

New Species of *Brachycephalus* (Anura: Brachycephalidae) from the Atlantic Rain Forest in São Paulo State, Southeastern Brazil

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ABSTRACT.—A new species of brachycephalid frog is described from São Luís do Paraitinga, in the Atlantic Rain Forest of São Paulo State, southeastern Brazil. The new species is characterized by male SVL = 10.8–12.1 and female SVL = 12.6–14.0 mm; and general color orange with dorsal reddish irregular markings, lateral surfaces with small dark brown spots, and belly with brownish spots and small dots. Comparisons with other brachycephalid species and osteological data are provided.

RESUMO.—Uma nova espécie de braquicefalídeo é descrita da localidade de São Luís do Paraitinga, na Floresta Atlântica do estado de São Paulo, sudeste do Brasil. A nova espécie é caracterizada pelo comprimento rostro-cloacal que nos machos varia de 10,8 a 12,1 mm e nas fêmeas de 12,6 a 14,0 mm; coloração geral alaranjada com manchas irregulares no dorso de cor avermelhada, superfícies laterais, com pequenas manchas marrom-escuro e ventre com manchas marrons. São incluídas comparações com as outras espécies de braquicefalídeos e dados osteológicos.

The family Brachycephalidae as currently understood contains 17 genera (Frost, 2007). Within the family Brachycephalidae, the genus *Brachycephalus* is endemic to the Atlantic Rain Forest of southeastern and southern Brazil inhabiting leaf litter on the forest floor (Pombal et al., 1998; Ribeiro et al., 2005; Alves et al., 2006). The species of the genus *Brachycephalus* described thus far have snout–vent lengths less than 18 mm, phalangeal loss, and reduced number of toes and are believed to have become evolutionarily miniaturized (Yeh, 2002). The known diversity of *Brachycephalus* included, until very recently, six species occurring in the states of Minas Gerais, Rio de Janeiro, São Paulo, and Paraná, namely, *Brachycephalus didactylus* (Izecksohn, 1971), *Brachycephalus ephippium* (Spix, 1824), *Brachycephalus hermogenesi* (Giaretta and Sawaya, 1998), *Brachycephalus nodoterga* Miranda-Ribeiro, 1920, *Brachycephalus pernix* Pombal, Wistuba, and Bornschein, 1998, and *Brachycephalus vertebralis* Pombal, 2001. However, in the last two years alone, as a result of herpetological surveys in the mountain slopes of the Serra do Mar and Serra da Mantiqueira, the following five new species were described, nearly doubling the known diversity of *Brachycephalus*: *Brachycephalus alipioi*

Pombal and Gasparini, 2006; *Brachycephalus brunneus* Ribeiro, Alves, Haddad, and Reis, 2005; *Brachycephalus ferruginus* Alves, Ribeiro, Haddad, and Reis, 2006; *Brachycephalus izecksohni* Ribeiro, Alves, Haddad, and Reis, 2005; and *Brachycephalus pombali* Alves, Ribeiro, Haddad, and Reis, 2006. Here, we contribute to the knowledge of the taxonomy and diversity of *Brachycephalus* by describing a new species from the slopes of the Serra do Mar in the state of São Paulo in southeastern Brazil.

MATERIALS AND METHODS

Specimens were anaesthetized and killed in 30% ethyl alcohol, fixed in 10% formalin, and preserved in 70% ethyl alcohol. The terminology used for external morphology and texture of the skin followed Lynch and Duellman (1997). The color codes used in color descriptions were those of Smithe (1975). Drawings were made using a Zeiss stereomicroscope fitted with a drawing tube. Ten standard metric traits were measured (in millimeters) with a micrometric ocular under a stereomicroscope: SVL (snout–vent length); HL (head length, from tip of snout to angle of jaw); HW (head width, greatest width of head located between angles of jaw); ND (nostril diameter); IND (internostril distance, between inner margins of nostrils); ED (eye diameter); IOD (interorbital distance, be-

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tween anterior corners of the eyes); END (eye-nostril distance, from anterior corner of the eye to posterior margin of nostril); THL (thigh length); and TBL (tibia length).

Morphological variation in cranial shape was assessed on the basis of 12 anatomical (cranial) landmarks defined in Clemente-Carvalho et al. (2008) among the following species of *Brachycephalus*: *Brachycephalus* sp. nov. ($N = 13$), *B. ephippium* ($N = 18$), *B. nodoterga* ($N = 14$), and *B. vertebralis* ($N = 21$). Specimens from both sexes of each species were pooled because relative warps analysis revealed no significant sexual dimorphism in cranial shape. Shape variables, the partial warps, which emphasize localized shape change in the neighborhood of anatomical landmarks, were computed following the thin-plate splines methodology described in Bookstein (1991, 1996). The partial warps are of dimension $2p - 6 = 18$, for $p = 12$ anatomical landmarks in this study. Dimension reduction of the 18 partial warps generated for the skull of the *Brachycephalus* taxa examined was achieved by canonical variates analysis, which maximizes variation among groups relative to variation within groups (Mardia et al., 2006). Confidence regions around sample centroids for canonical axes using parametric bootstrap (Ringrose, 1996) were computed by simulating 1,000 replicate data matrices (Von Zuben et al., 1998). Canonical axes were visualized in shape space by multivariate regression of partial-warp scores on canonical variates scores (Rohlf et al., 1996).

Specimens used in the description or examined for comparisons are in AL-MN (Adolpho Lutz collection, deposited in the Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil), CFBH (Célio F. B. Haddad collection, deposited in the Departamento de Zoologia, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo, Brazil), MHNCI (Museu de História Natural do Capão da Imbuia, Curitiba, Paraná, Brazil), MNRJ (Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil), MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil), ZSM (Zoologische Staatssammlung München, Germany), and ZUEC (Museu de História Natural, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil). Specimens examined are listed in Appendix 1.

Brachycephalus pitanga sp. nov.

Holotype.—CFBH 16746 (Fig. 1), adult female, one of a series collected in Núcleo Santa Virgínia at the Parque Estadual da Serra do Mar, municipality of São Luís do Paraitinga, in an area with predominant secondary Lower Montane Rain Forest (sensu Oliveira-Filho and Fontes, 2000) and an abandoned *Eucalyptus*



FIG. 1. *Brachycephalus pitanga*, CFBH 16746 (holotype), adult female in dorsal view.

plantation, about 50 m from the river Ipiranga (23°20'40"S, 45°08'15"W), São Paulo state, southeastern Brazil, approximately 920 m above sea level, on 13 January 2003 by A. M. Haddad and C. F. B. Haddad.

Paratopotypes.—CFBH 16747, adult male collected with the holotype; 16751, 16754, 16758, 16762–16766, adult males and CFBH 16750, 16752, 16753, 16755–16757, 16759–16761, adult females, collected on 30 November to 01 December 2002 by R. J. Sawaya and C. F. B. Haddad.

Diagnosis.—(1) SVL in males 10.8–12.1 and SVL in females 12.6–14.0 mm; (2) skin on head and dorsum with dermal ossification; (3) general color in life orange with dorsal red irregular markings, lateral surfaces with small dark brown spots, and belly with brownish spots and small dots.

Comparison with Other Species.—Like other species of the genus *Brachycephalus*, *B. pitanga* is diminutive in size and lacks phalanges in the manus and pes; the pectoral girdle is completely ossified with epicoracoids closely juxtaposed and articulating throughout their lengths, omosternum present and sternum absent. The skin on head and dorsum of *B. pitanga* has dermal ossification. This characteristic distinguishes the new species from *B. brunneus*, *B. ferruginus*, *B.*



FIG. 2. Live *Brachycephalus pitanga*, Núcleo Santa Virginia, Parque Estadual da Serra do Mar, municipality of São Luís do Paraitinga, São Paulo state, southeastern Brazil.

izecksohni, *B. pernix*, and *B. pombali* whose texture of skin on head and dorsum is smooth with no dermal ossification. The dorsal red color of *B. pitanga* (Fig. 2) distinguishes this new species from *B. alipioi*, *B. ephippium*, *B. nodoterga*, and *B. vertebralis* whose general color is orange without red marks. *Brachycephalus pitanga* is most similar to *B. vertebralis* from which it differs by color in life as stated above. *Brachycephalus pitanga*, like all *Brachycephalus* species thus far described, differs from *B. didactylus* and *B. hermogenesi* by having snout shape in dorsal view rounded, and general color orange. *Brachycephalus didactylus* and *B. hermogenesi* have snout short, pointed in dorsal view with a pointed tip, and general color brownish.

Description of the Holotype.—Body robust (Figs. 1, 3). Head as wide as long, slightly narrower than body; head length 39% of SVL; snout short with length almost equal to eye diameter, rounded in lateral and dorsal views (Fig. 3); nostrils protuberant, directed anterolaterally; canthus rostralis indistinct; loreal region weakly concave; lips nearly sigmoid; eye slightly protruding in dorsal and lateral views, eye diameter 25% of HL; tympanum absent; vocal sac not expanded externally; vocal slits present; tongue longer than wide, posterior half not adherent to floor of mouth; choanae relatively small and round; vomerine odontophores absent. Pectoral girdle arciferal and robust (Fig. 4); procoracoid and epicoracoid completely ossified; procoracoid and epicoracoid synostotically united with clavicle, coracoid, and scapula; suprascapula broadly expanded, its anterior half ossified as cleithrum; omosternum well developed, cartilaginous, its distal portion expanded; sternum absent. Forearm relatively slender, humeral region approximately as long as forearm; Fingers II and III robust and distinct; Fingers I and IV very small; tip of Finger II slightly rounded, tip of Finger III pointed;

relative lengths of Fingers II < III; subarticular tubercles and inner and outer metacarpal tubercles absent (Fig. 3). Legs relatively short, thigh robust; thigh length 35% of SVL, tibia length 90% of thigh length; Toes II–IV distinct; Toe II reduced, Toe I and V externally absent; tip of Toe II slightly rounded, tips of Toes III and IV pointed; relative lengths of Toes II < III < IV; subarticular tubercles and inner metatarsal tubercles absent; outer metatarsal tubercle distinct, large, and ovoid (Fig. 3). Skin on head and dorsum granular and with dermal ossification (Figs. 1, 3); skin on dorsolateral surfaces of body, flanks, and dorsal surface of thighs granular; skin on venter and ventral surfaces of the legs smooth; skin on ventrolateral surfaces of body and area around the cloacal opening granular.

Variation in Cranial Shape.—Cranial shape was compared between *B. pitanga* and the other species that occur in the state of São Paulo, namely *B. ephippium*, *B. nodoterga*, and *B. vertebralis*. *Brachycephalus hermogenesi*, which also occurs in São Paulo, was not included in the morphometric analysis because this species differs markedly in body shape from the other *Brachycephalus* species (Izecksohn, 1971; Alves et al., 2006). The ordination of the *Brachycephalus* taxa in the reduced space of the first two canonical variates, which describes principal directions of variation ($\approx 93\%$) in cranial shape among groups, is given in Figure 5. In this figure, each dot represents the centroid for a given taxon and 95% confidence ellipses are the estimated bounds of variation in cranial shape generated by the parametric bootstrap. This inferential procedure demonstrated that there is no overlap between the 95% confidence ellipses; therefore, the new species of *Brachycephalus* is unambiguously discriminated on the basis of cranial shape from the other known species occurring in the state of São Paulo. The deformation warpings between anatomical landmarks in the skull represent dilations and compressions in various locations and directions in the two-dimensional perspective of the skull. These graphs indicate that major differences in cranial shape among the species of *Brachycephalus* sampled are associated primarily with the rostral region, represented by landmarks 1 through 5, and the parotic plate region, represented by landmarks 7 through 9.

Color in Life of the Holotype.—General background color orange (spectrum orange, #17); central area of body and head with reddish (spectrum red, #11) irregular markings covered with small dark brown (sepia, #219) spots and dots. Arms and legs orange (spectrum orange, #17).

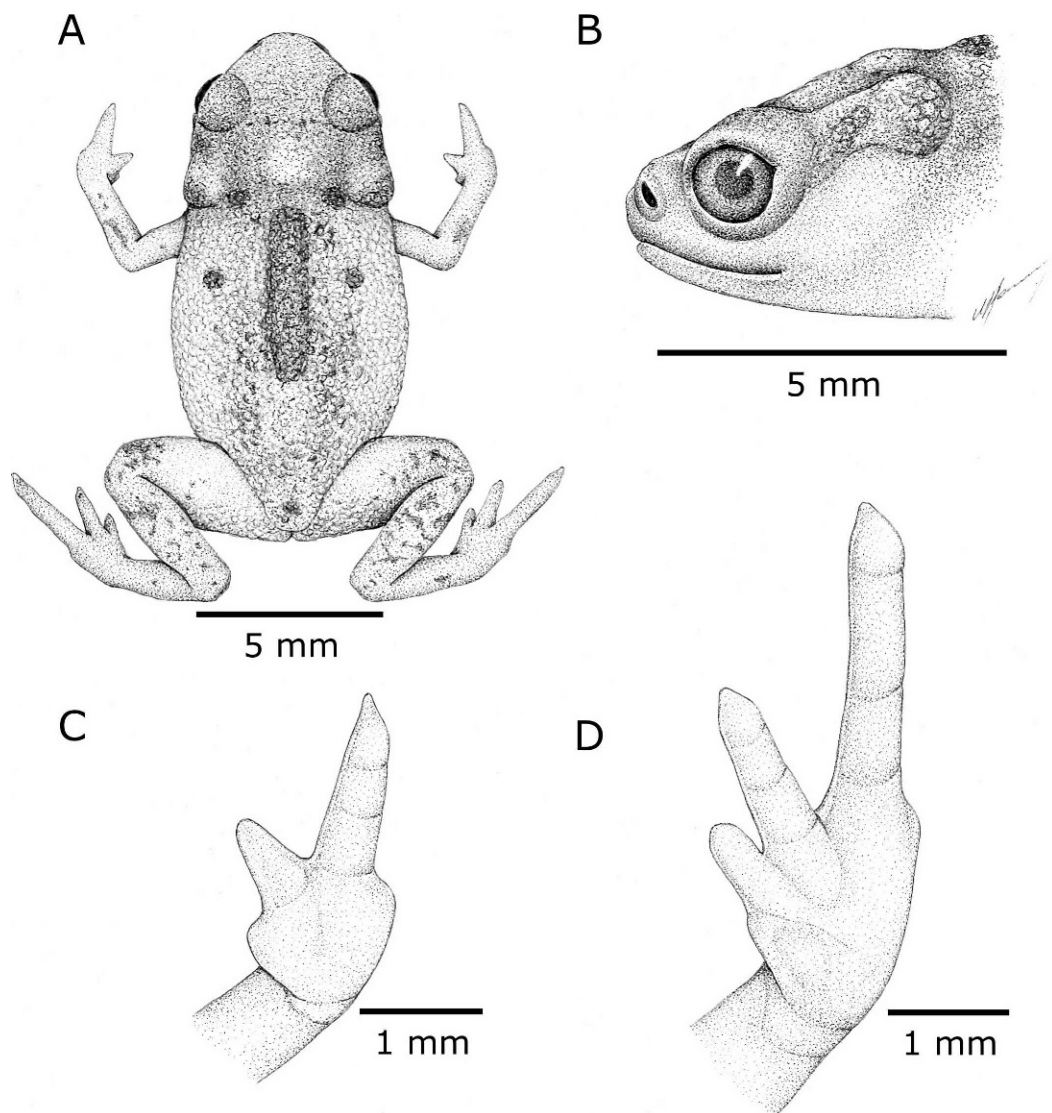


FIG. 3. *Brachycephalus pitanga*, CFBH 16746 (holotype). (A) Dorsal view of body, (B) lateral view of head, (C) left hand, and (D) left foot.

Coloration in Preservative of the Holotype.—General background color pale cream (cream color, #54); central area of body and head markings orange-brown (amber, #36).

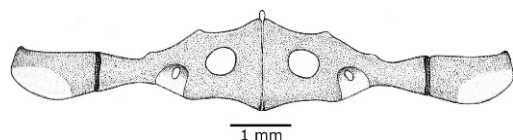


FIG. 4. *Brachycephalus pitanga*. Ventral view of pectoral girdle with scapula and suprascapula deflected.

Measurements of the Holotype.—In millimeters. SVL 13.4; HL 3.1; HW 4.8; IND 1.5; ED 1.4; IOD 2.1; END 1.1; THL 5.4; TBL 4.9.

Variation.—Measurements for nine males and 10 females are given in Table 1. In large specimens, the first finger is more distinct. Color in life varies among specimens from almost entirely orange to almost entirely red, as shown in Figure 6. In juvenile specimens, the general background color usually is darker than in the adults.

Natural History.—*Brachycephalus pitanga* is a diurnal species. Active males, females, and juveniles have been observed calling and

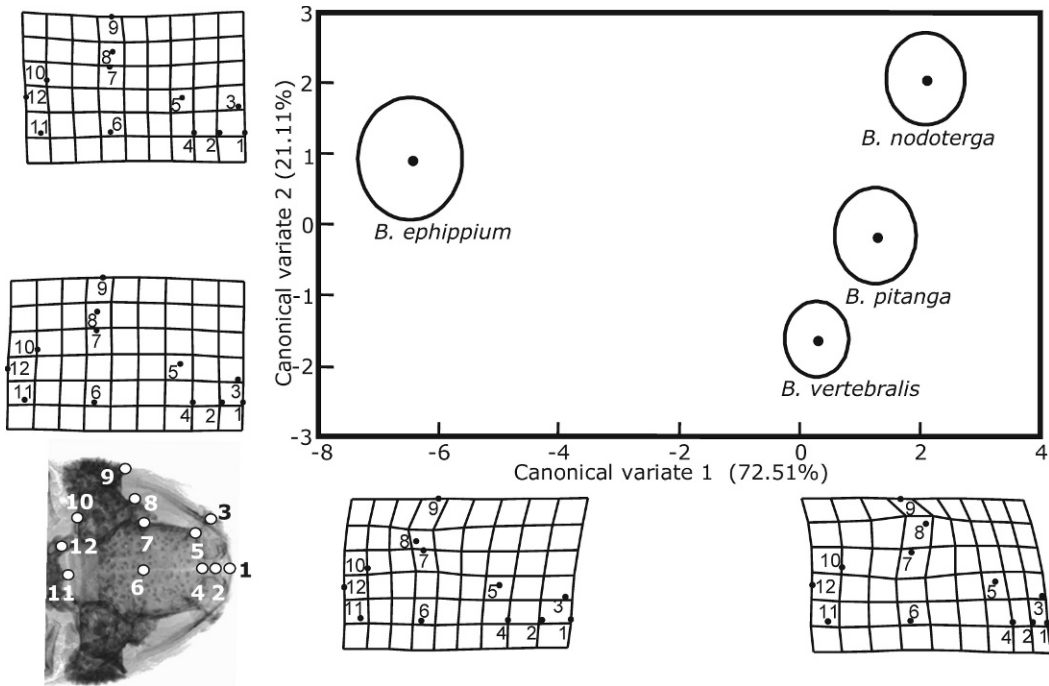


FIG. 5. Bivariate plot of centroids (denoted as dots) and 95% confidence regions for canonical variates 1 and 2 (and percent variance explained) derived from a canonical variate analysis of partial-warp scores for the skull of four species of *Brachycephalus*. Numbers in deformation grids refer to morphological landmarks shown on the skull of *Brachycephalus*. Estimated changes in cranial shape are shown as deformation implied by the first and second canonical variates for positive and negative deviations from the mean.

walking on the humid leaf-litter. Males call throughout the year, but during the dry season, they call mainly early in the morning in fogging or drizzling days. During dry days activity is reduced and the frogs can be observed amid the leaf-litter. At the type locality, at approximately 900 m elevation, *B. pitanga* occurs in very high population densities. Sampling the litter by means of four quadrats of 25 m² we found 15, 31, 52, and 50 specimens, corresponding to 60, 124, 208, and 200 individuals per 100 m², respectively. The frogs were released in the same locality after sampling. Some individuals open the mouth when disturbed ($N = 4$, from 37 specimens photographed), which we interpreted as a defensive display.

Distribution.—*Brachycephalus pitanga* is known from the type locality in Núcleo Santa Virgínia at the Parque Estadual da Serra do Mar reserve, municipality of São Luís do Paraitinga, and in old-growth Lower Montane Rain Forest in the Fazenda Capricórnio and patches of Upper Montane Rain Forest (sensu Oliveira-Filho and Fontes, 2000), both in municipality of Ubatuba and adjacent mountains close to the type locality, São Paulo State, southeastern Brazil.

Etymology.—"Pitanga" is a Tupi-Guarani indigenous word that means "reddish," and is

also the name of the red fruit of *Eugenia uniflora* (Family Myrtaceae), endemic from Brazil and widespread in the Atlantic Forest. It is used here in allusion to the reddish color of the dorsum of *B. pitanga*.

REMARKS

The description of *B. pitanga* adds to the growing known taxonomic diversity of *Brachycephalus*, which now amounts to 12 species (Ribeiro et al., 2005; Alves et al., 2006; Pombal and Gasparini, 2006). The shape variables (partial warps) computed using the thin-plate methodology (Bookstein, 1991, 1996) and the inferential procedure developed for canonical variates analysis (Ringrose, 1996; Von Zuben et al., 1998) demonstrated that *B. pitanga* can be completely discriminated on the basis of cranial shape from the other species of *Brachycephalus* that occur in the state of São Paulo. This finding is relevant because anuran morphology has been regarded as conservative both among and within species (Heyer, 1979; Austin et al., 2002; Loughheed et al., 2006). The geometric-statistical formalism of shape analysis, and in particular the thin-plate splines and partial-warps procedures, has only recently being used

TABLE 1. Measurements in millimeters of the type series of *Brachycephalus pitanga* (\bar{x} = mean; SD = standard deviation). Character abbreviations listed in the Material and Methods.

	Males (N = 9)			Females (N = 10)		
	\bar{x}	SD	Range	\bar{x}	SD	Range
SVL	11.5	0.4	10.8–12.1	13.4	0.4	12.6–14.0
HL	4.5	0.2	4.3–4.9	5.2	0.1	5.1–5.3
HW	4.7	0.2	4.5–5.0	5.1	0.1	4.8–5.3
ED	1.3	0.1	1.0–1.3	1.4	0.0	1.2–1.4
ND	0.2	0.0	0.1–0.2	0.2	0.0	0.2–0.2
IOD	2.4	0.1	2.2–2.6	2.7	0.0	2.6–2.7
IND	1.2	0.0	1.1–1.3	1.4	0.1	1.3–1.5
END	0.6	0.0	0.5–0.7	0.7	0.1	0.6–0.8
THL	5.1	0.3	4.6–5.5	5.5	0.4	5.0–6.2
TBL	4.5	0.2	4.0–4.8	5.0	0.2	4.8–5.2

in studies of anuran morphology and evolution and has proven very informative to uncover patterns of inter- and intraspecific variation (Larson, 2004; Fabrezi, 2006; Stayton and Ruta, 2006; Clemente-Carvalho et al., 2008). The results presented here show that this method can be very valuable to the morphological

characterization and diagnosis of new species as well.

Brachycephalus pitanga, like most species in the genus *Brachycephalus*, occurs in isolation from the other congeneric species in the mountain ranges of southern and southeastern Brazil at elevations from 600 m to 1,800 m (Pombal et al.,



FIG. 6. Intrapopulation variation in color in life of *Brachycephalus pitanga*, Núcleo Santa Virgínia, Parque Estadual da Serra do Mar, municipality of São Luís do Paraitinga, São Paulo state, southeastern Brazil. Upper row from left to right (museum numbers CFBH 16750, CFBH 16763, and CFBH 16755); lower row from left to right (CFBH 16757, CFBH 16756, and CFBH 16759).

1998; Pombal, 2001; Ribeiro et al., 2005; Alves et al., 2006; Pombal and Gasparini, 2006). The only known exceptions are *B. didactylus* and *B. hermogenesi*—formerly described in the genus *Psyllophryne*—in that the former occurs at sea level (Izecksohn, 1971) and the latter occurs from sea level to elevations of 600 m (Giaretta and Sawaya, 1998). Therefore, from a biogeographic standpoint, most known species of *Brachycephalus* effectively have an insular distribution. Thus, it is quite conceivable that, given this insular pattern of distribution, additional surveys in the slopes of the mountain ranges of southern and southeastern Brazil will reveal new, undescribed species.

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APPENDIX 1

Additional Specimens Examined.—*Brachycephalus alipioi*: Espírito Santo, Vargem Alta MNRJ 26042 (holotype), CFBH 3566–3567, MNRJ 26043–26055 (paratopotypes). *Brachycephalus brunneus*: Paraná, Campina Grande do Sul CFBH 7879 (holotype), 7880–7890