

SHORT COMMUNICATION

Reproductive biology, size and diet of *Hypsiboas cinerascens* (Anura: Hylidae) in two urban forest fragments in Central Amazonia, Brazil

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Palavras-chave: distribuição, fecundidade, sítio de ovipostura, sítio de vocalização.

Hypsiboas cinerascens (Spix, 1824) occurs in wetlands near streams in forests (Lima *et al.* 2012) and is common in continuous *terra firme* forests (Rojas-Ahumada and Menin 2010) and forest fragments (Tsuji-Nishikido and Menin 2011) in central Amazonia. The species is a member of the *Hypsiboas punctatus* Group and is widely distributed in South America—Guianas and Amazon Basin in Brazil, Colombia, Venezuela, Ecuador, Peru, and Bolivia (Frost 2013). The only published information about reproduction indicates that breeding is annual and peaks in the rainy season when males call in small groups (Zimmerman and Bogart 1984) from hidden positions under leaves (Hero 1990, Lima *et al.* 2012). According to reports, this species feeds on small arthropods (Parmelee

1999, Lima *et al.* 2012). Herein, we describe aspects of reproductive biology (reproductive period, daily pattern of calling activity, oviposition site and number of eggs), size, diet, and distribution of *H. cinerascens* in two urban forest fragments in central Amazonia, northern Brazil.

The study took place in two areas in the city of Manaus, state of Amazonas, northern Brazil: (1) the campus of the Universidade Federal do Amazonas (UFAM campus: 03°04'34" S, 59°57'30" W) and (2) Parque Municipal do Mindu (Parque do Mindu: 03°07' S, 59°05' W). The forest fragment of the UFAM campus comprises about 600 ha of *terra firme* forest (a nonseasonally flooded forest), secondary forest, campinarana sites, and deforested areas (more details in Tsuji-Nishikido and Menin 2011). The Parque do Mindu fragment (which is traversed by a polluted stream) contains 30.9 ha of *terra firme* forest, campinarana sites, and deforested areas (Ribeiro *et al.* 2007). The regional climate is characterized by a rainy season from

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November–May and a dry season from June–October (Marques Filho *et al.* 1981). The mean annual temperature is approximately 26° C (Marques Filho *et al.* 1981) and the mean annual rainfall between 1985 and 2004 was 2489 mm.

We sampled adult *Hypsiboas cinerascens* in nocturnal surveys (17:30–22:00 h) biweekly from September 2010 to May 2012 by simultaneous visual encounter and auditory sampling (Heyer *et al.* 1994). Data were collected in 10 riparian transects (250 m long) along the edges of first-order streams on the UFAM campus. Each time a frog was captured in the visual survey, we recorded (1) snout–vent length (SVL) with vernier calipers (0.05 mm), (2) gender (male or female), (3) call site, and (4) using a measuring tape, distance to the nearest calling male. The call site was characterized by the following variables—type of substrate (shrubs, palm leaves, fallen trunks), position of individuals in relation to the water surface (parallel, perpendicular up or down and diagonal), perch height on vegetation in relation to the water surface, and distance from the edge of the stream. The pattern of spatial distribution of the males was evaluated using nearest-neighbor distances in the Dispersion Index (variance/mean) and t-test (Brower and Zar 1984). Peripheral males, at distances greater than 30 m from one another were considered as belonging to a different group (Menin *et al.* 2004).

The reproductive period was determined by the presence of calling males and gravid females throughout the study period. The daily pattern of calling activity was determined over the course of three surveys, conducted between February and April 2011, for a 12 h period in one transect established at Parque do Mindu. The number of calling males was estimated on an hourly basis for each survey. Behavioral characteristics were observed through continuous observations (Martin and Bateson 1993). We determined fecundity (as number of eggs) by counting ovarian eggs ($N = 3$ females) and eggs from an individual clutch ($N = 1$) collected in the field.

To determine the diet of *Hypsiboas cinerascens*, 20 calling males and three females were caught, and shortly thereafter, anesthetized, killed, and fixed in 10% formalin. The individuals were collected on September 2010, March 2011, April 2011, August 2011 and February 2012. The stomachs were removed from the frogs in the laboratory, and the contents identified to order or family following the identification keys of Triplehorn and Johnson (2011). The length and width of each prey item were measured with an eyepiece reticule. The volume of each prey was estimated using the spheroid volume formula (Colli *et al.* 1992): $V = (\pi \cdot \text{length} \cdot \text{width}^2)/6$. We made individual-based rarefaction curve performing 1000 randomizations and the Mao Tau Sobs index, using EstimateS 8.2 (Colwell 2009), to validate the number of prey categories found in the diet of this species. Voucher specimens were deposited at Coleção Zoológica Paulo Bührnheim (CZPB) at the Universidade Federal do Amazonas (CZPB-UFAM 241–263).

Male *Hypsiboas cinerascens* had an average SVL of 32.3 mm (SD = 1.6, $N = 17$, range 28.7–36.2 mm). We observed five females during the study period (September 2010, March 2011 and August 2011), but only three were collected. The SVL of females is slightly larger (mean = 33.3 mm, SD = 1.02, $N = 3$, range 32.0–34.5 mm; $t = 0.86$, $p = 0.39$) than that of males and the females contained 165, 272, and 298 eggs in their abdominal cavities. The only clutch observed in the field contained 222 eggs. The eggs are black at the animal pole and whitish at the vegetal pole (Figure 1). All females ($N = 5$) were observed perching on plants at a distance of about 77–150 cm (mean = 112.3 cm, SD = 29.8) from calling males. Courtship was observed once in December 2011. The female was motionless in a shrub about 80 cm from the calling male. Over the course of 2.5 h, the male moved 2.3 m toward the stream; he was followed by the female, but the individuals did not touch one another. Axillary amplexus (Figure 1) occurred in the water and the eggs were deposited about 15 min after amplexus.

Calling males were observed throughout the entire study period (September 2010–March 2012; Figure 2) in seven of the ten transects sampled at UFAM campus, and in the Parque do Mindu transect. The daily calling activity began at dusk, at about 17:30–18:30 h and continued until 03:00 h. The most calling males were heard between 19:00 and 21:30 h (Figure 3), with males calling from groups composed of an average of five individuals (SD = 6.98, $N = 79$, range 2–17). We observed isolated calling males only five times. The number of groups in the transects varied from two to five (mean = 2.91, SD = 0.95, $N = 35$). The mean distance between nearest groups was 67 m (SD = 23.93 m, $N = 8$, range 40–110 m).

Males called from marginal shrubs ($N = 12$), palm leaves ($N = 5$) or fallen tree trunks ($N = 13$) in the streams or in marginal flooded areas, often with the plane of the body parallel to the water surface and hidden among leaves. The mean height of call site was 75 cm (SD = 68, $N = 37$, range 0–280 cm) and between 0 and 340 cm from the edge of stream (mean = 163 cm, SD = 107, $N = 37$).

The mean distance between the nearest calling males was 2.92 m (SD = 2.97, $N = 29$, range 23–900 cm). The Dispersion Index was 3.01, in agreement with uniform distribution ($t = 17.28$, $df = 28$, $p < 0.05$). We also observed silent males ($N = 3$) near calling males, although no male-male interaction (vocal or physical combat) was observed.

Of the 23 individuals examined, 15 had stomach contents (65%). Twenty-five prey items of eight prey categories were identified in the stomachs (Table 1), and probably the diet of this species is based in a greater number of prey items from that observed in this study (Figure 4). Acari were the most common prey, but the most important item, by volume, was Orthoptera (Table 1). The number of items per stomach varied from one to seven (mean = 1.7 prey/frog). Plant material was observed in all stomachs, including small seeds and unidentified plant parts.



Figure 1. Amplexant pair (A), amplexant pair during oviposition (B), and clutch (C) of *Hypsiboas cinerascens* at the campus of the Universidade Federal do Amazonas, Manaus, Brazil.

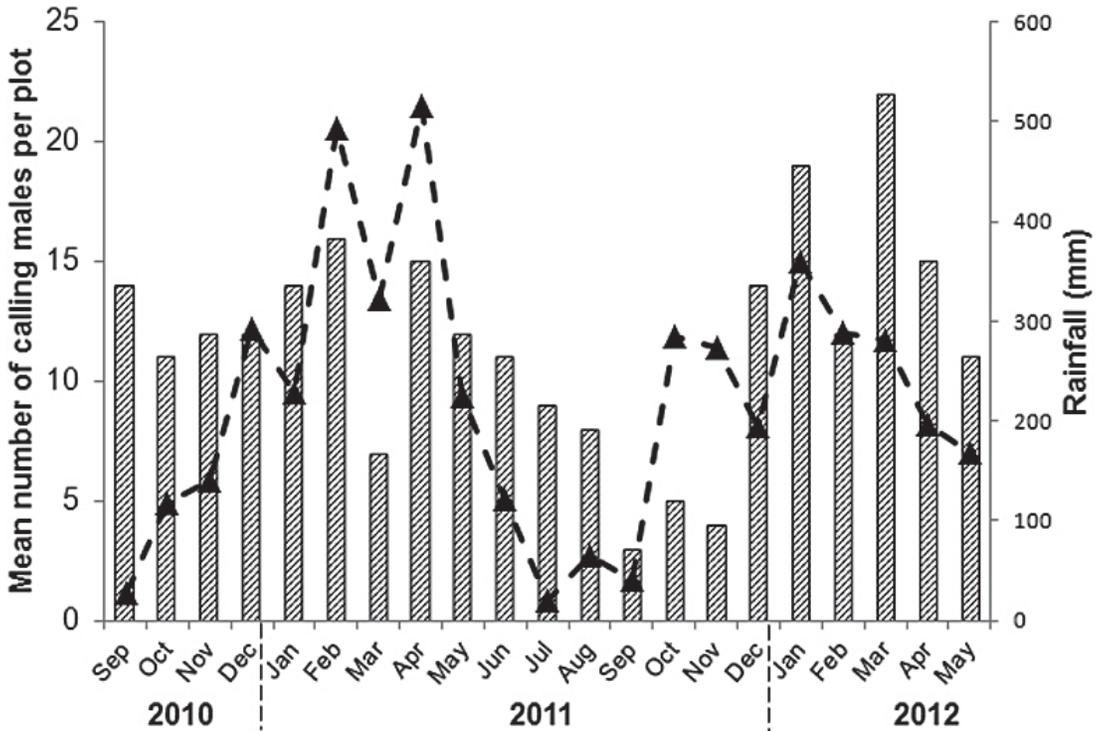


Figure 2. Mean number of calling males (bars) of *Hypsiboas cinerascens* per plot at the campus of the Universidade Federal do Amazonas, Manaus, Brazil, and monthly rainfall (dashed line) from September 2010 to May 2012.

The sizes of male and female adults of *Hypsiboas cinerascens* found in our study are similar to those found for other populations in the Amazonian region (Hoogmoed 1979, Aichinger 1992, Duellman 2005, Lima *et al.* 2012), but smaller than those in populations from Iquitos, Peru (Rodríguez and Duellman 1994) and Santa Cecilia, Ecuador (Crump 1974, Duellman 1978).

Male *Hypsiboas cinerascens* often called in small groups throughout the year with a peak in the rainy season. This pattern differs from that of Ecuadorian and Peruvian populations, in which reproduction has been reported to occur only in the rainy season (Crump 1974, Aichinger 1992). As observed by Haddad and Sawaya (2000) in *Hypsiboas leucopygia*, male-male aggressive

behavior or territorial calls were not observed in *H. cinerascens*, in contrast to aggressive behavior reported for *H. albopunctatus*, *H. bischoffi*, *H. goianus*, *H. leptolineatus*, *H. raniceps*, and the *H. boans* Group (Martins and Moreira 1991, Martins *et al.* 1998, Guimarães and Bastos 2003, Menin *et al.* 2004, Toledo *et al.* 2007, Reinke and Deiques 2010). The uniform distribution observed in the groups, the presence of a prepupal spine (Duellman 2005, pers. obs.), and the presence of silent males near calling males suggests the occurrence of territoriality in this species. The regular spacing among males may prevent physical combats as observed in *H. goianus* (Menin *et al.* 2004).

Axillary amplexus occurred only at the oviposition site, differing from *H. goianus*, in

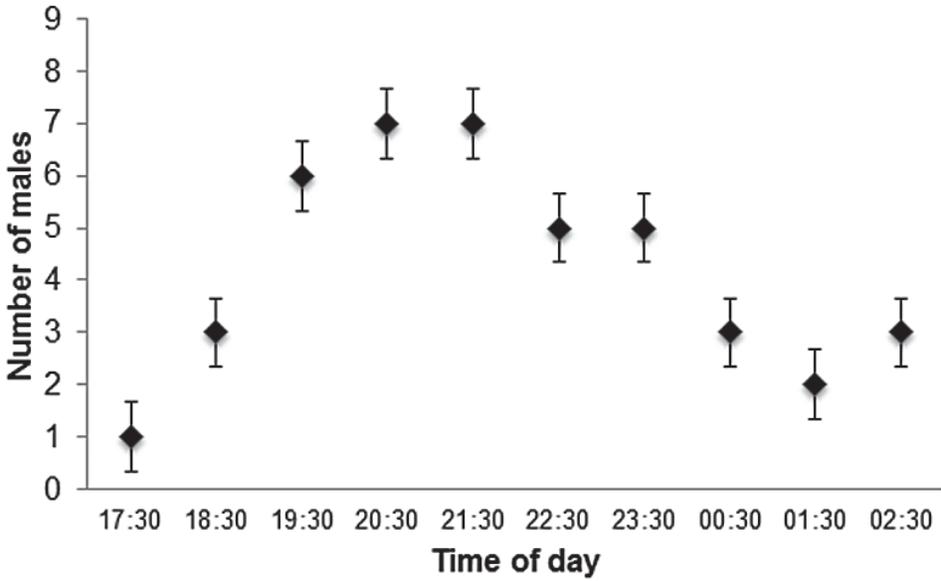


Figure 3. Hourly patterns of calling activity of males of *Hypsiboas cinerascens* in one plot at the Parque Municipal do Mindu forest fragment, Manaus, Brazil. Figures represent mean number and standard deviation of calling males.

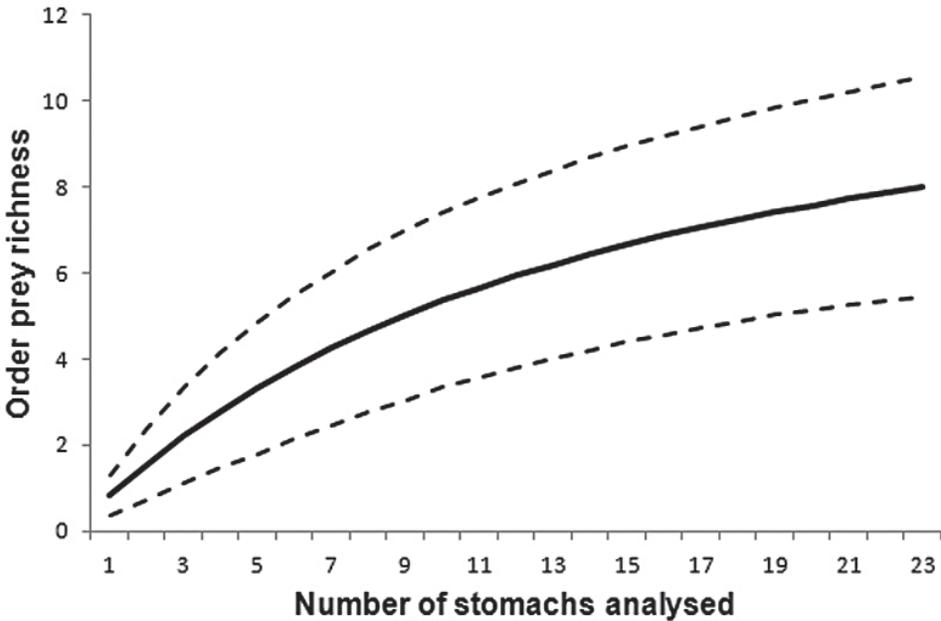


Figure 4. Rarefaction curve with their respective standard deviation based on number of stomachs analyzed of *Hypsiboas cinerascens* at the campus of the Universidade Federal do Amazonas, Manaus, Brazil.

Table 1. Number, volume and frequency of prey taxa in the diet of *Hypsiboas cinerascens* at the campus of the Universidade Federal do Amazonas, Manaus, Brazil. *N* = 15 stomachs.

Category of prey	Number	Volume (%)	Frequency
Acari	8	6.48	6 (40%)
Diptera	1	3.02	1 (2.35%)
Hemiptera	6	9.26	3 (20%)
Hymenoptera (Formicidae)	1	0.03	1 (2.35%)
Isoptera	1	0.10	1 (2.35%)
Coleoptera	2	2.37	2 (6.7%)
Orthoptera	4	61.30	3 (20%)
Unidentified Insecta	2	17.40	2 (6.7%)

which amplexus occurs in or near the calling site (Menin *et al.* 2004). The oviposition site of *H. cinerascens* resembles that described for other populations of this species, and the clutch is a gelatinous mass floating in the water (Duellman 1978). The number of eggs found in our study (~240 eggs) is less than that found in other studies carried out with this species in pristine forests (Crump 1974, Duellman 1978, 2005: 350–520 eggs (mean = 426); Aichinger 1992: 302–475 eggs (mean = 400); Rodríguez and Duellman 1994: 840 eggs; Lima *et al.* 2012: 400 eggs) and *H. punctatus* (310 eggs; Duellman 2005). Despite the few females found in this study, gravid females were found in both dry months (August and September) and rainy months (March and December) as reported by Duellman (1978). This feature, coupled with the annual pattern of calling activity of males, indicate annual reproduction as suggested by Hero (1990) and Lima *et al.* (2012), a pattern rarely found in most Amazonian hylid species (Bernarde 2007, Lima *et al.* 2012).

The diet and mean number of prey per stomach of *Hypsiboas cinerascens* found in our study is similar to that found by Parmelee (1999) in 12 individuals, which contained 10 prey items, from Cuzco Amazónico, in Peru. This author also recorded a low number of prey per stomach

(1.4), with orthopterans as the most important item in volume, a fact also reported for other hylid species (Parmelee 1999). Ants, dipterans and mites were also found in the stomachs of *H. cinerascens* from Peru (Parmelee 1999). The presence of a high percentage of empty stomachs (35%) in *H. cinerascens* and the low number of prey found in the stomachs may be related to differences in time of feeding, as suggested for sympatric species of *Dendropsophus* (Menin *et al.* 2005). Males of *H. cinerascens* perhaps feed after calling or alternate feeding nights with calling nights, fact recorded for other anuran species (Ryan 1985, Anderson *et al.* 1999).

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References

- Aichinger, M. 1992. Fecundity and breeding sites of an anuran community in a seasonal tropical environment. *Studies on Neotropical Fauna and Environment* 27: 9–18.
- Anderson, A. M., D. A. Haukos, and J. T. Anderson. 1999. Diet composition of three anurans from the Playa Wetlands of Northwest Texas. *Copeia* 1999: 515–520.
- Bernarde, P. S. 2007. Ambientes e temporada de vocalização da anurofauna no Município de Espigão do Oeste, Rondônia, Sudoeste da Amazônia - Brasil (Amphibia: Anura). *Biota Neotropica* 7: 87–92.
- Brower, J. E. and J. H. Zar. 1984. *Field and Laboratory Methods for General Ecology*. 2nd edition. Dubuque. William C. Brown Publishers. 288 pp.
- Colli, G. R., A. F. B. Araújo, R. Da Silveira, and F. Roma. 1992. Niche partitioning and morphology of two syntopic *Tropidurus* (Sauria: Tropiduridae) in Mato Grosso, Brazil. *Journal of Herpetology* 26: 66–69.
- Colwell, R. K. 2009. EstimateS: statistical estimation of species richness and shared species from samples. Version 8.2. URL <http://viceroy.eeb.uconn.edu/estimates>.
- Crump, M. L. 1974. Reproductive strategies in a tropical anuran community. *Miscellaneous Publications, University of Kansas Museum of Natural History* 61: 1–68.
- Duellman, W. E. 1978. The biology of an Equatorial herpetofauna in Amazonian Ecuador. *Miscellaneous Publications, University of Kansas Museum of Natural History* 65: 1–352.
- Duellman, W. E. 2005. *Cusco Amazónico, the lives of amphibian and reptiles in an Amazonian rainforest*. Ithaca. Cornell University Press. 433 pp.
- Frost, D. R. 2013. Amphibian Species of the World: an Online Reference. Version 5.6 (9 January 2013). Electronic database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA. Captured on 7 March 2013.
- Guimarães, L. D. and R. P. Bastos. 2003. Vocalizações e interações acústicas em *Hyla raniceps* (Anura, Hylidae) durante a atividade reprodutiva. *Iheringia, Série Zoologia* 93: 149–158.
- Haddad, C. F. B. and R. J. Sawayá. 2000. Reproductive modes of Atlantic forest hylid frogs: a general overview and the description of a new mode. *Biotropica* 32: 862–871.
- Hero, J. M. 1990. An illustrated key to tadpoles occurring in the Central Amazon rainforest, Manaus, Amazonas, Brasil. *Amazoniana* 11: 201–262.
- Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. A. C. Hayek, and M. S. Foster (eds.). 1994. *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians*. Washington. Smithsonian Institution Press. 364 pp.
- Hoogmoed, M. S. 1979. Resurrection of *Hyla ornatissima* Noble (Amphibia, Hylidae) and remarks on related species of green tree frogs from the Guiana area. *Zoologische Verhandelingen* 172: 1–46.
- Lima, A. P., W. E. Magnusson, M. Menin, L. K. Erdtmann, D. J. Rodrigues, C. Keller, and W. Hödl. 2012. *Guia de sapos da Reserva Adolpho Ducke, Amazônia Central / Guide to the frogs of Reserva Adolpho Ducke, Central Amazonia*. 2nd edition. Manaus. Editora INPA. 187 pp.
- Marques Filho, A. O., M. N. G. Ribeiro, H. M. Santos, and J. M. Santos. 1981. Estudos climatológicos de Reserva Florestal Ducke, Manaus, Amazonas. IV. Precipitação. *Acta Amazonica* 11: 759–768.
- Martin, P. and P. Bateson. 1993. *Measuring Behavior: an introductory guide*. 2nd edition. Cambridge. Cambridge University Press. 222 pp.
- Martins, M. and G. Moreira. 1991. The nest and the tadpole of *Hyla wavrini* Parker (Amphibia, Anura). *Memórias do Instituto Butantan* 53: 197–204.
- Martins, M., J. P. Pombal Jr., and C. F. B. Haddad. 1998. Escalated aggressive behaviour and facultative parental care in the nest building gladiator frog, *Hyla faber*. *Amphibia-Reptilia* 19: 65–73.
- Menin, M., R. A. Silva, and A. A. Giarretta. 2004. Reproductive biology of *Hyla goiana* (Anura, Hylidae). *Iheringia, Série Zoologia* 94: 49–52.
- Menin, M., D. C. Rossa-Feres, and A. A. Giarretta. 2005. Resource use and coexistence of two syntopic hylid frogs (Anura, Hylidae). *Revista Brasileira de Zoologia* 22: 61–72.
- Parmelee, J. R. 1999. Trophic ecology of a tropical anuran assemblage. *Scientific Papers of the Natural History Museum, University of Kansas* 11: 1–59.

- Reinke, M. and C. H. Deiques. 2010. História natural da espécie *Hypsiboas leptolineatus* (Anura: Hylidae) no Parque Nacional de Aparados da Serra, RS, Brasil. *Neotropical Biology and Conservation* 5: 188–196.
- Ribeiro, I. A. S., M. G. P. Araújo, and A. F. Santana. 2007. Palmeiras no Parque Municipal do Mindu, Manaus, Amazonas, Brasil. *Revista Brasileira de Biociências* 5: 888–890.
- Rodríguez, L. O. and W. E. Duellman. 1994. Guide to the frogs of the Iquitos region, Amazonian Peru. *Special Publication of the Natural History Museum, University of Kansas* 22: 1–80 + 12 plates.
- Rojas-Ahumada, D. P. and M. Menin. 2010. Composition and abundance of anurans in riparian and non-riparian areas in a forest in Central Amazonia, Brazil. *South American Journal of Herpetology* 5: 157–167.
- Ryan, M. J. 1985. *The Túngara frog. A Study in Sexual Selection and Communication*. Chicago and London. The University of Chicago Press. 230 pp.
- Toledo, L. F., O. G. S. Araújo, L. D. Guimarães, R. Lingnau, and C. F. B. Haddad. 2007. Visual and acoustic signaling in three species of Brazilian nocturnal tree frogs (Anura, Hylidae). *Phyllomedusa* 6: 61–68.
- Triplehorn, C. A. and N. F. Johnson. 2011. *Estudo dos Insetos*. São Paulo. Cengage Learning. 809 pp.
- Tsuji-Nishikido, B. M. and M. Menin. 2011. Distribution of frogs in riparian areas of an urban forest fragment in Central Amazonia. *Biota Neotropica* 11: 63–70.
- Zimmerman, B. L. and J. P. Bogart. 1984. Vocalizations of primary forest frog species in the Central Amazon. *Acta Amazonica* 14: 473–519.

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