [PP] = 1.0). Within the *P. cinereus* group, there was also strong support for placement of *P. serratus* as the sister taxon to the remaining members of the group, and this species includes two genetically divergent lineages. The remaining haplotypes in the *P. cinereus* group were almost evenly distributed between two large clades; one of these was recovered with strong

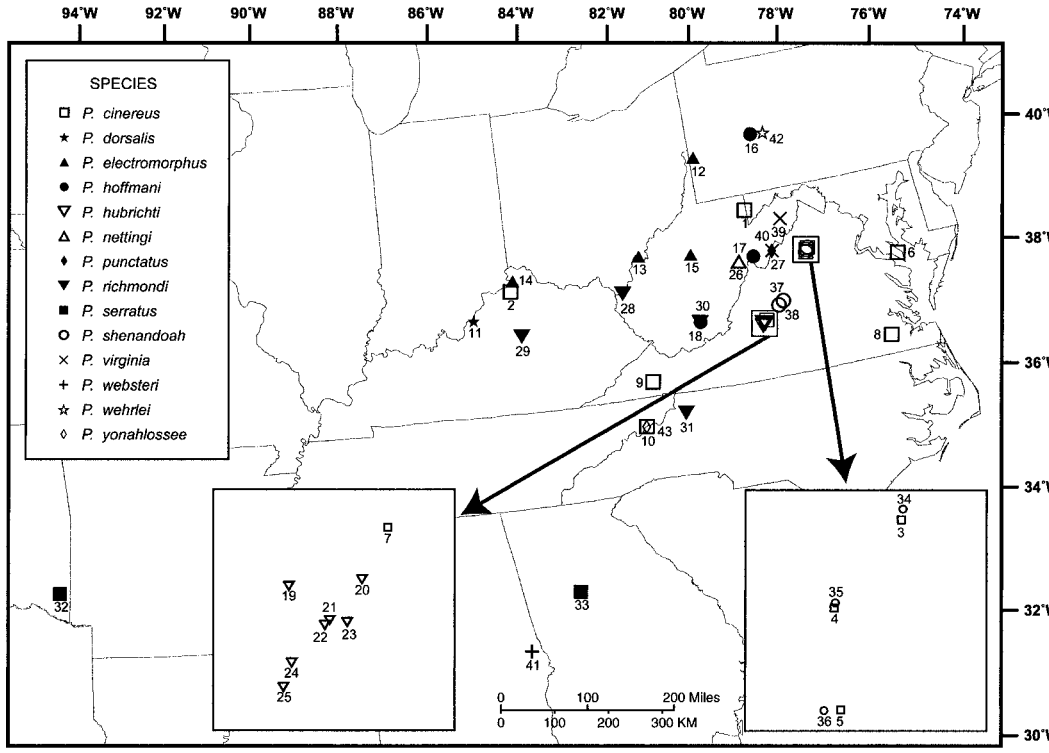

FIG. 1. Geographic distribution of the taxa included in this study.

MP-BS and PP support (94 and 0.99, respectively), with a strongly supported (*P. hubrichti* + *P. nettingi*) clade (ML-BS and PP support values = 84 and 0.99, respectively). Two other species comprised the sister clade of (*P. electromorphus* + *P. richmondi*), which was supported by MP-BS and PP values of 96 and 1.0, respectively. The second major clade included the strongly supported (*P. virginia* + *P. hoffmani*) clade (ML-BS = 97, MP-BS = 96, PP = 1.0), and a second (*P. shenandoah* + *P. cinereus*) clade (MP- and ML-BS = 88, PP = 1.0); the entire clade was only weakly supported by the Bayesian analyses (PP = 0.82). Within *P. cinereus*, two clades were recovered (samples 1–2, 7, 9–10; and 3–6, 8). The second group corresponded to Hass's Group III based on allozymes. Samples 1 and 10 were the sole representatives of Hass' Groups IV and I, respectively. Samples 2, 7, and 9 were members of Hass' Group II. The supposed *P. shenandoah* haplotypes (samples 37–38) from Thurow's (1999) Pompey Mountain and The Priest localities were not placed with the other samples of *P. shenandoah* but instead clustered within *P. cinereus* Group II, as did other samples from the same area analyzed by Hass (1985). We constrained a tree topology to include all haplotypes from the Thurow populations to form a clade with the SNP *P. shenandoah* haplotypes and conducted a ML search with 100 replicates under this constraint. The consensus tree obtained was compared with the unconstrained tree using the Shimodiara-Hasegawa test (1999) in PAUP (under the same model of evolution invoked invoked for the original ML search), and revealed a significantly less likely constraint tree (ln L = –9950.10358) relative to the best tree (ln L = –5038.34542; $P < 0.05$).

DISCUSSION

Accurate identification of species and populations of questionable status is the first step in any conservation program (Avise, 1989), and two phylogenetically based methods are available for use with DNA sequence data to determine species identification (Birstein et al., 1998). One of these requires sequencing of phylogenetically informative regions of DNA and tree-reconstruction to identify the sister taxon of the unknown samples of interest; this method has been deployed to identify origins of samples ranging from whales to marine turtles (Baker et al., 1996; Encalada et al., 1994). The second method requires identification of diagnostic nucleotide positions in DNA sequences as indicators of species origin (reviewed by Birstein et al., 1998),

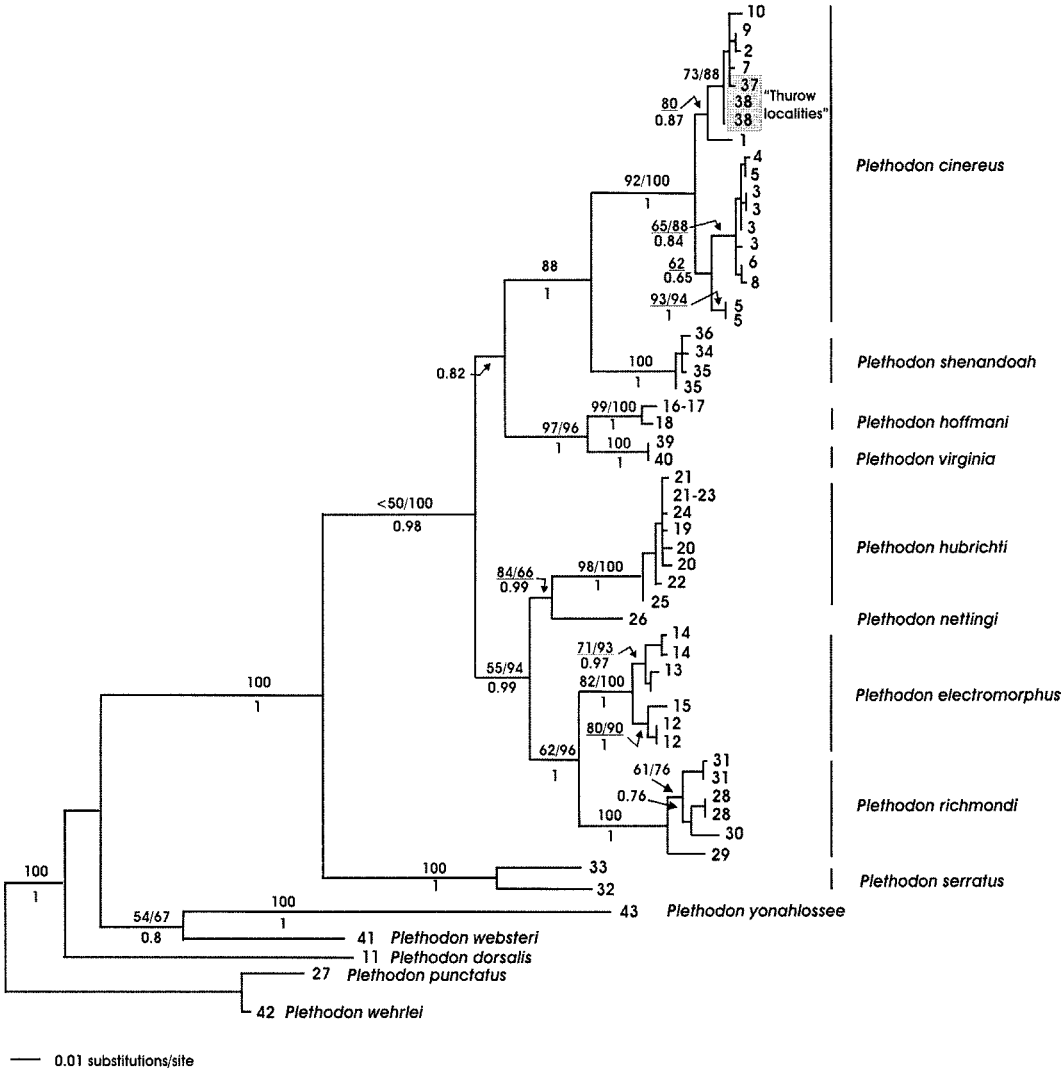

FIG. 2. Single Maximum Likelihood tree (ln L = –5038.34542) obtained for all ingroup haplotypes used in this study. Numbers above the branches represent ML bootstrap support/MP bootstrap support (only one number is presented when these are equal), and numbers below the branches correspond to Bayesian posterior probabilities. Numbers on the terminals represent sampled localities and match those in Figure 1, and the shaded box identifies the "Thurow localities" included in this study.

but both require the development of a database for implementation. In the tree-building approach, the database needed should include a representation of all possible taxa that could be related to, or identical to, the unknown samples. In this study, we have sampled all currently recognized species within the *P. cinereus* group as defined by Highton (1999); there is strong evidence for monophyly of this group (Mahoney, 2001), and widely distributed species were sampled from several localities (Table 1). This sampling design should be adequate to evaluate the phylogenetic affinity of the "Thurow samples" with a high degree of certainty.

Trees recovered by all three search algorithms used in this study revealed strong support for a *P. cinereus* clade that included samples collected from scattered sites within the range of the four groups of *P. cinereus* (Table 1, Fig. 1), with both samples from the "Thurow localities" nested strongly within this species (Fig. 2). When the haplotypes from these two populations are constrained to be part of the *P. shenandoah* clade, the Shimodaira-Hasegawa test reveals a significantly

less likely tree topology. We conclude that at least these two populations are members of the geographically widespread, and nonthreatened, *P. cinereus*.

It is possible that either Thurow's supposed *P. shenandoah*-like animals were present at these localities but missed by the collectors or are present only at his third locality (where fieldwork was conducted on several occasions but no salamanders were found). We view this as an unlikely interpretation, because the species *P. cinereus* and *P. shenandoah* are not difficult to distinguish in the field (Highton and Worthington, 1967), and those doing our field collecting (RH, FH, and REJ) have had considerable experience with both species. An alternative is that both *P. shenandoah*-like animals are still present at these localities but are either at such low densities as to be extremely difficult to find, or hybridization to sympatric *P. cinereus* (Thurow, 1999) has been so extensive that the *shenandoah*-like haplotypes are rare. Thurow did not give any indication of rarity of these phenotypes at any of the sites described in his original paper; he made two visits to each, and found salamanders resembling both species and intermediates each time at all three places (1999:270–271). One of Thurow's concerns was that all of these purported *shenandoah*-like populations were in various stages of being genetically introgressed and swamped by *P. cinereus* (1999:272), but this conclusion was based only on a qualitative assessment of salamander phenotypes. Thurow published photographs of some of the salamanders he examined from all three localities (1999:figs. 2–4), but these specimens were not properly vouchered in any museum collection, and subsequent attempts to locate them have not been successful (Thurow is deceased). In an earlier study of the genetic integrity of the three populations of *P. shenandoah* originally described from Shenandoah National Park, Carpenter et al. (2001) found no evidence of extensive introgression of *P. cinereus* mtDNA haplotypes into any of the populations of *P. shenandoah*. Although some of the salamanders figured by Thurow do resemble pure *P. shenandoah* from Shenandoah National Park, as these were illustrated by Highton and Worthington (1967), we can find no solid reason to consider the Thurow conclusions valid. The current recovery program (USFWS, 1994) should remain focused on populations in SNP.

This work does reveal some interesting features of patterns of evolution of the *P. cinereus* group throughout its range, and highlights some remaining issues and some taxa in need of further study. Our results confirm and extend Mahoney's (2001) report of a monophyletic *P. cinereus* group; her study (based on part of the mtDNA ND4 gene region and associated tRNAs) included more species representing eastern and western groups of *Plethodon*, but fewer representatives of the *P. cinereus* group. Our sampling was more extensive within the *P. cinereus* group, and included several species of the other groups of eastern *Plethodon* (Table 1). Our tree topologies always recovered a strongly supported *P. cinereus* clade, regardless of which species or combination of species were used to root the tree (Fig. 2).

Within the *P. cinereus* clade, several of the relationships recovered in this study confirm results reported by Highton (1999) based on UPGMA and neighbor-joining (NJ) trees generated from matrixes of pairwise genetic distance coefficients calculated from electromorph frequencies generated from 24 allozyme loci. In that work, Highton discovered that populations originally included under the name *P. hoffmani* included two parapatric species, and he described the unnamed species as *P. virginia*. Similarly, he discovered that *P. richmondi* also consisted of two species, and named the second of these as *P. electromorphus*. These pairs of species were the most similar on Highton's UPGMA and NJ trees (1999:figs. 2 and 3, respectively), and our mtDNA haplotype tree recovers (*P. hoffmani* + *P. virginia*) and (*P. richmondi* + *P. electromorphus*) clades with strong support (Fig. 2). Highton's trees recover *P. serratus* in a basal or nearly basal position, and our topologies always provide strong support for its position as sister clade to all other species in the *P. cinereus* group (Fig. 2).

Some major differences are apparent between this study and the topologies of Highton's (1999) clustering algorithms. Most notably these include placement of *P. hubrichti* and *P. nettingi*; *P. hubrichti* is recovered as another basal (i.e., most differentiated) taxon in the UPGMA tree, whereas *P. nettingi* is recovered as sister taxon to *P. shenandoah*, albeit with very weak bootstrap support (= 51; Fig. 2). Highton's NJ tree recovered *P. nettingi* and *P. hubrichti* as sister species, albeit with very low support (BS = 29; Highton, 1999:fig. 3), which is consistent with our topology. In our trees, *P. shenandoah* and *P. cinereus* are sister species, as they are in Highton's NJ tree, but on his UPGMA tree, *P. shenandoah* is the sister species of *P. nettingi*. Because Highton's study did not involve outgroup rooting, and support for many nodes in both of his trees was weak, more data will be required to determine the phylogeny of these species.

Finally, the mtDNA tree topology suggests the likely presence of considerable internal population structure for three of the four species represented by six or more haplotypes. Specifically, *P. cinereus*, *P. electromorphus*, and *P. richmondi* all appear to have sufficient intraspecific

haplotype diversity to warrant further study, whereas only *P. hubrichti* (with a very small range) appears reasonably conservative for the locus examined here.

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A New Species of *Scinax* (Anura: Hylidae) from the Andes of Venezuela

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ABSTRACT.—A new species of *Scinax* is described from Venezuela. It is the only species known from the Venezuelan Andes, between 600 and 1700 m. It differs from other species in the genus by its yellow coloration in life, two dorsolateral stripes, and unpatterned posterior surfaces of thighs. A description of the call is provided. The new species is not assigned to any species group.

RESUMEN.—Se describe una nueva especie de *Scinax* para Venezuela. Esta especie es la única que se conoce de los Andes de este país, entre los 600 y los 1700 msnm. Difiere de cualquier otro *Scinax* por su coloración amarilla, dos bandas dorsolaterales poco conspicuas, y ausencia de patrón en la parte posterior de los muslos. Se describe el canto. La nueva especie no es asignada a ningún grupo de especies.

Tree frogs of the genus *Scinax* Wagler, 1830, are one of the most taxonomically confusing groups among Neotropical anura. On several occasions, species names have been transferred from the genus *Hyla* Laurenti, 1768. Fouquette and Delahoussaye (1977) resurrected the genus *Ololygon* Fitzinger, 1843, for species in the *Hyla rubra* group, characterized by their unique sperm morphology. Duellman and Wiens (1992) placed all *Ololygon* in *Scinax*, based on the priority of that genus name over *Ololygon*. Although Köhler and Böhme (1996) supposed that the gender of *Scinax* was masculine, Frost (2000) argued convincingly that *Scinax* must be treated as feminine.

The genus *Scinax* contains 12 species in Venezuela: *Scinax baumgardneri* (Rivero, 1961), *Scinax boesemani* (Goin, 1966), *Scinax danae* (Duellman, 1986), *Scinax exiguus* (Duellman, 1986), *Scinax fuscomarginatus* (A. Lutz, 1925), *Scinax garbei* (Miranda-Ribeiro, 1926), *Scinax kennedyi* (Pyburn, 1973), *Scinax nebulosus* (Spix, 1824), *Scinax rostratus* (Peters, 1963), *Scinax ruber* (Laurenti, 1768), *Scinax wandae* Pyburn and Fouquette, 1971, and *Scinax x-signatus* (Spix, 1824; Barrio-Amorós, 1998; Barrio-Amorós and Fuentes 2003). *Scinax trilineatus* was synonymized with *S. fuscomarginatus* by Martins

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TABLE 1. Main body measurements taken from the type series and referred material of *Scinax manriquei*. Data on left column under each sex shows the range (maximum and minimum size); right column shows mean and standard error (SE) of the mean. Abbreviations of measurements are described in the Material and Methods. All measurements are expressed in millimeters (mm).

	Males (N = 18)		Females (N = 4)	
	Range	Mean ± SE	Range	Mean ± SE
SVL	23.0–30.0	27.7 ± 1.7	29.5–32.5	31.1 ± 1.1
TL	11.4–15.9	14.2 ± 0.9	13.9–15	14.5 ± 0.8
FeL	9.0–13.0	11.5 ± 0.9	12.6–13.5	13.0 ± 0.4
FL	9.2–12.5	11.2 ± 0.8	11–13.2	9.5 ± 0.8
HeL	8.0–10.2	9.5 ± 0.3	10.2–11.1	10.8 ± 0.4
HW	8.5–10.8	9.1 ± 0.3	9.8–10.8	10.1 ± 0.1
InD	1.7–2.1	1.9 ± 0.1	2–2.8	2.3 ± 0.3
UEW	2.4–4.0	2.9 ± 0.4	2.8–3.2	3.0 ± 0.2
IOD	2.4–3.8	3.2 ± 0.3	3.2–3.9	3.4 ± 0.3
EN	2.0–3.2	2.9 ± 0.3	3–3.5	3.2 ± 0.2
ED	2.4–4.0	3.2 ± 0.4	3–3.9	10.9 ± 0.3
TD	0.8–1.1	0.9 ± 0.1	1.1–1.5	1.2 ± 0.4
FD	0.7–1.4	1.1 ± 0.2	1.1–1.8	1.5 ± 0.2
4TD	0.5–1.3 (N = 17)	1.0 ± 0.2	1–1.8	1.5 ± 0.3
ETS	3.0–4.9	4.1 ± 0.4	4.4–5.0	4.7 ± 0.3
1FiL	2.9–4.3 (N = 16)	3.9 ± 0.4	4.1–4.7	4.4 ± 0.3
2FiL	3.0–5.8 (N = 15)	4.4 ± 0.3	4.8–5.2	5.0 ± 0.3

(1998), with no further explanation. All of these are lowland species, inhabitants of savannas and rain forests. Most can be found in clearings or secondary forest, as well as in human habitations (Hoogmoed and Gorzula, 1979; Duellman, 1997). No species are known from Andean cloud forests or from any montane environments in the Andes of Venezuela (Barrio-Amorós, 1998). Only *S. fuscomarginatus* (= *S. trilineatus*), *S. danae*, and *S. exiguus* are known from elevations between 1000 m and 1350 m in eastern Venezuela, and *S. danae* is the only cloud forest dweller in the Guiana Shield (Duellman, 1986). During a revision of the genus *Scinax* in Venezuela, it became apparent that there was an undescribed species from the Andes. The presence of the same species was also mentioned by Nieto-Castro (1999) under the name *Scinax* sp.2, in Colombia. The existence of this treefrog in montane habitats occupying an elevational range from 600 to at least 1700 m is significant.

MATERIALS AND METHODS

Specimens used in the description and examined (Appendix 1) for comparisons are deposited in CVULA (Colección de Vertebrados, Universidad de los Andes, Mérida, Venezuela), EBRG (Museo de la Estación Biológica Rancho Grande, Maracay, Venezuela), ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia) and MHNLS (Museo de Historia Natural La Salle, Caracas, Venezuela). Measurements were taken to 0.1 mm using dial calipers (Table 1). All measurements are in millimeters (mm). Abbreviations for measurements are SVL, snout–vent length; TL, tibia length; FeL, femur length; FL, foot length; HW, head width; HeL, head length; InD, internarial distance; UEW, upper eyelid width; IOD, interorbital distance; EN, anterior edge of eye to nostril; ED, eye diameter; TD, tympanum diameter; FD, disc width of Finger III; 4TD, disc width of Toe IV; ETS, distance between the anterior edge of the eye to the tip of snout; ETD, eye-tympanum distance; 1FiL, length of Finger I from the interior edge of the thenar tubercle to the tip of the Finger disc; 2FiL, length of Finger II, from the junction of Finger I and Finger III to the tip of finger disc. Measurements are based on adult animals, determined by examination of gonads. Descriptions of color of live specimens are based on color slides. The order of characters in the diagnosis and description follows Duellman (1986) and De la Riva (1993), with some modifications in the order. Webbing formula follows Myers and Duellman (1982). Calls were recorded with a Sony TCM-353V cassette tape recorder and microphone (Sony F-V5), and analyzed with the program Cool Edit Pro 1.2 for Windows. Comparative data are taken from Duellman (1972b, 1986), Fouquette and Pyburn (1972), Pyburn (1992), and Pyburn and Fouquette (1971).

FIG. 1. *Scinax manriquei* in life.

Scinax manriquei sp. nov.
Figure 1

Holotype.—CVULA IV-1094, an adult female, from Mesa Quintero, Guaraque, estado Mérida, Venezuela, 1700 m, 08°07′N, 71°36′W, collected

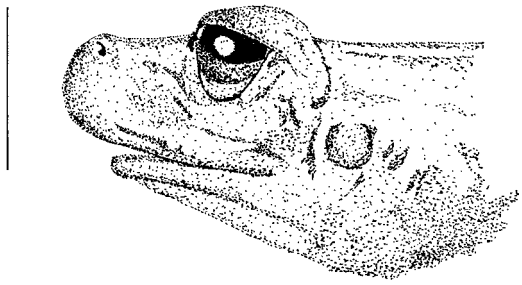

FIG. 2. Head of *Scinax manriquei*. CVULA IV-5138. Line = 5 mm.

4 July 1979 by H. Hita, D. Kontack, and A. de Pascual.

Paratypes.—CVULA IV-5137–38, Campamento Siberia (CADAFE), estado Táchira, 1000 m collected by A. Cardoso, J. Péfaur, and N. Sierra on 28 May 1990; EBRG 1681 and 1685, SW Bramón, Delicias, 600 m, estado Táchira, collected by C. Parrish on 8 April 1982; CVULA IV-2423–24, 9 km southwest of Quebrada Azul, vía La Azulita-La Trampa, 1400 m, estado Mérida, collected by J. Péfaur and A. de Pascual on 27 June 1980.

Referred Specimens.—Venezuela: Estado Mérida: Vía Chiguará-La Trampa (CVULA IV-1107–09). Estado Táchira: SW Bramón, Delicias (EBRG 1679–80, 1682–84). Vía Doradas-Represa Uribante-Caparo (CVULA IV-5810–12). La Alcantarilla, Uribante, 1075 m (CVULA IV-3850). Colombia: Norte de Santander: municipio Chinácota, vereda Paramillo, 1450–1500 m (ICN 15085–94). Norte de Santander: municipio Chinácota, vereda Tenería, 1400–1420 m. (ICN 33528).

Diagnosis.—A species of *Scinax* characterized by (1) finger webbing basal; (2) toe webbing vestigial between Toes I and II, last phalange of Toes II and IV free, and extending to nearly the base of disc on Toe V; (3) tibia length about 51.2% of SVL; (4) dorsal color in life yellow to brown, with indistinct whitish dorsolateral stripes, and brown to gray in preservative with more distinctive dorsolateral stripes; (5) dorsal skin smooth: (6) iris bronze in life, grayish yellow in preservative; and (7) tympanum small (28% of eye diameter).

Scinax manriquei (Fig. 1) is a small to moderately sized species (N = 18, mean = 27.7 mm SVL in males; N = 6, mean = 31.1 mm SVL in females). It can be distinguished easily from other *Scinax* from Venezuela and nearby countries by the following characters (the character of the other species in parentheses): from *S. baumgardneri*, *S. blairi*, *S. boesemani*, and *S. danae* by the presence of dorso-lateral stripes (absent); from *S. x-signatus* by the absence of an X-shaped marking on dorsum (present) and by the absence of

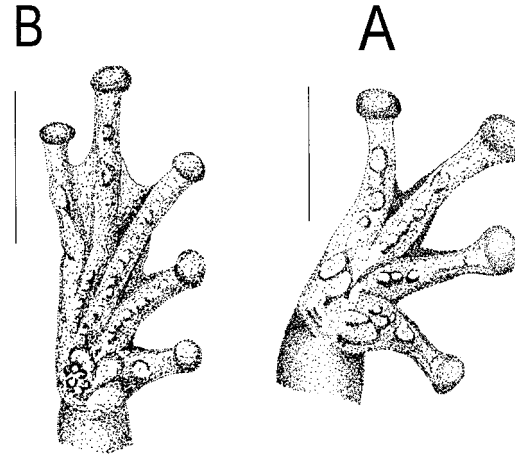

FIG. 3. *Scinax manriquei*. (B) Ventral view of foot. (A) Ventral view of hand. CVULA IV-5138. Line = 5 mm.

a distinct pattern on the posterior surface of the thighs (orange with black pattern on thighs); from *S. rubrus* by the absence of a distinct pattern on the posterior surface of the thighs (orange or yellow with black pattern on thighs); from *S. lindsayi* by the presence of dorso-lateral stripes (lacking) and differences in call; from *S. exiguus* by head slightly longer than wide (much longer than wide), snout rounded in dorsal view (subacuminate), larger size, up to 32.5 mm (size not over 23.0 mm) and completely parapatric distribution; from *S. fuscomarginatus* (as *S. trilineatus*) by absence of three dorso-lateral stripes and larger size (pattern as expressed and body size of 22.5 mm); from *S. wandae* by the presence of two light-colored dorsolateral stripes (two dark dorsolateral stripes and two dark lateral stripes), snout rounded dorsally (pointed) and larger size, males up to 30 mm (males to 26.9 mm); and from species of the *S. rostratus* group (*S. garbei*, *S. kennedyi*, *S. nebulosus*, and *S. rostratus*) by not having an acuminate snout in lateral profile nor an orange or yellow pattern with bands or spots on posterior sides of thighs (profile and patterns on thighs as described).

Description.—Body moderately slender; head slightly longer than wide; snout longer than eye diameter; rounded in dorsal view, projecting beyond margin of lip in profile; canthus rostralis almost rounded; loreal region slightly concave; nostrils oriented antero-laterally; tympanum round (sometimes vertically ovoid), slightly smaller than the disk of Finger III, 28% of eye diameter; supratympanic fold indistinct (Fig. 2); dentigerous processes of prevomers small, oblique, between and posteriorly to moderately large round to ovoid choanae, bearing three to five teeth; tongue cordiform, barely free behind;

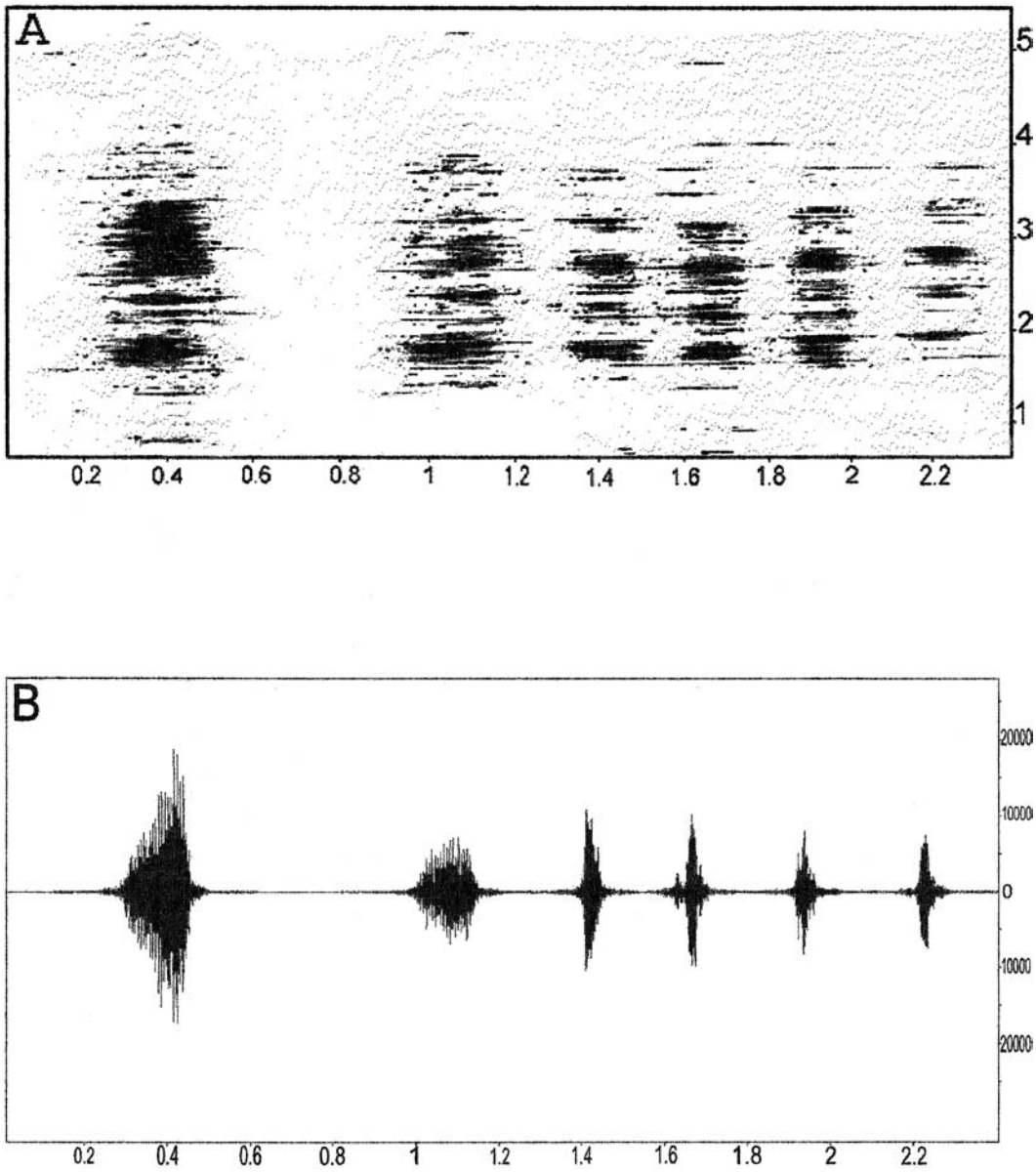

FIG. 4. (A) Audiospectogram and (B) oscilogram of a complex call of *Scinax manriquei*. Duration in milliseconds (bottom); frequency in Hz (right).

vocal slits extending from midlateral base of tongue to angle of jaws; vocal sac single, large, subgular, terminating posteriorly at thoracic fold at axillae level. Axillary membranes absent; ulnar tubercles absent. Fingers long with moderately sized discs; length of Fingers I<II<IV<III; width of disc of third finger bigger than tympanum; thenar tubercle small, flat, elliptical, not very distinct; smaller palmar tubercle flat, bifid, inconspicuous; subarticular tubercles of fingers single, round; supernumerary tubercles minute; all fingers webbed basally, nuptial pads absent (Fig. 3). Hind limbs moderately long; calcars and tarsal fold absent; toes bearing elliptical discs, slightly smaller than those of fingers; length of Toes I<II<III<V<IV; inner metatarsal tubercle large, elliptical; outer metatarsal tubercle small, round; subarticular tubercles moderately large, round; supernumerary tubercles small, round or oval; Toe webbing formula II 2-3 III 2-3 IV 3-1½ V (Fig. 3). Skin on dorsal surfaces of head, body and limbs smooth; skin on belly and

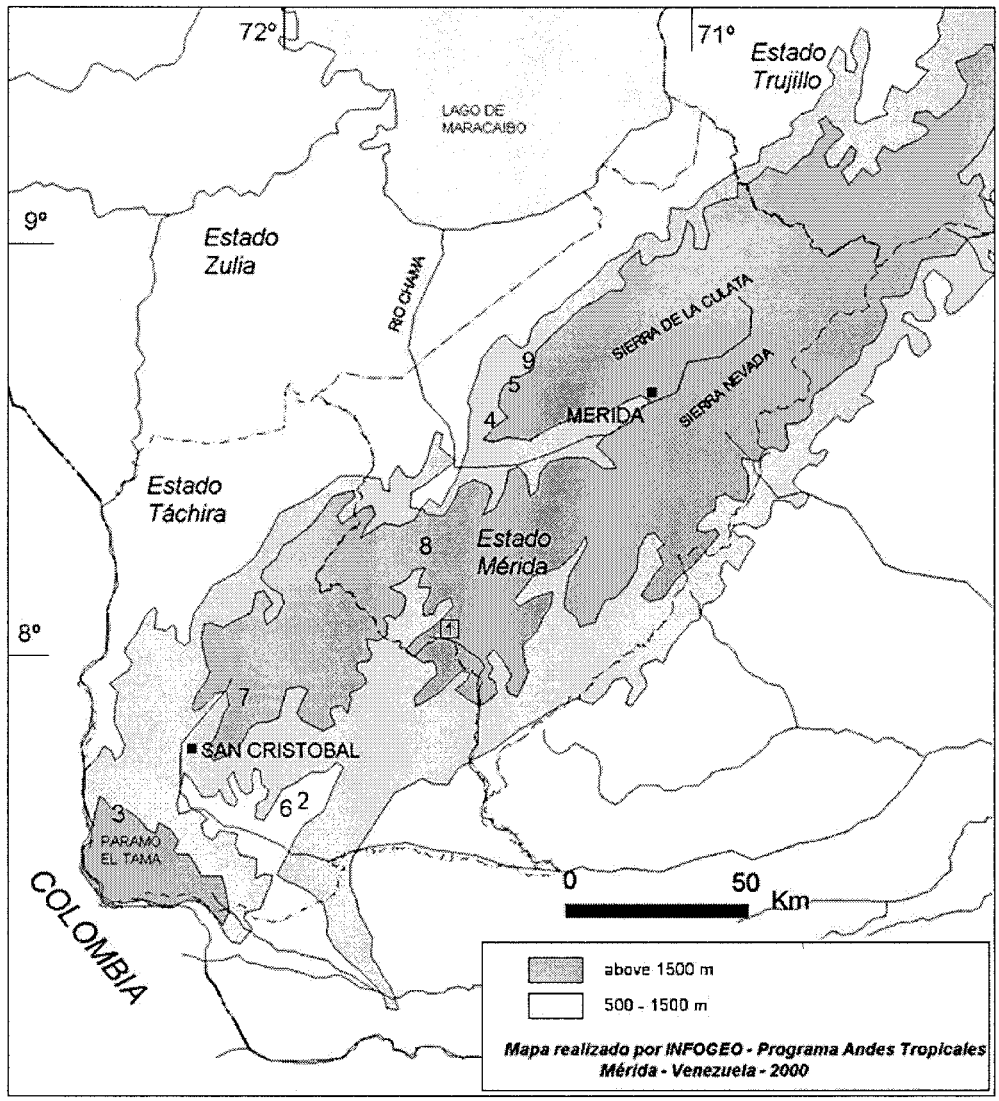

FIG. 5. Distribution of *Scinax manriquei* in the Andes of Venezuela. Numbers shown localities in text. 1—(open square) Mesa Quintero, Guaraque, estado Mérida, type locality. 2—Campamento Siberia (CADAFE), estado Táchira. 3—southwest Bramón, Delicias, estado Táchira. 4—Chiguará. 5—road between Chiguará and El Guamo. 6—Vía Doradas-Represa Uribante, estado Táchira. 7—Parque Nacional Chorro del Indio, estado Táchira. 8—La Macana, near Santa Cruz de Mora. 9—9 km southwest La Azulita, estado Mérida. 10 = Chinácota, Norte de Santander, Colombia.

posteroventral surface of thighs slightly granular; cloacal opening directed posteroventrally, at upper level of thighs.

Measurements of Holotype.—All measurements in millimeters (mm). SVL = 29.5; TL = 13.9; FeL = 13; FL = 13; HeL = 10.9; HW = 9.8; InD = 2.2; UEW = 3; IOD = 3.2; EN = 3; ED = 3.1; TD = 1.2; FD = 1.3; 4TD = 1.8; ETS = 4.4; 1FiL = 4.6; 2Fil = 5.

Coloration in Life.—Based on slides of living, uncollected specimens. Dorsal coloration lemon yellow, occasionally with pale white, inconspicuous dorsolateral stripes (which are more evident in preserved individuals). Throat yellow, more intense on vocal sac; venter and underside of tibiae and feet pale white. No trace of any pattern on posterior surfaces of thighs nor of transverse bars on hind limbs. Upper lip slightly lighter than the yellow of the body. Upper eyelids greenish-yellow. Iris bronze. Dorsum irregularly speckled with fine brown spots. In some specimens, the dorsolateral stripes are more visible and the

dorsal spots may be completely absent. There is no variation between day and night coloration.

Coloration in Alcohol.—Dorsum of body, head and limbs pale brown with two indistinct pale white dorsolateral stripes, beginning behind upper eyelids and extending to the posterior half of the body. Canthal and supratympanic stripes distinctly dark brown. Throat and venter white with a few melanophores on the lower lip. Eyelids greenish-gray and iris grayish-yellow.

There are two phases of coloration in preservative: Phase A, as in the holotype, is pale brown with whitish dorsolateral stripes, (CVULA IV-1107–09 and 3850). CVULA IV-1107, a female, is the darkest with a dirty gray venter. Phase B is pale white to pale yellow, with black melanophores, almost indistinct dorsolateral stripes, and pale white venter with no trace of melanophores (CVULA IV-5137, 5138); the melanophores at the upper thighs seem to align forming ill defined bars (especially in CVULA IV-5137). Some specimens (CVULA IV-2423 and 2424) do not show any sign of dorsolateral stripes. The following specimens belong to Phase B: EBRG 1679-83, EBRG 1685; CVULA IV-5810–12. Specimen EBRG 1680 has two dark brown and symmetrical dots on posterior dorsum, just above the cloacal opening. The dark or sometimes light brown canthal and supratympanic stripes are present in all individuals.

Vocalization.—Recordings were made the night of 22 July 2000 (15°C). At least two kinds of calls were produced. One was only a single note (with duration of 251 msec and a frequency of 3410 Hz). The other was a complex call produced by a different individual (??) with between three and six notes (Fig. 4). The first note was the longest and highest in frequency (251 msec, 3410.4 Hz), separated from the next by a gap of 479 msec. The second note resembled the first, only slightly shorter and with a lower frequency (249 msec, 1965.4 Hz). Next was a series of shorter notes (60, 81, 59, and 46 msec, with gaps of 129, 87, 161, and 201 msec) with frequencies of 1941.5, 3082, 1941.3, and 3324.2 Hz, respectively. The entire sequence was 1744 msec long. Advertisement calls are given in a complex array repeatedly in appropriate (wet or raining) nights. During drier nights the calls are sporadic and simple.

Distribution.—*Scinax manriquei* is known from several localities in the Andes of Venezuela and from two localities in Colombia (Fig. 5). Venezuelan records are from both slopes of the Táchira depression. It is known from a single locality on El Tamá massif, an outreach of the Colombian Cordillera Oriental, at Bramón, 600 m. This frog seems to be widely distributed throughout the Cordillera de Mérida (Mérida and Táchira states) and found both on the northwest face of the Cordillera toward the Maracaibo lake basin (La Azulita at 1400 m, near Santa Cruz de Mora, 1600 m or in Chiguará at 1400 m) and the southeast face toward the upper drainage system of the Orinoquia lowlands or Llanos (Mesa de Quintero at 1700 m, Uribante dam, 1000 m). In Colombia, it is known from two adjacent localities in cloud forest from 1400–1500 m, close to the Venezuelan border (Chinácota).

Natural History.—This species was observed during November 1999 and July 2000 at La Macana, near Santa Cruz de Mora at 1600 m and on the road between Chiguará and El Guamo, at about 1200 m in Mérida state (C. Gottberg, pers. com.), as well as at the field station Siberia in Táchira, at 1400 m (by AC). The habitat at all locations is characterized by a high degree of human perturbation. *Scinax manriquei* was usually found in vegetation above ditches surrounded by tall grasses as well as on artificial water tanks, ponds, and trails. It is syntopic at La Macana and El Guamo with *Hyla minuta* and *Hyla luteocellata*, as well as two unidentified species of *Eleutherodactylus*. In Chiguará, the senior author found an individual in a solitary bromeliad that grew on a fence post 2 m above ground. A few months earlier, a *Flectonotus pygmaeus* was found inside the same plant. In 1992, one of us (AO) also discovered the species near the Parque Nacional Chorro El Indio, southeast of San Cristóbal (Táchira state) at an elevation of 1400 m. This location was less disturbed than the above sites in that some forest remnants were present. Two females of the examined series contain oviductal eggs, one had 143 (CVULA IV-3850), taken in 17 July 1985; and the other had 456 (EBRG 1685), taken in 8 April 1982. April is considered the end of the rainy season in Northern Venezuela, and July is usually one of the wettest months in the year; so far, we cannot conclude a definite breeding season for *S. manriquei*, although it has been heard calling in July and November (end of rainy season).

Etymology.—We name this species in honor of Róger Manrique, a great friend, enthusiastic naturalist, and excellent nature artist, who has accompanied us on several field trips and guided us to many previously unknown corners of the Andes.

Discussion

Fouquette and Delahoussaye (1977) and Duellman and Wiens (1992) discussed species groups within *Scinax*. Although tadpoles are not known, *Scinax manriquei* does not belong to *catharinae* group because it is not known to oviposit in streams. It does not belong to the *perpusillus* group because members of this group have short, truncate snouts in dorsal view, a distinctive dorsal

color pattern and lay eggs in terrestrial bromeliads. It does not belong to the *rizibilis* group because they have paired vocal sacs. And it does not belong to the *rostratus* group (as explained in the diagnosis) because they have an acuminate snout in lateral profile and an orange or yellow pattern with bands or spots on the posterior thighs. Duellman and Wiens (1992) are unaware of any evidence to support the monophyly of the *S. ruber*, *staufferi*, and *x-signatus* groups. Until the species groups of *Scinax* are better defined, we cannot assign *S. manriquei* to any species group.

This is the first *Scinax* reported from the Andes of Venezuela. To date, the hylid fauna from the Cordillera de Mérida was considered impoverished (Duellman, 1972a, 1979), especially when compared to neighboring Colombian ranges. Nevertheless, explorations of mountain ranges in the states of Mérida and Táchira during recent years have produced unexpected findings, such as a montane *Scinax*. We are aware of only one other *Scinax*, *Scinax oreites*, that ascends high into the Andes. It occupies an altitudinal range from 1600 m to 2400 m in the Andes of northern Perú (Duellman and Wiens, 1993).

Following Barrio-Amorós (1998), hylids known from the Venezuelan Andes include *Flectonotus pygmaeus*, *Gastrotheca helenae*, *Gastrotheca nicefori*, *Hyla crepitans*, *Hyla jahni*, *Hyla lanciformis*, *Hyla lascinia*, *Hyla luteocellata*, *Hyla meridensis*, *Hyla minuta*, *Hyla platydactyla*, and *Hyla pelidna*. Few montane amphibian species are found on both sides of the Táchira depression. To date, only *Bufo sternosignatus*, *G. nicefori*, *F. pygmaeus*, *Eleutherodactylus tubernasus*, *Centrolene andinum*, and perhaps *H. lascinia* are known to inhabit both Norte de Santander, Colombia, El Tamá massif (Venezuelan-Colombian boundary), and the Cordillera de Mérida (Duellman, 1989; Ruiz-Carranza and Lynch, 1995; Ruiz-Carranza et al., 1996, Vélez, 1999).

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APPENDIX 1

Specimens Examined

Scinax baumgardneri.—Venezuela: Estado Amazonas: Capihuara, Casiquiare; examined alive.

Scinax boesemani.—Venezuela: Estado Bolívar: km 86 cerca Las Claritas, on the road beetwen El Dorado and Santa Elena de Uairén, MHNLS 10843–47.

Scinax manriquei.—Venezuela: see type series and referred specimens.

Scinax nebulosa.—Venezuela: Estado Bolívar: km 86 near Las Claritas, on the road beetwen El Dorado and Santa Elena de Uairén, MHNLS 10278.

Scinax rostrata.—Venezuela: Estado Anzoátegui: Momo, MHNLS 8708–12; Estado Delta Amacuro: Caño Winikina, MHNLS 12340–44; Estado Falcón: La Barranca, Coro, MHNLS 12350; Sierra de San Luis, MHNLS 12351.

Scinax rubra.—Venezuela: Estado Bolívar: San Martín de Turumbán, MHNLS 7710–12; Estado Delta Amacuro: Caño Winikina, MHNLS 12186, 12189–191, 7838–39; Boca Caño Macareo, EBRG 3521–40.

Scinax fuscomarginata.—(= *trilineata*) Venezuela: Estado Bolívar: km 13 on the road beetwen El Dorado and Santa Elena de Uairén, CVULA 6493–5.

Scinax x-signata.—Venezuela: Estado Anzoátegui: El Tejar, Puerto Píritu, MHNLS 10115–16; Estado Apure: 7 km northeast La Victoria, Distrito Páez, CVULA 4936; Mantecal, CVULA 2667; Hato El Frío, MHNLS 12113–19; Estado Barinas: 40 km S Barinas, CVULA 0562; Hato La Candelaria, CVULA 0759; El Irel, CVULA 1998–9, 2561, 2694, 2772, 2988–89; El Caimital, Barrancas, CVULA 2125; San Silvestre, CVULA 4852; Estado Cojedes: Hato Habana, 38 km SE de Las Vegas, MHNLS 6464, 6478–80; Estado Portuguesa: Colonia Agrícola Turén, CVULA 0415–17; Estado Táchira: San Antonio Caparo, CVULA 4765; Estado Trujillo: Monay, CVULA 3371, 3414; Estado Nueva Esparta: Juan Griego, Isla de Margarita, EBRG 3636.