

Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species

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Abstract. Whereas morphological analysis of populations recognized as *Leptodactylus mystaceus* indicates there is one species with modest geographic variation, analysis of advertisement calls indicates there are at least two or perhaps three species involved. The differences found in advertisement calls are sufficient to act as species isolating barriers to recognize at least two species, which action is taken. A consequent result is the description of a new sibling species. The significance of sibling species in the genus *Leptodactylus* is discussed briefly.

Introduction

Heyer (1978: 41) stated that the distribution of *Leptodactylus mystaceus* (as *L. amazonicus*) was characterized as occurring "Throughout the greater Amazon Basin, Guianas, northern Atlantic Forest, and cerrados bordering the Amazon Basin". At that time, recordings of advertisement calls were available only from a single site. Since then, recordings of *L. mystaceus* have been made at several other localities. Analysis of these recordings, together with those of two other members of the *L. mystaceus* complex, form the basis of the characterization of the acoustic variation within the group.

Materials and methods

Museum abbreviations follow Leviton et al. (1985) with the exception that MHNSM is the Museo de Historia Natural, Universidad San Marcos, Peru.

The recordings analyzed and associated data of morphologically identified *L. mystaceus* are (arranged roughly from East to West and North to South):

Brazil: Pará; near Altamira. ASN-AJC (Archivo Sonoro Neotropical, Adão J. Cardoso recording) Tape 64 (cut 1, 3), December 1986, 21:35 h, 25°C air temperature, voucher specimen ZUEC 7196, recorded by Adão J. Cardoso. ASN-AJC Tape 67 (cut 7), 7 December 1986, 25.5°C air temperature, voucher specimen ZUEC 7249, recorded by Adão J. Cardoso.

Brazil: Pará; Parque Nacional da Amazônia (Rio Tapajós). USNM (tape archive at National Museum of Natural History, Smithsonian Institution) Tape 53 (cut 6), 21 January 1981, 18:00-23:00 h, 24.6°C air temperature, voucher specimen USNM 288748, recorded by Ronald I. Crombie.

Brazil: Amazonas; Biological Dynamics of Forest Fragments sites, N of Manaus. USNM Tape 149 (cuts 1 and 2), 12 January 1984, 20:23 h, unvouchered, recorded by Barbara L. Zimmerman. USNM Tape 161 (cut 1), 9 April 1987, 19:30 h, unvouchered, recorded by Barbara L. Zimmerman.

Venezuela: Amazonas; Neblina base camp. USNM Tape 70 (cut 2), 24 February 1985, 19:00 h, 25.2°C air temperature, voucher specimen USNM 332867, recorded by Reginald B. Cocroft. USNM Tape 70 (cut 3), 24 February 1985, 20:15 h, 24.4°C air temperature, voucher specimen USNM 332868, recorded by Reginald B. Cocroft.

Ecuador: Napo; Limoncocha. USNM Tape 16 (cut 3), 20 June 1971, 22.9°C air temperature, unvouchered, recorded by W. Ronald Heyer. USNM Tape 16 (cut 4), 20 June 1971, 23.2°C air temperature, unvouchered, recorded by W. Ronald Heyer. USNM Tape 18 (cut 2), 15 July 1971, about 20:00 h, 22.9°C air temperature, unvouchered, recorded by W. Ronald Heyer. USNM Tape 18 (cut 3), 15 July 1971, 20:30-21:00 h, 22.9°C air temperature, voucher specimen LACM 92111, recorded by W. Ronald Heyer.

Brazil: Mato Grosso; Chapada dos Guimarães. ASN-AJC Tape 32 (cut 1), 12 November 1982, 23:00 h, 22°C air temperature, voucher specimen ZUEC 5093, recorded by Adão J. Cardoso.

Brazil: Amazonas; Rio Juruá, Barro Vermelho. USNM Tape 254 (cut 1), 23 October 1991, 18:30 h, 27°C, voucher specimen INPA 3272; recorded by Claude Gascon.

Brazil: Acre; Rio Juruá, Porongaba. USNM Tape 256 (cut 5), 19 February 1993, 18:30 h, 26°C, unvouchered, recorded by Claude Gascon.

Brazil: Acre; Rio Acre, Xapuri. ASN-AJC Tape 54 (cut 4), 24 December 1983, 20:10 h, voucher specimen ZUEC 5745, recorded by Adão J. Cardoso. ASN-AJC Tape 113 (cut 8), 16 November 1991, 17:00 h, 24°C air temperature, unvouchered, recorded by Adão J. Cardoso.

Peru: Madre de Dios; Tambopata. USNM Tape 204 (cut 3), 1 January 1989, 17:45 h, unvouchered, recorded by Reginald B. Cocroft. USNM tape 204 (cut 4), 1 January 1989, 18:10 h, 25.5°C air temperature, voucher specimen USNM 332861, recorded by Reginald B. Cocroft. USNM Tape 204 (cut 5), 1 January 1989, 18:30 h, 25.4°C air temperature, voucher specimen USNM 332862, recorded by Reginald B. Cocroft. USNM Tape 205

(cut 10), 4 January 1989, 19:10 h, 24.2°C air temperature, 24.8°C substrate, voucher specimen USNM 332863, recorded by Reginald B. Cocroft. USNM Tape 205 (cuts 11 and 12), 4 January 1989, 19:35 h, 23.8°C air temperature, 25.0°C substrate, voucher specimen USNM 332864, recorded by Reginald B. Cocroft.

For convenience, the above recordings are referred to by abbreviated specific localities throughout the rest of the text.

Leptodactylus elenae Paraguay: Itapua; El Tirol. USNM Tape 180 (cut 7), 16 November 1976, voucher specimen USNM 253384, recorded by Mercedes S. Foster.

Leptodactylus notoaktites Brazil: Paraná; 12 km W of São João da Graciosa on PR 410 to Curitiba. USNM Tape 10 (cut 11), 26 December 1978, 20:45 h, 16.9°C, voucher specimen USNM 217791, recorded by W. Ronald Heyer. Brazil: Paraná; Morretes. ASN-AJC Tape 23 (cut 3), 3 February 1982, 18:00 h, 24°C air temperature, voucher specimen ZUEC 4717, recorded by Adão J. Cardoso.

Advertisement calls were analyzed using a Kay Digital Sona-Graph model 7800, Uniscan II equipment, or "Canary" software (Charif et al., 1993) on a Macintosh IIfx computer.

Measurements were analyzed using SAS software (1988) for an IBM personal computer.

Advertisement calls

Altamira ($n = 20$ calls analyzed). Calls of single notes; call rate 1.6-2.2 per s; call duration 0.14-0.20 s; dominant frequency modulated from 720 to 2120 Hz, with or without a brief terminal drop in frequency; call intensity low initially, rising to a peak about 2/3 duration of call; call distinctly pulsed, of about 9-12 pulses, pulses very distinct at beginning of call, call usually pulsatile terminally; harmonic structure present (fig. 1).

Tapajós ($n = 5$, entire recording has a microhylid frog calling simultaneously with the *Leptodactylus*, precluding some analyses). Calls of single notes; call rate 1.4 per s; call duration 0.14-0.16 s; dominant frequency modulated from 680 to 1320 Hz, no drop in terminal frequency; call of lower intensity initially and terminally; call very distinctly pulsed throughout, of 9 pulses; harmonic structure present (fig. 2).

Manaus ($n = 30$). Calls of single notes, call rate 1.8-2.2 per s; call duration 0.16-0.23 s; dominant frequency modulated from 600 to 1440 Hz, with or without a brief terminal drop in frequency; call intensity low initially, rising to maximum intensity from 1/2 to 2/3 duration of call; call distinctly pulsed, of about 10-13 pulses, pulses either distinct throughout call or distinct through most of call and pulsatile at end of call; harmonic structure present (fig. 3).

Neblina ($n = 20$). Calls of single notes; call rate 1.6 per s; call duration 0.16-0.23 s; dominant frequency modulated from 600 to 1440 Hz, with or without a brief terminal drop in frequency; call intensity low initially, rising to maximum intensity from 1/2 to 2/3 duration of call; call distinctly pulsed, of about 12-16 pulses, pulses

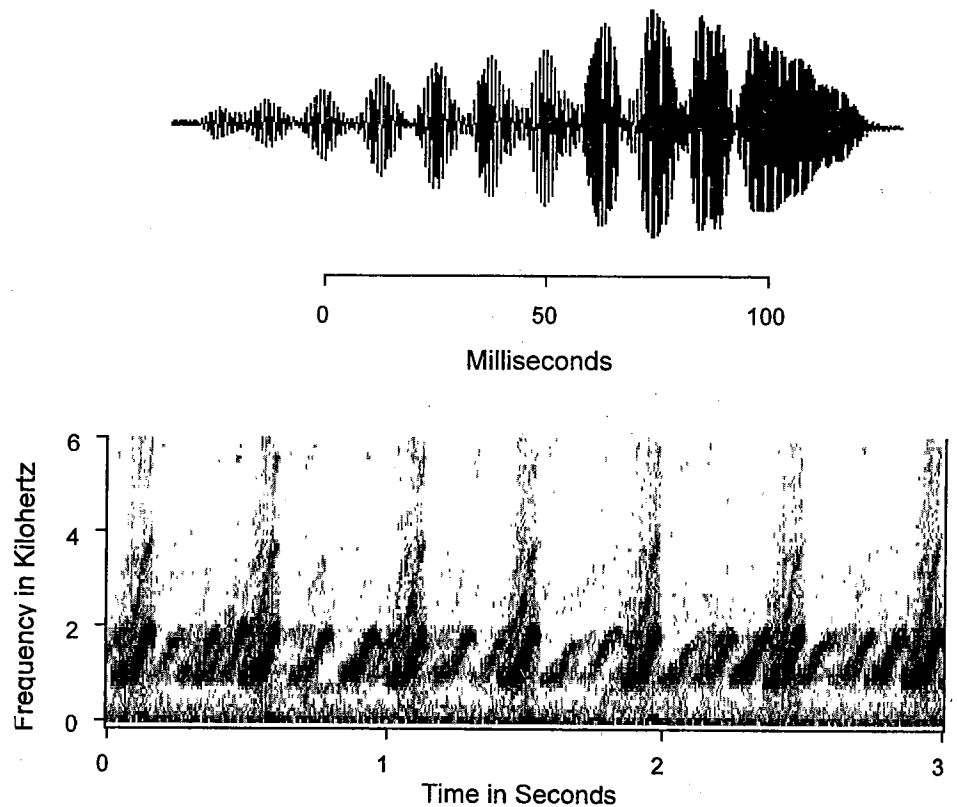


Figure 1. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Altamira, Pará, Brazil, ASN-AJC Tape 67, cut 7.

very distinct at beginning of call, terminal 1/4-3/5 of call pulsatile; harmonic structure present (fig. 4).

Limoncocha ($n = 40$). Calls of single notes; call rate 1.0-2.0 per s; call duration 0.15-0.25 s; dominant frequency modulated from 560 to 1760 Hz, with or without a brief terminal drop in frequency; call intensity low initially, reaching maximum about 1/2 to 2/3 duration of call; call distinctly pulsed, of about 11-17 pulses, either distinctly pulsed, throughout or (usually) terminal 1/5 to 1/7 of call pulsatile; harmonic structure present (fig. 5).

Chapada dos Guimarães ($n = 29$). Calls of single notes; call rate 1.0-1.6 per s; call duration 0.18-0.27 s; dominant frequency modulated from 620 to 1480 Hz, with or without a brief terminal drop in frequency; call intensity low initially, rising to maximum about 1/2 to 2/3 call duration; call distinctly pulsed, of 10-13 pulses, pulses either more or less distinct throughout call or terminal 1/3 to 1/4 of call pulsatile; harmonic structure present (fig. 6).

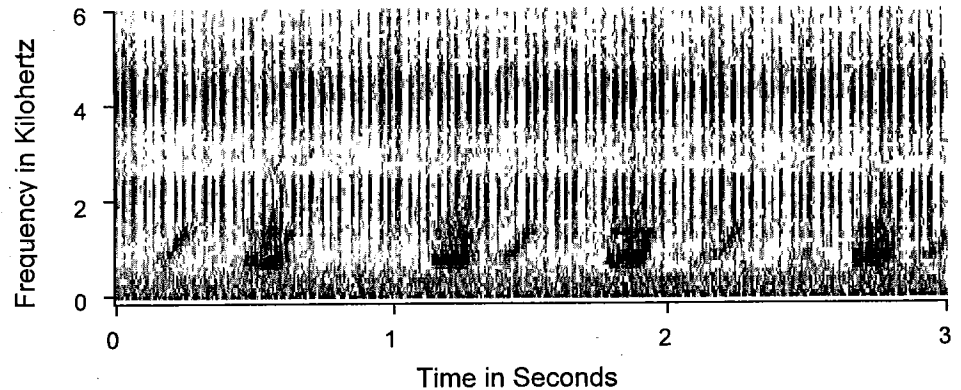


Figure 2. Audiospectrogram of advertisement call of *Leptodactylus mystaceus* cluster member from Parque Nacional da Amazônia (Rio Tapajós), Pará, Brazil, USNM Tape 53, cut 6.

Barro Vermelho ($n = 10$). Calls of single notes; call rate 1.4 per s; call duration 0.26-0.32 s; dominant frequency modulated from 520 to 1470 Hz, call without terminal drop in frequency; call intensity low initially, rising to maximum from 3/4 to just before end of call; call not distinctly pulsed, of either single pulse or two partial pulses; harmonic structure present (fig. 7).

Porongaba ($n = 9$). Calls of single notes; call rate 1.6 per s; call duration 0.19-0.22 s; dominant frequency modulated from 550 to 1470 Hz, call without terminal drop in frequency; call intensity low initially, rising to maximum about mid-call; call not distinctly pulsed, of either single pulse or two partial pulses; harmonic structure present (fig. 8).

Xapuri ($n = 126$). Calls of single notes; call rate 1.7-3.1 per s; call duration 0.09-0.13 s; dominant frequency modulated from 510 to 1510 Hz, calls with brief terminal drop in frequency; call intensity low initially, rising to maximum from about mid-call to 3/4 call duration; call not distinctly pulsed, of either single pulse or two partial pulses; harmonic structure present (fig. 9).

Tambopata ($n = 60$). Calls of single notes; call rate 1.4-2.3 per s; call duration 0.09-0.13 s; dominant frequency modulated from 560 to 1480 Hz, with or without a brief terminal drop in frequency; call intensity low initially, rising to maximum about 2/3 call duration; call not distinctly pulsed, of either single pulse or two partial pulses; harmonic structure present (fig. 10).

Leptodactylus elenae ($n = 10$). Calls of single notes; call rate 1.2 per s; call duration 0.21-0.24 s; dominant frequency modulated from 800 to 1520 Hz, call without terminal drop in frequency; call intensity low initially, rising to maximum about 3/4 to 4/5 duration of call; call of single pulse; harmonic structure present (fig. 11).

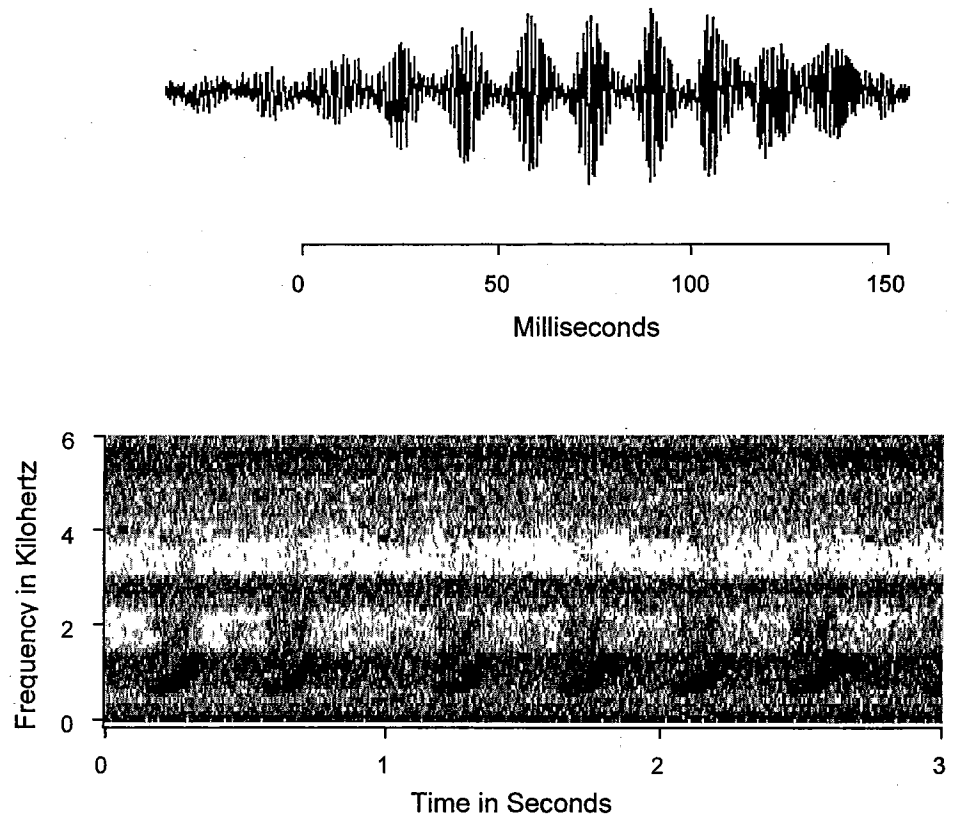


Figure 3. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Biological Dynamics of Forest Fragments sites, N of Manaus, Amazonas, Brazil, USNM Tape 149, cut 1.

Leptodactylus notoaktites ($n = 15$). Calls of single notes; call rate 1.5-2.0 per s; call duration 0.06-0.09 s; dominant frequency modulated from 470 to 1990 Hz, call without terminal drop in frequency; call intensity low initially, rising to maximum intensity about 2/5 call duration; call of single pulse; harmonic structure present (fig. 12).

Advertisement call comparisons

All calls have (1) the same general range of call rates, (2) harmonic structure, (3) the same kind of frequency and intensity modulation, and (4) the same broadcast frequency range with some differences among ranges. There are consistent differences in pulsation.

Among the pulsed calls, intrapopulation variation exists in whether there is a brief terminal drop in frequency. The exception, the calls from Tapajós which all lack a terminal

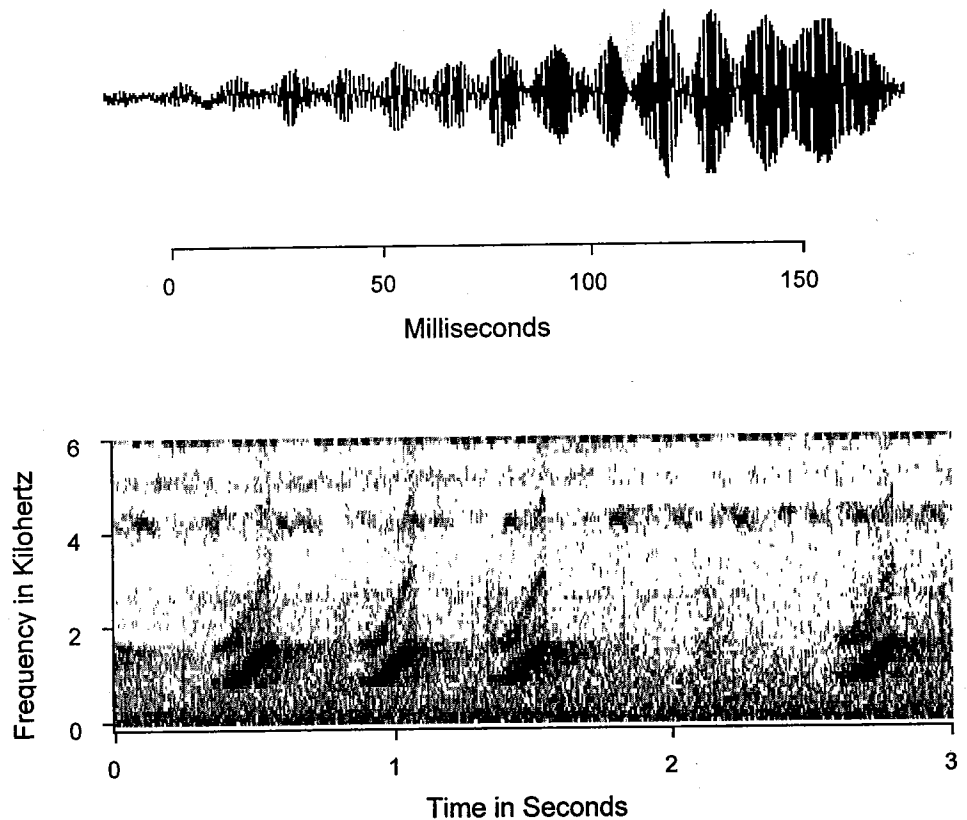


Figure 4. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Neblina base camp, Amazonas, Venezuela, USNM Tape 70, cut 3.

drop in frequency, may be related to the small number of calls available for analysis from the Tapajós locality ($n = 5$). The most distinctive frequency range exhibited by pulsed calls is that from Altamira, in which the broadcast frequency range is 720-2120 Hz, whereas the combined frequency range of all other pulsed calls is 560-1760 Hz. The number of pulses per call is similar among all pulsed calls.

In contrast, there is more variation among non-pulsed calls. The calls of *L. notoaktites* are the shortest and overlap the duration minimally with those from Xapuri and Tambopata, which also are relatively short. None of these calls overlap in duration with the longer calls from Barro Vermelho, Porongaba, or the call duration of *L. elenae*, the latter three of which are quite similar. Only at Tambopata was evidence found of intrapopulation variation in the presence of a brief terminal drop. All Xapuri calls have a brief terminal frequency drop; all Barro Vermelho, Porongaba, *L. elenae*, and *L. notoaktites* calls lack this feature.

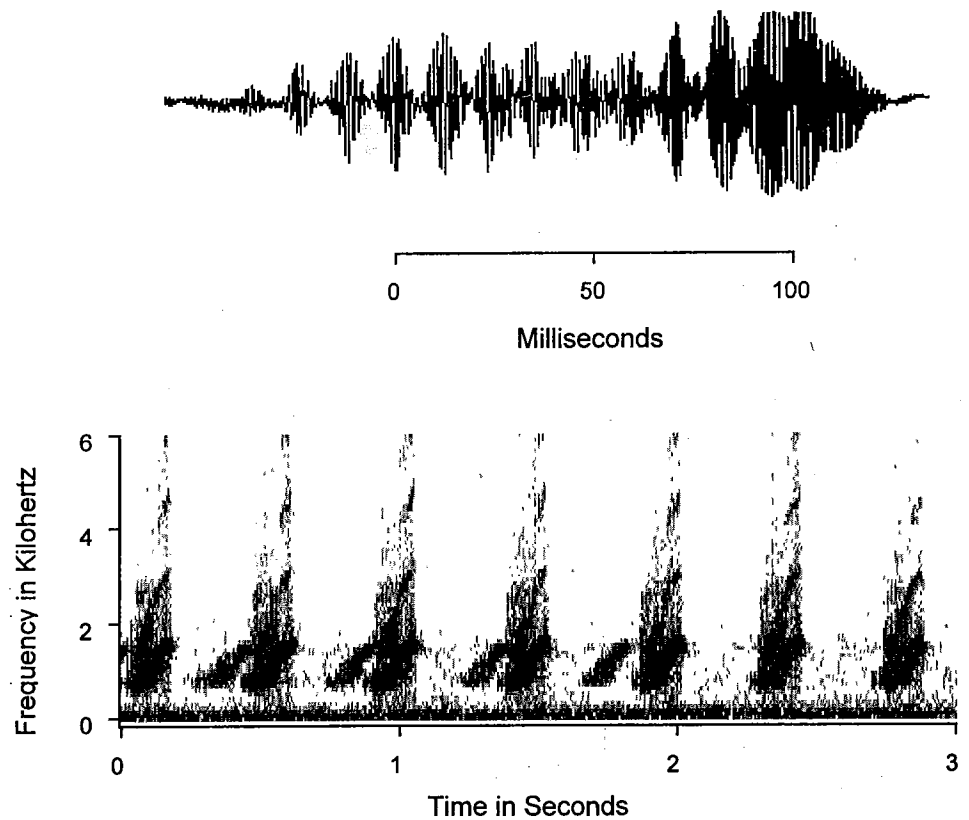


Figure 5. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Limoncocha, Napo, Ecuador, USNM Tape 16, cut 4.

The advertisement calls can be placed into the following groups: (1) Pulsed calls (Altamira, Tapajós, Manaus, Neblina, Limoncocha, Chapada dos Guimarães); (2) Very short, non-pulsed calls, lacking terminal frequency drop (*L. notoaktites*); (3) Short, non-pulsed calls, at least some calls with terminal frequency drop (Xapuri, Tambopata); (4) Relatively long, non-pulsed calls, lacking terminal frequency drop (Barro Vermelho, Porongaba, *L. elenae*).

Morphological comparisons and species delimitations

Leptodactylus elenae, *mystaceus* (as *amazonicus*, see Heyer, 1983, for nomenclatural change justification), and *notoaktites* differ morphologically (Heyer, 1978). All specimens reported herein, except for *L. elenae* and *notoaktites*, are morphologically like *mystaceus*.

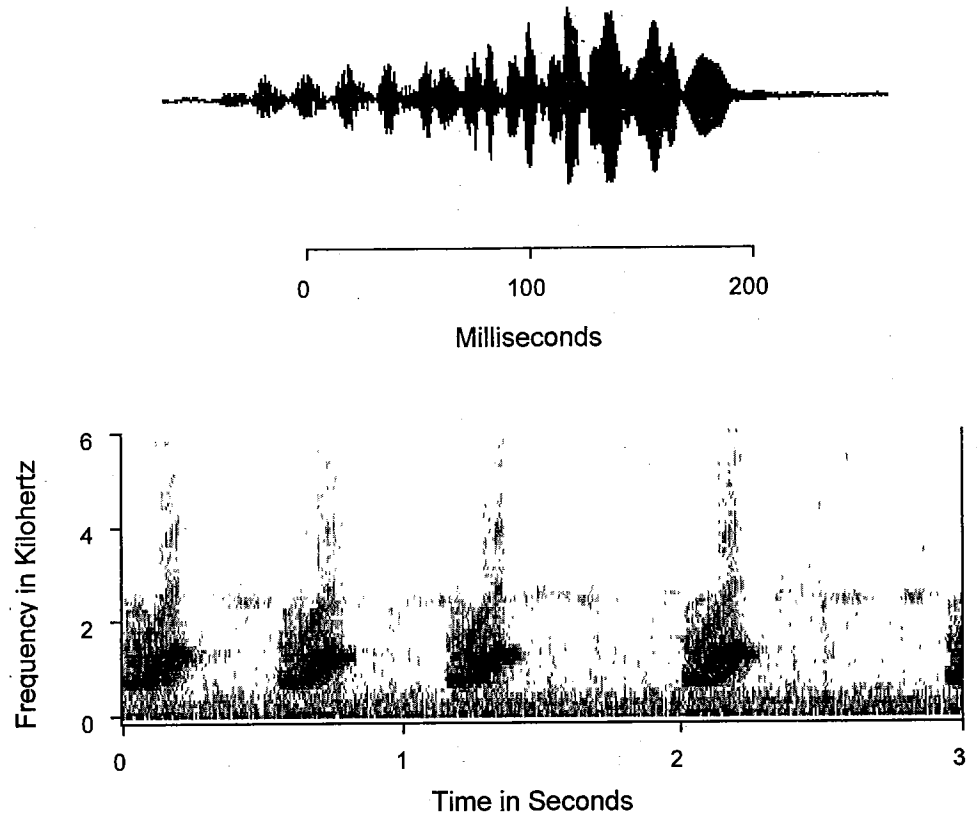


Figure 6. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Chapada dos Guimarães, Mato Grosso, Brazil, ASN-AJC Tape 32, cut 1.

Leptodactylus notoaktites, from the Atlantic Forest Morphoclimatic Domain of Brazil, with a distinct call and distinct morphology, poses no problem in terms of species limits within the *L. mystaceus* complex: it is a distinct species.

Leptodactylus elenae, from the Gran Chaco of Argentina and adjacent open formation domains in Bolivia, Brazil, and Paraguay (the Tocache Nuevo, San Martín, Peru locality record (Heyer, 1978: 46) most probably represents another species in the *L. fuscus* group), is morphologically distinct from all other populations discussed in this paper, but its advertisement call shares many features with the calls from the populations from Barro Vermelho and Porongaba. In comparing call details, it is clear that the Barro Vermelho and Porongaba calls are more similar to each other than either is to the call of *L. elenae* (compare figs 7, 8 and 11). Geographically, the range of *L. elenae* most closely approaches either the pulsed call type (such as at Chapada dos Guimarães) or the short, non-pulsed calls, usually with a terminal frequency drop (Tambopata and Xapuri), from which the calls of *L. elenae* are distinct. The combination of morphological distinct-

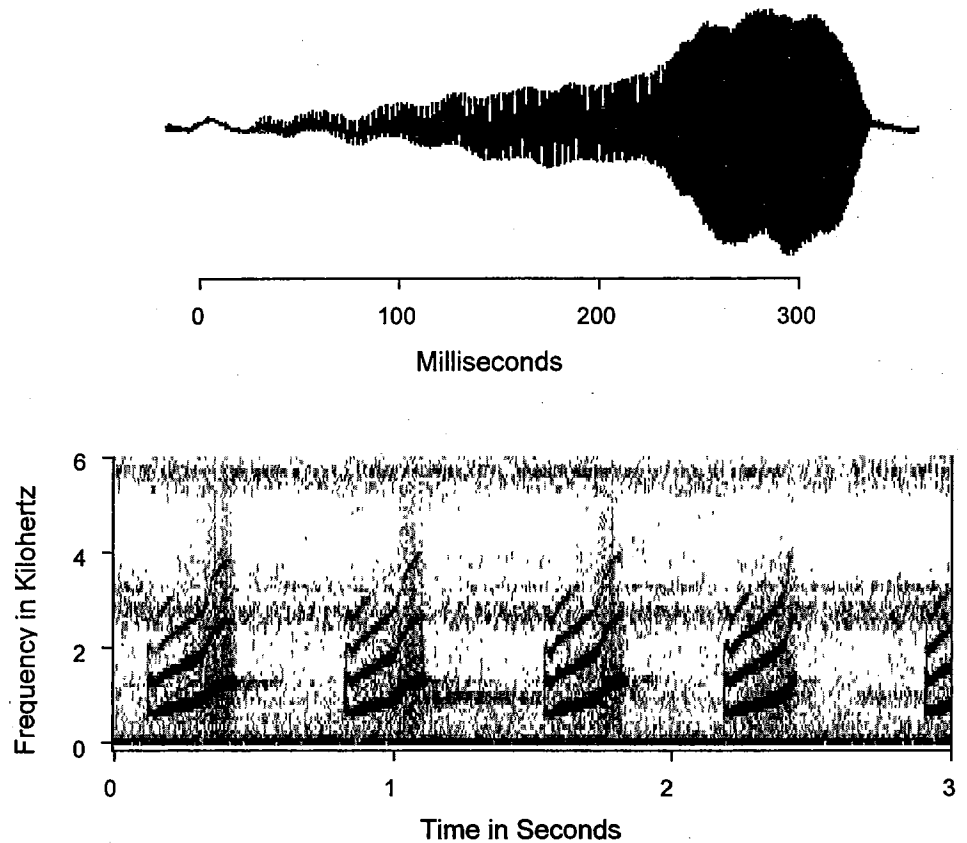


Figure 7. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Barro Vermelho, Amazonas, Brazil, USNM Tape 254, cut 1.

ness, geographic distribution, and advertisement call characteristics support recognition of *L. elenae* as a distinct species within the *L. mystaceus* complex.

Morphological variation within the Leptodactylus mystaceus cluster

The remaining questions relate to whether morphological differentiation exists among frogs with the (A) pulsed call types (from Altamira, Tapajós, Manaus, Neblina, Limoncocha, Chapada dos Guimarães), (B) short, non-pulsed calls, usually with terminal frequency drop (Xapuri, Tambopata), (C) relatively long, non-pulsed calls, lacking terminal frequency drop (Barro Vermelho, Porongaba), and whether (A), (B), (C) represent one, two or three distinct species.

The specimens associated with (A), (B), and (C) above are similar morphologically and one of us (WRH) would, without hesitation, place them into a single species, ascribing the differences to intraspecific variation. In trying to find differences among frogs associated

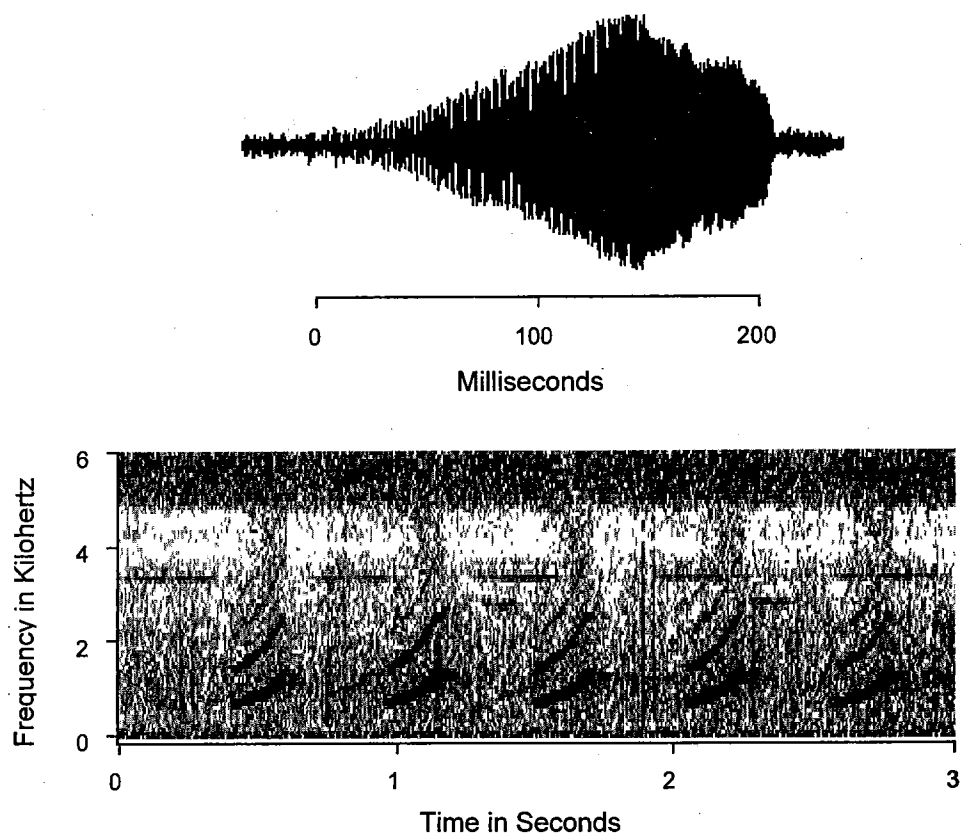


Figure 8. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Porongaba, Acre, Brazil, USNM Tape 256, cut 5.

with (A), (B), and (C), the frogs associated with (C) are the most distinctive. Specimens from the Rio Juruá, including those from Barro Vermelho and Porongaba have, as a group, the most boldly marked patterns, in which the dorsolateral folds are usually boldly outlined in dark, contrasting with the surrounding dorsal patterns; there are usually many, distinct, dark (almost black) spots on the lower flanks; and the general contrast in dark and light patterns, such as on the posterior thigh, is great. However, not all of the individuals from the Rio Juruá exhibit these features, and some individuals (especially juveniles) from locations associated with groups (A) and (B) are as boldly patterned as the most boldly patterned specimens from the Rio Juruá. It should also be noted that the sample size for Rio Juruá specimens is small ($n = 11$ adults and juveniles). All individuals examined (A), (B), (C), lack light tubercles on the dorsal shank and have either many or several prominent white tubercles on the sole of the foot. The Juruá specimens (C) and

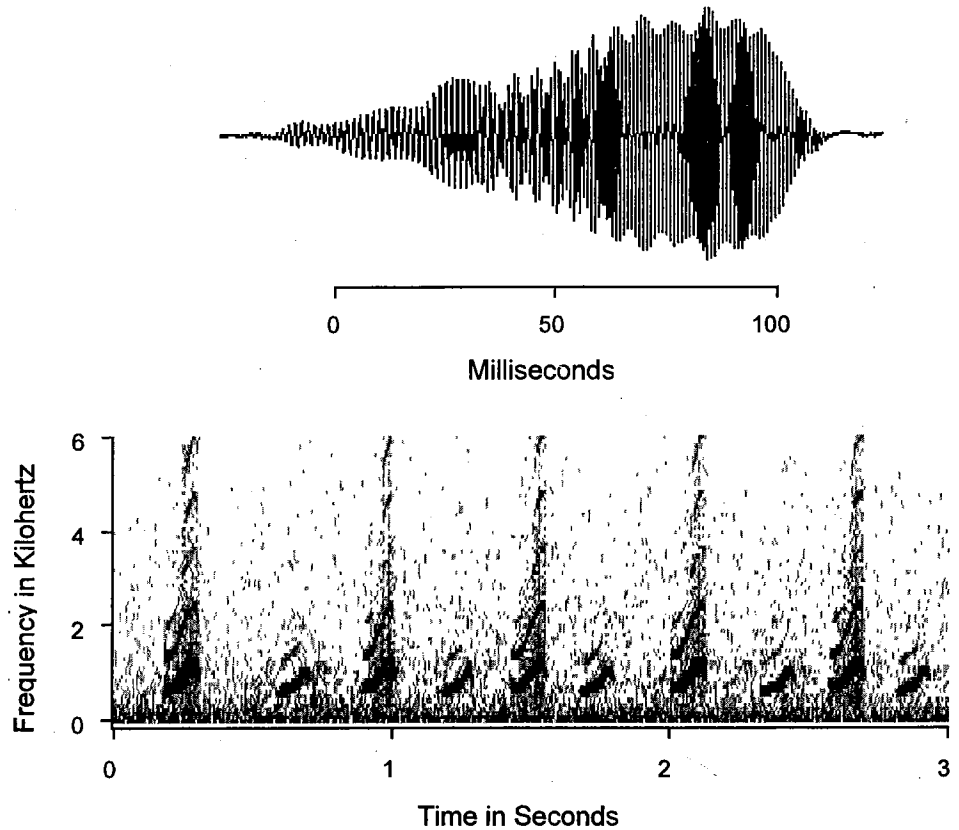


Figure 9. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Xapuri, Acre, Brazil, ASN-AJC Tape 113, cut 8.

those associated with (A) are alike in that most individuals lack tubercles on the posterior surface of the tarsus and a few individuals have one to a few weak light tubercles on the posterior surface of the tarsus, where they are concentrated distally. Individuals from Xapuri and Tambopata (B) share the same tarsal characteristics as noted for (A) and (C), but, in addition, some individuals have more weakly differentiated, light tubercles on the outer tarsus, so that tubercles are scattered over the entire posterior surface of the tarsus. These are the only differences encountered in examination of the specimens involved.

Discriminant function analysis of morphometric data has proven useful in distinguishing among closely related species of *Leptodactylus* (Heyer, 1978, 1994). Morphometric data from adult males and females were assembled to use in discriminant function analyses in an attempt to ascertain differences among populations. Data from the Guianas, Colombia, and Ecuador (Heyer, 1978), certain to be associated with the pulsed call (A)

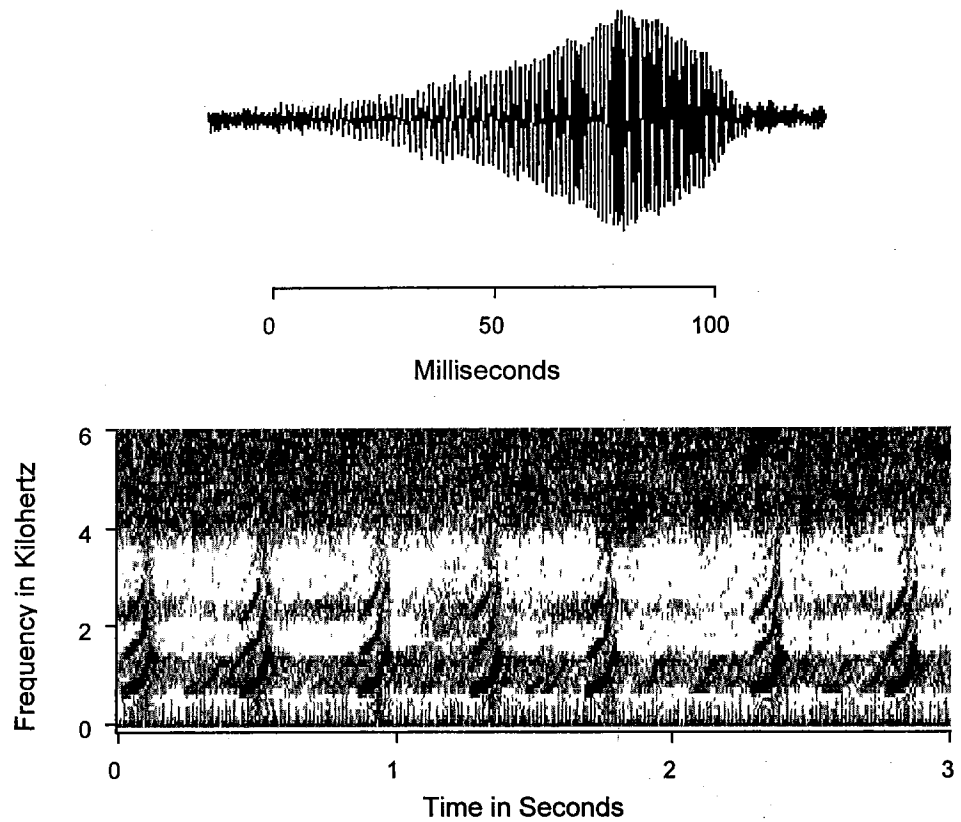


Figure 10. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus mystaceus* cluster member from Tambopata Reserved Zone, Madre de Dios, Peru, USNM Tape 204, cut 4.

were combined with data from specimens from Pará, Brazil, known to have pulsed calls. The same measurements (following methodology in Heyer, 1978) were taken from specimens from the Rio Juruá, including Barro Vermelho and Porongaba (C) and Xapuri and Tambopata (B). The variables analyzed are SVL (snout-vent-length), head length, head width, thigh length, shank length, foot length. Because of size differences between males and females, the discriminant function analyses were run separately by sex.

The three species hypothesis would be supported by discriminant function analysis if the three groups (A, B, C) were robustly differentiated. If not, then the analysis of the measurement data would either support the one or two species hypothesis. Similarly, the two species hypothesis would be supported if the two groups (A, B + C) were robustly differentiated. If not, then by default, the single species hypothesis would be supported by the discriminant function analyses.

The sample sizes of the groups and an example of the kind of variation observed in the variables are shown in table 1.

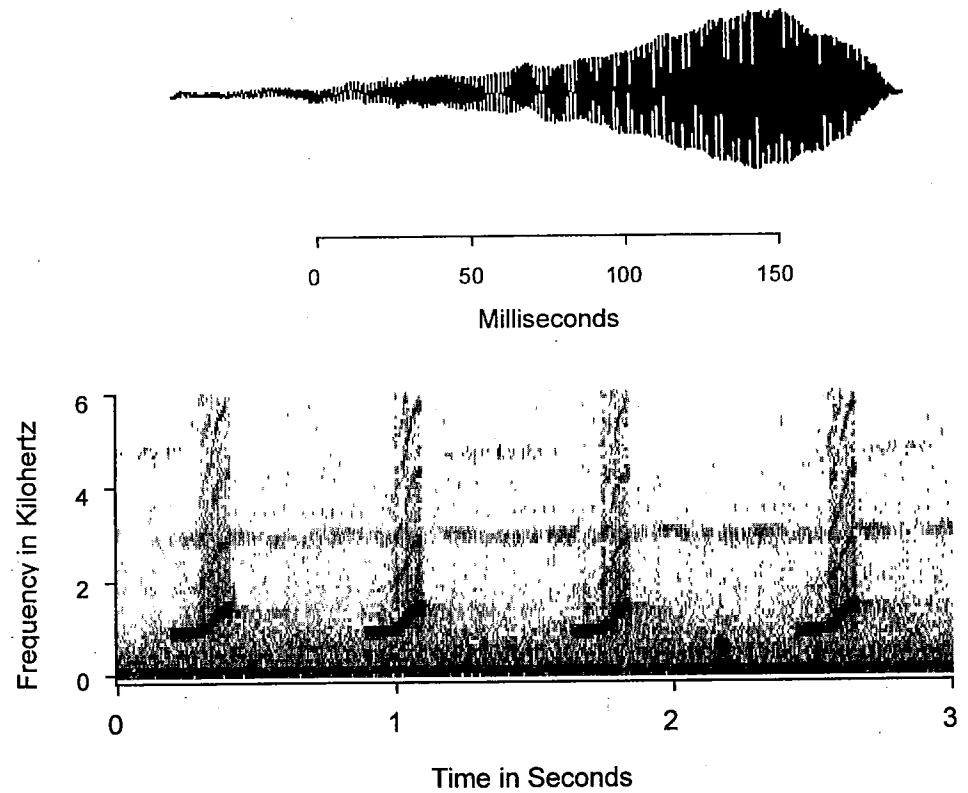


Figure 11. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus etenae*, USNM Tape 180, cut 7.

Table 1. Sample sizes and SVL (Snout-vent length) parameters for males and females of three groups of the *Leptodactylus mystaceus* cluster. A = specimens from the Guianas, Pará-Brazil, Venezuela, Colombia, and Ecuador; B = specimens from Xapuri, Acre, Brazil and Tambopata, Madre de Dios, Peru; C = specimens from the Rio Juruá, Brazil.

	<i>n</i>	SVL (mm)		
		Minimum	Mean	Maximum
A - males	69	42.4	47.4	52.2
females	60	44.8	50.0	56.1
B - males	21	45.9	49.3	52.2
females	4	45.8	49.8	53.5
C - males	3	46.1	47.1	48.6
females	3	43.7	47.0	49.1

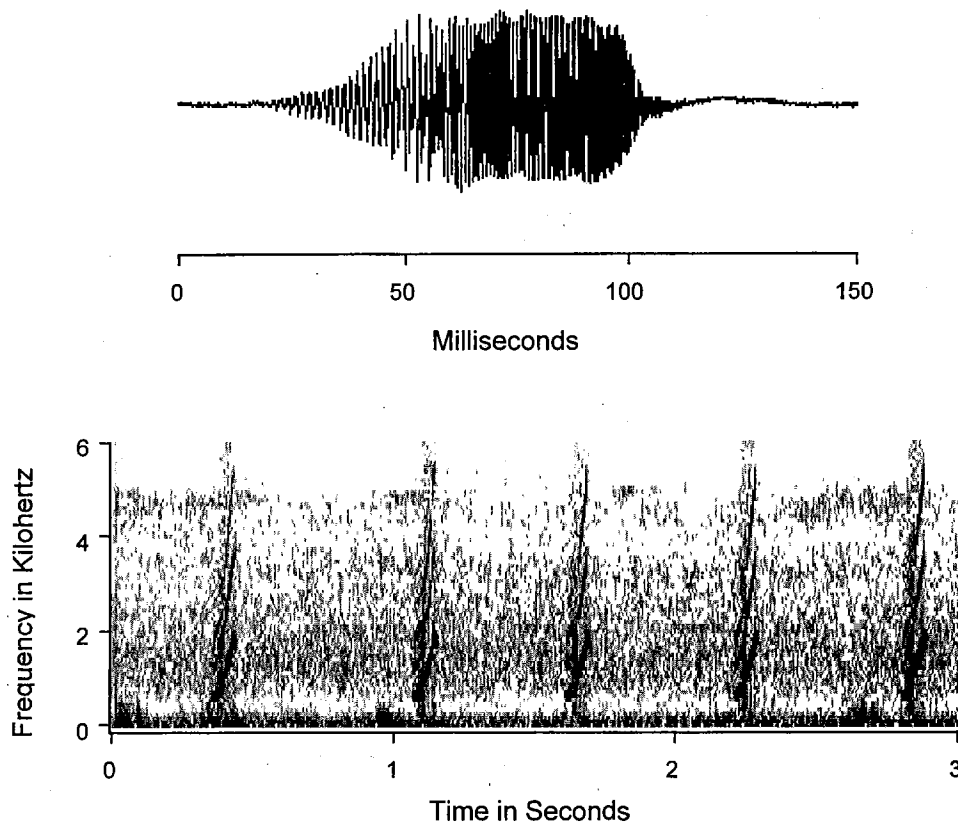


Figure 12. Audiospectrogram (below) and wave form (above) of advertisement call of *Leptodactylus notoakites*, USNM Tape 10, cut 11.

For the three species hypothesis, none of the variables for the female data are significant as univariate variables, indicating that the data set is too weak to use the results. It is not known whether this weakness is due to small sample sizes (for B, C) or whether the measurement data of the three groups involved really are very similar. The results of the male data set do not provide strong support, or perhaps even weak support, for the three species hypothesis (table 2). When the discriminant function means for the entire data set (including the specimen being posteriorly classified) are used, the posterior classification (resubstitution summary) results in a total error count estimate of 9% (table 2, upper results). However, when the data for the individual being posteriorly classified are removed and the discriminant function means calculated without those data and then the individual is posteriorly classified based on those means, the posterior classification (cross-validation summary) results in a total error count estimate of 52% (table 2, lower results). If the posterior classification results were robust, the

Table 2. Discriminant function analysis results for male data for the three species hypothesis. A = specimens from Guianas, Pará-Brazil, Venezuela, Colombia, and Ecuador; B = specimens from Xapuri, Acre, Brazil and Tambopata, Madre de Dios, Peru; C = specimens from the Rio Juruá, Brazil.

Resubstitution summary using quadratic discriminant function, number of observations classified into groups			
	A	B	C
A	56	13	0
B	2	19	0
C	0	0	3
Cross-validation summary using quadratic discriminant function, number of observations classified into groups			
	A	B	C
A	54	15	0
B	7	14	0
C	0	3	0

Table 3. Discriminant function analysis results for male data for the two species hypothesis. A = specimens from Gustetnas, Pará-Brazil, Venezuela, Colombia, and Ecuador; B + C = specimens from Xapuri, Acre, Brazil, Tambopata, Madre de Dios, Peru, and Rio Juruá, Brazil.

Resubstitution summary using quadratic discriminant function, number of observations classified into groups		
	A	B + C
A	54	15
B + C	2	22
Cross-validation summary using quadratic discriminant function, number of observations classified into groups		
	A	B + C
A	52	17
B + C	5	19

two methods of posterior classification would yield identical, or nearly so, results of almost all individuals being correctly classified to group of origin. The magnitude of the differences involved indicate that the posterior classification results are not robust and that there is no demonstrable support for the three species hypothesis based on the discriminant function analysis.

The female data for the two species hypothesis also are not significant as univariate variables. The male data results yield somewhat better posterior classification results than for the three species hypothesis (table 3). The classification based on group discriminant function means for all specimens results in a total error count estimate of 15% (table 3, upper results). The classification based on group discriminant function means calculated for all specimens but the individual being posteriorly classified results in a total error count estimate of 23% (table 3, lower results). The results of these methods are closer than for the three group analysis, but there are a rather unacceptable number of individuals incorrectly classified for the results to be interpreted as strongly supporting the two species hypothesis.

The available measurements data, analyzed by discriminant function analyses support the one or two species hypotheses better than the three species hypothesis. No further interpretation is warranted from the results.

The morphological data, including pattern, tubercles, and measurement data, do not demonstrate any strong support for either the two or three species hypotheses, but are best interpreted as demonstrating some geographic variation within a single species.

Character conflict and species limits

The advertisement call and morphological data are in conflict in terms of delimiting species boundaries for the members of the *L. mystaceus* cluster from the greater Amazon Basin. The morphological data are most consistent with recognition of a single species, which demonstrates some (modest) intraspecific variation. The call data are unequivocal in supporting at least two species, and perhaps three.

Such conflict is unusual in the genus *Leptodactylus*. Usually, both morphological and call data delimit species (e.g. Heyer, 1978, 1979). The single previously documented example of similar character conflict is for the species pair *L. geminus* - *gracilis*, in which advertisement calls differ, but the morphologies do not (Barrio, 1973). The important point to keep in mind in determining how many species are involved for the problem at hand is not whether we, as scientists, can recognize differences in morphologies of preserved specimens, but what the reality in nature is. There has been sufficient work done with the anuran communication system to know the kinds of differences among calls that frogs can consistently distinguish and respond accordingly to. The advertisement call broadcasts mating readiness on the part of the male for such frogs as members of the genus *Leptodactylus*. Female frogs are drawn to and select mates among calling males, so female response to advertisement calls is of utmost importance. Females that have been tested behaviorally select among very narrow tolerances in parameters of calling frequency (in Hz) and temporal packaging of the call (for an introduction to this topic, see Fritzsche et al., 1988).

The differences between the pulsed and non-pulsed calls analyzed herein are of the kind of difference that female frogs can unequivocally select between and therefore act as an isolating factor at the species level (see discussion in Gerhardt, 1988:

464-469). Whereas frog advertisement calls are typically quite stereotyped, there are a few instances reported of intraspecific advertisement call variation approaching the magnitude of differences between the pulsed and non-pulsed calls reported herein. For example, Littlejohn (1965: 240) reported call variation in *Litoria verreauxi* in which the same individual gave distinctly pulsed calls and barely partially pulsed calls that were almost unpulsed. However, for female *Litoria verreauxi*, the critical choice to make is between calling male *L. verreauxi* and *Litoria ewingi* where they occur in sympatry. In areas of sympatry, the pulse rate of *L. ewingi* is much slower for the same length signal than for *L. verreauxi* (Littlejohn, 1965). The pulses of *L. verreauxi* in areas of sympatry are tightly packed. The variant almost unpulsed call of *L. verreauxi* is the end of the continuum of pulse packing and female *L. verreauxi* should not distinguish among calls of the tightly packed versus almost non-existent pulses.

Members of the *Leptodactylus melanonotus* species group are notorious for uttering a wide variety of calls, at least some of which are advertisement calls (e.g. *L. petersii*, Heyer, 1994, figs 16-19). In contrast, members of the *Leptodactylus fuscus* species group (which contains the *Mystaceus* complex) have much more stereotyped advertisement calls. Specially for the *Leptodactylus mystaceus* complex, we have only heard males give a single kind of advertisement call at any of the sites we have heard them calling. For example, on the night of 19 June 1971, WRH sat in a pasture at Limoncocha, Ecuador from 17:00-23:30 h to listen to the calling dynamics of *L. leptodactyloides* (a member of the *L. melanonotus* group) and *L. mystaceus*. Whereas *L. leptodactyloides* gave at least two types of calls noted as warm-up chirps and regular calls in the field notes (unpublished field notes on file at USNM), *L. mystaceus* gave only one kind of call. The only variation in *L. mystaceus* calls were how loud the calls were and how often the calls were given by individual males.

Given the extensive distribution and geographical concordance of localities with the different call types, together with the lack of variation of call types within individuals or populations from the same site, the call data of the *L. mystaceus* cluster best support the conclusion that the calls represent species level differences at least for the pulsed and non-pulsed calls. Thus, there are minimally two species: (1) a species represented by populations with non-pulsed calls from Tambopata, Xapuri, and along the Rio Juruá; and (2) a species represented by populations with pulsed calls throughout the northern, central, eastern Amazon Basin and certain adjacent areas. The differences between call duration exhibited by the frogs from Tambopata and Xapuri and those from the Rio Juruá are at the level where they could be representing either species differences or representing geographic intraspecific variation. The general distinctiveness of the frogs from the Rio Juruá gives additional support for some level of differentiation of frogs from that area relative to all others. The number of calls available from the Rio Juruá is small and there is considerable geographic distance (ca. 600 km) between the localities along the Rio Juruá and either locality of Tam-

bopata or Xapuri, in which there could be intermediate call conditions. Although we recognize that the frogs from the Rio Juruá and those from Tambopata-Xapuri could represent distinct species, we conservatively recognize a single species of non-pulsed calling frogs. Additional call data are needed to determine if these are separate species.

The morphological and advertisement call data are in conflict with respect to interpreting species level differentiation in the *L. mystaceus* cluster. Although we lack data on female mate choice and molecular based estimates of genetic relationships for this cluster, there are reasons to expect that such data may not provide any evidence beyond that already presented here as to the number of species involved. Female frogs can be very selective, exhibiting significant choice among dialects of the same species (Ryan and Wilczynski, 1988; Ryan et al., 1992). Given the background of previous studies, we would expect females of the *L. mystaceus* cluster to select between at least the pulsed and non-pulsed call types. Such female choice evidence by itself would support either the hypothesis that the calls represent two species or the hypothesis that the calls represent two dialects of a single species, however. If molecular analyses showed trenchant differences between the pulsed and non-pulsed call type populations, that would support species level differentiation between them. However, if the speciation event associated with pulsed and non-pulsed call types was recent, molecular techniques might not distinguish the genetic differentiation signal from genetic variation.

The conclusion that at least two species need to be recognized in the *L. mystaceus* cluster results in the following name associations. The populations with pulsed calls bear the name *L. mystaceus*; the type of *Rana mystacea* Spix, 1824, is from the Rio Solimões (Heyer, 1983), an area of pulsed calls (fig. 14). The populations with non-pulsed calls have no available name and are hereby described as:

Leptodactylus didymus, new species

Holotype (fig. 13). USNM 332861, an adult male from Peru: Madre de Dios; Tambopata Reserved Zone, 12°50'S 69°17'W, collected by Reginald B. Cocroft on 1 January 1989.

Paratopotypes. USNM 222274, 247358-247367, 247645-247653, 268970, 332862-332866.

Referred specimens. Brazil, Acre, Xapuri, MZUSP 68986-68991, USNM 314910-314914, ZUEC 5745; Brazil, Acre, Porongaba, Rio Juruá, INPA 4247, 4253; Brazil, Amazonas, Altamira, Rio Juruá, INPA 3581, 5013; Brazil, Amazonas, Barro Vermelho, Rio Juruá, INPA 3048, 3272; Brazil, Amazonas, Vai-Quem-Quer, Rio Juruá, INPA 5290, 5428; Brazil, Amazonas, Vira-Volta, Rio Juruá, INPA 5555, 5706, 5717.

Peru, Madre de Dios, Tambopata Reserved Zone, MHNSM 4098-4099, 4370-4374, 11008, 11098.

Diagnosis. The species of *Leptodactylus* with a distinct pair of dorsolateral folds, a distinct light stripe on the posterior surface of the thigh, posterior surface of tarsus

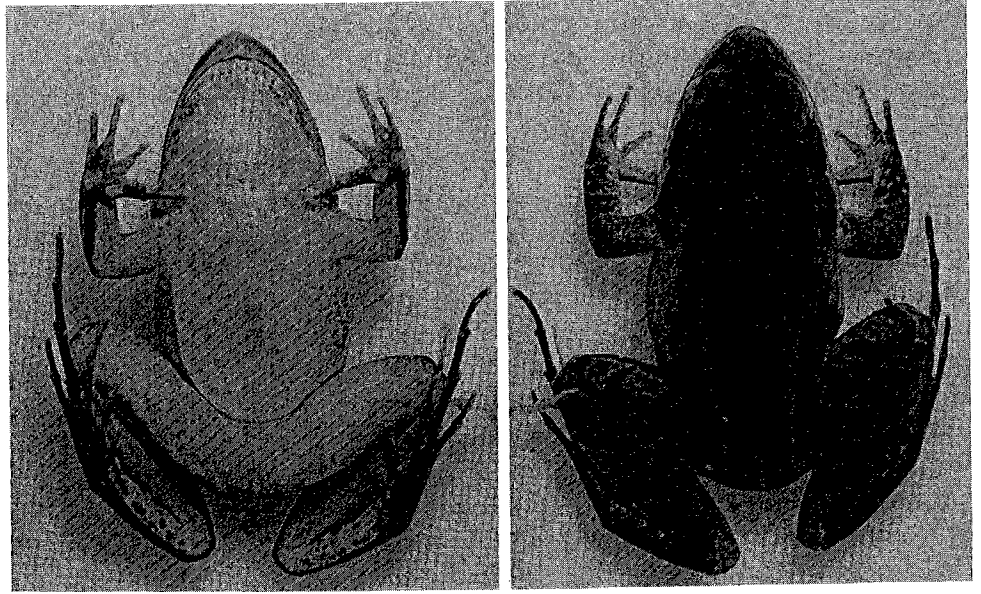


Figure 13. Holotype of *Leptodactylus didymus*.

smooth (or within distinct, non-obvious light tubercles), and sole of foot with prominent light tubercles are *L. didymus*, *mystaceus*, *notoaktites*, and *spixi*. Some individuals of *L. notoaktites* lack white tubercles on the sole of the foot and some have a light mid-dorsal stripe; no *L. didymus* have such a stripe. Most individuals of *L. spixi* have distinct white tubercles on the posterior surface of the tarsus and some have a mid-dorsal light stripe. *Leptodactylus didymus* and *mystaceus* cannot be distinguished morphologically and can be distinguished only by advertisement call, which is non-pulsed in *didymus* and distinctly pulsed in *mystaceus*.

Description of holotype. Snout subovoid-subelliptical from above, rounded with protruding shelf in profile; canthus rostralis indistinct; loreal region slightly obtusely concave; tympanum distinct, greatest diameter about 2/3 eye diameter; vomerine teeth in arched series posterior to choanae; vocal slits present; vocal sac median with weak lateral expansions; finger lengths III > I ≫ II ≅ IV; inner metacarpal tubercle ovoid, outer larger and somewhat triangular in shape; nuptial asperities absent; dorsum smooth anteriorly, weakly granular posteriorly; one pair of dorsolateral folds from just behind eye to leg; supratympanic fold distinct from eye to arm insertion; large, flat gland above shoulder posterior to tympanum and supratympanic fold; ventral surfaces smooth; belly discoidal fold distinct posteriorly, indistinct anteriorly and laterally; tips of toes just swollen; toes free, lacking fringe or web; subarticular tubercles moderately well developed; outer metatarsal tubercle round, about 1/4 ovoid inner metatarsal tubercle; tarsal

fold indistinct; metatarsal fold absent; dorsal shank smooth; outer tarsus with about six small, weak fleshy white tubercles; sole of foot with rows of large, distinct, fleshy white tubercles in line with toes.

SVL 46.7 mm, head length 18.6 mm, head width 15.8 mm, eye mid-nostril distance 4.6 mm, greatest tympanum (horizontal) diameter (including annulus) 3.6 mm, thigh 22.3 mm, shank 25.1 mm, foot 25.1 mm.

Well defined light upper lip stripe from tip of snout, extending under eye and under tympanum to shoulder insertion. Dorsum brown with indistinct darker markings including interorbital bar, scapular chevron, sacral blotch, and indistinct oblong spots between sacrum and vent. Dorsolateral fold with inner light highlight most distinct posteriorly and outer dark brown highlight most distinct anteriorly. Darker brown mid-dorsal pin stripe from in front of eyes to past sacrum. Short distinct light stripe above vent. Flanks lighter than dorsum, with just heavier mottling next to belly. Upper arms with weak darker brown cross bars; dark brown spot on front of upper arm; posterior upper arm with dark brown bar continuous with dark ulnar bar; light stripe on top of dark ulnar bar. Upper legs with distinct brown cross-bars; anterior surface of thigh with almost continuous dark brown horizontal bar; anterior surface and especially posterior surface of shank with distinct dark brown spot-like extensions of dark dorsal bars; outer tarsus brown with abrupt abutment of cream at tarsal fold location; posterior surface of thighs with series of darker spot-like extensions of dorsal bars, below which a light tan area in turn bordered by dark brown horizontal bar, below which a distinct light horizontal stripe abruptly abutting the dark brown bar. Chin bordered with brown flecks, few brown flecks on chest and posterior throat; otherwise venter uniform creamy white.

Etymology. From the Greek *didymos*, double or twin, referring to the morphological similarity between this species and *L. mystaceus*.

Distribution. *Leptodactylus didymus* is known from a few localities in the southwest part of the Amazon Basin (fig. 14). Relatively few localities of *didymus* or *mystaceus* are verified with advertisement call recordings (fig. 14). (After the manuscript was finished, we learned that Marquez et al. (1995) reported on the advertisement call of *L. mystaceus* from Bolivia. The call is pulsed and the other call parameters indicate that the Bolivian specimens reported from Puerto Almacén are clearly *L. mystaceus*. The call vouchered locality is included in fig. 14. The calls from Cuzco Amazónico, Peru (relatively near Tambopata) reported by Marquez et al. (1995) represent *L. didymus*.)

Based on the available data, *L. mystaceus* occurs in the northern and eastern sector of the Amazon basin, including the northernmost portion of the Atlantic Forest system of coastal Brazil and the gallery forest system of Amazon forests extending as far as the Chapada dos Guimarães in Mato Grosso, Brazil. Because of the geographic gap indicated by the northernmost Venezuelan locality in fig. 14 (Maracay, near Rancho Grande), the specimens (AMNH 70665-66) were re-examined (by WRH) to make certain they were



Figure 14. Distribution of members of the *Leptodactylus mystaceus* cluster. Dots = localities of *L. mystaceus* from which recordings are available; solid triangles = localities of *L. didymus*, from which recordings are available; open triangles = localities of *L. didymus*, for which recordings are not available; circles = museum records of either *L. didymus* or *mystaceus* for which recordings are not available.

not *L. poecilochilus*, a somewhat similar species known to occur in northern Venezuela. The Maracay specimens are undoubtedly *L. mystaceus*, based on morphology. Additional recordings are needed to clarify the limits of distribution of *L. didymus*.

Sibling species in *Leptodactylus*

Sibling species according to Mayr (1963: 34) are defined as "morphologically similar or identical natural populations that are reproductively isolated." One could argue that there are many species pairs or complexes within the genus *Leptodactylus* that are morphologically similar. An ecologist might have a different interpretation of morphological similarity than a systematist, for instance. If the strictest criterion is applied, that of virtual morphological identity (such that one can not separate specimens at hand), there are two sibling species pairs known in *Leptodactylus*: (1) *L. gracilis* and *geminus* (Barrio, 1973), and (2) *L. mystaceus* and *didymus*, reported here. Sibling species are of interest because they may afford a perspective on the speciation process for the taxa involved. In fact, the question might be raised as to why there are not many more cases of sibling species known in frogs and in the genus *Leptodactylus* in particular. Certainly, there is no reason as far as reproductive isolation is concerned for frogs of the genus *Leptodactylus* to have to recognize each other visually; call recognition is both sufficient and effective. We think that whereas it is likely that more sibling species of *Leptodactylus* will be discovered, the total number of sibling species in the genus is relatively small. This is because advertisement calls are now known for most species of *Leptodactylus*, and in many cases, geographic samples of calls are available in which it is clear that the calls represent the same taxon.

In order to determine why cases of sibling species are infrequent in *Leptodactylus*, the first approach is to compare and contrast the two known cases for similarities and differences. Both species pairs have derived reproductive biologies of egg placement in foam nests out of water. However, this feature is shared with many other *Leptodactylus*. The two species pairs are not from the same part of South America. The *L. gracilis* - *geminus* pair occurs in Argentina, Uruguay, and southern Brazil. To date, the distributions of the sibling pairs are parapatric (Cei, 1980; fig. 14) but more collecting is needed in the potential contact zones to determine whether the pairs are in fact parapatrically distributed or demonstrate narrow sympatry. The relative ages of the speciation events are not known for either pair. Advertisement calls can be modified with seemingly little genetic change; in fact the same vocal structures can produce different calls by behavioral changes involving either differential laryngeal muscle tensions or calling rates. Are the speciation events in the sibling pairs so recent that morphological differentiation is lagging behind and not yet in evidence? Alternatively, is there something about the morphologies that are particularly suited to the environments in the places they occur so that stabilizing selection is main-

taining them? Obviously, so little is known about the sibling species of *Leptodactylus* that consideration of the topic only raises questions and does not provide resolution. There are some potentially fruitful lines of inquiry in understanding the significance of sibling species in *Leptodactylus* which should lead to rewarding results, however.

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References

- Barrio, A. (1973): *Leptodactylus geminus* una nueva especie del grupo *fuscus* (Anura, Leptodactylidae). *Physis* 32: 199-206.
- Cei, J.M. (1980): Amphibians of Argentina. *Monit. Zool. Italiano, Monogr.* 2: 1-609.
- Charif, R.A., Mitchell, S., Clark, C.W. (1993): Canary 1.1 user's manual. Ithaca, New York, Cornell Laboratory of Ornithology.
- Fritsch, B., Ryan, M.J., Wilczynski, W., Hetherington, T.W., Walkowiak, W., Eds (1988): The evolution of the amphibian auditory system. New York, John Wiley & Sons.
- Gerhardt, H.C. (1988): Acoustic properties used in call recognition by frogs and toads. In: The evolution of the amphibian auditory system, p. 455-483. Fritsch, B., Ryan, M.J., Wilczynski, W., Hetherington, T.W., Walkowiak, W., Eds, New York, John Wiley & Sons.
- Heyer, W.R. (1978): Systematics of the *fuscus* group of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae). *Nat. Hist. Mus. Los Angeles County, Sci. Bull.* 29: 1-85.
- Heyer, W.R. (1979): Systematics of the *pentadactylus* species group of the frog genus *Leptodactylus* (Amphibia: Leptodactylidae). *Smithsonian Contrib. Zool.* 301: 1-43.
- Heyer, W.R. (1983): Clarification of the names *Rana mystacea* Spix, 1824, *Leptodactylus amazonicus* Heyer, 1978 and a description of a new species, *Leptodactylus spixi* (Amphibia: Leptodactylidae). *Proc. Biol. Soc. Wash.* 96: 270-272.
- Heyer, W.R. (1994): Variation within the *Leptodactylus podicipinus* - *wagneri* complex of frogs (Amphibia: Leptodactylidae). *Smithsonian Contrib. Zool.* 546: 1-124.
- Leviton, A.E., Gibbs, R.H. Jr., Heal, E., 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.
- Littlejohn, M.J. (1965): Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution* 19: 234-243.
- Marquez, R., De La Riva, I., Bosch, J. (1995): Advertisement calls of Bolivian Leptodactylidae (Amphibia, Anura). *J. Zool.* in press.

- Mayr, E. (1963): Animal species and evolution. Cambridge, Mass., Harvard University Press.
- Ryan, M.J., Perrill, S.A., Wilczynski, W. (1992): Auditory tuning and call frequency predict population-based mating preferences in the Cricket Frog, *Acris crepitans*. Amer. Nat. **139**: 1370-1383.
- Ryan, M.J., Wilczynski, W. (1988): Coevolution of sender and receiver: effect on local mate preference in Cricket Frogs. Science **240**: 1786-1788.
- SAS Institute Inc. (1988): SAS/STAT™ user's guide, release 6.03 edition. Cary, North Carolina, SAS Institute Inc.

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