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Source: *Journal of Herpetology*, 50(4):604-615.

Published By: The Society for the Study of Amphibians and Reptiles

DOI: <http://dx.doi.org/10.1670/15-156>

URL: <http://www.bioone.org/doi/full/10.1670/15-156>

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Comparative Morphometrics in Leptodactyline Frogs (Anura, Leptodactylidae, Leptodactylinae): Does Burrowing Behavior Relate to Sexual Dimorphism?

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ABSTRACT.—Fossorial habits occur in many animal lineages and usually involve both morphological and physiological adaptations that may evolve independently. Burrowing behavior in some species of the anuran subfamily Leptodactylinae involves the specialized use of the hind limbs and/or head. The aim of this study was to identify the morphometric characters associated with burrowing behavior in species of this subfamily. We then hypothesized that, as this habit is usually associated with males, we would find sexual dimorphism in head and hind-limb morphology in the burrowing species but not in the nonburrowing species. We compared 500 specimens from 24 species using phylogenetic statistical analyses and phylogenetic mapping of sexually dimorphic characters. The results demonstrated the following: 1) There was no correlation between the measurements of the limbs, head, or tarsal tubercle and burrowing behavior in the analyzed species; 2) there was no sexual dimorphism related to burrowing behavior reflected in measurements of the head or hind limbs; and 3) sexual dimorphism in the morphometric characters always was derived. Modifications of the ridged snout and increased ossification in the nasal region of the males of the fossorial species appear to be sufficient adaptations for burrowing.

RESUMEN.—Los hábitos fosoriales ocurren en muchos linajes de animales y usualmente implican tanto adaptaciones morfológicas y fisiológicas que a menudo evolucionan independientemente. El comportamiento cavador en anuros de la subfamilia Leptodactylinae implica el uso especializado de miembros posteriores y/o cabeza. El objetivo de este trabajo fue identificar caracteres morfométricos asociados con hábitos cavadores en especies de este subfamilia. Hipotetizamos que, como este comportamiento usualmente está ligado a machos, existe dimorfismo sexual en la morfología de los miembros y cabeza en especies con hábitos fosoriales y no en las especies sin hábitos fosoriales. Comparamos 500 ejemplares de 24 especies a través de análisis estadísticos filogenéticos, y optimizamos en una filogenia los caracteres de dimorfismo sexual. Los resultados muestran que: (1) No hubo correlación entre medidas de cabeza, miembros posteriores, o tubérculo tarsal y comportamiento cavador en las especies analizadas; (2) No hubo dimorfismo sexual en medidas de la cabeza o miembro posterior relacionada a comportamiento cavador; y (3) El dimorfismo sexual en los caracteres estudiados fue siempre derivado. Las modificaciones en el hocico en forma de espátula, y una hiperosificación en la región nasal en los machos de las especies fosoriales serían suficientes adaptaciones para la excavación.

Fossorial behavior occurs in many animal lineages and usually involves morphological and physiological adaptations, often evolving independently (White, 2005). Burrowing ability in frogs has been linked to modifications in the musculoskeletal system, mostly in the hind limbs and pectoral-cranial region of the body (Trueb, 1970; Emerson, 1976; Heyer, 1978; Trueb and Duellman, 1978; Davies, 1984; Kley and Kearney, 2007; Ponssa, 2008; Ponssa et al., 2011; Reilly and Jorgensen, 2011; Jorgensen and Reilly, 2013), along with external features such as hyperdevelopment of the inner metatarsal tubercles (Emerson, 1976; Trueb and Duellman, 1978; Kley and Kearney, 2007), formation of a rigid, chisel-like snout, and shortening of hind limbs (Heyer, 1978; Ponssa and Barrionuevo, 2012). In some taxa, fossorial behavior is associated with sexual dimorphism. Differences in size or shape between females and males can result from different selective pressures. Accordingly, sexual dimorphism associated with burrowing can affect the ability of each sex to perform that function. For example, in the North African amphisbaenian *Trogonophis wiegmanni*, Martin et al. (2012) proposed a relationship between sexual dimorphism of the head and burrowing ability. In colubrid snakes, the degree of sexual dimorphism in length of the tail might serve as an index to the degree of fossoriality (Clark, 1966).

Among anurans, the subfamily Leptodactylinae provides a good opportunity to investigate relationships among morphology, burrowing behavior, and sexual dimorphism because burrowing behavior is related to the reproductive mode in the

Leptodactylinae. The subfamily includes 97 species of Neotropical frogs (Frost, 2015) including the genera *Adenomera*, *Lithodytes*, *Hydrolaetare*, and *Leptodactylus*, which is composed of four species groups: *Leptodactylus latrans*, *L. melanonotus*, *L. pentadactylus*, and *L. fuscus* (Heyer, 1969). *Leptodactylus latrans* and *Lithodytes lineatus* are nonfossorial species that place their foam nests on top of water or in natural depressions, respectively (Regös and Schlüter, 1984). On the other hand, some species of *Leptodactylus* and *Adenomera* exhibit burrowing behavior associated with clutch deposition mode. For example, species of the *L. melanonotus* and *L. pentadactylus* species groups, such as *L. podicipinus* (Pantanal, Brazil) and *L. labyrinthicus*, deposit foam nests in constructed depressions (Prado et al., 2002; Rodrigues da Silva et al., 2005). Species of the *L. fuscus* species group and species of *Adenomera* place their foam nests on land in a nuptial chamber (Fernandez and Fernandez, 1921; Heyer, 1969, 1974). Burrowing behavior in *Leptodactylus* and *Adenomera* involves the use of the hind limbs and/or head (Philibosian et al., 1974; Pisanó et al., 1993; Prado et al., 2002; Maranhão dos Santos and Oliveira Amorim, 2005; Rodrigues da Silva et al., 2005). Heyer (1978) theorized that a correlation should exist between hind-limb, head lengths, and burrowing behavior in species where males exhibit this behavior. He hypothesized that males that construct incubation chambers have longer heads and shorter legs than do females (Heyer, 1978). Interestingly, Ponssa and Barrionuevo (2012) did not find sexual dimorphism in the head dimensions of *Ly. latinasus*, a member of the *L. fuscus* group. Ponssa et al. (2011) found a shorter tarsus in males than in females in *L. podicipinus* (*L. melanonotus* group) from Pantanal, Brazil.

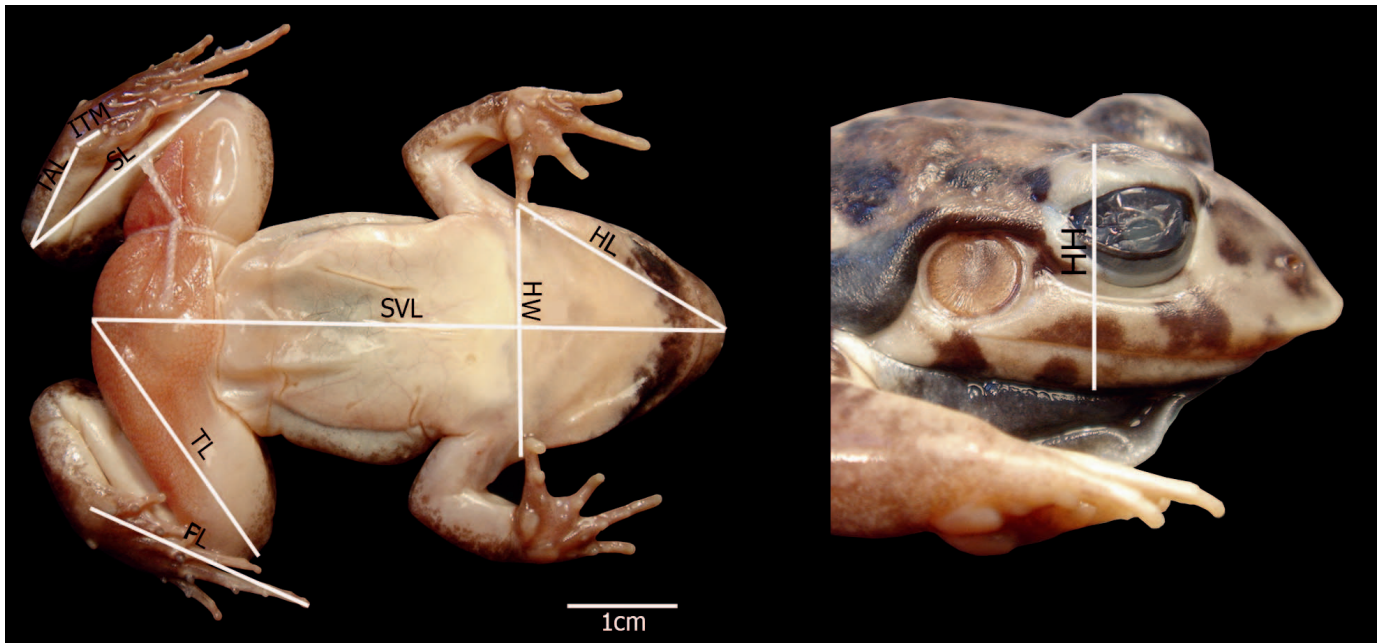


FIG. 1. Specimen of *Leptodactylus bufonius* (L440) showing the external analyzed measurements. (a) Ventral view. (b) Lateral view of the head. HH = head height; HL = head length; HW = head width; ITM = inner metatarsal tubercle; FL = feet length; SL = shank length; SVL = snout-vent length; TL = thigh length; TAL = tarsus portion length.

The goal of this study was to explore morphometric features that reflect fossorial behavior in species of *Leptodactylinae*. We expected that species where the males burrow, such as those of the *L. fuscus* group, some of the *L. pentadactylus* and *L. melanonotus* groups, and species of *Adenomera*, would display greater dimorphism (shorter limbs and longer heads) than related nonburrowing species. We optimize sexual dimorphism in the studied character states on the most recent phylogenies that include the taxa studied here (de Sá et al., 2014).

MATERIALS AND METHODS

To demonstrate the occurrence of morphometric differences in features associated with fossorial behavior, we compared the anatomies of burrowing species to those of related nonburrowing species. We considered nonburrowing species to be those that place their foam nests on water, edges of ponds, or natural water-filled basins, and burrowing species to be those that place foam nests in nuptial chambers or constructed depressions. Species of *Adenomera* and four groups of the genus *Leptodactylus* (*L. latrans* group, *L. melanonotus* group, *L. pentadactylus* group, *L. fuscus* group) were included. Details of voucher specimens are included in Appendix I and include 500 specimens of 24 species. All specimens were sexually mature adults, sexual maturity being determined by observations of gonads in females and by the presence of secondary sexual characters (spines on thumbs, vocal sacs, and vocal slits) in males. Unfortunately, behavioral studies were not available for many leptodactyline species, which limited the possible scope of the analysis of trait evolution. Although the phylogenetic sampling included only a limited number of species from the clade *Adenomera* + *Leptodactylus*, we are confident our results are representative of all species groups.

External dimensions were measured with a Mitutoyo (Kanagawa, Japan) CD-30C and CD-15B digital caliper (± 0.01 mm) and included snout-vent length (SVL) and head length

(HL), width (HW), and height (HH), as well as lengths of thigh (TL), shank (SL), tarsus (TAL), feet (FL), and inner metatarsal tubercle (ITM) (Fig. 1; Table 1). We selected these features because they were associated with burrowing behavior in this and other anuran groups (Emerson, 1976; Heyer, 1978; Kley and Kearney, 2007).

Statistical Analyses.—Values of all morphometric traits were converted to their natural logarithms prior to analysis to meet the requirements of normality and homoscedascity (Zar, 1999). As species are not independent data points, but related by their phylogenetic history, we used phylogenetically informed statistical analyses (Felsenstein, 1988; Harvey and Pagel, 1991) to explore how burrowing behavior of males and females correlated with dimorphism when phylogeny was taken into account. We conducted a phylogenetic size regression to obtain size-corrected morphometric parameters of all the morphological variables according to Revell (2009). We used the phylogenetic framework provided in de Sá et al. (2014), reduced to include only the taxa of our study (Fig. 2). As real branch lengths are not available for all species included in our analysis, we set arbitrary branch lengths according to Grafen (1989). We tested the adequacy of the arbitrary branch lengths by diagnostic plots of independent contrasts (Garland et al., 1991, 1992). This analysis was carried out in PDAP (Phenotypic Diversity Analysis Package; Midford et al., 2010) and implemented in Mesquite (Maddison and Maddison, 2015). The resulting correlations were not significant, suggesting that assigned branch lengths were adequate. We used Brownian motion as our model for evolutionary change. We generated eight phylogenetically size-corrected measurements using the ‘*phyl.resid*’ function (Revell, 2009) in the ‘*phytools*’ package (Revell, 2012) of R (R Development Core Team, 2015). A phylogenetic principal component analysis (PCA) was performed on the phylogenetically size-corrected measurements to reduce dimensionality using *phytools*’s ‘*phyl.pca*’ function (Revell, 2009). We concentrated on the first two

TABLE 1. Mean \pm SD morphometric measurements made on specimen of the 21 species of the genus *Leptodactylus* and the three species of *Adenomera* included in this study. Abbreviations: SVL = snout-vent length, HW = head width, HL = head length, HD = head depth, TL = thigh length, SL = shank length, TPL = tarsus portion length, PL = pees length, IMTL = inner metatarsal tubercle.

Species	SVL		HW		HL		HD	
	Female	Male	Female	Male	Female	Male	Female	Male
<i>A. andreae</i>	22.38 \pm 2.14	22.47 \pm 1.5	8.11 \pm 0.54	8.49 \pm 0.54	8.48 \pm 0.73	8.52 \pm 0.76	3.44 \pm 0.58	3.18 \pm 0.39
<i>A. hylaedactyla</i>	22.47 \pm 7.37	22.80 \pm 1.18	8.31 \pm 0.61	8.67 \pm 0.33	8.57 \pm 2.64	8.83 \pm 0.51	3.53 \pm 2.81	3.40 \pm 0.65
<i>A. marmorata</i>	20.07 \pm 1.54	17.25 \pm 1.04	7.4 \pm 0.46	6.7 \pm 0.4	8.08 \pm 0.54	7.55 \pm 0.59	2.68 \pm 0.54	2.48 \pm 0.28
<i>L. bufonius</i>	54.59 \pm 4.51	50.94 \pm 2.24	20.01 \pm 2.3	20.28 \pm 1.87	20.3 \pm 1.36	21.75 \pm 1.9	8.7 \pm 1.03	7.85 \pm 1.01
<i>L. chaquensis</i>	79.34 \pm 6.25	80.36 \pm 4.25	30.48 \pm 2.76	32.84 \pm 3.4	31.12 \pm 1.91	32.14 \pm 2.79	12.07 \pm 0.93	12.1 \pm 1.01
<i>L. cunicularis</i>	43.01 \pm 5.9	41.69 \pm 1.55	15.54 \pm 2.15	15.38 \pm 0.87	16.22 \pm 2.09	17.07 \pm 0.96	6 \pm 1.01	5.74 \pm 0.46
<i>L. elenae</i>	38.95 \pm 2.44	41.21 \pm 1.81	13.84 \pm 0.87	15.66 \pm 1.26	15.88 \pm 0.7	16.6 \pm 0.7	5.85 \pm 0.22	6.06 \pm 0.43
<i>L. furnarius</i>	41.69 \pm 3.88	37.33 \pm 2.56	13.08 \pm 1.31	12.57 \pm 0.98	16.24 \pm 1.96	15.26 \pm 1.16	5.31 \pm 0.46	4.9 \pm 0.78
<i>L. fuscus</i>	47.88 \pm 2.29	45.92 \pm 0.95	17.15 \pm 2.38	17.46 \pm 1.37	19.26 \pm 1.29	20.35 \pm 0.82	7.08 \pm 0.57	6.53 \pm 0.48
<i>L. gracilis</i>	48.27 \pm 2.7	46.4 \pm 1.95	15.92 \pm 1.2	15.54 \pm 0.76	18.48 \pm 0.81	17.73 \pm 0.59	7.6 \pm 0.37	6.95 \pm 0.43
<i>L. knudseni</i>	129.22 \pm 8.94	126.15 \pm 13.54	49.34 \pm 5.46	50.75 \pm 5.9	50.4 \pm 6.87	48.72 \pm 6.25	16.76 \pm 2.24	17.42 \pm 1.41
<i>L. labyrinthicus</i>	137.91 \pm 10.17	145.63 \pm 14.19	57.58 \pm 4.58	61.59 \pm 6.72	56.54 \pm 5.98	64.62 \pm 10.18	18.53 \pm 2.36	21.09 \pm 2.26
<i>L. latinasus</i>	30.36 \pm 2.75	30.41 \pm 1.59	11.71 \pm 1.34	11.07 \pm 0.52	12.93 \pm 0.72	13.46 \pm 0.64	4.14 \pm 0.4	3.79 \pm 0.36
<i>L. latrans</i>	90.52 \pm 9.15	99.19 \pm 9.57	30.57 \pm 3.45	36.49 \pm 3.66	34.66 \pm 3.34	39 \pm 2.93	11.59 \pm 1.48	14.54 \pm 1.85
<i>L. leptodactyloides</i>	44.63 \pm 2.92	35.92 \pm 2.93	15.92 \pm 0.84	13.03 \pm 1.38	17.85 \pm 1.06	14.41 \pm 1.91	5.71 \pm 0.41	4.79 \pm 0.49
<i>L. mystaceus</i>	48.38 \pm 3.96	47 \pm 3.98	17.05 \pm 1.65	16.99 \pm 1.6	18.25 \pm 1.49	19.01 \pm 1.14	5.96 \pm 0.95	5.92 \pm 0.67
<i>L. mystacinus</i>	58.09 \pm 3.93	58.58 \pm 3.2	23.4 \pm 2.4	23.11 \pm 2.1	23.69 \pm 1.52	24.25 \pm 0.91	8.77 \pm 0.7	8.52 \pm 0.8
<i>L. natalensis</i>	40.35 \pm 4.23	33.77 \pm 3.59	14.37 \pm 1.6	13.44 \pm 1.19	15.6 \pm 1.46	14.68 \pm 1.27	4.91 \pm 0.57	5.58 \pm 2.76
<i>L. notoaktites</i>	50.68 \pm 2.63	49.15 \pm 2.71	17 \pm 0.59	16.9 \pm 1.83	19.59 \pm 0.83	19.98 \pm 1.83	6.31 \pm 0.8	5.87 \pm 0.45
<i>L. pentadactylus</i>	125.92 \pm 22.49	128.3 \pm 14.93	47.93 \pm 9.51	57.3 \pm 8.95	43.54 \pm 7.85	51.95 \pm 5.33	15.69 \pm 3.7	18.97 \pm 2.98
<i>L. podicipinus</i>	36.63 \pm 1.62	33.25 \pm 2.2	12.87 \pm 0.47	12.33 \pm 1.37	13.59 \pm 1.14	13.16 \pm 1.63	5.39 \pm 0.34	5.39 \pm 0.45
<i>L. syphax</i>	77.47 \pm 3.71	73.08 \pm 4.55	30.09 \pm 1.6	27.69 \pm 1.34	32.81 \pm 2.02	30.77 \pm 2.31	11.04 \pm 0.77	9.96 \pm 0.7
<i>L. troglodytes</i>	47.41 \pm 3.43	47.22 \pm 1.93	17.13 \pm 1.05	17.63 \pm 1.12	17.96 \pm 1.36	19.58 \pm 0.84	6.98 \pm 0.71	6.81 \pm 0.8

principal components that, based on their loadings, were interpreted as measures of inner metatarsal tubercle dimension (PC1, females and males), head, thigh, shank, tarsus, and pes portion length (PC2, females and males), and width and height of head (PC2, males; Table 2). The scores from the components with eigenvalues >0.05 were entered in a phylogenetic multivariate analysis of variance (MANOVA) Geiger package of R (Harmon et al. 2008) to investigate the relationships

between morphological scores (dependent variable) and burrowing behavior (factor, considering two levels: burrowing and nonburrowing). We excluded four species (*Adenomera andreae*, *A. marmorata*, *Leptodactylus elenae*, and *L. pentadactylus*) for which reproductive modes are unknown. Of the 24 species sampled, published information about burrowing behavior was available for 14 species, but determination of which sex exhibits burrowing behavior is known for only 12 species

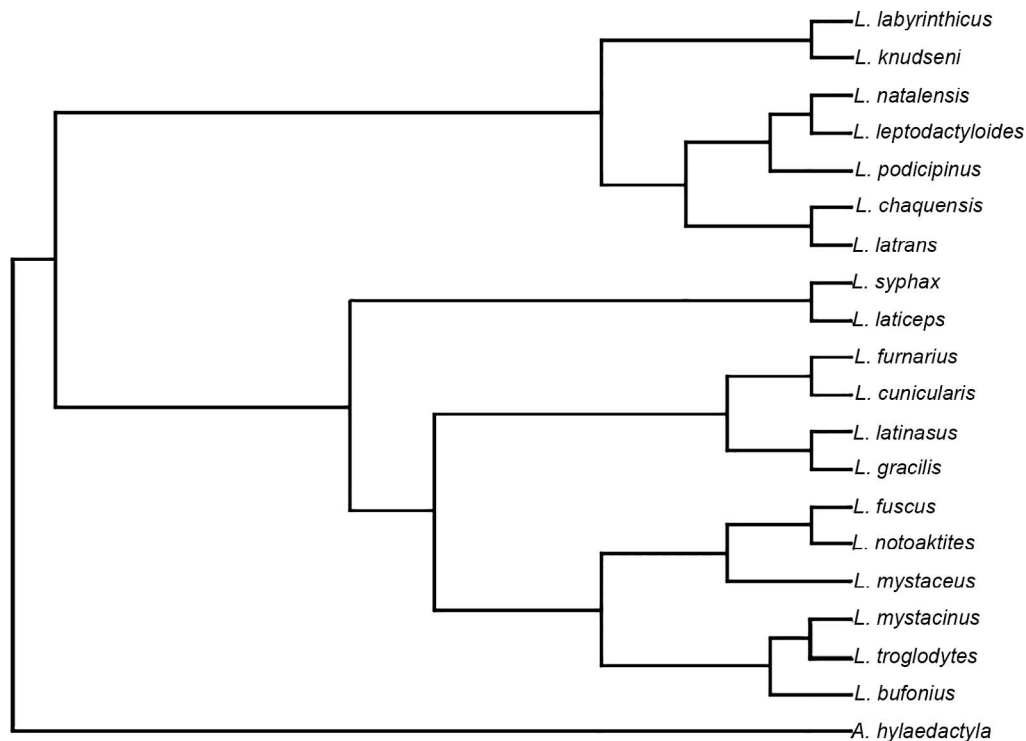


FIG. 2. Phylogenetic framework provided in de Sá et al. (2014) reduced to include only the taxa of our study.

TABLE 1. Extended.

TL		SL		TPL		PL		IML	
Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
9.4 ± 1.15	8.86 ± 0.91	10 ± 1.3	9.84 ± 1.2	6.17 ± 0.89	6.18 ± 0.51	9.89 ± 1.04	10.05 ± 0.67	0.78 ± 0.15	0.78 ± 0.25
8.85 ± 1.02	9.41 ± 0.60	10.05 ± 1.98	10.17 ± 0.45	6.49 ± 2.49	6.32 ± 0.48	10.39 ± 5.13	11.05 ± 0.99	0.74 ± 1.57	0.78 ± 0.13
8.65 ± 0.54	7.71 ± 0.49	9.44 ± 0.52	8.49 ± 0.42	6.09 ± 0.27	5.63 ± 0.38	9.93 ± 0.7	8.71 ± 0.42	0.65 ± 0.28	0.46 ± 0.08
20 ± 1.7	19.79 ± 1.22	22.82 ± 1.11	21.17 ± 0.85	13.13 ± 0.87	12.34 ± 0.64	21.52 ± 0.97	20.06 ± 0.91	2.16 ± 0.25	2.07 ± 0.24
31.79 ± 2.34	32.3 ± 1.93	35.95 ± 2.41	37.15 ± 1.78	19.29 ± 1.22	19.45 ± 1.58	37.86 ± 2.92	38.33 ± 2.42	3.3 ± 0.46	3.21 ± 0.34
21.54 ± 3.46	20.82 ± 0.99	25.05 ± 3.78	24.64 ± 1.33	13.71 ± 2.18	13.76 ± 0.84	24.57 ± 4.18	24.29 ± 0.69	1.51 ± 0.21	1.73 ± 0.16
15.78 ± 1	16.89 ± 0.72	18.95 ± 0.84	19.66 ± 1	10.5 ± 0.34	10.69 ± 0.66	19.57 ± 1.06	20.2 ± 1.16	1.4 ± 0.14	1.53 ± 0.17
20.23 ± 2.08	18.1 ± 1.2	24.9 ± 2.66	21.94 ± 1.37	14.21 ± 1.55	12.7 ± 1	26.21 ± 2.53	23.22 ± 1.29	1.34 ± 0.31	1.27 ± 0.27
19.31 ± 1.29	18.53 ± 0.97	22.83 ± 1.49	21.84 ± 1.21	12.77 ± 0.88	12.34 ± 1.21	22.13 ± 1.48	21.28 ± 1.29	2.06 ± 0.2	1.99 ± 0.19
22.11 ± 1.91	20.97 ± 1.38	27.59 ± 1.5	26.91 ± 0.87	14.31 ± 0.67	14.87 ± 0.83	26.09 ± 1.32	27.25 ± 1.08	1.81 ± 0.24	1.77 ± 0.27
53.86 ± 7.37	50.79 ± 16.54	55.14 ± 7.56	55.59 ± 5.48	33.4 ± 4.95	34.14 ± 2.59	51.64 ± 5.27	51.53 ± 6.87	6.15 ± 0.79	5.98 ± 0.78
55.17 ± 7.02	58.08 ± 5.2	59.84 ± 4.33	62.77 ± 5.48	36.46 ± 2.85	39.32 ± 2.65	56.47 ± 4.05	60.15 ± 4.05	7.56 ± 0.82	8.02 ± 1.11
11.88 ± 0.49	11.38 ± 0.84	13.9 ± 0.83	13.4 ± 0.46	7.92 ± 0.53	7.63 ± 0.33	14.93 ± 1	14.42 ± 0.68	1.21 ± 0.15	1.25 ± 0.09
42.35 ± 3.69	46.66 ± 5.13	46.67 ± 4.75	50.22 ± 5.21	26.61 ± 2.95	29.16 ± 3.06	48.71 ± 5.74	51.12 ± 5.17	3.79 ± 0.46	4.18 ± 0.59
19.1 ± 0.79	14.7 ± 1.3	20.98 ± 1.44	16.09 ± 1.33	11.94 ± 1.16	9.4 ± 0.5	23.3 ± 2.69	16.9 ± 1.16	1.69 ± 0.46	1.36 ± 0.36
20.86 ± 2.26	20.02 ± 1.51	24.26 ± 2.98	23.07 ± 1.92	13.59 ± 1.54	13.8 ± 1.13	23.37 ± 2.62	22.74 ± 1.83	1.71 ± 0.36	1.82 ± 0.28
21.34 ± 1.39	22.22 ± 1.3	23.92 ± 0.84	23.17 ± 0.95	13.87 ± 0.72	13.7 ± 0.9	22.46 ± 1.54	22.77 ± 0.94	2.32 ± 0.33	2.5 ± 0.27
16.84 ± 1.65	15.37 ± 1.87	17.74 ± 1.58	16.28 ± 1.93	10.04 ± 0.71	9.22 ± 0.95	19.35 ± 1.6	17.98 ± 1.56	1.41 ± 0.14	1.3 ± 0.15
23.04 ± 1.53	21.28 ± 1.22	27.52 ± 1.5	25.54 ± 1.75	16.06 ± 1.22	14.81 ± 1.26	28.23 ± 1.64	26.47 ± 1.74	1.75 ± 0.32	1.93 ± 0.18
52.7 ± 7.35	59.73 ± 9.6	54.65 ± 8.95	60.79 ± 10.11	33.02 ± 6.2	36.72 ± 5.8	53.41 ± 7.36	60.42 ± 10.28	5.18 ± 0.36	7.07 ± 1.15
14.3 ± 1.35	13.31 ± 1.06	14.92 ± 0.88	14.07 ± 1.29	8.44 ± 0.49	8.18 ± 0.72	16.74 ± 1.26	16.32 ± 1.44	1.37 ± 0.14	1.3 ± 0.16
31.55 ± 1.27	29.77 ± 1.98	31.81 ± 1.22	30.51 ± 1.96	19.74 ± 1.33	17.73 ± 2.71	26.82 ± 2.5	25.78 ± 1.84	3.88 ± 0.54	3.74 ± 0.27
18.99 ± 1.27	19.18 ± 0.84	19.57 ± 1	18.68 ± 0.8	12.57 ± 0.79	12.63 ± 0.75	17.55 ± 1.16	17.77 ± 1.54	1.72 ± 0.25	1.6 ± 0.19

(Table 3). These 14 species, in addition to the nonburrowers as well as *L. chaquensis* (Prado et al., 2000; Prado and Haddad, 2003), *L. laticeps* (Ceï, 1980), *L. latrans* (Fernandez, 1923), *L. leptodactyloides* (Rodrigues da Silva, 2009), *L. podicipinus* (Ceï, 1980, Prado et al., 2000), and *L. syphax* (Rodrigues da Silva, 2009), were all incorporated into the analysis to detect interspecific differences between burrowing and nonburrowing species. While *L. podicipinus* from Pantanal Brazil is described as having burrowing behavior (Prado et al., 2002), we included only Argentine specimens, which are considered in the nonburrowing group (Ceï, 1980).

To detect differences between sexes in morphometric variables we performed an analysis of covariance (ANCOVA) for each species, with SVL entered as the covariate to reduce the total influence of size. The analyses were performed with the STATISCA software (vers. 7.0, StatSoft, Inc. 2004).

Females and males were jointly analyzed using a sexual dimorphism index for each variable of interest to determine whether there is a relation between a threshold index and burrowing habit. We calculated the sexual dimorphism index as the ratio of female average divided by the male average (values ln transformed) for each variable. These index values were size- and phylogeny-corrected with ‘phyl.resid’ function in the R package phytols (Revell, 2009). We then included the values of the sexual dimorphism index in a phylogenetic PCA, which considers species as data points, using phytols’s ‘phyl.pca’ function. The scores from the two first components explained 94% of the variance and were used to perform a phylogenetic ANOVA, using phytols’s ‘phylANOVA’ function of R (Revell, 2012) to investigate the relationship between the sexual dimorphism index scores (dependent variable) and burrowing behavior (factor). This last analysis was performed only for the species for which behavioral data exist.

Phylogenetic Mapping.—Based on the significance of the ANCOVA test performed between the sexes in each species, we codified discrete characters to run an ancestral character-state reconstruction (using parsimony) on the phylogeny proposed by

de Sá et al. (2014). For this analysis we used TNT software (Goloboff et al., 2003) and considered only unambiguous changes. The resulting cladogram was edited using Winclada software 1.00.08 (Nixon, 2002). The state for each measured character (width, length and height of the head; length of thigh, shank, tarsus, feet, and inner metatarsal tubercle) were defined as: 0 = absence of sexual dimorphism; 1 = presence of sexual dimorphism, with males being larger than females; and 2 = presence of sexual dimorphism, with females being larger than males.

RESULTS

Length of the Head, Limbs, and Inner Metatarsal Tubercles—The phylogenetic MANOVA testing for differences between burrowing and nonburrowing species in the measurements of head, limbs, and inner metatarsal tubercle indicated nonsignificant differences in females (Wilk’s $\lambda F_{1,212} = 0.87, P = 0.78$) and males (Wilk’s $\lambda F_{1,200} = 0.92, P = 0.88$).

Burrowing Behavior and Sexual Dimorphism—A direct correlation between burrowing behavior and sexual dimorphism was not

TABLE 2. Loadings from phylogenetic PCA.

Character	PC1		PC2	
	Female	Male	Female	Male
Head width	-0.36	-0.50	0.38	-0.64
Head length	-0.46	-0.69	0.29	-0.65
Head depth	-0.58	-0.55	0.39	-0.62
Thigh length	-0.51	-0.76	0.32	-0.73
Shank length	-0.55	-0.79	0.43	-0.82
Tarsus portion length	-0.51	-0.75	0.37	-0.79
Pees length	-0.46	-0.77	0.43	-0.79
Inner metatarsal tubercle	-0.98	0.21	0.98	0.19
Eigenvalue	0.23	0.07	0.19	0.07
Explained variance	0.68	0.21	0.63	0.24

TABLE 3. Published data about reproductive mode implying aspects of burrowing or no-burrowing habits in species of Leptodactylinae.

Species	Group	Burrowing (b)/ no burrowing (nb)	Sex ^a	Snout (s)/ hindlimbs (h)	Observations	Literature
<i>Adenomera bokermanni</i>		b	-	-		Haddad and Prado (2005)
<i>A. hylaedactyla</i>		b	m			Menin et al. (2009)
<i>Lithodytes lineatus</i>		nb	-	-		Regös and Schlüter (1984); Schlüter et al. (2009)
<i>Leptodactylus bufonius</i>	<i>L. fuscus</i>	b	m	s/h		Cei (1949); Pisanó et al. (1993); Reading and Jofré (2003)
<i>L. cunicularis</i>	<i>L. fuscus</i>	b	-	-		Haddad and Prado (2005)
<i>L. fallax</i>	<i>L. pentadactylus</i>	b	m?	-		Lescure (1979); Rodrigues da Silva and Giaretta (2009)
<i>L. furnarius</i>	<i>L. fuscus</i>	b				Giaretta and Kokubum (2004)
<i>L. fuscus</i>	<i>L. fuscus</i>	b	f/m	s/h	"With movements of her legs, she loosened the sand, which she then pushed with her nose; while with her front limbs and with her chin, she compacted the loose sand as she stretched and contracted her body"	Cei (1949); Martins (1988); Luca et al. (2008); Giaretta and Kokubum (2004)
<i>L. gracilis</i>	<i>L. fuscus</i>	b				Gallardo (1964)
<i>L. knudseni</i>	<i>L. pentadactylus</i>	b	-	-		Gascon (1991)
<i>L. labyrinthicus</i>	<i>L. pentadactylus</i>	b	f/m	h	"the male excavate soil with his hind-limbs. After amplexus, the female completed the excavation by pushing away mud with her snout and hands"	Rodrigues da Silva et al. (2005)
<i>L. laticeps</i>	<i>L. fuscus</i>	nb				Cei (1980)
<i>L. latinasus</i>	<i>L. fuscus</i>	b	m			Gallardo (1958, 1964)
<i>L. leptodactyloides</i>	<i>L. melanonotus</i>	nb				Rodrigues da Silva (2009); Barreto Pereira et al. (2015); Caldwell and Lopez (1989); Gallardo (1964)
<i>L. mystaceus</i>	<i>L. fuscus</i>	b				Haddad and Prado (2005), Oliveira Filho and Giaretta (2008)
<i>L. mystacinus</i>	<i>L. fuscus</i>	b	m	s/h		Maranhão dos Santos and de Oliveira Amorim (2005)
<i>L. natalensis</i>	<i>L. melanonotus</i>		m	s/h		Haddad and Prado (2005)
<i>L. notoaktites</i>	<i>L. fuscus</i>	b				Martins (1996) in Prado et al. (2002)
<i>L. podicipinus</i>	<i>L. melanonotus</i>	b/nb	m	s	Population from Pantanal, Brazil exhibit digging behaviour	
<i>L. savagei</i>	<i>L. pentadactylus</i>	nb	-	-		Muedeking and Heyer (1976)
<i>L. syphax</i>	<i>L. fuscus</i>	nb				Rodrigues da Silva and Giaretta (2009)
<i>L. troglodytes</i>	<i>L. fuscus</i>	b	m	s		Kokubum et al. (2009)

^a m = male; f/m = female and male; m? = indicates that the authors infer the male participation in the chamber or depression construction. - = unknown.

evident in the measurements considered. Females and males differed morphometrically in species, both with and without burrowing behavior, such as *L. leptodactyloides* (nonburrower), *L. pentadactylus*, *L. syphax* (nonburrower), *L. furnarius* (burrower), *L. fuscus* (burrower), *L. latinasus* (burrower), *L. mystaceus* (burrower), and *L. troglodytes* (burrower) (results of ANCOVA, with sex as a factor and SVL as a covariable; see Table 4). All species (burrowing and nonburrowing), except for *L. knudseni* (burrower), *L. labyrinthicus* (burrower), *L. laticeps* (nonburrower), and *Adenomera marmorata*, present sexual dimorphism in at least one dimension of the head, limbs, or tarsal tubercle (Table 5).

Sexual dimorphism indices are displayed in Table 6. The results of the phylogenetic PCA reveal that the first two components describe 87% of the variation in the sexual dimorphism index. Length of the external metatarsal tubercle is highly correlated with PC1 and head width with PC2. The PCA ordering does not reflect a threshold separating the burrowing or the nonburrowing species (Fig. 3). The phyloge-

netic ANOVA did not reveal a significant link between burrowing behavior and the sexual dimorphism index (PC1 Wilk's $\lambda F_{1,17} = 2.20, P = 0.42$; PC2 Wilk's $\lambda F_{1,17} = 0.4, P = 0.75$).

Phylogenetic Mapping.—Sexual dimorphism and burrowing behavior were scored using eight discrete characters (Table 7). The characters obtained from the analysis were optimized (Fig. 4) on the topology of de Sá et al. (2014). The absence of burrowing behavior (character 8) optimizes as a synapomorphy of (*L. syphax* + *L. laticeps*), and the head height sexually dimorphic character 2 resulted in synapomorphy of the clade (*L. latinasus* [*L. gracilis* (*L. jolyi* + *L. sertanejo*)]). Burrowing behavior appears to be plesiomorphic in the Leptodactylinae. The burrowing habit is lost at the base of the clades (*L. syphax* + *L. laticeps*) of the *L. fuscus* species group, and in (*L. latrans* [*L. chaquensis* + *L. macrosternum*]). Morphometric character mapping on the phylogeny presented by de Sá et al. (2014) shows that absence of sexual dimorphism is a plesiomorphic character for almost all the analyzed traits (Fig. 4). Character

TABLE 4. Results of the analyses of covariance test (ANCOVA) for differences in head, leg, and tarsal tubercle between females and males of species of the genera *Leptodactylus* and *Adenomera*. *P-values of significant differences.

Species	F	P (significance)
<i>A. andreae</i>	2.05	0.13
<i>A. hylaedactyla</i>	0.78	0.63
<i>A. marmorata</i>	0.62	0.74
<i>L. chaquensis</i>	0.86	0.58
<i>L. ocellatus</i>	2.21	0.12
<i>L. leptodactyloides</i>	7.58	0.00*
<i>L. natalensis</i>	1.5	0.24
<i>L. podicipinus</i>	1.37	0.31
<i>L. pentadactylus</i>	11.63	0.00*
<i>L. knudseni</i>	1.74	0.19
<i>L. labyrinthicus</i>	5.21	0.06
<i>L. laticeps</i>	0.84	0.58
<i>L. syphax</i>	7.58	0.00*
<i>L. bufonius</i>	2	0.15
<i>L. cunicularis</i>	1.24	0.44
<i>L. elenae</i>	2.29	0.11
<i>L. furnarius</i>	4.27	0.00*
<i>L. fuscus</i>	4.17	0.01*
<i>L. gracilis</i>	1.68	0.19
<i>L. latinasus</i>	4.58	0.01*
<i>L. notoaktites</i>	1.2	0.39
<i>L. mystaceus</i>	3.14	0.02*
<i>L. mystacinus</i>	1.85	0.18
<i>L. troglodytes</i>	5.68	0.00*

0 (width of the head) displays an ambiguous optimization in the genus; the absence of dimorphism in this character state appears to be a synapomorphy of the *L. fuscus* species group, of the clade ((*L. podicipinus* + *L. diedrus*) [*L. griseigularis* + *L. discodactylus*]) [*L. nesiotus* [*L. petersii* + *L. leptodactyloides*]*L. natalensis* + *L. pustulatus*)], and in (*L. knudseni* [*L. fallax* + *L.*

labyrinthicus]) (Fig. 4). Character 2 (head height) is larger in females than in males of *L. troglodytes* and is optimized as a synapomorphy of the clade (*L. latinasus* [*L. gracilis* [*L. sertanejo* + *L. jolyi*]]). The other sexually dimorphic characters occur independently throughout the cladogram (Fig. 4).

DISCUSSION

Morphometric analyses and mapping of sexually dimorphic characters revealed three interesting results: 1) There was no significant correlation between the measurements of the limbs, head, or tarsal tubercle and burrowing behavior among analyzed species; (2) sexual dimorphism in head, hind limbs, or inner tarsal tubercle measurements was not related to burrowing behavior; and (3) sexual dimorphism in the examined morphometric characters always is derived, as previously noted by Heyer (1978). Burrowing behavior is plesiomorphic in the subfamily Leptodactylinae, but this hypothesis must be considered with caution because of the scarcity of species whose burrowing or nonburrowing habits are known. Despite the significant physical differences found between some burrowing and nonburrowing species and the presence of sexual dimorphism in some burrowers, we cannot link differences directly to burrowing behavior. Neither the traditional nor the phylogenetically informed analyses demonstrate a pattern of correlation between the measurements, sexual dimorphism, and burrowing behavior. Sexual dimorphism in some species cannot be interpreted as an adaptation to burrowing behavior. The analyses indicate that phylogenetic components actually obscure the relationship between morphometry and ecology (burrowing behavior) in *Leptodactylus* and *Adenomera* species. Interestingly, the character mapping indicates that only a few characters are optimized as putative synapomorphies of clades and that most of these characters

TABLE 5. Results of univariate effects of ANCOVA, showing the degree of significance of sexual dimorphism in different measurements in 21 *Leptodactylus* species and three *Adenomera* species. *P-values of significant differences.

Species	Head width		Head length		Head depth		Thigh length		Shank length		Tarsus portion length		Pees length		Inner metatarsal tubercle	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
<i>A. andreae</i>	3.07	0.09	0.00	0.98	1.41	0.25	4.44	0.05*	0.54	0.47	0.00	0.93	0.4	0.53	0.17	0.68
<i>A. hylaedactyla</i>	4.46	0.04*	0.8	0.38	0.46	0.51	2.99	0.1	0.06	0.8	0.58	0.45	0.69	0.41	0.37	0.55
<i>A. marmorata</i>	0.33	0.57	1.52	0.23	0.36	0.56	1.46	0.24	1.35	0.26	0.54	0.47	2.45	0.13	2.02	0.17
<i>L. bufonius</i>	6.78	0.01*	7.87	0.01*	0.00	0.93	2.98	0.1	6.71	0.02*	0.72	0.41	5.74	0.03*	0.41	0.53
<i>L. chaquensis</i>	4.57	0.04*	0.58	0.45	0.00	0.97	0.11	0.74	1.99	0.17	0.00	0.95	0.02	0.87	1.04	0.32
<i>L. cunicularis</i>	0.44	0.51	10.54	0.07*	0.08	0.77	0.057	0.81	0.63	0.44	1.92	0.19	1.02	0.33	8.22	0.01*
<i>L. elenae</i>	7.9	0.01*	0.39	0.54	0.47	0.5	2.76	0.17	0.01	0.92	0.02	0.9	0.43	0.52	2.32	0.14
<i>L. furnarius</i>	5.99	0.02*	2.99	0.09	0.04	0.84	0.52	0.48	1.07	0.31	0.002	0.96	3.04	0.09	2.25	0.15
<i>L. fuscus</i>	2.46	0.13	10.55	0.00*	1.29	0.27	0.44	0.51	0.89	0.36	0.14	0.71	0.75	0.4	0.16	0.69
<i>L. gracilis</i>	0.16	0.7	2.12	0.16	11.11	0.00*	0.99	0.33	0.04	0.83	1.38	0.25	2.24	0.15	1.71	0.2
<i>L. knudseni</i>	3.49	0.07	0.00	0.97	3.35	0.08	0.36	0.55	0.68	0.42	1.39	0.25	0.21	0.65	0.00	0.96
<i>L. labyrinthicus</i>	0.26	0.61	4.67	0.05	0.89	0.36	0.16	0.69	0.18	0.67	2.18	0.16	1.35	0.26	0.00	0.96
<i>L. laticeps</i>	0.00	0.94	2.00	0.17	2.31	0.15	0.18	0.67	0.09	0.76	0.14	0.71	0.21	0.65	0.50	0.49
<i>L. latinasus</i>	6.44	0.02*	3.8	0.07	7.99	0.01*	4.34	0.05	4.37	0.05	5.1	0.04*	2.57	0.13	0.75	0.4
<i>L. leptodactyloides</i>	0.52	0.47	0.18	0.67	1.86	0.18	8.71	0.00*	5.00	0.03*	2.28	0.15	13.23	0.00*	0.65	0.43
<i>L. mystaceus</i>	1.47	0.24	6.91	0.01	0.87	0.36	0.2	0.66	0.36	0.55	6.07	0.02*	0.03	0.86	6.89	0.01*
<i>L. mystacinus</i>	0.66	0.43	0.95	0.34	1.04	0.32	2.12	0.16	4.67	0.04*	0.23	0.63	0.41	0.53	1.55	0.23
<i>L. natalensis</i>	1.14	0.3	0.005	0.94	5.71	0.02*	2.67	0.12	0.74	0.4	0.26	0.61	0.25	0.62	0.68	0.42
<i>L. notoaktites</i>	0.11	0.74	0.94	0.34	0.61	0.45	5.79	0.03*	5.13	0.03*	3.02	0.1	3.34	0.08	1.59	0.22
<i>L. ocellatus</i>	13.29	0.00*	3.97	0.06	9.41	0.00*	0.19	0.66	0.33	0.57	0.078	0.78	1.51	0.23	0.46	0.50
<i>L. pentadactylus</i>	51.61	0.00*	36.71	0.00*	16.05	0.00*	8.82	0.00*	6.1	0.02*	8.07	0.01*	6.37	0.02*	30.6	0.00*
<i>L. podicipinus</i>	4.03	0.06	4.88	0.04*	4.32	0.053	0.91	0.35	3.84	0.06	0.69	0.41	3.85	0.06	0.00	0.99
<i>L. syphax</i>	0.53	0.48	0.18	0.67	1.87	0.19	8.71	0.00*	5.00	0.03*	2.29	0.15	13.23	0.00*	0.65	0.43
<i>L. troglodytes</i>	2.98	0.1	8.89	0.00*	0.35	0.56*	0.74	0.4	0.35	0.56	0.08	0.78	0.14	0.71	1.6	0.22

TABLE 6. Dimorphism indices for each variable in the studied species. HW = head width; HL = head length; HD = head depth; TL = thigh length; SL = shank length; TPL = tarsus portion length; PL = pees length; IMTL = inner metatarsal tubercle.

Species	HW	HL	WD	TL	SL	TPL	PL	IMTL
<i>A. andreae</i>	-0.02	0.01	0.06	0.03	0.01	0.00	0.00	-0.13
<i>A. hylaedactyla</i>	-0.02	-0.03	0.02	-0.02	0.01	0.00	0.01	0.05
<i>A. marmorata</i>	-0.02	0.01	0.00	0.00	0.00	0.00	0.01	0.09
<i>L. bufonius</i>	0.00	-0.01	0.00	0.00	0.00	-0.01	0.00	-0.21
<i>L. chaquensis</i>	-0.04	0.01	0.00	0.00	0.01	0.01	0.02	-0.10
<i>L. cunicularis</i>	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.18
<i>L. elenae</i>	-0.02	-0.02	0.02	0.00	0.00	0.00	0.01	0.07
<i>L. furnarius</i>	0.00	0.02	0.02	0.01	0.00	0.00	0.01	-0.03
<i>L. fuscus</i>	-0.02	0.00	0.04	-0.02	0.00	0.02	-0.02	0.28
<i>L. gracilis</i>	-0.01	0.02	-0.03	0.05	-0.01	-0.01	0.00	0.05
<i>L. knudseni</i>	0.20	0.01	-0.04	0.00	0.00	0.00	0.01	0.08
<i>L. labyrinthicus</i>	0.00	0.00	0.00	-0.01	-0.01	-0.01	0.00	0.03
<i>L. laticeps</i>	0.01	-0.01	0.07	0.02	0.02	0.02	0.01	-0.04
<i>L. latinus</i>	-0.04	0.00	-0.04	0.00	0.01	0.00	0.02	0.11
<i>L. latrans</i>	0.05	0.03	0.01	0.01	0.03	0.03	0.03	0.56
<i>L. leptodactyloides</i>	0.02	-0.01	0.00	-0.02	-0.01	-0.02	-0.01	-0.51
<i>L. mystaceus</i>	0.00	-0.01	-0.02	0.01	0.01	-0.01	0.01	-0.08
<i>L. mystacinus</i>	0.00	0.01	0.01	0.00	0.01	0.01	0.01	-0.02
<i>L. natalensis</i>	0.00	-0.02	-0.13	-0.04	-0.03	-0.02	-0.04	0.21
<i>L. notoaktites</i>	0.00	0.00	0.02	0.02	0.02	0.02	0.02	-0.13
<i>L. pentadactylus</i>	-0.05	-0.03	-0.07	-0.02	-0.02	-0.02	-0.01	-0.07
<i>L. podicipinus</i>	0.00	0.00	-0.05	-0.01	-0.01	-0.02	-0.02	0.15
<i>L. syphax</i>	0.02	0.02	0.02	0.00	0.00	0.03	0.00	0.03
<i>L. troglodytes</i>	-0.01	-0.02	0.00	0.00	0.00	0.00	0.01	0.21

occur as independent appearances without direct correlation to burrowing behavior.

Construction of depressions or underground incubation chambers has rarely been observed for most of the burrowing leptodactylines. Data regarding burrowing behavior have been reported for 16 species of *Leptodactylus* and *Adenomera* (Table 3).

According to Emerson (1976), there are two burrowing patterns in frogs: 1) hind feet-first burrowing, and 2) head-first burrowing. Burrowing species of *Leptodactylus* belong to the second group (Emerson, 1976), as they primarily use their heads to excavate, though some species also use their hind limbs. The external and osteological morphologies related to burrowing

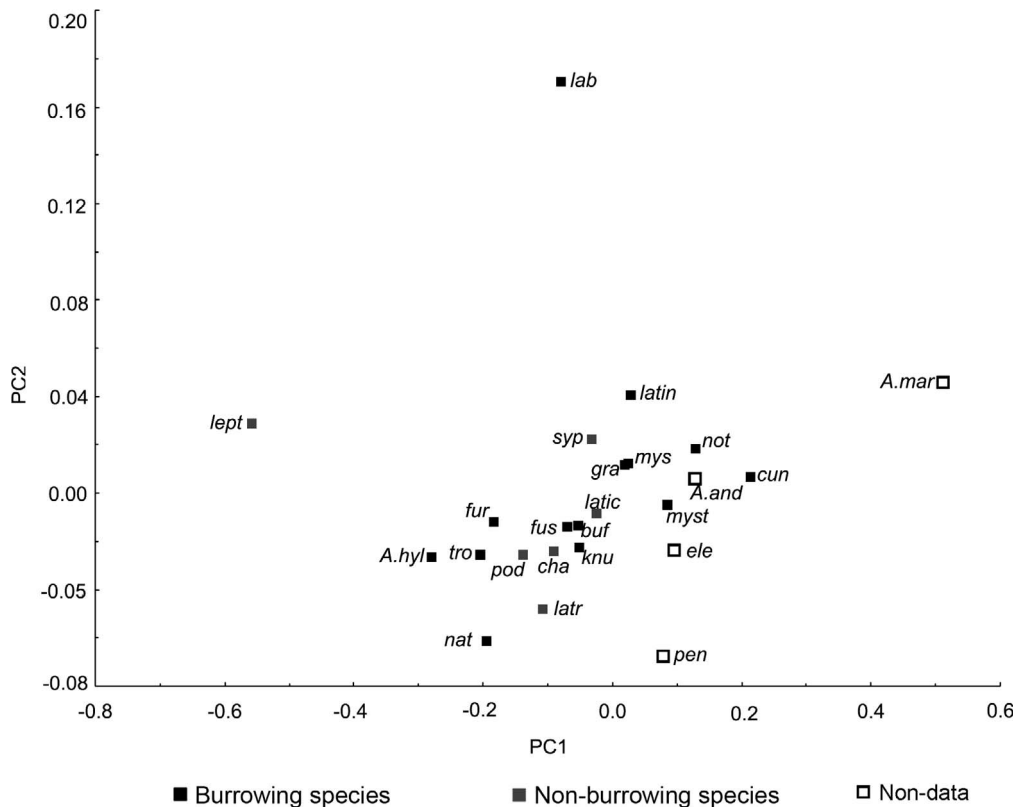


FIG. 3. Phylogenetic PCA ordering of the sexual dimorphism index of the burrowing, nonburrowing, and nondata species. The PCA ordering of the sexual dimorphism index does not reflect cluster of burrowing or nonburrowing species.

TABLE 7. Matrix with species and character defined for each measurement (width, length, and depth of the head; thigh, shank, tarsus, pees, and inner metatarsal tubercle length). The character states were defined as: 0 = absence of sexual dimorphism; 1 = presence of sexual dimorphism, greater in males than in females; 2 = presence of sexual dimorphism, greater in females than in males. The species measured in this work that are in the hypothesis of de Sá et al. (2014) were scored for this matrix. The last character refers to absence (0) or presence (1) of burrowing behavior.

Species	Head width	Head length	Head depth	Thigh length	Shank length	Tarsus length	Pees length	Metatarsal tubercle	Burrowing behavior
<i>A. hylaedactyla</i>	1	0	0	0	0	0	0	0	1
<i>A. andreae</i>	0	0	0	2	0	0	0	0	?
<i>L. bufonius</i>	1	1	0	0	2	0	2	0	1
<i>L. chaquensis</i>	1	0	0	0	0	0	0	0	0
<i>L. curricularis</i>	0	1	0	0	0	0	0	1	1
<i>L. elenae</i>	1	0	0	0	0	0	0	0	?
<i>L. furnarius</i>	2	0	0	0	0	0	0	0	1
<i>L. fuscus</i>	0	1	0	0	0	0	0	0	1
<i>L. gracilis</i>	0	0	2	0	0	0	0	0	1
<i>L. knudseni</i>	0	0	0	0	0	0	0	0	1
<i>L. labyrinthicus</i>	0	0	0	0	0	0	0	0	1
<i>L. laticeps</i>	0	0	0	0	0	0	0	0	0
<i>L. latinasus</i>	2	0	2	0	0	2	0	0	1
<i>L. mystaceus</i>	0	0	0	0	0	1	0	1	1
<i>L. mystacinus</i>	0	0	0	0	2	0	0	0	1
<i>L. natalensis</i>	0	0	1	0	0	0	0	0	1
<i>L. notoaktites</i>	0	0	0	2	2	0	0	0	1
<i>L. latrans</i>	1	0	1	0	0	0	0	0	0
<i>L. pentadactylus</i>	1	1	1	1	1	1	1	1	?
<i>L. podicipinus</i>	0	2	0	0	0	0	0	0	0/1
<i>L. syphax</i>	0	0	0	2	2	0	2	0	0
<i>L. troglodytes</i>	0	1	2	0	0	0	0	0	1
<i>L. leptodactyloides</i>	0	0	0	2	2	0	2	0	0

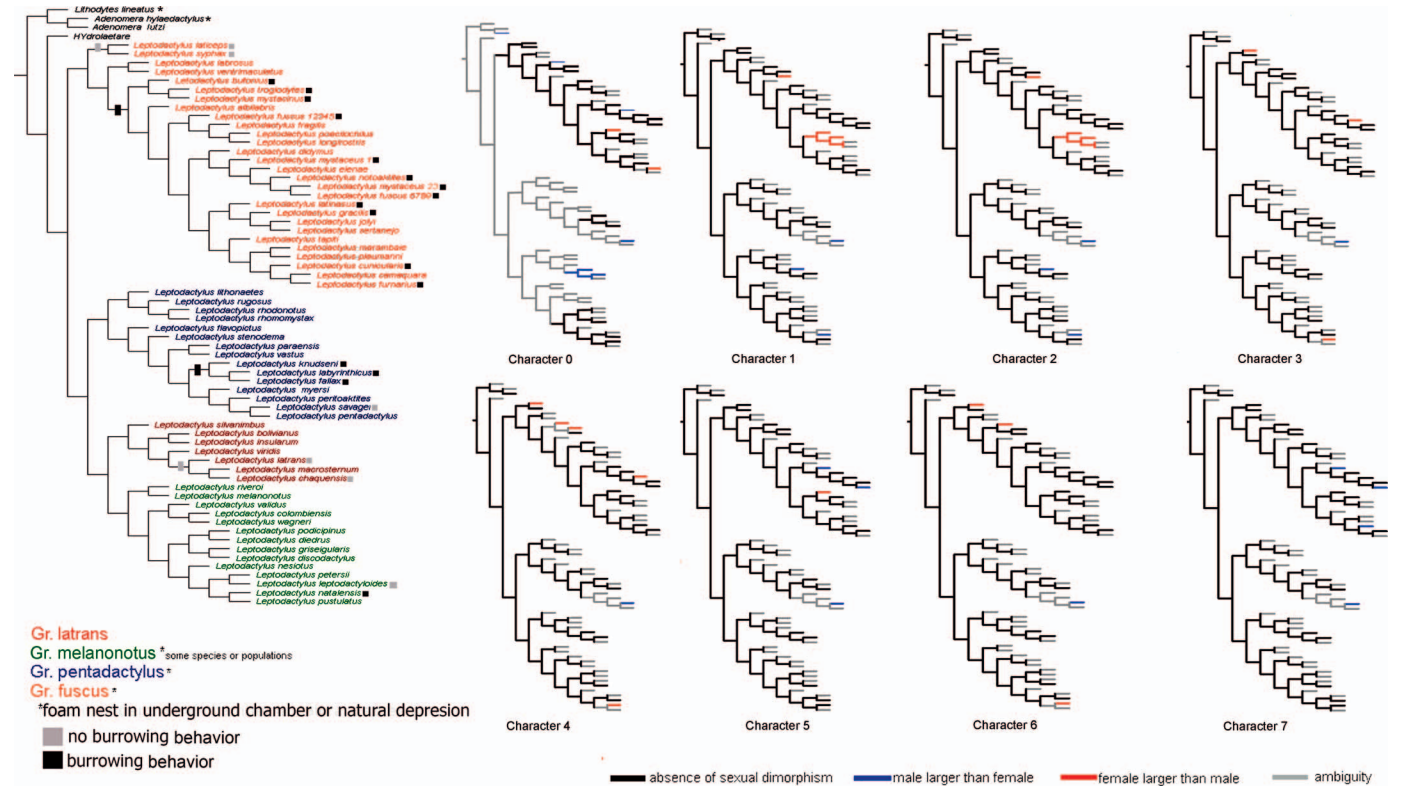


FIG. 4. *Leptodactylus* phylogeny by de Sá et al. (2014) in which relationships among the analyzed taxa are illustrated. The eight trees show the change of character states. Note that in this hypothesis, *L. laticeps* and *L. syphax* are transferred to the *L. fuscus* group; *L. silvanimbus* is related to the *L. latrans* group; and *L. riveroi* and *L. discodactylus* are included in the *L. melanonotus* group. Character 0 = head width; character 1 = head length; character 2 = head height; character 3 = thigh length; character 4 = shank length; character 5 = tarsus portion length; character 6 = foot length; character 7 = inner metatarsal tubercle.

behavior have been previously described (Ponssa, 2008; Ponssa and Barrionuevo, 2012). Sexual dimorphism in head dimensions was detected in several species studied, with head length being the most informative character for our inquiry. When the phylogenetic hypothesis is included in the analyses, however, the relationship between sexual dimorphism and burrowing behavior vanishes. Heyer (1978) suggested that dimorphism in nonburrowing species, such as *L. chaquensis* and *L. latrans*, could indicate other adaptations such as food niche separation or mating call broadcasting. On the other hand, Heyer (1978) also suggested that a longer head in males than in females of species of the *L. fuscus* group may reflect the development of a rigid, chisel-like snout important in the construction of incubating chambers. Several species with fossorial behavior, such as *L. bufonius*, *L. fuscus*, and *L. troglodytes*, exhibit sexual dimorphism in head length in which the head is longer in males than in females. By contrast in *L. podicipinus*, whose males exhibit burrowing behavior (Martins, 1996; Prado et al., 2002), the head is significantly longer in females than in males. Some species in which males have a pointed or sharp snout, such as *L. latinasus*, *L. elenae*, *L. furnarius*, *L. gracilis*, *L. mystaceus*, *L. mystacinus*, *L. notoaktites*, *Adenomera andreae*, *A. hylaedactyla*, and *A. marmorata* (Menin et al., 2009; Ponssa and Barrionuevo, 2012; de Sá et al., 2014), do not show any sexual dimorphism in head length. We can only conclude that sexual dimorphism in head shape appears in some species of the groups with terrestrial or burrowing tendency, such as the *L. pentadactylus* and *L. fuscus* species groups, but not in all of them. The other genus associated with burrowing, *Adenomera*, does not exhibit sexual dimorphism in head length, but the males do have a rigid, chisel-like and hyperossified snout area (Ponssa and Heyer, 2007; de Sá et al., 2014). We can infer that both characters, rigid chisel-like and hyperossified snout area, are independent acquisitions linked with burrowing behavior in *Adenomera* and *Leptodactylus* of the *L. fuscus* group.

Hind limb measurements also failed to reveal a correlation between shape and burrowing function. The eight species that exhibit unambiguous sexual dimorphism in leg length are scattered across different burrowing and non-burrowing groups. Six of these species show longer hind limb segments in females than in males but *L. pentadactylus* and *L. mystaceus* do not show differences. Similarly, in Crawfish Frogs, *Lithobates areolatus*, morphological features do not appear to be associated with burrowing (Engbrecht et al., 2011). Emerson (1976) hypothesized that fundamental morphological differences exist between jumping and burrowing species such as the lack of femur extension in burrowers, the different points of force concentration, and the asynchronous cycle of hind limb movement. Moreover, the increased length of any hind limb element improves jumping ability in frogs (Zug, 1972; Dobrowolska, 1973; Emerson, 1978, 1985, 1988; Choi and Park, 1996; Choi et al., 2003; Gomes et al., 2009), behavior that usually is associated with predator avoidance (Heyer, 1978). Jorgensen and Reilly (2013) found that relative hind limb length was not significantly different among most locomotor modes. We note, however, that burrowers/walkers/hoppers have relatively shorter hind limbs than do jumping-climbing and jumping-terrestrial frogs. Our failure to find a significant correlation between leg length and burrowing suggests that legs designed for jumping would scarcely require modification for effective use in burrowing with the hind feet. In fact, Nomura et al. (2009) found that hind-limbs-first burrowing behavior is plesiomorphic in anurans and requires fewer modifications in the anuran bauplan.

Emerson (1971) and Zug (1972) hypothesized that shortening of hind limbs in frogs would have detrimental effects on jumping ability. In *Leptodactylus*, burrowing behavior has not had a significant adverse effect on the ability to jump, as some species are able to cover nine body lengths per jump (Emerson, 1979, 1988). Reilly and Jorgensen (2011) proposed a hypothesis of the evolution of the locomotor modes in anurans wherein the walker/hopper locomotor mode is basal for Anura. In this study the authors utilized the locomotor modes previously defined by Zug (1978) and Emerson (1979). Burrowing species of the Leptodactylinae hardly fit that classification because they also are skilled jumpers (Ponssa, pers. obs.). Similarly, species of Pelodytidae are burrowing/walking/hopping frogs with long hind limbs, but their functional role in burrowing must be further explored (Jorgensen and Reilly, 2013).

When two functions requiring different morphology or physiology contend for optimization, the expected result is some intermediate phenotype that offers reasonable performance in both tasks but optimal performance in neither (Shine et al., 2003). Either the retained jumping function or the phylogenetic history could explain the lack of a clear pattern of shortening limbs in burrowing species of *Leptodactylus* and *Adenomera*. Other structures involved in locomotion, such as the muscles, tendons, and skeleton systems of limbs and girdles, will need to be further explored in these genera, because the combination of morphological and functional attributes of each individual species will determine the mode of their locomotion.

In the burrowing species of the genus *Leptodactylus*, there are no exaggerated morphometric characteristics that allow us to infer burrowing behavior. The species of the *L. fuscus* group, certain members of the *L. melanonotus* and *L. pentadactylus* species groups, and those of the genus *Adenomera* are fossorial. These frogs are not considered totally fossorial, but their burrowing abilities provide them with a microhabitat for reproduction and perhaps contribute to their thermoregulation, hydration, and overwintering (Brown et al., 1972; Hoffman and Katz, 1989; Wells, 2008; Nomura et al., 2009; Hoffman et al., 2010). Therefore, these species do not exhibit exaggerated character states typically related to an underground lifestyle as do species of the genera *Rhinophryus* or *Dermatonotus* (Trueb and Gans, 1983; Nomura et al., 2009). The greater ossification of the nasal region (Ponssa, 2008) and the ridged snout (Heyer, 1978; Ponssa and Barrionuevo, 2012) found in males of burrowing species of Leptodactylinae appear to be adequate adaptations for their limited burrowing ability.

Acknowledgments.—The authors thank the following people for providing specimens or working space in their collections: J. Faivovich, S. Nenda, E. Lavilla, S. Kretschmann, M. Canepa, R. Heyer, H. Zaher, C. Castro Mello, and J. Pombal Jr.; to M. F. Vera Candiotti for her comments on the analysis; and to J. Menzies for helping with the spelling and grammar. Funding was provided by CONICET (PIP 0284), Foncyt (PICT 2013-0404).

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Accepted: 9 January 2016.

APPENDIX I

Specimens examined. Abbreviations of collections are: FML, Fundación Miguel Lillo, Argentina; MNRJ, Museu Nacional Universidade Federal do Rio de Janeiro, Brasil; MZUSP, Museu de Zoologia Universidade de São Paulo, Brasil; L: personal collection of María Laura Ponssa.

Adenomera andreae (10 females, 10 males): MNRJ56768, MNRJ56769, MNRJ56770, MNRJ56773, MNRJ56771, MNRJ56772, MNRJ56896, MNRJ56897, MNRJ56898, MNRJ56899, MNRJ56900, MNRJ56901, MNRJ56902, MNRJ56903, MNRJ48277, MNRJ48261, MNRJ24799, MNRJ413, MZUSP80845, MZUSP98942.

A. hylaedactyla (11 females, 10 males): MNRJ54560, MNRJ37066, MNRJ54463, MNRJ51400, MNRJ51401, MNRJ53220, MNRJ53221, MNRJ 53218, MNRJ53213, MNRJ31006, MNRJ31007, MNRJ31202, MNRJ24627, MNRJ24025, MNRJ24026, MNRJ24027, MNRJ52895, MNRJ52888, MNRJ38814, MZUSP87814, MZUSP87830.

A. marmorata (10 females, 10 males): MNRJ56156, MNRJ57951, MNRJ56483, MZUSP140428, MZUSP140426, MZUSP140417, MZUSP140420, MZUSP140409, MZUSP140413, MZUSP140421, MZUSP140410, MZUSP140387, MZUSP140423, MZUSP140390, MZUSP140389, MZUSP140396, MZUSP140344, MZUSP140356, MZUSP1400350, MZUSP140368.

Leptodactylus bufonius (*L. fuscus* group) (10 females, 10 males): FML01059-52, FML00841-48, FML09177, FML0442-1, FML0442-41, FML04642-1, FML04642-3, FML04642-2, FML04642-5, FML04642-7, FML04642-4, FML04642-6, FML00442-30, FML00442-26, FML 27912, FML 27913, FML 27914, FML 27915, FML 27916, FML 27917.

L. chaquensis (*L. latrans* group) (10 females, 10 males): FML7680, FML 4334, FML8897, FML24152, FML25086, FML5337, FML4367-2, FML4245-1, FML 11179, FML15985, FML1848, FML7793, FML25099, FML16007, FML25100, FML4647-1, FML4647-5, FML4647-4, FML 27907, L 766.

L. cunicularis (*L. fuscus* group) (9 females, 5 males): MNRJ24863, MNRJ24864, MNRJ24865, MNRJ24866, MNRJ24867, MNRJ24868, MNRJ24872, MNRJ26313, MNRJ51954, MNRJ24869, MNRJ24870, MNRJ24871, MZUSP134488, MZUSP134489.

L. elenae (*L. fuscus* group) (10 females, 10 males): FML16022, FML12674, FML12683, FML24011, FML24013, FML14725, FML14724, FML14689, FML14699, FML14725, FML10638, FML10637, FML12681, FML12684, FML12682, FML08263, FML08262, FML15989, FML15988, FML15987.

L. furnarius (*L. fuscus* group) (12 females, 12 males): MNRJ56566, MNRJ56567, MNRJ38471, MNRJ38817, MNRJ27533, MNRJ32879, MZUSP140489, MZUSP140436, MZUSP106480, MZUSP132710, MNRJ106126, MNRJ106130, MNRJ43249, MNRJ17635, MNRJ32875,

MNRJ27534, MNRJ55592, MNRJ34696, MNRJ82973, MZUSP82974, MZUSP74331, MZUSP106124, MZUSP106127, MZUSP106128.

L. fuscus (*L. fuscus* group) (10 females, 10 males): FML09406, FML09398, FML24607, FML5723, FML4358, FML5273, FML16090, FML08253, FML08255, FML08254, FML08256, FML08257, FML01278-3, FML01278-6, FML01278-5, FML 27908, FML09405, FML 27909, FML 27910, FML 27910.

L. gracilis (*L. fuscus* group) (12 females, 10 males): FML04896-3, FML04896-4, FML04896-2, FML12036, FML12021, FML12038, FML15099, FML15125, FML15088, FML 03925, FML02632, FML22279, FML 22971, FML22289, FML02550, FML22323, MACN-HE38386, MACN-HE38388, MACN-HE38389, MACN-HE38387, MACN-HE25679, MACN-HE25680.

L. knudseni (*L. pentadactylus* group) (10 females, 11 males): MZUSP87666, MZUSP125009, MZUSP61556, MZUSP56592, MZUSP60134, MZUSP124716, MZUSP100025, MZUSP16677, MZUSP16683, MZUSP67086, MZUSP71187, MZUSP68293, MZUSP67087, MZUSP66087, MZUSP98632, MZUSP54667, MZUSP54668, MZUSP56593, MZUSP56594, MZUSP124713, MZUSP124714.

L. labyrinthicus (*L. pentadactylus* group) (8 females, 5 males): MZUSP21746, MZUSP38517, MZUSP9330, MZUSP25151-1, MZUSP25152, MZUSP24534, MZUSP56603, MZUSP54754, MZUSP69958, MZUSP21747, MZUSP58016, MZUSP10812, MZUSP25951, MZUSP54753.

L. laticeps (*L. pentadactylus* group) (10 females, 10 males): FML05295, FML04909, FML5294, FML5293, FML5249, FML02527-4, FML02434, FML06099, MACN-HE42547, MACN-HE26501, MACN-42549, MACN-HE42548, FML03645-1, FML05250, FML4666, FML02527-7, FML09267, FML00389, FML415-2, FML415-1.

L. latinasus (*L. fuscus* group) (10 females, 10 males): FML 27918, FML 27919, FML 27920, FML 27921, FML 27922, FML 27923, FML 27924, FML 27925, FML 27926, FML 27927, FML 27928, FML 27929, FML 27931, FML 27931, FML 27932, FML 27933, FML 27934, FML 27935, FML 27936, FML 27937.

L. leptodactyloides (*L. melanonotus* group) (10 females, 10 males): MZUSP24916, MZUSP40433, MZUSP40435, MZUSP40436, MZUSP40437, MZUSP40439, MZUSP40438, MZUSP40440, MZUSP40441, MZUSP4483, MZUSP4484, MZUSP4486, MZUSP85264, MZUSP85265, MZUSP85266, MZUSP85267, MZUSP85268, MZUSP85269, MZUSP85270, MZUSP85271.

L. mystaceus (*L. fuscus* group) (15 females, 12 males): MNRJ56708, MNRJ56709, MNRJ56710, MNRJ56711, MNRJ56713, MNRJ56714, MNRJ56914, MNRJ56918, MZUSP91105, MZUSP91100, MZUSP91105, MZUSP91088, MZUSP91106, MZUSP64239, MNRJ56915, MNRJ52823, MNRJ56696, MNRJ56710, MNRJ56695, MNRJ501, MZUSP91114, MZUSP64245, MZUSP64242, MZUSP64203, MZUSP64207, MZUSP64210, MZUSP64209.

L. mystacinus (*L. fuscus* group) (10 females, 10 males): FML05460, FML03567-1, FML05709-1, FML04805-2, FML03290-3, FML01272-2,

FML02356-3, FML02356-4, FML05259-1, FML03840, FML05308, FML05259-2, FML09709, FML09708, FML09710, FML15945, FML15946, FML05340, FML05878, FML4805-1.

L. natalensis (*L. melanonotus* group) (11 females, 12 males): MNRJ4913, MNRJ4914, MNRJ4915, MNRJ4916, MNRJ4917, MNRJ55765, MNRJ55766, MNRJ59125, MNRJ33085, MNRJ29797, MZUSP63109, MZUSP56751, MNRJ38139, MNRJ27930, MNRJ27798, MNRJ 32865, MNRJ4908, MNRJ4989, MZUSP63102, MZUSP63112, MZUSP63105, MZUSP56740, MZUSP56736.

L. notoaktites (*L. fuscus* group) (10 females, 10 males): MNRJ18396, MNRJ17698, MNRJ029062, MZUSP88069, MZUSP82606, MZUSP88068, MZUSP88067, MZUSP24149, MZUSP24150, MZUSP70371, MNRJ48296, MNRJ29061, MNRJ50903, MNRJ40217, MZUSP139394, MNRJ134606, MZUSP25420, MZUSP134055, MZUSP76402, MZUSP125405.

L. latrans (*L. latrans* group) (17 females, 16 males): FML04878, FML04261, FML04887, FML03555, FML20765, FML24023, FML00190, FML02621, FML20769, FML04336-1, FML07108, FML360-5, FML568-2, MNRJ34955, MNRJ49573, MNRJ39309, MNRJ49549, MNRJ43233, MNRJ43232, MNRJ43234, MNRJ43236, MNRJ34660, MNRJ46523, MNRJ132, MNRJ50949, MNRJ42181, MNRJ30504, MNRJ44108, MNRJ49550, MNRJ49548, MNRJ43235, MNRJ28347, MNRJ28348.

L. pentadactylus (*L. pentadactylus* group) (10 females, 10 males): MNRJ48124, MNRJ47898, MNRJ48244, MNRJ52980, MNRJ44219, MNRJ56628, MNRJ56629, MNRJ796, MNRJ without number (Santa Fe, Alto Itacoai, Amazonas. Vi/1950), MNRJ4559, MNRJ51146, MNRJ51145, MNRJ44220, MNRJ without number (Brotas, S. Salvador, E. da Bahia), MNRJ2559, MNRJ without number (Mata Yatoarana, Oriximina, Para. 25-26/i/1973), MNRJ without number (Bello Horizonte. Minas Gerais. x/1947), MNRJ without number (Obidos, Para.1/ii/1973), MNRJ without number (Agua Limpa prope Juiz de Fora, Minas Gerais. i/1947); MNRJ without number (Salvador, Estado da Bahia maior do Baixao de Brotas, menor da Baixa de Canela. Maior 29, Brazil).

L. podicipinus (*L. melanonotus* group) (10 females, 10 males): FML03577-12, FML03577-10, FML03577-1, FML03577-2, FML03577-6, FML15386, FML03576-3, FML10026, FML24016, FML03577-18, FML10027, FML03575, FML12198, FML08119, FML03577-5, FML03577-4, FML01861-2, FML01861-1, FML11791, FML13188.

L. syphax (*L. pentadactylus* group) (10 females, 10 males): MZUSP89380, MZUSP89361, MZUSP89375, MZUSP89383, MZUSP89366, MZUSP89386, MZUSP89379, MZUSP89377, MZUSP89365, MZUSP89372, MZUSP89378, MZUSP89358, MZUSP89376, MZUSP89368, MZUSP89396, MZUSP89369, MZUSP89373, MZUSP89359, MZUSP71575, MZUSP71573.

L. troglodytes (*L. fuscus* group) (10 females, 10 males): MNRJ38658, MNRJ45224, MNRJ31230, MNRJ31228, MNRJ31229, MNRJ38583, MNRJ38582, MNRJ59132, MNRJ38599, MNRJ38600, MNRJ45233, MNRJ59139, MNRJ36818, MNRJ52740, MNRJ54606, MNRJ38163, MNRJ57873, MNRJ46734, MNRJ46733, MNRJ50703.