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Vocalizations, morphological variation, and morphometry of *Pristimantis gutturalis* (Anura: Strabomantidae)

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Abstract

Vocalizations, morphological variation, and morphometry of *Pristimantis gutturalis* (Anura: Strabomantidae). *Pristimantis gutturalis* is a species of the *P. conspicillatus* group described from French Guiana, with distribution so far restricted to the eastern Guiana Shield. Some aspects of this species are yet understudied, including its vocal repertoire which is unknown to date. Although in its original description it was properly characterized with regard to morphology, the description of its coloration in life consisted of brief remarks based on field notes on two females only. Moreover, little morphometric data were presented in its description, with detailed measurements provided for the holotype (a female) only. Subsequent studies have only briefly addressed this species and have not presented significant new data on it. In order to improve the knowledge on *P. gutturalis*, based on data from the state of Amapá, northern Brazil, in the present study we describe for the first time the advertisement and territorial calls of this species, assess its morphological and chromatic variation, provide detailed morphometric data on males and one female, and briefly comment on its natural history. Additionally, we compare the advertisement call of *P. gutturalis* with calls of other species of the *P. conspicillatus* group, and make some remarks on the acoustics of this group.

Keywords: Advertisement call, Bioacoustics, Eastern Guiana Shield, Phenotypic variation, *Pristimantis conspicillatus* group, Taxonomy, Territorial call.

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Resumo

Vocalizações, variação morfológica e morfometria de *Pristimantis gutturalis* (Anura: Strabomantidae). *Pristimantis gutturalis* é uma espécie do grupo de *P. conspicillatus* descrita da Guiana Francesa, com distribuição restrita ao Escudo da Guiana oriental. Alguns aspectos dessa espécie são ainda pouco estudados, incluindo seu repertório vocal que é desconhecido até o momento. Embora na sua descrição original ela tenha sido devidamente caracterizada no que diz respeito à morfologia, a descrição de sua coloração em vida consistiu de breves observações baseadas em notas de campo sobre duas fêmeas apenas. Além disso, poucos dados morfométricos foram apresentados na sua descrição, com medidas detalhadas fornecidas apenas para o holótipo (uma fêmea). Estudos subsequentes apenas abordaram brevemente essa espécie e não apresentaram novos dados significativos sobre ela. A fim de melhorar o conhecimento sobre *P. gutturalis*, com base em dados provenientes do estado do Amapá, norte do Brasil, no presente estudo nós descrevemos pela primeira vez os cantos de anúncio e territorial dessa espécie, avaliamos sua variação morfológica e cromática, fornecemos dados morfométricos detalhados sobre machos e uma fêmea, e brevemente comentamos sobre sua história natural. Além disso, nós comparamos o canto de anúncio de *P. gutturalis* com os cantos de outras espécies do grupo de *P. conspicillatus* e fazemos algumas observações sobre a acústica desse grupo.

Palavras-chave: Bioacústica, Canto de anúncio, Canto territorial, Escudo da Guiana Oriental, Grupo de *Pristimantis conspicillatus*, Taxonomia, Variação fenotípica.

Introduction

Pristimantis Jiménez de la Espada, 1870 is a highly diverse group of direct-developing frogs, composed of 591 species widely distributed in the Neotropics (Frost 2022). Although the genus encompasses 13 species groups, most of its species remain unassigned to any group to date (*sensu* Padial *et al*. 2014, González-Durán *et al*. 2017, Zumel *et al*. 2021). Among these species groups, the *P. conspicillatus* group (*sensu* Padial *et al.* 2014) is the largest with 42 species, ranging from Costa Rica to the eastern Guiana Shield and southern Bolivia, and extending to Amazon, Cerrado, and Atlantic Forest regions in Brazil (Padial *et al.* 2014, 2016, Oliveira *et al.* 2017, 2020, Acevedo *et al.* 2020, Taucce *et al.* 2020, Roberto *et al.* 2022).

Despite this great diversity, the acoustic knowledge on the *Pristimantis conspicillatus* group is still deficient, since only 24 of its species have their advertisement calls described. One of the members of this group whose vocalization remains unknown is *Pristimantis gutturalis* (Hoogmoed, Lynch, and Lescure, 1977), a species described from French Guiana and restricted to the eastern Guiana Shield, occurring in southeastern Suriname, French Guiana, and in the Brazilian state of Amapá (Hoogmoed *et al.* 1977, Lescure and Marty 2000, Ouboter and Jairam 2012, Frost 2022). In its original description, the characterization of its coloration in life was brief, consisting of few remarks based on field notes on two females only (Hoogmoed *et al.* 1977). Moreover, its morphometry was poorly described, with detailed measurements provided for the holotype (a female) only (Hoogmoed *et al.* 1977).

Although *Pristimantis gutturalis* has been addressed in two works subsequent to its description (Lescure and Marty 2000, Ouboter and Jairam 2012), these studies provided only brief accounts that did not present significant new data, and hence some important aspects of this species still remain understudied. Aiming to contribute to the knowledge on *P. gutturalis,* based on data collected in the state of Amapá, northern Brazil, herein we describe for the first time its advertisement and territorial calls, assess its morphological and chromatic variation,

provide morphometric measurements for ten males and one female, and briefly comment on aspects related to its natural history. In addition, we provide comprehensive comparisons between the advertisement call of *P. gutturalis* and calls of other species of the *P. conspicillatus* group, and make remarks on the acoustics of this group.

Materials and Methods

Study Area and Data Collection

We carried out fieldwork in the state of Amapá, northern Brazil, on March 2018 at the Cancão Municipal Natural Park, in the municipality of Serra do Navio [00°54'54.70" N, 52°00'25.20" W, 139 m a.s.l.; ca. 372 km straight line away from the type locality of *Pristimantis gutturalis* (Lower Matarony River, French Guiana)]; and in two adjacent sites (ca. 400 km straight line away from the species type locality) located ca. 300 m from the south bank of the Araguari River, in the municipality of Porto Grande, on 04 and 09 March 2022 (site 1: 00°44'13.20" N, 51°30'0.82" W, 87 m a.s.l.), and on 11 March 2022 (site 2: 00°44'2.70" N, 51°29'54.03"W, 101 m a.s.l.). We obtained vocalizations only in Porto Grande, using a RØDE NTG1 microphone connected to a Tascam DR-100 digital recorder (site 1; sampling rates of 44.1 and 96.0 kHz; 16 bits resolution), and a CSR YOGA HT-81 microphone connected to a Zoom H1N digital recorder (site 2; sampling rate of 96.0 kHz; 16 bits resolution). See Appendix I for further details on recordings. We collected three adult males (CECC 1116, 3250, 3305) and one adult female (CECC 1959) from Serra do Navio, and seven adult males from Porto Grande (CECC 3795–96, 3811–15). We euthanized specimens with 5% lidocaine, fixed them in 10% formalin, and preserved them in 70% ethanol. We deposited specimens in the Herpetological Collection of the Universidade Federal do Amapá (CECC), and deposited recordings in this same collection as well as and in the Fonoteca Neotropical Jacques Vielliard (FNJV).

Acoustic Analyses

We recorded two types of calls, and classified them based on their particular functions associated to the social context involved, following Toledo *et al.* (2015) and Köhler *et al.* (2017). We classified one of these call types as the advertisement call, since it consisted of a conspicuous vocalization continuously emitted by all males. We classified the other type as the territorial call, since it was less frequent than the advertisement call and was not emitted by all individuals, being observed that males emitted this call type when there were many conspecifics calling nearby. Prior to analyses, we applied 400 Hz high-pass and 6000 Hz low-pass filters to the recordings in Raven Pro 1.5 software (K. Lisa Yang Center for Conservation Bioacoustics 2022) to remove background noises. After this step, we normalized (peak -1.0 dB) recordings that presented low amplitude level using Audacity v. 2.2.2 software (Audacity Team 2021). Then, we analyzed calls in Raven under the following settings: window size = 512 samples; 3 dB filter bandwidth = 124 or 270 Hz; window type = Hann; overlap = 80.1% (locked); hop size = 1.06 or 2.31 ms; DFT size = 512 samples; grid spacing = 86.1 or 188 Hz. Terminology and definitions of call traits essentially followed Köhler *et al.* (2017). We obtained dominant, maximum, and minimum frequency values through the "Peak Frequency", "Frequency 95%", and "Frequency 5%" functions, respectively (see Charif *et al.* 2010). We calculated note rate as "number of notes within a call - 1/ duration between the onset of the first note to duration the onset of the last note of the call". We produced call figures in R platform v.3.6.2 (R Core Team 2021) using seewave v.2.1.6 (Sueur *et al.* 2008) and tuneR v.1.3.3 (Ligges *et al.* 2018) packages with the following settings: window = Hanning; overlap = 85%; FFT = 512; a relative amplitude scale of 46 dB [indicated by colors, with red being the maximum amplitude (i.e. 0 dB)] was used to produce spectrograms.

Morphological Examination

We examined all collected specimens (10 adult males and one adult female). We assessed morphological and chromatic variation through comparisons with previous descriptions (Hoogmoed *et al.* 1977, Lescure and Marty 2000, Ouboter and Jairam 2012). In the Morphological variation subsection (Results section) we omitted traits that did not differ from previous descriptions, and also included traits that were not previously reported. Terminology and definitions of the morphological and chromatic traits described herein followed Duellman and Lehr (2009). We identified specimens as males by the presence of vocal sac, nuptial pads, and emission of advertisement calls, with the exception of the specimen CECC 1959 which we identified as female by its considerably larger size, lack of vocal sac, and absence of vocalizations.

Morphometric Measurements

Based on Padial *et al.* (2016), we measured the following traits in all collected individuals: snout–vent length (SVL), head length (HL, from posterior margin of lower jaw to tip of snout), head width (HW, at level of rictus), eye length (EL, measured horizontally); eye to nostril distance (EN), internarial distance (IND), eye-eye distance (EE), tympanic membrane height (TYH), tympanic membrane length (TYL), arm length (FA, from posterior margin of thenar tubercle to elbow), tibia length (TL), thigh length (TH, from vent to knee), and foot length (FL, from proximal border of inner metatarsal tubercle to tip of fourth toe). Additionally, following Watters *et al.* (2016) we measured the snout–nostril length (SN) and the snout length (SL). We took all measurements with a digital caliper (0.01 mm precision). Following the argument of Padial *et al.* (2016) that the measurement accuracy of both interorbital distance and upper eyelid width is greatly influenced by preservation conditions, we did not include these two traits. In this sense, we noticed minute fixation artifacts that could affect the accuracy of the measurement of the width of the discs of fingers III and IV, and of toe IV, and for this reason we did not include these three traits neither.

Results

Specimen Identification

Based on comparisons with the species original description (Hoogmoed *et al.* 1977), we identified our specimens as *Pristimantis gutturalis* since they have the following diagnostic traits: (1) a distinct white median longitudinal stripe on the throat, (2) four darker bars on the upper lip and continued on the lower lip (i.e. labial bars), being two originated from the lower margin of the eye, one from the nostril, and one from the region between eye and nostril, (3) cranial crests absent, (4) plain (i.e. uniform) color pattern on the posterior surfaces of thighs, (5) presence of a dark supratympanic stripe, (6) chest and anterior part of belly mottled, and the rest of the belly immaculate, (7) darker, oblique cross-bands on the upper surface of limbs, (8) finger I > finger II, (9) no webbing between fingers and toes, (10) fingers and toes lacking lateral fringes, (11) tarsal fold absent, (12) tympanum large, with both tympanic annulus and tympanic membrane distinct, (13) weak supratympanic fold, obscuring the upper edge of the tympanum, (14) canthus rostralis distinct, rounded, posteriorly convex and anteriorly concave, (15) traces of dorsolateral folds, (16) discoidal fold present, (17) skin on dorsum finely shagreen, with larger pustules interspersed, (18) skin of throat, belly and ventral surfaces of limbs smooth, with the exception of the proximoventral portion of thighs which is areolate.

Call Descriptions

Advertisement call.—The advertisement call of *Pristimantis gutturalis* (N = 11 males, 196 calls analyzed, 223 notes analyzed) (Figure 1A–

C) may be composed of one (N = 173 calls), two (N = 19 calls), or three (N = 4 calls) notes. Calls last 54 ± 39 ms (24–324 ms) and are spaced from each other by intervals of 850 ± 490 ms (150–2700 ms). Notes last 33 ± 4 ms (24–50 ms) and have pulsatile structure (*sensu* Köhler *et al.* 2017), so that pulse units cannot be properly counted or measured. The note structure may vary, with some notes being highly pulsatile (Figure 1B), whereas others are somewhat less pulsatile so that some amplitude peaks may be slightly noticeable (Figure 1C). In calls composed of two or three notes, these notes are spaced from each other by short intervals of 99 ± 1 ms (76–122 ms), being emitted at rates of 7.7 ± 0.3 notes/s (7.2–7.9 notes/s). Notes always have two emphasized frequency bands not harmonically related to each other [hereinafter referred to as the first (FFB) and the second (SFB) frequency bands]. A third frequency band located above the SFB may be present in some notes, but it has much less energy than the other bands (see Figure 1A). The FFB peaks at 1698 ± 76 Hz (1378–2250 Hz), the SFB peaks at 2864 ± 181 Hz (2498–3359 Hz), and the third frequency band, when present, peaks at 4108 ± 295 Hz (3750–5426 Hz). The dominant frequency of the notes can correspond to either the FFB or the SFB: it corresponded only to the FFB in one individual, only to the SFB in eight individuals, and it alternated its correspondence between FFB and SFB along the vocalizations of two individuals. The minimum frequency of the notes is at 1572 ± 105 Hz (1378–2438 Hz), and the maximum frequency is at 3262 ± 186 Hz (3000–3962 Hz). See Appendix II for a compilation of the main advertisement call traits reported to date for species of the *P. conspicillatus* group.

Territorial call.—The territorial call of *Pristimantis gutturalis* (N = 9 males, 95 calls analyzed) (Figure 2A–C) is audibly different from the advertisement call, has lower amplitude level than this latter, and is structurally highly variable, encompassing the three main structural patterns described next.

Pattern 1 (N = 7 males, 52 calls) (Figure 2A): a call comprising 2.6 ± 0.3 (2–4) fused highly pulsatile notes, with total duration of 83 ± 10 ms (48–142 ms) and spaced from other territorial calls by intervals of 318 ± 240 ms (15–778 ms). Spectrally, it usually has two emphasized bands in which the dominant frequency can alternate [N = 45 calls; FFB peaking at 1730 ± 112 Hz (1464–1875 Hz), and SFB at 2746 ± 118 Hz (2412–3188 Hz); a weak third band may be present too], but the energy may also be irregularly distributed over its bandwidth [N = 7 calls; dominant frequency at 2590 ± 332 Hz (1723–2813 Hz)]. Its minimum frequency is at 1776 ± 334 Hz (1313–2250 Hz), and its maximum frequency is at 3258 ± 176 Hz (3000–3750 Hz).

Pattern 2 (N = 7 males; 43 calls) (Figure 2B): a call consisting of a single highly pulsatile note with duration of 79 ± 10 ms (35–132 ms), spaced from other territorial calls by intervals 366 ± 133 ms (123–1890 ms). The energy is usually irregularly distributed over its bandwidth [N = 35 calls; dominant frequency at 2749 ± 51 Hz (2625–2813 Hz)], but it may also have two emphasized bands in which the dominant frequency can alternate [N = 8 calls; FFB peaking at 1571 ± 232 Hz (1313–1875 Hz), and SFB at 2683 ± 164 Hz (2498–2813 Hz); a weak third band may be present too]. Its minimum frequency is at 1965 ± 318 Hz (1464–2438 Hz), and its maximum frequency is at 3128 ± 168 Hz (2813–3445 Hz).

Pattern 3 (1 male; N = 3 calls) (Figure 2C): a call consisting of a single note that is pulsatile in its initial or final half, while its other half is pulsed. It has duration of 49 ± 13 ms (40–64 ms) and is spaced from other territorial calls by intervals of 155 ± 177 ms (30–280 ms). Spectrally, it may have two emphasized bands in which the dominant frequency can alternate [N = 2 calls; FFB at 1594 ± 183 Hz (1464–1723 Hz), and SFB at 2627 ± 305 Hz (2412–2842 Hz)], or the energy may be irregularly distributed over its bandwidth (N = 1 call; dominant frequency at 2498 Hz). Its minimum

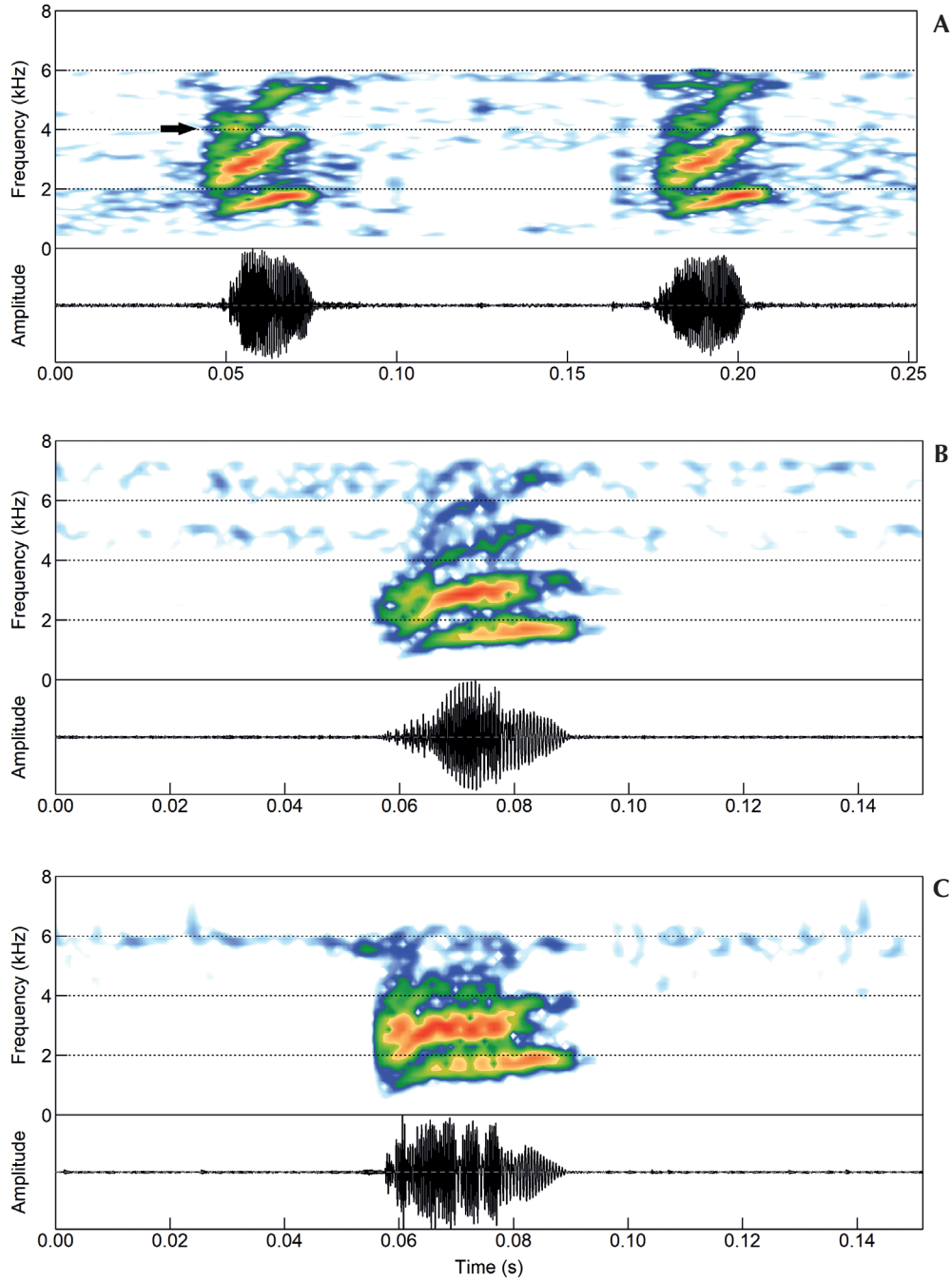

Figure 1. Audiospectrograms (top) and respective oscillograms (bottom) depicting the advertisement call of *Pristimantis gutturalis*. (**A**) Advertisement call composed of two notes (black arrow indicates the weak third band). (**B–C**) Advertisement calls composed of a single note each; notice that the note in C is somewhat less pulsatile than the one in B. Recording labels: (A) FNJV59100 (B) FNJV59093; (C) FNJV59101.

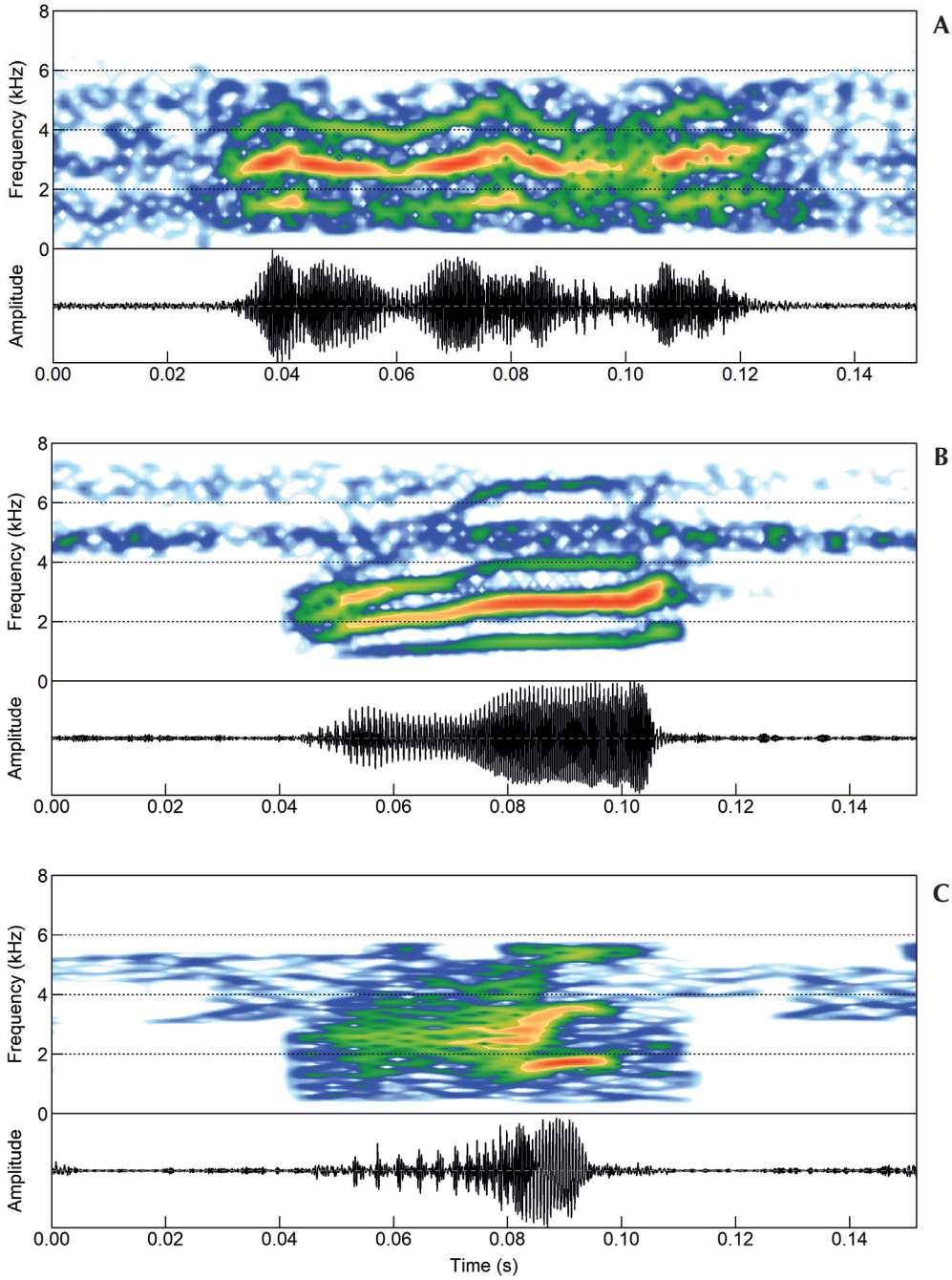

Figure 2. Audiospectrograms (top) and respective oscillograms (bottom) depicting the territorial call of *Pristimantis gutturalis*. (**A**) Territorial call composed of 3 fused pulsatile notes (pattern 1). (**B**) Territorial call composed of a single pulsatile note (pattern 2). (**C**) Territorial call composed of a note that is pulsed in its initial half and pulsatile in its final half (pattern 3). Recording labels: (A) FNJV59094; (B) FNJV59101; (C) FNJV59099.

frequency is at 1579 ± 217 Hz (1378–1809 Hz), and its maximum frequency is at 3359 ± 86 Hz (3273–3445 Hz).

The same individual may emit more than one territorial call pattern. There was no specific emission pattern of territorial calls, with these being emitted before, after, or between advertisement calls, between an advertisement call and another territorial call, or even in a sequence of territorial calls.

Morphological Variation

External morphology.—There is little variation in the external morphology. Dorsal skin is coarsely shagreen bearing interspersed pustules, which are mostly on the upper eyelid and snout regions. Relative length of fingers: III > IV > I > II. A variation was observed regarding toes III and V, with these toes having equal sizes in some specimens, and with the toe V being slightly longer in other specimens. Relative length of toes: IV > V ≥ III > II > I. Moreover, all specimens have ulnar tubercles, and all males have double, poorly developed nuptial pad (features not previously reported).

Coloration in life.—There is great variation in dorsal coloration within our sample (Figure 3) and in comparison to the previous descriptions (which state that the dorsum varies among greyish brown, brown, dark brown, and reddish brown, having red, white or black markings, and a blackish W-shaped mark on the scapular region followed by one or two chevrons; Hoogmoed *et al.* 1977, Lescure and Marty 2000, Ouboter and Jairam 2012). A specimen (CECC 3811; Figure 3A) showed an unusual dorsal pattern, consisting of a large dark brown blotch covering the entire dorsum, extending from the interorbital region to the cloacal region, and also covering the dorsal surface of thighs and shanks; the anterodorsal surface of its head stood out in contrast to the rest of its dorsum by being light beige. Another specimen (CECC 3812; Figure 3B) showed a white median longitudinal stripe over its dorsum, extending from the tip of the snout to the cloacal region. One specimen (CECC 3305; Figure 3C) presented bright orange blotches on its back and anterodorsal surface of its head. The other specimens showed small variations among themselves regarding the dorsal pattern, but within the variation range previously reported (Figure 3D–F). The dark labial bars may be more (Figure 3C–E) or less (Figure 3A–B, F) conspicuous. Iris coloration may be golden with the upper half brighter and the lower half darker (Figure 3 A–B, D, F), or entirely silver (Figure 3C, E). There is a median horizontal broad dark-reddish streak in the iris of all specimens. Tympanum coloration varies between shades of brown, being its upper portion somewhat darker. Flank coloration varies from creamish to brown, and may present some darker speckles, spots, or blotches. Belly coloration varies from creamish white to creamish yellow (Figure 4). Some specimens had reddish pigmentation on the proximoventral portion of thighs (Figure 4A–E). In all individuals, the areas adjacent/lateral to the white stripe on the throat have a darker pigmentation that varies in intensity (i.e. may be more or less pigmented). In most specimens the white stripe on the throat was delimited only by this dark pigmentation laterally spread (Figure 4B–C, F), but in some specimens conspicuous dark longitudinal bars bordering this white stripe on both sides could be distinguished (Figure 4A, D–E). The dark mottling on the chest and anterior part of the belly may be more or less conspicuous (Figure 4).

Coloration in preservative.—Brown blotches on the back became lighter or turned grayish brown. The orange blotches on the back and anterodorsal surface of the head of the individual CECC 3305 became light brown. The dorsal surfaces of limbs vary among creamish yellow, dark brown, and light brown, and the oblique cross-bands or spots became less evident. The dark supratympanic stripe may partially or completely fade. The white median longitudinal stripe on the throat became less conspicuous.

Figure 3. Dorsolateral views of some living adult males of *Pristimantis gutturalis* examined herein, depicting their chromatic variation. (**A**) CECC 3811; SVL 24.5 mm. (**B**) CECC 3812; SVL 22.0 mm. (**C**) CECC 3305; SVL 26.4 mm. (**D**) CECC 3796; SVL 25.1 mm. (**E**) CECC 3815; SVL 24.8 mm. (**F**) CECC 3795; SVL 21.1 mm. Photographs by Carlos Eduardo Costa-Campos.

Figure 4. Ventral views of some living adult males of *Pristimantis gutturalis* examined herein, depicting their chromatic variation. (**A**) CECC 3305; SVL 26.4 mm. (**B**) CECC 3796; SVL 25.1 mm. (**C**) CECC 3812; SVL 22.0 mm. (**D**) CECC 3814; SVL 25.0 mm. (**E**) CECC 3815; SVL 24.8 mm. (**F**) CECC 3795; SVL 21.1 mm. Photographs by Carlos Eduardo Costa-Campos.

Morphometry

Morphometric traits (in mm) of each specimen are provided in Table 1. Male SVL ranges from 21.1 to 26.4 mm, and the SVL of the female (CECC 1959) is of 39.9 mm. Male body proportions: TL 51–59% of SVL, FL 38–46% of SVL, HL 91–99% of HW, EN 107–133% of EL, EL 29–38% of HW, TYL 75–100% of TYH. Compared to males, the body proportions of the female did not differ much: TL 44% of SVL, FL 37% of SVL, HL 92% of HW, EN 104% of EL, EL 40% of HW, TYL 91% of TYH.

Natural History

At both studied localities we found specimens of *Pristimantis gutturalis* within the *terra-firme* rainforest, which is characterized by emergent trees and closed canopy. Males vocalized during the rainy season after 18:00 h, perched on branches and trunks ca. 60–150 cm above the ground, in a nearly vertical position with the head oriented toward the ground. In previous fieldwork carried out during the dry season, we found specimens of this species foraging in the leaf litter by morning and perched on branches and trunks close to the ground by night, but they were not vocally active. Syntopic anuran species at the Cancão Municipal Natural Park were *Allobates femoralis* (Boulenger, 1884)*, Ameerega pulchripecta* (Silverstone, 1976)*, Anomaloglossus baeobatrachus* (Boistel and de Massary, 1999), *Dendrobates tinctorius* (Cuvier, 1797), *Osteocephalus oophagus* Jungfer and Schiesari, 1995, *P. chiastonotus, P. inguinalis* (Parker, 1940)*, P. ockendeni* (Boulenger, 1912)*, P. zeuctotylus*, *Ranitomeya amazonica* (Schulte, 1999), *Rhinella castaneotica* (Caldwell, 1991)*,* and *Rhinella lescurei* Fouquet, Gaucher, Blanc and Vélez-Rodriguez, 2007. Syntopic anuran species at the municipality of Porto Grande were *Adenomera andreae* (Müller, 1923)*, Ameerega pulchripecta, P. chiastonotus, Rhinella castaneotica, Leptodactylus stenodema* Jiménez de la Espada, 1875*,* and *Osteocephalus oophagus*.

Discussion

Acoustic Comparisons With Species of the Pristimantis conspicillatus *Group*

The advertisement call traits described here for *Pristimantis gutturalis* allow distinguishing it from all members of the *P. conspicillatus* group whose calls have been described. By having 1–3 notes, the call of *P. gutturalis* differs from those of *P. charlottevillensis, P. chiastonotus, P. iiap, P. paulodutrai, P. rupicola, P. skydmainos* (1 note; Rodríguez 1994, Kaiser *et al.* 1995, Flores and Rodríguez 1997, Heyer and Carvalho 2000, Lescure and Marty 2000, Padial *et al.* 2016, Taucce *et al.* 2020), *P. incertus, P. latro, P. pictus, P. ventrigranulosus, P. vilarsi,* and *P. zeuctotylus* (combined range of 4–8 notes; Kaiser *et al.* 1995, Lescure and Marty 2000, Heyer and Barrio-Amorós 2009, Maciel *et al.* 2012, Oliveira *et al.* 2017, 2020). As its notes last 24–50 ms, the call of *P. gutturalis* differs from the calls of *P. vilarsi* (note duration of 3–21 ms; Heyer and Barrio-Amorós 2009), *P. achatinus* ("kree" notes; see Lynch and Myers 1983), *P. chiastonotus, P. iiap, P. samaipatae,* and *P. skydmainos* (combined range of note duration of 59–450 ms; Lynch and Myers 1983, Rodríguez 1994, Márquez *et al.* 1995, Flores and Rodríguez 1997, Köhler 2000, Lescure and Marty 2000, Padial and De la Riva 2009, Padial *et al.* 2016). The note rate of the call of *P. gutturalis* (7.2–7.9 notes/s) differentiates it from *P. buccinator, P. peruvianus* (combined range of note rate of 1.0–1.6 notes/s; Rodríguez 1994, Duellman 2005), *P. dundeei, P. koehleri, P. ventrigranulosus,* and *P. vilarsi* (combined range of note rate of 11.8–20.7 notes/s; Heyer and Muñoz 1999, Heyer and Barrio-Amorós 2009, Padial and De la Riva 2009, Maciel *et al.* 2012, Giaretta *et al.* 2018). The intervals between the notes of the call of *P. gutturalis* (76–122 ms) are shorter than the interval between notes of the calls of *P. buccinator* and *P. peruvianus* (combined range of inter-note interval of 180–779 ms; Rodríguez 1994), and longer than those of the calls of *P.*

Table 1. Measurements of morphometric traits (mm) and body proportions (ratios) of specimens (10 males and one female) of *Pristimantis gutturalis* from the municipalities of Serra do Navio (CECC 1116, 1959, 3250, 3305) and Porto Grande (CECC 3795–96, 3811–15), both in the state of Amapá, Brazil. Values of the "Overall" column are given as mean ± SD (minimum–maximum). [1] Female. [2] Values taken from Hoogmoed *et al.* 1977.

	CECC 1116	CECC 3250	CECC 3305	CECC 3795	CECC 3796	CECC 3811	CECC 3812	CECC 3813	CECC 3814	CECC 3815	Overall (males)	CECC 1959[1]	Holotype[1,2]
SVL	23.3	21.1	26.4	21.1	25.1	24.5	22.0	22.8	25.0	24.8	23.6 ± 1.8 (21.1–26.4)	39.9	30.4
HL	6.7	7.1	8.6	6.6	8.1	7.7	6.9	7.7	7.5	7.7	7.5 ± 0.6 (6.6–8.6)	10.9	11.6
HW	7.2	7.3	9.3	7.0	8.9	7.8	7.1	7.9	8.0	7.9	7.8 ± 0.8 (7.0–9.3)	11.8	9.9
EL	2.5	2.7	3.2	2.0	2.7	2.6	2.7	2.7	2.7	2.7	2.7 ± 0.3 (2.0–3.2)	4.7	3.9–4.1
EN	2.9	2.9	3.6	2.3	3.0	3.4	2.9	2.9	3.0	3.6	3.1 ± 0.4 (2.3–3.6)	4.9	4.1–4.3
IND	0.9	1.0	1.0	0.9	1.0	1.0	1.0	0.9	0.9	0.9	0.95 ± 0.05 (0.9–1.0)	2.0	-
EE	4.1	2.9	4.6	3.1	3.6	3.9	3.8	3.7	3.5	4.0	3.7 ± 0.5 (2.9–4.6)	6.3	-
SN	0.4	0.7	0.5	0.3	0.4	0.5	0.5	0.6	0.4	0.5	0.5 ± 0.1 (0.3–0.7)	1.1	1.6
SL	3.6	3.5	4.3	3.5	4.0	4.1	3.5	3.5	3.9	4.1	3.8 ± 0.3 (3.5–4.3)	5.9	5.9–6.1
TYH	1.3	1.2	1.3	1.1	1.1	1.3	1.2	1.2	1.1	0.8	1.2 ± 0.2 (0.8–1.3)	2.2	-
TYL	1.0	1.0	1.2	0.9	0.9	1.0	1.0	0.9	0.9	0.8	1.0 ± 0.1 (0.8–1.2)	2	1.8
FA	4.5	4.0	3.6	3.4	5.0	4.4	3.6	5.1	5.1	6.9	4.6 ± 1.0 (3.4–6.9)	9.2	-
TL	13.2	12.4	15.6	11.7	13.0	13.4	11.8	12.4	12.8	12.9	12.9 ± 1.1 (11.7–15.6)	17.6	17.3
TH	10.9	9.9	12.3	9.8	10.9	10.8	10.0	10.9	10.6	10.9	10.7 ± 0.7 (9.8–12.3)	15.8	-
FL	9.8	9.7	10.9	8.6	10.1	9.7	9.2	9.9	10.3	9.5	9.8 ± 0.6 (8.6–10.9)	14.9	-
TL/SVL	0.57	0.59	0.59	0.55	0.52	0.55	0.54	0.54	0.51	0.52	0.55 ± 0.03 (0.51–0.59)	0.44	-
FL/SVL	0.42	0.46	0.41	0.41	0.40	0.40	0.42	0.43	0.41	0.38	0.41 ± 0.02 (0.38–0.46)	0.37	-
HL/HW	0.93	0.97	0.92	0.94	0.91	0.99	0.97	0.97	0.94	0.97	0.95 ± 0.03 (0.91–0.99)	0.92	-
EN/EL	1.16	1.07	1.13	1.15	1.11	1.31	1.07	1.07	1.11	1.33	1.15 ± 0.09 (1.07–1.33)	1.04	-
EL/HW	0.35	0.37	0.34	0.29	0.30	0.33	0.38	0.34	0.34	0.34	0.34 ± 0.03 (0.29–0.38)	0.40	-
TYL/TYH	0.77	0.83	0.92	0.82	0.82	0.77	0.83	0.75	0.82	1.00	0.83 ± 0.08 (0.75–1.00)	0.91	-

achatinus ("ribit" call; see Lynch and Myers 1983) and *P. incertus* (combined range of inter-note interval of 10–50 ms; Lynch and Myers 1983, Kaiser *et al.* 1995). Since the call of *P. gutturalis* is composed of pulsatile notes (i.e. pulses cannot be distinguished), it differs from the calls of *P. buccinator, P. charlottevillensis, P. fenestratus, P. giorgii, P. iiap, P. koehleri, P. latro, P. moa, P. paulodutrai, P. peruvianus, P. pictus, P. pluvian, P. relictus, P. samaipatae, P. ventrigranulosus* [notes composed of 2–23 pulses (combined range); Rodríguez 1994, Kaiser *et al.* 1995, Heyer and Carvalho 2000, Köhler 2000, Padial and De la Riva 2009, Maciel *et al.* 2012, Padial *et al.* 2016, Oliveira *et al.* 2017, 2020, Roberto *et al.* 2022], *P. ramagii, P. vilarsi* (notes described as pulsed; Heyer and Barrio-Amorós 2009, Oitaven *et al.* 2017), *P. rupicola* (notes may be either pulsed or unpulsed; Taucce *et al.* 2020), and *P. skydmainos* (notes are unpulsed; Rodríguez 1994, Flores and Rodríguez 1997). By having its first emphasized frequency band at 1378–2250 Hz, the call of *P. gutturalis* differentiates from the calls of *P. incertus* (first band at 3000 Hz; Kaiser *et al.* 1995) and *P. rupicola* (first band at 2410–3490 Hz; Taucce *et al.* 2020). By having its second emphasized frequency band at 2498–3359 Hz, the call of *P. gutturalis* differentiates from the calls of *P. buccinator, P. charlottevillensis, P. dundeei, P. incertus, P. paulodutrai, P. pluvian, P. relictus, P. rupicola, P. skydmainos, P. ventrigranulosus,* and *P. vilarsi* [second band at 3375–6000 Hz (combined range); Rodríguez 1994, Kaiser *et al.* 1995, Flores and Rodríguez 1997, Heyer and Muñoz 1999, Heyer and Carvalho 2000, Maciel *et al.* 2012, Giaretta *et al.* 2018, Oliveira *et al.* 2020, Taucce *et al.* 2020, Roberto *et al.* 2022]. By its lower value of maximum frequency (3000–3962 Hz), the call of *P. gutturalis* differs from the call of *P. dundeei* (maximum frequency of 4125–5250 Hz; Giaretta *et al.* 2018). The call of *P. gutturalis* further distinguishes from the call of *P. charlottevillensis* by not having a harmonic structure (up to three harmonics in *P. charlottevillensis;* Kaiser *et al.* 1995). For a compilation of the main call traits reported for species of the *P. conspicillatus* group, see Appendix II.

Remarks on the Acoustics of the Pristimantis conspicillatus *Group*

The low note rate reported for *P. achatinus* [0.2–0.4 notes/s ("kree" notes); Lynch and Myers 1983] and *P. relictus* (0.5–2.2 notes/s; Roberto *et al.* 2022) does not seem to be correct in view of the short inter-note intervals that can be noticed in their respective call figures (see Figure 8 in Lynch and Myers 1983; Figure 3 in Roberto *et al.* 2022). The strikingly low values of the first frequency band reported by Rodríguez (1994) (therein referred to as fundamental frequency) for *P. fenestratus* [190 Hz (172–207 Hz)] and *P. peruvianus* [260 Hz (234–280 Hz)] are probably incorrect, and may be due to the limited technology available at the time for call recordings and analyses. The presence of more than one emphasized frequency band in the notes of the advertisement call was found to be a common feature among the species of the *P. conspicillatus* group. Although this feature was not described for *P. chiastonotus, P. zeuctotylus,* and *P. ramagii,* it is possible to notice in the spectrogram figures of the first two (Figure 5 in Lynch and Hoogmoed 1977; p. 367 in Lescure and Marty 2000) and of the latter (Figure 1 in Oitaven *et al.* 2017) that at least two emphasized bands are present. Given the wide range of the dominant frequency reported for *P. fenestratus* (1710–3591 Hz; Padial and De la Riva 2009), *P. giorgii* (1660–4142 Hz; Oliveira *et al.* 2020), and *P. ramagii* (2217–4898 Hz; Oitaven *et al.* 2017), and considering the call figures provided in these studies, it can be inferred that, as in *P. gutturalis,* the dominant frequency alternated its correspondence between the emphasized bands in the calls of these species. As demonstrated herein, these frequency bands may be useful for the acoustic distinction among species of the *P. conspicillatus* group, and therefore we highlight the importance of providing values for each band separately when describing calls of species of this group.

Aggressive vocalizations have been also reported for two other members of the *Pristimantis conspicillatus* group. Lynch and Myers (1983) described "chirp" notes for *P. achatinus* and attributed to them a territorial function. As these "chirps" were mainly emitted during a physical male combat (see Lynch and Myers 1983), we argue that they would actually be better classified as fighting calls (*sensu* Toledo *et al.* 2015). Oitaven *et al.* (2017) described the territorial call of *P. ramagii*, and stated that this vocalization differs from the advertisement call, and is rarely emitted and by a few individuals only. Likewise, the territorial call of *P. gutturalis* was not emitted by all vocally active males and was less frequent than the advertisement call. Although without following a specific emission pattern, territorial calls were emitted when there were many individuals actively calling relatively close to each other (ca. 80–100 cm). This call type showed great variation regarding both temporal and spectral structures (see Figure 2). Modifications in traits of aggressive vocalizations may be directly related to aspects of the social context (e.g. Schwartz 1989; Reichert and Gerhardt 2013, Brasileiro *et al*. 2021), but in-depth behavioral studies are needed to elucidate the specific factors associated to the variation reported here.

Morphology and Morphometry

In the original description of *Pristimantis gutturalis* its coloration in life was briefly described based on field notes on two females only (Hoogmoed *et al*. 1977), and subsequent accounts on this species (Lescure and Marty 2000, Ouboter and Jairam 2012) did not bring substantial new data. Herein we comprehensively assessed its morphological and chromatic variation, and showed that despite little morphological variation, this species has a high chromatic variation, especially with regard to its dorsal pattern (see Figure 3). Although Hoogmoed *et al*. (1977) included the "*Dorsum greyish brown with a blackish W-shaped mark on the scapular region, followed by one or two chevrons*" in the diagnosis section of *P. gutturalis,* we advocate that this trait should not be used to diagnose this species in view of the striking variation in dorsal coloration demonstrated herein.

Hoogmoed *et al*. (1977) described morphometric traits only for the holotype of the species (a female), and provided some body proportions (ratios) for the type series. However, the sample sizes provided in their table of proportions do not exactly match the number of individuals informed as part of the type series (e.g. the ratios tympanum/eye length and tibia/SVL, were allegedly to be based on 4 males and 16 females, but the type series actually comprises only 19 individuals, being 2 males, 8 females, and 9 juveniles). In the diagnosis section the authors reported an SVL of 19.0–20.3 mm for young males and of 17.9–40.9 mm for females, but made it explicit that only one adult female was known. The only subsequent morphometric information provided for this species merely stated that it reaches a maximum size of ca. 41 mm (Lescure and Marty 2000, Ouboter and Jairam 2012). Given these inconsistencies and the paucity of data on the morphometry of this species, herein we provided a thorough morphometric characterization of 10 males and one female of *P. gutturalis*. Examined males are considerably smaller than the examined female, and this latter is also larger than the female holotype (Table 1).

Conclusions

We described the advertisement and territorial calls of *Pristimantis gutturalis,* assessed its morphological and chromatic variation, and described its morphometry. We commented on the acoustics of the *P. conspicillatus* group, and showed that the advertisement call of *P. gutturalis* can be distinguished from calls of all species in this group. We found great chromatic variation, especially with regard to the dorsal pattern, thus contradicting the species description by demonstrating that the dorsal pattern should not be considered as diagnostic. Data presented here

contribute to a better understanding on the vocal repertoire and phenotypic variation of *P. gutturalis,* and may help in future identifications of new populations of this species. Since studies have pointed to a potential cryptic diversity within the *Pristimantis conspicillatus* group (e.g., Oliveira *et al.* 2017, Trevisan *et al.* 2020), providing detailed taxonomic data is crucial to establish clear species boundaries, and thus support future studies in unveiling hidden diversity.

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References

Acevedo, A. A., O. Armesto, and R. E. Palma. 2020. Two new species of *Pristimantis* (Anura: Craugastoridae) with notes on the distribution of the genus in northeastern Colombia. *Zootaxa 4750:* 499–523.

Audacity Team. 2021. *Audacity(R): Free Audio Editor and Recorder. Version 2.2.2.* URL: https://audacityteam.org

Brasileiro, A. C., P. Cascon, and D. C. Passos. 2021. How aggressive calls of a Neotropical treefrog vary among different levels of social tension? *Ethology, Ecology and Evolution 33:* 468–475.

Charif, R. A., A. M. Waack, and L. M. Strickman. 2010. *Raven Pro 1.4 User's Manual*. Version 1.4. Ithaca. Cornell Lab of Ornithology.

Duellman, W. E. 2005. *Cusco Amazónico. The Lives of Amphibians and Reptiles in an Amazonian Rainforest*. Ithaca. Cornell University Press. 433 pp.

Duellman, W. E. and E. Lehr. 2009. *Terrestrial-Breeding Frogs (Strabomantidae) in Peru*. Münster. Natur und Tier - Verlag GmbH. 382 pp.

Flores, G. and L. O. Rodríguez. 1997. Two new species of the *Eleutherodactylus conspicillatus* group (Anura: Leptodactylidae) from Peru. *Copeia 1997:* 388–394.

Frost, D. R. (ed.). 2022. *Amphibian Species of the World: an Online Reference. Version 6.1.* Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. Captured on 31 March 2022.

Giaretta, A. A., B. F. V. Teixeira, C. S. Bernardes, and P. Marinho. 2018. Distribution, call and habitat of *Pristimantis dundeei* (Anura, Craugastoridae). *Neotropical Biodiversity 4:* 134–137.

González-Durán, G. A., M. Targino, M. Rada, and T. Grant. 2017. Phylogenetic relationships and morphology of the *Pristimantis leptolophus* species group (Amphibia: Anura: Brachycephaloidea), with the recognition of a new species group in *Pristimantis* Jiménez de la Espada, 1870. *Zootaxa 4243:* 42–74.

Heyer, W. R. and C. L. Barrio-Amorós. 2009. The advertisement calls of two sympatric frogs, *Leptodactylus lithonaetes* (Amphibia: Anura: Leptodactylidae) and *Pristimantis vilarsi* (Amphibia: Anura: Strabomantidae). *Proceedings of the Biological Society of Washington 122:* 282–291.

Heyer, W. R. and C. M. Carvalho. 2000. The enigmatic advertisement call of *Eleutherodactylus ramagii* (Amphibia: Anura: Leptodactylidae). *Amphibia-Reptilia 21:* 117–121.

Heyer, W. R. and A. M. Muñoz. 1999. Validation of *Eleutherodactylus crepitans* Bokermann, 1965, notes on the types and type locality of *Telatrema heterodactylum* Miranda-Ribeiro, 1937, and description of a new species of *Eleutherodactylus* from Mato Grosso, Brazil (Amphibia: Anura: Leptodactylidae). *Proceedings of the Biological Society of Washington 112:* 1–18.

Hoogmoed, M. S., J. D. Lynch, and J. Lescure. 1977. A new species of *Eleutherodactylus* from Guiana (Leptodactylidae, Anura). *Zoologische Mededelingen 51:* 33–42.

K. Lisa Yang Center for Conservation Bioacoustics. 2022. *Raven Pro: Interactive Sound Analysis Software. Version 1.5.* URL: https://ravensoundsoftware.com

Kaiser, H., C. M. Dwyer, W. Feichtinger, and M. Schmid. 1995. A new species of *Eleutherodactylus* (Anura: Leptodactylidae) from Tobago, West Indies, and its morphometric and cytogenetic characterization. *Herpetological Natural History 3:* 151–163.

Köhler, J. (2000) Amphibian diversity in Bolivia: A study with special reference to montane forest regions. *Bonner Zoologische Monographien 48:* 1–243.

Köhler, J., M. Jansen, A. Rodríguez, P. J. R. Kok, L. F. Toledo, M. Emmrich, F. Glaw, C. F. B. Haddad, M-O. Rödel, and M. Vences. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa 4251:* 1–124.

Lescure, J. and C. Marty. 2000 *Atlas des Amphibiens du Guyane*. Paris. Musée Nationalle d'Histoire Naturelle. 388 pp.

Ligges, U., S. Krey, O. Mersmann, and S. Schnackenberg. 2018. *tuneR: Analysis of Music and Speech.* URL: https://CRAN.R-project.org/package=tuneR

Lynch, J. D. and M. S. Hoogmoed. 1977. Two new species of *Eleutherodactylus* (Amphibia: Leptodactylidae) from northeastern South America. *Proceedings of the Biological Society of Washington 90:* 424–439.

Lynch, J. D. and C. W. Myers. 1983. Frogs of the *fitzingeri* group of *Eleutherodactylus* in eastern Panama and Chocoan South America (Leptodactylidae). *Bulletin of the American Museum of Natural History 175:* 481–572.

Maciel, N. M., W. Vaz-Silva, R. M. Oliveira, and J. M. Padial. 2012. A new species of *Pristimantis* (Anura: Strabomantidae) from the Brazilian Cerrado. *Zootaxa 3265:* 43–56.

Márquez, R., I. De La Riva, and J. Bosch. 1995. Advertisement calls of Bolivian Leptodactylidae (Amphibia, Anura). *Journal of Zoology 237:* 313–336.

Oitaven, L. P. C., J. R. O. Santos, A. D. O. Silva, P. G. Gambale, and J. G. B. Moura. 2017. Description of vocalisations and analysis of intra and inter-individual variation in *Pristimantis ramagii* (Boulenger, 1888) in an upland swamp, northeast Brazil. *Herpetology Notes 10:* 197–203.

Oliveira, E. A., L. R. Rodrigues, I. L. Kaefer, K. C. Pinto, and E. J. Hernández-Ruz. 2017. A new species of *Pristimantis* from eastern Brazilian Amazonia (Anura, Craugastoridae). *ZooKeys 687:* 101–129.

Oliveira, E. A., L. A. Silva, K. L. A. Guimarães, M. Penhacek, J. G. Martínez, L. R. R. Rodrigues, D. J. Santana, and E. J. Hernández- Ruz. 2020. Four new species of *Pristimantis* Jiménez de la Espada, 1870 (Anura: Craugastoridae) in the eastern Amazon. *PLoS One 15:* 1–28.

Ouboter, P. E. and R. Jairam. 2012. *Amphibians of Suriname*. Leiden. Brill. 388 pp.

Padial, J. M. and I. De la Riva. 2009. Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura). *Zoological Journal of the Linnean Society 155:* 97–122.

Padial, J. M., T. Grant, and D. R. Frost. 2014. Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa 3825:* 1–132.

Padial, J. M., G. Gagliardi-Urrutia, J. C. Chaparro, and R. C. Gutiérrez. 2016. A new species of the *Pristimantis conspicillatus* Group from the Peruvian Amazon (Anura: Craugastoridae). *Annals of Carnegie Museum 83:* 207–218.

R Core Team. 2021. *R: A language and environment for statistical computing. Version 3.6.2.* URL: https:// www.R-project.org.

Reichert, M. S. and H. C. Gerhardt. 2013. Gray tree frogs, *Hyla versicolor*, give lower-frequency aggressive calls in more escalated contests. *Behavioral Ecology and Sociobiology 67:* 795–804.

Roberto, I. J., D. Loebmann, M. L. Lyra, C. F. B. Haddad, and R. W. Ávila. 2022. A new species of *Pristimantis* Jiménez de la Espada, 1870 (Anura: Strabomantidae) from the "Brejos de Altitude" in Northeast Brazil. *Zootaxa 5100:* 521–540.

Rodríguez, L. 1994. A new species of the *Eleutherodactylus conspicillatus* Group from Peru, with comments on its call. *Alytes 12:* 49–63.

Schwartz, J. J. 1989. Graded aggressive calls of the spring peeper, *Pseudacris crucifer*. *Herpetologica 45:* 172–181.

Sueur, J., T. Aubin, and C. Simonis. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics 18:* 213–226.

Taucce, P. P. G., J. S. Nascimento, C. C. Trevisan, F. S. F. Leite, D. J. Santana, C. F. B. Haddad, and M. F. Napoli. 2020. A new rupicolous species of the *Pristimantis conspicillatus* group (Anura: Brachycephaloidea: Craugastoridae) from central Bahia, Brazil. *Journal of Herpetology 54:* 245–257.

Toledo, L. F., I. A. Martins, D. P. Bruschi, M. A. Passos, C. Alexandre, and C. F. B. Haddad. 2015. The anuran calling repertoire in the light of social context. *Acta Ethologica 18:* 87–99.

Trevisan, C. C., H. Batalha-Filho, A. A. Garda, L. Menezes, I. R. Dias, M. Solé, C. Canedo, F. A. Juncá, and M. F. Napoli. 2020. Cryptic diversity and ancient diversification in the northern Atlantic Forest *Pristimantis* (Amphibia, Anura, Craugastoridae). *Molecular Phylogenetics and Evolution 148:* 1055–7903.

Watters, J. L., S. T. Cummings, R. L. Flanagan, and C. D. Siler. 2016. Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa 4072:* 477–495.

Zumel, D., D. Buckley, and S. R. Ron. 2021. The *Pristimantis trachyblepharis* species group, a clade of miniaturized frogs: description of four new species and insights into the evolution of body size in the genus. *Zoological Journal of Linnean Society 40:* 1–40.

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Appendix I. *Recordings (.wav) analyzed in the present study and associated metadata. All recordings were made in the municipality of Porto Grande, state of Amapá, Brazil (see Material and Methods section for coordinates of Site 1 and Site 2). Abbreviation of the recording equipment (recorder / microphone): TRN (Tascam DR-100 / RØDE NTG1); ZYH (Zoom H1N / YOGA HT-81).*

Recording label	Locality	Time	Date	Air temperature (°C)	Recording equipment	Voucher CECC
FNJV59093	Site 1	18:47	09 March 2022	24.8	TRN	3796
FNJV59094	Site 1	17:22	09 March 2022	24.6	TRN	3795
FNJV59095	Site 1	17:46	04 March 2022	-	TRN	-
FNJV59096	Site 1	17:57	04 March 2022	-	TRN	-
FNJV59097	Site 1	18:28	04 March 2022	-	TRN	-
FNJV59098	Site 1	18:33	04 March 2022	-	TRN	-
FNJV59099	Site 1	18:44	04 March 2022	-	TRN	-
FNJV59100	Site 1	18:55	04 March 2022	24.4	TRN	3814
FNJV59101	Site 2	17:53	11 March 2022	24.8	TRN	3813
FNJV59102	Site 2	18:16	11 March 2022	-	ZYH	-
FNJV59103	Site 2	18:34	11 March 2022	-	ZYH	-
FNJV59104	Site 2	19:02	11 March 2022	-	ZYH	-

Appendix II. *Summary of main advertisement call traits reported to date for the species of the* Pristimantis conspicillatus *group. Data reported herein for* P. gutturalis *are in bold. Values are given as mean ± SD (minimum–maximum). N = calls analyzed/notes analyzed/individuals. [*] Considering notes within a call; [a] "Kree notes". [b] "Ribit call". [c] "Bleep notes". [d] Originally given as notes/min, but herein converted. [e] Middle note duration. [f] Last note interval. [g] See Discussion section. [h] "...a series of quickly repeated whistle-like notes...". [i] As* Eleutherodactylus terraebolivaris. [j] *As* Eleutherodactylus ramagii. [k] *As* Eleutherodactylus fenestratus. [l] *As* Eleutherodactylus *sp.*

Species	Call duration (ms)	Notes/ call	Note duration (ms)	Note rate (notes/s)[*]	Inter-note interval (ms)[*]	Peak of the FFB (Hz)	Peak of the SFB (Hz)	Dominant frequency (Hz)	Minimum frequency (Hz)	Maximum frequency (Hz)	References
P. achatinus[a] (*N* = -/36/2)	-	(1–6)?	320 (150–450)	(0.2–0.4)	170 (70–350)?	1500 or 1600 (1300–1800)	3100 (3000–3400)	= SFB	-	-	Lynch and Myers 1983
P. achatinus[b] (*N* = 20/-/5)	150 (130–180)	2	-	-	30 (10–50)	-	-	(2400–3300)?	-	-	Lynch and Myers 1983
P. achatinus[c] (*N* = -/-/5)	-	-	-	-	-	-	-	-	-	-	Lynch and Myers 1983
P. buccinator (*N* = 6/-/-)	-	(1–6)	(50–66)	1.6[d]	618 (403–779)	(1846–2131)?	3595 (3564–3630)	= SFB	-	-	Rodríguez 1994
P. charlottevillensis (*N* = -/13/-)	51 (43–68)	1	51 (43–68)	-	-	2000	4000	= SFB	-	-	Kaiser *et al.* 1995
P. chiastonotus (*N* = -/-/-)	74	1	74	-	-	-	-	2620–3450[g]	-	-	Lescure and Marty 2000
P. dundeei (*N* = 2/-/2)	(290–530)	(5–8)	(20?–50)	(13.7–14.7)	-	(2067–2153)	(3692–3875)	= SFB	-	-	Heyer and Muñoz 1999
P. dundeei (*N* = -/-/1)	55 (470–642)	10.5 (9–12)	34 (33–34)[e]	18.9 (18.7–19.2)	32 (30–34)[f]	2023 (2006–2039)	4125 (4125–4125)	= SFB	2156 (2062–2250)	5063 (4875–5250)	Giaretta *et al.* 2018
P. dundeei (*N* = -/-/5)	266 ± 74 (136–314)	4.6 ± 1.0 (2–6)	43 ± 3 (40–47)[e]	17.7 ± 1.7 (16.2–20.7)	36 ± 5 (31–41)[f]	1901 ± 79 (1818–2012)	3656 ± 210 (3375–3937)	= SFB	1934 ± 169 (1750–2203)	4375 ± 145 (4125–4500)	Giaretta *et al.* 2018
P. fenestratus (*N* = 3/-/-)	-	3 (1–3)	45	-	(73–84)	190 (172–207)?	3100 (2896–3450)	= SFB	-	-	Rodríguez 1994
P. fenestratus (*N* = 10/-/2)	(160–360)	(2–3)	(50–90)	(7.6–9.1)	-	(1720–2067)	(3531–3617)	= SFB	-	-	Heyer and Muñoz 1999
P. fenestratus (*N* = -/2/1)	75	1	75	-	-	-	-	3270	1300?	5000?	Köhler 2000
P. fenestratus (*N* = 22/55/6)	265 ± 82 (157–458)	2.6 ± 0.6 (2–4)	63 ± 11 (50–91)	10.1 ± 1.5 (7.7–12.7)	-	1747 ± 158 (1542–2048)	-	3086 ± 581 (1710–3591)[g]	-	-	Padial and De la Riva 2009

Appendix II. *Continued.*

Species	Call duration (ms)	Notes/ call	Note duration (ms)	Note rate (notes/s)*	Inter-note interval (ms)*	Peak of the FFB (Hz)	Peak of the SFB (Hz)	Dominant frequency (Hz)	Minimum frequency (Hz)	Maximum frequency (Hz)	References
P. giorgii (*N* = 13/42/7)	171 ± 78 (131–278)	(3–4)	40 ± 12 (17–74)	-	-	1512 ± 259 (663–1872)	-	3007 ± 511 (1660–4142)[g]	-	-	Oliveira *et al.* 2020
***P. gutturalis* (*N* = 196/223/11)**	**54 ± 39 (24–324)**	**1.2 ± 0.3 (1–3)**	**33 ± 4 (24–50)**	**7.7 ± 0.3 (7.2–7.9)**	**99 ± 1 (76–122)**	**1698 ± 76 (1378–2250)**	**2864 ± 181 (2498–3359)**	**= FFB or SFB**	**1572 ± 105 (1378–2438)**	**3262 ± 186 (3000–3962)**	**Present study**
P. iiap (*N* = -/30/3)	75 (63–84)	1	75 (63–84)	-	-	1857 (1739–2129)	3690 (3136–4010)	= SFB	-	-	Padial *et al.* 2016
P. incertus (*N* = 10/-/-)	138 (128–160)	4	19 (11–26)	-	26 (15–32)	3000	6000	= FFB	-	-	Kaiser *et al.* 1995[i]
P. koehleri (*N* = 21/119/6)	421 ± 160 (173–644)	5.7 ± 1 (3–8)	36 ± 7 (20–54)	14.1 ± 1.8 (11.8–17.3)	-	1854 ± 72 (1732–1971)	3662 ± 129 (3245–3971)	= SFB	-	-	Padial and De la Riva 2009
P. latro (*N* = 7/49/6)	455 ± 69 (402–581)	7	40 ± 5 (31–46)	-	-	1381 ± 36 (1342–1449)	3069 ± 254 (2636–3272)	= SFB	-	-	Oliveira *et al.* 2017
P. moa (*N* = 20/75/6)	294 ± 48 (212–380)	4.3 ± 0.6 (3–5)	45 ± 9 (23–64)	-	-	1510 ± 111 (1321–1660)	3118 ± 159 (2657–3400)	= SFB	-	-	Oliveira *et al.* 2020
P. paulodutrai (*N* = -/10/1)	(27–32)	1	(27–32)	-	-	(2150–2330)	(3540–3970)	= SFB	1500?	4960?	Heyer and Carvalho 2000[j]
P. peruvianus (*N* = 7/-/-)	-	2 (1–5)	30	-	185 (180–380)	260 (234–280)?	3070 (2812–3140)	= SFB	-	-	Rodríguez 1994
P. peruvianus (*N* = -/-/-)	-	>1[h]	150	1.0[d]	-	1300?	1600?	-	-	-	Duellman 2005
P. pictus (*N* = 15/56/4)	235 ± 21 (216–302)	(4–5)	26 ± 4 (17–33)	-	-	1677 ± 113 (1406–1915)	2889 ± 160 (2487–3272)	= SFB	-	-	Oliveira *et al.* 2020
P. pluvian (*N* = 8/16/3)	152 ± 8 (141–165)	2	56 ± 7 (47–73)	-	-	1852 ± 92 (1618–1936)	3645 ± 248 (3484–4312)	= SFB	-	-	Oliveira *et al.* 2020
P. ramagii (*N* = 138/-/9)	151 ± 52 (73–245)	2.6 ± 0.7 (1–4)	36 ± 8 (19–69)	-	-	-	-	3942 ± 491 (2217–4898)[g]	-	-	Oitaven *et al.* 2017
P. relictus (*N* = 181/150/3)	60 ± 80 (20–660)	1.9 ± 1.2 (1–8)	30 ± 10 (20–50)	1.4 ± 0.8 (0.5–2.2)	-	2033 ± 55 (1895–2153)	3923 ± 126 (3618–4221)	= SFB	-	-	Roberto *et al.* 2022

Appendix II. *Continued.*

Species	Call duration (ms)	Notes/ call	Note duration (ms)	Note rate (notes/s)*	Inter-note interval (ms)*	Peak of the FFB (Hz)	Peak of the SFB (Hz)	Dominant frequency (Hz)	Minimum frequency (Hz)	Maximum frequency (Hz)	References
P. rupicola (*N* = -/168/15)	31 ± 19 (11–86)	1	31 ± 19 (11–86)	-	-	3010 ± 0230 (2410–3490)	5850 ± 500 (4000–6900)	= FFB	-	-	Taucce *et al.* 2020
P. samaipatae (*N* = 3/-/-)	-	2?	(66–100)	6.0 ± 0.6 (5.7–6.6)[d]	-	(1575–1797)	(3009–3352)	= SFB	-	-	Márquez *et al.* 1995[k]
P. samaipatae (*N* = 3/-/1)	228 ± 3 (225–231)	2	83 ± 66 (73–90)	-	-	-	-	3180	1200?	6800?	Köhler 2000
P. samaipatae (*N* = 98/160/12)	292 ± 168 (82–1062)	2 ± 0.2 (1–3)	89 ± 16 (59–141)	8.5 ± 2.1 (2.7–14.9)	-	1705 ± 64 (1535–1834)	3327 ± 176 (2922–3853)	= SFB	-	-	Padial and De la Riva 2009
P. skydmainos (*N* = -/4/-)	60	1	60	-	-	-	-	4430 (4424–4500)	-	-	Rodríguez 1994[l]
P. skydmainos (*N* = -/6?/1?)	60	1	60	-	-	2120	4240	= SFB	-	-	Flores and Rodríguez 1997
P. ventrigranulosus (*N* = 22/108/3)	289 ± 60 (202−411)	4.9 ± 0.8 (4−7)	44 ± 9 (24−74)	17.1 ± 1.1 (15.6−19.8)	-	3386 ± 314 (2127–3652)	4021 ± 270 (3452–4335)	= SFB	-	-	Maciel *et al.* 2012
P. vilarsi (*N* = -/1/1)	521	8	9 ± 6 (3–21)	14	-	(1959–2256)	(3799–4284)	= SFB	-	-	Heyer and Barrio-Amorós 2009
P. zeuctotylus (*N* = -/-/-)	489	4	45	-	-	-	-	(2140–3250)[g]	-	-	Lescure and Marty 2000