Rediscovering the rare short-winged unicorn katydid *Toledopizia salesopolensis* (Piza) (Tettigoniidae: Conocephalinae) from South and Southeastern Brazil: First description of male and bioacoustics

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Abstract

Toledopizia Chamorro-Rengifo & Braun, 2010 is a poorly known monotypic genus of Copiphorini. The only known specimen is the female type of T. salesopolensis (Piza, 1980). In this contribution, we present an updated description of this species, describing the unknown male, and provide biological and bioacoustic data. We also describe color variation, update the distribution data, and extend the known distribution of the species to two localities in Paraná State and another two in São Paulo State.

Keywords

behavior, calling song, Copiphorini, male genitalia, new record

Introduction

Toledopizia Chamorro-Rengifo & Braun, 2010 is a monotypic genus of brachypterous katydids with a conspicuously long and pointed fastigium of vertex that is five or six times longer than the antennal scape; the pronotum is small, with shallow lateral lobes without humeral sinus and with an exceptionally long ovipositor that is almost as long as the body (Chamorro-Rengifo and Braun 2010). The genus was proposed by Chamorro-Rengifo and Braun (2010) to allocate the species Toledopizia salesopolensis (Piza, 1980), a species described based on a unique female from Reserva Biológica de Boraceia in Salesópolis municipality, São Paulo State, Brazil (Piza 1980). The species was first allocated in the genus Acantheremus Karny, 1907 and was compared to another species currently in the genus Copiphora Serville, 1831), Copiphora azteca Saussure & Pictet, 1898 (Piza 1980). Piza (1980) believed that the specimen had short wings because it was probably a juvenile.

The genus is very similar to another monotypic genus from Guatemala, Mayacephalus Cadena-Castañeda, Monzón-Sierra & Cortés-Torres, 2016. However, this last genus was proposed as a

the presence of a humeral sinus, well-developed furrows on the pronotal disk, and the genicular lobes of fore and mid femora being unarmed (Cadena-Castañeda et al. 2016). Another very similar genus is Daedalellus Uvarov, 1940, but its members have a shorter and rounded fastigium of vertex, which is three times longer than the antennal scape (Nickle 2001, Cadena-Castañeda et al. 2016).

In this contribution, we present four new records of Toledopizia salesopolensis from the Coastal Atlantic Forest of Brazil. Additionally, we provide the first description of the male and the first description of the bioacoustics of this species.

Methods

The katydids were sampled in four sites throughout the dense ombrophilous forest of the coastal Atlantic forest: Graciosa Moutain range (-25.339522, -48.892949) in the Graciosa road, which crosses the sea's ridge of Paraná State in the Morretes municipality; Guaricana old road (-25.726172, -49.008736), which drives to the sea's ridge of Parana in the São José dos Pinhais municipality; Extrema municipality in the Mantiqueira Mountain range (-22.879661, -46.304542); and São Lourenço da Serra in sea's ridge of São Paulo State (-22.927950, -46.904334) (Fig. 1A, B). The katydids were collected at night with the help of flashlights and sweep nets. Males were located following the stridulation, and females by active searching.

After collection, individuals were brought to the laboratory, placed in individual plastic jars, and fed with fish food flakes, insects, and grass seeds. To gather bioacoustic samples, males were placed alone in a tulle box and recorded with the help of a Tascam DR-22WL recorder; samples were in .wav sound format at a sampling frequency of 96 kHz, 24 bits, +1 dB / -3 dB. Songs were analyzed using Raven PRO 64 1.5.0 (Raven 2014), with the following configuration: sampling DFT 1024, hamming 512, and window size 256. For each male, we analyzed each sylseparated taxon based on the following combination of characters: lable of five echemes and the subsequent 40 echemes. A sample

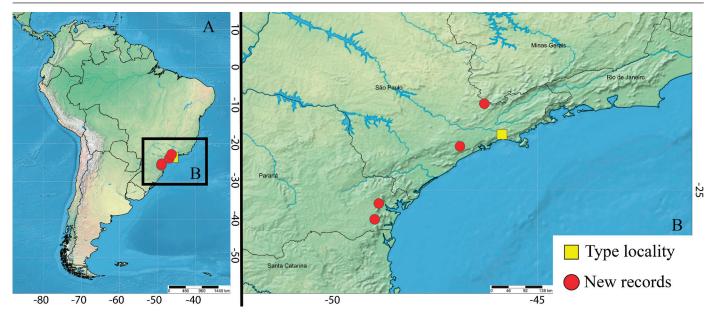


Fig. 1. Occurrence map of Toledopizia salesopolensis. A. South America; B. Sampled areas. Red circles: new records; yellow square: type-locality.

of the male calling song will be available at Orthoptera Species File after publication. The sound parameters are presented as "mean \pm standard deviation". Oscillograms and spectrograms were obtained with the help of the software RStudio (RStudio Team 2015) and the packages Tuner 1.0 (Ligges et al. 2013) and Seewave (Sueur et al. 2008). The configuration used was as follows: window size = 512, zero-padding = 16, overlap = 90%, and collevels = seq (-46,0,0,5). We used the terminology proposed by Ragge and Reynolds (1998): (i) syllable = a sound produced during the opening and closing of the tegmina; (ii) hemisyllable = a sound produced by only the closing stroke; and (iii) echeme = a group of syllables of first order.

The individuals were then euthanized, dissected in order to remove the internal organs and maintain the color, and filled with cotton to maintain the shape. Male genitalia were removed with the help of scissors and insect pins, then left for 6 hours in 10% KOH, washed with 1% acetic acid for five minutes, and transferred to vials with glycerine that were placed on the respective specimen pin. The terminology adopted to the phallic complex followed Chamorro-Rengifo and Lopes-Andrade (2014), to the sternum followed Mendes and Rafael (2021), and to the wing venation followed Desutter-Grandcolas et al. (2017).

The specimens were analyzed under an Olympus SZH10 stereomicroscope with an Olympus DF Planapo IX lens. Photos were taken with the aid of a Leica digital camera, DFC295, coupled with the Leica stereoscopic microscope and stacked with Zerene Stacker software (Version 1.04 Build). Photos of the habitus were made using a Nikon D5300 attached to a sigma 105 mm macro lens, then stacked with Zerene software. Photos in vivo were not stacked.

We use the following abbreviations in the text for measurements (in mm): body length, BL (distance from the fastigium to the apex of abdomen, excluding terminalia); tegmina length, TL; fastigium length, FL; head width, HW; pronotal disc length, PL; lateral lobe of pronotum length, LLPL; lateral lobe of pronotum height, LLPH; femur III length, FiiiL; tibia III length, TiiiL; subgenital plate length, SPL; cercus length, CL; ovipositor length, OL; stridulatory file length, SFL; number of teeth in the stridulatory file, TN.

The specimens are deposited at the Museu Nacional (MNRJ), Rio de Janeiro, Brazil, and "Coleção Entomológica Padre Jesus Santiago Moure" (DZUP), Curitiba, Brazil.

Results

Taxonomy

Family Tettigoniidae Krauss, 1902 Subfamily Conocephalinae Kirby & Spence, 1826 Tribe Copiphorini Karny, 1912

Genus Toledopizia Chamorro-Rengifo & Braun, 2010

Type species.—Toledopizia salesopolensis (Piza, 1980)

Emmended diagnosis.—Toledopizia is characterized by the following combination of characters: medium-sized brachypterous katydids; very long and acuminated fastigium of vertex, at least five times longer than scapus (Fig. 2), dorsally with a row of tiny tubercles at each border (Fig. 3A, B); pronotum lacking humeral sinus and no furrows marked on pronotal disk (Fig. 3C); in males, posterior border slightly more produced behind than in females (Fig. 2A, B); tegmina not surpassing the third abdominal tergite in females, and the sixth in males; all genicular lobes armed with a conspicuous spine; ovipositor very long, almost as long as body, and almost straight (Fig. 2C).

Comments.—Toledopizia differs from other Copiphorini genera by the following characteristics: Mayacephalus by the presence of humeral sinus, dorsal furrows on the pronotum, and fore and mid genicular lobes unarmed; Daedalellus and Brachycaulopsis Fontana, Mariño-Pérez & Woller, 2013 by the shorter and apically rounded fastigium of vertex; Caetitus Antunes, Chamorro-Rengifo & Takiya, 2018 by the fastigium of vertex shorter, with three spines, genae bearing spine-like projections and pronotum, notably produced behind; and Copiphora and Acantheremus by the macropterous condition.

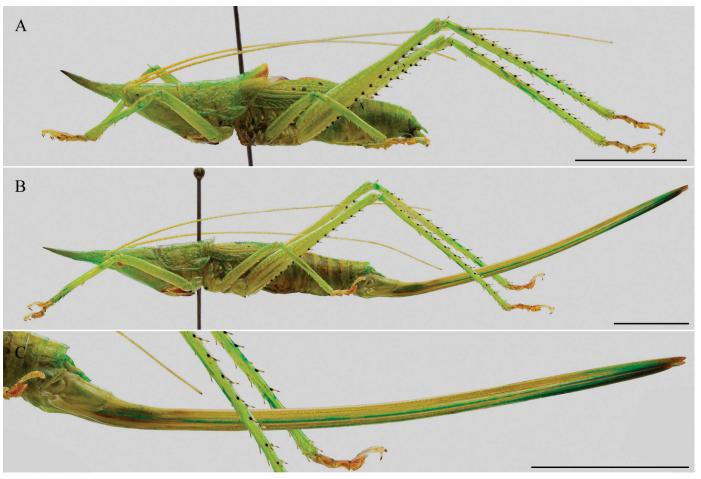


Fig. 2. Habitus of Toledopizia salesopolensis. A. Male; B. Female; C. Ovipositor. Scale bars: 1 cm.

Toledopizia salesopolensis (Piza, 1980)

Acantheremus salesopolensis Piza, 1980: 111 (Fig. 1) (original description).

Toledopizia salesopolensis - Chamorro-Rengifo and Braun 2010: 53 (Fig. 2); Cigliano et al. 2021 (Orthoptera SpeciesFile online).

Holotype—BRAZIL • ♀; São Paulo, Salesópolis, Reserva Biológica de Boraceia; 25 Dec. 1963; F. Werner and H. Reichardt leg.; Departamento de Zoologia da Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Piracicaba, São Paulo, Brazil.

Material examined.—BRAZIL • 1♀; São Paulo, São Lourenço da Serra; 23°55′40.6″S, 46°54′15.6″W; Nov.2017; P.W. Engelking leg.; MNRJ • 2♂; São Paulo, Extrema; 22°52′46.8″S, 46°18′16.4″W; Mar.2018; P.W. Engelking leg.; MNRJ • 2♂; Paraná, São João dos Pinhais, Estr. Guaricana; 25°43′19″S, 49°0′30″W; 09.Apr.2021; M. Fianco & D.N. Barbosa leg.; active night collecting; DZUP • 1♂; Paraná, Morretes, Serra da Graciosa; 25°20′41″S, 48°53′28″W; 22.Apr.2021; M. Fianco & A.L. Mott Jr. leg.; active night collecting; DZUP • 1♂1♀; same data as for preceding; 08.Apr.2021; M. Fianco, A.L. Mott Jr. & C.C. Borda leg.; DZUP.

Additional description.—Body slender, general color green, fasti-gium of vertex quite long, ovipositor as long as body (Fig. 2A–C). Head (Fig. 3A, B) opisthognathous; eye small (smaller than scapus); fastigium of vertex triangular, acuminated, and very long

(at least five times longer than scapus), with a row of tiny tubercles on each side; antennae filiform and long, almost twice as long as body. Pronotal disk (Fig. 3C) without furrows, anterior and posterior borders truncated—in males, posterior border more produced behind, with posterior margin slightly elevated. Lateral lobes of pronotum longer than tall, with an arched and oblique anterior margin, anteroventral angle widely obtuse; ventral margin anterior half slightly concave, posterior half convex; posteroventral angle widely obtuse; humeral sinus inconspicuous. Wings small, tegmina (Fig. 3D, E) marked with black dots, not surpassing third abdominal tergite in females and sixth in males; anal area and surface below CuA reddish; ScP and R almost straight; R branching on apex, branches parallel; M straight, branching near half of tegmen; A1 (Fig. 3G) slightly curvate. Stridulatory file 1.7 mm long, bearing 159 teeth. Hind wing (Fig. 3F) short, not surpassing tegmina. Prosternum bearing two thin spines near anterolateral margin. Mesosternal lobes almost triangular (Fig. 3H), with convex anterolateral margins, and almost straight lateral and posterior borders. Metasternal lobes (Fig. 3H) almost triangular, bearing two protuberances anterolaterally; posterolateral margin forming a straight angle. Lateral lobes of metasternum similar to those of mesosternum. Legs with all femora dorsally smooth and all genicular lobes armed with a conspicuous spine; fore femora armed internally and mid femora externally; hind femora on both ventral margins with 14 teeth, increasing in size apically; hind tibia on both dorsal margins armed basally and apically with black spines, ventral spines concolorous. Tenth tergite (Fig. 3I) with hind margin in males and females produced into two conspicuously

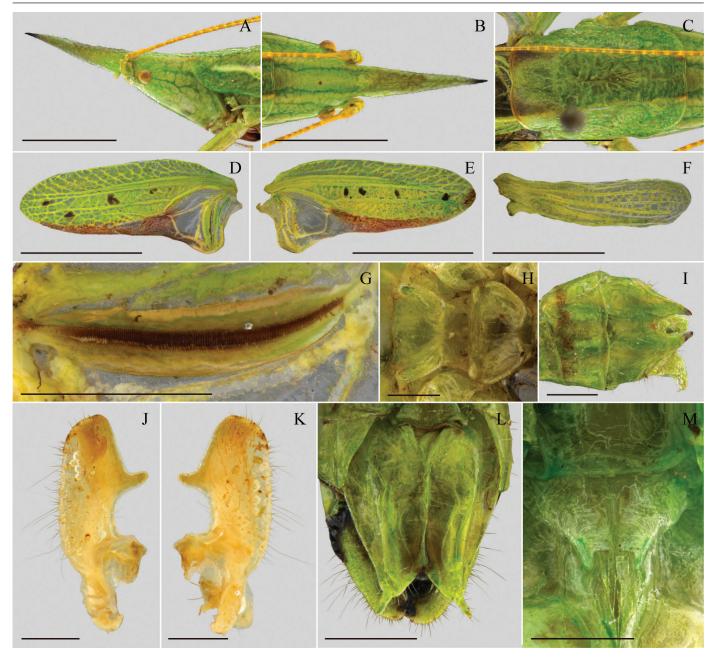


Fig. 3. Specific characters of *Toledopizia salesopolensis*. A, B. Head in lateral (A) and dorsal view (B); C. Pronotum, dorsal view; D, E. Male left (D) and right tegmen (E); F. Posterior wing; G. Stridulatory file (A1 vein); H. Meso and metabasisternum; I. Tergite X; J, K. male left cercus in ventral (J) and dorsal view (K); L, M. male (L) and female subgenital plate (M). Scale bars: 6 mm (A–C); 7 mm (D–F); 1 mm (G–I, M); 0.5 mm (J, K); 1.5 mm (L).

acuminated lobes. Male cerci (Fig. 3J, K) robust and blunt, with an inward finger-like projection medially and a ventral mediodistal fold. Phallic complex (Fig. 4) bearing two parallel irregular bars comprising the titillator's sclerites, the only sclerotized appendage of the external face of the phallus. Male subgenital plate (Fig. 3L) with a well-developed medial keel throughout all extension and a V-like sinus on posterior border. Females with long and conical cerci; subgenital plate short (Fig. 3M), posterior margin produced into two thin lobes separated by U-like sinus; ovipositor very long, as long as body, and nearly straight.

Chromatic pattern.—Specimens can be found with two color patterns: green and brownish-yellow (Figs 2, 5). On both morpho-

types, tip of fastigium of vertex blackish; dorsal surface of pronotum and fastigium of vertex slightly darker than surroundings; lateral carinae yellowish in green morph, and light yellow in brownish-yellow morph, brownish near posterior margin in males; tegmina marked with black spots and reddish on the area between CuA and hind margin behind stridulatory area; abdomen lighter than other body parts and dorsally translucent, evidencing the dorsal vessel. In addition, green morphotype has conspicuously yellowish tarsi.

Biology.—All individuals sampled seem to have a particular relation to bamboo (see Fig. 5A, B, and Fig. 6B, male resting at bamboo leaves) (Poaceae: Bambusoideae). Even with several sampling

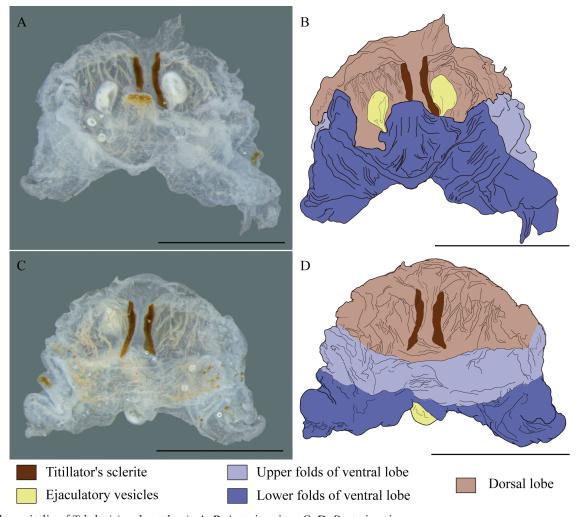


Fig. 4. Male genitalia of Toledopizia salesopolensis. A, B. Anterior view; C, D. Posterior view.

efforts in the Coastal Atlantic Forest, the individuals were only collected in areas with bamboo. We hypothesize that females use the bamboo stalks or roots to oviposit since the Copiphorini generally oviposits in grasses (Poaceae). Individuals were found resting and feeding in the leaves of grasses, including bamboo, in the field, but males call while in stems inside vegetation. We hypothesize that they go to more open areas to forage, but males could avoid predation with this behavior. The species is omnivorous; individuals were seen feeding on seeds of grasses in the field, but in laboratory conditions, they also fed on insects, using the forelegs to catch the preys (Fig. 6A).

Bioacoustics.—Males stridulate only at night, producing long 19-21; SPL: 1.4-1.5; CL: 2; OL: 38.0-40.2. echeme sequences (Fig. 7A) with a duration of about 45.3 \pm 12.3 min (5-82 min, n = 18). Each echeme (Fig. 7B) is of moderate duration, of about 1.3 s \pm 0.16 (0.8–1.7 s, n = 128), consisting of 22 \pm 3 syllables (19–29, n = 128) emitted continually, with a duration of 40 ± 4 ms (20–49 ms, n = 336) each and a mute interval of 17 ms \pm 3 (7–38 ms, n = 321). The echemes are separated by a constant mute interval with a duration of 1.3 ± 0.3 s (0.6–2.7 s, n = 140). The peak frequency is 17.4 ± 0.7 kHz (15.5–22.5 kHz, n = 464), and the bandwidth 95% is of 6.4 ± 0.5 kHz (4.7–9.4 kHz, n = 464); the total bandwidth ranges from 12 ± 2 kHz to 25 ± 3 kHz (8.4-27 kHz, n = 464) (Fig. 7C, D). The sequence of echemes is produced in a crescendo of intensity that stabilizes after 5 min.

The echemes are also in a crescendo of intensity that generally stabilizes after the fourth syllable, but each syllable is produced in a constant intensity. At the field, they call from small to mediumthick branches or stems, in heights ranging from 50 cm to 1.5 m. Additionally, we heard many individuals singing at the same time in chorus behavior.

Measurements (mm).—Males: BL: 25.0-29.1; TL: 9.0-10.8; FL: 5.3-8.0; HW: 4.0-4.7; PL: 7-9; LLPL: 5.6-8.0; LLPH: 3.5-4.5; FiiiL: 14.1–18.0; TiiiL: 14.7–18.0; SPL: 2.7–3.5; CL: 1.5–2.5; SFL: 2; TN: 159. Females: BL: 30.0-35.1; TL: 8.0-8.4; FL: 6.2-8.0; HW: 5-6; PL: 6.9-8.0; LLPL: 6; LLPH: 3.8-4.0; FiiiL: 19.5-19.7; TiiiL:

Discussion

As mentioned before, the two nominal species T. salesopolensis and Mayacephalus dickmanorum Cadena-Castañeda, Monzón-Sierra & Cortés-Torres, 2016 are very similar due to the long and pointed fastigium of the vertex, the long and straight ovipositor, and the brachypterous condition (Cadena-Castañeda et al. 2016). Both species can be distinguished by the presence or absence of a humeral sinus of pronotum, spines on the genicular lobes of the fore and mid femora, and by the form of the male and female postabdomen. In T. salesopolensis, the tenth abdominal tergite of both sexes is



Fig. 5. Toledopizia salesopolensis in vivo. A, B. Male on bamboo leaf, green morph; C. Male on leaf, yellow morph.

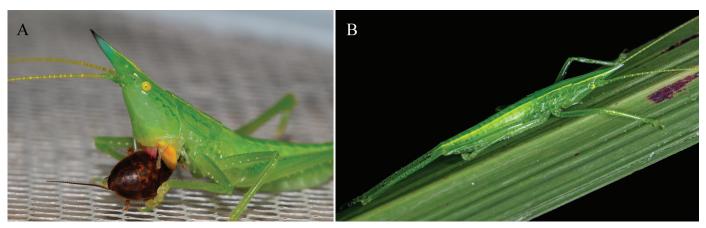


Fig. 6. Toledopizia salesopolensis in vivo. A. Nymph feeding on a cockroach; B. Male on a bamboo leaf.

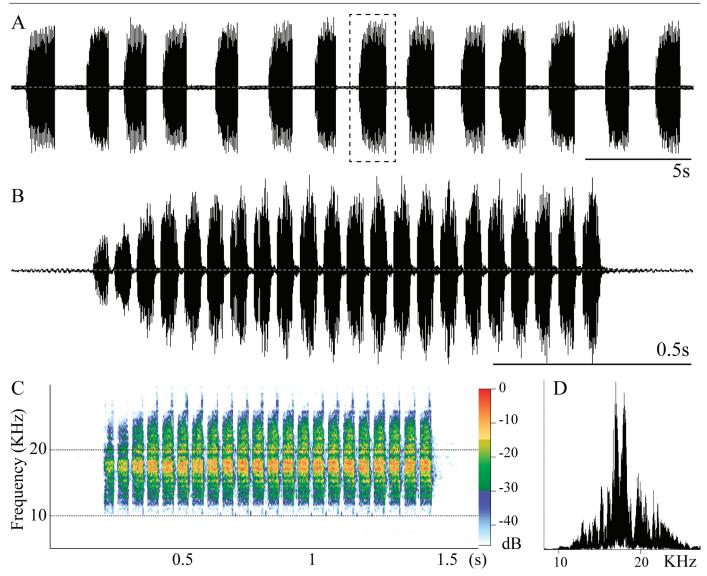


Fig. 7. Calling song of *Toledopizia salesopolensis*. A. Sonogram of an echeme sequence; B. sonogram of one echeme; C. 2-D spectrogram, ranging from 5 to 30 kHz, of one echeme; D. spectrogram of an echeme sequence, in linear intensity scale.

produced into two acute lobes, the male cercus has a medial finger-like projection and a ventral mediodistal fold, the male subgenital plate has the hind margin with a V-like sinus and a conspicuous medial keel, and the female subgenital plate has the hind margin with a U-like sinus flanked by two small posterior projections; in *M. dickmanorum*, the tenth tergite of both sexes has no modification, the male cercus bears a small subapical inward denticule dorsally and a finger-like ventral branch, the male subgenital plate has no medial keel and the posterior border with a U-like sinus, and the female subgenital plate has the posterior border with a V-like sinus flanked by two conspicuously upcurved and long projections (Cadena-Castañeda et al. 2016). The two species may be congeneric, but we decided to maintain both genera as valid taxa because we do not know the phallic complex of *M. dickmanorum*, and there are no phylogenetic analyses comprising both genera.

Among more than 55 genera of the tribe Copiphorini, the bioacoustics of only a few species has been studied (e.g., some species of *Artiotonus* Montealegre-Z., Morris, Sarria-S. & Mason, 2011, *Copiphora, Euconocephalus* Karny, 1907, *Lirometopum* Scudder, 1875, and *Vestria* Stål, 1874 etc.; see Naskrecki 2000, Montealegre-Z et al.

2011, Tiwari and Diwakar 2018). Like the other Copiphorini species, except for some *Neoconocephalus* Karny, 1907 species, males call only during the night and continue calling after midnight (Naskrecki 2000, Fianco in prep.). The energy of the calls of *T. salesopolensis* ranges from audible to ultrasound, but most of the energy is concentrated in the audible range for humans, just like the sounds produced by species of *Neoconocephalus*, *Copiphora*, and *Lirometopum*. However, the dominant frequency is quite higher (ca. of 5–12 kHz higher), and most of the species of these three last genera do not call in the ultrasound range (see exception in Montealegre-Z and Postles (2010)), as occurs in *Artiotonus* and *Copiphora gorgonensis* Montealegre-Z. & Postles (Montealegre-Z and Postles 2010, Montealegre-Z et al. 2011).

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