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ARE *Leptodactylus didymus* AND *L. mystaceus* PHYLOGENETICALLY SIBLING SPECIES (AMPHIBIA, ANURA, LEPTODACTYLIDAE)?

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INTRODUCTION

The *Leptodactylus fuscus* species group consists of 25 currently recognized species; within this species group and distributed throughout the Amazon Basin, Atlantic Forests, Gran Chaco, and cerrados is the *L. mystaceus* species complex. This species complex consists of *L. didymus*, *L. elenae*, *L. mystaceus*, *L. notoaktites*, and *L. spixi*. Adult morphologies have been used to distinguish these species from each other except for *L. didymus* and *L. mystaceus* (Heyer, 1978; Heyer et al., 1996). *Leptodactylus didymus* and *L. mystaceus* are morphologically indistinguishable; the species are recognizable only by the characteristics of their advertisement calls: non-pulsed in *L. didymus* and pulsed in *L. mystaceus* (Heyer et al., 1996).

Traditionally, *L. mystaceus* and *L. didymus* have been considered "sibling species." The concept of "sibling species" was originally introduced by Mayr (1942: 151) to describe pairs or groups of morphologically identical or nearly identical species; however, in subsequent work Mayr (1976) interchangeably used the terms "sibling and cryptic species" to describe morphologically similar species. Mayr (1942: 151) considered sibling species to be important in understanding the full complexity of animal speciation. In order to differentiate these two terms, herein we take a narrow cladistic methodological approach (i.e., dichotomous speciation) by which we restrict the term "sibling" species to two taxa that share a most recent common ancestor; whereas, the term cryptic (derived from the Greek *Kruptos*, meaning 'hidden'; Allaby, 1991) species refers to "hidden" diversity and does not necessarily imply close phylogenetic relationship. Thus, the sibling species pair of *L. didymus* and *L. mystaceus* assumes two postulates: (1) the taxa shared a most recent common ancestor not shared with other species in the *L. mystaceus* species complex and (2) the two taxa could represent a recent speciation event (i.e., not enough time has passed to reach morphological differentiation, although this is not a requisite).

Herein, we analyze the genetic diversity among taxa in this species complex to determine if the sibling species *L. didymus* and *L. mystaceus* are sister taxa. If the assumptions about sibling species are correct, then we would expect that the two taxa involved would be genetically closer between themselves than with any other closely related species.

MATERIAL AND METHODS

Molecular sequence data were obtained for *L. didymus*, *L. elenae*, *L. mystaceus*, *L. notoaktites*, and *L. spixi*; in addition, data were collected for *L. fuscus* and *L. mystacinus* (other *fuscus* species group members) to use as outgroups. We obtained a total of 2553 base pairs (bp) for each taxon, 786 bp corresponding to the 12S rDNA gene, 814 bp to the 16S rDNA gene, and 953 bp to the ND1 gene. The sequence data have GenBank accession numbers are AY948952 – 948959, AY905695, AY905716-17, AY911264, and AY911285-911286. Voucher specimens are presented in the *Appendix*. Sequences were aligned using Clustal X (Thompson et al., 1997). Alignment of ND1 coding sequences included the known complete ND1 coding sequence for *Rana catesbeiana* (Nagae, 1988). Maximum Parsimony (MP) and Maximum Likelihood (ML) exhaustive search analyses were performed with PAUP* (Swofford, 1998). ML analyses used the GTR+G model recommended by Modeltest 3.04 (Posada and Crandall, 1998), with empirical base frequencies. Analyses were performed using the two *Leptodactylus* taxa as outgroups and also tested the effect of alternatively using a single outgroup taxon at the time on the recovered trees. ND1 sequences also included *Rana* as an outgroup and for MP analyses, the third position was down-weighted relatively to first and second positions and gaps positions were alternatively treated as missing data and as a fifth character; transition substitutions were down-weighted relative to transversion substitutions.

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RESULTS

The results of the different phylogenetic analyses are best illustrated by the trees in Fig. 1. Neither in the MP (with gaps as a fifth character, Fig. 1*A*) or the ML (Fig. 1*B*) analyses do *L. didymus* and *L. mystaceus* exhibit sister species relationships within the *mystaceus* species complex. Support for clades was assessed using bootstrap (1000, pseudoreplicates; Felsestein, 1985), decay indices enforcing topological constraints (Bremer, 1988), and Bayesian posterior probabilities (Bayes et al., 2001) (Fig. 1).

Analyses of data partitions separately (i.e., 12S, 16S, and ND1 data matrices) and alternatively using the two *Leptodactylus* outgroups or using only one at the time (either *L. fuscus* or *L. mystacinus*) also resulted in tree topologies where *L. didymus* and *L. mystaceus* do not exhibit sister taxa relationships. MP weighted analyses as well as alternative treatment of gaps as missing data in combined and separate analyses also retrieved similar trees in which *L. didymus* and *L. mystaceus* do not exhibit sister taxa relationships.

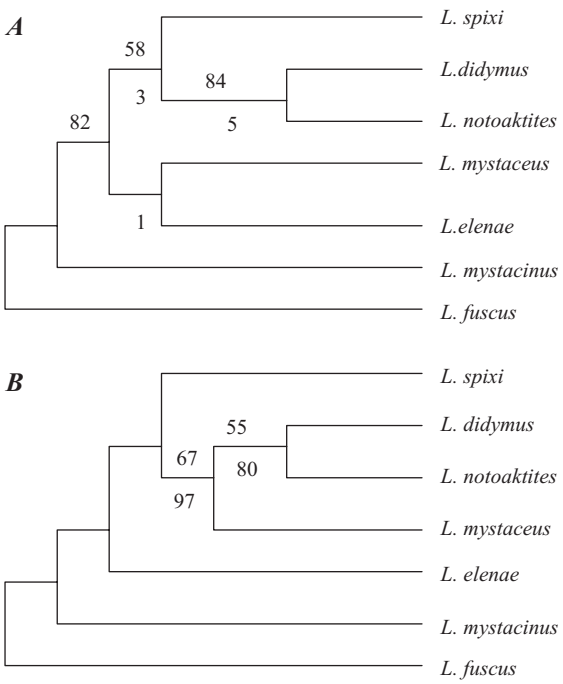

Fig. 1. ***A.*** MP most parsimonious tree recovered (length = 1000, C.I. = 0.75, gaps as fifth character. Bootstrap support above 50% is indicated above branches, number below branches corresponds to clade decay indexes. ***B.*** ML tree, bootstrap support above 50% is indicated above branches, number below branches corresponds to Bayesian posterior probabilities.

DISCUSSION

The present molecular analyses of the *L. mystaceus* complex shows that *L. didymus* and *L. mystaceus* are not sibling species as defined in this paper (i.e., a sister species relationship was not recovered in any of the analyses), despite their being morphologically indistinguishable. The topology recovered enforcing a sister taxa relationship between *Leptodactylus didymus* and *L. mystaceus* is 5 steps longer than the most parsimonious tree; however a Kishino – Hasewaga test comparing the two likelihood topologies was not statistically significant. These two taxa are treated as distinct species based on their call differences (Heyer et al., 1996), a common isolating mechanism occurring in anurans. The genetic differentiation between these two species is comparable (about 10%) to that between each of them with other species in the complex that have differentiated morphologically (9 – 12% between *L. mystaceus* and other species, 8 – 12% between *L. didymus* and other species, see Table 1).

These results are interesting because they highlight an unusual case among vertebrates in which the species involved show behavioral (e.g., call) and genetic (Table 1) differentiation, but do not differ morphologically.

Two alternative hypotheses need to be considered to explain this case.

1. Morphological convergence. Either *Leptodactylus didymus* and *L. mystaceus* are morphologically converging on each other or they both may be converging onto

TABLE 1. Genetic distances among taxa analyzed in this study.

	spixi	*didymus*	*fuscus*	*mystaceus*	*notoaktites*	*elenae*	*mystacinus*
spixi	0.00						
didymus	0.10	0.00					
fuscus	0.11	0.13	0.00				
mystaceus	0.10	0.12	0.14	0.00			
notoaktites	0.07	0.08	0.12	0.09	0.00		
elenae	0.09	0.11	0.12	0.10	0.08	0.00	
mystacinus	0.11	0.13	0.13	0.12	0.11	0.10	0.00

the morphology of at least a third species occurring in the Amazon basin. This morphological convergence could be justified if either one of these two species, or a third unidentified taxon at this point, are proven to produce skin toxins that would make them, if not toxic, at least strongly distasteful, giving them a selective advantage by avoiding predation. Alternatively, their morphological characteristics may be providing unique camouflage advantages in the habitat they occupy. Extensive field-work would be needed to test either of these alternatives.

2. Retention of ancestral morphological patterns. The two taxa involved are exhibiting morphological adult patterns inherited from a) a most recent common ancestor to both of them or b) to an ancestor to the *L. mystaceus* species complex, or a subclade of it. We have no evidence in support of the first alternative. Our data show that the two taxa involved are not sibling species; that is they do not share a most recent common ancestor. There is also no evidence in support of the second scenario, particularly considering that all other taxa in the *L. mystaceus* species complex can be differentiated morphologically among themselves and from the *L. didymus – L. mystaceus* pair.

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APPENDIX. Voucher specimen data used in molecular analyses.

Leptodactylus didymus. USNM 268970, Peru, Madre de Dios, Tambopata Reserve.

Leptodactylus elenae. USNM 319643, Argentina, Salta, Embarcacion, 4.0 km NE of junction with road into, on National Route 34.

Leptodactylus fuscus. MZUSP 67073, Brazil; Roraima; Caracaranã, near Normandia.

Leptodactylus mystaceus. MZUSP 70371, Brazil, Pará, Serra de Kukoinhokren.

Leptodactylus mystacinus. RdS 789, Uruguay, Departamento de San Jose, Sierra de Mahoma.

Leptodactylus notoaktites. USNM 303191, Brazil, Saõ Paulo, ca. 5 km S of Luiz Antonio, Fazenda Jatai.

Leptodactylus spixi. USNM 534008, Brazil, Sergipe, Crasto.