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The relationships of *Leptodactylus diedrus* (Anura, Leptodactylidae)

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Leptodactylus diedrus shares some external features with the monotypic genus *Vanzolinius*. Thirty eight characters of morphology and behavior are analyzed for 14 taxa which include species of *Leptodactylus* chosen to encompass the extremes of variation found in the genus, *Vanzolinius discodactylus*, and three outgroup taxa, *Adenomera marmorata*, *Lithodytes lineatus* and *Physalaemus pustulosus*. New osteological and myological data are provided for *Leptodactylus diedrus* and *Leptodactylus riveroi*, and the advertisement call of *L. diedrus* is described and figured for the first time. Cladistic analyses of the data set yield support, but not completely convincingly so, for a sister-group relationship of *Leptodactylus diedrus* with *Vanzolinius discodactylus*. Additional studies are required to test the robustness of this hypothesis, as well as resolve encountered problems of paraphyly of both the genus *Leptodactylus* and previously defined species groups within it.

INTRODUCTION

The recently described frog species *Leptodactylus diedrus* Heyer, 1994 was included as a member of the *Leptodactylus podicipinus-wagneri* complex within the *Leptodactylus melanonotus* species group. However, two features of external morphology of *L. diedrus* distinguish it from other members of the *L. melanonotus* species group: complete absence of dorsolateral folds and toe tips expanded into small disks, with the largest disks having a single dorsal longitudinal groove. The monotypic genus *Vanzolinius* also lacks dorsolateral folds and the toe tips are expanded into small disks, with 1-5 longitudinal dorsal grooves (HEYER, 1997). The primary purpose of this paper is to evaluate whether *L. diedrus* is a *Leptodactylus* or *Vanzolinius*.

The original descriptions of *Leptodactylus riveroi* Heyer and Pyburn, 1983 and *Leptodactylus silvanimbus* McCranie, Wilson, & Porras, 1980 could not associate these species with

the species groups previously defined by HEYER (1969). Recent evidence indicates that *L. silvanimbus* is a member of the *L. melanonotus* species group (HEYER et al., 1996). A secondary purpose of this paper is to evaluate the relationships of *L. riveroi* and *L. silvanimbus* to the other species groups of *Leptodactylus* through analysis of morphological and behavioral characters.

Finally, this paper includes new information on myological and osteological characters for *L. diedrus*, *L. riveroi* and *L. silvanimbus* and describes for the first time the advertisement call of *L. diedrus*.

MATERIALS AND METHODS

CHOICE OF TAXA

As the relationships of key species are the focus of this study, individual species are used as the operational taxonomic units (OTUs) for analysis. In addition to the focal species *Leptodactylus diedrus*, *L. riveroi*, *L. silvanimbus* and *Vanzolinius discodactylus*, representatives of the four species groups of *Leptodactylus* are included: *Leptodactylus bufonius* and *Leptodactylus fuscus* (members of the *Leptodactylus fuscus* species group); *Leptodactylus chaquensis* and *Leptodactylus insularum*¹ (members of the *Leptodactylus ocellatus* species group); *Leptodactylus leptodactyloides* and *Leptodactylus melanonotus* (members of the *Leptodactylus melanonotus* species group); and *Leptodactylus pentadactylus*² (member of the *Leptodactylus pentadactylus* species group).

Representatives of the most closely related genera to *Leptodactylus* (LYNCH, 1971; HEYER, 1974, 1975) were initially included as outgroup taxa: *Adenomera marmorata* and *Lithodytes lineatus*. Preliminary analyses using these two taxa as outgroups indicated that they often appeared within *Leptodactylus* on the cladograms and neither consistently performed as an effective outgroup taxon. Consequently, a third outgroup taxon was included in the analysis: *Physalaemus pustulosus*.

CHOICE OF CHARACTERS

The characters used in a similar previous study (HEYER, 1974) were screened to determine whether they demonstrated variation among the taxa included in this study. Those characters derive from external morphology of adults, adult jaw, hyoid, and thigh musculature, adult osteology, and external larval morphology. In addition to that suite of characters, other larval, osteological, and advertisement call features are examined herein.

1. Jesús MANZANILLA (personal communication) has a study in progress with Enrique LA MARCA and Abraham MUIARES demonstrating that two members of the *Leptodactylus bolivianus* complex occur in Venezuela. The available names suggest that *L. bolivianus* and *L. insularum* are appropriate to apply to these two species. All of the data for *L. insularum* reported on in this paper are based on specimens from Costa Rica and Panama.

2. There are two species currently embraced in *Leptodactylus pentadactylus* (Ulisses GALATTI, personal communication). All of the data for *L. pentadactylus* reported in this paper are based on the taxon from Costa Rica and Panama.

CHOICE OF ANALYTIC PROCEDURES

There are no strong internal arguments from the data to treat the characters with three or more states as ordered or unordered. Either option carries with it assumptions about the evolution of character state change (SWOFFORD, 1993). Rather than make an a priori choice, the data set is analyzed in two ways: (1) as completely unordered states, and (2) with as many ordered characters as reasonable. Morphoclines are used to order character states. Ordered character states are analyzed as unpolarized.

The data matrix analyzed was created in MacClade (MADDISON & MADDISON, 1992); trees were generated and evaluated with PAUP version 3.1.1 (SWOFFORD, 1993). Specific options are indicated as appropriate in association with the results.

SPECIMENS EXAMINED

The specimens supplementing those examined previously (HEYER, 1974: 4-5, tab. 1, 1994, 1997; WASSERSUG & HEYER, 1988) are listed in app. 1.

ANALYSIS OF CHARACTERS

The character state descriptions are the same as those used previously (HEYER, 1974, although the character numbering is different), unless otherwise indicated.

ADULT MORPHOLOGY

Character 1. Vocal sac

State 0: vocal sac absent. State 1: no vocal sac visible externally, present internally. State 2: indications of lateral vocal folds. State 3: well developed paired lateral vocal sacs. State 4: well developed, large, single vocal sac.

The greatest difficulty assigning the taxa of this study to these states is whether certain species have state 1 or 2. Some individuals of *Adenomera marmorata*, *Vanzolinius discodactylus*, *Leptodactylus diedrus*, *L. leptodactyloides* and *L. melanonotus* appear to have either state 1 or 2. However, most individuals demonstrate either one state or the other and in those cases, the commoner condition is used. Only for *L. diedrus* and *L. leptodactyloides* are both states about equally represented.

When used as ordered states, the order is 4-0-1-2-3.

Character 2. Tympanum visibility

State 0: tympanum well developed, easily seen externally. State 1: tympanum partially concealed, but still visible externally.

Character 3. Male thumb

State 0: thumb without modifications. State 1: thumb with one horny spine (state 2 of HEYER, 1974, in part). State 2: thumb with two horny spines (state 2 of HEYER, 1974, in part). State 3: thumb with pair of nuptial pads.

When used as ordered states, the order is 3-0-1-2.

Character 4. Dorsolateral folds

This character was included in character 5 of HEYER (1974); however, as much of the variation observed in the taxa of this study were subsumed in only two states, new state characterizations are defined for this study.

State 0: no dorsolateral folds. State 1: one short pair. State 2: one well-developed pair. State 3: three to five well developed pairs.

When used as ordered states, the order is 0-1-2-3.

Character 5. Toe disks

The state characterizations used previously (HEYER, 1974, character 6) do not adequately encompass the variation found in the taxa being analyzed and are not followed here.

State 0: toe tips narrow. State 1: toe tips noticeably expanded without any dorsal modification. State 2: toe tips expanded, usually in form of small disks with a single dorsal groove in larger disks. State 3: toe tips with small disks, dorsal surfaces with 2-5 grooves (rarely 1). State 4: toe tips disked with a pair of dorsal scutes.

When used as ordered states, the morphocline 0-1-2-3 is used with state 4 additionally linked to state 1.

Character 6. Tarsal decoration

State 0: tarsal fold. State 1: tarsal tubercle.

Character 7. Toe webbing

This is equivalent to character 9 of HEYER (1974) in part.

State 0: toes without web or fringes. State 1: toes with weak basal fringes and webbing. State 2: toes with fringes extending length of toes except for tips. State 3: females with weakly developed lateral toe fringes and males either with ridges or weakly developed fringes (see HEYER et al., 1996).

When used as ordered states, the order is 0-1-2-3.

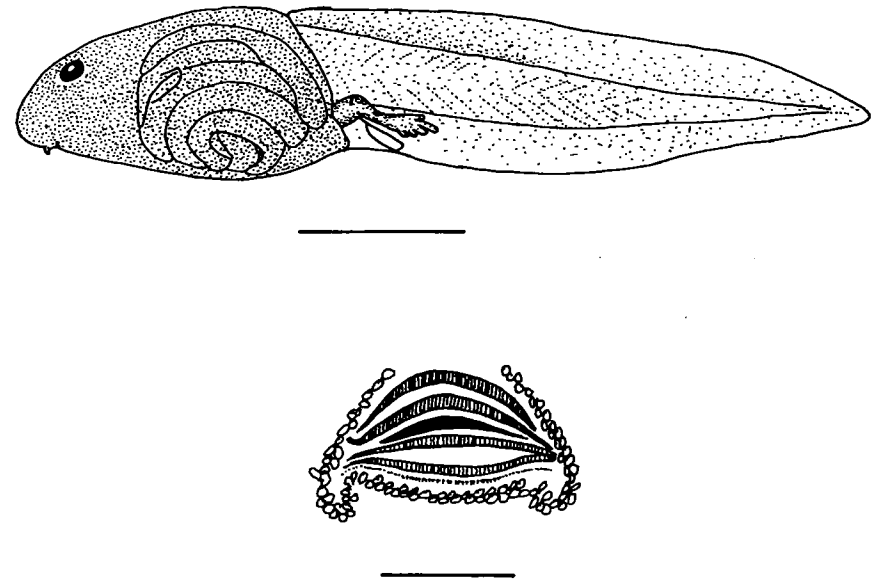


Fig 1. - Semi-diagrammatic representation of lateral view and oral disk of stage 37 specimen from KU 121362, reported in the literature as *Vanzolinius discodactylus*. Upper line scale is 5 mm, lower 1 mm.

LARVAL CHARACTERS

The larvae described as *Vanzolinius discodactylus* from Ecuador, Pastaza, Mera, by DUELLMAN (1978) may or may not be *V. discodactylus* larvae (HEYER, 1997). The larval character states used in this analysis are from the Mera specimens. Because there is reasonable doubt regarding the identification, phylogenetic analyses are performed both with the data from these specimens and with larval data treated as missing for *V. discodactylus*. Although DUELLMAN (1978) described the larvae in question, they have never been figured. To enhance the interpretations made in this paper, larval figures are presented (fig. 1).

Larval data that supplement those reported in HEYER (1974) are from: HEYER et al., (1990) for *Adenomera marmorata*; LAMAR & WILD (1995) for *Lithodytes lineatus*; LIMA (1992) for *Leptodactylus riveroi*; MCCRANIE et al. (1986) for *Leptodactylus silvanimbus*; SCHLÜTER & REGÖS (1996) for *Lithodytes lineatus*; and WASSERSUG & HEYER (1988) for *Adenomera marmorata*, *Leptodactylus chaquensis*, *L. fuscus*, *L. pentadactylus* and *Physalaemus pustulosus*.

Character 8. Larval tongue papillae

WASSERSUG & HEYER (1988) reported that *Adenomera marmorata* had 2 pustules, *Leptodactylus chaquensis* and *L. pentadactylus* 3 papillae, and *L. fuscus* and *Physalaemus pustulosus* 4 tongue papillae. The following represent new observations: *Leptodactylus riveroi* has one simple papilla; *Leptodactylus silvanimbus* has 1 bifid papilla; *Leptodactylus leptodactyloides* and *Vanzolinius discodactylus* have 1 papilla with a broadened fan tip; *Leptodactylus insularum* and *L. melanonotus* have 2 pustules; and *Leptodactylus bufonius* has 4 papillae. It is unclear whether the simple, bifid, and broadened fan tip conditions of larvae having a single papilla should be considered as 1, 2, or 3 distinct character states. Given that uncertainty, a single character state is used in this study (surveying more individuals and taxa in future studies should clarify the situation). All three taxa with 2 papillae in this data set have pustules, rather than elongate papillae. While the coding is unambiguous for this particular study, future comparisons in larger data sets may have to distinguish pustular and papillate states.

State 0: one tongue papilla. State 1: two pustules. State 2: three papillae. State 3: four papillae.

When used as ordered states, the order is 0-1-2-3.

Character 9. Larval labial denticle rows

The variation used previously (HEYER, 1974, character 11) is at a coarse scale; those state definitions are not followed here.

State 0: no labial denticle rows (formula 0/0). State 1: apparent ontogenetic development of larval denticle rows from Gosner stages 26-42 of denticle formulae 0/0 to 2(2)/3(1); this pattern is unusual and likely represents a distinct evolutionary trajectory within the study set of taxa. State 2: labial row A-2 continuous, row P-1 interrupted; formula 2/3(1). State 3: labial row A-2 continuous, row P-1 continuous; formula 2/3. State 4: labial row A-2 interrupted, row P-1 interrupted; formula 2(2)/3(1). State 5: labial row A-2 interrupted, row P-1 continuous; formula 2(2)/3.

Only *Leptodactylus leptodactyloides* shows variation among these states (formula 2[2]/3).

There is no obvious morphocline among the states and it seems reasonable to assume that any given state could be derived from any other state. This character is therefore always treated as unordered.

Character 10. Larval vent

State 0: median. State 1: dextral.

Character 11. Larval pattern

State 0: lacking melanophores (red in life). State 1: uniform, light. State 2: uniform, dark. State 3: mottled.

This character is always treated as unordered.

Character 12. Larval ecomorph

This character follows the definitions of ALTIG & JOHNSTON (1989).

State 0: lentic nektonic. State 1: lentic facultative carnivore. State 2: endotrophic nidicolous.

There is no obvious ordering pattern among the states, which are always treated as unordered.

LARVAL SCHOOLING AND FEMALE ATTENDANCE

While these behavioral characters likely contain phylogenetic information, the data are known for too few taxa at this time to analyze. More sustained observations are needed for most taxa. For the taxa of this data set, both larval schooling and female attendance have been documented for *Leptodactylus insularum* (WELLS & BARD, 1988, as *Leptodactylus bolivianus*), and *L. leptodactyloides* (COCROFT & MORALES in DOWNIE, 1996). Larval aggregations, but apparently without female attendance, are known for *Leptodactylus riveroi* (LIMA, 1992) and *Leptodactylus silvanimbus* (HEYER et al., 1996).

EGG CHARACTERS

Character 13. Egg pigment

State 0: eggs with melanin. State 1: eggs lacking melanin.

Character 14. Egg deposition

State 0: eggs deposited in a foam nest on top of the water. State 1: eggs deposited in a foam nest away from the water; documentation of this state is from LAMAR & WILD (1995) for *Lithodytes lineatus* and RODRIGUEZ & DUELLMAN (1994) for *Vanzolinius discodactylus*.

ADULT MUSCULATURE: JAW MUSCLES

All taxa lack the adductor mandibulae externus superficialis and have the adductor mandibulae posterior subexternus muscle (s only condition of STARRETT, 1968).

Character 15. Depressor mandibulae muscle

State 0: bulk of muscle arises from squamosal, a small slip from the dorsal fascia, and a few fibers from the annulus tympanicus (dfsQat condition of STARRETT, 1968). State 1: bulk of muscle arises from dorsal fascia, a small slip from the squamosal, and attachment by a few

fibers to the annulus tympanicus may or may not be present (DFsq, DFsqat conditions of STARRETT, 1968).

The conditions of the species examined for this study which were previously unreported are: DFsq in *Leptodactylus diedrus* and *L. riveroi*; DFsqat in *Leptodactylus leptodactyloides* and *L. silvanimbus*.

ADULT MUSCULATURE: HYOID MUSCLES

Character 16. Geniohyoideus medialis muscle

State 0: muscle continuous medially, dividing posteriorly where the posteromedial processes of the hyoid articulate with the body of the hyoid; the hyoglossus muscle is completely covered ventrally by the geniohyoideus medialis. State 1: muscle divided ventrally, exposing hyoglossus; posterior half of muscle covered ventrally by sternohyoideus.

Character 17. Geniohyoideus lateralis muscle

State 0: no attachment of muscle to hyale. State 1: muscle attached to hyale, indicated by lateral flaring of the muscle. State 2: distinct slip of geniohyoideus lateralis attaches to hyale anterolaterally.

The male examined of *Leptodactylus diedrus* has state 1, the female state 0.

When used as ordered states, the order is 0-1-2.

Character 18. Anterior petrohyoideus muscle

State 0: muscle inserts entirely on edge of the hyoid apparatus. State 1: muscle inserts entirely on the ventral surface of the hyoid body.

Character 19. Sternohyoideus muscle origin

State 0: single medial slip originates from meso- and xiphisterna. State 1: medial slip divides into two slips, one originating from anterior portion of the mesosternum, another from the posterior meso- and/or xiphisternum.

Character 20. Sternohyoideus muscle insertion

State 0: muscle inserts in narrow band near the lateral edges of the hyoid. State 1: some fibers insert near the lateral edges of the hyoid and some insert near midline of hyoid posteriorly. State 2: muscle inserts in a narrow band with fibers attached near midline posteriorly.

When used as ordered states, the order is 0-1-2.

Character 21. Omohyoideus muscle

State 0: muscle inserts partly on hyoid plate and partly on fascia between the posterolateral and posteromedial processes of the hyoid. State 1: muscle inserts entirely on hyoid plate ventrally. State 2: muscle absent.

This character is always treated as unordered.

ADULT MUSCULATURE: THIGH MUSCLES

Complex of distal thigh muscle tendons

Variation is minimal with intraspecific variation as great as interspecific variation in the data set. The data are not included for analysis. New dissection data indicate that *Leptodactylus diedrus*, *L. riveroi* and *L. silvanimbus* have state 0 of character 22 and *Leptodactylus leptodactyloides* has state 1 of character 22 as defined by HEYER (1974: 15).

Character 22. Iliacus externus muscle

State 0: muscle short, extending less than half the distance anteriorly on the iliac bone from where the leg muscles join the iliac to the anterior extremity of the iliac (short state of LIMESSES, 1964: fig. 2). State 1: muscle long, extending from three quarters to full length of iliac (long B state of LIMESSES, 1964: fig. 2).

Tensor fasciae latae

All taxa of the data set have state C-2 as defined by LIMESSES (1964: fig. 4).

Character 23. Semitendinosus muscle

State 0: interior (with respect to femur) and exterior portions of the semitendinosus unite in a common tendon distally. State 1: exterior portion is well developed, but smaller than interior portion and attaches by a tendon to the interior portion.

Character 24. Sartorius muscle

State 0: muscle very narrow (not quite as narrow as fig. 6A of LIMESSES, 1964, but much narrower than her fig. 6B). State 1: muscle moderate (narrow type of LIMESSES, 1964: fig. 6B). State 2: intermediate condition between states 1 and 3. State 3: muscle broad (wide type of LIMESSES, 1964).

When used as ordered states, the order is 0-1-2-3.

Character 25. Accessory head of adductor magnus muscle

State 0: no distinct tendon of accessory head. State 1: accessory head absent.

Adductor longus muscle

All taxa of the data set have state 0 of character 29 defined by HEYER (1974: 18).

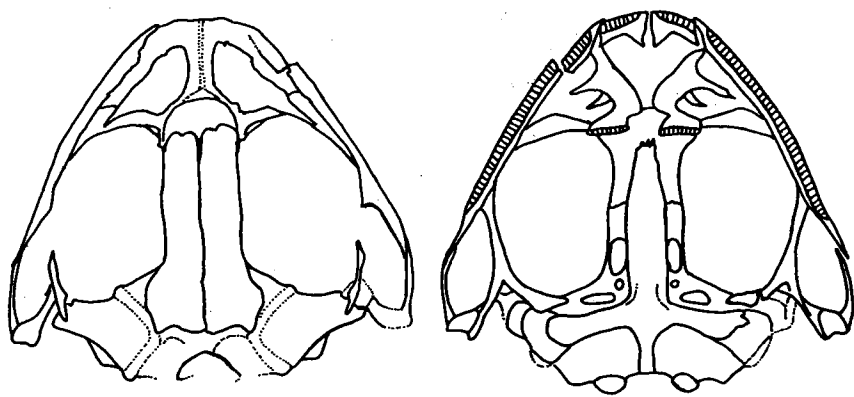


Fig. 2. – Dorsal and ventral views of skull of *Leptodactylus diedrus*, AMNH 115721. Scale line: 5 mm. Drawn from cleared and stained specimen. Only features clear in perspective are indicated by solid line; some features are obscured by partially cleared muscle masses. Dentigerous areas indicated by hatching. Note that right maxilla and left pterygoid bones are broken.

ADULT OSTEOLOGY

The previously undescribed skulls of *Leptodactylus diedrus* (fig. 2) and *Leptodactylus riveroi* (fig. 3) are representative of the features described below.

Median contact of vomers

Only *Leptodactylus bufonius* within the data set exhibits median contact of vomers. All other taxa have vomers separated medially. The character is not analyzed further.

Character 26. Posterolateral projection of frontoparietal

State 0: no or minimal projection, such as a bump or swelling (fig. 2). State 1: distinct, but relatively short posterolateral projection (fig. 3).

Character 27. Anterior articulation or overlap of vomers

State 0: anterior process of vomer does not articulate or overlap premaxilla or maxilla. State 1: anterior process of vomer articulates or overlaps premaxilla or maxilla.

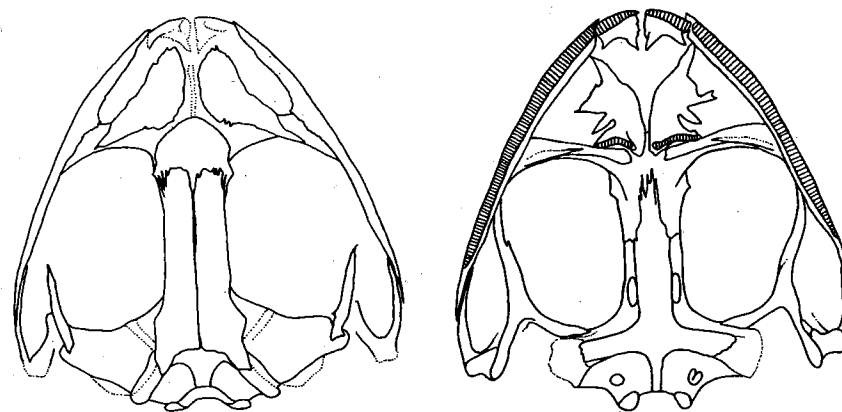


Fig. 3. – Dorsal and ventral views of skull of *Leptodactylus riveroi*, MZUSP 60101. Scale line: 5 mm. Drawn from cleared and stained specimen. Only features clear in perspective are indicated by solid lines; some features are obscured by partially cleared muscle masses. Dentigerous areas indicated by hatching.

Character 28. Sphenethmoid and optic foramen relationship

State 0: posterior extent of sphenethmoid widely separated from optic foramen. State 1: posterior extent of sphenethmoid closely approximates optic foramen. State 2: posterior extent of sphenethmoid borders foramen.

When used as ordered states, the order is 0-1-2.

Character 29. Anterior extent of sphenethmoid

State 0: sphenethmoid anterior extent no more than to middle of vomerine bones. State 1: sphenethmoid anterior extent beyond middle of vomerine bones.

Character 30. Pterygoid and parasphenoid overlap

State 0: no overlap in an anterior-posterior plane. State 1: elements overlap but are not in contact. State 2: pterygoid and parasphenoid overlap and are in contact.

When used as ordered states, the order is 0-1-2.

Character 31. Alary process of hyoid

State 0: alary process narrow, usually stalked. State 1: alary process broad and winglike.

Character 32. Sacral diapophyses

State 0: sacral diapophyses expanded. State 1: sacral diapophyses rounded.

Character 33. Terminal phalanges

State 0: simple or knobbed, single or slightly bifurcate. State 1: T-shaped, expanded.

ADVERTISEMENT CALLS

Data are derived from the following sources: BARRIO (1966) for *Leptodactylus chaquensis*; DREWRY et al. (1982) for *Physalaemus pustulosus*; HEYER (1978) for *Leptodactylus bufonius* and *L. fuscus*; HEYER (1979) for *Leptodactylus pentadactylus* (Middle American calls only); HEYER (1994) for *Leptodactylus leptodactyloides*; HEYER (1997) for *Vanzolinius discodactylus*; HEYER et al. (1996) for *Leptodactylus melanonotus* and *L. silvanimbus*; HEYER & PYBURN (1983) for *Leptodactylus riveroi*; HEYER et al. (1990) for *Adenomera marmorata*; MARQUEZ et al. (1995) for *Lithodytes lineatus*; and STRAUGHAN & HEYER (1976) for *Leptodactylus insularum* (as *L. bolivianus*) and *L. melanonotus*.

The advertisement call of *Leptodactylus diedrus* (fig. 4) has the following characteristics: call rate 0.7 calls per s (40.8 per min) (based on recording length of 175 s; rest of data based on analysis of 20 individual calls); call duration 0.18-0.30 s ($x = 0.23$) (end of call difficult to determine precisely); calls of single pulsed notes, 2-6 major pulses per note ($x = 3.6$), 6-12 total major and partial pulses per note ($x = 9.0$); call frequency modulated, frequency rising through call; harmonics well developed, particularly second; broadcast (i.e., fundamental) frequency range 490-1170 Hz (modal values 510-1100 Hz), peak broadcast frequency 780-860 Hz (modal value 860 Hz), frequency range of second harmonic 1160-2080 Hz (modal values 1260-[no clear modal value, 1880, 1960, 2000, 2010, 2080 Hz with equal low numbers of occurrences]), peak frequency of second harmonic 1640-1810 Hz (modal value 1720 Hz).

Character 34. Notes per call

State 0: one note per call. State 1: multiple notes per call.

Both *Leptodactylus chaquensis* and *L. leptodactyloides* are known to have two call types. There is one note per call in both call types for both species.

Character 35. Pulse structure

State 0: call consists of a note of a single pulse. State 1: each note with 2 consistent, well-defined pulses. State 2: each note with 2-5 strong pulses, one or more of the strong pulses partially pulsed. State 3: each note of more than 6 pulses. State 4: entire note partially pulsed.

Leptodactylus pentadactylus from Middle America have calls demonstrating both states 0 and 4.

When used as ordered states, the morphocline 0-1-2-3 was used with state 4 treated as unordered. A stepmatrix was used to enter the ordering information.

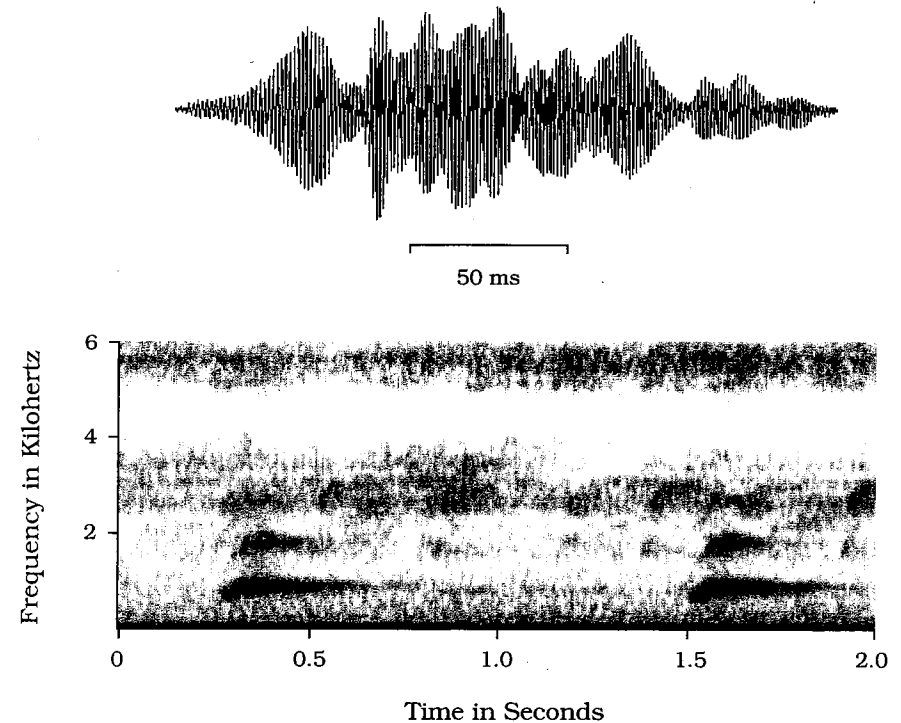


Fig. 4. – Advertisement call of *Leptodactylus diedrus*. Wave form of first call represented in the audiospectrogram. Audiospectrogram unfiltered; wave form filtered around 290-2300 Hz. USNM Tape 255, Cut 1. Brazil, Amazonas, Altamira. Recorded 17 November 1991, 16.00 h, 80°F, by Claude GASCON from a pot-hole filled pond in the forest. Voucher specimen INPA 5020.

Character 36. Frequency modulation

State 0: no or negligible frequency modulation (not readily discernible by human ear). State 1: rising frequency modulation, extremely sharp (rise in frequency so fast not readily discernible to human ear), with or without a slight drop in frequency at end of call. State 2: rising frequency modulation, moderate (rising whistle to human ear), with or without slight drop in frequency at end of call. State 3: rising and falling frequencies throughout call. State 4: falling frequencies throughout call.

This character is always treated as unordered, as it seems most likely that any given state could be derived from any other state.

Character 37. Call duration

State 0: very short calls, < 0.1 s duration. State 1: moderately short calls, 0.1-0.2 s duration. State 2: moderate length calls, 0.2-0.5 s duration. State 3: long calls, > 0.5 s duration.

The described call lengths of *Leptodactylus insularum* are 0.07 s for a Costa Rican sample and 0.10 s for a Panamanian sample; the species is coded as state 0 for analytic purposes. The call duration of *Leptodactylus diedrus* exactly straddles the durations of states 1 and 2 and is considered polymorphic for this character. *Leptodactylus chaquensis* has two types of advertisement calls reported, one type falling into state 1, the other into state 3.

When used as ordered states, the order is 0-1-2-3.

Character 38. Harmonic structure

State 0: no (or weak) harmonic structure. State 1: distinct harmonic structure.

Leptodactylus chaquensis and *L. leptodactyloides* have two call types; one call type of each species has a harmonic structure, the other lacks harmonic structure. The calls of *Leptodactylus insularum* reported from Costa Rica lack harmonic structure, those reported from Panama have harmonic structure.

Multiple advertisement calls

Some species of *Leptodactylus* have a distinct call given initially or two call types given sporadically throughout a calling bout. Other species have a single advertisement call. Unfortunately, this character is unknown for many of the taxa in this data set, so it is not included in the phylogenetic analysis. A single advertisement call is documented for *Leptodactylus fuscus* and *L. pentadactylus*. Multiple advertisement calls are documented for *Leptodactylus chaquensis*, *L. leptodactyloides*, *L. melanonotus* and *L. silvanimbus*.

CHARACTERIZATION OF ROBUSTNESS OF DATA

In order to evaluate the robustness of the ordered and partially ordered data sets, data were used for all 14 taxa and 38 characters. The character states for *Vanzolinius discodactylus* are those for the presumed larvae (tab. 1). *Physalaemus pustulosus* was used as the outgroup taxon to root the tree. The default options were used for the branch-and-bound search procedure. As multiple states are not allowed (in PAUP) for ordered characters, the first character states in the matrix (tab. 1-3) were used for multiple states of characters 1 and 35 for the analyses using partially ordered data.

The search using the entirely unordered data set yielded 194 most parsimonious trees of a length of 122. The search using the partially ordered data set yielded a single shortest tree of a length of 138.

Three tree statistics have been proposed that allow evaluation of the data in terms of phylogenetic signal: (1) the Consistency Index (CI); (2) the Permutation Tail-Probabilities

Tab. 1. - Standard data matrix used in parsimony phylogenetic analyses. Characters of adult morphology (1-7), larval characters (8-12) and egg characters (13-14).

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Leptodactylus bufonius</i>	3	0	0	0	0	0	0	3	4	0	3	0	1	1
<i>Leptodactylus fuscus</i>	3	0	0	3	0	0	0	3	4	0	3	0	1	1
<i>Leptodactylus leptodactyloides</i>	1,2	0	2	1	1	0	2	0	3,5	0	2	0	0	0
<i>Leptodactylus melanonotus</i>	1	0	2	0	0	0	2	1	5	0	2	0	0	0
<i>Leptodactylus chaquensis</i>	2	0	2	3	0	0	2	2	2	0	2	0	0	0
<i>Leptodactylus insularum</i>	1	0	2	2	0	0	2	1	3	0	2	0	0	0
<i>Leptodactylus pentadactylus</i>	1	0	1	2	0	0	0	2	4	0	3	1	0	0
<i>Leptodactylus diedrus</i>	1,2	0	2	0	2	0	2	?	?	?	?	?	1	?
<i>Leptodactylus riveroi</i>	0	0	2	2	0	0	2	0	4	0	2	0	?	?
<i>Leptodactylus silvanimbus</i>	1	0	2	0	0	0	3	0	5	0	2	0	0	?
<i>Adenomera marmorata</i>	2	0	0	0	1	0	0	1	0	0	1	2	1	1
<i>Lithodytes lineatus</i>	1	0	0	2	4	0	0	?	1	0	0	0	1	1
<i>Vanzolinius discodactylus</i>	1	0	0	0	3	0	2	0	5	0	2	0	1	0
<i>Physalaemus pustulosus</i>	4	1	3	0	0	1	1	3	5	1	3	0	1	0

Tab. 2. - Standard data matrix used in parsimony phylogenetic analyses. Characters of adult musculature: jaw muscles (15), hyoid muscles (16-21) and thigh muscles (22-25).

Taxon	15	16	17	18	19	20	21	22	23	24	25
<i>Leptodactylus bufonius</i>	1	0	2	0	0	0	0	1	0	3	0
<i>Leptodactylus fuscus</i>	1	0	1	0	1	0	0	1	0	1,3	0
<i>Leptodactylus leptodactyloides</i>	1	0	0	0	0	0	0	1	0	1	0
<i>Leptodactylus melanonotus</i>	1	0	0	0	0	0	0	1	0	1	0
<i>Leptodactylus chaquensis</i>	1	0	0	0	0	0	0	1	0	1	0
<i>Leptodactylus insularum</i>	1	0	0	0	1	0	0	1	0	1	0
<i>Leptodactylus pentadactylus</i>	1	0	0	0	0	0	0	1	0	3	0
<i>Leptodactylus diedrus</i>	1	0	0,1	0	0	0	0	1	0	1	0
<i>Leptodactylus riveroi</i>	1	0	0	0	0	0	0	1	0	1	0
<i>Leptodactylus silvanimbus</i>	1	0	0	0	0	0	0	1	0	2	1
<i>Adenomera marmorata</i>	1	1	2	0	0	2	1	1	0	1	0
<i>Lithodytes lineatus</i>	1	0	0	0	1	1	0	1	1	1	0
<i>Vanzolinius discodactylus</i>	1	0	0	0	1	0	0	1	0	1	0
<i>Physalaemus pustulosus</i>	0	0	0	1	1	2	2	0	1	0	0

Tab. 3. - Standard data matrix used in parsimony phylogenetic analyses. Characters of adults osteology (26-33) and of advertisement calls (34-38).

Taxon	26	27	28	29	30	31	32	33	34	35	36	37	38
<i>Leptodactylus bufonius</i>	0	1	2	1	1	0	1	0	0	0	2	1	0
<i>Leptodactylus fuscus</i>	0	0	2	1	2	0	1	0	0	0	2	1	0
<i>Leptodactylus leptodactyloides</i>	0	1	0	0	1	0	1	0	0	0	1	0	0,1
<i>Leptodactylus melanotus</i>	0	0	0	1	1	0	1	0	0	1	1	0	0
<i>Leptodactylus chaquensis</i>	1	0	0	0	2	0	1	0	0	3	0,3	1,3	0,1
<i>Leptodactylus insularum</i>	0	0	0	0	1	0	1	0	0	0	2	0	0,1
<i>Leptodactylus pentadactylus</i>	1	1	1	1	1	0	1	0	0	0,4	2	2	0
<i>Leptodactylus diedrus</i>	0	1	0	0	1	0	1	1	0	2	2	1,2	1
<i>Leptodactylus riveroi</i>	1	1	0	0	1	0	1	0	1	1	3	3	0
<i>Leptodactylus silvanimbus</i>	0	0	0	0	1	0	1	0	0	4	0	1	1
<i>Adenomera marmorata</i>	0	1	2	1	1	0	1	1	0	0	1	0	0
<i>Lithodytes lineatus</i>	0	0	0	0	1	0	1	1	0	0	0	1	1
<i>Vanzolinius discodactylus</i>	0	1	0	0	1	0	1	1	0	2	1	1	0
<i>Physalaemus pustulosus</i>	0	0	0	1	0	1	0	0	0	3	4	2	1

(PTP) test; and (3) skewness of tree-length distributions. HILLIS (1991) suggested that the g_1 statistic provided an appropriate means of characterizing skewness of tree-length distributions to determine the amount of phylogenetic signal contained in the underlying data. He calculated critical values for g_1 statistics of tree-length distributions for six, seven, and eight taxa. Those critical values do not apply for different numbers of taxa. One approach to using the results published by HILLIS (1991) would be to use multiple subsamples of 8 taxa from the total of 14 taxa used in this study. As a sample of 8 taxa would only account for 57 % of the taxa, the skewness properties are not analyzed herein.

SANDERSON & DONOGHUE (1989) demonstrated that CI is correlated with number of taxa, but not number of characters beyond a minimum number. They provided a formula based on analyses of 60 data sets that gives an expected CI for comparative purposes based on number of taxa (valid for a range between about 5 and 60 taxa). The expected CI for 14 taxa is 0.63. The CI excluding uninformative characters for the 194 most parsimonious trees of the unordered data set is 0.60; for the single most parsimonious tree of the partially ordered data set, 0.52. The two data sets have a bit more homoplasy than predicted solely by the number of taxa.

The PTP test (run on PAUP* test version 4.0 with 100 replicates) evaluates the most parsimonious trees produced from a data matrix against randomized data having the same structure as the data matrix being evaluated (FAITH & CRANSTON, 1991). The data matrix (tab. 1-3) differs significantly from random data at the $P = 0.01$ level for both the entirely unordered and partially ordered data sets.

These results indicate that the unordered and partially ordered data sets do contain phylogenetic information, but exactly how strong the phylogenetic signal is within each is not known.

RELATIONSHIPS OF *LEPTODACTYLUS DIEDRUS*

The strict consensus tree for the 194 most parsimonious trees of the unordered character state data set is entirely unresolved. As expected, applying some structure to the data set by ordering some characters yields more resolved most parsimonious cladograms. This greater resolution may or may not correlate with increased phylogenetic signal; for purposes of the rest of the discussion, the assumption is made that the partially ordered data set enhances the phylogenetic signal contained in the data. Further discussion is limited to partially ordered data sets.

The single most parsimonious tree resulting from the partially ordered data matrix indicates that *Leptodactylus diedrus* and *Vanzolinius discodactylus* share a sister-group relationship, nested within other species of *Leptodactylus* (fig. 5). The bootstrap 50 % majority-rule consensus tree for these data is mostly unresolved, with only two clades having any support: the trichotomy *Adenomera marmorata* – *Leptodactylus bufonius* – *Leptodactylus fuscus* with bootstrap support of 52 % and *Leptodactylus diedrus* – *Vanzolinius discodactylus* with bootstrap support of 68 %.

The same cladogram (fig. 5) indicates that *Leptodactylus* is paraphyletic, with *Adenomera marmorata* occurring within rather than outside of *Leptodactylus* lineages. In order to focus on the relationships of *Leptodactylus diedrus*, further analyses use *Physalaemus pustulosus* as the outgroup taxon for tree-rooting purposes and *Adenomera marmorata* and *Lithodytes lineatus* are deleted. The analysis based on 12 taxa (fig. 6a) results in only one major change among the relationships of *Leptodactylus* and *Vanzolinius* species: the position of *Leptodactylus pentadactylus* switches from being a member of the clade of all other species of *Leptodactylus* and *Vanzolinius* except for the species *Leptodactylus bufonius* and *L. fuscus*, to forming a clade with those latter two species. The single most parsimonious tree has a length of 114 with a CI excluding uninformative characters of 0.57. The bootstrap results indicate that there is support for more lineage structure (fig. 6b) than when data are included for *A. marmorata* and *L. lineatus*.

There are an additional 15 trees of a length of 115 using the 12-taxon partially ordered data matrix. The strict consensus tree of the 114 and 115 length trees, as well as the bootstrap 50 % majority-rule consensus tree are identical in structure to fig. 6b. The bootstrap support values for the 114 and 115 length trees are almost identical to those of fig. 6b, with 71 % for the *Leptodactylus diedrus* – *Vanzolinius discodactylus* clade.

As indicated previously, the larval information for *Vanzolinius discodactylus* as used in the matrix (tab. 1) may be correct or not. In order to determine whether larval information for *V. discodactylus* impacts the relationships between it and *L. diedrus*, two additional analyses were run on the partially ordered matrix. In both cases, *Physalaemus pustulosus* was used as the outgroup and the default options for the branch-and-bound search were followed. In the

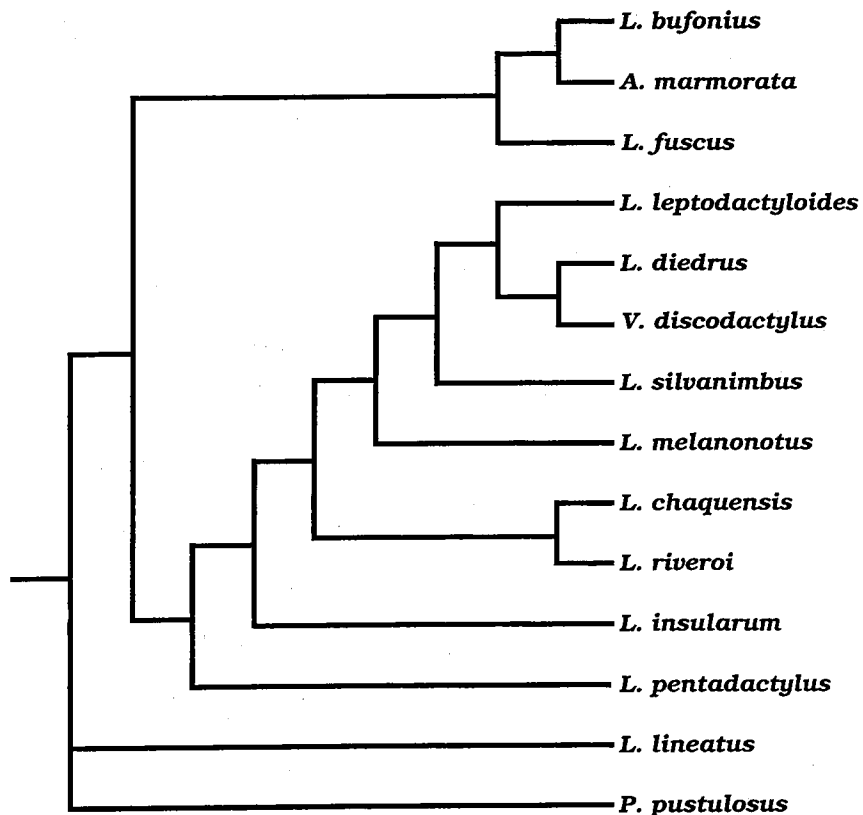


Fig. 5. – Single most parsimonious tree using the partially ordered state data matrix (tab. 1-3), *Physalaemus pustulosus* used as outgroup taxon.

first analysis, the larval states for *V. discodactylus* were treated as unknown. A single most parsimonious tree of a length of 114 resulted, identical in structure to that using the larval states as in tab. 1 (that is, the tree is identical to that of fig. 6a, with slight differences in the retention and rescaled consistency indices). The bootstrap support for the *L. diedrus* – *V. discodactylus* clade is 65 %. In the second analysis, all larval characters (characters 8-12) were deleted. Three most parsimonious trees resulted, of length 99 and CI excluding uninformative characters 0.56. The strict consensus tree has a different structure, with the clade *L. diedrus* – *V. discodactylus* forming a basal trichotomy with the outgroup taxon, *Physalaemus pustulosus*, and the rest of *Leptodactylus* (fig. 7a). The results of the bootstrap 50 % majority-rule consensus tree (fig. 7b) are similar to other bootstrap results.

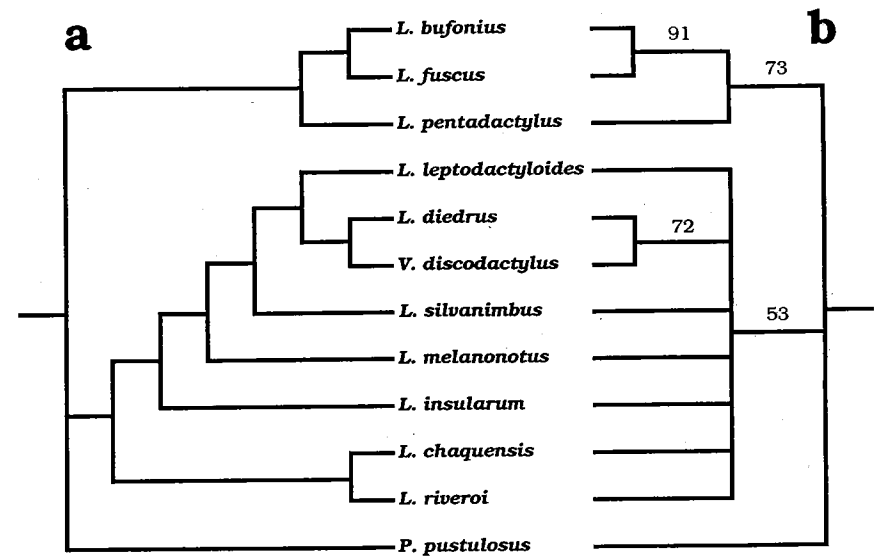


Fig. 6. – (a) Single most parsimonious tree using the partially ordered data set (tab. 1-3), deleting *Adenomera marmorata* and *Lithodytes lineatus*, with *Physalaemus pustulosus* used as outgroup taxon. (b) Bootstrap 50 % majority-rule consensus tree for data set of (a).

Character support for the clade *Leptodactylus diedrus* – *Vanzolinius discodactylus* is moderately strong. For example, in the cladogram of fig. 6a, there are four apomorphic character states that support the clade. Three of these are unique and unreversed states (toe disk, terminal phalanges, advertisement call pulse conditions). The one non-unique state (of egg pigmentation) occurs in a total of three nodes within the tree.

In summary, the results infer a sister-group relationship between *Leptodactylus diedrus* and *Vanzolinius discodactylus*, but the evidence is not 100 % convincing. Of particular concern is the lack of very strong bootstrap support for this relationship.

DISCUSSION

This study raises more questions than it answers:

(1) *Adenomera marmorata* and *Lithodytes lineatus* do not consistently form sister-group relationships to the rest of the *Leptodactylus* species analyzed. Often they form sister-group relationships with certain *Leptodactylus* species, causing paraphyly of *Leptodactylus* as currently understood.

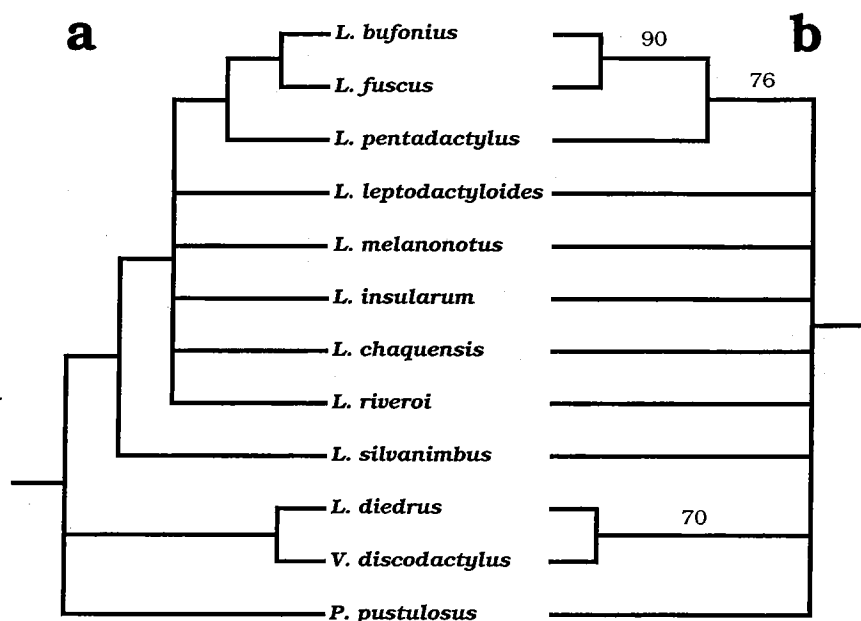


Fig. 7. – (a) Strict consensus tree of three most parsimonious trees using the partially ordered data set (tab. 1-3), deleting characters 8-12 and taxa *Adenomera marmorata* and *Lithodytes lineatus*, with *Physalaemus pustulosus* used as outgroup taxon. (b) Bootstrap 50% majority-rule consensus tree for data set of (a).

(2) The results of the phylogenetic analyses using the data matrix of tab. 1-3 do not consistently provide support for the previously defined species groups. For example, in fig. 6a, *Leptodactylus chaquensis* and *L. insularum*, considered to be members of the same species group, do not cluster together. The results (e.g., fig. 6a) do indicate that both *Leptodactylus riveroi* and *L. silvanimbus* show relationships to the previously defined *L. melanonotus* and *L. ocellatus* species groups (HEYER, 1969, 1994), rather than representing basal speciation events within *Leptodactylus*.

(3) There is some support for a sister-group relationship of *Leptodactylus diedrus* and *Vanzolinius discodactylus*, but not overwhelmingly so. The support is certainly not strong enough to make any taxonomic changes based on that purported relationship at this time. BURTON (1998) found a synapomorphy in the hand musculature of *Hydrolaetare schmidtii* and *Vanzolinius discodactylus*, from which he concluded that *Hydrolaetare* and *Vanzolinius* show a sister-group relationship exclusive of *Leptodactylus* within the family. BURTON (1998) did not examine either *L. diedrus* or any member of the previously defined *L. melanonotus* or *L. ocellatus* species groups in his study, however.

In order to answer the questions this study has raised, more taxa need to be evaluated. There are about a half dozen species of *Adenomera* and one species each of *Hydrolaetare* and *Lithodytes* that should be evaluated to determine whether *Leptodactylus* as currently understood is paraphyletic. There are about 60 species of *Leptodactylus*, many of which have relatively complete data available (advertisement calls and larvae known). A study evaluating relationships within *Leptodactylus* should include many more species than were included in this study (species were specifically chosen for this study that embraced the diversity found in the genus).

From a practical perspective, however, the relationships of species within *Leptodactylus* probably will not be completely resolved using the kinds of characters included in this study. While there may be a few more characters that could be added from morphology, behavior, and karyotypes, those additional potential characters will be relatively few compared to the number of additional *Leptodactylus* that should be added to understand their relationships. Thus, the data matrix would likely have more taxa than characters, which while it potentially could reveal much, would preclude complete resolution of the relationships. Using molecular characters in concert with the kinds of characters analyzed in this paper is an obvious approach to a more robust understanding of the relationships involved.

RESUMEN

Leptodactylus diedrus comparte algunas de las características externas del monotípico género *Vanzolinius*. Treinta y ocho caracteres morfológicos y comportamentales fueron analizados para 14 taxa, incluyendo *Vanzolinius discodactylus*, 10 especies de *Leptodactylus* (abarcando los extremos de variación del género), y tres especies utilizadas como grupo hermano, *Adenomera marmorata*, *Lithodytes lineatus* y *Physalaemus pustulosus*. Se reportan nuevas características osteológicas y miológicas para *L. diedrus* y *L. riveroi*. Además se describe e ilustra el canto nupcial de *L. diedrus*. Los análisis cladísticos de los datos obtenidos apoyan, aunque no fuertemente, una relación de grupos hermanos entre *L. diedrus* y *V. discodactylus*. Se necesitan estudios adicionales para verificar la robustez de esta hipótesis, y para resolver los problemas de parafilia del género *Leptodactylus* y los grupos de especies definidos previamente dentro del género.

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APPENDIX 1

SPECIMENS EXAMINED FOR THIS STUDY SUPPLEMENTING
THOSE EXAMINED PREVIOUSLY
(HEYER, 1974, 1994, 1997; WASSERSUG & HEYER, 1988)

Wet metamorphosed specimens: *Adenomera marmorata*, USNM 209077-209120, Estação Biológica de Boracéia, São Paulo, Brazil; *Leptodactylus bufonius*, USNM 319557-319561, 319598-319606, ca. 60 km NE Joaquín V. González, Salta, Argentina; *Leptodactylus chaquensis*, USNM 341260-341274, Filadelfia, Boquerón, Paraguay, and USNM 341275-341283, Parque Nacional Defensores del Chaco, Alto Paraguay, Paraguay; *Leptodactylus diedrus*, AMNH 115705 (female, muscles examined), Estirón, Río Ampiyacu, Peru, and INPA 3591, 3633, 5016, 5020, 5160, 5215, 5228, Altamira, Rio Jurúa, Amazonas, Brazil; *Leptodactylus fuscus*, USNM 202456-202490, São Carlos, Rondônia, Brazil; *Leptodactylus insularum*, USNM 227621-227624, Rincón de Osa, Puntarenas, Costa Rica; *Leptodactylus leptodactyloides*, USNM 321214 (female, muscles examined), Cocha Cashu Biological Station, Madre de Dios, Peru; *Leptodactylus melanonotus*, USNM 209823-209826, 226409-226410, near Aldama, Tamaulipas, Mexico; *Leptodactylus pentadactylus*, USNM 298079-298080, 347153-347156, Isla Popa, Bocas del Toro, Panama; *Leptodactylus riveroi*, MZUSP 60100, Gaviõesinho, Reserva INPA-WWF, Amazonas, Brazil; *Leptodactylus silvanimbus*, USNM 212048-212050 (paratypes, male 212048 thigh muscles examined), 348631 (female, jaw and throat muscles examined), Belén Gualcho, Ocotepeque, Honduras; *Lithodytes lineatus*, USNM 283881-283915, Sucúa, Morona-Santiago, Ecuador; *Physalaemus pustulosus*, USNM 227670, Rincon de Osa, Puntarenas, Costa Rica (thigh muscles examined).

Wet larval specimens: *Leptodactylus bufonius*, USNM 307186, 54 km NE Joaquín V. González, Salta, Argentina; *Leptodactylus chaquensis*, USNM 302515, Estancia Caiman, Mato Grosso do Sul, Brazil; *Leptodactylus insularum*, USNM 330407, near Cañas, Guanacaste, Costa Rica; *Leptodactylus leptodactyloides*, USNM 313545, Limoncococha, Napo, Ecuador; *Leptodactylus melanonotus*, USNM 330843, near Cañas, Guanacaste, Costa Rica; *Leptodactylus riveroi*, USNM 313505, Reserva Ducke,

Amazonas, Brazil; *Leptodactylus silvanimbus*, USNM 509811, Belén Gualcho, Ocotepeque, Honduras; *Vanzolinius discodactylus*, KU 121361-121362, Mera, Pastaza, Ecuador.

Dry skeletons: *Leptodactylus chaquensis*, USNM 29773 (female), Estancia Caiman, Mato Grosso do Sul, Brazil; *Leptodactylus leptodactyloides*, USNM 227606 (male), Limoncocha, Napo, Ecuador; *Leptodactylus pentadactylus*, USNM 297785 (male), Gamboa, Panamá, Panama; *Leptodactylus silvanimbus*, USNM 226386 (male), Belén Gualcho, Ocotepeque, Honduras.

Cleared and stained skeletons (also examined for jaw, throat, and thigh muscles prior to skeletal preparation): *Leptodactylus diedrus*, AMNH 115721 (male), Estirón, Rio Ampiyacu, Loreto, Peru; *Leptodactylus riveroi*, MZUSP 60101 (male), Gaviõesinho, Reserva INPA-WWF, Amazonas, Brazil; *Physalaemus pustulosus*, USNM 509952, near Lambeau Crown Trace, St. Paul, Tobago (examined for jaw and throat muscles only).

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