12-2006

Genetic Resolution of the Enigmatic Lesser Antillean Distribution of the Frog *Leptodactylus Validus* (Anura, Leptodactylidae)

Keneth Yanek

W. R. Heyer

Rafael O. de Sá
University of Richmond, rdesa@richmond.edu

Recommended Citation

Yanek, Keneth, W. Ronald Heyer, and Rafael O. de Sá. "Genetic Resolution of the Enigmatic Lesser Antillean Distribution of the Frog *Leptodactylus Validus* (Anura, Leptodactylidae)." *South American Journal of Herpetology* 1, no. 3 (December 2006): 192-201. doi:10.2994/1808-9798(2006)1[192:grotel]2.0.co;2.

South American Journal of Herpetology, 1(3), 2006, 192-201

GENETIC RESOLUTION OF THE ENIGMATIC LESSER ANTILLEAN DISTRIBUTION OF THE FROG *LEPTODACTYLUS VALIDUS* (ANURA, LEPTODACTYLIDAE)

KENETH YANEK[1,3]; W. RONALD HEYER[2] AND RAFAEL O. DE SÁ[1,4]

[1] *Department of Biology, University of Richmond, Richmond, Virginia 23237, USA.*
[2] *Department of Vertebrate Zoology, National Museum of Natural History, PO Box 37012, Smithsonian Institution, Washington, D.C. 20013-7012, USA.*
[3] *KY current address: Division of Transplant, Department of Surgery, Virginia Commonwealth University, Richmond, Virginia 23298-0254, USA. E-mail: kyanek@mcvh-vcu.edu*
[4] *Corresponding Author: rdesa@richmond.edu*

ABSTRACT: *Leptodactylus validus* has an unusual distribution, inhabiting Trinidad, Tobago, and the Lesser Antilles, but not the mainland of South America. This distribution is inconsistent with other distribution patterns observed for these islands. Although slight variation in adult morphology has been observed among the different island populations of *L. validus*, call data suggest the presence of a single species. Calls of *L. pallidirostris* from Venezuela and Brazil suggested that this taxon might be conspecific with *L. validus*. Sequence data from the 12S and 16S mt rDNA genes indicate that *L. validus* represents a single species throughout its distribution and is conspecific with *L. pallidirostris*. Dispersal of *L. validus* from Trinidad and Tobago to the Lesser Antilles was likely mediated by human activities.

KEYWORDS: Anura, *Leptodactylus validus*, systematics, synonymy, distribution.

INTRODUCTION

The frog species *Leptodactylus validus* Garman, 1888 as currently understood occurs on the continental islands of Trinidad and Tobago and certain oceanic islands of the Lesser Antilles and is absent from the mainland of South America. This distribution is inconsistent with the distribution patterns observed for other faunal elements of this region. Heyer (1994) described four possible distribution patterns expected for a species in this region: 1) occurring on the mainland of South America, Trinidad and Tobago, and the Lesser Antilles, 2) present in mainland South America, Trinidad and Tobago but not on the Lesser Antilles, 3) found only in Trinidad and Tobago, or 4) found only in the Lesser Antilles. In order to resolve the enigmatic distribution pattern of *L. validus*, Heyer (1994) suggested that either *L. validus* was present on the South American mainland or, alternatively, that *L. validus* might represent two or more closely related, morphologically similar species throughout its distribution. The most likely candidate for a currently recognized species of *Leptodactylus* occurring on the mainland adjacent to Trinididad and Tobago that might be conspecific with *L. validus* is *Leptodacytlus pallidirostris* Lutz, 1930. The available data (Heyer, 1994) are equivocal as to whether *L. pallidirostris* and *L. validus* are conspecific or not.

The purpose of this paper is to analyze molecular data for intensive samples of *L. validus* throughout its distribution and to assess the relationships of *L. validus* with closely related species. The research protocol using sequence data from the 12S and 16S mt rDNA genes is designed to evaluate whether one of the four common distribution patterns for the Lesser Antillean fauna (see above) better describes the currently understood distribution of *L. validus*.

MATERIAL AND METHODS

Frozen tissue samples (liver and muscle) of *Leptodactylus validus* from Trinidad (n = 15), Tobago (n = 2), and the Lesser Antillean Islands of St. Vincent (n = 13) and Grenada (n = 20) in addition to *L. pallidirostris* samples (from Brazil n = 1 and Guyana n = 1) were included in this study. Samples of *L. podicipinus* (Cope, 1862) (from Brazil n = 4) and *L. wagneri* (Peters, 1862) (from Brazil n = 1 and Ecuador n = 2) were also included among the ingroup taxa from the *L. melanonotus* species group. *Leptodactylus chaquensis* Cei, 1950 (n = 1) (*L. ocellatus* spe-

cies group) and *L. knudseni* Heyer, 1972 (n = 1) (*L. pentadactylus* species group) were used as outgroups. The four species groups currently recognized in the genus – *'fuscus,' 'melanonotus,' 'ocellatus,'* and *'pentadactylus,'* are based primarily on morphological data (Heyer, 1969, 1974; Maxson and Heyer, 1988; Frost, 2006). Locality data for the voucher specimens examined are provided in Appendix I.

DNA isolation, amplification, and purification

Total genomic DNA was isolated using standard protocols (Hillis *et al.*, 1996). Fragments of mitochondrial DNA (mtDNA) from the 12S and 16S mt rDNA genes were amplified using polymerase chain reaction (PCR; Palumbi, 1996) in an MJ Research PTC-200 thermocycler and a Stratagene SCS-2 temperature cycler. Double stranded amplifications of nearly the complete 12S (~880 base pairs) and 16S (~1,500 bp) rRNA genes were amplified using four sets of primers (Goebel *et al.*, 1999). PCR reactions for the anterior ~570 bp of the 12S gene were performed using the primers 12S Tphef 5'ATAGC(A/G)CTGAA(A/G)A(C/T)GCT(A/G)AGATG3' and 12S RdS1 5'GGTACCGTCAAGTCCTTTGGGTT3' with JumpStart Taq DNA Polymerase (Sigma). PCR reactions consisted of a 2.5-min denaturation at 94°C, 1-minute of annealing at 55°C, and a 2-min extension at 72°C, followed by 30 cycles of a 1-min denaturation at 94°C, 1 minute of annealing at 55°C, and an extension period of 1.5-min at 72°C. A second set of primers, 12SA-L 5'AAACTGGGATTAGATACCCCACTAT3' and 12SB-H 5'GAGGGTGACGGGCGGTGTGT3', was used to amplify a second 12S gene fragment of ~390 bp in length, beginning approximately 80 bp prior to the end of the fragment amplified by the 12S Tphef/RdS1 primers. This latter fragment was amplified using PCR Master Mix (Promega) and lowering the annealing temperature to 53°C.

The primers 12L13 5'TTAGAAGAGGCAAGTCGTAACATGGTA3' (Feller and Hedges, 1998) and 16H10 5'TGATTACGCTACCTTTGCACGGT3' (Hedges, 1994) were used to amplify a segment of the 16S rDNA gene of approximately ~1,030 bp in length using Master Mix (Promega). PCR conditions consisted of a 2-minute denaturation at 94°C, a 1-min annealing period at 50°C, and a 72°C extension for 1.5-min, followed by 34 cycles of a 1-min denaturation at 94°C, 1-min of annealing at 50°C, and a 1.5-min extension at 72°C. An additional 16S fragment ~550 bp long was amplified under the same reaction parameters using the primers 16SaR-L 5'CGCCTGTTTACCAAAAACAT3' and 16Sd 5'CTCCGGTCTGAACTCAGATCACGTAG3' with Master Mix (Promega), which overlapped the end of the L13/H10 fragment by approximately 70 bp. Amplified products were purified using the Wizard Plus Miniprep DNA Purification System (Promega).

Sequencing

Purified templates were sequenced using SequiTherm Excel II DNA sequencing kits (Epicentre) in an MJ Research PTC-200 thermocycler. Amplified fragments were sequenced in both directions. Single-stranded sequencing reactions were performed using primers labeled with an infrared fluorescent dye (5' IRD800; Li-Cor). The primers used for sequencing reactions were identical in sequence to those used for amplification except 16Sd, which was replaced by the primer 16SbR-H 5'CCGGTCTGAACTCAGATCACGT3' (Palumbi *et al.*, 1991). Sequencing reactions consisted of a 2.5-min denaturation at 95°C, followed by 30 cycles of a 30 second denaturation at 95°C, 30 seconds of annealing at 58°C, and 30 seconds of extension at 70°C. Reaction products for gene fragments < 650 bp in length were run on 41 cm 6% acrylamide gels and those exceeding 650 bp were run on 66 cm 4% acrylamide gels. Gels were .25 mm in thickness and sequences were collected using a Li-Cor 4000L automated DNA sequencer.

Sequence Alignment

Image data from each single strand sequence, along with the chromatographs constructed by the BaseImagIR Ver.4.2 software (Li-Cor Biotechnology Division), were imported into the software program AlignIR (Li-Cor Biotechnology Division) and aligned with their complementary sequence. For each strand, bands from the aligned image files and their corresponding chromatographs were visually inspected and corrected for mismatches. Sequences were aligned with ClustalX (Thompson *et al.*, 1997) using the multiple alignment option. Alignment ambiguities were improved manually under a parsimony criterion while considering secondary structure constraints (Kjer, 1995; Hickson *et al.*, 1996).

Phylogenetic Analyses

Phylogenetic analyses were performed for independent and combined data sets using PAUP* 4.0b10 (Swofford, 2002). Identical haplotypes were represented by a single sample. Pair-wise genetic distances were calculated under the general time reversible model with gamma distributed rate variation.

Maximum parsimony (MP) analyses were performed using heuristic algorithms using the tree-bisection reconnection (TBR) branch swapping option. All analyses were run with bases as unordered character states; gaps were treated alternatively as missing data or as a fifth character state, in the latter insertions and deletions (indels) represent informative evolutionary changes (Simmons and Ochoterena, 2000; Simmons *et al.*, 2001). Weighted analyses were performed with transversional (tv) changes assigned twice the weight of transitions (ti). Stem and loop positions were identified using secondary structure models for *Xenopus laevis* (Cannone *et al.*, 2002). Additional MP and maximum likelihood (ML) analyses were performed on all three datasets weighting loop positions twice relative to stem positions.

Strict and 50% majority rule (50% MR) consensus trees were derived for analyses resulting in multiple equally parsimonious trees. Statistical stability of internal branches was assessed via nonparametric bootstrapping (Felsenstein, 1985) based on 1000 pseudoreplicates (50% MR, heuristic search).

Modeltest (Ver. 3.06, Posada and Crandall, 1998) was used to select the best-fitting model of sequence evolution for each dataset and the likelihood parameters to be implemented in ML analyses (Fisher, 1922; Felsenstein, 1981) using PAUP*. Hierarchical Likelihood Ratio Tests (hLRTs) resulted in selection of the Tamura-Nei model (1993) with among-site rate heterogeneity (TrN+G) for all datasets. Using the Akaike Information Criterion (AIC, Bozdogan, 1987), Modeltest identified the General Time Reversible Model (Rodriguez *et al.*, 1990) with invariant sites and gamma-distributed rate heterogeneity (GTR+I+G) for the 12S and combined datasets, whereas the General Time Reversible Model with gamma-distributed rate variation across sites (GTR+G) was selected for the 16S dataset. For ML analyses heuristic searches were conducted using TBR branch swapping and nonparametric bootstrapping (100 pseudoreplicates, 50% majority rule). Bootstrap values < 75% were considered well supported, between 55% and 74% moderately supported, and values > 55% were considered to have low support.

Additional likelihood analyses were performed based on Bayesian inference using MrBayes (Ver. 3.0b4, Huelsenbeck and Ronquist, 2001). The model of sequence evolution for each dataset was selected using Modeltest under AIC. The number of substitution types was set to 6, enabling the rates to vary, thus being subject to the constraint of time-reversibility (Tavare, 1986). Seven simultaneous MCMC chains were run to determine the number of samples to discard based on convergence of log-likelihood values. Analyses were initiated using randomly selected starting trees, and topologies were sampled every 10 generations for 2.0×10^6 generations. The resulting 50% majority-rule consensus tree was rooted using the outgroup samples. For the Bayesian analyses, credibility values for a clade were considered statistically significant when posterior support values were < 99%.

Results

Analyses of the 12S, 16S, and combined datasets resulted in *Leptodactylus validus* and *L. pallidirostris* forming a monophyletic group. *Leptodactylus wagneri* and *L. podicipinus* samples formed exclusives clades in all analyses. Pair-wise genetic distances among the *L. validus* and *L. pallidirostris* samples were < 1% in all datasets. Corrected pair-wise genetic distances among samples for each data set are given in Table 1.

Herein, we present the combined data set and the results of the combined analyses given that the independent analyses of 12S and 16S data overall did not differ from the combined analyses. Exclusive 12S and 16S haplotypes are given in Tables 2 and 3 respectively. In the combined sequence data the 50 *L. validus* samples grouped into 11 haplotypes (Table 4); these haplotypes have 16 (0.71%) variable sites. Haplotype A includes samples from Grenada (n = 9) and St. Vincent (n = 8), haplotype B consists of samples from Grenada (n = 11) and St. Vincent (n = 5), whereas haplotype C includes samples from Trinidad (n = 8) and haplotype D contains the samples from Tobago (n = 2). Haplotypes E-K each consists of a single sample from Trinidad.

Plots of pair-wise uncorrected p-distances versus K2p distances for 12S and 16S are given in Fig. 1A

Table 1: Percentage values of pair-wise genetic distances for: the 12S data set (top graph), 16S data set (middle graph), and combined data set (bottom graph). Distances corrected using the general time-reversible model with gamma distributed rates for variable sites.

12S Data	*L. validus*	*L. pallidirostris*	*L. podicipinus*	*L. wagneri*	*L. chaquensis*
L. validus	0-0.49				
L. pallidirostris	0.12-0.62	0.25			
L. podicipinus	10.51-11.15	10.52-10.85	0.12-0.88		
L. wagneri	5.04-5.69	5.04-5.72	10.53-11.21	0.24-1.52	
L. chaquensis	12.23-12.81	12.19-12.68	12.18-12.72	10.95-11.81	
L. knudseni	16.93-17.50	17.00-17.01	15.11-15.57	15.84-16.60	12.10
16S Data	*L. validus*	*L. pallidirostris*	*L. podicipinus*	*L. wagneri*	*L. chaquensis*
L. validus	0-0.36				
L. pallidirostris	0.14-0.73	0.51			
L. podicipinus	15.22-16.79	15.34-16.43	0-2.1		
L. wagneri	9.12-9.92	8.84-9.57	13.98-15.39	0.22-1.73	
L. chaquensis	20.43-20.96	20.20-20.79	17.66-17.99	21.25-22.31	
L. knudseni	26.10-26.75	25.93-26.62	26.02-26.91	28.39-28.76	20.70
Combined Data	*L. validus*	*L. pallidirostris*	*L. podicipinus*	*L. wagneri*	*L. chaquensis*
L. validus	0-0.32				
L. pallidirostris	0.14-0.64	0.41			
L. podicipinus	13.5-14.54	13.45-14.21	0.05-1.64		
L. wagneri	7.64-8.04	7.41-7.78	12.79-13.59	0.23-1.65	
L. chaquensis	17.21-17.55	16.93-17.50	15.50-15.86	16.91-17.90	
L. knudseni	22.44-22.74	22.27-22.66	21.57-22.18	23.18-23.57	17.20

Table 2: 12S rDNA sequence haplotypes for *L. validus* samples from: Grenada (Gren), St. Vincent (StVn), Tobago (Tobo), and Trinidad (Trin). Haplotypes A and B represent multiple samples. Asterisks indicate samples used to represent a haplotype. Haplotypes C-E each consist of a single sample as listed below the table.

A	B
Gren006881	Gren196977
Gren006882	Gren196979
Gren006883	Gren196980
Gren006939	Gren197000
Gren196978	Gren197001
Gren196999	Gren197002
Gren197005	Gren197003
Gren197006	Gren197004
Gren197017	Gren197007
StVn056421	Gren197008
StVn056490	Gren197044
StVn056561	StVn196894
StVn056562	StVn196896
StVn056612	StVn196897
*StVn056613	StVn196899
StVn196895	StVn196900
StVn196898	Trin175410
Tobo186596	Trin175620
Tobo186597	*Trin196726
Trin196729	Trin196727
	Trin196730
	Trin196731
	Trin196732
	Trin196733
	Trin196734
	Trin196735
	Trin196888

(C) Trin175424
(D) Trin196728
(E) Trin196886

and 1C respectively, whereas comparison of uncorrected p-distances with corrected GTR divergences are provided in Fig. 1B and 1D. These graphs show a nearly linear distribution.

Phylogenetic analyses of the combined data set were performed under MP, ML, and Bayesian analyses as described above; overall tree topologies from MP and ML trees do not differ from Bayesian trees and consequently are not shown. The MP analysis of the combined data with gaps as missing data resulted in three equally parsimonious trees (L = 765; CI = 0.85). Among the 2247 bp aligned, 527 (23.5%) characters were variable and 349 (15.5%) were parsimony-informative. The strict consensus tree placed the *L. pallidirostris* and *L. validus* samples in a monophyletic group with 100% support. Within this clade, the *L. pallidirostris* sample from Brazil appeared basal to a well-supported clade that united the *L. pallidirostris* sample from Guyana with a well-supported *L. validus* subclade. Within the *L. validus* subclade there is good support for a subclade formed by the two Grenada/St. Vincent haplotypes (A and B); relationships among other *L. validus* samples are unresolved. The analysis with gaps as a fifth character recovered three minimum-length trees (L = 815; CI = 0.84); the strict consensus tree was identical to the consensus tree obtained when gaps were considered as missing data. An analysis applying a 2:1 (tv:ti) weighting scheme recovered three minimum-length

Table 3: 16S rDNA sequence haplotypes for *L. validus* samples from: Grenada (Gren), St. Vincent (StVn), Tobago (Tobo), and Trinidad (Trin). Samples A-C represent multiple samples. Asterisks indicate samples used to represent a haplotype. Haplotypes D-G each consist of a single sample as listed below the table.

A	B	C
Gren006881	Trin175424	*Tobo186596
Gren006882	*Trin196726	Tobo186597
Gren006883	Trin196727	
Gren006939	Trin196728	
Gren196977	Trin196729	
Gren196978	Trin196730	
Gren196979	Trin196731	
Gren196980	Trin196732	
Gren196999	Trin196733	
Gren197000	Trin196734	
Gren197001	Trin196888	
Gren197002		
Gren197003		
Gren197004		
Gren197005		
Gren197006		
Gren197007		
Gren197008		
Gren197017		
Gren197044		
StVn056421		
StVn056490		
StVn056561		
StVn056562		
StVn056612		
*StVn056613		
StVn196894		
StVn196895		
StVn196896		
StVn196897		
StVn196898		
StVn196899		
StVn196900		

(D) Trin196886
(E) Trin175410
(F) Trin175620
(G) Trin196735

Table 4: Combined sequence haplotypes for *L. validus* samples from: Grenada (Gren), St. Vincent (StVn), Tobago (Tobo), and Trinidad (Trin). Haplotypes A-D represent multiple samples. Samples used to represent each haplotype are indicated by an asterisk. Haplotypes E-K each consist of a single sample as listed below the table.

A	B	C	D
Gren006881	Gren196977	*Trin196726	*Tobo186596
Gren006882	Gren196979	Trin196727	Tobo186597
Gren006883	Gren196980	Trin196730	
Gren006939	Gren197000	Trin196731	
Gren196978	Gren197001	Trin196732	
Gren196999	Gren197002	Trin196733	
Gren197005	Gren197003	Trin196734	
Gren197006	Gren197004	Trin196888	
Gren197017	Gren197007		
StVn056421	Gren197008		
StVn056490	Gren197044		
StVn056561	StVn196894		
StVn056562	*StVn196896		
StVn056612	StVn196897		
*StVn056613	StVn196899		
StVn196895	StVn196900		
StVn196898			

(E) Trin175410
(F) Trin175424
(G) Trin175620
(H) Trin196728
(I) Trin196729
(J) Trin196735
(K) Trin196886

trees (L = 1041; CI = 0.856). Weighting loop positions (n = 1135) relative to stem positions (n = 1112) with gaps as missing data also resulted in three minimum-length trees (L = 1269; CI = 0.850). The strict consensus trees from these analyses were identical to the strict consensus trees obtained from the unweighted combined dataset. Using gaps as a fifth character under the same weighting scheme resulted in a single tree (L = 1379; CI = 0.839) identical to the consensus tree of the unweighted analyses. The ML analysis of the combined data under the TrN+G model parameters resulted in a bootstrap 50% MR consensus tree similar to the one from the MP analyses, with slight differences in relationships among samples within the *L. podicipinus* clade. A similar tree was obtained using the GTR+I+G model parameters, with better resolution demonstrated among samples within the *L. podicipinus* clade. ML analyses considering secondary structure and weighting loop:stem positions (2:1), using both evolutionary models resulted in bootstrap 50% MR consensus trees identical to the tree from the analysis using the GTR+I+G model.

The Bayesian analysis was performed under the GTR+I+G model settings. Convergence of the log likelihood values among the seven MCMC chains occurred within 40,000 generations of sampling, consequently the first 4,000 trees sampled were discarded. The resulting consensus tree from MrBayes (Fig. 2) was similar to the unweighted ML tree obtained using the GTR+I+G model parameters, with a better resolution of relationships among *L. validus* samples. However, with the exception of the subclade consisting of samples from Grenada/St. Vincent (haplotypes A and B), there was little posterior support for relationships among other *L. validus* samples in the clade.

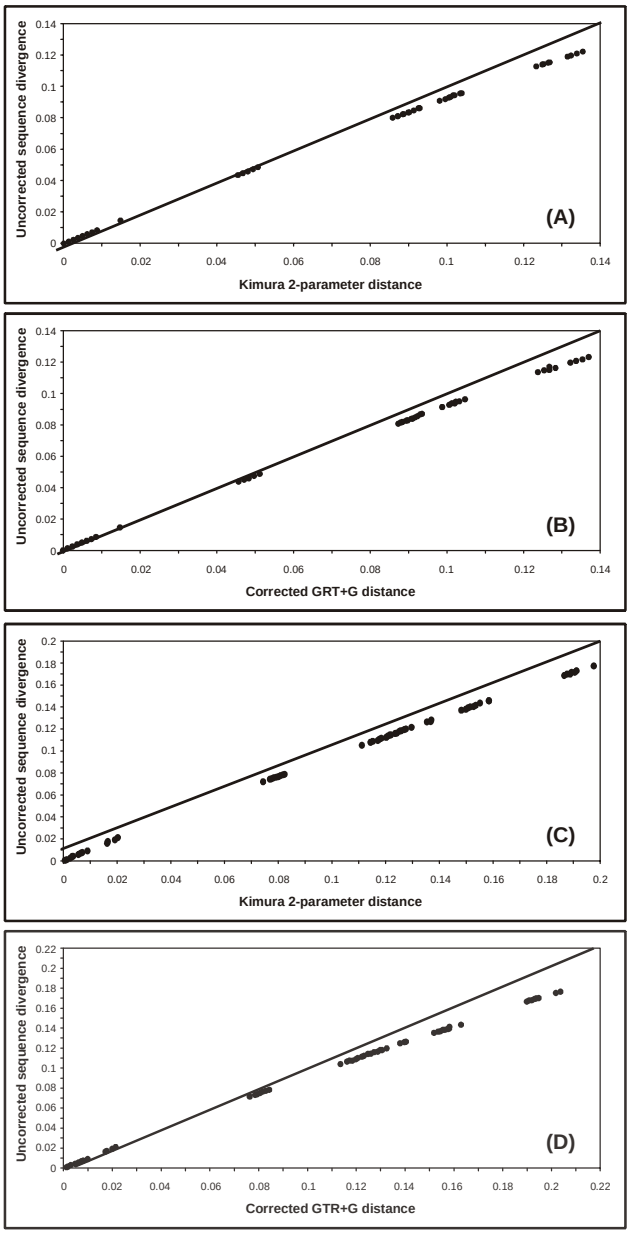

Figure 1. Plots depicting relative rates of transitional saturation for the 12S (A) and 16S (C) Gene sequences; and substitutional saturation for 12S (B) and 16S (D) using pair-wise genetic distances corrected under the GTR evolutionary model.

Discussion

Morphological characters and call data have been previously used to assess variation among species within the *Leptodactylus podicipinus-wagneri* complex (Heyer, 1994). However, morphological data were insufficient to resolve all species boundaries in this complex. The status of *L. pallidirostris* and *L. validus* within this complex remained one of the major unresolved problems (Heyer, 1994).

Slight adult morphological variation exists between populations from the Lesser Antilles and those from Trinidad and Tobago. The overall morphologies of *L. pallidirostris* and *L. validus* are very similar to each other. *Leptodactylus pallidirostris* is distributed throughout Venezuela, Guyana, Suriname, French Guiana, and northern Brazil. Available call data for *L. validus* from Trinidad and Tobago are similar to calls analyzed from Brazilian and Venezuelan populations of *L. pallidirostris* (Heyer, 1994). The overall data presented in Heyer (1994) were equivocal as to whether *L. pallidirostris* and *L. validus* were conspecific or represented distinct species.

All analyses performed in this study strongly support a monophyletic group consisting of the *L. pallidirostris* and *L. validus* samples; moreover, *L. pallidirostris* samples did not cluster as an exclusive monophyletic clade. These results support the conspecificity of the two taxa. However, saturation of nucleotide substitutions among samples can affect the estimation of evolutionary distances and potentially result in misleading tree topologies (Swofford *et al.*, 1996; Page and Holmes, 1998; Nei and Kumar, 2000; de Peer *et al.*, 2002). Our assessment of sequence saturation indicates a low degree of both transitional and overall substitutional saturation for these sequences (Fig. 1).

The combined analyses reveal some genetic structuring among populations within this clade, i.e., genetically distant samples are also geographically distant. For example, closer relationships are demonstrated among samples of *L. validus* from the Lesser Antilles. Likewise, the *L. pallidirostris* sample from Guyana appears more closely related to the *L. validus* samples than the *L. pallidirostris* sample from Brazil. The following maximum genetic distances were obtained for *L. validus* and *L. pallidirostris* samples: < 0.37% with the sample from Guyana and < 0.73% for the sample from Brazil. Also, less than 0.5% sequence divergence is observed among the *L. validus* samples. Levels of sequence divergence are not absolute predictors of species diversity; however, these low levels of sequence divergence are consistent with *L. pallidirostris* and *L. validus* being conspecific. In this scenario, *L. validus* is a single species distributed throughout the Lesser Antilles, Trinidad and Tobago, and adjacent mainland South America.

Advertisement calls are commonly analyzed in anuran systematic studies. These calls are almost always

species-specific in *Leptodactylus* (see Heyer *et al.*, 2005 for an exception) and in anurans in general, and usually serve as a reliable indicator of species boundaries in frogs (Heyer and Straughan, 1976; Heyer, 1978, 1979, 1994; Heyer *et al.*, 1996; Wieczorek and Channing, 1997; Camargo *et al.*, 2006). The available call data from Brazilian and Venezuelan populations of *L. pallidirostris* are very similar to the advertisement call of *L. validus* (Heyer, 1994), providing additional support for the conspecific status of the two species.

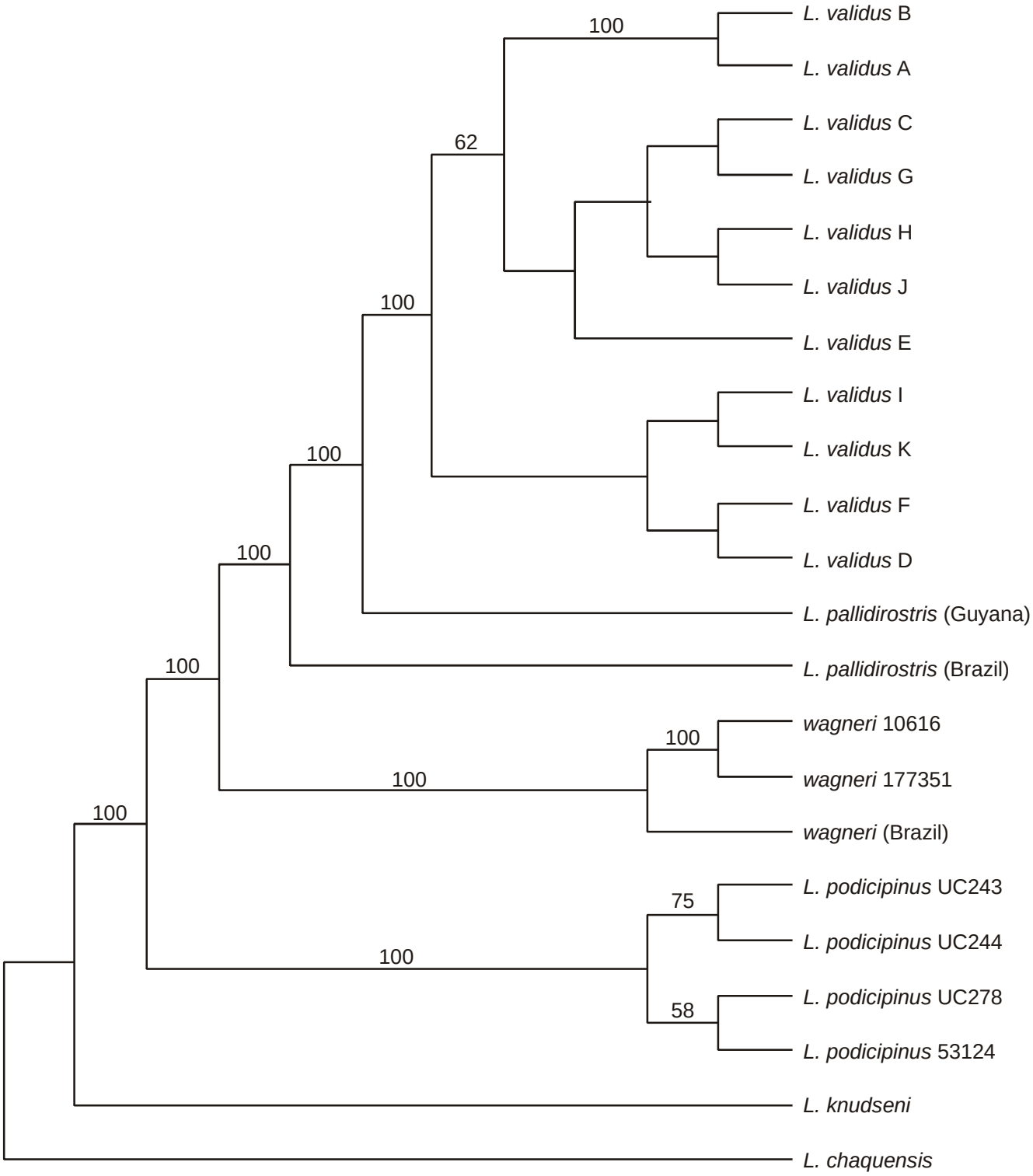

Figure 2. Bayesian consensus tree of combined dataset. Posterior probability values > 50% are shown above branches. See text for clade descriptions.

Heyer (1994) also indicated that some of the Venezuelan samples examined and assigned to *L. pallidirostris* closely resembled Trinidad and Tobago samples designated as *L. validus*, supporting their conspecificity.

Leptodactylus pallidirostris was described by Lutz in 1930 from Kartabo, Guyana, who repeatedly referred to the species' resemblance to *L. validus.* This is in agreement with the present study which proposes that *L. pallidirostris* and *L. validus* represent a single taxon. *Leptodactylus validus* was described by Garman in 1888 "1887", therefore this name has priority over *L. pallidirostris.* Consequently, *L. pallidirostris* Lutz, 1930 is placed in the synonymy of *L. validus* Garman, 1888.

The taxonomic resolution of *L. pallidirostris* as a synonym of *L. validus* results in the resolution of the previous distributional enigma involving *L. validus sensu* Heyer, 1994. Murphy (1997) considered all five of the Lesser Antillean amphibians and reptiles that occur in Trinidad and Tobago to have been recent introductions from the Lesser Antilles. Murphy (1997) indicated that most, if not all, of the introductions were the result of human activity. The distribution and minimal genetic variation of *L. validus* is consistent with human mediated introductions, but in this case the direction was most likely from Trinidad and Tobago to the Lesser Antilles. As Trinidad and Tobago are continental islands, gene flow in *L. validus* from Trinidad and Tobago with the mainland populations likely occurred as late as about 20,000 years ago (Murphy, 1997).

Resumen

Leptodactylus validus tiene una distribución particular, encontrandose en Trinidad, Tobago, y las Antillas Menores, pero no en América del Sur. Esta distribución es inconsistente con los patrónes de distribución para otros grupos en estas islas. A pesar que se ha obervado variación en la morfología adulta de *L. validus* en diferentes islas, los datos de canto sugieren la presencia de una sola especie. Cantos de *L. pallidirostris* de Venezuela y Brasil sugieren que esta especie podría ser coespecífica con *L. validus.* Datos moleculares de secuencias de los gene mt 12S y 16S sugieren que *L. validus* consiste de una sola especie en su distribución y que esta especie es coespecífica con *L. pallidirostris.* La dispersión de *L. validus* de Trinidad y Tobago a las Antillas Menores podría haber ocurrido atraves de actividades humanas.

Acknowledgments

We acknowledge funding support for this study through National Science Foundation Awards #9815787 and #0342918 to RdS and WRH. KY thanks the Graduate School of Arts and Sciences, University of Richmond, for financial support. We thank the colleagues and institutions that kindly provided tissue specimens and/or helped in collecting samples.

Literature Cited

Bozdogan, H. 1987. Model selection and Akaike's information criterion (AIC): The general theory and its analytical extensions. Psychometrica 52(3):345-370.

Camargo, A., R.O. de Sá and W.R. Heyer. 2006. Phylogenetic analyses of mtDNA sequences reveal three cryptic lineages in the widespread neotropical frog *Leptodactylus fuscus* (Schneider, 1799) (Anura, Leptodactylidae). Biological Journal of the Linnean Society 87:325-341.

Cannone J.J., S. Subramanian, M.N. Schnare, J.R. Collett, L.M. D'Souza, Y. Du, B. Feng, N. Lin, L.V. Madabusi, K.M. Muller, N. Pande, Z. Shang, N. Yu and R.R. Gutell. 2002. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BioMed Central Bioinformatics 3:15. URL:http://www.rna.icmb.utexas.edu/.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17(6):368-376.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Feller, A.E. and S.B. Hedges. 1998. Molecular evidence for the early history of living amphibians. Molecular Phylogenetics and Evolution 9(3):509-516.

Fisher, R.A. 1922. On the mathematical foundations of theoretical statistics. Philosophical Transactions of the Royal Society A222:309-368.

Frost, D.R. 2006. Amphibian Species of the World: an Online Reference. Version 4 (17 August, 2006). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.php. American Museum of Natural History, New York, USA.

Garman, S. 1888 "1887". West Indian Batrachia in the Museum of Comparative Zoology. Bulletin of the Essex Institute 19:13-16.

Goebel, A.M., J.M. Donnelly and M.E. Atz. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. Molecular Phylogenetics and Evolution 11(1):163-199.

Hedges, S.B. 1994. Molecular evidence for the origin of birds. Proceedings of the National Academy of Sciences of the United States of America 91:2621-2624.

Heyer, W.R. 1969. The adaptive ecology of the species groups of the genus *Leptodactylus* (Amphibia, Leptodactylidae). Evolution 23:421-428.

Heyer, W.R. 1974. Relationships of the *marmoratus* species group (Amphibia, Leptodactylidae) within the subfamily Leptodactylinae. Contributions in Science, Los Angeles County Museum 253:1-45.

HEYER, W.R. 1978. Systematics of the *fuscus* group of the frog genus *Leptodactylus* (Amphibia: Leptodactylidae). Science Bulletin, Natural History Museum of Los Angeles County 29:1-85.

HEYER, W.R. 1979. Systematics of the *pentadactylus* species group of the frog genus *Leptodactylus* (Amphibia: Leptodactylidae). Smithsonian Contributions to Zoology 301:1-43.

HEYER, W.R. 1994. Variation within the *Leptodactylus podicipinus-wagneri* complex of frogs (Amphibia: Leptodactylidae). Smithsonian Contributions to Zoology 546:1-24.

HEYER, W.R. AND I.R. STRAUGHAN. 1976. A functional analysis of the mating calls of the Neotropical frog genera of *Leptodactylus* complex (Amphibia: Leptodactylidae). Papéis Avulsos de Zoologia 29:221-245.

HEYER, W.R., J.M. GARCÍA-LOPEZ AND A.J. CARDOSO. 1996. Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species. Amphibia-Reptilia 17:7-31.

HEYER, W.R., R.O. DE SÁ AND A. RETTIG. 2005. Sibling species, advertisement calls, and reproductive isolation in frogs of the *Leptodactylus pentadactylus* species cluster (Amphibia, Leptodactylidae). Russian Journal of Herpetology, 12(Supplement):35-39.

HICKSON, R.E., C. SIMON, A. COOPER, G.S. SPICER, J. SULLIVAN AND D. PENNY. 1996. Conserved sequence motifs, alignment, and secondary structure for the third domain of animal 12S rRNA. Molecular Biology and Evolution 13(1):150-169.

HILLIS, D.M., C. MORITZ AND B.K. MABLE (EDS.). 1996. Molecular Systematics, Second Edition. Sinauer Associates Inc., Sunderland, Massachusetts.

HUELSENBECK, J.P. AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

KJER, K.M. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. Molecular Phylogenetics and Evolution 4(3):314-330.

LUTZ, A. 1930. Segunda memoria sobre especies brasileiras do genero *Leptodactylus*, incluindo outras alliadas. Memórias do Instituto Oswaldo Cruz 23(1):1-20.

MAXSON, L.R. AND W.R. HEYER. 1988. Molecular systematics of the frog genus *Leptodactylus* (Amphibia: Leptodactylidae). Fieldiana: Zoology, New Series 41:1-13.

MURPHY, J.C.E. 1997. Amphibians and Reptiles of Trinidad and Tobago, Krieger Publishing Co, Malabar, Florida, 245 pp.

NEI, M. AND S. KUMAR (EDS.). 2000. Molecular Phylogenetics and Evolution, Oxford University Press, Oxford, UK.

PAGE, R.D.M. AND E.C. HOLMES (EDS.). 1998. Molecular Evolution, A Phylogenetic Approach, Blackwell Science, Oxford, UK.

PALUMBI, S.R. 1996. Nucleic Acids II: The polymerase chain reaction. In Hillis, D.M., Moritz, C. and Mable, B.K. (eds.), Molecular Systematics, Second Edition, pp. 205-221. Sinauer Associates Inc., Sunderland Massachusetts.

PALUMBI, S., A. MARTIN, S. ROMANO, W.O. MCMILLAN, L. STICE AND G. GRABOWSKI. 1991. The simple fool's guide to PCR, version 2.0. Privately published document compiled by S. Palumbi, Department of Zoology and Kewalo Marine Laboratory. University of Hawaii, Honolulu, HI.

POSADA, D. AND CRANDALL, K.A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817-818.

RODRIGUEZ, F., J.L. OLIVER, A. MARTIN AND J.R. MEDINA. 1990. The general stochastic model of nucleotide substitution. Journal of Theoretical Biology 142:485-501.

SIMMONS, M.P. AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49:369-381.

SIMMONS, M.P., H. OCHOTERENA AND T. CARR. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Systematic Biology 50:454-462.

SWOFFORD, D.L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

SWOFFORD, D.L., G.J. OLSEN, P.J. WADDELL AND D.M. HILLIS. 1996. Phylogenetic Inference. In Hillis, D.M., Moritz, C. and Mable, B.K. (eds.), Molecular Systematics, Second Edition, pp. 407-514. Sinauer Associates Inc., Sunderland, Massachusetts.

TAMURA, K. AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

TAVARE, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17:57-86.

THOMPSON, J.D., T.J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN AND D.G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Research 25:4876-4882.

WIECZOREK, A.M. AND A. CHANNING. 1997. The taxonomic status of Broadley's reed frog. African Journal of Herpetology 46(2):110-116

Received 01 August 2006
Accepted 01 October 2006

Appendix I

Museum catalogue numbers, field numbers, and locality data for the samples utilized in this study. Museum abbreviations: BWMC = Bobby Witcher Memorial Collection, Avila University, LSUMZ = Louisiana State University, Museum of Natural Science, QCAZ = Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito, USNM National Museum of Natural History, Smithsonian Institution.

Species	Museum Number	Field Number	Locality
L. chaquensis	USNM 319708	USFS 186524	Argentina: Tucumán; ca. 40 km SE San Miguel de Tucumán.
L. knudseni	QCAZ 13244		Ecuador: Provincia de Orellana; Parque Nacional Yasuní.
L. pallidirostris	USNM 535774	USFS 207682	Guyana: Northwest District; Baramita.
L. pallidirostris	USNM 302408	WRH 8626	Brazil: Roraima; Igarapé Cocal..
L. podicipinus		UC 243	Brazil: Mato Grosso do Sul; Estância Mimosa; Mun. de Bonito.
L. podicipinus		UC 244	Brazil: Mato Grosso do Sul; Estância Mimosa; Mun. de Bonito.
L. podicipinus		UC 278	Brazil: São Paulo; Chácara Renascer; Bauru.
L. podicipinus	USNM 053124	USFS 303207	Brazil: São Paulo; Fazenda Jatai.
L. validus	BWMC 06881		Grenada: St. Andrew; Spring Gardens Estate.
L. validus	BWMC 06882		Grenada: St. Andrew; Birch Grove.
L. validus	BWMC 06883		Grenada: St. Andrew; Birch Grove.
L. validus	BWMC 06939		Grenada: St. George; Beausejour.
L. validus	USNM 314793	USFS 196977	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314794	USFS 196978	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314795	USFS 196979	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314796	USFS 196980	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314798	USFS 197044	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314813	USFS 196999	Grenada: St. George; Grand Anse Bay.
L. validus	USNM 314814	USFS 197000	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314815	USFS 197001	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314816	USFS 197002	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314817	USFS 197003	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314818	USFS 197004	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314819	USFS 197005	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314820	USFS 197006	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314821	USFS 197007	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314822	USFS 197008	Grenada: St. George; inland from Grand Anse Bay.
L. validus	USNM 314831	USFS 197017	Grenada: St. George; inland from Grand Anse Beach.
L. validus	USNM 314512	USFS 56421	St. Vincent: St. Andrew; near Vermont.
L. validus	USNM 314513	USFS 56490	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314514	USFS 56612	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314515	USFS 56613	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314718	USFS 196894	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314719	USFS 196895	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314720	USFS 196896	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314721	USFS 196897	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314722	USFS 196898	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314723	USFS 196899	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314714	USFS 196900	St. Vincent: St. George; Arnos Vale.
L. validus	USNM 314516	USFS 56561	St. Vincent: St. George; Rose Cottage.
L. validus	USNM 314517	USFS 56562	St. Vincent: St. George; Rose Cottage.
L. validus	USNM 523940	USFS 186596	Tobago: St. Paul; Delaford, Louis d'Or River.
L. validus	USNM 523941	USFS 186597	Tobago: St. Paul; Delaford, Louis d'Or River.
L. validus	USNM 286948	USFS 175410	Trinidad: St. George; Simla Research Station.
L. validus	USNM 286959	USFS 175424	Trinidad: St. George; north of Simla Research Station.
L. validus	USNM 306105	USFS 175620	Trinidad: St. George; near Brasso Seco Village.
L. validus	USNM 314671	USFS 196886	Trinidad: St. George; west of Carapo.
L. validus	USNM 314672	USFS 196888	Trinidad: St. George; west of Carapo.
L. validus	USNM 314627	USFS 196726	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314628	USFS 196727	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314629	USFS 196728	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314630	USFS 196729	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314631	USFS 196730	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314632	USFS 196731	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314633	USFS 196732	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314634	USFS 196733	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314635	USFS 196734	Trinidad: St. Patrick; near Chatham Beach.
L. validus	USNM 314636	USFS 196735	Trinidad: St. Patrick; near Chatham Beach.
L. wagneri	LSUMZ H-13653	JPC 12969	Brazil: Acre; ca. 5km N Porto Walter, inland from the Rio Juruá.
L. wagneri	LSUMZ H-12885	JPC 10616	Ecuador: Sucumbios; Estación Científica near Cuyabeno.
L. wagneri	USNM 320988	USFS 177351	Ecuador: Pastaza; Coca, 1km ENE of Tiguino.