

# Clutches, tadpoles and advertisement calls of *Synapturanus mirandaribeiroi* and *S. cf. salseri* in Central Amazonia, Brazil

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Species of the genus *Synapturanus* are small, burrowing frogs that inhabit the leaf litter of the rainforest floor. This genus is distributed in the northern region of South America and contains three species: *Synapturanus mirandaribeiroi*, *S. salseri* and *S. rabus*. In this study we describe the clutch, tadpole and advertisement call of two sympatric species (*S. cf. salseri* and *S. mirandaribeiroi*) that occur in Central Amazonia, Brazil. Both lay terrestrial eggs and their tadpoles hatch at a late developmental stage (approximately stage 42). Clutches and tadpoles of the two species are similar in size and appearance. The shape and coloration of newly metamorphosed individuals are similar to those of adults. The advertisement call of *S. cf. salseri* is a long, single, clearly pulsed, relatively pure tone between 900 and 1290 Hz, while that of *S. mirandaribeiroi* is a single, brief, nearly pure tone between 1094 and 1710 Hz. Advertisement calls of *Synapturanus* spp. are similar to those of *Myersiella microps*, and may be homologues. Both genera inhabit the forest floor, although their distributions are allopatric. Similarities in habitat and reproductive mode among *Synapturanus* spp. and *Myersiella microps* further corroborate the hypothesis of a close phylogenetic relationship between these genera.

*Key words:* Anura, eggs, larvae, Microhylidae, phylogeny, vocalizations

## INTRODUCTION

Frogs of the genus *Synapturanus* Carvalho, 1954 are small (17–33 mm), burrowing frogs which inhabit the leaf litter of the rainforest floor (Pyburn, 1976). This genus contains three species distributed in the northern region of South America. *Synapturanus mirandaribeiroi* Nelson and Lescure, 1975 occurs in lowland forests of northern Brazil, Colombia, Venezuela, Suriname and the Guyanas (Barrio & Brewer-Carías, 1999; Lescure & Marty, 2000; Frost, 2005). The type locality of this species is Kanashen (a Waiwai Indian village and mission) on the upper Essequibo River, Rupununi District, Guyana (Frost, 2005). *Synapturanus salseri* Pyburn, 1975 also occurs in lowland forests of Colombia, Venezuela and Brazil (Señaris et al., 2003; Frost, 2005), and the type locality of the species is Timbó, Vaupés, Colombia (Frost, 2005). *Synapturanus rabus* Pyburn, 1976, occurs in southeastern Colombia and adjacent Ecuador, and its type locality is also Vaupés, in Colombia (Frost, 2005).

Due to their fossorial habit, it is difficult to locate *Synapturanus* species in the wild. Thus, their natural history is poorly known. The call of *S. mirandaribeiroi* was described by Nelson (1973) and Lescure & Marty (2000), based on individuals collected in Suriname and Guyana, respectively. The call of *S. salseri* was described by Pyburn (1975) for the type locality of Vaupés, Colombia, and by Zimmerman & Bogart (1984) for the Manaus region (Brazil). Those two populations are approximately 1100 km apart and differences in advertisement calls were

noted (Zimmerman & Bogart, 1984). Pyburn (1975) briefly described the clutch, tadpoles and newly metamorphosed individuals of *S. salseri*. Hero (1990) gave a brief description of unidentified tadpoles found in the Manaus region, which could have been of either *S. mirandaribeiroi* or *S. salseri*. Apart from that one uncertain identification, nothing is known about clutches or tadpoles of *S. mirandaribeiroi*.

*Synapturanus salseri* and *S. mirandaribeiroi* are sympatric in Colombia (Pyburn, 1975). *Synapturanus mirandaribeiroi* and a species morphologically similar to *S. salseri* (hereafter *S. cf. salseri*, due to the differences in calls, tadpoles and adults) are both found near to Manaus, in the central Amazon region of Brazil, in reserves belonging to the Biological Dynamics of Forest Fragments Project (Zimmerman & Rodrigues, 1990) and in the Reserva Florestal Adolpho Ducke (Lima et al., 2006). Their vocalizations have been heard during periods of rainfall from October to May (Zimmerman & Bogart, 1984), and the species reproduces in nests formed by cavities below the leaf-litter layer (Pyburn, 1975).

Here we describe the tadpole, clutch, newly metamorphosed individuals and advertisement call of *S. cf. salseri* and *S. mirandaribeiroi* in central Amazonia, Brazil.

## MATERIALS AND METHODS

The study was conducted in the Reserva Florestal Adolpho Ducke (RFAD, 02°55'–03°01'S, 59°53'–59°59'W, 10,000 ha) located to the north of the city of Manaus,

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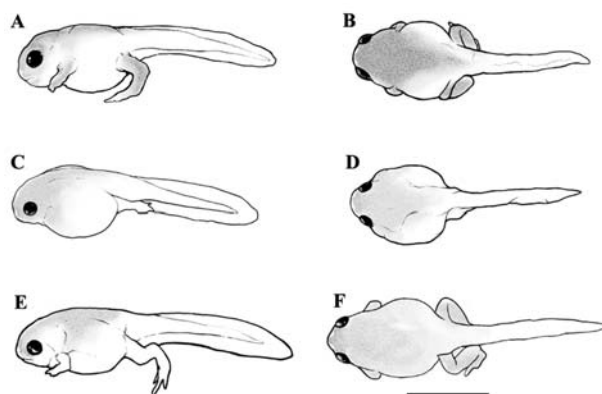
**Table 1.** Measurements (in mm) of tadpoles of *S. mirandaribeiroi* and *S. cf. salseri*. Mean±standard deviation; range in parentheses. \*indicates  $n=1$ .

Characteristics	<i>S.</i>		
	<i>mirandaribeiroi</i> Stage 42 ( $n=6$ )	<i>S. cf. salseri</i> Stage 41 ( $n=7$ )	Stage 42 ( $n=4$ )
Total length	15.61±0.45 (15.00–16.10)	12.61±0.64 (11.70–13.70)	17.32±1.04 (15.90–18.40)
Body length	6.31±0.23 (6.00–6.60)	5.37±0.22 (5.00–5.60)	6.67±0.30 (6.30–7.00)
Tail length	9.30±0.52 (8.60–10.00)	7.24±0.45 (6.70–8.10)	10.65±0.83 (9.60–11.60)
Body height	3.95±0.30 (3.60–4.50)	3.63±0.21 (3.30–4.00)	4.05±0.17 (3.90–4.30)
Body width	4.38±0.34 (4.00–4.90)	4.78±0.13 (4.60–5.00)	4.80±0.16 (4.60–5.00)
Maximum tail height	1.23±0.12 (1.10–1.40)	1.68±0.18 (1.50–2.00)	1.40±0.18 (1.20–1.60)
Tail muscle height	1.22±0.10 (1.10–1.30)	0.93±0.11 (0.70–1.00)	1.15±0.13 (1.00–1.30)
Tail muscle width	1.40±0.17 (1.20–1.60)	1.08±0.07 (1.00–1.20)	1.32±0.15 (1.20–1.50)
Interorbital distance	1.41±0.15 (1.20–1.60)	1.14±0.05 (1.10–1.20)	1.37±0.15 (1.20–1.50)
Eye–nostril distance	0.47±0.03 (0.43–0.50)	0.39±0.01 (0.37–0.40)	0.61±0.07 (0.53–0.67)
Maximum eye diameter	0.98±0.07 (0.90–1.10)	0.67±0.05 (0.60–0.70)	0.85±0.06 (0.80–0.90)
Maximum nostril diameter	0.32±0.03 (0.27–0.37)	0.34±0.03 (0.30–0.40)	0.28±0.02 (0.27–0.30)
Vent tube length	-	0.73±0.09 (0.60–0.80)	0.40*

Amazonas state, northern Brazil. The vegetation consists of “terra firme” rainforest, a well-drained forest not subject to seasonal flooding. The climate is characterized by a rainy season from November to May and a dry season during the rest of the year (Marques Filho et al., 1981). Mean annual temperature is approximately 26 °C (Marques Filho et al., 1981) and mean annual rainfall was 2489 mm between 1985 and 2004.

Fieldwork was carried out from October 2003 to November 2005. A 0.5 m<sup>2</sup> area was marked around the point at which each male ( $n=50$ ) was calling and the leaf and root layers were removed. This area was carefully excavated to a depth of approximately 10–20 cm. Seventeen adults were preserved in 10% formalin. Four clutches were kept in plastic basins with soil similar to that of the nest and maintained at approximately 25 °C in the laboratory. Seventeen tadpoles in developmental stages 41 and 42 (Gosner, 1960) were preserved in 5% formalin. Clutches were moistened daily until tadpoles reached metamorphosis. Sixteen newly metamorphosed individuals were preserved after reaching stage 46 (Gosner, 1960).

Larval description and variation was based on examination of specimens in Gosner stages 41 and 42 of *S. cf.*

**Fig. 1.** Tadpoles of *Synapturanus mirandaribeiroi* (stage 42: A and B) and *S. cf. salseri* (stage 41: C and D; stage 42: E and F). A, C and E: lateral view; B, D and F: dorsal view. Scale bar = 5 mm.

*salseri* and stage 42 of *S. mirandaribeiroi*. Descriptive terminology and morphometric measures followed those of Altig & McDiarmid (1999) and Anstis (2002). External measurements were taken with a grid calibrated for the 10× objective lens of a stereomicroscope to the nearest 0.01 mm.

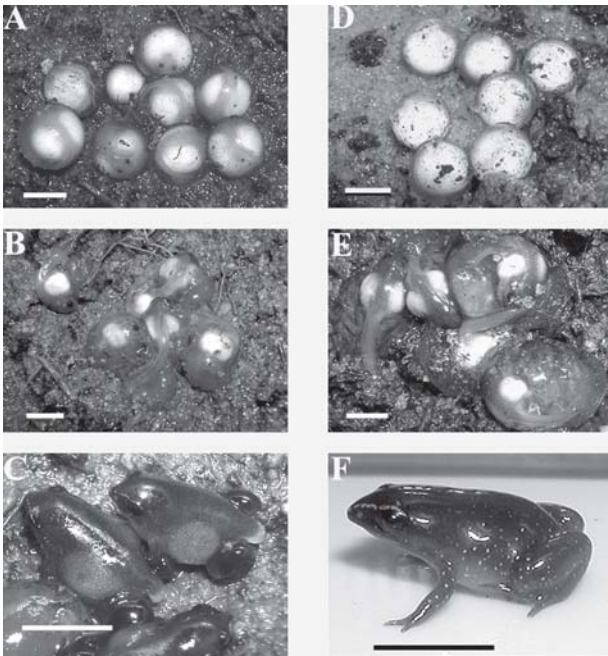
Vocalizations were recorded from October to November 2003 with a Sony TC-D5M tape recorder coupled to an external condenser microphone (TECT Model UEM-83). Air temperature was measured with a mercury thermometer (0.5 °C accuracy). Calls were analysed with Spectrogram 7 software (Horne, 2002) using 16 bit resolution, 22 and 44 kHz sampling frequencies and 1024 points FFT (fast fourier transform). Six individuals of *S. mirandaribeiroi* were recorded and one individual was recorded and collected (INPA-H 11834). Two individuals of *S. cf. salseri* were recorded and one individual was recorded and collected (INPA-H 11836).

The snout–vent length (SVL) of adult specimens was measured with a Vernier calliper (0.02 mm accuracy). Voucher specimens are housed in the Amphibians and Reptiles Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H), Manaus, Amazonas, Brazil (Appendix 1).

## RESULTS

### *Synapturanus mirandaribeiroi* Nelson and Lescure, 1975

*Description of the tadpole.* The description is based on six tadpoles in Gosner’s stage 42 (Gosner, 1960; INPA-H 15838) (Table 1). Body wider than deep, depressed in lateral view (Fig. 1A), ovoid in dorsal view (Fig. 1B). Body and tail 40.4% (range 37.9–42.7%) and 59.6% (range 57.3–62.1%) of total length, respectively. Snout truncated in dorsal view and rounded in profile. Eyes directed laterally. Aperture of nostrils is not visible. External nostrils visible as rounded whitish spot located dorsolaterally. Maximum nostril diameter 31.9% (range 23.3–37.7%) smaller than eye–nostril distance. Interorbital distance 30% (range 17.0–43.8%) greater than maximum eye diameter. Spiracle absent. Oral disc simple slit; feeding mouthparts absent.



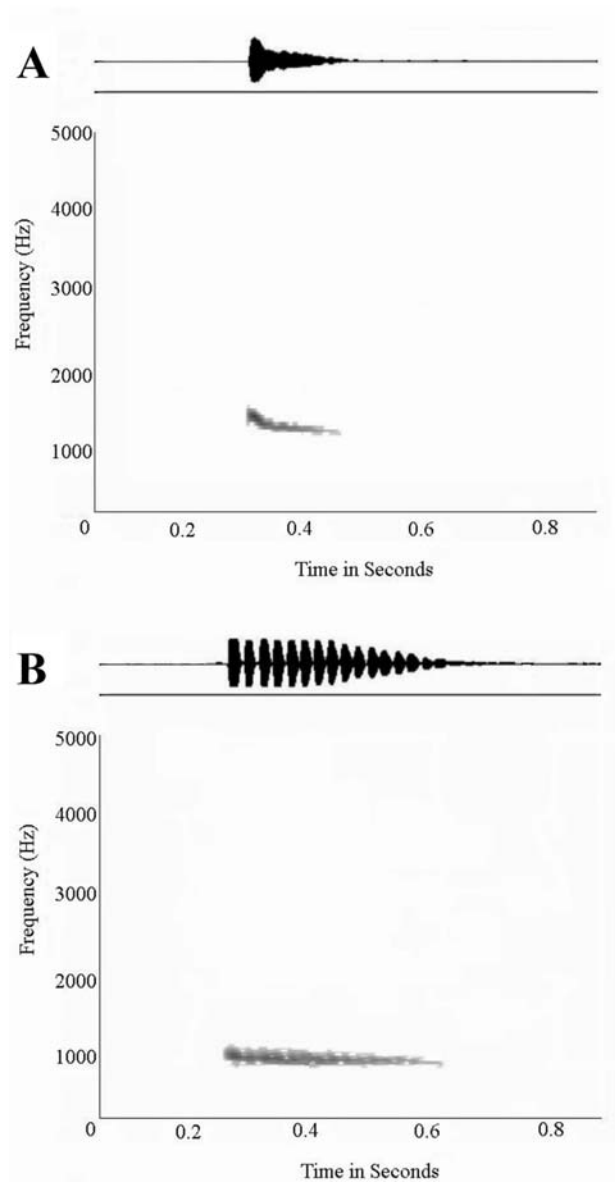
**Fig. 2.** Clutch, tadpoles and newly metamorphosed individuals of *Synapturanus mirandaribeiroi* (A–C) and *S. cf. salseri* (D–F). Scale bar = 5 mm.

Abdomen with large yolk sac. Vent tube absent at this stage.

Caudal muscle robust; higher than dorsal and ventral fins in the anterior third of the tail. Fins retained in only one tadpole; all other tadpoles had vestigial or no fins. Fins shallow; dorsal and ventral fin similar in height. Dorsal fin originating at the tail–body junction, ventral fin originating at the posterior ventral terminus of the body. Tail slightly arched, maintaining the same height throughout the anterior two-thirds, gradually narrowing into a rounded tip.

Head and dorsum reddish-brown in life, becoming brighter laterally, with a mottled brown pattern on the flanks and limbs; abdomen white to cream; skin and tail transparent; caudal musculature light brown. In preservative, dorsal colour greyish and abdomen cream. Chromatophores are distributed on the head, on the dorsal region of the body and on dorsal surfaces of the hind limbs and forelimbs. Chromatophores diminish gradually to the flanks and are absent on the ventral surfaces of body and tail, which are transparent. Presence of yolk throughout larval development. Newly metamorphosed individuals with brown dorsum, flanks comparatively brighter and a mottled brown pattern on the flanks and limbs.

**Adults** (see pictures in Lima et al., 2006). Seven males (SVL  $27.95 \pm 1.48$  mm; range 25.60–29.40 mm) and two females (SVL 29.20 and 31.12 mm) were collected. All but one of the seven males had a glandular boss on the wrist, the pale wrist boss contrasting with the darker arm colour. Dorsal coloration mottled with brown, light brown, orange or cream with many small cream spots on the dorsum and legs. A fine yellow line passes laterally from the snout over the eye. Belly whitish with creamish yellow on the



**Fig. 3.** Oscillogram (upper) and sonogram (lower) of the advertisement call of A) *Synapturanus mirandaribeiroi* (recorded on 25 November 2003, 1739, air temperature 27.5 °C) and B) *Synapturanus cf. salseri* (recorded on 28 October 2003, 1731, air temperature 26.5 °C).

edges of abdomen; the throat region has a similar pattern to the dorsum, but lighter. Tympanum completely concealed in all individuals.

**Reproductive biology.** One clutch with nine eggs was found on 13 November 2003 (Fig. 2A). Each egg was surrounded by a gelatinous capsule. In the laboratory the tadpoles hatched after reaching stage 42 (Fig. 2B). Only six individuals completed metamorphosis (SVL  $8.18 \pm 0.23$  mm; range 7.80–8.50 mm; stage 46) (Fig. 2C). Another clutch was found on 10 November 2005 and consisted of five hatched tadpoles plus a tadpole inside the egg. Both clutches were found in burrows, approximately 5–10 cm below the soil surface and within 5–20 cm of an adult male.

**Advertisement call.** The advertisement call recorded for seven males of *S. mirandaribeiroi* was a single, brief,

nearly pure tone concentrated between 1100 and 1700 Hz (Fig. 3A). The note reduced in frequency from beginning to end. Mean note duration was  $138.9 \pm 17.3$  ms (range 106–190 ms,  $n=55$  notes). There were two weak harmonics (not visible in Fig. 3A) at 2800 Hz and 3800 Hz. Mean interval between notes was  $5993.3 \pm 1437.9$  ms (range 3132–11080 ms,  $n=47$ ) and the call rate was 9 notes/minute (mean  $9.4 \pm 1.6$  notes/minute; range 7–12 notes/minute,  $n=7$ ).

### *Synapturanus* cf. *salseri* Pyburn, 1975

**Description of the tadpole.** The description is based on four tadpoles in Gosner's stage 42 (INPA-H 15837) (Table 1). Body wider than deep, depressed in lateral view (Fig. 1E) and ovoid in dorsal view (Fig. 1F). Body and tail 38.5% (range 36.9–40.0%) and 61.5% (range 60.0–63.0%) of total length, respectively. Snout truncated in dorsal view and rounded in profile. Eyes directed laterally. Nostril aperture not visible. External nostrils consisting of a rounded whitish spot located dorsolaterally. Maximum nostril diameter 52.8% (range 43.3–59.7%) smaller than eye–nostril distance. Interorbital distance 37.2% (range 25.0–46.6%) greater than maximum eye diameter. Spiracle absent. Oral disc a simple slit; no feeding mouthparts. A large yolk sac in the abdomen. Vent tube medial, present in one individual at this stage.

Caudal muscle robust; higher than dorsal and ventral fins in the anterior third of the tail. Fins retained in two tadpoles; the other individuals had vestigial or no fins. Fins shallow; dorsal and ventral fins similar in height. Dorsal fin originating after one-third of the tail length; ventral fin originating at the posterior ventral terminus of the body. Tail slightly arched, maintaining the same height throughout the anterior two-thirds, gradually diminishing to the rounded tip.

Head and dorsum reddish-brown in life, lightening laterally, with a mottled brown pattern on the flanks and limbs; abdomen white to cream; skin and tail transparent; caudal musculature light brown. In preservative, dorsal colour greyish tan and abdomen cream or yellowish cream. Chromatophores are distributed on the dorsal region of the head and body, dorsal surfaces of the limbs and anterior region of the tail. The chromatophores diminish gradually to the flanks and are absent on ventral surfaces of the body and tail, which are opaque. Presence of yolk throughout larval development. Newly metamorphosed individuals (Fig. 2F) had brown coloration with numerous light cream to orange spots dorsally over the dorsum, arms and legs.

**Variation.** In stage 41 (Fig. 1C,D; INPA-H 15834), body and tail 42.6% (range 40.9–43.7%) and 57.4% (range 56.2–59.1%) of total length, respectively. Nostrils located in frontal area of the snout. Vent tube medial, present in all individuals. Fins present in all individuals. Chromatophores unevenly scattered, distributed on head and dorsal region of the body; tail opaque.

**Adults** (see pictures in Lima et al., 2006). Eight males (SVL  $28.59 \pm 1.62$  mm; range 26.10–30.60 mm) were collected. All of the males had a glandular boss on the wrist, the pale wrist boss contrasting with the darker arm colour. Dorsal coloration mottled with brown, light brown and orange,

with many small cream spots on the dorsum and legs. Belly white with pink borders; throat region has a similar pattern to the dorsum, but lighter and more pink. Tympanum completely concealed in all individuals.

**Reproductive biology.** Five clutches of *S. cf. salseri* were found on 28 and 30 October 2003 and on 12 April 2004, each containing approximately eight eggs (mean  $8.4 \pm 0.55$  eggs). There was a transparent gelatinous capsule surrounding each egg (Fig. 2D). Two clutches were preserved and the tadpoles were in stage 41 of Gosner (1960). In the remaining clutches, the tadpoles hatched at a late stage of development (approximately stage 42, Fig. 2E), and newly metamorphosed individuals had an SVL of  $9.05 \pm 0.38$  mm (range 8.40–9.60 mm;  $n=10$ ; stage 46) (Fig. 2F). All clutches were found in burrows, approximately 5–10 cm below the soil surface and within 20 cm of an adult male.

**Advertisement call.** The advertisement call recorded for three males of *S. cf. salseri* was a single, long, clearly pulsed, relatively pure tone concentrated between 900 and 1290 Hz (Fig. 3B). The note reduced slightly in frequency range from beginning to end. Mean note duration was  $283.7 \pm 30.1$  ms (range 254–354 ms,  $n=14$  notes). There were two weak harmonics (not visible in Fig. 3B) at 2300 Hz and 3400 Hz. Mean interval between notes was  $8895.4 \pm 2584.9$  ms (range 6506–15,464 ms,  $n=11$ ) and the call rate was 6 notes/minute (mean  $5.9 \pm 1.1$  notes/minute, range 5–7 notes/minute,  $n=3$ ).

## DISCUSSION

The clutches and froglets of *S. cf. salseri* described here are similar to those described by Pyburn (1975), which were collected in the type locality in Colombia. However, the tadpoles differ in two characteristics. Pyburn (1975) found a downward curve at the end of the tail in individuals in stage 41. This characteristic was not observed in individuals from Manaus. Moreover, the total length of the individuals at stage 41 from Colombia was much greater (23.7 mm, one individual) than that observed in this study ( $12.6 \pm 0.6$  mm,  $n=7$ ). The advertisement call of *S. cf. salseri* described here is similar in duration, frequency and call rate to that described by Zimmerman & Bogart (1984) for other specimens from Manaus, Brazil. However, the frequency is lower than that described by Pyburn (1975) for specimens from the type locality (1400 Hz in Colombia versus 900 and 1290 Hz described here). Both studies also noted the two weak harmonic frequency bands. The observed differences may reflect differences between both populations, but the form from Manaus could also be a new species.

The presence of adult males near clutches was also noted by Pyburn (1975) for *S. salseri* from Colombia, suggesting that perhaps males from both species perform parental care.

The clutch, tadpoles and newly metamorphosed individuals of *S. mirandaribeiroi* are described here for the first time. They resemble those of *S. cf. salseri*, differing however in total length (tadpoles of *S. cf. salseri* are longer; see Table 1) and eye diameter (greater in *S.*

*mirandaribeiroi*; see Table 1). The advertisement call of *S. mirandaribeiroi* is similar in duration and frequency to that described by Nelson (1973) from Suriname and Lescure & Marty (2000) from Guyana. Nelson (1973) also noted the presence of two weak harmonic frequency bands.

The lack of a spiracle in both species suggests that respiration takes place by diffusion. The lack of feeding mouthparts is probably related to the presence of yolk in their gut throughout larval development. The absence of a beak and keratinized mouthparts has been observed in another neotropical microhylid frog, *Syncope antenori* (Krügel & Richter, 1995). The lack of spiracle and the absence of feeding mouthparts have also been found in *Assa darlingtoni*, a Myobatrachidae frog from Australia that shows terrestrial reproduction (Ingram et al., 1975). The tadpoles of *Synapturanus* spp. resemble other microhylid tadpoles in having unperforated nares that do not open into the internal nares until metamorphosis (Krügel & Richter, 1995).

Newly metamorphosed individuals of both *S. cf. salseri* and *S. mirandaribeiroi* closely resemble the adults in both body form and coloration. The newly metamorphosed individuals of *S. cf. salseri* can be differentiated from those of *S. mirandaribeiroi* by size (*S. cf. salseri* are bigger, see Table 1) and coloration (only individuals of *S. cf. salseri* have numerous light-cream to orange spots dorsally over the back, arms and legs).

Besides the differences in tadpole morphology and calls (see above), the adult males of *S. cf. salseri* reported in this study have greater SVL (28.6 mm; range 26.1–30.6 mm) than those described from the type locality in Colombia (mean 25.1 mm; range 23.7–26.4 mm; Pyburn, 1975). The pattern of dorsal coloration (brown with light cream spots over the back, arms and legs) and ventral (white/cream with throat region light brown) is similar to that described for specimens from Colombia. However, in individuals from Colombia, the cream spots in the dorsum are more evident (see Fig. 1 in Pyburn, 1975). Traditionally, the species under study has been considered as *Synapturanus salseri* (Zimmerman & Bogart, 1984; Zimmerman & Rodrigues, 1990; Lima et al., 2006), but the many differences found between the populations of the type locality and that from Manaus indicate that these two populations may be separate species.

Both *S. cf. salseri* and *S. mirandaribeiroi* have terrestrial eggs, tadpoles that hatch at a late stage of development and metamorphosis in terrestrial nests (Hero, 1990; Wild, 1995; this study). The only other neotropical Microhylidae genus known to present terrestrial development is *Myersiella* Carvalho, 1954, a species that occurs in coastal Atlantic forest of southeastern Brazil (Frost, 2005) and whose larva also hatches before reaching metamorphosis (Izecksohn et al., 1971). The advertisement calls of *Synapturanus* spp. are similar to those of *Myersiella microps*, as observed by Wild (1995) and Hartmann et al. (2002), both being composed of a one-note whistle. Carvalho (1954) and Wild (1995), based on characteristics of skull, call and reproduction, suggested *Myersiella* and *Synapturanus* to be closely related gen-

era, and it has been proposed that the similarities between their calls are a result of shared basal characters (Nelson, 1973). The similarities in calls, habitat and reproductive mode corroborate the hypothesis of a close phylogenetic relationship between those genera, as proposed by Wild (1995).

## ACKNOWLEDGEMENTS

We thank W.E. Magnusson and M. Gordo for reviewing a previous version of this manuscript, J. Lopes and J. Nascimento for field assistance, B. Marshall for English review, K. Mokross for the drawing, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) for collecting permits (#027/02, 036/03, 099/04, 095/05 and 062/05) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)/PNOPG (#550651/01-2, #471453/03-0) for financial support. This work was supported by graduate fellowships from CNPq to D.J.R. and Coordenação de Aperfeiçoamento de Pessoal em Nível Superior (CAPES) to M.M.

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## APPENDIX 1: VOUCHER SPECIMENS

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Accepted: 22 August 2006