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A New Species of *Grigiotermes* (Apicotermitinae, Termitidae) from the Northern Atlantic Forest, Delimited by Morphological and Molecular Data

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Abstract

In this paper, we described a new species of *Grigiotermes*, *G. piassava* sp. n., from the Brazilian Atlantic Forest. The imago and worker caste are described, including the gut morphology. The inclusion of the new species in *Grigiotermes* was based on morphological and DNA data. Apparently, the distribution of the species is limited to the northern region of the Atlantic Forest, located above the São Francisco River, which is considered one of the most threatened sectors of the biome.

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Introduction

The subfamily Apicotermtinae is represented in South America by 17 genera and 57 species (Constantino, 2023). In the Neotropical region, this taxon still represents one of the most significant taxonomic gaps for termites, possibly because the identification of its species often relies on gut dissection, requiring a certain level of expertise in the anatomy and taxonomy of termites (Kambhampati & Eggleton, 2000; Bourguinon et al., 2010).

The genus *Grigiotermes* was initially proposed by Mathews (1997), who designated *G. metoecus* Mathews, 1977 as a type species described in the same study. Later, Fontes (1983) transferred the species *Anoplotermes bequaerti* Snyder & Emerson, 1949 (described in Snyder, 1949) to *Grigiotermes*, based on gut morphology characters.

Bourguignon et al. (2016) revised the species of the genus and considered *G. metoecus* a junior synonym of *Anoplotermes hageni* Snyder & Emerson, 1949 and *G. bequaerti* a junior synonym of *Anoplotermes turricola* Silvestri, 1901. In addition, the authors proposed transferring *A. turricola* to a new genus, *Patawatermes* Bourguignon & Roisin 2016. Currently, *Grigiotermes* has only one species, *G. hageni*.

In this paper, we describe a new species for the genus *G. piassava* sp. n., including the description of the imago and the worker gut anatomy. Morphological and DNA data justify the inclusion of the new species in the genus.

Material and Methods

All the specimens examined are housed in the Museu de Zoologia da Universidade de São Paulo (MZUSP), São



Paulo, Brazil, and at the Federal University of Paraíba Termite Collection (FUPTC), João Pessoa, Paraíba, Brazil. The lists of material examined for the new species are sorted by country (uppercase), state or province (bold), municipality (italics), and specific locality (when informed). Collection data are listed as date, name of the collector, and lot number (in parentheses); an asterisk after the lot number indicates samples with imagos.

The terminology of Noirot (2001) was adopted for the gut coiling, gizzard, and enteric valve cuticular ornaments. The mandible terminology follows the proposal of Constantini et al. (2020), which included more details of the molar region. The terms used for pilosity are comparative: bristles are stiff hairs with well-marked bases; hairs are shorter and thinner than bristles, without conspicuous bases.

The morphometric characters used here, the acronyms in their descriptions, and their corresponding numbers with Roonwal's system (Roonwal, 1970) (in parentheses) are as follows. For imagos: length of head to medium base of clypeus, LH (8); maximum width of head with eyes, WH (17); maximum diameter of compound eye, excluding ocular sclerite, MDE (49); maximum diameter of lateral ocellus, MaDO (55); minimum diameter of lateral ocellus, MiDO (56); median length of pronotum, LP (66); maximum width of pronotum, WP (68); minimum length of forewing without scale, MiLW (75); length of tibia, LT (85). For workers: maximum head width, WH (17); length of tibia, fore, LfT, and hind, LT (85).

Line drawings were made using a camera lucida attached to a stereomicroscope. Images of the imagos and workers were obtained with a digital camera coupled to a Leica M205 A stereomicroscope, for the enteric valve, a Zeiss Primo Star microscope was used. Images with different depths of focus were further processed and merged with Leica Application Suite 4.8 software.

The distribution map was created using QGis 2.14, and the Biome maps follow the propose of IBGE (2012).

Molecular Analysis

We extracted total genomic DNA from sample FUPTC-10943 (workers and imago) using the DNeasy Blood & Tissue Kit from Qiagen, Germany. We performed PCR amplifications for two partial genes, COI and COII. However, obtaining the COI sequence for the imago was impossible. We used the forward primer LCO-1490 and the reverse primer HCO-2198 for COI, as Folmer et al. (1994) described. For COII, we used the forward primer A-tLeu and the reverse primer B-tLys, as described in Inward et al. (2007). PCR conditions followed Schyra and Korb (2019). We sequenced the PCR products using bidirectional sequencing in an ABI 3130 Genetic Analyzer from Applied Biosystems, and checked the sequence quality of the strands. We also assembled and edited the sequences using GENEIOUS v 9.1.3 (Kearse et al., 2012). The whole mitogenomes of Apicotermitinae from GenBank (March 10, 2023) were aligned with our COI-COII sequence using MAFFT v.7.017 (Katoh & Standley, 2023). *Acholotermes chirotus* and *Astalotermes murcus* were used as outgroups (GenBank accession numbers: MW773487 and KY224676).

We selected the best available evolutionary model using jModel Test 3.0.4 (GTR+I+G) (Posada, 2008) and constructed a maximum likelihood tree (ML) using IQ-TREE (Trifinopoulos et al., 2016) web server (ultrafast bootstrap (BS) algorithm with 10,000 replicates) and a Bayesian (BI) tree using MrBayes 3.2.6 (Ronquist et al., 2012). In the last analysis, we assessed parameter convergence using Tracer v.1.6.082, and adequate sample sizes (ESS) were within acceptable ranges (ESS-200). After discarding the first 10% of the sample trees as burn-in, we calculated a consensus tree using the remaining trees according to the majority rule and the bipartitions' posterior probabilities (PP). We deposited our sequences in GenBank (NCBI) under accession numbers OQ865343 (COI), OQ865214 (COII), and OR344909 (COII - imago).

To identify whether the sampled alate belonged to the species *Grigiotermes piassava* sp. n., two distinct delimitation methods were used: distance and tree (Vences et al., 2021). Based on the distance method, we performed the Assemble Species by Automatic Partitioning (ASAP) analysis (Puillandre et al., 2021) online (https://bioinfo.mnhn.fr/abi/public/asap/), using the Kimura-2-Parameter (K2P) distance model (Kimura, 1980) with default settings. Only the partition that presented the lowest ASAP score was considered (Supplementary material).

The tree delimitation was performed using the Poisson Tree Process method (Zhang et al., 2013) (PTP and bPTP) through the iTaxo Tools program (Vences et al., 2021). The ultrametric tree used for the analyses was constructed using the BEAST v1.10.4 program (Drummond et al., 2012), with relaxed clock lognormal input, the Yule speciation process model, GTR+G, and constant population size. The number of generations run on CIPRES v. 3.3 (Miller et al., 2010) was 1,106. We used the Tracer v1.7.2 program (Rambaut et al., 2018) to check the effective sample size (ESS) values considered adequate (ESS > 200), and Tree Annotator v1.10.4 (Drummond et al., 2012) to generate a maximum clade credibility tree, discarding 10% as burn-in.

Description

Grigiotermes piassava Carvalho & Vasconcellos, sp.n.

urn:lsid:zoobank.org:act:DF8F7591-34F6-467C-B4AC-25FA2FC41DFB

Holotype. Worker, in a separate vial with paratypes from the same colony, part of lot MZUSP-27599, 23.xi.2021, A. Vasconcellos and collaborators coll.

Type-locality. Tamandaré Municipality, Biological Reserve of Saltinho (S 8.727, W 35.178), Pernambuco State, Brazil.

Paratypes. BRAZIL. Alagoas. Murici: 14.iv.2021, A.S.N. Carvalho and collaborators coll. (FUPTC-10943*, MZUSP-27600*); **Paraíba**. João Pessoa: 16.ix.1994, E.G. Silva coll. (FUPTC-672, MZUSP-27601); **Pernambuco**. Igarassu: 10.iii.2016, A.A.V.O. Couto coll. (FUPTC-8790); *Tamandaré*: 03.xi.2021, A. Vasconcellos and collaborators coll. (FUPTC-11078, MZUSP-27599); *Caruaru*: 19.xi.1997, no collector information (FUPTC-1084).

Etymology. The species epithet "piassava" was made in apposition from the word "piaçava." Piaçava is a broom made with fibers of palm trees, very commonly used in countryside regions of Brazil, and the enteric valve shape of the new species is similar.

Imago external morphology (Figs 1a, 1b): Head rounded, slightly convex in profile, postclypeus slightly inflated, eyes relatively small, with the diameter equal to half of the head height, not touching the ventral margin of the head, ocelli far from the eyes by a distance nearly equal to their diameter. In dorsal view, fontanelle rounded, two elongated pale spots between the fontanelle and the ocelli, two marked ovaleted spots between the ocelli and the antennal sockets. Antennae with 15 articles (2>3<4<5). Pronotum with the exact head

width, sinuous in profile, with the anterior margin prominent. Head and body densely covered with bristles and hairs. Head, pronotum, tergites and sternites dark brown. Female slightly larger than male; Mandibles (Fig 2), left: apical tooth slightly larger than M1+2, M3 inconspicuous, premolar process conspicuous, molar process not hidden by molar prominence, molar prominence poorly developed, right: apical tooth with the double of the M1 size and the posterior edge forming a closed angle with M1, edge between M1 and M2 forming a concave blade, M2 poorly developed, molar plate concave, without ridges. Measurements, in millimeters, of 4 imagos (2 males and 2 females): Male: LH: 0.86-0.97, WH: 1.50, MDE: 0.28-0.30, MaDO: 0.14, MiDO: 0.11, LP: 0.65-0.67, WP: 1.21-1.31, MiLW: 12.1, LTf: 0.87-0.88; Female: LH: 0.9-1.0, WH: 1.60-1.65, MDE: 0.31-0.32, MaDO: 0.13-0.14, MiDO: 0.11-0.12, LP: 0.12-0.75, WP: 1.37-1.42, LfT: 0.92, LhT:1.25.



Fig 1. Imago of Grigiotermes piassava sp. n. (female): head in lateral view (A), dorsal view (B) (FUPTC-10943).



Fig 2. Imago mandibles of *Grigiotermes piassava* sp. n.; A: apical tooth; M: marginal teeth, PP: premolar process, MP: molar process, Mpr: molar process, Mpr: molar plate.

Worker external morphology (Figs 3a-3c): Monomorphic, head rounded, postclypeus inflated, fontanelle slightly visible, antenna with 14 articles (2>3<4<5), tibia slightly inflated. Head covered with sparse bristles and setae, pronotum with a row of bristles at the anterior margin, body covered with sparse bristles. Mandibles (Fig. 4), left: apical tooth with the double of M1+2 size, with the posterior edge curved, M1+2 triangular and blunt, situated at the first third of the distance between the apical and M3, M3 edge forming a right angle, molar process not occluded by the molar prominence, molar prominence well developed. Right: apical tooth with double the M1 size and the posterior edge forming a closed angle with M1, edge between M1 and M2 forming an open angle, molar plate concave, without ridges. Measurements, in millimeters of five workers from five samples: WH: 0.94–1.15; Lft: 0.72–0.88; Lht: 0.53–0.6



Fig 3. Worker of Grigiotermes piassava sp. n.: lateral view (A), head in lateral (B), and dorsal views (C) (FUPTC-10943).



Fig 4. Worker mandibles of *Grigiotermes piassava* sp. n.; A: apical tooth; M: marginal teeth, PP: premolar process, MP: molar process, Mpr: molar plate.

Digestive tube (Figs 5a–5d): esophagous insertion in the crop displaced ventrally (Fig 5c), gizzard regressed, without any trace of the columns or pulvilli. Mixed segment short and not inflated (Fig 5c), P1 enlarged and short, inserted distally at the P3 (Figs 5c, 5d), without an enteric valve seating. Enteric valve symmetric, with six armed ridges, proximal portion of pads ornamented with hexagonal scales (Fig 6a), distal portion everted in the P3 lumen, well-developed spines, and pectinated margins (Fig 6b). P3 globose, P4 elongated, with a well-developed U-turn.



Fig 5. Digestive tube of *Grigiotermes piassava* sp. n. worker: dorsal (A), left (B), ventral (C), and right (D) views. Abbreviations: C = crop, MT = mesenteric tongue (mixed segment), P1 = ileum, P3 and P3b = paunch, P4 = colon, P5 = rectum.



Fig 6. Enteric valve of Grigiotermes piassava sp. n.: sagittal section view (A), view of the lumen in situ (B).

Biological Notes

All samples were collected in the soil up to a depth of 20 cm, both at the edge and in the interior of the forest fragments. Some samples contained juveniles and imagos, suggesting that the species lives in diffuse colonies in the soil profile.

Phylogenetic Relationships

We recovered *G. piassava* sp. n. as the sister taxon of *G. hageni* with high support (Bs=100% and PP=1.00) (Fig 7) for both phylogenetic trees.



Fig 7. Phylogenetic tree including representative species of Apicotermitinae and *Grigiotermes piassava* sp. n. and *Grigiotermes hageni* (re branch), indicates the posterior probabilities at the nodes.

Comparisons

The imago of *G. piassava* sp. n. is larger than *G. hageni*, has elongated spots close to the fontanelle, and is darker. The worker is externally very similar to *G. hageni*, but can be recognized by the position of the P1 insertion (dorsally oriented in *G. hageni*, more ventrally oriented in *G. piassava* sp. n.), the absence of the enteric valve seating (present in *G. hageni*), and the shape of the enteric valve (plates pectinated in *G. piassava* sp. n., not pectinated in *G. hageni*).

Among other Neotropical Apicotermitinae species, *G. piassava* sp. n. shares some similarities in gut coiling with *Patawatermes* (Bourguignon et al., 2016), particularly the dilated P1, likely by a plesiomorphic condition. These species appear to share a common ancestor (Fig 7). *G. piassava* sp. n. can be clearly distinguished from *Patawatermes* by the absence of the enteric valve seating (Fig 5d) and the fused spines in the distal row (Fig 6b); in *Patawatermes*, the spines are loose.

Discussion

The taxonomy of Neotropical Apicotermitinae has grown in recent years, describing more than ten new genera since 2010 (Bourguignon et al., 2010; Carrijo et al., 2023). However, the genera delimitations are still strongly associated with the enteric valve shapes, eight of the 17 genera are monotypic. In this work, we combined morphological and molecular evidence (Fig 7), and both evidences agree that the new species can be well accommodated in *Grigiotermes*.

All records of *G. piassava* sp. n. were restricted to the northern Atlantic Forest, above the São Francisco River, including montane forests ("Brejos de Altitude"), considered one of the most threatened sectors of the biome. Three studies evaluated the composition of Apicotermitinae species in the Atlantic Forest using analyses of internal morphology: Cancello et al. (2014), Constantini (2018), and Vasconcellos et al. (2023). In addition, several studies have been carried out with termites in Caatinga dry forests (Vasconcellos et al., 2010; Vasconcellos & Moura, 2014), located on the west side



Fig 8. Geographic registries of Grigiotermes piassava sp. n.

of the Atlantic Forest in northeastern Brazil. In none of these studies was *G. piassava* sp. n. recorded. The northern Atlantic Forest is a center of endemism, being a distinct biogeographic unit from the other sectors of the biome (Prance, 1982; Silva et al., 2021). Despite its high biological value, a large part of the northern Atlantic forest was converted into sugarcane monocultures and areas for urban expansion, with only 5.4% of its original coverage remaining (Almeida & Souza, 2023). The description and distribution of *G. piassava* sp. n. reinforces the importance of this biographical sector for the conservation of the Atlantic Forest's biodiversity as a whole.

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Authors' Contribution:

Antonio Carvalho: Investigation, Writing, Visualization; Mauricio M. Rocha: Investigation, Writing, Visualization, Supervision;

Ricardo Koroiva: Formal analysis, Writing;

Sara R.P. Monteiro: Writing, Visualization;

Alexandre Vasconcellos: Resources, Conceptualization, Writing, Supervision.

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8

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