A New Species of *Hypsiboas* from the Atlantic Forest of Southeastern Brazil (Amphibia: Anura: Hylidae)

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We describe a new species of *Hypsiboas* of the *Hypsiboas pulchellus* species group from highland streams of the Atlantic Forest of southeastern Brazil. Vocalizations, egg-mass, and tadpole are also described. We compared the new species with other species of the *Hypsiboas pulchellus* species group. The new species is most similar to *Hypsiboas semiguttatus*, its sister species, and *Hypsiboas curupi*, from which it differs in advertisement call and larval morphology. We provide information on natural history, phylogenetic relationships, embryos, geographic distribution, and conservation.

ITH 31 described species, the *Hypsiboas pulchellus* group is the most speciose group of the recently resurrected genus Hypsiboas Wagler, 1830 (Faivovich et al., 2005; Garcia et al., 2007). This group is widely distributed in South America (B. Lutz, 1973; Duellman et al., 1997; Faivovich et al., 2004, 2005), and its reproduction occurs in streams or ponds in forests or open areas, generally in mountainous regions (B. Lutz, 1973; Duellman et al., 1997; Kwet and Di-Bernardo, 1999). Even though some morphological characterizations of the Hypsiboas pulchellus group had been advanced by B. Lutz (1973) and Duellman et al. (1997; e.g., body moderately robust, hypertrophied forelimbs, projecting prepollical spines in adult males, a supralabial bright stripe, and a dark dorsolateral line delimited superiorly by a bright stripe), some of these are plesiomorphies for the group (e.g., projecting prepollical spines in adult males) and the polarity of others is unclear (Faivovich et al., 2004, 2005). The group includes several species with a confusing taxonomic history, which have been the focus of recent papers (Duellman et al., 1997; Garcia et al., 2001, 2003, 2007).

Hypsiboas semiguttatus was described by A. Lutz (1925) from a specimen collected in the highlands of Serra do Mar at Santa Catarina State, Brazil. Kwet and Di-Bernardo (1999) and Garcia et al. (2003) considered *Hypsiboas semiguttatus* as a widely distributed species. Herein, we describe a new species from the highland streams of the Atlantic Forest at Serra do Mar, State of São Paulo, Brazil. The new species closely resembles *Hypsiboas semiguttatus* and other species from the same clade. Vocalizations, clutch, and tadpole are also described and we provide information on embryos, natural history, geographic distribution, and conservation. We also include the species within the phylogenetic framework of the *H. pulchellus* group advanced by Faivovich et al. (2004, 2005).

MATERIALS AND METHODS

Adult specimens were manually collected, fixed in 10% formalin, and maintained in 70% ethyl alcohol. Adults were measured with calipers to the nearest 0.1 mm. Measurements of adults follow Duellman (2001). Abbreviations used for the measurements of adults are: SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), EN

(eye-nostril distance), NS (nostril-snout distance), IN (internarial distance), IO (interorbital distance), TD (tympanum diameter), FAL (forearm length), FAB (forearm breadth), HAL (hand length), THL (thigh length), TIL (tibia length), TAL (tarsal length), FL (foot length), and DAD (diameter of disk of finger III). Standards for measuring snout shape follow Heyer et al. (1990). Webbing formulae follows the terminology of Savage and Heyer (1967) as modified by Myers and Duellman (1982). Tadpoles and eggs were fixed and preserved in 5% formalin. Tadpole measurements were made using a caliper to the nearest 0.1 mm and an ocular micrometer in a Zeiss stereomicroscope. Measurements of tadpoles follow McDiarmid and Altig (1999). Tadpole stages follow Gosner (1960) and tooth row formulae follow Altig (1970). Cement gland shape follows terminology of Nokhbatolfoghahai and Downie (2005). The specimens are housed in the collection Célio F. B. Haddad (CFBH), Departmento de Zoologia, Universidade Estadual Paulista, Rio Clara, São Paulo, Brazil.

Vocalizations were recorded in the field with Marantz PMD222 or Sony TCM 82V tape recorders equipped with Sennheiser K6, AudioTechnica AT835b, or Le Son SM-48 microphones. The vocalizations were analyzed and edited using the Raven software (Charif et al., 2004). Calls were edited at sampling frequencies of 22 and 44 kHz, and analyzed with fast Fourier transform (FFT) of 128 points. In order to characterize the geographic distribution, we searched for populations of the new species in other locations surrounding the type locality, mainly from the drainages in some regions of the Serra de Paranapiacaba, a segment of the Serra do Mar, in Southeastern Brazil.

To study the relationships of the new species with the *Hypsiboas pulchellus* group, and considering previous results of Faivovich et al. (2004, 2005), we designed an analysis solely for this group. The specific sampling for the group is the same as that included by Faivovich et al. (2005), with the addition of the new species. For the outgroups we included one exemplar of every species group of *Hypsiboas* recognized by Faivovich et al. (2005), plus two species of *Aplastodiscus*, one of which is used as the root.

Whole cellular DNA was extracted from ethanol-preserved tissues using the DNeasy (QIAGEN, Valencia, CA) isolation

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kit. Amplification was carried out in a 25-µl-volume reaction using puRe Taq Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ). We used the following primer pairs for DNA amplification and sequencing (see Faivovich et al., 2005, for primer sequences): MVZ59-MVZ50, L13-Titus I, L2A-H10, AR-BR (12s, tRNA Valine, 16s), MVZ15-H14149(H) (Cytochrome b), Rhod1A-1C (Rhodopsin), R1 GFF-GFR (RAG1), SIA 1-2 (Seventh in Absentia), 28SV-JJ (28s large ribosomal subunit). For all the amplifications, the PCR program included an initial denaturing step of 30 seconds at 94°C, followed by 35 or 45 cycles of amplification (94°C for 30 seconds, 48-60°C for 60 seconds, 72 for 60°C seconds), with a final extension step at 72°C for 6 min. Polymerase chain reaction (PCR)-amplified products were desalted and concentrated using an ArrayIT PCR Product Purification Kit (TeleChem International Inc., Sunnyvale, CA), and labeled with fluorescent-dye labels terminators (ABI Prism Big Dye Terminators v. 3.0 cycle sequencing kits; Applied Biosystems, Foster City, CA). The labeled PCR products were cleaned using cleanSEQ (Agencourt Biosciences, Beverly, MA). The products were sequenced with an ABI 3730XL (Applied Biosystems, Foster City, CA). The samples were sequenced in both directions. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher 3.0. (Gene Codes, Ann Arbor, MI). Complete sequences were edited with BioEdit (Hall, 1999). The fragment of tyrosinase could not be amplified for this species; it was included in the analysis as well for all other terminals for which it has been previously sequenced (Faivovich et al., 2005).

The phylogenetic analysis was done using the program POY (vers. 3.0.12a, W. C. Wheeler, D. S. Gladstein, and J. De Laet, American Museum of Natural History, New York, NY). The rationale for using parsimony as an optimality criterion was advanced by Farris (1983) and recently discussed, among others, by Goloboff (2003) and Goloboff and Pol (2005). The preference for the treatment of sequence data as dynamic homologies simultaneously with tree search, as opposed to static homology hypotheses (multiple alignments) independent of tree search, has been justified by Wheeler (1996, 2002) and De Laet (2005).

We obtained a quick consensus estimation (Goloboff and Farris, 2001), as done by Faivovich et al. (2005), and used that topology as a constraint for building 200 Wagner trees followed by a round of tree bissection and reconnection (TBR) branch swapping. The final trees were submitted to a final round of swapping without the constraint, using iterative pass optimization (Wheeler, 2003). Bremer supports (Bremer, 1988) were calculated with POY; parsimony Jacknife values (Farris et al., 1996) were estimated using TNT (Goloboff, 2003) on the basis of the implied alignment generated by POY. Tree edition was done with Winclada (ver. 0.9.9 [Beta], K. C. Nixon, Winclada, Ithaca, New York, 1999).

Hypsiboas caipora, new species

Figure 1

Holotype.—CFBH 7312, Brazil, São Paulo, Serra do Mar, Serra de Paranapiacaba, Municipality of Pilar do Sul, adult male, 23°56′S, 47°40′W, approx. 780 m above sea level, 11 June 2004, A. P. Antunes, D. C. Briani, and A. T. Silva.

Paratypes.—CFBH 5738–5742, 7313–7318, 8292–8297 (adult males); 5743, 10873 (adult females); 7319–20 (juveniles);

August 2002–December 2005, A. P. Antunes, C. F. B. Haddad, F. L. Pincinato, F. Rohe, R. Belmonte-Lopes, and E. Dahora.

Diagnosis.—A species included in the Hypsiboas pulchellus group and characterized by the following combination of characters: (1) small to medium size (SVL 29.7-37.5 mm in males and 37.6-44.3 mm in females); (2) body moderately robust; (3) beige, light brown, or golden color on background, with or without dark brown spots or stripes on head, vertebral, and/or dorsolateral areas, or with large asymmetrical blotches that in some specimens cover almost whole dorsum; (4) flanks with small bright spots, cream or golden; (5) uniform pattern on the concealed parts of thigh; (6) advertisement call with a metallic sound and distinct acoustic parameters (18 analyzed calls of at least eight males), composed by 14-48 pulses (32 \pm 10), frequency range of 1.33–3.89 kHz, dominant frequency of 2.50–2.93 kHz (2.67 \pm 0.12), and duration of 149.3–408.2 ms (249.0 \pm 73.2); (7) tadpoles with a rostral gap in marginal papillation and tooth row formula 2(2)/4(1); (8) reproduction in mountain streams of the Atlantic Forest.

Comparisons.—Compared with Hypsiboas alboniger, H. cordobae, H. cymbalum, H. joaquini, H. melanopleurus, and H. riojanus, H. caipora is smaller, without overlap in SVL (B. Lutz, 1973; Duellman et al., 1997; Garcia et al., 2003). Hypsiboas balzani, H. cordobae, H. cymbalum, H. ericae, H. guentheri, H. joaquini, H. marginatus, H. marianitae, H. prasinus, and H. pulchellus have or may have a predominant green dorsal coloration (B. Lutz, 1973; Duellman et al., 1997; Caramaschi and Cruz, 2000; Garcia et al., 2003), not observed in H. caipora. The new species differs from Hypsiboas alboniger, H. andinus, H. balzani, H. bischoffi, H. caingua, H. cordobae, H. marianitae, H. prasinus, H. pulchellus, *H. riojanus*, and *H. secedens*, by the absence of any pattern on posterior surface of thighs (B. Lutz, 1973; Duellman et al., 1997). Unlike Hypsiboas caipora, H. melanopleura and H. palaestes lack blotches on the flanks. Hypsiboas freicanecae has a metallic triangle on dorsum of snout (Carnaval and Peixoto, 2004), absent in *H. caipora*. From *Hypsiboas beckeri*, H. buriti, H. cipoensis, H. goianus, H. latistriatus, H. leptolineatus, H. phaeopleura, H. polytaenius, and H. stenocephalus, H. caipora differs by the absence of well-defined longitudinal stripes on the dorsum and by its more robust body. Besides being larger, *Hypsiboas joaquini* shows white bright blotches on limbs, absent in H. caipora; moreover, H. joaquini inhabits open areas, whereas H. caipora inhabits forested areas. Hypsiboas caipora is most similar with H. semiguttatus and mainly with H. curupi, from which it differs in duration of advertisement call of the former, and dominant frequency and larval morphology of later. The duration of the advertisement call in Hypsiboas semiguttatus (437.7-917.0 ms, n = 19; Garcia et al., 2007) is longer than that of *H. caipora* (149.3–408.2 ms, n = 18) and has pulses more irregularly spaced. Furthermore, Hypsiboas caipora has more robust body and its bright spots on flanks are less evident than in H. semiguttatus. The advertisement call of Hypsiboas caipora has higher frequencies, with dominant frequency of 2.50–2.93 kHz (2.67 \pm 0.12, n = 18), while in *H. curupi* the dominant frequency is 1.2–2.2 kHz (1.7 \pm 0.4, n = 12; Garcia et al., 2007). In addition, the tadpole in H. caipora has rostral papillation with a gap (continuous papillation in *H*.



Fig. 1. Hypsiboas caipora. Holotype (CFBH 7312)-adult male in life.

curupi), and 2/4 labial tooth row formula (vs. 3/5 in *H. curupi*; Faivovich, 1996, as *Hyla semiguttata*).

Description of holotype.-Body moderately robust; head slightly wider than long; head length about 35% of SVL; snout rounded in dorsal view and nearly rounded to truncate in profile; pupil horizontal; canthus rostralis distinct; nostrils slightly protuberant, oval and directed laterally; internarial distance slightly smaller than eye-nostril distance; loreal region concave; vocal sac subgular; tympanum distinct, its diameter nearly 35% of eye diameter (Figs. 2A-2B). Dorsal and flank skin texture smooth; ventral skin texture slightly granular; scars on the dorsum and flanks. Hypertrophied forearms and projecting prepollical spines; ulnar fold present with small tubercles linearly distributed on posterior region of forearms; no nuptial pads; hand large; palmar surface with many small supernumerary tubercles; inner and outer metacarpal tubercles indistinct; relative length of fingers I <II < IV < III; subarticular tubercles single and rounded; discs of fingers well developed; width of disc on finger III larger than tympanum diameter (about 115%); hand webbing formulae I–II (2⁺–3⁻) III (2.5–2⁺) IV; disc of finger I smaller than in other fingers (Fig. 2C). Hind limbs elongate and slender; thigh length slightly longer than tibia length and about 55% of SVL; plantar surface with many small tubercles; relative length of toes I < II < V < III < IV; discs of toes well developed; foot webbing formulae I (2⁻-2⁺) II (1–2.5) III (1.5–3^{1/2}) IV (2.5–1) V; inner metatarsal tubercle present and oval; outer metatarsal tubercle absent; subarticular tubercles single and rounded (Fig. 2D).

Coloration in life.—Dorsal background color light brown to slightly golden, with supernumerary small dark brown spots and large asymmetrical dark brown blotches. Iris golden; region between eye and nostril with dark brown canthal stripe; supralabial stripe cream to golden, extending from the snout to the insertion of arm, less evident on snout tip and fragmented near the tympanum; a dorsolateral dark brown stripe from eyelid to inguinal region delimited superiorly by a bright cream to golden stripe. Flanks brown to grayish, with small bright spots, cream to golden; limbs background color light brown with supernumerary small dark brown spots, except on the prepollex; nearly round dark brown blotches on the surfaces of limbs, mainly on tibia and forearm; ulnar fold cream; a longitudinal dark brown line delimited anteriorly by a thin beige line on thigh, from vent to knee; lack of any pattern on the hidden surfaces of thigh; outer region of tibia and forearm with a



Fig. 2. Hypsiboas caipora. Holotype (CFBH 7312). (A) Head in dorsal view; (B) head in lateral view; (C) left hand in ventral view; (D) left foot in ventral view.

longitudinal stripe often dark brown. Region surrounding the vent with small beige spots; ventral surfaces white to cream; gular region darker compared to the ventral coloration; bones green.

Coloration in preservative.—In 70% ethanol dorsal and limbs coloration become grayish-brown. Supralabial and dorsolateral stripes become white. After some time, blotches on dorsum become less evident, as well as the green color, which tends to disappear.

Measurements of holotype (in mm).—SVL 33.0; HL 11.6; HW 12.0; ED 4.2; EN 2.6; NS 1.5; IN 2.7; IO 6.8; TD 1.5; FL 6.7; FB 3.3; HAL 11.1; THL 18.4; TIL 18.1; TAL 11.2; FL 15.3; DAD 1.7.

Variation.—Variation in size of *Hypsiboas caipora* is shown in Table 1. Among 19 paratypes three have uniform beige to light brown dorsal pattern (Fig. 3A); within the same light colored dorsal background, there are individuals with sinuous dorsolateral dark brown lines barely visible and with variable extension, from inguinal region to the insertion of arms, recorded in specimens not collected (Fig. 3B); eight have dark brown spots and/or stripes concentrated on head and on vertebral and/or dorsolateral area (Figs. 3C–3F); five have ornamented pattern with dark spots that coalesce to form blotches of irregular size and shape on dorsum, frequently covering part of head, except snout (Figs. 3G–3J); in three individuals the dark brown blotches cover almost all dorsum (Figs. 3K–3L). The space between eye and nostril generally has the background color

 Table 1. Measurements in Millimeters (Range, Mean, and Standard Deviation) of *Hypsiboas caipora*, New Species.

്ര (<i>n</i> = 18)				
Characters	Range	Mean	Standard deviation	çç (n = 2)
SVL	29.7–37.5	33.4	2.3	37.6–44.3
HL	10.3-12.4	11.6	0.7	13.2-14.9
HW	10.9-12.7	11.8	0.6	13.8–15.2
ED	3.2-4.4	4.0	0.2	4.0-4.8
EN	2.2-3.1	2.5	0.2	3.0-3.5
NS	1.3-1.8	1.5	0.2	1.7-1.9
IN	2.4-2.9	2.7	0.2	3.0-3.3
IO	6.2-6.9	6.4	0.2	7.0-8.3
TD	1.3-1.7	1.5	0.1	1.8-2.2
FAL	5.7-7.2	6.4	0.3	7.7–7.9
FAB	2.6-4.2	3.2	0.3	3.4-3.5
HAL	10.3-12.4	11.1	0.5	12.5-13.6
THL	16.0-20.0	17.9	0.8	20.3-21.5
TIL	15.5-19.2	17.3	0.8	20.1-20.3
TAL	9.5-11.9	11.0	0.6	12.7-12.9
FL	13.1–16.3	14.5	0.8	16.0-19.8
DAD	1.5–2.0	1.7	0.1	1.9–2.3

(beige to light brown) and may have some small dark brown spots (Fig. 3L). Figure 3 shows the variation on the color pattern from specimens photographed in the field. The dorsolateral dark and bright stripes have variable width and may be fragmented in the inguinal region. Gular region sometimes darker compared to ventral coloration. The concentration of biliverdin in tissues is variable among specimens, determining that in some specimens bones do not appear green at all. Females do not show hypertrophied forearms and the prepollex is smaller. Females can have a slightly olivaceous coloration distributed on background (n = 2), not observed in males. Variation in hand and foot webbing formulae are respectively: I–II $(2^+-2^+)-(2^--3^-)$ III $(2.5-2^{-})-(2^{-}-2^{-})$ IV and I $(1.5-2^{+})-(2^{-}-2^{-})$ II $(1-2^{+})-(1-2.5)$ III $(1-2^{\frac{1}{2}})-(1.5-2^{-})$ IV $(2^{\frac{1}{2}}-1)-(2.5-1)$ V. The dissection of the referred specimen CFBH 14198 indicates the absence of the slip of the muscle depressor mandibulae that originates at the level of the m. dorsalis scapulae.

Vocalizations.—The advertisement call is a metallic sound and consists of one short single note (single advertisement call; Fig. 4A) and less frequently of two notes (complex advertisement call; Fig. 4B). At an air temperature of 16– 20°C, the single advertisement calls (18 calls analyzed of at least eight males) are composed of 14–48 pulses (32 ± 10), with frequency between 1.33–3.89 kHz, dominant frequency of 2.50–2.93 kHz (2.67 \pm 0.12), and duration of 149.3– 408.2 ms (249.2 \pm 73.2). Harmonics were not always evident in the spectrogram. The advertisement call has, in general, ascendant frequency and intensity, with one peak of energy concentrated near the final part of the call. The pulses are frequently overlapped.

The complex advertisement call (two analyzed calls of one male) at an air temperature of 4°C consists of one first note, apparently the same note of the single advertisement call, with duration of 335–385 ms, 32–35 pulses, frequency range of 1.44–3.68 kHz, and dominant frequency of 2.42–2.75 kHz. The second note had a duration of 146–193 ms,

13–15 pulses, frequency range of 1.54–3.68 kHz, and dominant frequency of 2.40–2.75 kHz (Fig. 4B). This call was recorded for isolated and aggregated males, but was conspicuous at courtship.

Territorial calls were emitted during agonistic interactions between males and consisted of a single note (single territorial call; Fig. 4C) or two notes with short duration (complex territorial call; Fig. 4C). At an air temperature of 19°C, duration of the single territorial call (four calls analyzed of one male) was $31.5-42.4 \text{ ms} (36.6 \pm 4.5)$, frequency range of 1.21-3.33 kHz, and dominant frequency of 2.07–2.42 kHz (2.21 \pm 0.17). In the complex territorial call (two analyzed calls of one male), at an air temperature of 19°C, the first note had a duration of 31.5-34.5 ms, frequency range of 1.28-2.89 kHz, and dominant frequency of 1.90-2.07 kHz. The duration of the second note was 25.4-26.9 ms, frequency range of 1.35-2.93 kHz, and dominant frequency of 1.89-2.07 kHz. The duration of the complex territorial call was approximately 144-158 ms. Territorial calls had descendent intensity and frequency.

Distress calls (Fig. 4D) were recorded when a male was collected by its legs. The calls were variable, even in the same individual, mainly in relation to duration (62–640 ms), dominant frequency (2.75–7.57 kHz), and number of pulses (37–256). This call has harmonic structure with sound energy distributed over a wide range of frequencies (1.0–15.9 kHz).

Natural history.—Adult males were registered calling in all months along the year. They call at night from the marginal vegetation of streams inside the forest, generally at heights varying from 0.5 to 1.5 m above water. Males start to emit advertisement calls during the sunset and cease during the sunrise, with one peak in the dusk and the other in the morning. Males vocalize sporadically; intervals between the advertisement calls were long, lasting from few minutes to hours. Eventually males vocalize in chorus, apparently stimulated by nearby calling individuals. Most adult males have scars on the dorsum, likely caused by prepollical spines of other males during territorial fights, as commonly occur in other species of Hypsiboas (for a review see Faivovich et al., 2005). Amplected females carried the males to the water. Then they submerged and placed the spawn generally in roots of the marginal vegetation. Reproduction seems to occur throughout the year, and from 2002 to 2006 was recorded in January, March, May, July, August, September, November, and December.

During the day, reproductive males hid mainly in the leaf litter, near the margins of the streams and rarely emitted vocalizations. Defensive behaviors comprise primarily crypsis and immobility; secondarily, they include liquid cloacal discharges, escaping to ground or water, inflating the lungs, aggression with the prepollical spine, tanathosis, distress calls, and a peculiar odor. An adult male was preyed by the snake *Tropidophis paucisquamis* (Serpentes: Tropidophiidae) in the field.

Tadpoles aggregate in the backwaters near the margins of streams and apparently they are more active at night. Newly metamorphosed froglets were observed on the vegetation along the stream in December and January, and have a total length of 18.0–19.9 mm (n = 3).

Egg-mass and embryos.—Eggs are individually surrounded by gelatinous capsules which are generally attached to roots of the marginal vegetation. One clutch obtained in the field in



Fig. 3. Variation in dorsal coloration patterns of *Hypsiboas caipora*. (A) Uniform; (B) with sinuous dark brown lines on the dorsolateral region; (C–F) with dark brown spots and/or stripes, concentrated on head, vertebral, and/or dorsolateral region; (G–J) ornamented with asymmetrical dark brown blotches; and (K–L) almost all dark brown dorsum with beige to light brown blotches.



Fig. 4. Spectrogram (above) and oscillogram (below) of the vocalizations of *Hypsiboas caipora*. (A) Single advertisement call (December 2003; air temperature 20°C); (B) complex advertisement call (August 2003; air temperature 4°C); (C) single and complex territorial calls (November 2004; air temperature 19.5°C); and (D) distress calls (October 2004; air temperature 13°C). Graphics in the same scale, except the scale of frequency in D.

January 2004 was collected on the day following oviposition, and the embryos had a total length of about 5 mm. All the eggs were fertilized, giving rise to 72 tadpoles, some of which were raised in the lab to confirm the identification of the tadpole employed in the description. Another clutch obtained in captivity had 64 eggs. Eggs have a diameter of 185

8.5 mm and body length about 4.3 mm (n = 2) show two lateral gill tufts, each one originating from the region between eye and oral disc, with a length of about 2.5 mm. *Tadpole.*—One tadpole of *Hypsiboas caipora* was collected in the stream at the type locality, at developmental stage 28

the stream at the type locality, at developmental stage 28 (Gosner, 1960), and has the following measurements (mm): total length 34.1; body length 14.2; tail length 20.8; body height 8.7; body width 9.1; caudal height 9.5; nostril diameter 0.5; snout–spiracle distance 10.6; eye diameter 1.8; internarial distance 2.5; interorbital distance 4.2; eye–nostril distance 2.6; oral disc width 4.5.

Body ovoid and robust (Fig. 5), corresponding to approximately 40% of total length; body wider than high; snout rounded in lateral and dorsal views; eye large and dorsolateral, its diameter about 15% of body length; nostrils oval with a small elliptic projection on its inner margin. Caudal height about 45% of tail length; tail musculature well developed; height of ventral fin about 75% of dorsal fin; origin of dorsal fin on body. Spiracle medium sized, wide, sinistral, and directed posterodorsally; cloacal tube large and opening laterally on the right side of ventral fin. Oral disc well developed, ventral, subterminal, not visible in dorsal view, and about 50% of body width; marginal papillae surrounding the oral disc, except for a rostral gap (about 10% of oral disc); labial tooth row formula is 2(2)/4(1)(Fig. 5D), being P(4) shorter than other rows and located near the border of marginal papillae; small flaps with labial teeth on the lateral areas of the oral disc; free margin of both jaw sheaths serrated, anterior larger than the inferior. Coloration in life brownish with dark brown, gray, and reddish blotches; coloration in preservative gravish-brown.

Geographic distribution and conservation.—Three populations of Hypsiboas caipora are known from the municipalities of Pilar do Sul (23°58'S, 47°41'W; 23°56'S, 47°42'W; 23°56'S, 47°40'W), Sete Barras (24°07'S, 47°59'W), and São Miguel Arcanjo (23°59'S, 47°52'W), located in the mountains of Serra de Paranapiacaba, segment of the Serra do Mar, southern of São Paulo State, Brazil (Fig. 6). In this section occur the more preserved remnants of the Atlantic Forest of Brazil. This species was found only inside the forest at the highlands, at an altitude of 700-800 m. Apparently, the reproduction of Hypsiboas caipora is restricted to some large streams of the Alto Paranapanema and Ribeira de Iguape river basins. The populations of Sete Barras and São Miguel Arcanjo, officially protected at the Parque Estadual de Carlos Botelho, were identified on the basis of analysis of vocalizations from individuals recorded in the field but not collected.

Etymology.—The name *caipora* is based on a specific epithet derived from the Tupi indigenous language meaning forest inhabitant (kaa =forest + pora =inhabitant; Bueno, 1982). It refers to the habitat where the new species is found: the Atlantic Forest at Serra do Mar in Southeastern Brazil. Also, it refers to a personage of Brazilian folklore related to the life in the forest (Cascudo, 1954).

Phylogenetic relationships.—The analysis resulted in two most equally parsimonious trees/alignment combinations with



Fig. 5. Tadpole of Hypsiboas caipora in stage 28. (A) Lateral view; (B) dorsal view; (C) ventral view; and (D) oral disc.

length 4365 steps that were hit 15 independent times (Fig. 7). Overall, relationships hypothesized among outgroups differ from those resulting from the much densely sampled analysis of Faivovich et al. (2005). However, we do not consider these results to be a rigorous test of their hypothesis, as our sampling of these groups is much restricted and these terminals are only intended to be outgroups of our group of interest.



Fig. 6. Geographic distribution of *Hypsiboas caipora*: (bold circle) type locality and (empty circles) other two populations.

The two most parsimonious trees differ only in the position of *H. leptolineatus*, being either the sister taxon of *H. latistriatus* + *H. polytaenius*, or the sister taxon of the group composed of *H. caipora*, *H. joaquini*, *H. semiguttatus*, and *H. curupi Hypsiboas caipora* was recovered as the sister taxon of *H. semiguttatus*, and together both make the sister taxon of *H. curupi* and *H. joaquini* (Garcia et al., 2007).

DISCUSSION

Hypsiboas caipora is a member of *H. pulchellus* group, as suggested by its overall similarity with *H. semiguttatus* and corroborated by the phylogenetic analysis. Besides the molecular synapomorphies that support this group, the only putative morphological synapomorphy so far suggested for this group is the absence of the slip of the muscle

depressor mandibulae that originates at the level of the m. dorsalis scapulae (Faivovich et al., 2005).

The description of *Hypsiboas caipora* adds a fourth species to the clade composed of *H. semiguttatus* and species previously confused with it. Garcia et al. (2007) stated that besides the molecular evidence they are still unaware of morphological synapomorphies supporting the monophyly of the clade composed of *H. joaquini* + *H. semiguttatus* + *H. curupi*, to which we should add *H. caipora*. The superficial similarity among these species is striking, and possibly further research will allow defining character states related to their color patterns.

Hypsiboas caipora, H. semiguttatus, H. joaquini, and H. curupi have several similar characters that make difficult their diagnoses. The dorsal coloration of H. semiguttatus and of H. curupi varies from uniform pattern to presence of dark brown dorsal spots or stripes in vertebral and/or dorsolateral area. Although this pattern occurs in *Hypsiboas caipora*, it is frequent the fusion of spots, forming asymmetrical dark brown blotches, which in some specimens cover almost whole dorsum. Generally, Hypsiboas caipora is smaller than its two closely related species, but overlap in SVL occurs, mainly with H. curupi. Thus, beyond the morphology, biological characters, as the advertisement call, the most easily recognized differential character, and habitat are essential in the characterization of these species. Still, some undescribed species externally resembling Hypsiboas semiguttatus occur in southern Brazil and Argentina.

The geographic distribution of the four species from this clade so far is known to be allopatric, and associated with mountain areas, reproducing along streams. *Hypsiboas joaquini* is known from the highlands of Serra Geral, in southern Brazil, in open formations and Araucaria Forest (Garcia et al., 2003). Recently, the geographic distribution of *Hypsiboas semiguttatus* was restricted to Serra do Mar of southern Brazil, a region with influence of Araucaria Forest



Fig. 7. One of the two most parsimonious trees (length 4365 steps) resulting from the phylogenetic analysis of the *Hypsiboas pulchellus* species group. Arrow points to the only difference between the two most parsimonious trees, the alternative placement of *H. leptolineatus*. Black circles indicate nodes recovered in the quick consensus estimation (see text). Numbers above nodes are Bremer supports; numbers below are Parsimony Jackknife frequencies. Asterisks indicate Parsimony Jackknife frequencies of 100%. Branch lengths proportional to number of transformations; not all loci are available for all terminals. The species *Hypsiboas* sp. has recently been described as *Hypsiboas curupi* by Garcia et al. (2007).

and Atlantic Forest, while *H. curupi*, previously considered *H. semiguttatus*, is known from the Araucaria Forest and Seasonal Forest of Misiones in Argentina and part of western border of southern Brazil (Garcia et al., 2007). *Hypsiboas caipora* is distributed at lower latitudes and inhabits Atlantic Forest, but the presence of few remnants and isolated trees of *Araucaria angustifolia* in the western slope of the Serra de Paranapiacaba shows the influence of Araucaria formation in this region, probably more intense in the beginning of Holocene (Viadana, 2002). The known geographic distribution of this clade overlaps with Araucaria formation and surrounding transitional areas, a formation mainly associated with the subtropical plateau of the southern Brazil and part of Argentina (Ab'Sáber, 2003).

The taxonomic distribution of the structure of the larval cement glands in Hylinae hylids is still poorly known, being mostly restricted to the observations of Nokhbatolfoghahai and Downie (2005), on a few species of Hypsiboas, Dendropsophus, and Trachycephalus. Within Hypsiboas, Nokhbatolfoghahai and Downie (2005) reported their type A morphology in *H. crepitans* (a member of the *H. faber* group) and H. geographicus (H. semilineatus group). Cement gland morphology has also been quite superficially described or illustrated in H. heilprini (H. albopunctatus group; Noble, 1927), H. rosenbergi (H. faber group; Breder, 1946), and H. pugnax (H. faber group; Chacon-Ortiz et al., 2004). Our observations of a Type A morphology in embryos and early larvae of H. caipora are the first report on cement gland morphology in a species of the *H. pulchellus* species group. Additional research is still needed to provide information on the presence and developmental pattern of the cement gland in hylids.

Relationships among Hypsiboas semiguttatus and related species of the H. polytaenius clade had been hypothesized earlier (Faivovich et al., 2004, 2005). The results of the present phylogenetic analysis, however, are noteworthy in that the *H. polytaenius* clade, here represented by *H.* latistriatus, H. leptolineatus, and H. polytaenius, is not recovered as monophyletic in the strict consensus (not shown, but evident from Fig. 7). The monophyly of this putative clade, solely supported by the characteristic dorsal pattern of longitudinal stripes, has never been openly disputed, although it has never been rigorously tested. The current analysis has the same three (of the nine) species from the group as Faivovich et al. (2005), so is not a rigorous test of monophyly. The only relevant difference between this and the previous analyses is the inclusion of *H. caipora*. These results indicate that further research is needed to better understand relationships among these nodes of the phylogenetic tree of the *H. pulchellus* group.

MATERIAL EXAMINED

Hypsiboas andinus. Argentina: Tucumán, Estancia Agua Negra, Sierra de Medina, CFBH 4036–4037; Catamarca, Puesto Río Blanco, CFBH 4040; Toma del Río Andalgalá, CFBH 4039; Tucumán, Dique La Angostura, Tafi del Valle, CFBH 4038.

Hypsiboas beckeri. Brazil: Minas Gerais, São Tomé das Letras, CFBH 7416–7420, 7422–7424.

Hypsiboas bischoffi. Brazil: Rio Grande do Sul, São Francisco de Paula, CFBH 3676; Santa Catarina, São Bento do Sul, CFBH 3010, 3014; Rancho Queimado, CFBH 3358; São Paulo, Barra do Turvo, CFBH 6343; Jundiaí, CFBH 0718; Ribeirão Branco, CFBH 2137, 6821; São Paulo, CFBH 5689. *Hypsiboas cipoensis*. Brazil: Minas Gerais, Serra do Cipó, Santa do Riacho, CFBH 0289.

Hypsiboas ericae. Brazil: Goiás, Alto Paraíso de Goiás, Chapada dos Veadeiros, CFBH 3599–3604.

Hypsiboas goianus. Brazil: Goiás, Silvânia, CFBH 2666, 4167-4168.

Hypsiboas joaquini. Brazil: Santa Catarina, Urubici, CFBH 3281–3289, 3624–3630.

Hypsiboas latistriatus. Brazil: São Paulo, Campos de Jordão, CFBH 9902, 9865; Rio de Janeiro, Itatiaia, CFBH 9866.

Hypsiboas leptolineatus. Brazil: Santa Catarina, São Domingos, CFBH 7166–7169.

Hypsiboas marginatus. Brazil: Rio Grande do Sul, São Francisco de Paula, CFBH 3078, 3090–3097, 3413.

Hypsiboas phaeopleura. Brazil: Goiás, Alto Paraíso de Goiás, Chapada dos Veadeiros, CFBH 3598.

Hypsiboas polytaenius. Brazil: Rio de Janeiro, Maringá, Distrito de Itatiaia, CFBH 5746–5752.

Hypsiboas prasinus. Brazil: Minas Gerais, Monte Verde, CFBH 7475; São Paulo, Jundiaí, Serra do Japi, CFBH 0002, 0694, 0729, 7413; São Paulo, Ribeirão Branco, CFBH, 6887, 6893; Santa Catarina, Lages, CFBH 0074.

Hypsiboas pulchellus. Brazil: Rio Grande do Sul, São Francisco de Paula, CFBH 3374–3379.

Hypsiboas semiguttatus. Brazil: Paraná, Piraquara, Mananciais da Serra, CFBH 3364, 3379–3381, 3704–3707, 5000.

Hypsiboas stenocephalus. Brazil: Minas Gerais, Cristina, CFBH 9918, 9862, 9863.

Hypsiboas curupi. Argentina: Misiones, San Vicente, CFBH 3444–3446, 4908–4910.

GenBank numbers of the sequences included in the phylogenetic analysis: All sequences generated by Faivovich et al. (2004, 2005). Refer to these publications for voucher collection number and locality: Aplastodiscus eugenioi (AY843669, AY843913, AY844660, AY844456, AY844875), A. perviridis (AY843569, AY843791, AY844543, AY844366, AY844025, AY844771, AY844201), Hypsiboas albopunctatus (AY549317, AY549370, AY844569, AY844041, AY844795), H. andinus (AY549319, AY549372, AY844573, AY844387, AY844799), H. balzani (AY549323, AY549376, AY844582, AY844395, AY844806, AY844226), H. benitezi (AY843606, AY843830, AY844583, AY844396, AY844227), H. bischoffi (AY549324, AY549377, AY844586, AY844398), H. caingua (AY549326, AY549379, AY844591, AY844057, AY844812, AY844234), H. caipora (EU077268, EU077267, EU077265, EU077266, EU077264, EU077263), H. cordobae (AY549330, AY549383, AY844600, AY844411, AY844066, AY844819, AY844244), H. ericae (AY549332, AY549385, AY844605, AY844416, AY844071), H. faber (AY549334, AY549387, AY844607, AY844825), H. guentheri (AY843631, AY549390, AY844612, AY844830, AY844253), H. joaquini (AY549339, AY549392, AY844616, AY844421, AY844834, AY844256), H. latistriatus (AY549360, AY549413, AY844668, AY844293), H. leptolineatus (AY549341, AY549394, AY844621, AY844424, AY844839, AY844260), H. marginatus AY844083, (AY549342, AY549395, AY844624, AY844426, AY844842, AY844263), H. marianitae (AY549344, AY549397, AY844625, AY844427, AY844843), H. polytaenius (AY843655, AY843895, AY844641, AY844443, AY844859), H. prasinus (AY549347, AY549400, AY844642, AY844100, AY844860), H. pulchellus (AY549352, AY549405, AY844644, AY844445, AY844102, AY844862, AY844278), H. punctatus (AY549353, AY549406, AY844645), H. riojanus (AY549355, AY549408, AY844648, AY844447, AY844865, AY844279), H. rufitelus (AY843662, AY843905, AY844652, AY844105, AY844867, AY844282), H. semiguttatus (AY549357, AY549410, AY844655, AY844452, AY844870, AY844285), H. semilineatus (AY843778, AY843779, AY843909, AY844656, AY844453, AY844108, AY844871, AY844286), Hypsiboas curupi (AY549359, AY549412, AY844880).

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