

## SIBLING SPECIES, ADVERTISEMENT CALLS, AND REPRODUCTIVE ISOLATION IN FROGS OF THE *Leptodactylus pentadactylus* SPECIES CLUSTER (AMPHIBIA, LEPTODACTYLIDAE)

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### INTRODUCTION

A recent re-evaluation of morphological and advertisement call variation in the large species of frogs of the *Leptodactylus pentadactylus* cluster discovered more examples of sibling species as defined by Ernst Mayr in his influential book *Animal Species and Evolution*. All previously documented instances of sibling species in frogs demonstrated advertisement call differentiation consistent with the calls serving as pre-mating isolating mechanisms. However, we find one instance of two species with non-distinguishable adult morphologies as well as non-distinguishable advertisement calls. Presumably, the new instances of sibling species reflect retention of ancestral adult morphologies and advertisement calls. Larval and habitat differentiation appear to be important factors in the speciation processes in this group of frogs.

Ernst Mayr defined sibling species as, “morphologically similar or identical natural populations that are reproductively isolated,” in his influential book *Animal Species and Evolution* (Mayr, 1963 : 34). He considered the phenomenon to be important to understanding the complexity and scope of speciation processes in animals.

Mayr’s concept of sibling species has been documented in the frog genus *Leptodactylus* (Barrio, 1973; Heyer et al., 1996). In these examples, reproductive isolation among sibling species was documented through analysis of advertisement calls. That is, advertisement calls are very different from each other among species whose adults are morphologically identical. Females use the calls to distinguish and select males of their own species to mate with in any mixed chorus where sibling species co-occur.

In the process of re-evaluating variation in the *Leptodactylus pentadactylus* species cluster, we discovered instances of sibling species that do not demonstrate reproductive isolation on the basis of advertisement calls.

Herein we summarize the nature of the differentiation involved and discuss the evolutionary implications of our findings.

### MATERIAL AND METHODS

Features for several color patterns, adult morphological characters, and measurements were analyzed in terms of geographic variation for the species currently known as *Leptodactylus knudseni*, *labyrinthicus*, and *pentadactylus* (Heyer et al., 2005). These data were used to postulate species boundaries for the taxa studied.

Molecular sequence data were obtained for 1807 base pairs of 12S and 16S mitochondrial rDNA genes using standard techniques (see Heyer et al., in press). The sequence data have GenBank accession numbers AY947855-82. Voucher specimen data are presented in the *Appendix*. The sequence data were analyzed with maximum likelihood and parsimony methods using PAUP\* (Swofford, 1998).

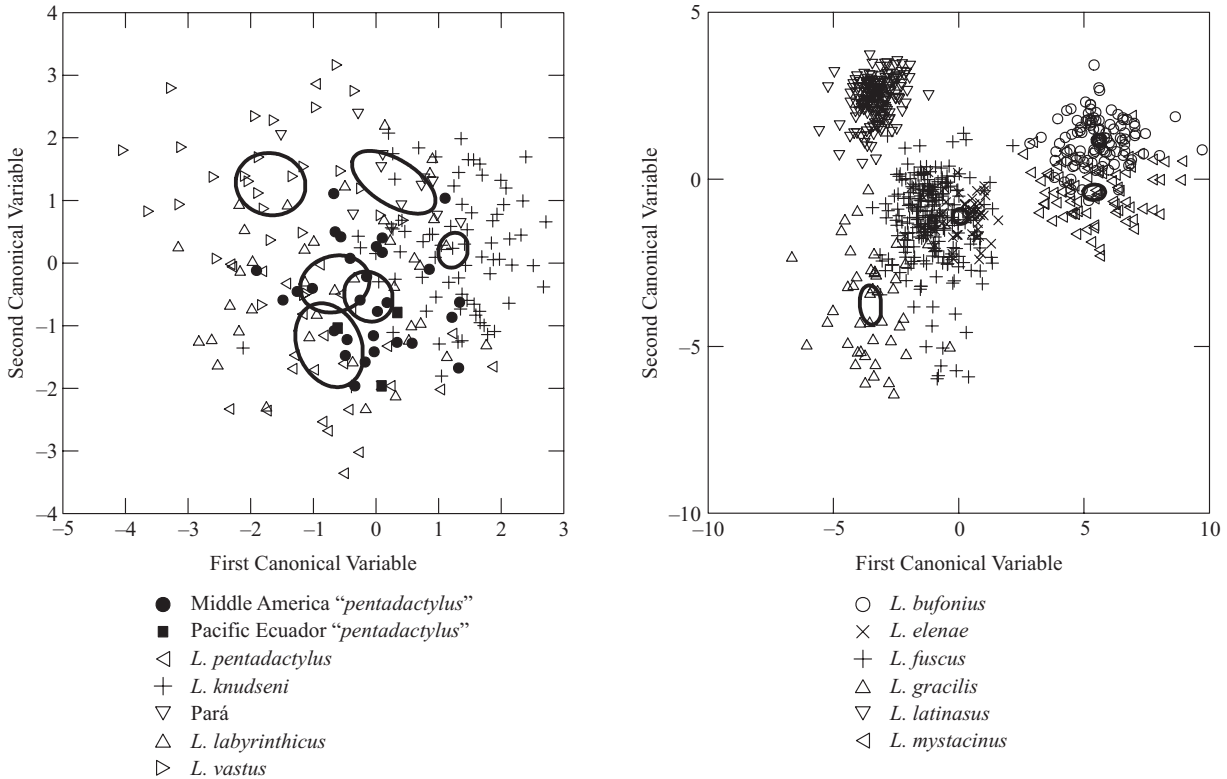
### RESULTS AND DISCUSSION

#### Taxonomic Novelties

In order to discuss the biological implications of our results it is necessary to give a brief synopsis of some of our taxonomic findings. A set of specimens from the State of Pará, Brasil identified in collections either as *L. knudseni* or *L. labyrinthicus* represent a new species. This new species will be referred to as the Pará species for purposes of this paper. The taxon currently recognized as *L. labyrinthicus* contains three species: *L. labyrinthicus*, *L. vastus*, and a new species from coastal Venezuela. Herein we use the available name *L. vastus* for the taxon occupying northeastern Brazil (justification in Heyer, in press). The taxon currently recognized as *L. pentadactylus* consists of four species: *L. pentadactylus* and three new species re-

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**Fig. 1.** Discriminant function analyses of male measurement data for comparable sets of species of the *Leptodactylus pentadactylus* species cluster (left) and the *Leptodactylus fuscus* group (right). The *L. fuscus* group species are those that occur together in the Gran Chaco of South America. Note that the data on the right are more structured than those on the left, for example, there is only a single cloud of points on the left and at least three clouds of points on the right and the sample confidence ellipses ( $p = 0.683$ ) are much smaller on the right than the left. There are 6 confidence ellipses on the left (none for Pacific Ecuador “*pentadactylus*” with  $N = 3$ ) and 6 on the right (the ellipses for *L. fuscus* and *L. latinasus* are not visible due to small sizes of ellipses and density of data points).

ferred to herein as Middle American “*pentadactylus*,” Pacific Colombia “*pentadactylus*,” and Pacific Ecuador “*pentadactylus*.” The status of *L. knudseni* is unchanged. We analyzed DNA from all these species except for the coastal Venezuela and Pacific Colombia “*pentadactylus*” species.

#### Lack of Adult Morphological and Advertisement Call Differentiation

There is much less morphological differentiation among members of the *L. pentadactylus* cluster than found in other major clades of *Leptodactylus* studied to date. The measurement data illustrate the sort of differentiation exhibited by all the adult morphological data (Fig. 1). In particular, adult morphologies can not distinguish *L. knudseni* from Middle American “*pentadactylus*” nor *L. labyrinthicus*, *L. vastus*, and the northern Venezuela species from each other (Heyer, in press).

The advertisement calls of *L. knudseni* can not be consistently differentiated from the calls of Middle American “*pentadactylus*” (Fig. 2). The calls of these two species are

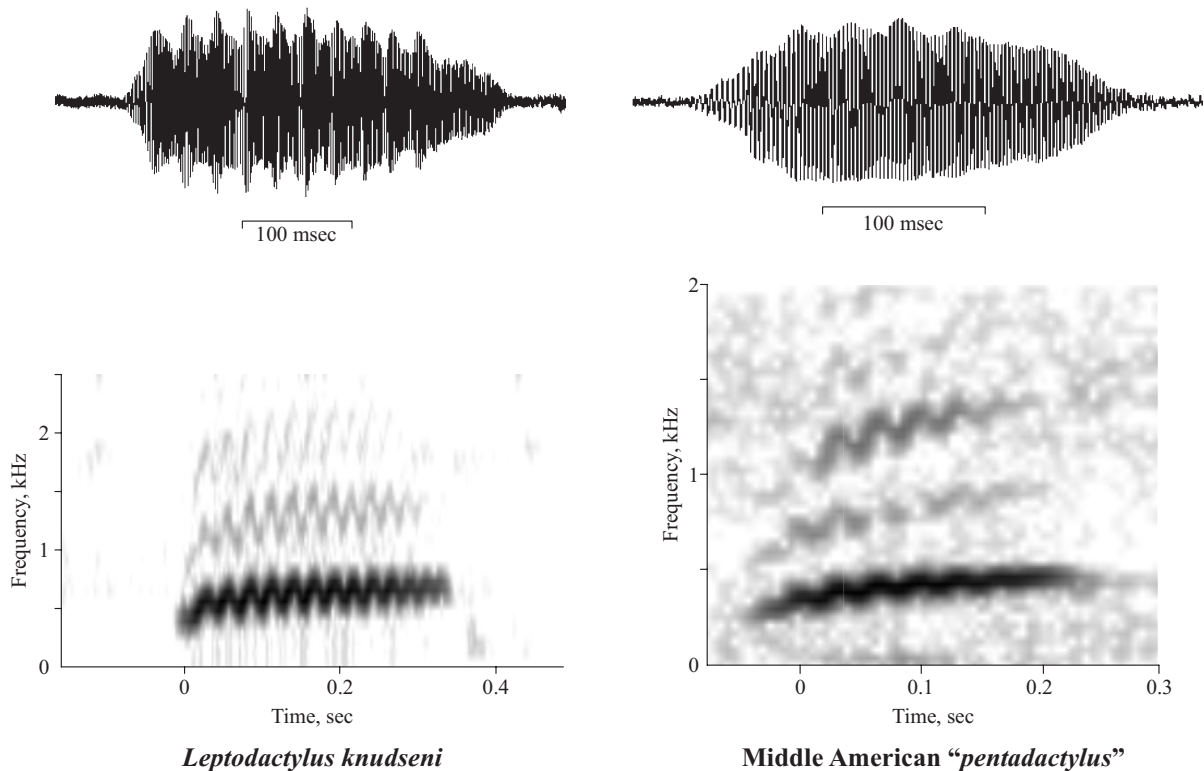
so similar to those of *L. labyrinthicus* that it is likely that females of any of these species would select males of the wrong species in a mixed chorus should they occur sympatrically.

Although there is no adult morphological or advertisement call differentiation among certain of the species, there are other features that are diagnostic. For example, *L. knudseni* and Middle American “*pentadactylus*” differ from each other in several larval internal buccal cavity characters as well as juvenile color patterns (Heyer, in press).

#### Genetic Relationships

The results of the mitochondrial rDNA gene analyses are best illustrated by the maximum likelihood bootstrap tree (Fig. 3) and the comparison of sequence differences among the samples analyzed (Table 1).

Contrary to expectations based on the morphological and advertisement call data, neither *L. knudseni*/Middle American “*pentadactylus*” nor *L. labyrinthicus*/*vastus* are



**Fig. 2.** Wave forms and audiospectrograms of *Leptodactylus knudseni* (USNM recording 228, cut 1) (left) and Middle American "pentadactylus" (USNM recording 106, cut 1) (right). The calls have the same duration, structure, and overlapping broadcast frequencies.

sister-species in the molecular cladogram (Fig. 3) that reflects their genetic relationships.

### Sibling Species in Frogs Revisited

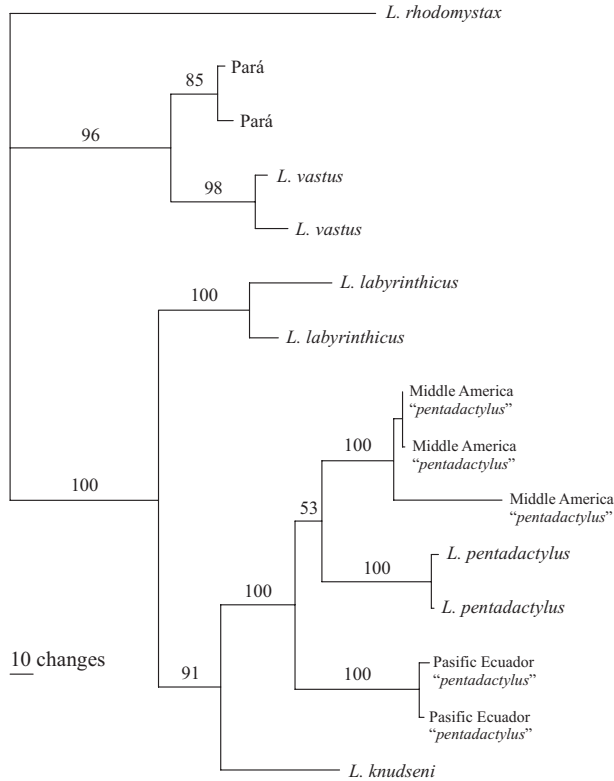
Our study demonstrates additional examples of sibling species as defined by Mayr (1963) in frogs of the genus *Leptodactylus*. Although sibling species in frogs are not common, they are not unexpected because they have been documented in several families of frogs. We did expect to find that advertisement calls of sibling species in frogs would serve as a reproductive isolating mechanism. Finding sibling species that do not differ in advertisement calls but demonstrate extensive molecular differentiation has not been previously reported in frogs that we are aware of. There are two implications of our findings.

1) Ancestral adult morphologies and advertisement calls are retained in several species of the *L. pentadactylus* cluster. The fact that advertisement calls do not differ among some species of the *L. pentadactylus* cluster requires rethinking of the role of advertisement calls in the speciation processes of frogs.

2) Other factors than adult morphology and advertisement calls play an important role in speciation within the *L. pentadactylus* cluster. There is much more larval varia-

tion within the *L. pentadactylus* cluster than observed in other major *Leptodactylus* clades. The variation includes differentiation in internal buccal cavity morphology, oral disk morphology, and aquatic versus terrestrial larvae (Heyer, in press). Habitat differentiation is pronounced among the species of the *L. pentadactylus* cluster in general and especially in those species lacking differences in adult morphology or advertisement calls (Heyer, in press). For example, *L. labyrinthicus* is closely associated with the Cerrado Morphoclimatic Domain, *L. vastus* with the Caatinga Morphoclimatic Domain, whereas the morphologically similar Pará species occurs only within the rain forests of the Amazonian Equatorial Morphoclimatic Domain as defined by Ab'Sáber (1977).

Our study indicates that the role of advertisement calls in frog speciation processes requires re-evaluation. We have been able to identify this finding because we analyzed three different data sets for the same taxa of frogs: morphological, advertisement call, and genetic. We suspect that additional examples of sibling species lacking differentiation in advertisement calls will be found in the future with analyses of variation in morphology, advertisement calls, and molecules simultaneously on the same taxa.



**Fig. 3.** Maximum likelihood bootstrap analysis of combined 12S and 16S mitochondrial rDNA data. Numbers are bootstrap values greater than 50% support.

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## REFERENCES

- Ab'Sáber A. N.** (1977), "Os domínios morfoclimáticos na América do Sul. Primeira aproximação," *Geomorfologia (São Paulo)*, No. 52, 1 – 22 + map.
- Barrio A.** (1973), "*Leptodactylus geminus* una nueva especie del grupo *fuscus* (Anura, Leptodactylidae)," *Physis*, **32**, 199 – 206.
- Heyer W. R.** (in press), "Variation and taxonomic clarification of the large species of the *Leptodactylus pentadactylus* species group (Amphibia: Leptodactylidae) from Middle America, northern South America, and Amazonia," *Arquivos de Zoologia*.
- Heyer W. R., de Sá R. O., and Muller S.** (2005), "On the enigmatic distribution of the Honduran endemic *Leptodactylus silvanimbus* (Amphibia: Anura: Leptodactylidae)," in: Donnelly M. A., Crother B. I., Guyer C., Wake M. H., and White M. E. (eds.), *Ecology and Evolution in the Tropics: A Herpetological Perspective*, Univ. of Chicago Press, Chicago, pp. 81 – 101.

**TABLE 1.** General Time-Reversible Distance Matrix

	Lr	Lk	P1	P2	Lv1	Lv2	Ll1	Ll2	MAp1	MAp2	MAp3	PE1	PE2	Lp1	Lp2
Lr	–														
Lk	0.30	–													
P1	0.30	0.14	–												
P2	0.31	0.14	0.01	–											
Lv1	0.31	0.17	0.04	0.05	–										
Lv2	0.32	0.15	0.05	0.05	0.01	–									
Ll1	0.31	0.13	0.18	0.18	0.19	0.18	–								
Ll2	0.30	0.10	0.15	0.16	0.20	0.19	0.03	–							
MAp1	0.34	0.09	0.15	0.15	0.19	0.18	0.12	0.10	–						
MAp2	0.34	0.09	0.15	0.15	0.19	0.18	0.12	0.10	0.00	–					
MAp3	0.36	0.12	0.18	0.18	0.19	0.18	0.08	0.14	0.03	0.03	–				
PE1	0.34	0.12	0.18	0.18	0.21	0.19	0.16	0.12	0.07	0.07	0.12	–			
PE2	0.34	0.12	0.18	0.18	0.21	0.19	0.16	0.13	0.07	0.07	0.11	0.00	–		
Lp1	0.35	0.11	0.21	0.22	0.25	0.23	0.16	0.13	0.06	0.06	0.10	0.09	0.09	–	
Lp2	0.34	0.10	0.20	0.21	0.24	0.22	0.16	0.13	0.06	0.06	0.10	0.09	0.09	0.00	–

Lr, *Leptodactylus rhodomystax*, MZUSP 940333; Lk, *Leptodactylus knudseni*, QCAZ 13077; P1, Pará species, MZUSP 70023; P2, Pará species, MZUSP 70075; Lv1, *Leptodactylus vastus*, MTR 976629 (MZUSP ???); Lv2, *Leptodactylus vastus*, USNM 284552; Ll1, *Leptodactylus labyrinthicus*, USNM 303175; Ll2, *Leptodactylus labyrinthicus*, UC 233; MAp1, Middle American "*pentadactylus*" species, USNM 347153; MAp2, Middle American "*pentadactylus*" species, USNM 298079; MAp3, Middle American "*pentadactylus*" species, USNM 534219; PE1, Pacific Ecuador "*pentadactylus*" species, QCAZ 17056; PE2, Pacific Ecuador "*pentadactylus*" species, QCAZ 19859; Lp1, *Leptodactylus pentadactylus*, USNM 303466; Lp2, *Leptodactylus pentadactylus*, MZUSP 70917.

- Heyer W. R., García-Lopez J. M., and Cardoso A. J.** (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.
- Leviton A. E., Gibbs Jr. R. H., Heal E., and Dawson C. E.** (1985), "Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology," *Copeia*, **1985**, 802 – 832.
- Mayr E.** (1963), *Animal Species and Evolution*, The Belknap Press of Harvard Univ. Press, Cambridge (MA), pp. xiv + 1 – 797.
- Swofford D. L.** (1998), *PAUP\*. Phylogenetic Analysis Using Parsimony (\* and Other Methods). Version 4*, Sinauer Assoc. Sunderland (MS).

## Appendix

Voucher specimen data used in molecular analyses. Museum designations follow Leviton et al. (1985) with the addition of Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito (QCAZ).

***Leptodactylus knudseni*.** QCAZ 13077 — Ecuador, Francisco Orellana, Parque Nacional Yasuní.

***Leptodactylus labyrinthicus*.** Ulisses Caramaschi field number 233 — Brasil, São Paulo, Piraju. USNM 303175 — Brasil, São Paulo, Fazenda Jataí, 5 km S of Luis Antonio.

**Middle American "*pentadactylus*" species.** USNM 298079, 347153 — Panama, Bocas del Toro, Isla Popa. USNM 534219 — Honduras, Colon, Quebrada Machin.

**Pacific Ecuador "*pentadactylus*" species.** QCAZ 17056 — Ecuador, Esmeraldas, Alto Tambo. QCAZ 19859 — Ecuador, Esmeraldas, Bosque Protector La Perla.

**Pará species.** MZUSP 70023 — Brasil, Pará, Aldeia A-Ukre. MZUSP 70075 — Brasil, Pará, Rio Vermelho.

***Leptodactylus pentadactylus*.** MZUSP 70917 — Brasil, Pará, Serra de Kukoinhokren. USNM 303466 — Brasil, Pará, near Cachoeira do Espelho, ca. 50 km (airline) S of Altamira.

***Leptodactylus rhodomystax* (outgroup taxon).** MZUSP 70375 — Brazil, Pará, Serra de Kukoinhokren.

***Leptodactylus vastus*.** MTR 976629 (to be deposited in MZUSP) — Brasil, Mato Grosso, Gaúcha do Norte. USNM 284552 — Brasil, Pernambuco, near Caruaru.