Testes Size in Leptodactylid Frogs and Occurrence of Multimale Spawning in the Genus *Leptodactylus* in Brazil

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ABSTRACT.—Although rare, some anurans show multimale spawning. Herein we present data on testes mass relative to body mass for 11 leptodactylids and report multimale spawning in *Leptodactylus chaquensis* and *Leptodactylus podicipinus*. Testes mass averaged 4.13% of body mass for *L. chaquensis* and 0.75% for *L. podicipinus*. Testes size of both leptodactylid species with multimale spawning show that they are large and close to values recorded for rhacophorid frogs with multimale breeding (0.71–7.79%). Large testes size in frogs with multimale spawning supports the sperm competition hypothesis.

Parker (1970) defined sperm competition as competition between sperm of two or more males to fertilize ova of a single female. Sperm competition has been invoked based on two criteria: (1) direct observation of multiple mating by individual females during a single reproductive episode, and (2) indirect inference of multiple mating through paternity analyses (Birkhead, 1995). Although this phenomenon is taxonomically widespread, including animals with external and internal fertilization, much more attention has been devoted to animals with internal fertilization (Byrne, 2002).

Fertilization is external in most frogs, and generally a single male grasps a female dorsally in amplexus until the female spawns (Kusano et al., 1991). The male sheds sperm onto the eggs almost simultaneously with spawning. Although sperm competition is poorly studied in anurans, some species are known to show multimale breeding, in which two or more males grasp, or associate with, a single female (e.g., Pyburn, 1970; Fukuyama, 1991; Jennions and Passmore, 1993; Kasuya et al., 1996; Kaminsky, 1997). Multiple paternity has recently been demonstrated through genetic analyses for the phyllomedusine *Agalychnis callidryas* (D'Orgeix and Turner, 1995) and the myobatrachid *Crinia georgiana* (Roberts et al., 1999). Indirect evidences showed that it also occurs in the rhacophorid *Chiromantis xerampelina* (Jennions and Passmore, 1993).

Because sperm number affects likelihood of paternity when females breed with more than one male, selection will favor males with large testes and large, high quality ejaculates (see Birkhead, 1995). Several studies have demonstrated that there is a positive relationship between testes size and intensity of sperm competition, estimated by the number of males a female is likely to breed with, in several vertebrate groups (e.g., Warner and Robertson, 1978; Kenagy and Trombulak, 1986, Birkhead and Moller, 1992). In frogs, testes size is also related to the intensity of sperm competition. Many species that show multimale breeding have relatively large testes compared with other species where this behavior is unknown (Kusano et al., 1991; Jennions and Passmore, 1993; Emerson, 1997).

To date, multimale spawning in anurans has been directly observed in six rhacophorids (Coe, 1967, 1974; Fukuyama, 1991; Feng and Narins, 1991; Jennions et al., 1992; Kasuya et al., 1996), three hylids (Pyburn, 1970; Roberts, 1994), one bufonid (Kaminsky, 1997), and one myobatrachid (Roberts et al., 1999). In this paper, we describe multimale spawning behavior in two foam nest-building species, *Leptodactylus chaquensis* and *Leptodactylus podicipinus* (Leptodactylidae) and compare testes size relative to body size among foam nest-building leptodactylids exhibiting different reproductive modes. Testes size was also compared among 33 frog species in five families: Bufonidae, Hylidae, Leptodactylidae, Ranidae, and Rhacophoridae.

MATERIALS AND METHODS

Multimale Spawning Behavior.—Observations were carried out in the Pantanal, a great floodplain with an area of about 140,000 km², in the municipality of Corumbá (19°34'S, 57°00'W), Mato Grosso do Sul state, southwestern Brazil. Climate is of the "Aw" type in Köppen's clas-

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sification, with a dry winter from May to September and a rainy summer from October to April. Floods are common, generally from January to April. The region is dominated by savanna-like formations ("cerrado") and gallery forests.

Field observations of mating behavior of *L. chaquensis* and *L. podicipinus* were made from October 2000 to April 2001. *Leptodactylus chaquensis* breeds during the rainy period at the study site, from October to March, in puddles and flooded areas, and males typically call during rain (Prado et al., 2000). Eggs embedded in foam nests are deposited on the water surface. *Leptodactylus podicipinus* breeds at the edges of permanent ponds and flooded areas throughout the year; eggs in foam nests are deposited in basins constructed by males (Prado et al., 2002). In both species, females attend nests and tadpoles (Prado et al., 2000; Martins, 2001). Calling males, amplectant pairs, and foam nests were located by active searching. The sites were marked, and behavioral sequences at breeding sites were recorded. Only individuals involved in multimale spawning were collected, measured to the nearest 0.1 mm, and preserved.

Testes Size.—Testes and body sizes were analyzed for 11 leptodactylids in two genera: *Leptodactylus* and *Physalaemus*. Both genera are characterized by depositing eggs embedded in foam nests. *Leptodactylus* species analyzed were *L. chaquensis, Leptodactylus fuscus, Leptodactylus labyrinthicus, Leptodactylus macrosternum, Leptodactylus mystacinus, Leptodactylus notoaktites, Leptodactylus ocellatus,* and *L. podicipinus.* Reproductive modes among the *Leptodactylus* species exhibit a continuum, from deposition of a foam nest on the surface of water, to deposition of foam inside burrows far from water (Heyer, 1969; Prado et al., 2002). *Physalaemus albonotatus, Physalaemus crombiei,* and *Physalaemus nattereri,* three species that deposit foam nests on the surface of water, were also investigated.

Data from *L. chaquensis, L. podicipinus, L. fuscus,* and *P. albonotatus* were obtained from males collected in the Pantanal (19°34'S, 57°00'W), Brazil, from December 1996 to November 1997, and in January 1999. Data on the seven other Brazilian leptodactylids were obtained from preserved specimens housed in the Célio F. B. Haddad collection, deposited in Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, São Paulo state, Brazil.

Body and testes mass was measured in the laboratory after frogs were blotted to remove excess liquid. Body and testes mass of each specimen was determined to the nearest 0.001 g for larger species, and 0.0001 g for the smaller ones on electronic balances. Our data on relative testes size in leptodactylids (two genera, 11 species) were compared to data published for species in the families Bufonidae (one genus, four species), Hylidae (one genus, one species), Ranidae (three genera, five species), and Rhacophoridae (four genera, eight species; Kusano et al., 1991; Jennions and Passmore, 1993). Leptodactylids were also compared to species in the family Hylidae (two genera, four species), housed in the Célio F. B. Haddad collection. Considering allometric relationships between organ size and body size, and to allow biological interpretation and comparison between studies and taxa (King, 2000), we calculated linear regressions of variables using log-transformed data (Zar, 1999). Only males captured during the breeding season and with well-developed secondary sexual characteristics (e.g., vocal sacs, nuptial excrescences) were included in this study, as testes size can vary seasonally (Ko et al., 1998).

Comparative studies are generally confounded by phylogenetic effects, and comparative methods based on reconstructed phylogenies are recommended (Harvey and Pagel, 1995). However, phylogenies of Brazilian anuran species are not available. Alternatively, we used taxonomic classification as an approximate evolutionary tree (Harvey and Purvis, 1991), and, to minimize the problem of nonindependence of species datapoints (Felsenstein, 1985), we included whenever possible, more than one genus in each analyzed family (Emerson, 1997). Testes mass relative to body mass was also compared between species that show multimale spawning and those where this behavior is unknown.

RESULTS

Multimale Spawning Behavior in Leptodactylus chaquensis.—On 13 November 2000, males of *L. chaquensis* were observed calling during the day in a recently formed puddle. We returned to this site at dusk and observed two males calling and fighting in the middle of a nest. An hour later, four males gathered around the nest and began to emit low calls. The largest male, which was always in the center of the nest, emitted louder calls, jumped toward the other frogs, and hit them with its head. Sometimes these encounters ended in wrestling bouts, with the largest male clasping the other around the pectoral region with the forelimbs, with throats and venters pressed together. Additional males arrived, and finally, eight males were within the nest (the largest in the middle and others on the periphery), when a female jumped within the nest and one of the males grasped her. The other males attempted to displace the amplectant male by pushing their heads between the pair; minutes later the pair broke apart. Then, the largest male grasped the female and immediately they start-
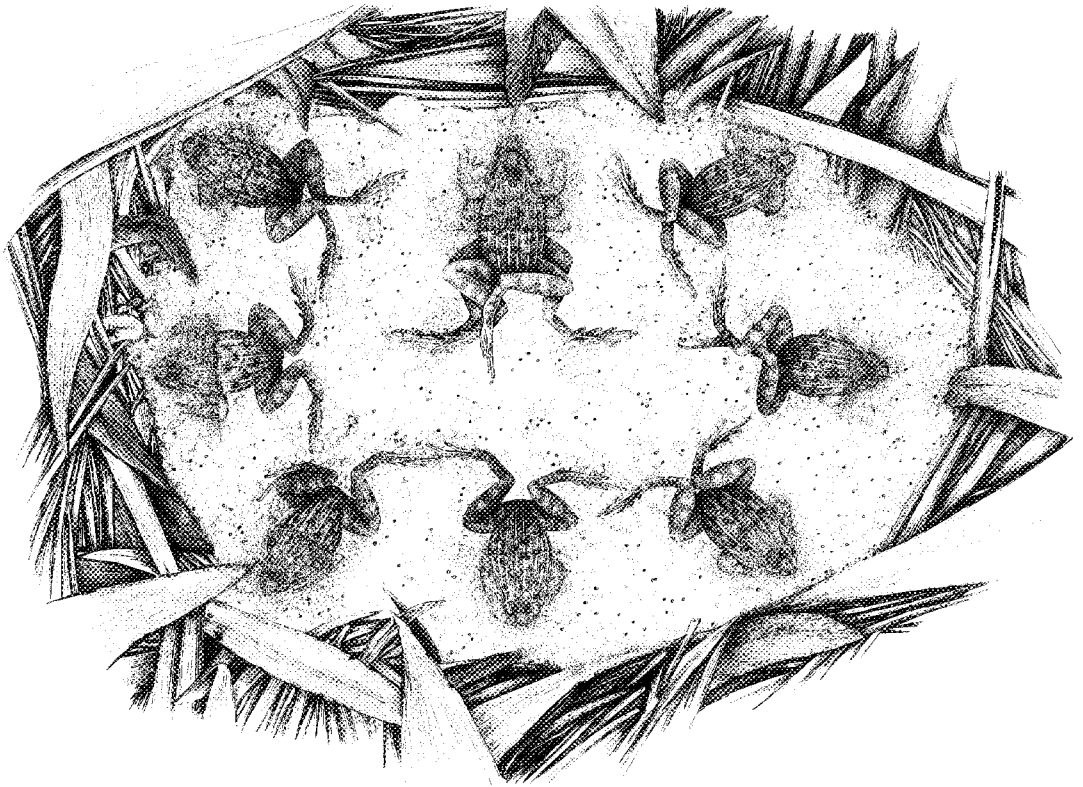
FIG. 1. Illustration of multiple spawning in *Leptodactylus chaquensis*, observed in the field on 13 November 2000, South Pantanal, Brazil. A couple spawning in the top center, surrounded by seven peripheral males churning into the foam nest in synchrony with the couple. Note that individuals have their bodies partially submerged in the foam. Drawn after slides and observations.

ed spawning in bouts of churning. At the same time the seven peripheral males began to churn into the foam nest in synchrony with the pair, with their legs kicking the foam in the same way of the amplexed male, but none of them touched the pair in amplexus (Fig. 1). All individuals within the nest were collected. The female SVL was 75.1 mm, the amplectant male measured 86.0 mm, and mean (± SD) SVL of the peripheral males was 72.5 ± 3.74 mm (range = 68.0–76.9 mm, N = 7).

In two other foam nests, we observed large males calling in the center of each nest surrounded by four males. Additionally, in December 2000, two nests were observed: one with four males and two females, the other with three males and one female. Male-male combats were recorded in three nests; however, pair formations or multimale spawning behavior were not observed.

Multimale Spawning Behavior in Leptodactylus podicipinus.—On 4 April 2001 at 2310 h, at the edge of a permanent pond, we found a male *L. podicipinus* calling inside a basin under a leaf.

We found a female inside the basin close to the calling male; around 0135 h the pair entered in amplexus and immediately began to spawn. We noticed that, in fact, there were two males grasping the female, a small one in the middle and the larger, resident male on the top (Fig. 2). We failed to notice the exact moment the second male approached, but no calling or movements were detected. The three frogs remained together, both males churning into the foam nest. The eggs were deposited during circular movements performed by the frogs. The larger male released its hold of the still amplectant pair, at which time, the frogs were collected. The female SVL was 36.8 mm, the smaller male measured 32.3 mm, and the larger 36.8 mm.

Testes Size.—Percentage of testes mass relative to body mass in species of the family Leptodactylidae ranged 0.04–4.13% (Table 1). Two species presented much larger relative testes size compared to the other leptodactylids; testes mass relative to body mass averaged 4.13% for *L. chaquensis*, and 0.75% for *L. podicipinus*. In the remaining leptodactylids, testes size ranged
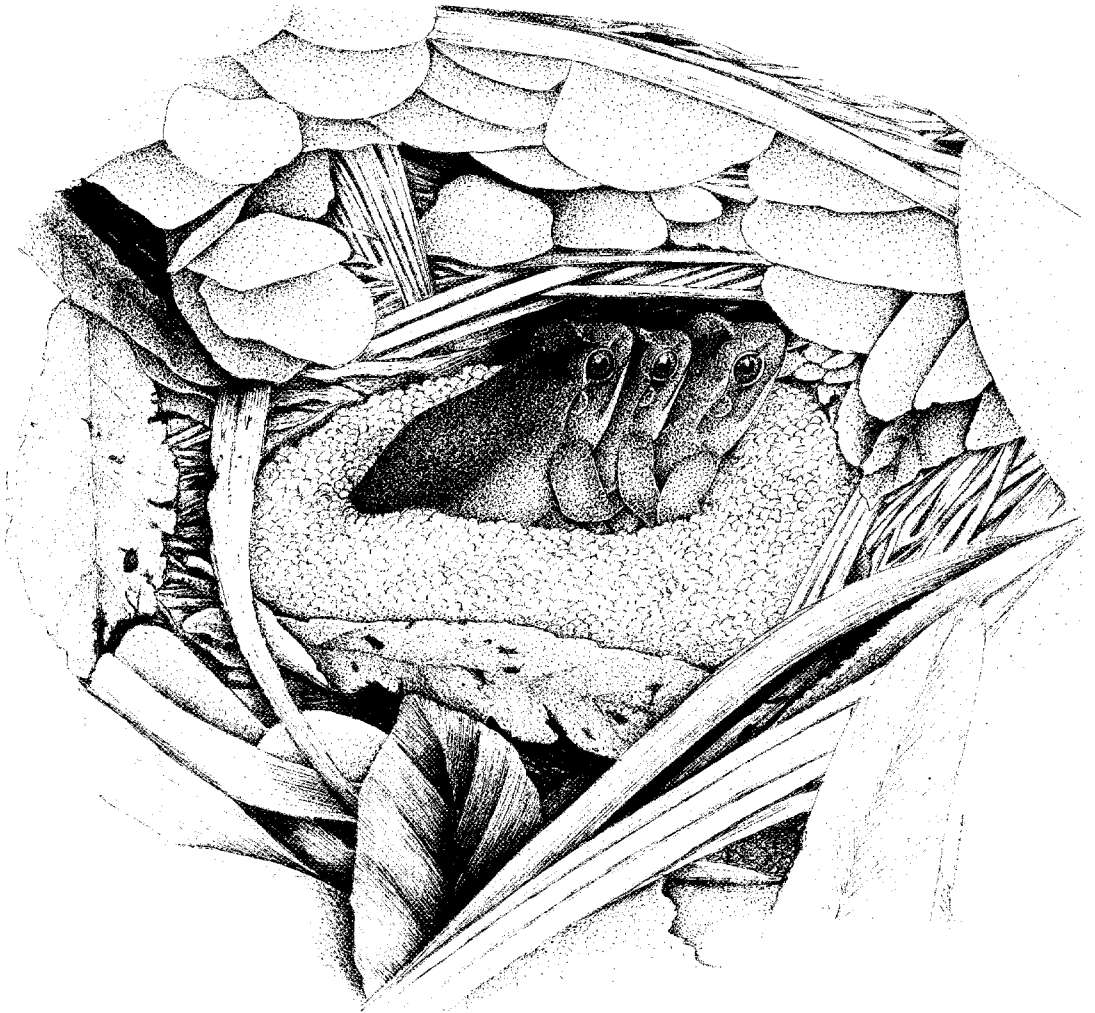
FIG. 2. Illustration of multiple spawning in *Leptodactylus podicipinus*, observed in the field on 4 April 2001, South Pantanal, Brazil. Two males grasping a female, a small one in the middle and the larger, resident male on the top. Drawn after slides.

from 0.04–0.19%. Testes mass relative to body mass was much smaller in *L. fuscus*, *L. labyrinthicus*, *L. mystacinus*, and *L. notoaktites* (range = 0.04–0.06%). Relative testes size varied less among species in the genus *Physalaemus* (range = 0.12–0.19%). Linear regression calculated for the leptodactylids revealed a significant positive relationship between body mass and testes mass ($r^2 = 0.63$, $P = 0.003$, $N = 11$; Fig. 3). Figure 3 shows that both *Leptodactylus* species with multimale spawning herein described lie above the 95% confidence interval of the calculated regression.

Considering the species analyzed in Table 1, testes size relative to body size varied less among bufonids (range = 0.13–0.39%), hylids (range = 0.14–0.53%), and ranids (range =

0.17–0.45%). Similar to leptodactylids, rhacophorid species exhibited a great variation in relative testes size (range = 0.25–7.79%). Rhacophorids that show multimale breeding presented larger relative testes size (Table 1). Among the rhacophorid multibreeders, percentage of testes mass relative to body mass range from 0.71% in *Polypedates leucomystax* to 7.79% in *Chiromantis xerampelina*. Testes mass correlated positively to body mass for the 33 frog species in five families analyzed ($r^2 = 0.53$, $P < 0.0001$, $N = 33$; Fig. 4): larger species tend to have larger testes. The testes of the leptodactylids *L. chaquensis* and *L. podicipinus*, and the rhacophorids *C. xerampelina*, *P. leucomystax*, *Rhacophorus arboreus*, *R. owstoni*, *R. schlegelii*, and *R. viridis* lie outside the upper 95% confidence interval of the

TABLE 1. Body mass, testes mass (combined mass of both testes), and percentage of testes mass relative to body mass for 33 anuran species in five families. Means and SD are shown. (1) Present study; (2) Jennions and Passmore (1993); (3) Kusano et al. (1991); (4) unpublished data from C. P. A. Prado. Species that exhibit multimale spawning in **bold**.

Families and species	N	Body mass (g)	Testes mass (mg)	Testes/body (%)
Leptodactylidae				
Leptodactylus chaquensis[1]	**21**	**32.8 ± 6.3**	**1358.0 ± 423.0**	**4.13 ± 0.99**
L. fuscus[1]	21	7.7 ± 1.1	5.0 ± 1.8	0.06 ± 0.02
L. labyrinthicus[1]	3	561.0 ± 183.5	220.0 ± 15.0	0.04 ± 0.01
L. macrosternum[1]	2	161.0 ± 13.3	180.0 ± 14.0	0.11 ± 0.02
L. mystacinus[1]	6	15.3 ± 2.0	9.4 ± 1.2	0.06 ± 0.01
L. notoaktites[1]	5	10.7 ± 1.3	5.0 ± 2.0	0.04 ± 0.02
L. ocellatus[1]	4	113.4 ± 17.4	130.0 ± 19.0	0.12 ± 0.02
L. podicipinus[1]	**20**	**4.3 ± 0.5**	**33.0 ± 13.6**	**0.75 ± 0.26**
Physalaemus albonotatus[1]	7	1.2 ± 0.4	1.4 ± 0.5	0.12 ± 0.04
P. crombiei[1]	6	1.0 ± 0.1	1.6 ± 0.6	0.15 ± 0.05
P. nattereri[1]	4	8.8 ± 0.1	17.0 ± 3.0	0.19 ± 0.04
Rhacophoridae				
Buergeria buergeri[3]	10	4.1 ± 0.5	21.2 ± 5.2	0.52 ± 0.14
B. japonica[3]	19	1.8 ± 0.3	4.3 ± 1.6	0.25 ± 0.08
Chiromantis xerampelina[2]	**12**	**13.4 ± 2.7**	**1030.0 ± 340.7**	**7.79 ± 2.38**
Polypedates leucomystax[3]	**3**	**7.2 ± 1.1**	**49.7 ± 36.3**	**0.71 ± 0.54**
Rhacophorus arboreus[3]	**22**	**13.9 ± 4.6**	**753.9 ± 427.9**	**5.15 ± 2.00**
R. owstoni[3]	3	7.6 ± 1.0	73.1 ± 29.1	0.95 ± 0.27
R. schlegelii[3]	**27**	**3.2 ± 0.5**	**33.5 ± 10.9**	**1.06 ± 0.35**
R. viridis[3]	21	9.1 ± 3.0	96.3 ± 38.5	1.11 ± 0.44
Bufonidae				
Bufo garmani[2]	5	42.7 ± 5.7	55.5 ± 19.6	0.13 ± 0.06
B. gutturalis[2]	9	38.7 ± 6.5	118.7 ± 38.1	0.31 ± 0.08
B. japonicus[3]	4	94.1 ± 19.1	331.7 ± 173.5	0.35 ± 0.15
B. maculatus[2]	11	9.8 ± 1.3	37.3 ± 11.9	0.39 ± 0.14
Hylidae				
Hyla japonica[3]	10	2.5 ± 0.3	6.8 ± 2.2	0.28 ± 0.10
Phyllomedusa hypochondrialis[4]	13	2.7 ± 0.5	12.0 ± 4.0	0.53 ± 0.13
Scinax acuminatus[4]	32	5.8 ± 0.8	8.4 ± 3.0	0.14 ± 0.05
S. fuscomarginatus[4]	11	0.6 ± 0.1	1.8 ± 0.5	0.30 ± 0.08
S. nasicus[4]	17	1.8 ± 0.8	6.0 ± 3.0	0.29 ± 0.16
Ranidae				
Leptopelis natalensis[2]	3	4.7 ± 0.8	9.1 ± 2.0	0.19 ± 0.04
Ptychadena anchietae[2]	10	5.6 ± 0.8	15.2 ± 2.0	0.28 ± 0.05
Rana ornativentris[3]	9	13.8 ± 3.8	36.0 ± 13.4	0.26 ± 0.05
R. porosa[3]	9	14.9 ± 3.9	24.8 ± 9.0	0.17 ± 0.05
R. tagoi[3]	14	5.9 ± 2.7	23.2 ± 11.9	0.45 ± 0.26

calculated regression (Fig. 4). Testes size of *L. fuscus*, *L. mystacinus*, and *L. notoaktites* were much smaller than predicted (Fig. 4). Testes mass relative to body mass averaged 0.033 ± 0.03 (range = 0.007–0.078, N = 6) in species known to show multimale breeding, and 0.003 ± 0.002 (range = 0.0004–0.011) in the remaining 27 species where this behavior was not observed. Ignoring phylogenetic relationships, testes mass relative to body mass was significantly larger in multimale breeders than in species where multimale spawning was not recorded yet (Mann Whitney U-test: W = 5.0, P < 0.001).

DISCUSSION

Testes size relative to body size showed much variation among leptodactylid species. Larger testes were observed for *L. chaquensis*, where testes mass averaged 4.13% of body mass, and for *L. podicipinus*, it averaged 0.75% of body mass. Compared to other 31 anuran species distributed in the families Bufonidae, Hylidae, Leptodactylidae, Ranidae, and Rhacophoridae, our results on testes size of both leptodactylid species show that they are large and close to values recorded for rhacophorid frogs. Extremely low values of testes mass relative to body mass were

FIG. 3. Relationship between log body mass and log testes mass for 11 leptodactylid species (log y = −2.37 + 0.89 log x). Curved lines represent 95% confidence intervals. Species, Lc: *Leptodactylus chaquensis*; Lf: *Leptodactylus fuscus*; Ll: *Leptodactylus labyrinthicus*; Lm: *Leptodactylus macrosternum*; Lmy: *Leptodactylus mystacinus*; Ln: *Leptodactylus notoaktites*; Lo: *Leptodactylus ocellatus*; Lp: *Leptodactylus podicipinus*; Pa: *Physalaemus albonotatus*; Pc: *Physalaemus crombiei*; Pn: *Physalaemus nattereri*. Note that multimale breeder species lie above the 95% confidence interval.

FIG. 4. Relationship between log body mass and log testes mass for 33 frog species in five families (log y = −2.0 + 0.88 log x). Curved lines represent 95% confidence intervals. Families and species, Bufonidae: Bga: *Bufo garmani*; Bg: *Bufo gutturalis*; Bj: *Bufo japonicus*; Bm: *Bufo maculatus*; Hylidae: Hj: *Hyla japonica*; Phh: *Phyllomedusa hypochondrialis*; Sa: *Scinax acuminatus*; Sf: *Scinax fuscomarginatus*; Sn: *Scinax nasicus*; Leptodactylidae: Lc: *Leptodactylus chaquensis*; Lf: *Leptodactylus fuscus*; Ll: *Leptodactylus labyrinthicus*; Lm: *Leptodactylus macrosternum*; Lmy: *Leptodactylus mystacinus*; Ln: *Leptodactylus notoaktites*; Lo: *Leptodactylus ocellatus*; Lp: *Leptodactylus podicipinus*; Pa: *Physalaemus albonotatus*; Pc: *Physalaemus crombiei*; Pn: *Physalaemus nattereri*; Ranidae: Len: *Leptopelis natalensis*; Pta: *Ptychadena anchietae*; Ro: *Rana ornativentris*; Rp: *Rana porosa*; Rt: *Rana tagoi*; Rhacophoridae: Bub: *Buergeria buergeri*; Buj: *Buergeria japonica*; Cx: *Chiromantis xerampelina*; Pol: *Polypedates leucomystax*; Rha: *Rhacophorus arboreus*; Rho: *Rhacophorus owstoni*; Rhs: *Rhacophorus schlegelii*; Rhv: *Rhacophorus viridis*. Note that multimale breeder species lie above the 95% confidence interval.

verified for *L. labyrinthicus*, *L. fuscus*, *L. mystacinus*, and *L. notoaktites* (0.04–0.06%). In 19 species from four families of Asian anurans, testes mass averaged 0.2–0.5% of body mass, except for the foam nest-building rhacophorids of the genera *Rhacophorus* and *Polypedates* (Kusano et al., 1991), where relative testes mass ranged 0.7–5%. For 18 species of African anurans, included in 10 genera and four families, relative testes mass was small (0.1–0.6%) in 17 species, the exception being the rhacophorid *Chiromantis xerampelina* with large testes (average 7.8%; Jennions and Passmore, 1993).

Observation of more than one male with a female during oviposition in *L. chaquensis* and *L. podicipinus* is indirect evidence on the occurrence of multimale breeding. Although peripheral males of *L. chaquensis* were not grasping the amplexed pair, as recorded for other species (e.g., *Chiromantis rufescens*: Coe, 1967; *Agalychnis saltator*: Roberts, 1994; *Crinia georgiana*: Roberts et al., 1999; *L. podicipinus*, this study), their movements were similar and synchronized with the reproductive movements of the amplectant pair, and they showed the same churning behavior described for other leptodactylid males during oviposition (e.g., Hödl, 1986, 1990; this study). The behavior observed in both species here studied indicates that males probably were shedding sperm to fertilize eggs. Multimale spawning is reported for the following foam nest-building rhacophorids herein compared to leptodactylids: *C. xerampelina* (Jennions et al., 1992), *P. leucomystax* (Feng and Narins, 1991), *R. arboreus* (Kasuya et al., 1996), and *R. schlegelii*

(Fukuyama, 1991). At least for one species, *C. xerampelina*, peripheral males in the same foam nest are capable of fertilizing the eggs (Jennions and Passmore, 1993). Jennions and Passmore (1993) predicted that multimale breeding may also occur in the rhacophorids *R. viridis* and *R. owstoni* as they exhibit relative large testes.

In species with external fertilization, two strategies are available for males to increase their fertilization success: (1) to increase the number of sperm released and (2) to maintain proximity to the female (Gross, 1985; Jennions and Passmore, 1993). Therefore, selection may favor males with high sperm production, consequently with large testes (Jennions and Passmore, 1993). The relative large testes in the rhacophorid multimale breeders, as well as in the two leptodactylids of the present study, are consistent with this prediction.

Several factors have been suggested as important for the occurrence of multimale spawning in the rhacophorids *R. arboreus* and *C. xerampelina*, as well as for its frequency: (1) conspicuousness of the foam nest; (2) time required for foam nest construction; (3) male-biased operational sex ratio (OSR); and (4) possibility of fertilization of eggs by sperm retained in the

foam nest (Kusano et al., 1991; Jennions and Passmore, 1993). *Leptodactylus chaquensis* exhibits all the above mentioned breeding features (C. P. A. Prado, pers. obs.). Furthermore, male-male combat and encounter calls in the nest are known both for *R. arboreus* (Kasuya et al., 1996) and for *L. chaquensis* (this study); these behaviors possibly allow males to locate the nest and join to other males and amplectant pair (Kasuya et al., 1996). However, presence of a foam nest is not essential for the occurrence of multimale breeding, since such behavior has been reported for anurans exhibiting different reproductive modes (e.g., Pyburn, 1970; Kaminsky, 1997; Roberts et al., 1999). For multimale breeders exhibiting different reproductive modes, other factors may influence the occurrence of simultaneous polyandry, as high male density, explosive breeding activity, competition for oviposition sites, rate of female arrival, and rainfall (e.g., Kaminsky, 1997; Byrne, 2002).

Leptodactylus podicipinus deposits foam nests in leaf-covered basins constructed by males prior to female arrival (Prado et al., 2002). We suggest that for *L. podicipinus* the number of additional males may be restricted by difficulties in finding the leaf-covered basins. This situation is somewhat similar to that described for the rhacophorid multimale breeder *R. schlegelii*. In this species, the amplexed female digs the soil, and constructs a hole where the foam nest is made (Fukuyama, 1991). One or two sneakers were more commonly observed in *R. schlegelii*; they follow amplectant pairs, and enter the nest excavating. Because of difficulties in finding the holes, and because they are narrow, participation of additional males in the spawning process is also limited for this rhacophorid (Kusano et al., 1991).

Leptodactylus chaquensis, C. xerampelina, and *R. arboreus* have much larger testes mass than all other species, including rhacophorids and leptodactylids. Jennions and Passmore (1993) suggested the existence of a positive relationship among the frequency of multimale breeding, average number of males participating in spawning, and relative testes mass in rhacophorids. *Chiromantis xerampelina* shows the highest frequency of multimale breeding (92.3%) and mean number of males present in the nest (5.5 ± 2.8), followed by *R. arboreus* (81.4%, 3.4 ± 1.9) and *R. schlegelii* (44.4%, 1.9 ± 1.3). *Leptodactylus chaquensis* and *R. arboreus* present exposed foam nests, male-male combat in the nest and exhibit similar relative testes size. *Leptodactylus podicipinus* and *R. schlegelii* deposit foam nests cryptically, and their testes size are also similar. Based on relative testes size and breeding behavior, we predict that frequency of multimale breeding and number of males participating in

the mating may be higher for *L. chaquensis* than for *L. podicipinus*.

Among the species analyzed here, *L. fuscus*, *L. mystacinus*, and *L. notoaktites* are members of the *"fuscus"* group (Heyer, 1969), where foam nests are deposited in subterranean chambers constructed by males prior to female arrival. Reproductive behavior has been studied for *L. mystacinus* (Sazima, 1975), and mainly for *L. fuscus* (e.g., Solano, 1987; Freitas et al., 2001), but multimale spawning remains unrecorded. The small testes relative to body size may be a common feature in the *"fuscus"* group, in which access to female by additional males is more difficult because of chamber structure, that presents a very small and hidden aperture, and male behavior, that uses its body to obstruct the chamber tunnel subsequent to female entrance (Martins, 1988).

Percentage of testes mass relative to body mass in *L. labyrinthicus* (0.04%), a member of the *"pentadactylus"* group (Heyer, 1969), was comparable to those in members of the *"fuscus"* group (0.04–0.06%). The *"pentadactylus"* group exhibits at least two reproductive modes (Prado et al., 2002): (1) foam nests in water-filled depressions at the edges of water bodies, and exotrophic tadpoles in water (e.g., *L. knudseni*: Hero and Galatti, 1990; *L. labyrinthicus*: Agostinho, 1994); (2) foam nests placed in burrows in the ground, and tadpoles develop inside the nests (e.g., *L. fallax*: Davis et al., 2000; *L. pentadactylus*: Hero and Galatti, 1990). However, reproductive behavior is poorly known in this group. *Leptodactylus chaquensis, L. macrosternum*, and *L. ocellatus* belong to the *"ocellatus"* group (Heyer, 1969), depositing eggs in foam nests on the surface of water. Testes size seems not to be related to the species groups, at least in the *"ocellatus"* group, with *L. chaquensis* exhibiting a testes mass much larger than the other two species of the same group, contrasting to the *"fuscus"* group.

Although sperm competition may be a major factor influencing relative testes size in vertebrates, Emerson (1997) tested two other hypotheses for differences in relative testes size among 90 species of frogs belonging to five families: (1) relative clutch size and (2) androgen level. Phyllomedusine hylid frogs known to have multiple-male mating (Pyburn, 1970; Roberts, 1994) have larger testes than hylids without multiple mating and variation in relative testes size among frogs support both the sperm competition and the clutch size hypotheses (Emerson, 1997). However, clutch size hypothesis was tested by Emerson (1997) only for ranids with no multimale breeding. Positive relationship between testes size and clutch size, as observed by Emerson (1997), may result from an indirect effect

of body size, as testes and clutch sizes increase with body size (e.g., Kusano et al., 1991; this study). For *Leptodactylus*, testes size does not seem to be related to clutch size; *L. labyrinthicus* (Agostinho, 1994) and *L. ocellatus* (Vaz-Ferreira and Gehrau, 1975) exhibit clutches as large as clutches of *L. chaquensis* and *L. podicipinus* (Prado et al., 2000), but much smaller relative testes size (Table 1). For the myobatrachid multimale breeder *Crinia georgiana*, Byrne (2002) commented that testes size relative to body size is at least four times greater than any other *Crinia* species.

Simultaneous polyandry is phylogenetically widespread among frog families, which exhibit different reproductive modes, and reproductive activity patterns (e.g., explosive or prolonged breeders), suggesting convergent evolution. Different selective factors may have favored evolution of polyandry in different species (Roberts et al., 1999). The evolution of simultaneous polyandry involves many adaptive changes in behavior (see Jennions et al., 1992) and may be restricted to taxa with reproductive modes where sperm are released in a limited space, such as (1) shallow ponds (e.g., *Bufo americanus*: Kaminsky, 1997; *Crinia georgiana*: Byrne, 2002); (2) foam nests (e.g., rhacophorids: Kusano et al., 1991; leptodactylids, present study); and (3) leaf nests (e.g., phyllomedusines: Pyburn, 1970). Observations of multimale breeding in many anurans, and wide variation in testes size and occurrence of several types of mating systems strongly indicate that sperm competition and multiple paternity may be more common in anurans, as noted before by Roberts et al. (1999).

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