Rustitermes boteroi, a new genus and species of soldierless termites (Blattodea, Isoptera, Apicotermitinae) from South America

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Abstract

We present the description of a new genus and species of soldierless termites from South America. Rustitermes boteroi Constantini, Castro & Scheffrahn, gen. et sp. nov. can be identified by the morphology of the enteric valve, with six slightly asymmetric cushions, each one forming a central pouch made of scales smaller than those between the cushions. The new genus features two characteristic rows of thick bristles on the interior margin of the fore tibia, and is supported by COI molecular sequence data. This species is distributed from Tobago to northern Argentina.

Keywords

Neotropics, enteric valve, soil-feeder, barcode sequence
Introduction

Soil-feeding termites represent a large part of the termite fauna in Neotropical ecosystems (Ackerman et al. 2009; Bourguignon et al. 2011; Palin et al. 2011; Cancello et al. 2014). Species in the subfamily Apicotermitinae are almost exclusively soil feeders (Bourguignon et al. 2016a), and preferential consumption of different soil components has been suggested as a driver of great diversity in these termites (Bourguignon et al. 2009).

The Apicotermitinae subfamily was first proposed by Grassé and Noirot (1954) and defined by Sands (1972) in the most extensive taxonomic work on this group of termites carried out in Africa. Both morphological and molecular data corroborate the monophyly of Apicotermitinae (Inward et al. 2007; Bourguignon et al. 2017).

The taxonomic work of Sands (1972) described 51 new species, redescribed 9 species, and established 16 new genera. Sands (1972) considered the genus *Anoplotermes* Müller to be exclusively Neotropical. At the time, this was the sole apicotermitine genus of the region.

Taxonomic study of the worker caste has been underwhelming in the Neotropical Region (Rocha et al. 2019), and because all New World Apicotermitinae are soldierless, this subfamily has been historically understudied. Before 2009, only five genera had been described (Fontes 1992; Bourguignon et al. 2010). In recent years however, the development of Apicotermitinae taxonomy in the Neotropical Region has been increased by the description of ten new genera, with enteric valve morphology being of essential diagnostic character for the establishment of new taxa (Scheffrahn 2013; Bourguignon et al. 2016b; Scheffrahn et al. 2017; Castro et al. 2018), although other characters may also be very useful, especially in taxa with less robust enteric valve armature (Acioli and Constantino 2015; Constantini et al. 2018). The diversity of soldierless termites can be high, reaching up to 31 morphospecies for a single primary tropical rainforest (Bourguignon et al. 2013).

Herein we describe *Rustitermes boteroi* gen. nov. et sp. nov. based on the morphology of the imago, worker caste and molecular COI data.

Material and methods

The material examined in this study is deposited at Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP); Colección de artrópodos terrestres de la Amazonía Colombiana of the SINCHI Institute in Leticia, Amazonas, Colombia (CATAC); and at the University of Florida Termite Collection at Fort Lauderdale Research and Education Center, Davie, Florida, United States (UF). All the specimens are preserved in 80–85% ethanol; colonies marked with (*) in the material examined contain alates.

Some type material of old species was consulted for comparisons and remarks with *R. boteroi* sp. nov., to avoid generating new synonyms for described species. The
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abbreviations of the cited institutions are: AMNH – American Museum of Natural History, New York, USA; USNM – Smithsonian National Museum of Natural History, Washington, D.C., USA; CMNH – Chicago Museum of Natural History, Chicago, USA. The species reviewed were: Anoploterme bolivianus Snyder (alate, USNM), Anoploterme brucei Snyder (alate and worker, AMNH), Anoploterme distans Snyder (worker, AMNH), Anoploterme gracilis Snyder (alate, AMNH), Anoploterme hondurensis Snyder (alate, AMNH), Anoploterme meridianus Emerson (worker, AMNH), Anoploterme punctatus Snyder (worker, AMNH), Anoploterme pyriformis Snyder (alate, AMNH), Anoploterme rotundus Snyder (alate and worker, AMNH and USNM), Anoploterme subterraneus Emerson (alates and workers, AMNH and USNM), Anoploterme tenebrosus (Hagen) (alate, AMNH), Aparaterme abbreviatus (Silvestri) (alate and worker, AMNH), Aparaterme cingulatus (Burmeister), and Aparaterme silvestrii (Emerson) (workers, CMNH).

The terminology used to describe worker mandibles follows Sands (1972) and Deligne (1999), with some modification, while worker digestive tube descriptions follow Noirot (2001).

In Sands (1972, fig. 2), a variation of the tooth that he calls the “marginal subsidiary” is represented, which may or may not be hidden by the molar prominence (depending on the position it occupies), and suggests that the development of this tooth is a useful generic characteristic. The same tooth is called “premolar” by Deligne (1999) and “molar” by Krishna (1968). Traditionally, the term “subsidiary” is used to designate the structure present at the base of the apical tooth in the left or right mandible in some non-Termitidae families, which could generate some misunderstanding in the literature. In addition, a process not reported in the literature, closer to M3, was observed in the left mandible of some Apicotermitinae alates (Sands 1972). Therefore, we propose to call this a “pre-molar process” for the structure closest to M3 and a “molar process” for the structure closest to the molar prominence, stressing that both processes are part of the molar region (see Fig. 2C, MP).

The mandibles were examined on a microscope slide in PVA medium, after adding a cover glass and pressing them gently into position, as shown in Figure 2C, D. Nevertheless, we examined the mandibles in every possible position before separating them from the head, in order to undertake a careful examination of the “pre-molar process” and “molar process”.

The terms used for pilosity are comparative: bristles are stiff hairs with well-marked bases; spine-like bristles are shorter and thicker than bristles; hairs are shorter and thinner than bristles and without conspicuous bases.

Workers and imagos were examined in a petri dish filled with 80% ethanol, whereas the dissection of the enteric valve (EV) was done with two no. 20 minuthen pins (BioQuip, Rancho Dominguez, CA).

The EV was detached from the paunch (P3) and all the food particles were removed by gentle manipulation. The extracted EV was inserted in a drop of PVA mounting medium (BioQuip, Rancho Dominguez, Cat. #6371A) and then gently massaged with the side of a minuthen pin for a few minutes until the EV became detached from the
muscles. Afterwards, the EV was transferred on to a microscope slide where, after adding another drop of the same mounting medium, the fully cleaned tubular EV was splayed open before final mounting.

The following morphometric characters were measured as defined by Roonwal (1970) and indicated in parentheses: for alates—maximum diameter of compound eyes (48); inter-eye distance (52); maximum diameter of ocellus (55); minimum diameter of ocellus (56); eye-ocellus distance (57); length of pronotum (65); width of pronotum (68); minimum length of forewing without scale (75); maximum length of forewing scale (76); for alates and workers—length of head to lateral base of mandibles (5); width of head (17); lengths of pro- and metatibia (85); width of protibia (86); protibia index (53, p.61).

Microphotographs were taken as multi-layer montages using a Leica M205C stereomicroscope for the worker head, fore tibia and mandibles; for the worker EV a Leica CTR 5500 compound microscope was used, controlled by the Leica Application Suite version 3 software.

The distribution map was created using ArcGIS desktop ver. 10.4.1 (ESRI, Redlands, CA). The list of examined material is sorted by country (uppercase), state or province, and locality. Collection data are organized as follows: latitude, longitude, collection date, altitude, collector name, collection, and collection number.

The COI barcoding region (Cytochrome c Oxidase subunit 1) was sequenced for four colonies of *R. boteroi* sp. nov. from Peru, Ecuador, French Guiana and Paraguay. DNA extraction and PCR were performed by the Canadian Centre for DNA Barcoding (BOLD systems), following standard high-throughput protocols (deWaard et al. 2008). The PCR employed the primers LepF1 and LepR1 (Hebert et al. 2003), which generated 622 to 652bp. To infer the relationship of *Rustitermes* gen. nov. with the other Neotropical Apicotermitinae, a Bayesian phylogeny was performed with the COI region. In addition to the four sequences of *Rustitermes boteroi* sp. nov. from colonies, UF.FG411 (BOLD:AAW5963), UF.PA534 (BOLD:ACB7291), UF.EC400 (BOLD:ABA4343) and UF.PU602 (BOLD:ACO6749), 49 GenBank and BOLD sequences were used in the analysis: 35 sequences of Neotropical Apicotermitinae (22 species, 14 genera); eight non-Neotropical Apicotermitinae genera, five non-Apicotermitinae Termitidae, and one Rhinotermitidae [*Heterotermes crinitus* (Emerson)] as outgroup. The tree was constructed under the same parameters as other recently published papers on Neotropical Apicotermitinae (Carrijo et al. 2015; Castro et al. 2018). Sequence alignment was performed under the MUSCLE algorithm; the substitution model used was the GTR+I+G, selected under the Akaike Information Criterion (AIC) by jModelTest2 (Darriba et al. 2012); the phylogeny was reconstructed with BEAST 1.8.0 (Drummond et al. 2012) under a Yule speciation process. Four 20,000,000 generations Markov chain Monte Carlo (MCMC) searches were conducted independently and combined. Sampling was conducted every 2000 generations. Convergence and stationarity were assessed with Tracer 1.5 (Rambaut et al. 2014) and the first 1000 trees of each run were discarded as burn-in.
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Taxonomy

Rustitermes Constantini, Castro & Scheffrahn, gen. nov.
http://zoobank.org/A6BB62D4-9A1E-4FAD-A0B3-B16B56D4CB87

Type species. Rustitermes boteroi sp. nov.

Diagnosis. Enteric valve with six slightly asymmetrical cushions. Each cushion forming a central pouch made of scales smaller than those between the cushions. Each cushion composed of about 60–80 scales, wider at the base and narrower at the apex. Posterior portion of pads truncated, with 35 to 50 rectangular scales arranged from the middle to the apex of the cushion and increasing in density in this same direction.

Imago (Fig. 1; Table 1). Fontanelle inconspicuous in both sexes, the region of the fontanelle depressed; medium spot slightly conspicuous. Left mandible with apical tooth a little bit larger than M1 + 2; M3 triangular with lateral margins forming an obtuse angle; non-conspicuous premolar process; molar process not hidden by molar prominence (Fig. 2C).

Head capsule with short, sparse bristles; coloration of head capsule dark brown; frontal marks slightly lighter than rest of head capsule, with poorly defined margins. Pronotum subhexagonal, with anterior margin straight, without central incision; lateral margins very straight and well-marked; pronotum with few sparse bristles and short hairs. Tergites and sternites with short hairs covering the plates. Fore coxa with a set of 4–5 prominent long bristles; inner face of fore tibia with two rows of 6–7 thick bristles.

Worker (Figs 2–4). Monomorphic. Small and rounded fontanelle; postclypeus rather slightly inflated; head capsule covered with medium and long bristles. Left mandible with prominent apical tooth compared to M1 + 2, triangular M3 with lateral margins forming a right/acute angle, molar process not concealed by molar prominence. Pronotum with long bristles, concentrated along margin of anterior and posterior lobes. Tergites and sternites with dense cover of long bristles, facing the posterior region or upwards. Fore coxa with a set of 4–5 thick bristles; inner face of fore femur with long bristles. Fore tibia moderately inflated, inner face of fore tibia with two rows of 6–7 thick bristles.

Mixed segment (MS) separated from ileum (P1) by a simple transverse junction; P1 of uniform width along entire length, forming an inverted C in ventral view. Enteric valve without armature, with six pyriform cushions of slightly different dimensions, the two largest and two smallest cushions adjacent to each other. The center of each cushion is formed into a lumen-facing pouch consisting of about 60 fringed scales. The remainder of the cushions consists of 50–75 (depending on size of cushion) larger fringed polygons. The cuticle between the cushions is composed of even larger fringed cuboidal scales. All cushions are wider at base (near P1) and narrower at apex (near P3). Tubular and short EV seating. Worker measurements highly variable among and within different colonies (Table 2).

Comparison and remarks. The digestive tube coiling of the new genus is similar to Hydrecotermes, but R. boteroi sp. nov. can be differentiated by the worker, which
Table 1. Measurements (mm) of imagos of Rustitermes boteroi sp. nov. from colony MZUSP 26677.

|                           | Female (n = 4) |           | Male (n = 5) |           |
|---------------------------|---------------|-----------|--------------|-----------|
|                           | Range         | Mean      | Range        | Mean      |
| Length of head            | 0.78–0.92     | 0.83      | 0.65–0.78    | 0.75      |
| Width of head with eyes   | 1.13–1.20     | 1.18      | 1.12–1.15    | 1.12      |
| Maximum diameter of compound eye | 0.27         | 0.27      | 0.27–0.28    | 0.27      |
| Inter-eye distance        | 0.87–0.95     | 0.93      | 0.87–0.88    | 0.87      |
| Maximum diameter of ocellus | 0.12         | 0.12      | 0.11–0.12    | 0.11      |
| Minimum diameter of ocellus | 0.08–0.09    | 0.09      | 0.08–0.09    | 0.09      |
| Eye-ocellus distance      | 0.08–0.11     | 0.1       | 0.08–0.1     | 0.09      |
| Length of pronotum        | 0.57–0.63     | 0.6       | 0.55–0.58    | 0.56      |
| Width of pronotum         | 1.00–1.08     | 1.04      | 0.97–0.98    | 0.97      |
| Length of forewing with scale | 11.60–12.13  | 11.82     | 10.40–10.53  | 10.47     |
| Width of fore tibia       | 0.13          | 0.13      | 0.13         | 0.13      |
| Length of fore tibia      | 0.88–0.95     | 0.93      | 0.83–0.88    | 0.86      |

has thick bristles along the inner margin of the fore tibia, absent in Hydrecotermes. In the workers, the enteric valve and the digestive tube may be similar to Aparatermes, but the cuticle between the cushions in Aparatermes does not have cuboidal scales. In Aparatermes the insertion of P1 in P3 occurs in dorsal view with a trilobate EV setting, in Rustitermes the enteric valve seating (EVS) is not trilobate. Also, the enteric valve of Aparatermes has small spines or pointy scales, which are absent in R. boteroi sp. nov.; in addition, the EV in Aparatermes has the posterior portion of the pads without scales. The imago presents a visible molar process and fore tibia with two rows of thick bristles.

Molecular analysis. The Bayesian phylogeny using the COI marker clearly separates Rustitermes gen. nov. from the other soldierless termites (Figure 5). The new genus was recovered as sister group of Patawatermes, but without high posterior probability support.

Etymology. Named in honor of Michael K. Rust, retired professor of urban entomology at the University of California, Riverside (UCR), and mentor of RHS. Mike encouraged RHS to publish his first taxonomic paper (Scheffrahn and Rust 1983).
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http://zoobank.org/95C95B44-17A3-464D-AC58-ADE9F3BC168D

Material examined. **Holotype.** Worker from colony labeled as UF no. PU602; the holotype is kept in a separate small vial in the same vial as the paratypes.

**Type locality.** PERU. Cucayali, Nueva Requena, -8.37007, -74.84366.

**Type repository.** University of Florida, Fort Lauderdale Research and Education Center, Termite Collection in Davie, Florida.

**Paratypes.** ARGENTINA. Corrientes, Santo Tome, (-28.57900, -56.0840), 1.JUL.1998, 93 m, J. Křeček coll. (UF no. AG360). BOLIVIA. Cochabamba, Chapare, Villa Tunari, (-18.15343, -60.03293), 26.MAY.2013, 408 m, Chase, Křeček, Mullins, Nishimura, Mangold, and Scheffrahn coll. (UF no. BO85). Beni, San Javier, (-14.70207, -64.89097), 29.MAY.2013, 152 m, Chase, Křeček, Mullins, Nishimura, Mangold, and Scheffrahn coll. (UF no. BO375); (-14.54909, -64.88964), 29.MAY.2013, Chase, Křeček, Mullins, Nishimura, Mangold, and Scheffrahn coll. (UF no. BO431, BO437). Santa Cruz, Roboré, (-18.15343, -60.03293), 31.MAY.2013, 408 m, Chase, Křeček, Mullins, Nishimura, Mangold, and Scheffrahn coll. (UF no. BO738). BRASIL. Alagoas, Quebrangulo, (-9.2288, -36.4259), 19.JUN.2000, 780 m, MP Silva coll. (MZUSP 13712). Bahia, Conde, (-11.7718, -37.7301), 15.JUN.2016, 78 m, JP Constantini coll. (MZUSP 26648). Espírito Santo, Pedro Canário, (-18.3557, -39.8445), 20.JUN.2016, 43 m, JP Constantini coll. (MZUSP 26652); 21.JUN.2016, (MZUSP 26676(a), 26677*). Paraíba, João Pessoa, (-7.1480, -34.8614), 01-20.JUN.2000, 66 m, A Vasconcellos coll. (MZUSP 13710, 13711). Pernambuco, Recife, Porto Dois Irmãos, (-7.9999, -34.9473), s/d, 88m, A Vasconcellos coll. (MZUSP 13702). COLOMBIA. Amazonas, La Chorrera, Lago grande (-2.07066, -72.170611), 28.APR.2011, 223 m, Scheffrahn, Chase, Mangold, Křeček, Myles, Nishimura and Setter coll. (UF no. EC400). FRENCH GUIANA. Cayenne, Sinnamary, (+5.06314, -52.98479), 13.FEB.2008, 102 m, J. Křeček coll. (UF no. FG411). PARAGUAY. Central, Ypacaraí, (-25.3804, -57.2001), 27.MAY.2012, 248 m, Scheffrahn, Chase, Mangold, Křeček and Myles coll. (UF no. PA8). PERU. Cucayali, Nueva Requena, (-8.37007, -74.84366), 29.APR.2014, 185 m, Carrijo, Chase, Constantino, Mangold, Mullins, Křeček, Kuswanto, Nishimura, and Scheffrahn coll. (UF no. PU602, PU613). TRINIDAD AND TOBAGO. Anse Fourni, Manson Hall, (+11.28467, -60.60133), 31.MAY.1996, 472 m, Chase, Mangold, Křeček, and Scheffrahn coll. (UF no. TT619). Guayaguayare, Rio Claro-Mayaro (+10.23516, -61.13266), 20.MAY.2003, 41 m, Chase, Mangold, Křeček, and Scheffrahn coll. (UF no. TT1614). VENEZUELA. Bolivar, Cantarrana, (+4.46750, -61.59694), 29.APR.2004, 874 m, J. Perozo coll. (UF no. VZ1443.1).
Diagnosis. Unarmed enteric valve with six slightly asymmetrical cushions, each one forms a central pouch made of about sixty scales, smaller than those between the cushions.

Imago. As described for the genus.

Worker (Figs 2–4; Table 2). Monomorphic, head capsule with long and short bristles, with more abundance of long bristles. Head capsule color varying between whitish and yellowish. Antennae with 14 articles densely covered with short hairs and some long bristles. Pronotum with long bristles, concentrated along the margins of the anterior and posterior lobes, with some sparse short bristles in the center of the pronotum. Inner face of fore tibia with two rows of 6–7 thick bristles. Inner face of fore femur with thick bristles. Mesotibia and metatibia with 25–35 long, thick bristles.

Figure 2. Rustiitermes boteroi sp. nov. A, B worker head capsule in dorsal and lateral view C imago mandibles D worker mandibles E worker right fore tibia F live habitus of worker. MP = molar process. Specimens from lot CATA 1797 (A, B), MZUSP 26677 (C, D), BO437 (E).
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Table 2. Measurements (mm) of 9 colonies (n = 10) of Rustitermes boteroi sp. nov. L = length, W = width.

|                | Length of head with postclypeus | Max. width of head | Hind tibia L | Fore tibia W | Fore tibia L | Ratio fore tibia W/L |
|----------------|---------------------------------|--------------------|--------------|--------------|--------------|---------------------|
| Holotype       | 0.95                            | 0.95               | 0.88         | 0.18         | 0.58         | 0.30                |
| PU602          | 0.79–0.89 (0.86)                 | 0.96–1.00 (0.99)   | 0.95–1.04 (0.99) | 0.14–0.16 (0.15) | 0.74–0.79 (0.78) | 0.18–0.21 (0.20)    |
| AG360          | 0.8–1.05 (0.86)                  | 0.85–1.18 (0.92)   | 0.53–0.75 (0.63) | 0.10–0.15 (0.13) | 0.43–0.53 (0.46) | 0.24–0.29 (0.28)    |
| BO431          | 0.82–0.96 (0.90)                 | 1.02–1.09 (1.04)   | 0.89–0.98 (0.95) | 0.14–0.18 (0.16) | 0.72–0.77 (0.75) | 0.18–0.23 (0.21)    |
| EC400          | 0.92–0.95 (0.89)                 | 1.0–1.04 (1.01)    | 0.98–1.04 (1.01) | 0.16–0.19 (0.18) | 0.77–0.82 (0.80) | 0.20–0.24 (0.23)    |
| MZUSP 13712    | 0.80–0.84 (0.83)                 | 0.98–1.12 (1.04)   | 0.77–0.88 (0.84) | 0.13–0.18 (0.15) | 0.63–0.72 (0.67) | 0.19–0.26 (0.22)    |
| FG411          | 0.80–0.85 (0.82)                 | 0.87–0.93 (0.89)   | 0.68–0.80 (0.74) | 0.17–0.20 (0.18) | 0.55–0.58 (0.57) | 0.33–0.35 (0.33)    |
| PA8            | 0.82–0.89 (0.85)                 | 0.88–0.95 (0.91)   | 0.88–0.96 (0.91) | 0.16–0.19 (0.18) | 0.68–0.75 (0.73) | 0.21–0.27 (0.24)    |
| TT1614         | 0.77–0.88 (0.82)                 | 0.86–0.91 (0.89)   | 0.88–0.96 (0.90) | 0.14–0.18 (0.16) | 0.70–0.74 (0.72) | 0.19–0.24 (0.22)    |
| CATA-0954      | 0.85–1.01 (0.91)                 | 0.93–1.02 (0.99)   | 0.77–0.85 (0.82) | 0.16–0.20 (0.18) | 0.65–0.71 (0.69) | 0.20–0.24 (0.22)    |

Figure 3. Digestive tube from left to right: dorsal, right, ventral and left views (gut regions indicated: C = crop, M = mesenteron, P1 = ileum, P3a and b = paunch, P4 = colon, P5 = rectum, EVS = enteric valve seating). Scale bar: 0.5 mm.

Enteric valve without armature, with six pyriform cushions of slightly different dimensions, each cushion consisting of 50–75 (depending on size of cushion) larger fringed polygons. The cuticle between the cushions is composed of even larger fringed cuboidal scales assembled close to P3.

Remarks. See remarks for genus.

Ecology and distribution. This species was collected mainly in soil, although it can also be found at the base of trees or occasionally under pieces of wood or fallen tree limbs above ground. Very common in pastures and open areas; found in young rubber crops in great abundance, less abundant in natural forests. Range: from Trinidad and Tobago to northern Argentina and the Atlantic Forest in Brazil (see discussion below); no known records for Chile and Uruguay (Figure 6).

Etymology. Named in honor of the great Colombian artist Fernando Botero.
Discussion

Despite its wide distribution and abundance in disturbed sites and open areas *R. boteroi* sp. nov. had not been previously described, indicating the lack of taxonomic work on soldierless termites. Currently, the use of the worker caste for the taxonomic identification of termites has been shown to be increasingly necessary, regardless of the presence of imago or soldier castes (Rocha et al. 2019).

Figure 4. Worker enteric valve of *Rustitermes boteroi* sp. nov. A EV fully stretched laterally, showing the six cushions (end cushion bisected) B EV detail of smallest cushion in A C whole mount EV lateral profile of cushions. Food flow in each image from bottom to top.
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Many other Apicotermitinae species present wide distributions in South America, such as Compositerms vindai Scheffrahn, which has been reported from Panama to Paraguay (Scheffrahn 2013), Aparatermes silvestrii (Emerson), reported from Trinidad and Tobago to Paraguay (Pinzón et al. 2019), Longustitermes manni (Snyder), reported from Honduras to Brazil (Bourguignon et al. 2010), Tonsuritermes tucki Constantini and Cancello, reported from Colombia and French Guiana to southern Brazil and Paraguay (Constantini et al. 2018); and, with older records, species such as Anoploterms meridianus Emerson, 1925 and Anoploterms parvus Snyder, 1923, recorded from Central America to Argentina (Bourguignon et al. 2010; Krishna et al. 2013; Constantino 2019). Possibly, many others common species are not yet described, and many others,
already described, have unknown ranges. Species with disjunct distributions based on few records probably have much larger distributions, as is the case with *Disjunctitermes* species (Scheffrahn et al. 2017). The New World Apicotermitinae are a typical example of both Linnean and Wallacean shortfalls (Bini et al. 2006).

According to the data presented, *R. boteroi* sp. nov. is widely distributed in the Guiana shield, the Amazon and the Atlantic forest. An effort is needed to identify Cerrado, Caatinga and Amazonian samples (there is abundant material deposited in MZUSP) to determine if this species is present in these areas.

Molecular phylogeny using the COI marker was useful to complement evidence of the separation of *R. boteroi* sp. nov. from the other Apicotermitinae genera. However, this marker alone is not enough to provide a resolved phylogeny allowing to understand the evolution of this group. The new world Apicotermitinae were determined to be monophyletic, but the relationship between most genera had very low branch support, making it impossible to provide deeper discussions.
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References

Acioli ANS, Constantino R (2015) A taxonomic revision of the neotropical termite genus Rustitermes (Isoptera, Termitidae, Apicotermitinae). Zootaxa 4032: 451–492. https://doi.org/10.11646/zootaxa.4032.5.1

Ackerman IL, Constantino R, Gauch HG, Lehmann J, Riha SJ, Fernandes ECM (2009) Termite (Insecta: Isoptera) species composition in a primary rain forest and agroforests in central Amazonia. Biotropica 41: 226–233. https://doi.org/10.1111/j.1744-7429.2008.00479.x

Bini LM, Diniz-Filho JAF, Rangel TFLVB, Bastos RP, Pinto MP (2006) Challenging Wallacean and Linnean shortfalls: Knowledge gradients and conservation planning in a biodiversity hotspot. Diversity and Distributions 12: 475–482. https://doi.org/10.1111/j.1366-9516.2006.00286.x

Bourguignon T, Leponce M, Roisin Y (2011) Beta-diversity of termite assemblages among primary French Guiana rain forests. Biotropica 43: 473–479. https://doi.org/10.1111/j.1744-7429.2010.00729.x

Bourguignon T, Šobotník J, Dahlsjö CAL, Roisin Y (2016a) The soldierless Apicotermitinae: insights into a poorly known and ecologically dominant tropical taxon. Insectes Sociaux 63: 39–50. https://doi.org/10.1007/s00040-015-0446-y

Bourguignon T, Šobotník J, Lepoint G, Martin JM, Roisin Y (2009) Niche differentiation among neotropical soldierless soil-feeding termites revealed by stable isotope ratios. Soil Biology & Biochemistry 41: 2038–2043. https://doi.org/10.1016/j.soilbio.2009.07.005

Bourguignon T, Scheffrahn RH, Kreček J, Nagy ZT, Sonet G, Roisin Y (2010) Towards a revision of the Neotropical soldierless termites (Isoptera: Termitidae): redescription of the genus Anoplotermes and description of Longustitermes, gen. nov. Invertebrate Systematics 24: 1–357. https://doi.org/10.1071/IS10012

Bourguignon T, Scheffrahn RH, Nagy ZT, Sonet G, Host B, Roisin Y (2016b) Towards a revision of the Neotropical soldierless termites (Isoptera: Termitidae): Redescription of the
genus *Grigiotermes* Mathews and description of five new genera. Zoological Journal of the Linnean Society 176: 15–35. https://doi.org/10.1111/zoj.12305

Bourguignon T, Šobotník J, Hanus R, Krasulová J, Vrkošlav V, Cvačka J, Roisin Y (2013) Deliningating species boundaries using an iterative taxonomic approach: The case of soldierless termites (Isoptera, Termitidae, Apicotermitinae). Molecular Phylogenetics and Evolution 69: 694–703. https://doi.org/10.1016/j.ympev.2013.07.007

Bourguignon T, Lo N, Šobotník J, Ho SYW, Iqbal N, Coissac E, Lee M, Jendryka MM, Sillam-Dussès D, Křížková B, Roisin Y, Evans TA (2017) Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the Tropics. Molecular Biology and Evolution 34: 589–597. https://doi.org/10.1093/molbev/msw253

Cancello EM, Silva RR, Vasconcellos A, Reis YT, Oliveira LM (2014) Latitudinal variation in termite species richness and abundance along the Brazilian Atlantic Forest hotspot. Biota tropica 46: 441–450. https://doi.org/10.1111/btp.12120

Carrijo TF, Scheffrahn RH, Křeček J (2015) *Compositermes bani* sp.n. (Isoptera, Termitidae, Apicotermitinae), a new species of soldierless termite from Bolivia. Zootaxa 3941: 294–298. https://doi.org/10.11646/zootaxa.4109.3.10

Castro D, Scheffrahn RH, Carrijo TF (2018) *Echinotermes biriba*, a new genus and species of soldierless termite from the Colombian and Peruvian Amazon (Termitidae, Apicotermitinae). ZooKeys 748: 21–30. https://doi.org/10.3897/zookeys.748.24253

Constantini JP, Carrijo TF, Palma-Onetto V, Scheffrahn R, Carnohan LP, Šobotník J, Cancell EM (2018) *Tonsuritermes*, a new soldierless termite genus and two new species from South America (Blattaria: Isoptera: Termitidae: Apicotermitinae). Zootaxa 4531: 1–383. https://doi.org/10.11646/zootaxa.4531.3.4

Constantino R (2019) Termite Database. University of Brasilia. http://www.termitologia.net/termite-database [July 16, 2019]

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 1–772. https://doi.org/10.1038/nmeth.2109

Deligne J (1999) Functional morphology and evolution of a carpenter’s plane-like tool in the mandibles of termite workers (Insecta Isoptera). Belgian Journal of Zoology 129: 201–218. http://biblio.naturalsciences.be/associated_publications/bjz/129-1/bjz_129_deligne_201-218.pdf [July 16, 2019]

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA barcodes. Analytical protocols. Methods in Molecular Biology (Clifton, N.J.) 410: 275–293. https://doi.org/10.1007/978-1-59745-548-0_15 [June 27, 2019]

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1–1969. https://doi.org/10.1093/molbev/mss075

Fontes LR (1992) Key to the genera of New World Apicotermitinae. In: Quintero D, Aiello A (Eds) Insects of Panama and Mesoamerica. Oxford University Press, New York, 242–248.

Grassé PP, Noirot C (1954) *Apicotermes arquieri* (Isoptère): ses constructions, sa biologie. Considérations générales sur la sous-famille des Apicotermitinae nov. Annales des Sciences Naturelles, Zoologie (11) 16: 345–388.
Rustitermes boteroi, a new genus of soldierless termite from South America

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Inward DJG, Vogler AP, Eggleton P (2007) A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Molecular Phylogenetics and Evolution 44: 953–967. https://doi.org/10.1016/j.ympev.2007.05.014

Krishna K (1968) Phylogeny and generic reclassification of the Capritermes complex (Isoptera, Termitidae, Termitinae). Bulletin of the American Museum of Natural History 138: 261–324.

Krishna K, Grimaldi DA, Krishna V, Engel MS (2013) Treatise on the Isoptera of the World. 4. Termitidae (part one). Bulletin of the American Museum of Natural History 377: 973–1494. https://doi.org/10.1206/377.4

Noirot C (2001) The gut of termites (Isoptera) comparative anatomy, systematics, phylogeny. II. – Higher termites (Termitidae). Annales de la Société entomologique de France (NS) 37: 431–471.

Palin OF, Eggleton P, Malhi Y, Girardin CAJ, Rozas-Dávila A, Parr CL (2011) Termite diversity along an Amazon-Andes elevation gradient, Peru. Biotropica 43: 100–107. https://doi.org/10.1111/j.1744-7429.2010.00650.x

Pinzón OP, Scheffrahn RH, Carrijo TF (2019) Aparatermes thornatus (Isoptera: Termitidae: Apicotermitinae), a new species of soldierless termite from Northern Amazonia. Florida Entomologist 102: 1–141. https://doi.org/10.1653/024.102.0123

Rambaut A, Suchard MA, Xie D, Drummond A (2014) Tracer v1.6. http://tree.bio.ed.ac.uk/software/tracer/ [July 10, 2019]

Rocha MM, Cuezzo C, Constantini JP, Oliveira DE, Santos RG, Carrijo TF, Cancell EM (2019) Overview of the morphology of neotropical termite workers: history and practice. Sociobiology 66: 1–32. https://doi.org/10.13102/sociobiology.v66i1.2067

Roonwal ML (1970) Measurements of termites (Isoptera) for taxonomic purposes. Journal of the Zoological Society of India 21: 9–66.

Sands WA (1972) The soldierless termites of Africa (Isoptera: Termitidae). Bulletin of the British Museum (Natural History), Entomology (suppl.) 18: 1–224.

Scheffrahn RH (2013) Compositermes vindai (Isoptera: Termitidae: Apicotermitinae), a new genus and species of soldierless termite from the Neotropics. Zootaxa 3652: 381–391. https://doi.org/10.11646/zootaxa.3652.3.6

Scheffrahn RH, Rust MK (1983) Tenuirostritermes cinereus (Buckley), a nasutitermitine termite from southcentral Texas (Isoptera: Termitidae). Sociobiology 8: 77–87.

Scheffrahn RH, Carrijo TF, Postle AC, Tonini F (2017) Disjunctitermes insularis, a new soldierless termite genus and species (Isoptera, Termitidae, Apicotermitinae) from Guadeloupe and Peru. ZooKeys 665: 71–84. https://doi.org/10.3897/zookeys.665.11599