VERÔNICA SARAIVA FIALHO

SISTEMÁTICA DE LISTROSCELIDINAE (ORTHOPTERA: TETTIGONIIDAE) DA MATA ATLÂNTICA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

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APROVADA: 25 de julho de 2012.

Maria Kátia Matiotti
da Costa

Cristiano Lopes Andrade

Karla Suemy Clemente Yotoko
Orientadora

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SUMÁRIO

RESUMO

FIALHO, Verônica Saraiva, M. Sc., Universidade Federal de Viçosa, julho, 2012. **Sistemática de Listroscelidinae (Orthoptera: Tettigoniidae) da Mata Atlântica**. Orientadora: Karla Suemy Clemente Yotoko. Coorientadores: Carlos Frankl Sperber e Jorge Abdala Dergam dos Santos.

A ordem Orthoptera é pouco estudada no Brasil. Esforços conjuntos entre especialistas do grupo no país alcançaram a aprovação do projeto intitulado Biota de Orthoptera do Brasil, para levantamentos e estudos da diversidade em cada bioma. Ao Laboratório de Bioinformática e Evolução da Universidade Federal de Viçosa, coube toda a parte de levantamento da diversidade, sistemática e evolução a partir de dados moleculares. O primeiro passo consistiu em padronizar os procedimentos para obtenção de sequências nucleotídicas de diferentes espécies de Orthoptera, desde as coletas, passando pelo armazenamento do material, no campo e no laboratório, até a padronização dos protocolos de extração e obtenção dos fragmentos amplificados (Apêndice A). Da padronização da metodologia de coleta para preservação do DNA, publicamos um artigo sobre a vantagem da utilização do álcool combustível como solução matadora e preservadora, ressaltando a diminuição dos custos e impedimentos logísticos com o transporte de substâncias inflamáveis. Estes resultados foram publicados na revista Zookeys, cujo artigo, publicado em conjunto com membros do Laboratório de Ortopterologia da UFV, consta no Apêndice B. O Capítulo 1, feito em conjunto com membros do Laboratório de Sistemática e Biologia de Coleoptera (LabCol), contém descrições de oito espécies e um novo gênero provenientes do bioma Mata Atlântica e uma completa revisão taxonômica do grupo, com redescrições, inclusão de novos registros de distribuição e fotografias detalhadas dos espécimes e suas estruturas. Ainda em conjunto com o LabCol, no Capítulo 2 utilizamos uma abordagem molecular baseada nos genes *CO*I e 18S para estabelecer as relações filogenéticas entre as espécies e gêneros coletados ao longo da Mata Atlântica, presentes no Capítulo 1. Nossos resultados moleculares nos permitem sugerir a descrição de duas espécies e um gênero, a serem adicionados aos já propostos no Capítulo 1. Além disso, propusemos uma região do *CO*I como *DNA Barcode*, para auxiliar no diagnóstico de espécimes. Como perspectivas futuras, ressaltamos *(i)* a necessidade de novas coletas visando capturar machos adultos para verificar as proposições de novos táxons, feitas no Capítulo 2 e *(ii)* a necessidade investigar mais profundamente a possibilidade de existência de *numts* (sequências mitocondriais que migraram para o núcleo) em várias das espécies de Listroscelidinae.

ABSTRACT

FIALHO, Verônica Saraiva, M. Sc., Universidade Federal de Viçosa, July, 2012. **Systematic of Listroscelidinae (Orthoptera: Tettigoniidae) of the Brazilian Atlantic Forest**. Adviser: Karla Suemy Clemente Yotoko. Co-advisers: Carlos Frankl Sperber and Jorge Abdala Dergam dos Santos.

In Brazil, the order Orthoptera is poorly studied. Joint efforts among Brazilian experts achieved the approval of the project entitled *Biota de Orthoptera do Brasil*, to list the species present and make studies of biodiversity in different biomes in the country. The *Laboratório de Bioinformática e Evolução*/UFV was asked to survey the diversity, systematics and evolution of the group from molecular data. The first step was to standardize procedures to obtaining nucleotide sequences of different species of Orthoptera, from collection, through storage of material (in field and laboratory) to the standardize protocols of extraction and attainment of the amplified fragments (Appendix A). We standardized collection methodology for the preservation of DNA, and published an article with members of the *Laboratório de Ortopterologia*/UFV about the advantage of using fuel alcohol as a killer and preservative solution, emphasizing the reduction of costs and logistical impediments to transporting flammable substances. This article corresponds to Appendix B of this dissertation. Chapter 1, written with members of the *Laboratório de Sistemática e Biologia de Coleoptera*/UFV (LabCol), contains descriptions of eight species and one new genus from the Central Corridor of the Atlantic Forest and a complete taxonomic revision of the group, with redescriptions, including new distribution records and detailed photographs of the specimens and their structures. Still in together with LabCol, in Chapter 2 we used a molecular approach based on COI and 18S genes to establish phylogenetic relationships among species and genera collected along the Central Corridor of the Atlantic Forest, present in Chapter 1. Our results allow us to suggest the molecular description of two species and one genus, to be added to those already proposed in Chapter 1. In addition, we proposed a region of the COI gene as a DNA barcode, to assist in the diagnosis of specimens. As future prospects, we note *(i)* the need for new collections aimed at capturing adult males to check the propositions of new taxa, made in Chapter 2 and *(ii)* the need to further investigate the possibility of numts (mitochondrial sequences that migrated to the nucleus) in several species Listroscelidinae.

INTRODUÇÃO GERAL

Em 2010 foi submetido ao Sistema Nacional de Pesquisa em Biodiversidade (SISBIOTA) do CNPq o projeto multi-institucional intitulado Biota de Orthoptera do Brasil (Edital MCT/CNPq/MMA/MEC/CAPES/FNDCT – Ação Transversal/FAPs Nº 47/2010), composto por três sub-projetos: *Diversidade Biológica, Sistemática e Biogeografia de Ortópteros*; *Filogenia e Identificação Molecular em Orthoptera (DNA Barcodes)* e *Determinantes da Biodiversidade de Orthoptera do Brasil*.

O projeto inclui a participação de quase todos os ortopterologistas do Brasil, sob coordenação do professor Carlos Frankl Sperber, da Universidade Federal de Viçosa (UFV). Esperam-se como resultados deste projeto a formação de profissionais especializados e a ampliação do conhecimento da diversidade de ortópteros brasileiros, a fim de contribuir e direcionar projetos de manejo e conservação. Dessa forma, o projeto envolve coletas e processamento dos espécimes, sistemática morfológica e molecular, além de estimativa de variáveis ecológicas para teste de hipóteses explicativas em diferentes escalas espaciais.

Com a aprovação do projeto, fez-se necessário padronizar os procedimentos de obtenção de sequências nucleotídicas para estudos de diversidade e evolução de Orthoptera, conforme previsão do sub-projeto *Filogenia e Identificação Molecular em Orthoptera (DNA Barcodes).*

O projeto requer coletas em todos os biomas brasileiros, exigindo um plano de trabalho estruturado e personalizado, de modo a possibilitar a execução das coletas sem infringir leis e respeitando restrições logísticas das coletas. Assim, a partir de janeiro de 2011, iniciei este processo de padronização (Apêndice A) no Laboratório de Bioinformática e Evolução (LBE/UFV), que culminou na publicação do artigo *Ethanol fuel improves arthropod capture in pitfall traps and preserves DNA*. Este trabalho retrata os principais achados dos testes utilizando álcool combustível como solução matadora e preservadora, conduzidos em parceria com o Laboratório de Ortopterologia (UFV), e encontra-se no Apêndice B desta dissertação.

Após a obtenção dos protocolos padronizados, participei de uma expedição de campo para coleta de esperanças no bioma Mata Atlântica (MA), junto à estudante de doutorado Juliana Chamorro Rengifo, do Laboratório de Sistemática e Biologia de Coleoptera (LabCol/UFV). Durante as coletas, interessei-me pelos tetigonídeos Listroscelidinae e decidi trabalhar com a taxonomia desses insetos, unindo a abordagem morfológica e a molecular.

Assim, o Capítulo 1 traz o inventário com a descrição de novos *taxa* e uma revisão taxonômica do grupo, focada nas espécies brasileiras. No Capítulo 2 concentra-se a análise molecular das espécies coletadas na MA, com inferências filogenéticas apoiadas nos dados discutidos no Capítulo 1 e a proposição de uma região para os *DNA Barcodes*. Por último, fiz as considerações finais a respeito de tudo que foi trabalhado.

CAPÍTULO 1

Systematics of spiny predatory katydids (Listroscelidinae: Tettigoniidae) from Brazilian Atlantic forest

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Abstract

We describe eight new katydids species of Listroscelidinae Redtenbacher, 1891 collected at the Brazilian Atlantic forest: *Cerberodon* **sp. nov. 1**, *Listroscelis* **sp. nov. 1**, *Listroscelis* **sp. nov. 2,** *Listroscelis* **sp. nov. 3**, *Listroscelis* **sp. nov. 4**, *Listroscelis* **sp. nov. 5**, *Listroscelis* **sp. nov. 6**. A monospecific genus is described: **Gen. nov. sp. 1**. Redescriptions of species and new records of distribution are also provided. We propose *Cerberodon cuiabensis* Piza as a **syn. nov.** of *Carliella mandibularis* Karny, and *Cerberodon angustifrons* Piza as a **syn. nov.** of *Listroscelis atrata* Redtenbacher. New distributional records of *Megatympanon speculatum* Piza are provided. We provide images of living individuals collected in field and an identification key for genera and species treated in this work.

Keywords

Cerberodon, Listroscelis, Gen. nov., *Megatympanon*, *Monocerophora*, National Parks, Nature Conservation Units, distributional records.

Introduction

Listroscelidinae Redtenbacher, 1891 are carnivorous katydids easily recognized by their large mandibles and long spines on the legs. It is presumed they are primarily insectivorous (Bruner 1915). The group was originally proposed as a tribe inside Conocephalinae Burmeister, 1838 ("Conocephaliden"), due to the foretibiae with enlarged spines and the small fastigium, compressed and narrow. Initially the tribe was composed for genera that actually belong to Listroscelidinae, Meconematinae Burmeister, 1838 and Hexacentrinae Karny, 1925. The name Listroscelini was erected based on *Listroscelis* Serville, 1831. In 1898, Saussure & Pictet grouped *Listroscelis* inside Listroscelites, separating this genus from the others. However it was checked *L. arachnoides* syn. (=*Arachnoscelis arachnoides*). Later on, the group gained the status of subfamily, Listrocelinae (Kirby 1906), but adding an erroneous term.

Bruner (1915) elaborated a taxonomic key including only genera that occur on American tropics and considered the group a family. After that, Karny (1924) redefined Meconeminae (=Meconematinae) and transferred some genera previously assigned to Listroscelinae.

Zeuner (1936a, b) re-evaluated the subfamilies of Tettigoniidae by examining traditional characteristics of the head, venation of the wings and structures of the prothoracic tracheal apparatus. Listroscelinae was included inside the group Conocephaloid (= Xiphidiinae), also with Salomoninae (enclosing Agraeciinae Redtenbacher, 1891), and Copiphorinae Karny, 1912. This classification agreed with that of Karny (1924). Conocephaloids was considered close related to Tettigonioids (Tettigoniinae, Decticinae, Saginae, Mecopodinae and Phyllophorinae). In the other hand, a close relationship between Listroscelinae and Tympanophorinae was proposed, based on the pattern of venation of forewings (Zeuner 1936a). Zeuner (1940) reorganized the subfamily and moves *Xiphidiopsis* Redtenbacher, 1891, *Phlugis* Stål, 1861 and *Phlugiola* Karny, 1907 to Meconeminae. The author suggested the possibility of gathering

Meconeminae with Listroscelinae, considering that the differences between them would be gradual.

The name Listroscelidinae begun to be used since the publication of the key by Rentz (1979) for subfamilies of Tettigoniidae. Following the later, the main characters supporting the group are the fore tibiae usually bearing 5-7 long spines and the fastigium of vertex narrow and usually sulcated, middle and fore femora of many species bearing heavy teeth. Nickle (1992) disagreed from Rentz about the number of spines, suggesting 4-8 on the fore tibiae as a diagnostic number. However, in the latter case *Phlugis* Stal, 1861 (Meconematinae) as included in group.

Listroscelidinae is exclusively Pantropical, with most species distributed in the Oriental and Austral regions (Eades et. al. 2012). On the southern North America, only the genus *Neobarrettia* Rehn 1901 is known (Conh 1957). The genus *Arachnoscelis* Karny, 1911 is known only from Central American (Costa Rica and Panamá) and Western South America (Colombia and Peru). Species of eight genera occurs in Brazil, which shelters the mighty species richness of Listroscelidinae in America. Five genus were proposed each based on a single specie: *Carliella* Karny, 1911, *Isocarliella* Mello-Leitão, 1940, *Liostethomimus* Karny, 1914, *Macrometopon* Bruner, 1915 and *Megatympanon* Piza, 1958. Three genus were originally based on at least two species: *Cerberodon* Perty, 1832 (three species), *Listroscelis* Serville, 1831 (four species) and *Monocerophora* Walker, 1869 (two species). In most cases, the species were collected in the Atlantic Forest biome.

Materials and Methods

Specimens and examination

Field work and collections were carried out at conservation units or reserves in the Southeastern and Northeastern Brazilian Atlantic Forest. Fifteen areas (Figure 1) were surveyed in the states of Bahia (BA), Espírito Santo (ES), Minas Gerais (MG) and Rio de Janeiro (RJ), but specimens of Listroscelidinae were collected only in ten Conservation Units (CU), as follows: 1, 2, 3, 4, in BA; 7, 10, 11, in MG; 9, in ES; and 13, 15, in RJ.

All Listroscelidinae were captured manually. Images of live katydids were taken in field. Then, specimens were killed and dried for preserving. Before drying, one middle- or hind leg of each specimen was preserved in ethylic alcohol (100%) under –20C°.

Listroscelidinae deposited in a few Brazilian entomological collections were also borrowed and examined (see acronyms below). Specimens were examined under a Zeiss Stemi DV4 stereomicroscope. Photos were taken under a Zeiss Discovery V8 or a Zeiss Stemi 2000–C stereomicroscope, equipped with a Canon EOS 1000D or a Zeiss MRc Axiocam, respectively. Images were stacked with CombineZM freeware (Hadley 2010) or Zeiss AxioVision 4.8, respectively, then edited in CorelDraw X5. Color images are available at the Orthoptera Species File (Eades et al., 2012). Images of type material deposited at the Museum of Vienna were provided by the curator. The identification key is for Listroscelidinae of the Atlantic Forest.

Abbreviations, labels and depositories

We use the following abbreviations for measurements (in mm) and counting of parts: TL, total body length; FF, length of fore femur; FT, length of fore tibia; HF, length of hind femur; HT, length of hind tibia; PL, pronotal length at midline; PW, maximum pronotal width; EyeW, minimum eye width; TegL, maximum tegmina length; TegW, maximum tegmina width; SL, length of the stridulatory file of male tegmen; NT, number of teeth in the stridulatory file; minT, minimum tooth length; maxT, maximum tooth length; OL, length of the ovipositor. We took the measurements of the largest parts of the body with graph paper, the stridulatory line, teeth and EyeW were measured with the metric ruler of the stereomicroscope. We counted the number of spines on the inner and outer margins of the left and right femora (F) and tibiae (T). For forelegs, sFF; for middle legs, sMF; sMT; sMTld (dorsolateral); and for hind legs, sHF; sHTd (dorsal); sHTv (ventral). We provide the range of the variation found for either the holotype and allotype, and the range of variation for males and females of the whole type series or examined specimens. Some measures were obtained from the literature.At the descriptions we use “fastigium” as an abbreviation for fastigium verticis and “frons” for frontal fastigium.

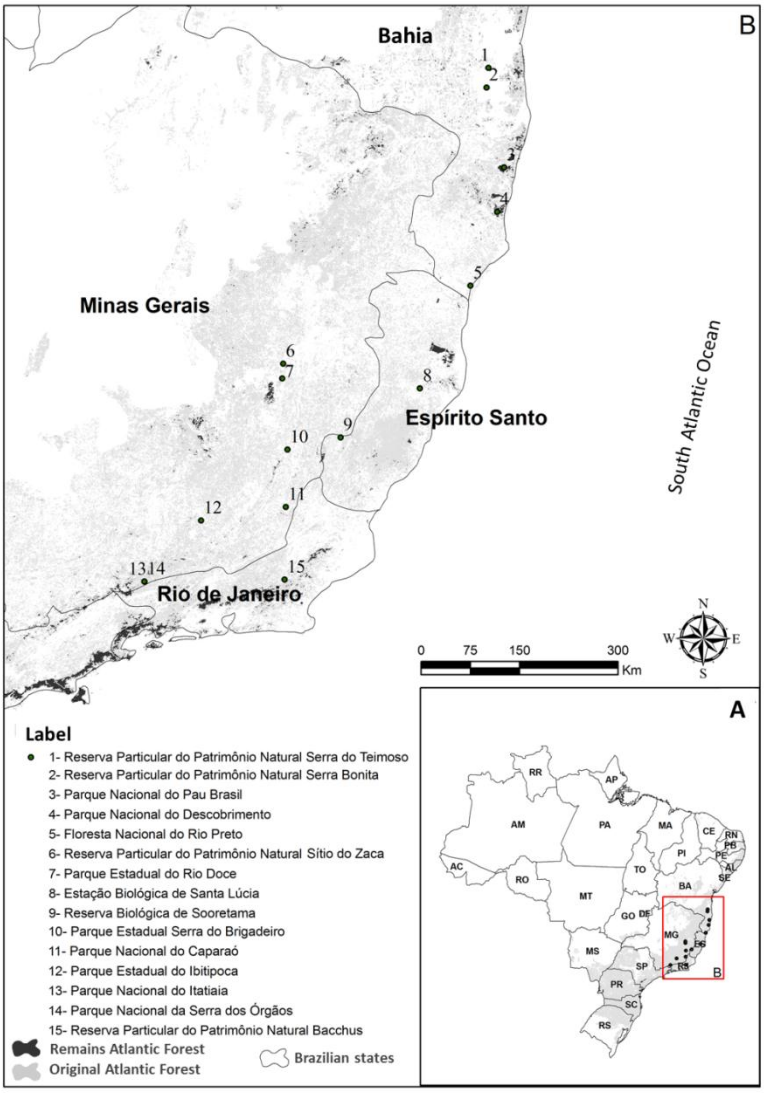

Figure 1: Map showing the sampled areas. A: The studied area on the map of Brazil. B: sampling points for this study. Dark grey represents the remaining fragments of the Atlantic Forest; ligh grey represents the original coverage of the biome; before the Portuguese colonization of Brazil in 1500. BA: state of Bahia; ES: state of Espírito Santo; MG: state of Minas Gerais; RJ: state of Rio de Janeiro.

Each specimen of the type series received an additional label containing the name of the species, authors and its status in the type series (holotype, allotype or others). We used red labels for holotypes and blue labels for paratypes, if any. We based each species description on the morphology of male holotype and female allotype, if any. We provided the observed variation, if any, in a separate section after each description. Specimens have been deposited in or belong to the following institutional collections (with acronyms used in this paper):

- **MNRJ** Museu Nacional Rio de Janeiro (Rio de Janeiro, RJ, Brazil)
- **UFES** Coleção de Insetos (Vitoria, ES, Brazil)

Descriptions and Redescriptions

Listroscelidini Redtenbacher, 1891

Cerberodon Perty, 1832

Type species: *Cerberodon viridis* Perty, 1832

Redescription. General coloration of the specimen body could include the following colors and its variations of dark and light: greenish, blackish, reddish and orangish. **Head.** Fastigium laterally compressed, narrower and shorter than the first antennomere. Eyes globose and frontally prominent, inserted separately of the basal margin of antennal sockets. Apex of antennal sockets at the same distance of half the length of the eyes. Sclerites of antennal sockets not in contact at the midline. Head in frontal view apparently globose and broad, due to the protruding vertex; width of the head eight-ninths its length. Frons subtriangular, with a rounded tiny projection at the middle of its length, where the ocellus is located, trough not easily visible. Face, genae and partial or total portion of clypeus strongly wrinkled, with a brain-like appearance. Mandibles robust with the apex elongated; in male, the apical portion of the left mandible is strongly angulated, projected and curved upwards. Mandibles with a basal process at the incise area. Maxillary and labial palpi greenish. **Thorax**. Pronotum with anterior margin slightly curved inwards, posterior margin almost straight. Prozone with a transverse furrow extending through the lateral lobe and sometimes reaching or the anterior margin. Mesozone with a straight transverse furrow strongly curved to the posterior margin of the pronotum; in lateral view, this furrow leading to a dorsal strong depression. Metazone with a transverse furrow extending laterally at the lateral lobe, reaching the lower margin. Lateral lobes with lower margin straight, posterior margin slightly oblique; corners rounded and without sinus humeralis. Wings fully developed or shortened; tegmina devoid of bright spot. The coxae could bear an acute or rounded spine at ventral margin, on the basal and distal portions. Legs robust and short; hind femora shorter than the length of the body. Mid and fore femora with a broad longitudinal furrow along the ventral surface. Both ventral margins of femora armed with long spines interspersed with thin spines. Fore tibiae slightly curved inwards and bearing a small hole or spur dorsally, below and close to each opening of the tympanum. Mid tibiae bearing four to seven spines. Hind tibiae with spines at dorsal and ventral margins. Genicular lobes of all legs ending in an acute spine. Tympanum at the outer margin of tibiae; area around tympanum slightly inflated; narrow opening, width one-eleventh the length of the opening. Each sternite with two stout spines, but the ones at the metasternum being comparatively flattened. **Abdomen.** Cerci, in male, bent in almost an acute angle, ending in an acuminate hook-like tip. Apical portion with almost the same length of the basal one, the former with tubercles and long bristles. Supranal (epiprocte) plate triangular or rounded. Paraprocts, in male, triangular; lateral outer vertex with a spine curved downwards. Subgenital plate of male wide; apical portion with a V-shape emargination. Styli short, almost or shorter than one-quarter the length of the plate. Subgenital plate of female triangular or subtriangular, with an emargination leading a V-shape cut almost one-quarter the length of plate at the longitudinal midline. **Male genitalia** with a membranous epiphallus and a sclerotized and long phallus, visible at dorsal and lateral view, projected outwards, ending in a rough lobe. This genus includes *C. viridis* Perty, 1832 and *Cerberodon* **sp. nov. 1**.

***Cerberodon* sp. nov. 1** (Figs 1 & 3)

Diagnosis. This species can be distinguished from *C. viridis* by the following combination of characters: (i) fore tibiae with ventral surface dark brownish when dead, or dark orange when live; (ii) male tegmina shorter than the length of the abdomen not covering the last two abdominal segments, and in female not covering the five last abdominal segments; (iii) supranal plate in male semitriangular.

Description (holotype ♂ & allotype ♀). Holotype: TL 27; PL 9; PW 7; EyeW 4; TegL 14; SL 2.39; NT 66; minT 0.04; maxT 0.08; HF 22; HT 26; sFF, inner margin 6, outer 6, small 2–0; sMF, inner margin, large 5, small 19, outer large 5, small 6; sHF, inner margin, large 8–10, small 2–8, outer large 10–12, small 0–1; sMT, inner margin 7, outer 4; sMTld 4; sHTd, inner margin 10–15, outer 12–13, sHTv, inner margin 9–12, outer 12. Allotype ♀ TL 35; PL 8; PW 8; EyeW 4; TegL 13; HF 22; HT 24; sFF, inner margin, large 5–6, small 2–3, outer large 5–6, small 1–2; sMF, inner margin, large 19, small 18, outer large 4, small 4; sHF, inner margin, large 11–13, small 0, outer large 11–12, small 0; sMTld; sHTd, inner margin, outer, sHTv, inner margin, outer; OL 28. Coloration pattern consisting of light greenish and dark brownish areas (Fig. 1A). **Head.** Dorsal area light greenish (Fig.1B). Fastigium dark brownish. Antennal scape dark brownish. Pedicele with frontal surface dark brownish and dorsal surface greenish. Antennomeres greenish. Eyes dark brownish, the surface around them blackish. Frons dark brownish, semitriangular, with an oval protruding tubercle at the middle, and with the ocelus pole (Fig. 1C). Face, genae and clypeus dark brownish. Mandibles light brownish with cutting edge darkish; outer margin wrinkled. Clypeus dark brownish with apical portion darker. Labrum pinkish with basal portion dark brownish. Mouthpart in ventral view dark brownish. **Thorax.** Pronotum greenish (Fig. 1D–E). Fore coxae with basal portion greenish and apical portion reddish; trochanters dark brownish. Fore femora greenish; lower margin reddish; spines whitish with inner base blackish. Tibiae greenish with base darker; spines light greenish. Mid femora greenish, lower margin and spines whitish, ventral area greenish. Hind femora greenish, lower margin lighter, spines dark brownish; tibiae greenish with spines light brownish. Tarsus dark brownish, the third one darker. Tarsus claws greenish with tip darker. Sternum mostly greenish, prosternum lighter (Fig. 1E); meso- and metasternum with posterior margin reddish (Fig. 1F). Tegmina with primary and secondary veins light greenish, spaces between veins dark brownish; stridulatory area darker. **Abdomen.** Tergites dark brownish; sternites slightly reddish. Supranal plate wide, broader than longer, subtriangular (Fig. 1H). Cerci of male with basal portion wider than the apical, the latter flattened dorsoventrally, strongly curved inwards and ending in an acute tip (Fig. 1H). Paraprocts with a protruding spine curved downwards. Subgenital plate of male with a deep V-shape emargination (Fig. 1I), being half the length of the plate; in female, with a V-shape emargination (Fig. 1J), being one-quarter the length of the plate. **Male genitalia** as shown in Fig. 1K–L. Ovipositor longer than the length of abdomen. **Alive**, the areas described as dark brownish are reddish, almost dark orangish (face, mandibles, posterior margin of the pronotum, ventral surface of fore femora, spines of tibiae, tarsi, part of the sternum, abdominal tergites, abdominal spiracles, and cerci in male, dorsal and ventral margin of the ovipositor). Eyes pinkish frontally, and whitish at dorsally; labrum lighter than after dead (Fig. 3A–F).

Type series. *Holotype male* labeled \Brasil, MG, Alto Caparaó, P.N Caparaó. 23–26, XI, 2011. J. Chamorro *leg*. [handwritten on with paper] \ 1/Listro/Caparao [typewritten on white paper] \ *Cerberodon* sp. nov. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on red paper]\. *Allotype female* labeled \Brasil, MG, Alto Caparaó, P.N Caparaó. 4–8, II, 2012. V. Fialho. [handwritten on with paper] \ *Cerberodon* sp. nov. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on blue paper].

Comments. Deimatic behavior was performed by male holotype, and was documented in Fig. 3D. Similar behaviors have been observed in *Neobarrettia* Rehn, 1901 (Cohn 1965), other Listroscelidinae, and species of different subfamilies as in *Mygalopsis ferruginea* Redtenbacher, 1891 (Sandow and Bailey 1978), *Acanthodis curvidens* (Stål, 1875) (Robison 1969), and *Panacanthus pallicornis* (Walker, 1869) (pers. obs. and images available on OSF).

***Cerberodon viridis* Perty, 1832,** Modified from description of Redtenbacher, 1891 (Figs 2 & 3)

Diagnosis. This species can be distinguished from *Cerberodon* sp. nov. 1 by the following combination of characters: (i) fore tibiae with ventral area blackish; (ii) tegmina longer than the abdomen; (iii) supranal plate of male rounded.

Redescription. (Base on images of material and additional specimens). Type and General coloration consisting of light greenish and maroonish or dark brownish areas (Fig. 2A). **Head.** Dorsal area greenish (Fig.2B). Fastigium dark brownish. Scape and pedicele with frontal portion maroonish, and dorsal one greenish; antennomeres light brownish. Eyes light brownish, with cuticle around the eyes blackish. Frons dark brownish, semitriangular and protruding (Fig. 2C). Face, genae and clypeus dark brownish (Fig. 2C). Mandibles light brownish with cutting edge darkish; outer margin wrinkled. Labrum brownish. Mouthparts in ventral view dark brownish. **Thorax.** Pronotum greenish (Fig. 2D, 2E). Legs, in general, greenish with spines dark brownish; coxae and trochanters with ventral area maroonish, the remaining greenish. Forelegs with ventral area of femora and areas close to spines of tibiae blackish. Tarsi and tarsal claws dark brownish. Sternum greenish, with some spots maroonish (Fig. 2F). Tegmina with primary and secondary veins greenish, spaces between veins dark brownish, anal and stridulatory area dark brownish. **Abdomen.** Tergites greenish; sternites in male light reddish, and in female whitish. Supranal plate wide, broader than longer. Cerci of male with basal portion wider than the apical one, strongly curved inwards and ending in an acute tip. Subgenital plate of male with a deep V-shape emargination, being shorter than half of the length of the plate; in female with a V-shape emargination (Fig. 2J), being one-quarter the length of the plate. **Male genitalia** as shown in figure 2L. Ovipositor longer than the length of the body. **Alive**, the areas described above as dark brownish, reddish or maroonish when dead, are dark reddish when alive (face, anterior and posterior margin of the pronotum, spines at the legs, mouthparts in ventral view, areas at the sternum, abdominal spiracles, abdominal sternites, dorsal and ventral margin of the ovipositor). Eyes pinkish at frontal, and whitish at dorsal; labrum pinkish, lighter than after dead (Fig. 3G–L).

Specimens examined. Specimens were collected in Brazil, Rio de Janeiro, Nova Friburgo, Reserva Particular do Patrimônio Natural Bacchus, 16–19, XI, 2011. All are labeled as follows: \Brasil, RG, R.P.P.N. Bacchus, 16–19, XI, 2011, J. Chamorro *leg*. [handwritten on with paper] \ *Cerberodon viridis* [handwritten on with paper]\ 1 Adult ♀: \3/Listro/Bacchus [typewritten on white paper] \ 1 immature male ♂ \ 4/Listro/Bacchus [typewritten on white paper] \ 2 immature female ♀ \ 1 and 2/Listro/Bacchus [typewritten on white paper]\. 2 male ♂ and 2 females ♀ (MNRJ): male 1 \ No. R. Anlé. Petrópolis. 1936 [handwritten on yellowish paper] \ *Cerberodon viridis* [handwritten on white paper] \ male 2 \ Petropolis 1.52. Frey Thomaz [handwritten on blue paper] no verso Inst. Osvaldo Cruz [handwritten on yellowish paper] \ female 1 \ Vista chinesa 79. col. OTERO [handwritten on yellowish paper] \ *Cerberodon viridis* [handwritten on white paper] \ female 2 \ Petropolis. Est. do Rio. BRASIL. [typewritten] janeiro 1958. Herta [handwritten] \ COLECÃO CAMPOS SEABRA [typewritten on white paper].

Variation. Measurements of females (n=2, including the allotype, in this case, only are shown the same measurements in Redtenbacher, 1891): TL 38–42 (including tegmina); PL 7–7.5; TegL 20–27; FF 15–16; FT 16–18; HF 22–24; OL 25.5–26. Other specimens, Measurements of males (n=2): TL 33; PL 8; PW 7; EyeW 5; TegL 26–27; SL 2.71–2.78; NT 65–67; minT 0.02; maxT 0.18–0.19; HF 22–23; HT 24–26; sFF, inner margin, large 7-7, small 9–13, outer large 6–7, small 2-19; sMF, inner margin, large 6–7, small 11–12, outer large 6–7, small 17–24; sHF, inner margin, large 10–14, small 0-2, outer large 9–14, small 0–3; sMTld 1–4; sHTd, inner margin 12–14, outer 16-16, sHTv, inner margin 13–17, outer 11–13. Measurements of females (n=3): TL 36–39; PL 7–9; PW 7; EyeW 4–5; TegL 22–27; HF 23–24; HT 25–28; sFF, inner margin, large 6-6, small 0-3, outer large 6-7, small 0-4; sMF, inner margin, large 6-6, small 14-20, outer large 6-7, small 18-23; sHF, inner margin, large 11-13, small 0-0, outer large 9-12, small 0-0; sMTld 3-4; sHTd, inner margin 13- 14, outer 15- 17, sHTv, inner margin 14-17, outer 11-12; OL 26-28.

Comments. Material type was collected from an unknown locality from Brazil. These are the first records of a specific locality where *C. viridis* inhabits.

Listroscelis Serville, 1831

Type species: *Listroscelis armata* Serville, 1834

Redescription. General coloration of the specimen body could include the following colors and its variations of dark and light: greenish, blackish, maroonish, yellowish and brownish. **Head.** Fastigium laterally compressed, narrower and shorter than the first antennomere. Eyes globose and protruding, located separately of the antennal sockets. Apex of the antennal sockets shorter half than the length of the eyes. Sclerites of antennal sockets not in contact at the midline. Head at frontal view lengthy, width five-ninths of the length; vertex slightly visible at frontal, not protruding. Frons subtriangular, with a rounded projection at the middle of the length, where the ocellus is located, not totally visible, in some cases the ocellus is at the base of the frons. Face smooth, or slightly wrinkled. Mandibles with apex elongated and curved inwards; in males of some species mandibles symmetric, in other species asymmetric, the left mandible with lateral lower portion lengthened and curved outwards, different than in *Cerberodon* where the apex is lengthened. Mandibles with a basal process at the cutting area. **Thorax.** Pronotum with anterior and posterior margins straight or slightly curved inwards. Prozone with a transverse furrow slightly extending at the lateral lobe, not reaching the lower margin. Mesozone with a transverse furrow, extending at the lateral lobe, reaching or not the lower margin. Metazone with a straight transverse furrow extending laterally at the lateral lobe, almost reaching the posterior margin. Lateral lobes with lower margin almost straight; posterior margin slightly oblique, with rounded corners without sinus humeral. Wings fully developed; tegmina with a yellowish bright spot at the base. Coxae could bear a spine that could be acute or rounded at ventral, on the basal and distal portion. Legs slender, hind femora have the same length of the body or slightly longer. Mid and fore femora with a ventral broad distinct longitudinal furrow at all the length. All femora armed with long spines interspersed or not with thin spines. Fore tibiae with area close to the spines blackish; a small hole or spur at dorsal, below and close to each opening of the tympanum. Mid tibiae at ventral have four to six spines. Hind tibiae have several spines at dorsal and ventral margin. Genicular lobes of all legs ending in an acute tip. Tympanum wide opening, width one-sixth the length of the opening; localized frontally at the tibiae; area around the tympanum conspicuously inflated. Each sternite with two spines with tip rounded; spines at the prosternum slender, and ones of the metasternum could be flattened or slender. **Abdomen. C**erci in males, with apex bent or slightly curved inwards or downwards; tubercles and bristles covering the entire surface. Supranal plate could be triangular, elongated or modified with lobes, emarginated or not. Subgenital plate of male variable, emarginated in different ways; styli with length variable. Paraprocts in males triangular and concealing the genitalia; a spine curved downward at the lateral outer vertex of the plate. Subgenital plate of female could be or not emarginated. **Male genitalia** with an epiphallus membranous and a phallus sclerotized, long and projected outwards, with rounded tips.

This genus includes *L. armata* Serville, 1831, *L. atrata* Redtenbacher, 1891, *L. carinata* Karny, 1907, *L. ferruginea* Redtenbacher, 1891, *Listroscelis* **sp. nov. 1**, *Listroscelis* **sp. nov. 2**, *Listroscelis* **sp. nov. 3**, *Listroscelis* **sp. nov. 4** and *Listroscelis* **sp. nov. 5**, *Listroscelis* **sp. nov. 6**.

***Listroscelis* sp. nov. 1** (Figs 4 & 12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) General coloration dark maroonish, dark brownish and light and dark greenish; (ii) face, genae and clypeus with transverse wrinkles arranged horizontally, parallel to the vertex; (iii) left mandible, in male, with the pre-apical lateral portion elongated and bent, apical portion projected upwards; (iv) yellowish spot being almost one-quarter the length of the tegmen; (v) supranal plate in male triangular.

Description (holotype ♂ & allotype ♀). Holotype ♂: TL 25; PL 7; PW 5; EyeW 3; TegL 14; SL 2.11; NT 73; minT 0.02; maxT 0.09; HF 23; HT 26; sFF, inner margin, large 4–5, small 24–25, outer large 5, small 30–32; sMF, inner margin, large 5, small 23–26, outer large 4, small 28; sHF, inner margin, large 11–12, small 21, outer large 12–13, small 12–19; sMTld 4; sHTd, inner margin 16–17, outer 15, sHTv, inner margin 10–13, outer 10–13. Allotype ♀: TL 28; PL 6; PW

5; EyeW 3; TegL 14; HF 23; HT 26; sFF, inner margin, large 4, small 11–14, outer large 5–6, small 25–26; sMF, inner margin, large 5–6, small 29–30, outer large 4–5, small 22–23; sHF, inner margin, large 7–15, small 5–12, outer large 11, small 4–10; sMTld 4; sHTd, inner margin 18, outer 15–16, sHTv, inner margin 9–10, outer 10–11; OL 21. General coloration consisting of dark maroonish and greenish areas (Fig. 4A). **Head.** Dorsal area dark maroonish (Fig. 4B); vertex with dorsal area dark yellowish, and sides blackish. Antennal scape and pedicele blackish with indistinct dark brownish spots. Antennomeres blackish; sclerites of antennal sockets blackish. Eyes dark yellowish with undefined dark brownish spots; a dark yellowish oval area at the dorsal inner surface. Frons oval and protruding, dark brownish, with a defined ocellus. Face and basal portion of clypeus dark maroonish, with transversal wrinkles arranged parallel to the vertex (Fig. 4C); middle and apical portion of clypeus yellowish, with undefined dark brownish spots. Labrum almost blackish with basal portion dark brownish. Mandibles blackish. Maxillary and labial palpi light greenish, with blackish spots at the apical portion of each palpus. Mouthparts in ventral view, excepting the appendages, dark brownish. **Thorax.** Pronotum dark maroonish (Fig. 4D–E). Epimeron with the area close to the prothoracic spiracle fluorescent yellowish. Tegmina with the portion between the costal region to the R vein, and between the Sc vein to the lower margin, greenish fluorescents and blackish; region between the R and Sc veins brownish. Primary veins brownish, secondary veins greenish, space between veins blackish. Hind wings dark brownish. Sternum blackish; spines and furrows light yellowish (Fig. 4F). Legs with ventral portion of coxae blackish; mid and hind coxae with undefined dark yellowish spots, lateral and ventral portions light brownish. Trochanters have similar color to that of the coxae. Femora and tibiae of all legs with similar coloration pattern as follows: dorsal portion light brownish, ventral one blackish and apical portion of ventral edge light brownish. Large spine of each femur with a whitish oval spot at the inner base. Tibiae light greenish with areas close to the spines light brownish; spines dark brownish. Hind femora at lateral with a slightly brownish stripe parallel to the ventral margin. Areas close to the tympanum light brownish (Fig. 4G). Tarsi have first, second and fourth tarsomeres light brownish, apical portion of each tarsus dark brownish; third one blackish with undefined dark brownish spots. Tarsus claws dark brownish. **Abdomen**. Tergites with central portion light brownish, and lateral portions dark brownish. Sternites blackish. Supranal plate of male triangular (Fig. 4H). Cerci of male stout and elongated, dismissing in size from the basal to the apical portion; tip projected and curved inwards (Fig. 4K). Subgenital plate of male with basal portion dark brownish and the remaining light brownish (Fig. 4I); an emargination leading a U-shape cut almost one-quarter the length of the plate at the longitudinal midline. Styli almost one-third the length of the plate. Ovipositor one-sixth times longer than the abdomen. **Male genitalia** as shown in Fig. 4L.

Variation. Measurements of males (n=8, including the holotype): TL 23–26; PL 7; PW 5–6; EyeW 2–3; TegL 13–14; HF 22–24; HT 24–26; sFF, inner margin, large 4–5, small 27–35, outer large 5, small 28–37; sMF, inner margin, large 4, small 29–33, outer large 3–4, small 24–35; sHF, inner margin, large 4–15, small 21–25, outer large 10–13, small 9–22; sMTld 4; sHTd, inner margin 16–18, outer 15–17, sHTv, inner margin 9-11, outer 15-17. Stridulatory file (n=2) SL: 2.11–2.23; NT: 73–78; minT 0.02–0.03. Measurements of females (n=2, including the allotype): TL 27–28; PL 6–7; PW 5; EyeW 3; TegL 12–14; HF 22–23; HT 25–26; sFF, inner margin, large 4–5, small 10–14, outer large 5–6, small 25–26; sMF, inner margin, large 5–6, small 14–30, outer large 4–6, small 22–24; sHF, inner margin, large 7–15, small 1–12, outer large 9–11, small 0–10; sMTld 4–4; sHTd, inner margin 16–18, outer 15–16, sHTv, inner margin 9–12 , outer 9–11; OL 20–21.

Type series. Specimens were collected in Brazil, Bahia, Camacan, Reserva Particular do Patrimônio Natural Serra Bonita, 10–12, I, 2012. All are labeled as follow: \Brasil, Camacan, BA, R.P.P.N Serra Bonita, 10–12, I, 2012, J. Chamorro *leg*. [handwritten on with paper]\. *Holotype male* also labeled \17–Listro–Bonita [typewritten on white paper] \ *Listroscelis* sp. nov. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on red paper]\. *Allotype female* labeled \7/Listro/Bonita \ *Listroscelis* sp. nov. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\. *Paratypes* all labeled: \ *Listroscelis* sp. nov. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\. 7 ♂ adults, codes: 1,2,3,4,5,13,14; 1♂ immature, code: 26; 1 ♀ adults, codes: 15; 1 ♀ immature, codes: 7,15.

Comments. Individuals of this species were abundant in field, unlike other species of the genus. **Alive** as shown in Figure 12A–C.

***Listroscelis* sp. nov. 2** (Figs 5 & 12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) general coloration light greenish with few areas light brownish; (ii) face, genae and clypeus smooth; (iii) mandibles symmetric; (iv) yellowish spot being almost one-twelfth the length of the tegmen; (v) supranal plate in male elongated, the width of the plate decreases rapidly from the middle to the tip; a deep emargination with an oval-shape at the longitudinal midline.

Description (holotype ♂ & allotype ♀). Holotype ♂: TL 26; PL 7; PW 5; EyeW 3; TegL 22; SL 2.22; NT 136; minT 0.01; maxT 0.06; HF 23; HT 25; sFF, inner margin, large 4–5, small 23–27, outer large 5, small 36; sMF, inner margin, large 5, small 32, outer large 4, small 27; sHF, inner margin, large 13–15, small 18–21, outer large 12–13, small 7–10; sMTl d 5; sHTd, inner margin 16–18, outer 15, sHTv, inner margin 10, outer 12-13. Allotype ♀: TL 27; PL 6; PW 5; EyeW 3; TegL 22; HF 12; HT 28 sFF, inner margin, large 4-5, small 19–21, outer large 4–5, small 26-28; sMF, inner margin, large 5, small 31, outer large 4, small 30; sHF, inner margin, large 13–15, small 12–13, outer large 12, small 7–10; sMTld 6; sHTd, inner margin 18, outer 16–17, sHTv, inner margin 10–11, outer 13; OL 26. General coloration consisting of light greenish and light brownish areas (Fig. 5A). **Head.** Dorsal area light greenish (Fig. 5B). Fastigium at dorsal portion whitish and at laterals brownish. Antennal scape and pedicele blackish; antennomeres dark brownish; sclerites of antennal sockets blackish. Eyes dark brownish with irregular blackish spots. Frons semi-oval, light brownish, with a thin tubercle (Fig. 5C). Face almost smooth, slightly wrinkled, light brownish; sides light greenish. Clypeus and labrum light brownish. Mandibles with basal portion and sides light brownish, the remaining including the cutting edge blackish. Maxillary and labial palpi greenish. Mouthparts in ventral view light brownish. **Thorax.** Pronotum light greenish (Fig. 5D–E). Sternites and spines light greenish (Fig. 5F). Legs including coxae and trochanters light greenish; spines at the femora and tibiae light brownish. Areas close to the tympanum light brownish (Fig. 5G). Tarsi light greenish. Tarsus claws light greenish with the tip dark brownish. Tegmina including primary and secondary veins light greenish; spaces between veins dark brownish. **Abdomen.** Tergites and sternites light greenish. Supranal plate of male as shown in Fig. 5H. Subgenital plate of male wide with a V-shape emargination at the longitudinal midline (Fig. 5I). Styli being one-fifth the length of the plate. Cerci with apical portion slightly curved downwards (Fig. 5H, 5J). Ovipositor one-thirteenth longer than the length of the abdomen. **Male genitalia** as shown in Fig. 5K.

Type series. *Holotype male* labeled \Brasil, ES, Linhares, ReBio de Sooretama. 29–XI – 2–XII, 2011. J. Chamorro *leg.* [handwritten on with paper] \ 6–Listro–Sooretama [typewritten on white paper] \ *Listroscelis* sp. nov. 2 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on red paper]\. *Allotype female*, same locality and data as the holotype, also labeled \1–Listro–Sooretama [typewritten on white paper] \ *Listroscelis* sp. nov. 2 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\.

Comments. Alive**,** both individuals had more vivid greenish coloration than after dead (Fig. 12D–E).

Listroscelis sp. nov. 3 (Figs 6)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) general coloration dark and light brownish and light greenish; (ii) face, genae and clypeus almost smooth, slightly wrinkled; (iii) mandibles symmetric; (iv) yellowish spot being almost one-sixteenth the length of the tegmen; (v) supranal plate in male triangular.

Description (holotype ♂): TL 26; PL 6; PW 5; EyeW 3; TegL 22; SL 2.68; NT 178; minT 0.02; maxT 0.08; HF 22; HT 24; sFF, inner margin, large 5-6, small 27, outer large 5, small 35–38; sMF, inner margin, large 5, small 29, outer large 5, small 28; sHF, inner margin, large 10–12, small 22–25, outer large 16–17, small 12–22; sMTld 4; sHTd, inner margin 18, outer 16–18, sHTv, inner margin 9–12, outer 11. General coloration consisting of dark and light brownish and light greenish areas (Fig. 6A). **Head.** Dorsal area dark brownish (Fig. 6B). Fastigium dorsally light yellowish and laterals dark brownish. Antennal scape and pedicele light brownish; antennomeres dark brownish. Frons semitriangular, dark brownish (Fig. 6C). Eyes reddish. Face dark brownish. Mouthparts in ventral view whitish. **Thorax.** Pronotum dark brownish (Fig. 6D–E). Forelegs with coxae and trochanters light brownish; femora light brownish, lower margin lighter. Spines at the femora and tibiae dark brownish with tip darker. Tibiae with apical portion dark brownish and the remaining light greenish; areas close to the spines blackish. Tympanum with cuticle around the openings blackish. Mid and hind coxae and trochanters yellowish. Midlegs light greenish. Hind femora and tibiae light greenish, apical and basal portion dark brownish. Mid and hind spines light brownish. Tarsi light brownish, the third one darker. Tarsus claws light brownish with tip dark brownish. Tegmina with primary and secondary veins light greenish; spaces between veins dark brownish; stridulatory area darker. Hind wings light greenish with primary veins dark brownish. Prothoracic spiracle with the upper and lateral portions dark brownish. Sternum yellowish with spines light greenish (Fig. 6F). **Abdomen.** Tergites light brownish; sternites dark brownish. Subgenital plate of male with a deep V-shape emargination (Fig. 6I); styli being one-third the length of the plate. Cerci laterally flattened, with apical portion bended inward (Fig. 6H, 6J). **Male genitalia** as shown in Fig. 6K.

Type series. *Holotype male* labeled \Brasil, BA, P.N. do Descobrimento. J. Chamorro *leg.* 13–15, I, 2012 [handwritten on white paper] \ 4/Listro/Descobrimento [typewritten on white paper] \ *Listroscelis* sp. nov. 3 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on red paper]\.

***Listroscelis* sp. nov. 4** (Figs 7 & 12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) general coloration dark and light brownish; (ii) face, genae and clypeus smooth in both sexes, but in male slightly wrinkled; (iii) left mandible, in male, with pre-apical lateral portion elongated and bent, apical portion projected upwards; (iv) yellowish spot being one-eleventh the length of the tegmina; (vi) Supranal plate in male triangular.

Description (holotype ♂ & allotype ♀). Holotype ♂: TL 28; ; PL 7; PW 5; EyeW 3; TegL 20; SL 2.20; NT 92; minT 0.04; maxT 0.11, HF 20; HT 21; sFF, inner margin, large 4–5, small 15–18, outer large 5, small 17; sMF, inner margin, large 5, small 22, outer large 5, small 21; sHF, inner margin, large 13, small 0–3, outer large 12, small 0; sMTld 5; sHTd, inner margin 16, outer 14–15, sHTv, inner margin 10, outer 11–13. Allotype ♀: TL 24; PL 6; PW 4; EyeW 3; TegL 20HF 19; HT 21; sFF, inner margin, large 4–5, small 14–16, outer large 4–5, small 20–27; sMF, inner margin, large 4, small 20, outer large 4–5, small 17; sHF, inner margin, large 0–13, small 0, outer large 0, small 0; sMTld 4;sHTd, inner margin 16, outer 14, sHTv, inner margin 7–9, outer 10; OL 16. General coloration consisting of dark and light brownish areas (Fig. 7A). **Head.** Dorsal area dark brownish, with a whitish stripe extending from the tip of the fastigium to the posterior margin of the head (Fig. 7B). Fastigium with laterals portions dark brownish. Eyes dark brownish with undefined darker spots. Antennal scape and pedicele in male almost blackish, pedicele with the inner area yellowish, in female dark brownish with undefined blackish spots. Antennomeres light brownish. Sclerites of antennal sockets blackish. Frons almost semitriangular, dark brownish (Fig. 7C). Face dark brownish, slightly wrinkled in male, in female smooth. Clypeus in male almost yellowish, with central portion whitish, in female with basal portion at the angles blackish; labrum almost blackish with basal portion yellowish. Mandibles blackish. Maxillary and labial palpi light greenish. Mouthparts in ventral view yellowish. **Thorax.** Pronotum dark brownish (Fig. 7D–E); lateral lobes with a blackish stripe at the lower and posterior margin. Epimeron with the area close to the prothoracic spiracle light brownish; prothoracic spiracle with the margin blackish. Sternites and spines yellowish (Fig.

7F). Legs with coxae and trochanters yellowish with undefined dark brownish spots, mid and hind coxae and trochanters darker. Femora and tibiae dark brownish. Tibiae with spines light brownish. Femora with spines dark brownish. Apical portion of femora darker. Hind femora with lower margin blackish. Tarsus dark brownish; tarsal claws dark brownish with tip blackish. Tegmina light brownish with primary veins light brownish, secondary veins light greenish, spaces between veins light brownish. **Abdomen.** Tergites light brownish and sternites dark brownish. Cerci stout, with tip strongly curved inwards (Fig. 7K–L). Subgenital plate of male with a slight emargination V-shape at the longitudinal midline (Fig. 7I); styli almost half the length of the plate. Ovipositor one-seventh longer than the abdominal length. **Male genitalia** as shown in Fig. 7L.

Type series. *Holotype male* labeled \Brasil, MG, Araponga, P.E. Brigadeiro. 12–15, XII, 2011. J. Chamorro *leg.* [handwritten on with paper] \ 2–Listro–Brigadeiro [typewritten on white paper] \ *Listroscelis* sp. nov. 4 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on red paper]\. *Allotype female*, same locality and data as the holotype, and additionally labeled \1–Listro–Brigadeiro [typewritten on white paper] \ *Listroscelis* sp. nov. 4 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\.

Comments. Alive, abdominal tergites and sternites are light greenish; the whitish stripe at the dorsal of the head is more vivid (Fig. 12F–G).

Listroscelis sp. nov. 5 (Figs 8 & 12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) general coloration dark and light brownish; (ii) face, genae and clypeus almost smooth, slightly wrinkled; (iii) mandibles symmetric; (iv) yellowish spot being one-tenth the length of the tegmen; (v) supranal plate broad, with an emargination leading an oval-shape cut of almost one-quarter the length of the plate at the longitudinal midline.

Description (holotype ♂ & allotype ♀). Holotype ♂: TL 32; PL 8; PW 6; EyeW 4; TegL 29; SL 3.86; NT 153; minT 0.04; maxT 0.16; HF 25; HT 26; sFF, inner margin, large 4–5, small 24–27, outer large 6, small 30–39; sMF, inner margin, large 5, small 37, outer large 5, small 27; sHF, inner margin, large 14, small 1–4, outer large 14, small 2–4; sMTld 0; sHTd, inner margin 16–19, outer 15–17, sHTv, inner margin 11–14, outer 11–13. Allotype ♀: TL 36; PL 8; PW 6; EyeW 3; TegL 29; HF 27; HT 28; sFF, inner margin, large 4–5, small 21–22, outer large 5, small 31–32; sMF, inner margin, large 4, small 32, outer large 4, small 27; sHF, inner margin, large 14, small 10–14, outer large 13–14, small 4–13; sMTld 3; sHTd, inner margin 16–17, outer 15–16, sHTv, inner margin 10–11, outer 13–14; OL 25. General coloration consisting of light and dark brownish areas (Fig. 8A). **Head.** Dorsal area dark brownish (Fig. 8B). Fastigium at dorsal portion yellowish and laterals light brownish. Eyes dark brownish with undefined darker spots. Antennal scape and pedicele light brownish with indistinct dark brownish spots; antennomeres black brownish. Frons semitriangular with a defined ocelus (Fig. 8C). Face almost smooth with few wrinkles. Clypeus yellowish, labrum almost light brownish with apical portion dark brownish, almost blackish. Mandibles blackish. Maxillary and labial palpi yellowish. Mouthparts in ventral view light brownish. **Thorax.** Pronotum dark brownish (Fig. 8D–E). Sternum and spines light brownish (Fig. 8F). Epimeron with area close to the prothoracic spiracle light brownish; prothoracic spiracle with the margin blackish. Legs including coxae and trochanters light brownish; femora darker than the tibiae; spines of femora dark brownish; spines of tibiae dark brownish with tip lighter. Area close to the tympanum dark brownish (Fig. 8G). Tarsi light brownish, the third one darker. Tarsal claws light brownish with tip darker. Tegmina light brownish; primary veins dark brownish, secondary veins light brownish, spaces between veins dark brownish. Hind wings light brownish. **Abdomen.** Tergites and sternites light brownish. Cerci stout, apical portion narrower and curved downwards (Fig. 8H–I, 8K). Subgenital plate broad with a V-shape emargination (Fig. 8I). Stily almost one-fifth the length of the plate. Ovipositor one-eleventh longer than the abdomen. **Male genitalia** as shown in Fig. 8L.

Variation. Measurements of females (n=2, including the allotype): TL 36–39; PL 8–9; PW 6; EyeW 3; TegL 29–31; HF 27–30; HT 20–33; sFF, inner margin, large 4–5, small 21–27, outer large 5, small 31–39; sMF, inner margin, large 5–4, small 32–33, outer large 4, small 27–34; sHF, inner margin, large 10–14, small 10–17, outer large 10–14, small 4–15; sMTld 6; sHTd, inner margin 16–18, outer 15–16, sHTv, inner margin 10–11, outer 13–16; OL 25–27.

Type series. Specimens were collected in Brazil, Bahia, Parque Nacional do Descobrimento, 13–15, I, 2012. All specimens are labeled as follow: \Brasil, BA, P.N. do Descobrimento. J. Chamorro *leg*. 13–15, I, 2012 [handwritten on white paper]\. Additionally labeled: *Holotype male* \23–Listro–Descobrimento [typewritten on white paper] \ *Listroscelis* sp. nov. 5 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on red paper]\. *Allotype female* \1–Listro–Descobrimento [typewritten on white paper] \ *Listroscelis* sp. nov. 5 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\. *Paratype female* \2–Listro–Descobrimento [typewritten on white paper] \ *Listroscelis* sp. nov. 5 Fialho, Chamorro-Rengifo & Lopes-Andrade 2012 [handwritten on blue paper]\.

Comments. Alive, individuals have almost the same colors than after dead (Fig. 12H).

***Listroscelis* sp. nov. 6** (Figs 9–12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) general coloration dark and light brownish and light greenish; (ii) face, genae and clypeus smooth; (iii) yellowish spot being one-quarter the length of the tegmen.

Description (holotype ♀): TL 25; PL 7; PW 4; EyeW 3; TegL 19; HF 22; HT 23; sFF, inner margin, large 4, small 6-8, outer large 4-5, small 8-9; sMF, inner margin, large 4, small 20, outer large 4, small 15; sHF, inner margin, large 14, small 5-7, outer large 11-12, small 0; sMTld 3; sHTd, inner margin 16, outer 16, sHTv, inner margin 10-11, outer 13-14; OL 19. General coloration consisting of dark and light brownish and light greenish areas (Fig. 9A). **Head.** Dorsal area dark brownish (Fig. 9B). Fastigium with the dorsal portion whitish, laterals dark brownish. Eyes dark brownish, slightly reddish, with undefined blackish spots. Antennal scape, pedicele and antennomeres dark brownish. Sclerites of antennal sockets blackish. Frons oval and dark brownish (Fig. 9C). Face dark brownish, sides lighter; area below to the eyes yellowish. Clypeus dark brownish with undefined blackish spots. Labrum almost yellowish with basal portion dark brownish. Mandibles blackish. Maxillary and labial palpi whitish. Mouthparts in ventral view yellowish. **Thorax.** Pronotum dark brownish (Fig. 9D–E); lateral lobes with a darker stripe at the lateral-posterior margin. Legs with coxae and trochanters light greenish. Fore femora light greenish, with lower margin with a black stripe; spines light greenish with the tip blackish; tibiae dark brownish. Mid femora greenish with lower margin almost whitish; spines dark brownish with inner base whitish; tibiae dark brownish. Hind femora dark brownish, with lower margin light greenish; spines light greenish with tip dark brownish; tibiae light brownish. Tarsi light brownish, the third one darker. Tarsal claws light brownish with tip darker. Tegmina greenish; primary veins light brownish, secondary veins light greenish, spaces between veins blackish. Sternites and spines light greenish (Fig. 9F); spines of meta-sternum flattened. **Abdomen.** Tergites dark brownish with undefined areas reddish; sternites light brownish. Ovipositor one-eleventh longer than the abdomen.

Variation. Measurements of females (n=3, including the holotype): TL 22–29; PL 6–7; PW 4–5; EyeW 3; TegL 19–22; HF 22; HT 23–24; sFF, inner margin, large 4, small 6–20, outer large 4–5, small 8–24; sMF, inner margin, large 4–5, small 20–24, outer large 4, small 15–18; sHF, inner margin, large 12–14, small 1–7, outer large 11–13, small 0; sMTld 4; sHTd, inner margin 13–17, outer 14–16, sHTv, inner margin 10–13, outer 10–14; OL 16–19.

Type series. Specimens were collected in Brazil, RJ, Parque Nacional de Itatiaia. 7–13, XI, 2011. *Holotype female* labeled \Brasil, RJ, P.N. Itatiaia. 7–13, XI, 2011 J. Chamorro *leg*. [handwritten on with paper] \ 6/Listro/Itatiaia [typewritten on white paper] \ *Listroscelis* sp. nov. 6 Fialho, Chamorro-Rengifo & Lopes-Andrade

2012 [handwritten on red paper]\. *Paratypes females* labeled as the holotype, with codes \ 3,8/Listro/Itatiaia [typewritten on white paper]\.

Comments. Alive, as show in Fig. 12I.

***Listroscelis carinata* Karny, 1907** (Figs 10 –12)

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) General coloration dark brownish and greenish; (ii) face, genae and clypeus slightly wrinkled; (iii) left mandible with the apical portion elongated outwards; (iv) yellowish spot being almost one-eighth the length of the tegmen; (v) supranal plate of male elongated, the width of the plate decreases rapidly from the middle to the tip; a deep emargination with an linear-shape at the longitudinal midline; tip of the plate acute.

Redescription (Based on images of material type and observation of other specimens). It is unknown how many specimens were measured by Kirby 1907, males (n=?, material type): TL 22–24; PL 6; TegL 20; HF 20–21. females (n=?, material type): TL 23–28; PL 5.9–6.2; TegL 17.5–21; HF 21–22; OL 16–23. General coloration consisting of dark brownish and dark greenish areas (Fig. 10A). **Head.** Dorsal portion dark brownish. Fastigium with dorsal portion yellowish, and sides brownish (Fig. 10B). Antennal scape and pedicele brownish. Antennomeres dark brownish; sclerites of antennal sockets blackish. Eyes dark brownish; a dark yellowish oval area at the dorsal inner surface. Frons oval and protruding, dark brownish. Head in frontal view apparently elongated, due to the outstretched labrum (Fig. 10C). Face and clypeus dark brownish. Labrum with basal portion light yellowish, the remaining dark brownish. Mandibles dark brownish; apical portion of both mandibles elongated, and acuminated, but the left one more elongated than the other. Maxillary and labial palpi dark brownish. **Thorax.** Pronotum dark brownish (Fig. 10D–E). Epimeron with the area close to the prothoracic spiracle light brownish. Tegmina dark brownish, with primary, secondary veins and spaces between them brownish. Hindwings light brownish. Legs with similar coloration pattern as follows: coxae and trochanters dark brownish, femora dark greenish, tibiae dark brownish. Additionally, fore femora with a dark brownish stripe at the lower margin of the lateral portion, in female this stripe is weaker. Spines of each femora and tibiae dark brownish. Areas close to the tympanum dark brownish. Tarsus of all legs dark brownish, the third-one darker. Tarsus claws dark brownish with the tip darker. **Abdomen.** Supranal plate as shown in Fig. 10H. Cerci of male elongated, forceps-shape, with tip curved downwards (Fig. 10K). Subgenital plate of male with an emargination leading a V-shape cut less than one-half the length of the plate at the longitudinal midline (Fig. 10I). Styli almost one-third the length of the plate. Ovipositor almost the same length as the body. Male genitalia as shown in Fig. 10L.

Variation. Not including data of material type. Measurements of male (n=1): TL 23; PL 8; PW 5; EyeW 2.5; TegL 21; SL 2.82; NT 158; minT 0.03; maxT 0.08; HF 21; HT 25; sFF, inner margin, large 3–3, small 0–6, outer large 3–3, small 14–0; sMF, inner margin, large 4–4, small 20–17, outer large 5–5, small 24–18; sHF, inner margin, large 11–12, small 5–5, outer large 10–10, small 7–9; sMTld 5–5; sHTd, inner margin 16–16, outer 16–18, sHTv, inner margin 13–16, outer 13–15. Measurements of females (n=6): TL 24–31; PL 6–7; PW 4–5; EyeW 2–3; TegL 18–20; HF 22–33; HT 23–25; sFF, inner margin, large 3–4, small 10–14, outer large 4–4, small 15–17; sMF, inner margin, large 4–4, small 13–21, outer large 4–5, small 19–21; sHF, inner margin, large 10-12, small 0–7, outer large 9–12, small 3–5; sMTld 5–5; sHTd, inner margin 16–17, outer 16–17, sHTv, inner margin 13–15, outer 10–14; OL 18–22.

It is unknown the type locality of *L. carinata*, the only information available is: “Minas Gerais, Espirito Santo”. From now on, it is known at least two localities where this species could be found.

Specimens examined. 1 adult male ♂ and 2 Adults ♀ (MNRJ). Specimens labeled are follow: male, \Collatina. E do. E. Santo. M. Rosa, Out. 36 [typewritten on yellowish paper] \ No. Proc. 58/512 [type– and handwritten on yellowish paper] \ *Listroscelis carinata* Karny, 1907 [handwritten on white paper]\. Female 1 \Collatina. E. do. Santo. M. Rosa, Out. 36 [typewritten on yellowish paper] \ No. Proc. 58/510 [type– and hindwritten on yellowish paper] \ *Listroscelis carinata* Karny, 1907 [handwritten on white paper]\. Female 2 \Collatina. E. do. Santo. M.

Rosa, Out. 36 [typewritten on yellowish paper] \ No. Proc. 58/511 [type– and handwritten on yellowish paper] \ *Listroscelis carinata* Karny, 1907 [handwritten on white paper]\. Specimens collected in Brazil, MG, Parque Estadual do Rio Doce, 9-12, XII, 2011. All are labelled \Brasil, MG, P.E. Rio Doce, J. Chamorro *leg*. [handwritten on with paper] \ 7/Listro/Riodoce [typewritten on white paper] \ *Listroscelis carinata* Karny, 1907 [handwritten on white paper]\.

Comments. Specimens examined of the MNRJ have lost the color, spite of that, it is noticed that the face and the abdominal sternites of the male are dark brownish. **Alive**, some parts of the body are aquamarine blue: ventral area of legs, pleura close to each coxae, abdominal sternites, pleurites, subgenital plate and cerci. Parts which are light greenish: dorsal and lateral area of legs, coxae, trochanters, and basal portion of the ovipositor. A whitish spot at the base of each spine of femora. Abdominal spiracles are whitish. Tergites light purple (Fig. 12J–L).

***Listroscelis atrata* Redtenbacher, 1891** (Fig. 11)

C. angustifrons Piza, 1960 **syn. nov.**

Diagnosis. This species can be distinguished from the others species of *Listroscelis* by the following combination of characters: (i) General coloration maroonish and blackish; (ii) face, genae and clypeus wrinkled; (iii) left mandible with lateral-subapical portion strongly elongated upwards; (iv) yellowish spot being at least one-tenth the length of the tegmen; (v) supranal plate of male rounded.

Redescription (Based on images of material type and observation of other specimens). Measurements of the holotype ♂: TL 22; PL 6; TegL 13; FF 14.5; TF 16. General coloration consisting of maroonish, blackish, and additionally some areas dark and light brownish and yellowish (Fig. 11A). **Head.** Dorsal portion maroonish. Fastigium with a yellowish stripe at dorsal, extending at frontal and at dorsal portion of the head (Fig. 11B). Antennal scape and pedicel at frontal blackish; at frontal and at dorsal indefinitely yellowish and blackish. Antennomeres blackish. Sclerites of antennal sockets blackish. Eyes blackish; a yellowish oval area at the dorsal inner surface. Frons triangular and protruding, and with a yellowish defined ocellus at the base. Face maroonish (Fig. 11C). Clypeus with a yellowish stripe at the laterals portion and midline. Labrum with basal portion yellowish, the remaining maroonish. Mandibles maroonish. Maxillary and labial palpi light brownish. Mouthparts in ventral view blackish. **Thorax.** Pronotum maroonish (Fig. 11D–E). Epimeron with the area close to the upper and anterior margin of the prothoracic spiracle blackish, lower and posterior margin dark brownish. Tegmina dark brownish, with primary, secondary veins light brownish and spaces between them light brownish. Hindwings light brownish. Coxae could bear a spine that could be acute or rounded at ventral, on the basal and distal portion. Legs with similar coloration pattern as follows: coxae and trochanters maroonish; femora with the area close to the inner and lower outer margin, and the ventral area blackish. Tibiae light brownish. Spines of each femora dark brownish, additionally, the spines at the fore- and midlegs with an oval yellowish spot at the inner base. Spines at the tibiae light brownish. Areas close to the tympanum darker. Tarsus of all legs light brownish, the third-one darker. Tarsus claws dark brownish, the tip not markedly darker. Sternites maroonish, spines longer and slender. **Abdomen.** Tergites dark brownish; sternites blackish. Supranal plate as shown in Fig. 11H. Cerci at lateral view seems a triangle, with lateral sides long, tip curved inwards (Fig. 11K). Subgenital plate with an emargination leading a U-shape cut less than one-sixth the length of the plate at the longitudinal midline (Fig. 11I). Styli long, almost four-fifth the length of the plate. **Male genitalia** as shown in Fig. 11L.

Variation. Measurements of males, not including holotype data (n=2): TL 25–26; PL 7–8; PW 5; EyeW 3; TegL 21–23; HF 24; HT 26; sFF, inner margin, large 4-4, small 9-13, outer large 4-6, small 17-26; sMF, inner margin, large 4–44, small 19–13, outer large 5–5, small 13–20; sHF, inner margin, large 10–12, small 2–5, outer large 9–13, small 0–13; sMTld 0–6; sHTd, inner margin 16–17, outer 17–17, sHTv, inner margin 15–15, outer 13–13. (n=1): SL; NT 62; minT 0.02; maxT 0.11.

Specimens examined. 1 adult male ♂ (UFES), specimen labeled as follow: \Brasil: ES, D. Martins. Zona rural. 12. XXX.1999 - manual. M.V. Amado col. [handwritten on whitish paper] \ *Listroscelis atrata* Redtenbacher, 1891 [typewritten on whitish paper]\. 1 adult male ♂ (in alcohol), specimen labeled as follow: \Alagados do Itabapoana – Mata. Presidente Kennedy – ES. Fevereiro – 2012. Furieri, K. S & Loiola, G. R [handwritten on whitish paper] \ *Listroscelis atrata* Redtenbacher, 1891 [typewritten on whitish paper]\.

***Monocerophora* Walker, 1869**

Type species: *Monocerophora minax* Walker, 1869 junior synonym of *Monocerophora longispina* (Burmeister, 1838).

Diagnosis. General coloration of the body could include the following colors and its variations of dark and light: brownish, blackish, purple and yellowish. **Head.** Fastigium laterally compressed, narrower and shorter than the first antennomere. Eyes globose, located laterally at the head, inserted at the same level of basal margin of antennal sockets. Apex of antennal sockets at the same height of dorsal margin of the eyes. Sclerites of antennal sockets not in contact at the midline. Head in frontal view apparently slender, due to the fastigium and frons are protruded upwards; vertex rarely visible at frontal; width six-tenths the length of the head. Frons long, triangular or lanceolated, projected, surpassing or not the apex of the sclerites of antennal sockets; a visible ocellus at the lower portion of the frons. Face smooth. Mandibles robust, with the tip elongated and curved inwards. Mandibles without a basal process at the cutting area. **Thorax.** Pronotum with anterior and posterior margins slightly curved inwards. Prozone with a transverse furrow extending at the lateral lobe, not reaching the lower margin. Mesozone with a transverse furrow extending at the lateral lobe reaching or not the lower margin. Metazone with a straight transversal furrow reaching the posterior margin of lateral lobes. Lateral lobes with lower margin straight; posterior margin slightly truncated. Wings fully developed; tegmina without a bright spot. Coxae could bear a spine that could be acute or rounded at ventral, on the basal and distal portion. Legs slender; hind femora have the same length of the body. Mid and fore femora at ventral with a broad longitudinal furrow at all the length. Femora of all legs with both ventral margins armed with long spines that are not interspaced by thin spines. Fore tibiae with a small hole or spur at dorsal, below and close to each opening of the tympanum; area close to the spines blackish. Hind tibiae with several spines at dorsal and ventral margin. Genicular lobes legs ending in a tip acute. Tympanum localized frontally at the tibiae; wide opening, width one-tenth the length of the opening; area around the tympanum not inflated. Each sternite with two slender spines with tip rounded. **Abdomen.** Cerci in males slightly curved downwards, sulcated at the outer portion; tubercles and bristles covering the entire surface. Supranal plate triangular. Paraprocts in males triangular, concealing the genitalia; a spine curved downwards at the lateral outer vertex of the plate. Subgenital plate of males elongated, with a short and slight emargination; styli long, almost have the same length of the plate. Subgenital plate of female wide, with a short U-shape emargination at the apical portion. **Male genitalia** with a membranous epiphallus and a sclerotized and subtriangular phallus, with rounded tip.

This genus includes the following species *M. longispina* (Burmeister, 1838) and *M. spinosa* (Karny, 1907).

Monocerophora longispina (Burmeister, 1838)

Listroscelis longispina Burmeister, 1838
Monocerophora minax Walker, 1869

Diagnosis. This species can be distinguished from *M. spinosa* by the following combination of characters: (i) fastigium projected forwards, with a tip rounded; (ii) male with frons strongly projected upwards, reaching the tip of the antennal pedicele, in female, the frons is almost one-third the length of the antennal scape; (iii) face and mandibles blackish.

Redescription. (type material ♂). (Based on images of holotype of *M. minax* ♂ and complemented by description of males and females collected). ♂: LT 33.8. General coloration consisting of dark and light brownish and blackish areas.

Head. Dorsal area blackish. Antennal scape, pedicele and antennomeres blackish. Eyes dark brownish with undefined blackish spots. Face almost smooth and blackish, central portion close to the clypeal suture wrinkled. Clypeus blackish. Labrum with basal portion yellowish, the remaining blackish. Maxillary and labial palpi yellowish. **Thorax.** Pronotum dark brownish. Fore- and midlegs with similar coloration as follow: femora dark brownish with apical portion lighter; ventral area and spines blackish. Tibiae with apical portion yellowish, central portion light brownish; apical portion blackish; spines blackish. Fore tibiae with areas close to the spines blackish. Hind legs dark brownish; femora with a blackish stripe at the ventral margin, extending from the apical portion to the middle of the length; dorsal area blackish. Tarsi dark brownish, the third one darker. Tegmen light brownish; primary and secondary veins light brownish; spaces between veins darker; stridulatory area slightly blackish. **Abdomen.** Cerci stout, the tip slightly curved inwards.

Observations on males and females collected. (Figs 13–15). **Head.** Fastigium at dorsal with lateral portions dark brownish (Fig 13B). Eyes with inner dorsal area light brownish. Mandibles stouter in male than in female (Fig 13C). Clypeus with basal suture yellowish; basal portion blackish and slightly wrinkled; central and apical portion maroonish; basal portion at the longitudinal midline whitish. **Thorax.** Pronotum with anterior, posterior and rear half of the lateral margin blackish (Fig 13D, E). Sternum almost blackish, with furrows of meso- and metasternum whitish; spines dark brownish (Fig. 13F). Tarsal claws light brownish with the tip darker. Hind wings and veins yellowish. **Abdomen.** Sternites in male, first to third light brownish, the remaining blackish; in female, sternites dark brownish. Supranal plate trapezoidal with the posterior margin rounded (Fig. 13H). Cerci sulcated at the outer portion (Fig. 13K). Subgenital plate two times longer than wider (Fig. 13I); styli slightly longer than the plate. **Male genitalia** as shown in Fig. 13L. Ovipositor almost with the same length of the body. **Alive**, coloration of individuals lighter. Eyes blackish, with inner lighter dorsal area separated by a yellowish stripe. Antennal scape with a longitudinal whitish stripe at the outer portion. Spiracle of the third thoracic segment yellowish, more noticeable than the others. Costal margin of the tegmina greenish (Fig. 15A–C).

Variation. Measurements of males (n=2, not including the holotype): TL 38–42; PL 8–11; PW 7–8; EyeW 4; TegL 45–55; SL 3.53–3.12; NT: 111–118; maxT 0.08–0.09; HF 35–37; HT 39–43; sFF, inner margin, large 4–4, outer large 3–4; sMF, inner margin, large 3, outer large 3; sHF, inner margin, large 8–11, outer large 8–9; sMTld 3; sHTd, inner margin 11–14, outer 10–12, sHTv, inner margin 16–20, outer 16–19. Measurements of females (n=3): TL 36–43; PL 9–10; PW 6–8; EyeW 3–4; TegL 46–51; HF 34–35; HT 37–40; sFF, inner margin, large 4–4, outer large 4–4; sMF, inner margin, large 3, outer large 3; sHF, inner margin, large 9–10, outer large 10–11; sMTld 3; sHTd, inner margin 11–12, outer 10–12, sHTv, inner margin 16–17, outer 17–18; OL 29–35.

The size of mandibles, size of head and length of fastigium in males are variables. Mandibles in some individuals can be stouter than in others, the head can be wider and the length of the fastigium can reach only the apex of the antennal scape.

Specimens examined. Specimens were collected in Brazil, Bahia, Jussari, Reserva Particular do Patrimônio Natural Serra do Teimoso; Camacan, Reserva Particular do Patrimônio Natural Serra Bonita; Porto Seguro, Parque Nacional do Pau Brasil, and Prado, Parque Nacional do Descobrimento. All are labeled as follow: 1 adult male ♂, 4 adult female ♀ and 1 immature female ♀: \Brasil, BA, Jussari, P.N. R.P.P.N Teimoso. 7–9, I, 2012. J. Chamorro *leg*. [handwritten on white paper] \ codes 1A, 2B, 3A, 3B and 5/Listro/Teimoso [typewritten on with paper]\. 1 adult male ♂, 3 adult female ♀, 1 immature female ♀: \Brasil, BA, Camacan, R.P.P.N Bonita. 10–12, I, 2012. J. Chamorro *leg*. [handwritten on white paper] \ codes 11, 16, 17, 18 and 25/Listro/Bonita [typewritten on with paper]\. 1 adult male ♂ and 1 adult female ♀: \Brasil, BA, Porto Seguro, P.N. Pau Brasil, J. Chamorro *leg*. [handwritten on white paper] \ 2 and 9/Listro/Pau [typewritten on with paper]\. 2 immature male ♂ and 1 immature female ♀: \Brasil, BA, Prado, P.N. Descobrimento. 13–15, I, 2012. J. Chamorro *leg*. [handwritten on white paper] \ codes 3, 8, 12/Listro/Descobrimento [typewritten on with paper]\.

***Monocerophora spinosa* (Karny, 1907)** (Figs 14–15)

Listroscelis spinosa Karny, 1907

Diagnosis. This species can be distinguished from *M. longispina* by the following combination of characters: (i) fastigium triangular with the tip rounded, surpassing the apex of the antennal sockets, being one-third the length of the scape, (ii) frons triangular, slightly lower than the sclerites of antennal sockets, (iii) face dark brownish, with sides light brownish.

Redescription. (Based on images of holotype ♂ and complemented by description of females collected). Measurements of the holotype ♂: TL 30; PL 9; TegL 50.5; FF 28; HF 32; HT 36. General coloration consisting of dark and light brownish and blackish areas (Fig. 14A). **Head.** Dorsal area with central portion blackish, sides light brownish (Fig.14B). Fastigium at dorsal with a fine stripe light brownish, extending from the tip of the fastigium to the posterior portion of the head; sides of the fastigium blackish. Eyes light brownish with undefined blackish spots; inner dorsal area yellowish. Antennal scape, pedicele and sclerites of antennal sockets blackish. Fastigium at frontal almost blackish. Frons dark brownish, almost blackish, with a defined ocellus light yellowish (Fig. 14C). Face smooth, dark brownish. Labrum with apical portion dark brownish. Mandibles blackish with basal portion dark brownish. Palpi brownish. **Thorax.** Pronotum dark brownish, with a blackish stripe at the longitudinal midline (Fig. 14D-E). Tegmina light brownish; primary and secondary veins light brownish, spaces between veins dark brownish; costal margin darker. Mid and fore femora dark brownish, apical portion lighter. Femora with spines light brownish, a whitish spot at the outer basal portion of each spine. Hind femora with a dark brownish stripe at the ventral margin. Tibiae light brownish, basal portion lighter; spines at the mid and fore tibiae with the tip light brownish. Tarsi light brownish, the third one darker. **Abdomen.** Sternites dark brownish (Fig.14F). Cerci slightly curved downwards, and slightly sulcated at the outer portion.

Observations on females collected. (Figs 14–15). **Head.** Antennomeres light brownish, posterior margin of each antennomere with a dark brownish stripe. Clypeus dark brownish with central portion whitish (Fig.14C). Maxillary palpi yellowish, labial palpi light brownish. Mouthparts in ventral view yellowish. **Thorax.** Pronotum with a blackish mark at the postero-lateral margin of the lateral lobe (Fig. 14D, E). Hind wings light brownish, veins with the same color**.** Sternum dark blackish; areas close to the furrows of the pro- and metasternum darker (Fig. 14F). Legs with coxae and trochanters dark brownish with undefined blackish spots, coxae and trochanters of forelegs darker. **Abdomen.** Sternites dark brownish, and pleurites blackish. Subgenital plate as shown in Fig. 14H. **Alive**, individuals (females) lighter than after dead. Eyes at dorsal light yellowish. Whitish area at the central portion of the clypeus is more vivid. Tibiae reddish. Abdomen with pleura light purple. Basal portion of the ovipositor pinkish (Fig 15D, E). Immature female as shown in Fig. 15F.

Variation. Measurements of females (n=3): TL 33–39; PL 9; PW 7–8; EyeW 3–4; TegL 46–49; HF 16–32; HT 18–36; sFF, inner margin, large 4–5, outer 4–5 large; sMF, inner margin, large 3–4 , outer large 3–4; sHF, inner margin, large 10–12; outer large 11–10; sMTld 3; sHTd, inner margin 11–13, outer 10–11, sHTv, inner margin 16–17, outer 17–17; OL 26–27.

Specimens examined. Specimens were collected in Brazil, Rio de janeiro, Itatiaia, Parque Nacional do Itatiaia, 7–13, XII, 2011. 5 females ♀, 3 adults, 2 immatures. All are labeled as follow: \Brasil, RJ, Itatiaia, P.N Itatiaia, 7–13, XII, 2011, J. Chamorro *leg*. [typewritten on white paper] \ *Monocerophora spinosa* (Karny, 1907) [typewritten on withe paper] \ with codes: 1,2,4, 5 and 7/Listro/Itatiaia typewritten on white paper]\.

Comments. Individuals were captured mostly in plants of the Arecaceae family.

Gen. nov.

Type species: Gen. nov. sp. 1

Description. General coloration of the body could include the following colors and its variations of dark and light: greenish, yellowish and reddish. **Head.** Fastigium dorsally flattened, triangular, smaller than the length of the first antennomere, linear sulcus at the midline. Eyes ovals and frontally prominent, inserted close to the upper edge of antennal sockets. Sclerites of antennal sockets not in contact at the midline. Head in frontal view apparently globose and broad, due to the protruding vertex; width of the head half of its length. Frons triangular, with a defined ocellus. Face, genae and clypeus smooth. Mandibles robust and elongated. Mandibles with a basal process at the cutting area. Maxillary and labial palpi lengthy, maxillary palpi reaching the third abdominal sternite; last three palpi almost with the same length. **Thorax.** Pronotum with anterior margin slightly curved inwards; posterior margin almost straight. Prozone with a transverse furrow, not extending at the lateral lobes. Mesozone without a transverse furrow. Metazone with a marked transverse furrow extending laterally at the lateral lobe, reaching the lower margin. Lateral lobes with lower margin slightly curved, posterior margin completely curved; corners rounded without sinus humeral. Wings short, not covering even the first abdominal segment. Coxae could bear a spine that could be acute or rounded at ventral, on the basal and distal portion. Legs slender and elongated; hind femora slightly longer than the length of the body. Mid and fore femora at ventral without a broad longitudinal furrow at all the length. Both ventral margins of femora armed with spines. Fore tibiae straight not curved inwards, with a spine at dorsal, on the outer side, below the tympanum. Hind tibiae with spines at dorsal and ventral margins. Genicular lobes of legs ending in an acute tip. Tympanum localized frontally at the tibiae; sclerites of the tympanum completely inflated; opening, length almost three times the width. Each sternite with two spines stout, spines at the meso- and metasternum flattened. **Abdomen**. Paraprocts, in male modified, with a lateral elongated process. Subgenital plate of male wide; apical portion emarginated. Subgenital plate of female three times longer than wide, apical portion triangular, without emargination. **Male genitalia** with a membranous epiphallus and without a sclerotized or defined phallus.

This new genus include Gen. nov. sp. 1 and a second species that is not described here because only were collected females and immature.

Gen. nov. sp. 1 (Figs 16 & 17)

Diagnosis. This species can be distinguished by the following combination of characters: (i) General color greenish; (ii) a red mark at the last two abdominal tergites; (iii) stridulatory file and apical portion of each tegmen with a red mark; (iv) tegmina short, covering only the meso- and metanotum; (v), paraprocts in male modified like an elongated structure with an acute tip.

Description (holotype ♂ & allotype ♀). Holotype: TL 18; PL 3; PW 3; EyeW 2; TegL 1.3; SL 0.83; NT ; minT 0.01; maxT 0.1; HF 18; HT 23; sFF, inner margin, large 4–4, outer large 6–6; sMF, inner margin, large 5–5, outer large 5–5; sHF, inner margin, large 11, outer large 14; sMTld 5–5; sHTd, inner margin 19, outer 21, sHTv, inner margin 11–25, outer 10–21. Allotype ♀: TL 17; PL 4; PW 3; EyeW 3; TegL 1.6; HF 20; HT 24; sFF, inner margin, large 4, outer large 7; sMF, inner margin, large 6, outer large 7; sHF, inner margin, large 14, outer large 15; sMTld 5; sHTd, inner margin 20–19, outer 22–20, sHTv, inner margin 12, outer 12; OL 12. General coloration yellowish (after death, individual was stored in alcohol and lost the natural color) (Fig.16A). **Head.** Fastigium triangular and with a longitudinal furrow at the midline; not visible from the dorsal view (Fig.16B,C). Antennal scape two times longer than the pedicele. Head in frontal view apparently enlarged due to the mandibles slender, and the highest vertex (Fig.16A). Antennas seem to be inserted at the middle of the face. Frons subtriangular and slightly inflated. Clypeus short, almost one-quarter the length of the labrum. Mandible with cutting edge blackish. **Abdomen**. Legs with femora at dorsal with bristles thicker over the entire surface; at ventral, with spines decreasing from the base to the apex. Fore tibiae with nine spines in male, and five in female. Tarsi with the fourth tarsomere longer, the same length as the first three together. Tegmina as shown in Fig.16E. **Abdomen.** Cerci in male reddish, cylindrical, the width decreases from after the middle to the apex; apical portion shorter than the basal, the former with tubercles and with more bristles than the later; tip rounded. Styli short, almost one-quarter the length of subgenital plate. (Fig.16I). **Male genitalia** as shown in Fig.16L. Ovipositor slightly shorter than the abdomen, with a blackish stripe at the outer surface.

Type series. *Holotype male* labeled \Brasil, BA, Porto Seguro, P.N Pau Brail. 4–6, II, 2012. J. Chamorro *leg*. [handwritten on with paper] \ 4/Listro/Pau [typewritten on white paper] \ Gen. nov. sp. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on red paper]\. *Allotype female* labeled \Brasil, BA, Porto Seguro, P.N Pau Brail. 4–6, II, 2012. J. Chamorro *leg*. [handwritten on with paper] \ 1/Desc/Pau [typewritten on white paper] \ Gen. nov. sp. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on blue paper]\. *Paratypes*, 3 Adult males ♂ labeled as follow \ Brasil, BA, Prado, P.N. Descobrimento. 13–15, I, 2012. J. Chamorro *leg*. [handwritten on with paper] \ codes: 14, 22, and 16/listro/Desc [handwritten on with paper] \ Gen. nov. sp. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on blue paper]\. 5 adult female labeled as follow 1 ♀ \Brasil, BA, Porto Seguro, P.N Pau Brail. 4–6, I, 2012. J. Chamorro *leg*. [handwritten on with paper] \ 8/listro/Pau [typewritten on white paper]\. 1 ♀ immature code 6/listro/Pau. 4 ♀ \Brasil, BA, Prado, P.N. Descobrimento. 13–15, I, 2012. J. Chamorro *leg*. [handwritten on with paper] \ codes: 5, 15, 17 and 18/listro/Des [typewritten on white paper] \, and additionally labeled Gen. nov. sp. 1 Fialho, Chamorro-Rengifo & Lopes-Andrade [handwritten on blue paper]\.

Variation. Measurements of males (n=4, including the holotype): TL 15–19; PL 3; PW 2–3; EyeW 2; TegL 1.3–2; HF 15–20; HT 20–24; sFF, inner margin, large 4–4, outer large 6–6; sMF, inner margin, large 5–7, outer large 5–6; sHF, inner margin, large 11–12, outer large 14; sMTld 5–5; sHTd, inner margin 19–23, outer 21–22, sHTv, inner margin 11–25, outer 10–21. Measurements of females (n=6, including the allotype): TL 16–21; PL 2.5–4; PW 2.5–3; EyeW 2–3; TegL 1–1.6; HF 18–20; HT 22–24; sFF, inner margin, large 4–5, outer large 5–7; sMF, inner margin, large 6, outer large 7; sHF, inner margin, large 11–14, outer large 13–15; sMTld 5; sHTd, inner margin 19–21, outer 18–22, sHTv, inner margin 12–15, outer 12–17; OL 12–15.

Comments. Alive, male light greenish. Eyes, abdominal sternites and lateral portion of last two abdominal tergites yellowish. Sclerites of the tympanum, tegmina, cerci, and spot at the last two abdominal segments reddish. Female without the reddish spot at the apex of the abdomen (Fig. 17).

Terpandrini Gorochov, 1990

Megatympanon Piza, 1958

Megatympanophon Rentz, 1979 **syn.**

Type species: *Megatympanon speculatum* Piza, 1958 (Fig.18)

This genus includes only *Megatympanon speculatum* Piza, 1958. As we consider the description of this species is complete, we do not create a new one or a redescription. We provide new records of distribution.

Redescription. General coloration of the body could include the following colors: yellowish, brownish and greenish. **Head**. Fastigium laterally compressed, narrower and shorter than the first antennomere. Eyes globose and laterally prominent, inserted close to the outer edge of the antennal sockets. Apex of antennal sockets at the same height of upper edge of the eyes. Sclerites of antennal sockets in contact at the midline, concealing the tip of the fastigium. Frons triangular; ocellus not visible. Face, genae and clypeus smooth. Mandibles not robust and without modifications. Maxillary and labial palpi yellowish. **Thorax.** Pronotum with anterior margin straight, not curved inwards, posterior margin strongly curved outwards, Mesozone covering basal and dorsal portion of the tegmina. Prozone with a transverse curved furrow, slightly extending at the lateral lobe but not reaching the lower or anterior margin. Mesozone with a V-shape transverse sulcus at the midline, and extending at the lateral lobe, not reaching the lower margin. Metazone with a transverse furrow slightly extending laterally, not reaching the lower margin. Lateral lobes with lower margin curved, posterior margin oblique; corners rounded with a sinus humeral due to the pronounced metazone. Wings fully developed; tegmina with no bright spot. Legs slender, femora almost the same length of the body. Mid and fore femora at ventral with a broad longitudinal furrow at all the length. Both ventral margins of femora armed with long spines interspersed with thin spines. Fore tibiae not slightly curved inwards; without a small hole or spur at dorsal, below and close to each opening of the tympanum. Mid tibiae with seven to nine spines. Hind tibiae with spines at dorsal and ventral margins. Genicular lobes of all legs with two acute spines, one at the middle of the outer margin and the other at the tip.

Tympanum localized frontally at the tibiae; area around the tympanum not inflated; opening narrowed, the lateral sclerites of the tympanum concealing the opening. Each sternite with two long and acute spines. **Abdomen.** Cerci, in male, cylindrical and curved inwards, with an acute tip. Supranal plate (epiprocte) short and subtriangular. Paraprocts, in male, not developed. Subgenital plate of male wide; apical portion with a V-shape emargination. Styli short, almost one-quarter the length of the plate. Subgenital plate of female with an emargination leading a U-shape cut almost one-third the length of plate at the longitudinal midline. **Male genitalia** with a membranous epiphallus and without a sclerotized phallus.

Specimens examined. 2 males and 1 female (MNRJ). Male 1 \ Petropolis E. Rio. Fev.- Março 1958. D'Albuquerque [handwritten on yellowish paper] \ *Megatympanon speculatum* [handwritten on whitish paper] \ male 2 \ Le Vallon Alto Mosela. Petrópolis – II a III. 958. Dalcy. col. [handwritten on yellowish paper] \ *Megatympanon speculatum* [handwritten on whitish paper] \ female 1 \ Serra da Caveira, 600 m. M. Itaguay. Est. do Rio. 25-2-1948. W. Zikán, col. [typewritten on yellowish paper] \. 1 male \ Brasil, SP, Salesópolis, Est. Biol. De Boracéia 20-27. iv. 2011. F. A. G de Mello, col. CNPq-SISBIOTA [typewritten on whitish paper] \ *Megatympanon speculatum* [handwritten on whitish paper]\.

Variation. Measurements of male (n=2): TL 35–38; PL 12; PW 6; EyeW 4; TegL 67–69; HF 33–34; HT 34–36; sFF, inner margin, large 6–7 small 0-3, outer large 7-7, small 3-6; sMF, inner margin, large 7–8, small 0-5, outer large 7–9, small 0–3; sHF, inner margin, large 12–14, small 0, outer large 12–14, small 0; sMTld 3–4; sHTd, inner margin 26–31, outer 23–30, sHTv, inner margin 22–24, outer 20–24. Stridulatory file (n=1) SL: 5.56; NT: 64; min 0.05; maxT 0.45. Measurements of female (n=1): TL 42; PL 12; PW 7; EyeW 5; TegL 39; HF 34; HT 36; sFF, inner margin, large 6–6, small 3–5, outer large 9–8, small 7–5; sMF, inner margin, large 7–7, small 3–8, outer large 6–7, small 6–9; sHF, inner margin, large 14, small 7, outer large 12, small4 ; sMTld 2; sHTd, inner margin 28, outer 29, sHTv, inner margin 17, outer 16; OL 26.

Nomenclatural changes

Carliella Karny, 1911

Carliella mandibularis Karny, 1911

Cerberodon cuiabensis Piza, 1982 **syn. nov.**

Type material of *C. cuiabensis* Piza, 1982, male and female were collected from Cuiabá, Mato Grosso, Brazil, the same locality type where was collected the holotype of *Carliella mandibularis* Karny, 1911. Instead *C. cuiabensis* was described as *Cerberodon*, the taxonomic characters are very different from those of this genus, and the descriptions are more agreed with the description of *Carliella* (Piza 1982). Type material of *C. cuiabensis* was examined by JCR in 2008 at the Esalq Museum, and the images are available at OSF.

Carliella differs from *Cerberodon* and the others genera by the vertex of the head not protruding, face slightly wrinkled, pronotum without a deep furrow at the metazone, abdomen with sternites blackish. Cerci of male stout, with apical portion abruptly curved inwards. Subgenital plate of male as wide as long, with a short U-shape emargination, subgenital plate of female short and triangular, without emargination.

Identification key of Listroscelidinae species of the Brazilian Atlantic Forest

1. Sclerites of the antennal sockets in contact at the midline. Opening of the tympanum narrow and concealing by the lateral sclerites. Fore tibiae without a small hole or spur dorsally, below and close to each opening of the tympanum. Spines at each sternum ending in an acute tip. Paraprocts not developed or modified...***Megatympanon speculatum*** (Fig. 18)

1' Sclerites of the antennal sockets not in contact at the midline. Opening of the tympanum wide, not concealing by lateral sclerites. Fore tibiae with a small hole or spur at dorsal, below and close to the opening of the tympanum, could be two or only one at the outer side. Spines at each sternum ending in a rounded tip. Paraprocts developed and could be spine-like modified…………………………..**2**

2. Paraprocts developed and modified, all the outer portion spine-like, or like a long process. Fore tibiae with a small hole or spur at dorsal, below and close to the outer opening of the tympanum……………..…....**Gen. nov. sp. 1** (Fig. 16-17)

2'. Paraprocts developed but only with a little spine at the vertex of the outer portion. Fore tibiae with a small hole or spur at dorsal, below and close to each opening of the tympanum……….…………………………………………………**3**

3. Face strongly wrinkled, brain-like. Males with apical portion of the left mandible strongly elongated and curved upwards. Females with subgenital plate elongated, with a deep V-shape emargination…………..........***Cerberodon***......**5**

3'. Face smooth or slightly wrinkled; the wrinkles are linear, never brain-like. Apical or lateral portion of the left mandible in male could be or not elongated. Females with subgenital plate without a deep V-shape emargination.……………**4**

4. Basal portion of the tegmina with a bright yellowish spot. Mandibles symmetric or not, the apical portion of the left one could be different than the right one…………………………………………………………..***Listroscelis***…...**6**

4'. Basal portion of the tegmina without a bright yellowish spot. Mandibles symmetric, the apical portion of the left is similar than the right one………………………………………..…….…….***Monocerophora***......**12**

5. Fore tibiae with ventral area blackish; tegmina, in both sexes, surpassing the length of the abdomen; supranal plate rounded………………..***C. viridis*** (Fig. 2-3)

5'. Fore tibiae with ventral area dark brownish at dead, or dark orange at live; tegmina shorter than the abdomen, not covering the two last abdominal tergites in male, and the five last abdominal tergites in female; supranal plate semitriangular…………………………………..…***Cerberodon* sp. nov. 1** (Fig. 1, 3)

6. Face darker than the lateral portion of the head, scape and pedicele never blackish…….…………………………………..……………..…..***L. ferruginea***

6'. Face not darker than the lateral portion of the head, but if so, scape and pedicele blackish...**7**

7. Fore femora with a blackish stripe at the ventral margin………………………**8**

7'. Fore femora without a blackish stripe at the ventral margin …...……………..**9**

8. Pronotum with a blackish stripe at the postero-lateral margin.………………………………………....***Listroscelis* sp. nov. 6** (Fig. 9, 12)

8'. Pronotum without a blackish stripe at the postero-lateral margin…………………………………………………***L. carinata*** (Fig. 10, 12)

9. Mandibles as symmetric, left mandible with pre-apical lateral portion elongated and curved, apical portion projected upwards…...……………….………………**10**

9'. Mandibles symmetric….…………………………………….…….……………**12**

10. General color brownish; ventral surface of femora not blackish…..…………………………….…………***Listroscelis* sp. nov. 4** (Fig. 7, 12)

10'. General color maroonish; ventral surface of femora blackish…….…………………………………………………....………………**11**

11. Yellowish spot at the tegmina being almost one-quarter the length of the tegmen. Tegmen width decreases markedly from almost half of its length to the apex……….………………………………...***Listroscelis* sp. nov. 1** (Fig. 4, 12)

11'. Yellowish spot at the tegmina being shorter than one-quarter the length of the tegmen. Tegmen width not decreases markedly from almost half of its length to the apex……………………………………………….…***L. atrata*** (Fig. 11)

12. Supranal plate of male triangular……….........***Listroscelis* sp. nov. 3** (Fig. 6)

12'. Supranal plate in male not triangular………………..……………………...**13**

13. Supranal plate of male decrease markedly from the middle of its length to the apex; with a linear-shape emargination………..***Listroscelis* sp. nov. 2** (Fig. 5, 12)

13’. Supranal plate of male not decrease markedly from the middle of its length to the apex; with an oval-shape emargination……………………..……..……***Listroscelis* sp. nov. 5** (Fig. 8, 12)

14. Frons projected like a spine, surpassing the length of antennal scape or only reaching its tip; face blackish…………..***Monocerophora longispina*** (Fig. 13, 15)

14’. Frons triangular, acuminated, the tip only reaching the apex of sclerites of antennal sockets. Face brownish……..……..***Monocerophora spinosa*** (Fig. 14-15)

Discussion

Taxonomic considerations

We defined that *Carliella*, *Cerberodon*, *Isocarliella*, *Macrometopon*, and *Monocerophora*, are grouped inside Listroscelidini. Gen. nov. is a genus most different and modified. The only and current member is *Listroscelis*. From now on, we are going to consider all the before mentioned genera as Listroscelidini. The tribe is equivalent to Listroscelidinae *sensu* Gorochov (1995), which includes *Carliella*, *Cerberodon*, *Listroscelis* and *Monocerophora*.

Species of this tribe bear a compressed and narrowed fastigium, similar to that of the unique North American Listroscelidinae *Neobarrettia*; in *Megatympanon* the fastigium is a little bit different. At the same than in *Neobarretia*, this structure grows at the anterior portion of the occiput, between the antennas (view from the dorsal). In *Monocerophora longispina* it is slightly upwardly directed, but it must be the result of the overdevelop growth of the frons. The form of the frons is different than in other groups of Tettigoniidae, it is a triangle or semitriangle. In cases *Cerberodon*, *Listroscelis* and *Isocarliella*, it seems to be a sulcated triangle, with the central portion protruding. As mentioned above, species of *Monocerophora* bear an overdeveloped frons. The frons differs from species of Terpandrini (*Neobarretia* and *Megatympanon*) and *Arachnoscelis*, in which this structure is not markedly differentiated. In Gen. nov. the fastigium is sulcated.

The growth of the vertex is remarkable; the most exaggerated case occurs in *Cerberodon*. This characteristic is observed also in *Neobarretia* and Gen. nov., and must be directly related with the frontal or fronto-lateral position of the eyes, and also with the predatory habit. The position of the eyes is similar than other predatory groups as Meconematinae, Saginae and Hexacentrinae.

The head is elongated, whit is more evident when seen at frontal; the labrum and mandibles are long, slightly similar to those of *Neobarrettia*. In some species, the development of the mandibles is outstanding. The form and development are related with sexual dimorphism, so it is an apomorfic character, and in this case, at the level of tribe, it can not be used as part of the definition of the group. The same occurs in *Arachnoscelis*, which includes species with different levels of development of mandibles. However, the way of development, as was described, is different between *Cerberodon* and *Listroscelis*. The mandible in *Carliella*, *Cerberodon* and *Listroscelis* bear a large, basal, ventral process, a state unique among Tettigoniidae (Naskrecki 2000). This process was also observed in the new genus, but not in *Monocerophora* species.

The fore tibiae are notably curved. The long movable spines at the fore tibiae are also typical, they are longer than in Terpandrini. There are two small dorsal spur below the tympanum, at the inner and outer sides. The spur was observed only in the unique individual of *Listroscelis* sp. nov. 3. Unfortunately, it seems that the spur can be easily lost. None of the other collected individuals conserved this structure, but it was possible to confirm the previous existence of the spur due to the remaining of its small cavity. Conh (1957) registered that none of the Listroscelidinae examined (including *Cerberodon*, *Listroscelis* and *Macrometopon*) bear the dorsal spur, but now we determine that the spur is an important character of the tribe. In *Gen. nov.*, similar to Decticini and *Neobarrettia*, there is only one spur at the outer side. It could be means that in Gen. nov. the spur of the inner portion was lost. By the contrary, in

Megatympanon, there is neither a spur nor a cavity. Conh (1957) explained that is was not clear if the spur is a derived and convergent or if it is an ancestral feature lost independently in the groups. At least for Listroscelidini + Gen. nov., the two spurs are an ancestral condition and the loss are independent event. The no identified species related with Gen. nov. sp. 1, also bears only one outer spur. The ventral spines at all femora interspaced with minute spines is also characteristic, but it is different in *Monocerophora*.

For most, the opening of the tympanum is elongated and wide, usually frontally localized, different from Terpandrini, where the openings are long but narrow, and almost concealed. The tympanum in Gen. nov. is most similar to *Arachnoscelis*.

The spines at the sternum are typically a characteristic of the subfamily, and in Listroscelidini with the exception of *Monocerophora*, the spines at the pro- and meso- sternum are slender, and the ones at the metasternum are usually slightly flattened, it is similar to *Neobarrettia*. In *Monocerophora* all the spines are slender, and in *Megatympanon* they are long and acute. Other type of armature, with different levels of development, is present at the ventral coxal, a spine at the distal border of the ventral side of the coxae, and other, on the proximal border. It was suggested that the sternal and coxal spines can be useful for catching the prey (Cohn 1957, Rentz 1995).

The metazone is typically elevated from the sulcus of the metazone. In *Monocerophora* this elevation is more conspicuous. *Neobarrettia* also bears this characteristic. On the contrary, in *Megatympanon* the metazone is so elongated that covers the basal dorsal portion of the wings, similar to some Terpandrini. The auditory spiracle is of a typical Conocephaloid (Zeuner, 1936a), elongated and wide openned.

Cerci, supranal plate and subgenital plate are variable throughout the tribe. Patterns are found at the genus level, except in *Listroscelis*. The most remarkable and characteristic modification shared is in the paraprocts and genitalia. The paraprocts are wide, concealing the internal genitalia, and bear a little spine at the outer vertex. The genitalia consists of a membranous epiphallus, and titillatores developed forming a projected and erected phallus. However Conh (1957) dissected a genitalia of *Macrometopon* and the phallus was not observed. These tow characteristics are different to Terpandrini, in which the paraprocts are not modified as in Listroscelidini.

A dark line at the longitudinal midline at the pronotum was observed in immature individuals of *Monocerophora* and *Listroscelis*, the difference is that in adult *Listroscelis* the line disappears. By the other hand, the lengthened mandibles, in the case of species of *Listroscelis* with overdeveloped growth, is only observed in the adult stage, so, it is difficult to differ specimens of those two genera. Those characteristics suggest a common origin but different pattern of development during the growing of the individuals. The mandibles in *Cerberodon* are also developed only in the adults (Fig X). Zeuner (1936a) suggested that the differences among *Meconema* Serville, 1831 and *Listroscelis* are superficial and it is the result of the carnivorous and herbivorous habitus. We only want to point out that *Meconema* (Meconematinae) also conserve the line at the pronotum, as the same as some species of Conocephalinae, and Pseudophyllinae.

The position of *Neobarrettia* inside Terpandrini should be revalued. First of all, it is the only genus of North America inside the tribe, the others, excepting *Megatympanon*, are in the Australian region. Second, significant features were already exposed suggesting that *Neobarrettia* is more similar to Listroscelidini, but also it must not be classified inside the tribe. The similarities among *Megatympanon* and *Terpandrus* Stål, 1874 were exposed by Rentz (2001).

Natural history and geographic considerations

Individuals of spiny predatory katydids are rarely seen in field. Until now, a maximum of two species for genus were collected in each locality. They are rare compared with subfamilies as Phaneropterinae, Pseudophyllinae and Conocephalinae. Added to this, the proportion of females and males captured is almost at least 5:1, complicating the identification of the species. In cases such as *Listroscelis* sp. nov. 6, *M. spinosa* (PN Itatiaia) and *L. carinata* (PN Rio Doce) no male was collected. They are aggressive, especially *Monocerophora* and

Cerberodon. By the other hand, individuals of Gen. nov. are very quickly, witch difficults any attempt to observe or collect them in field.

All specimens were collected inside well preserved forest, and particularly close to water bodies or wetter regions inside the forests. *Cerberodon* additionally have been collected close to water, in ravines. Generally Listroscelidinae were always located from 50 meters of height, until almost two meters, and as it was anteriorly mentioned. *M. spinosa* (PN Itatiaia) was collected almost always in Arecaceae, indicating some kind of specialization for certain characteristics of the forest. Morphological characteristics that also may hinder the distribution of these katydids is the ability to fly. *Cerberodon* sp. nov. 1 and species of Gen. nov. are weak fliers, and if the speciation of these species of Listroscelidinae were recent and with especial ecological requirements, they could have a restrict distribution. The situation is more critical given the fact that the Atlantic Forest is almost completely deforested. Despite specific research on biological and ecological factors have not been made, we propose that this group could be used as an indicator of ecosystem quality.

Despite of the under-representation of specimens collected by locality on the Atlantic Forest, some peculiarities of the distribution of Listroscelidinae would be identified. Species have not a wide distribution. Based on the data already collected and observations in field, the fact that *Listroscelis armata* Serville, 1831, was described from French Guiana, and later on it was also reported from BA (Burmeister, H. 1838, Serville 1839, Saussure. 1859, Carl. 1908), it is a doubtful situation. Individuals BA should be revised again.

Acknowledgements

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Captions

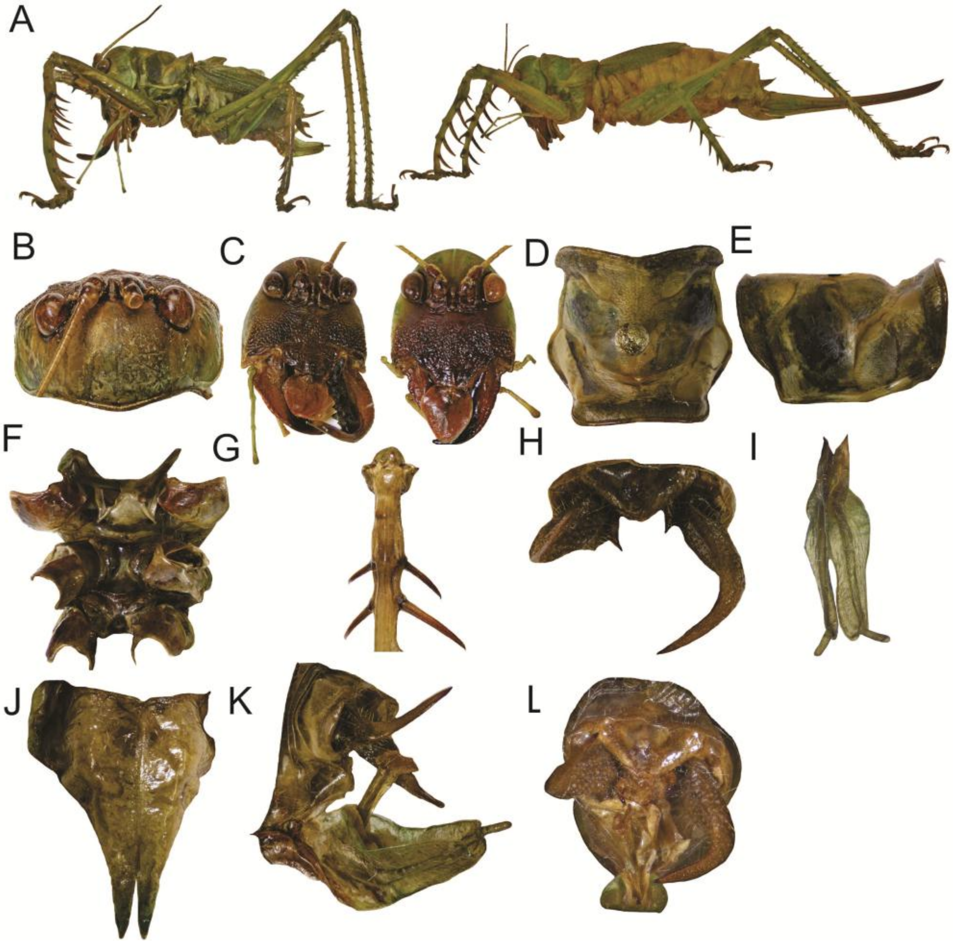

FIGURE 1. *Cerberodon* **sp. nov. 1**, male holotype (**A–I, K, L**) and female allotype (**A, C, J**). **A.** Lateral view of the body, male on the left and female on the right. **B.** Dorsal view of the head. **C.** Frontal view of the face, male on the left and female on the right. **D.** Dorsal view of the pronotum. **E.** Lateral view of the pronotum. **F.** Ventral view of the thorax. **G.** Frontal view of the tympanum. **H.** Dorsal view of the supranal plate. **I.** Ventral view of the subgenital plate. **J.** Ventral view of the subgenital plate. **K.** Lateral view of the abdomen apex. **L.** Posterior view of the abdominal apex.

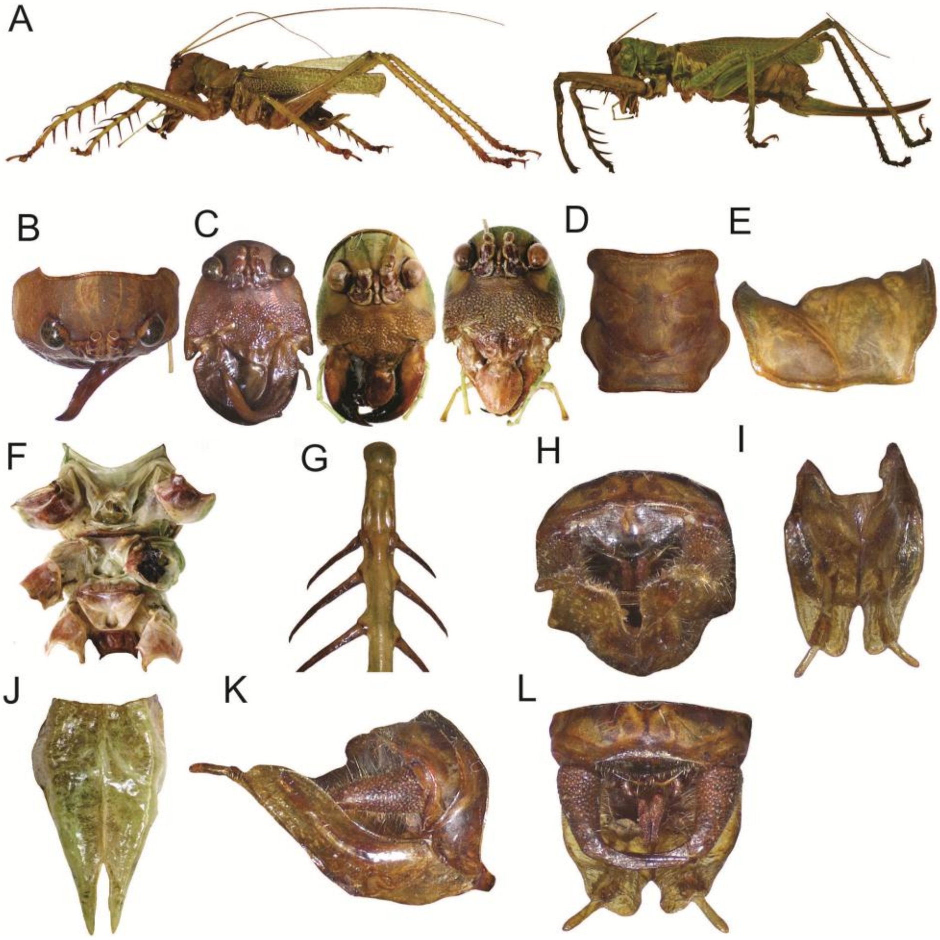

FIGURE 2. *Cerberodon viridis*, immature male (**A–I, K, L**) and female (**A, C, J**). **A.** Lateral view of the body, male on the left and female on the right. **B.** Dorsal view of the head. **C.** Frontal view of the face, male on the left, female on the right. **D.** Dorsal view of the pronotum. **E.** Lateral view of the pronotum. **F.** Ventral view of the thorax. **G.** Frontal view of the tympanum. **H.** Dorsal view of the supranal plate. **I.** Ventral view of the subgenital plate. **J.** Ventral view of the subgenital plate. **K.** Lateral view of the abdominal apex. **L.** Posterior view of the abdominal apex.

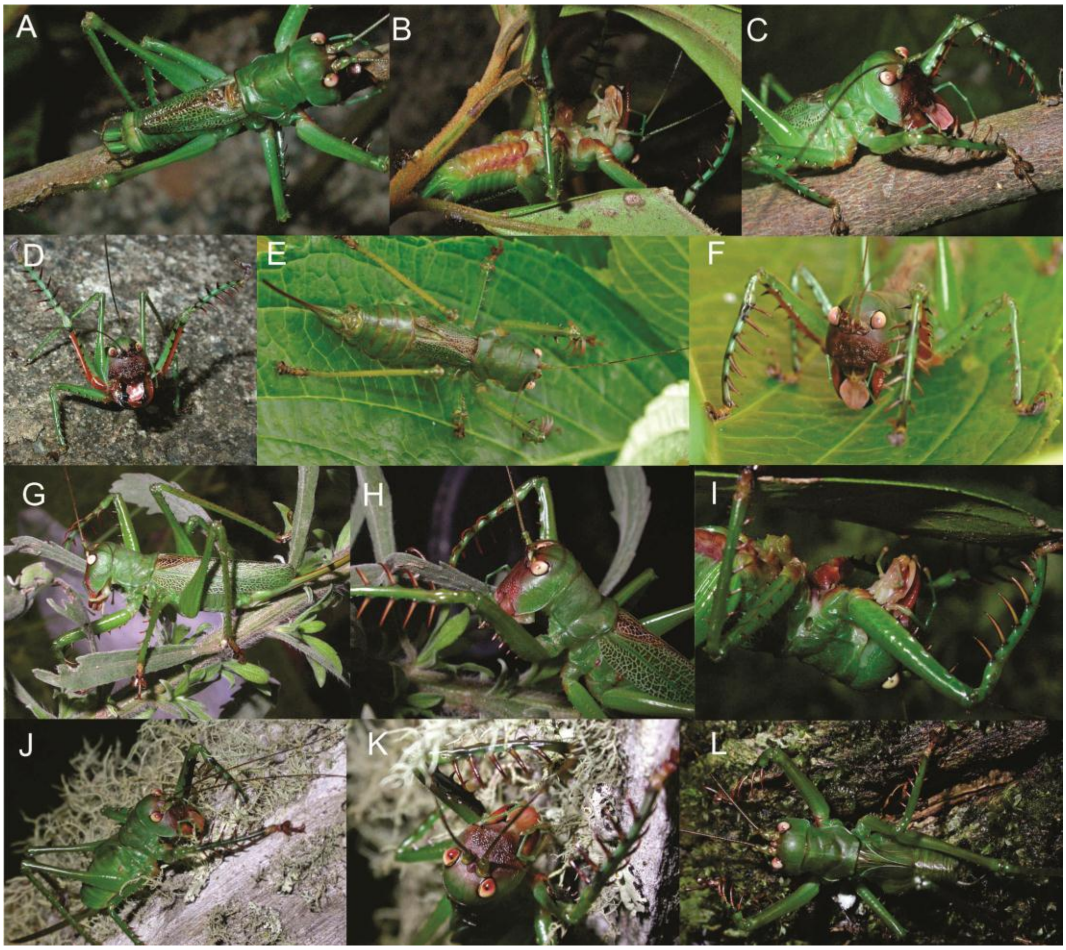

FIGURE 3. *Cerberodon* **sp. nov. 1**, live, male holotype (**A–D**), female allotype (**E, F**). *Cerberodon viridis*, live, female adult (**G–I**), female immature (**J, K**), male inmature (**L**).

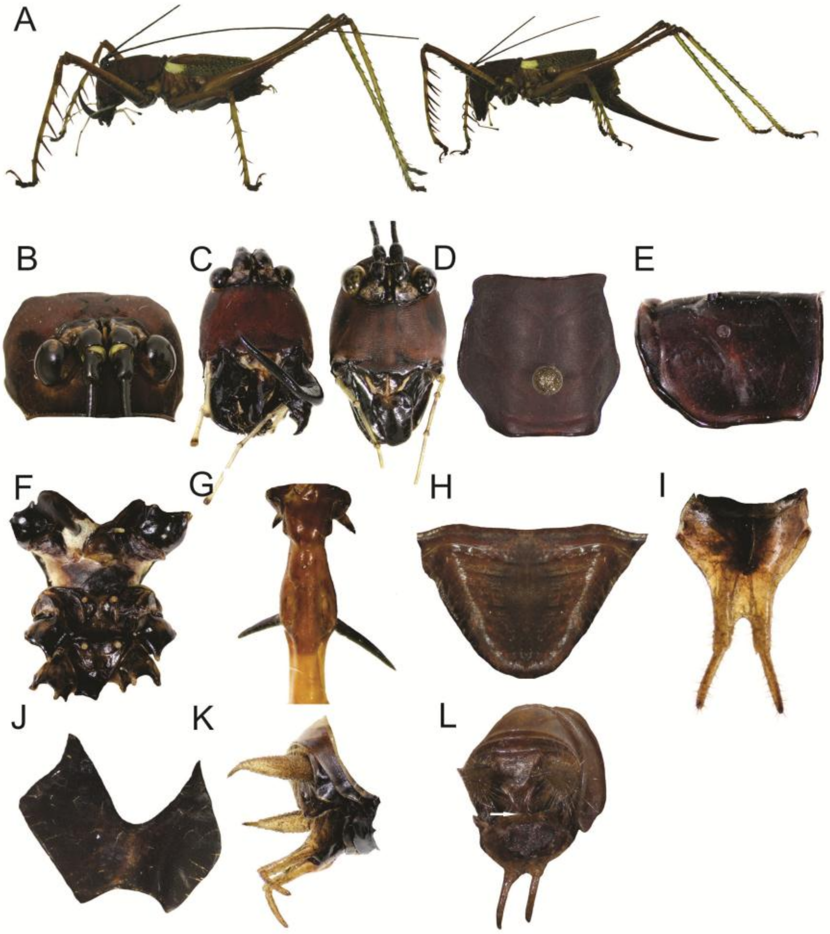

FIGURE 4. *Listroscelis* **nov. sp. 1**, male holotype (**A–I, K,L**) and female allotype (**B,C,J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

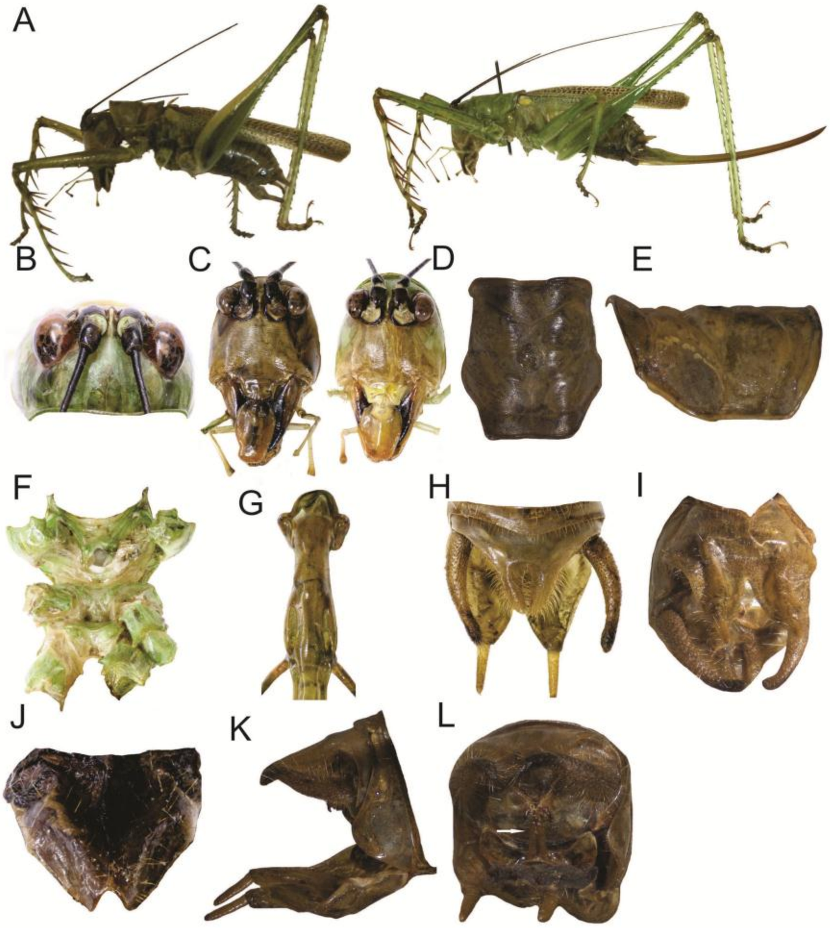

FIGURE 5. *Listroscelis* **sp. nov. 2**, male (**A–I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

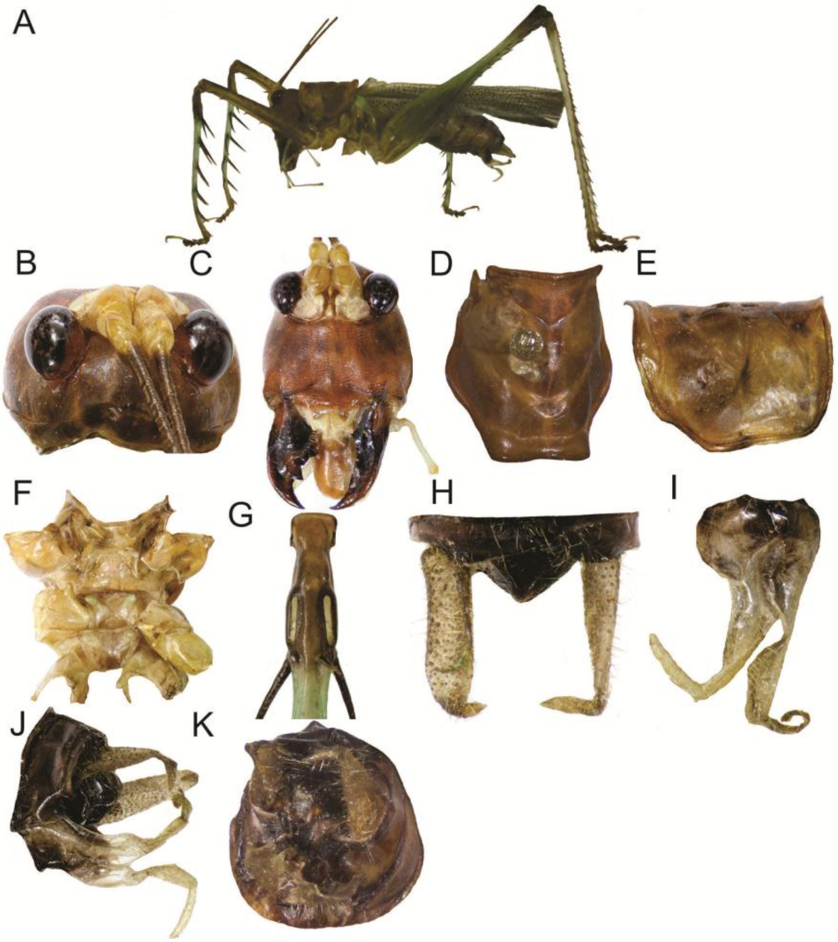

FIGURE 6. *Listroscelis* **sp. nov. 3**, male holotype (**A-K**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

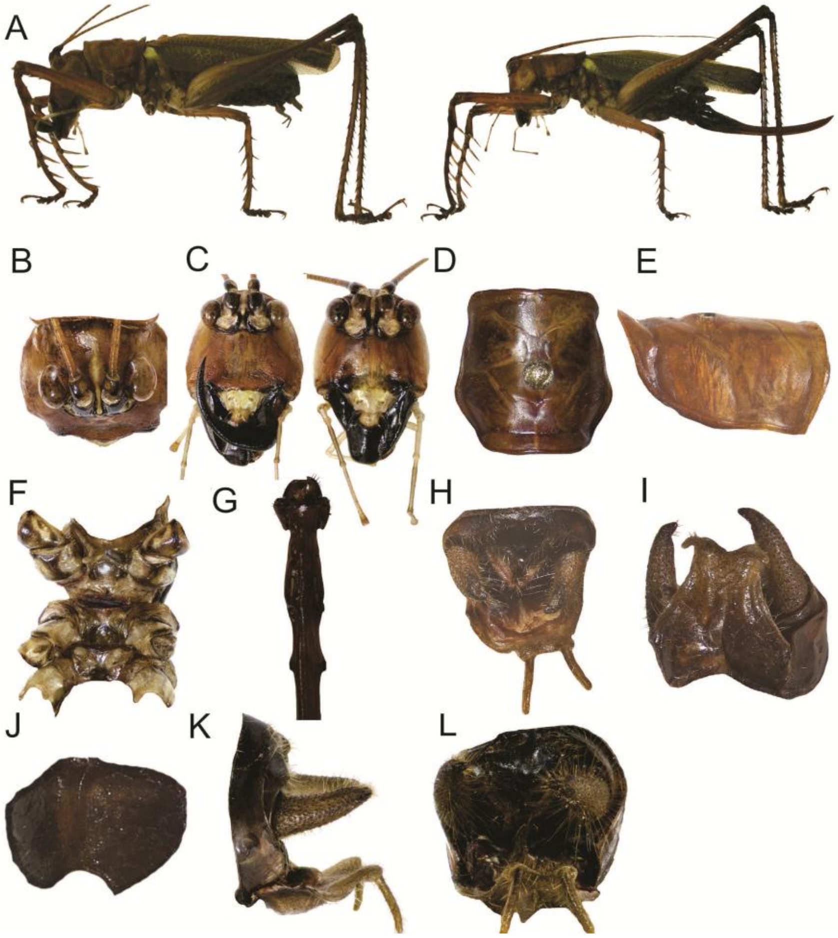

FIGURE 7. *Listroscelis* **sp. nov. 4**, male (**A–I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

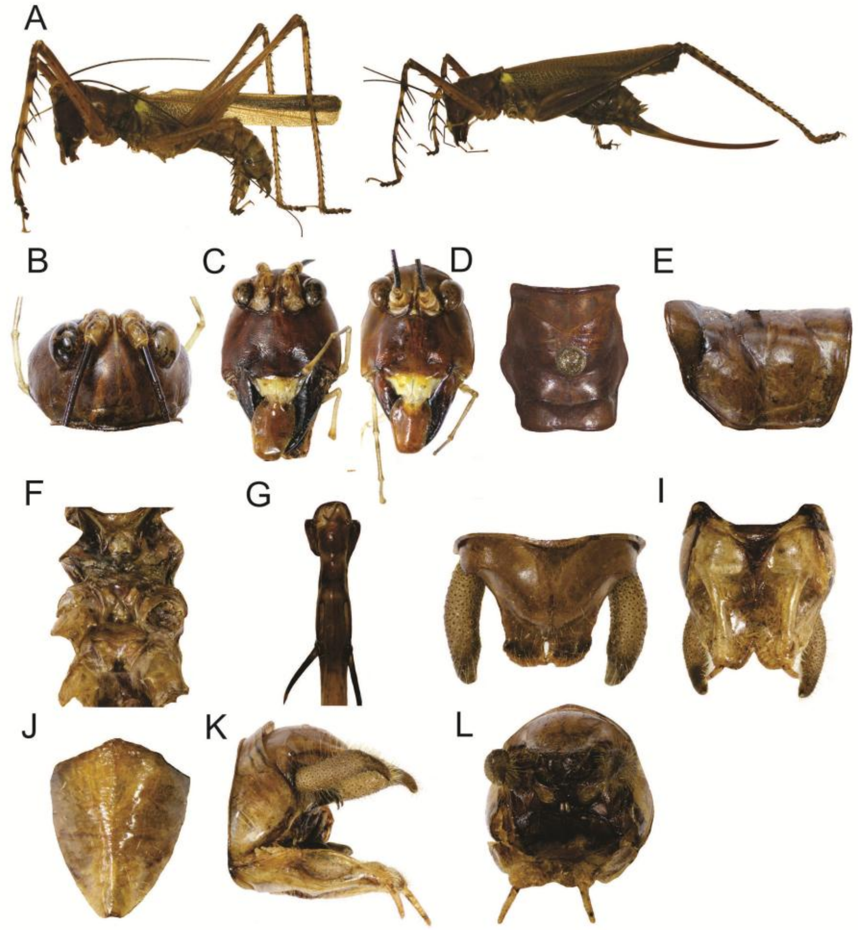

FIGURE 8. *Listroselis* **sp. nov. 5**, male (**A, B–I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the subgenital plate. **J**. Ventral view of the Subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

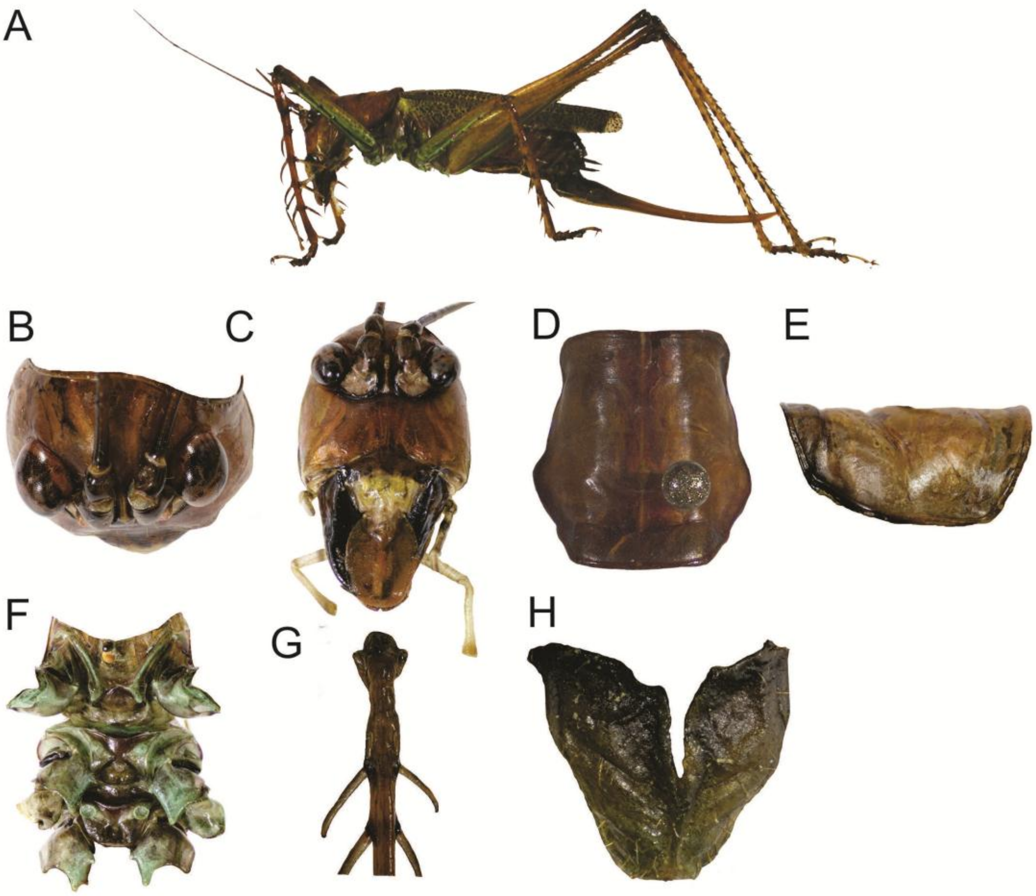

FIGURE 9. *Listroscelis* **sp. nov. 6**, female (**A-H**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Ventral view of the subgenital plate.

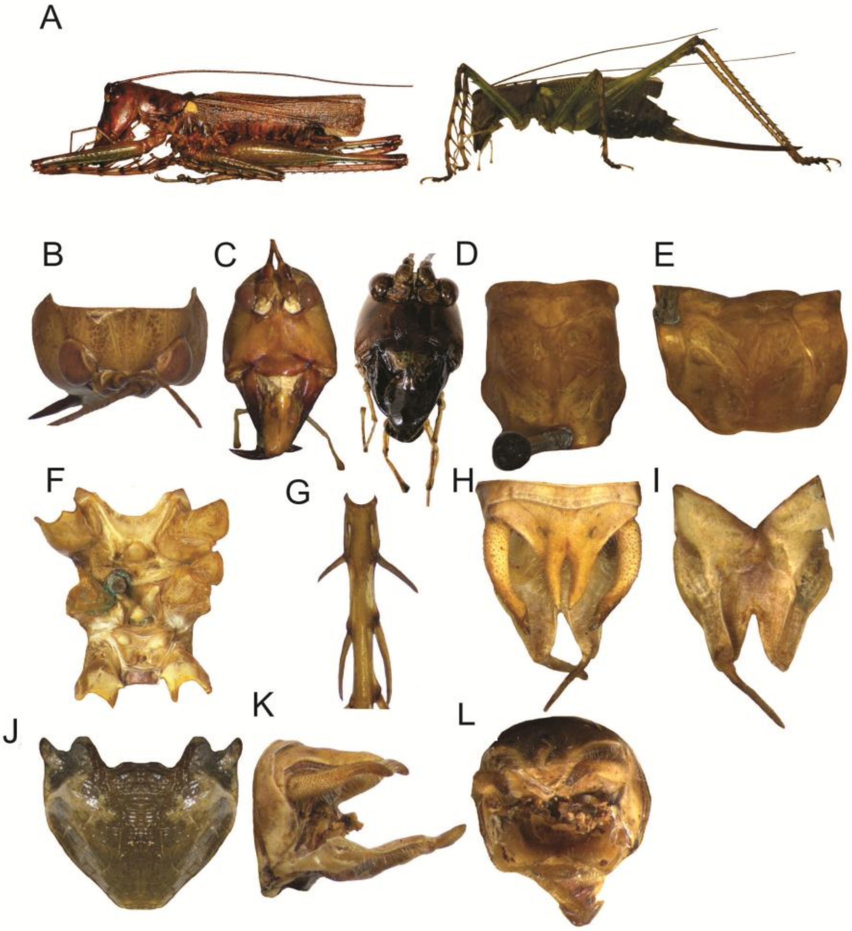

FIGURE 10. *Listroscelis carinata*, male holotype (**A),** male not holotype **(B-I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

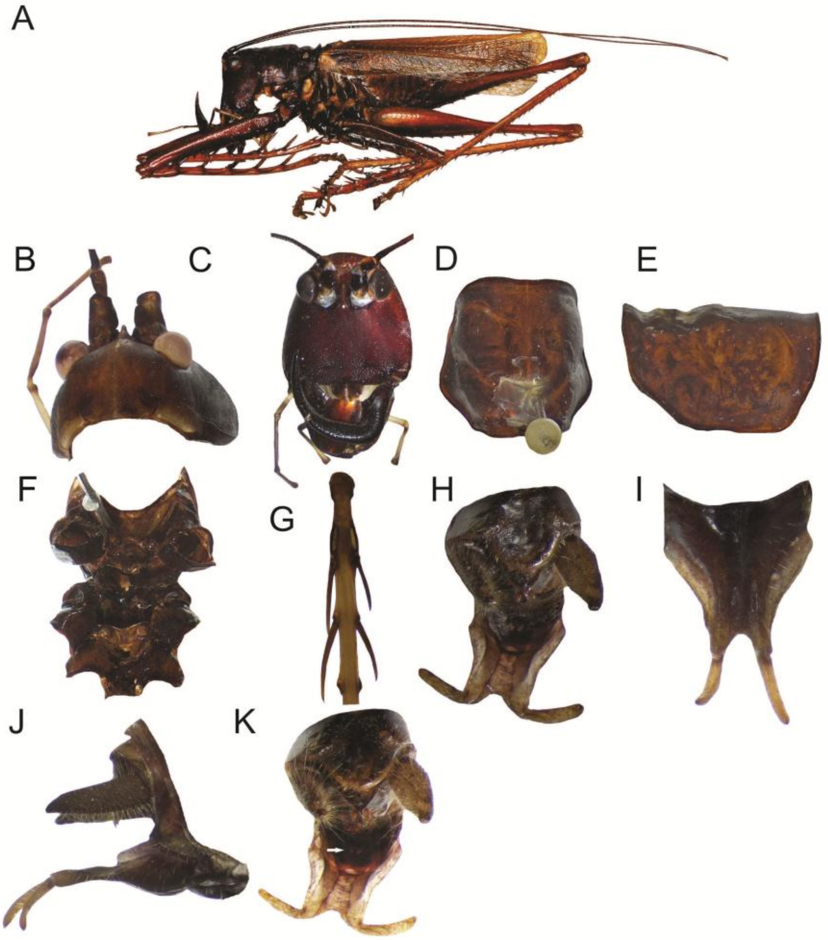

FIGURE 11. *Listroscelis atrata* **sp. nov.**, male holotype (**A),** male not holotype **(B-K**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

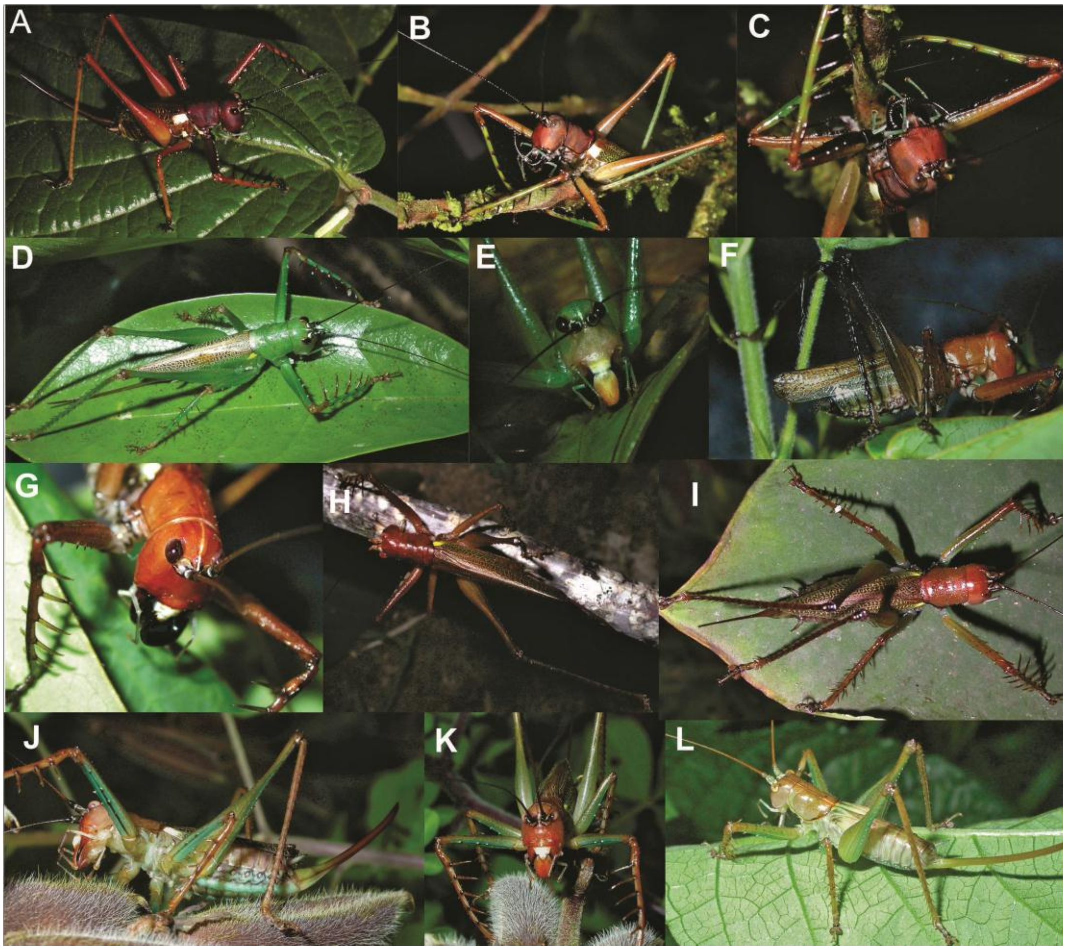

FIGURE 12. Individuals of *Listroscelis* live. *Listroscelis* **nov. sp. 1** female (A); male (B, C). *Listroscelis* **sp. nov. 2**, female (D–E). *Listroscelis* **sp. nov. 4**, male (F, G). *Listroscelis* **sp. nov. 5**, female (H). *Listroscelis* **sp. nov. 6**, female (I). *L. carinata*, female (J–L).

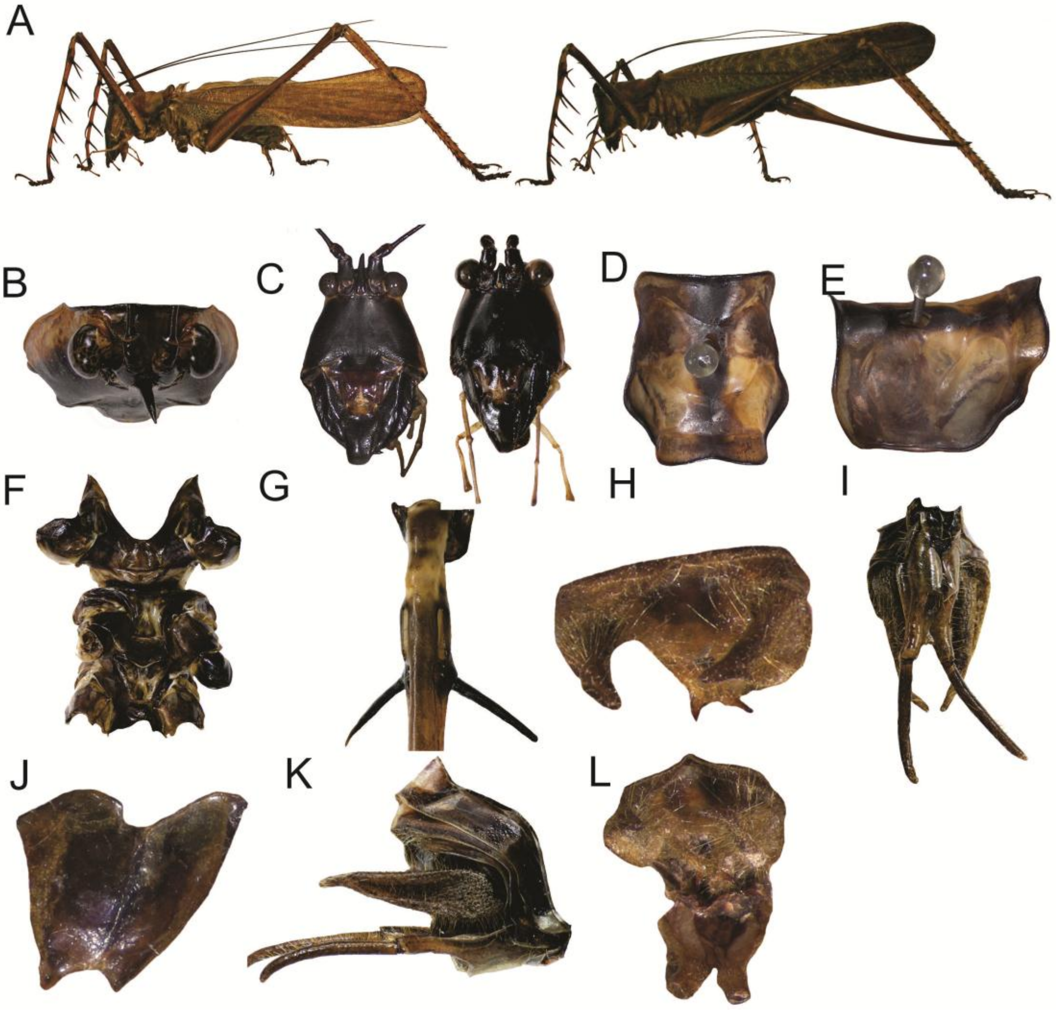

FIGURE 13. *Monocerophora longispina*, male (**A–I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

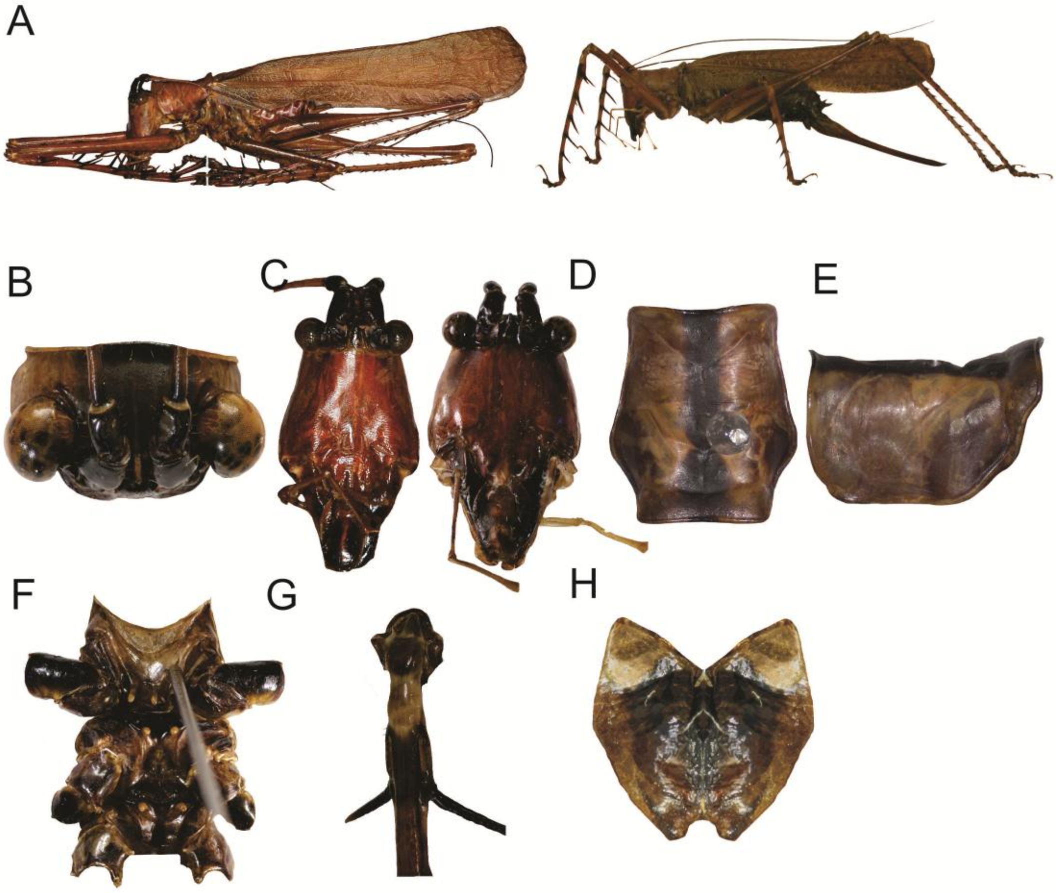

FIGURE 14. *Monocerophora spinosa*, male holotype (**A, C**), female (**A-H**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Ventral view of the subgenital plate.

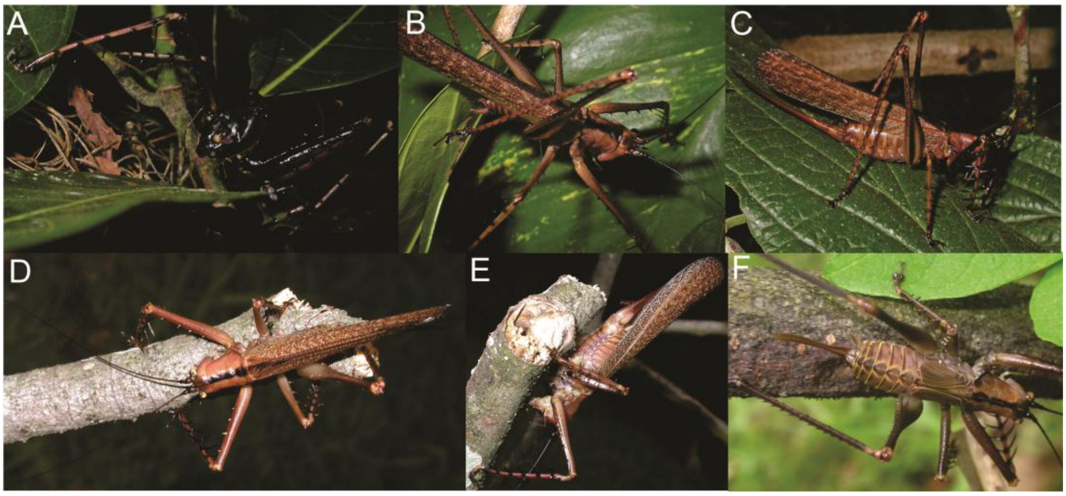

FIGURE 15. Individuals of *Monocerophora* live. *M. longispina*, male (**A, B**) and female (**C**). *M. spinosa*, female adult (**D, E**) and female immature (**F**).

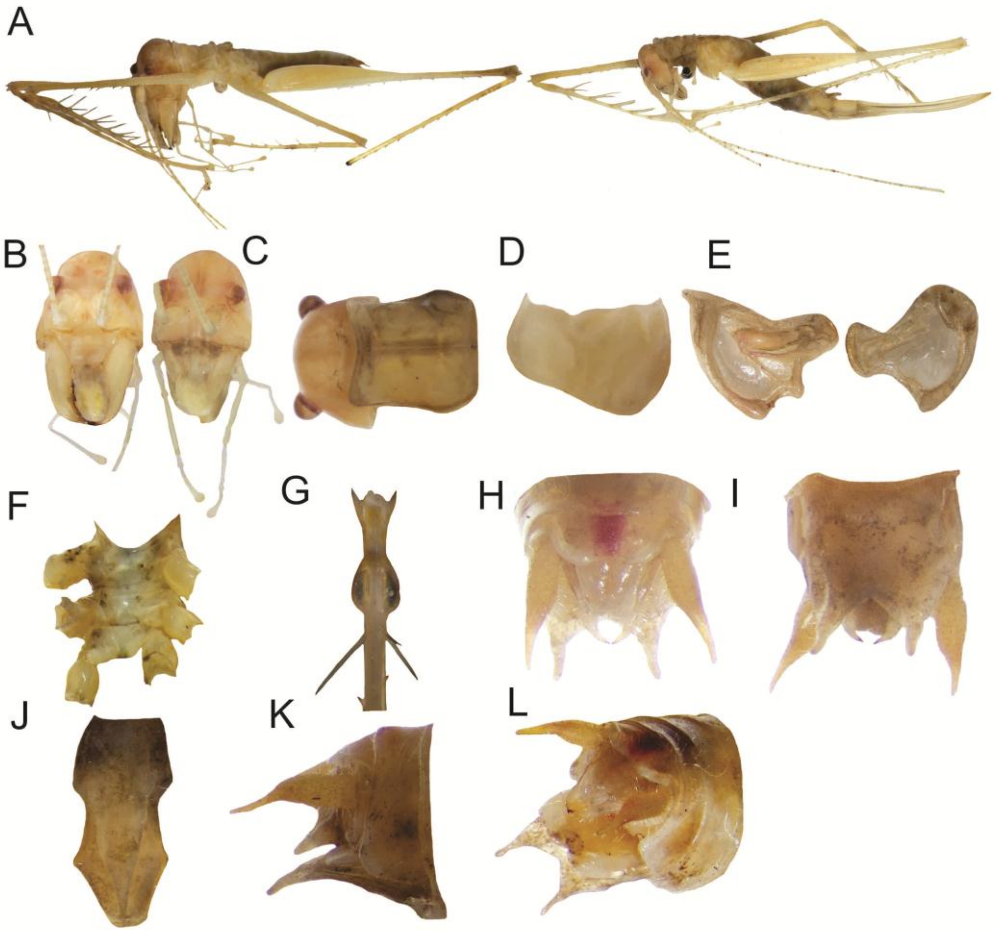

FIGURE 16. Gen. nov. sp. 1, male (**A–I, K, L**) and female (**B, J**). **A**. Lateral view of the body. **B**. Frontal view of the face, left male, right female. **C.** Dorsal view of the head and pronotum. **D**. Lateral view of the pronotum. **E**. Dorsal view of the tegmina. F. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

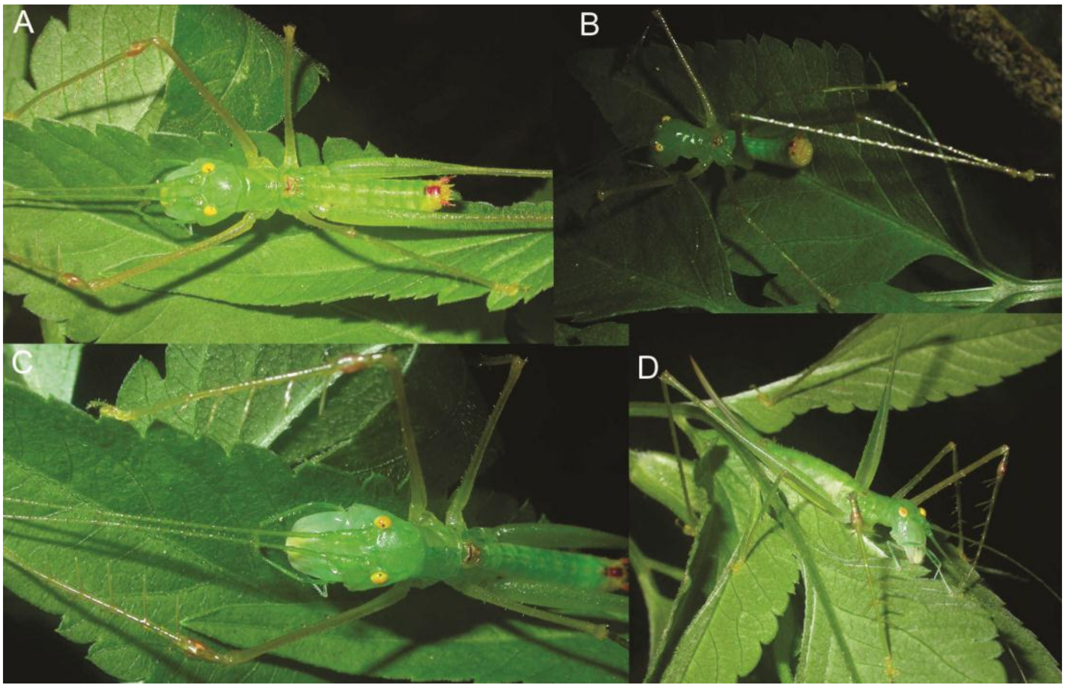

FIGURE 17. Individuals of **Gen. nov. sp. 1,** live, male (**A, C**) and female (**D**).

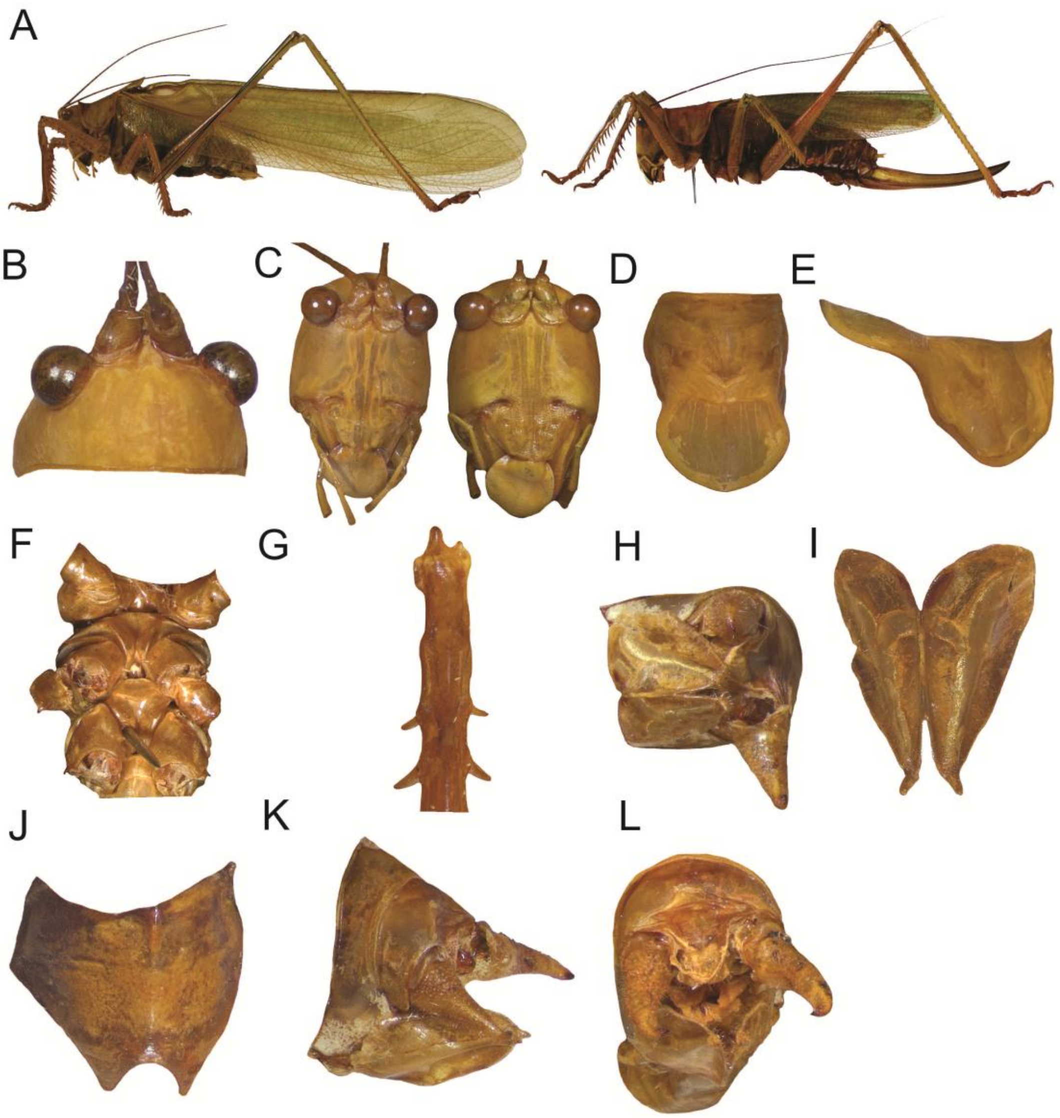

FIGURE 18. *Megatympanon speculatum*, male (**A–I, K, L**) and female (**B, C, J**). **A**. Lateral view of the body. **B**. Dorsal view of the head. **C**. Frontal view of the face, left male, right female. **D**. Dorsal view of the pronotum. **E**. Lateral view of the pronotum. **F**. Ventral view of the thorax (sternum). **G**. Frontal view of the tympanum. **H**. Dorsal view of the supranal plate. **I**. Ventral view of the Subgenital plate. **J**. Ventral view of the subgenital plate. **K**. Lateral view of the abdominal apex. **L**. Posterior view of the abdominal apex.

CAPÍTULO 2

Sistemática de Listroscelidinae (Orthoptera: Tettigoniidae): um caso de congruência de caracteres morfológicos e moleculares

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RESUMO

Apesar de ser um bioma muito diverso e bastante ameaçado, a biodiversidade presente na Mata Atlântica ainda é pouco conhecida. Dentre os Orthoptera, os Listroscelidinae (Tettigoniidae) são especialmente pouco estudados, devido à escassez de taxonomistas, e às dificuldades de coleta. Este trabalho teve como objetivo investigar as relações filogenéticas entre os Listroscelidinae coletados no bioma Mata Atlântica. Para isso, sequenciamos os genes COI e o 18S. As hipóteses filogenéticas obtidas com os dois genes separadamente foram complementares, uma vez que o COI apresentou resolução para os ramos mais recentes e o 18S para os ramos mais profundos. Além disso, os resultados foram congruentes com a taxonomia morfológica, indicando que estes marcadores são úteis como ferramentas complementares para estudos taxonômicos. Adicionalmente, propusemos uma região de aproximadamente 500 pares de bases na porção final do COI (DNA Barcodes) que pode ser utilizada por não especialistas tanto na identificação de espécimes quanto na detecção de possíveis espécies novas em Listroscelidinae.

Palavras-chave: COI; 18S; Unidades de Conservação; bioma Mata Atlântica; Filogenia Molecular; DNA Barcodes.

INTRODUÇÃO

A Mata Atlântica (MA) é considerada um *hot spot* de biodiversidade, pois apresenta elevadas taxas de endemismo, que podem ser explicadas pela existência de numerosos micro-habitats (Costa 1997, Myers et al. 2000, Moritz et al. 2000, Oliveira-Filho & Fontes 2000, Leigh et al. 2004). Entretanto, a taxa de endemismo e a diversidade genética dos organismos da MA são subestimadas, o que indica que ainda serão necessários muitos anos de estudos para que tenhamos uma ideia mais completa da biodiversidade que contém e dos mecanismos geradores desta biodiversidade. Para agravar a situação, a MA também é o bioma mais ameaçado do Brasil, resultado da ocupação antrópica que se concentra preferencialmente em áreas litorâneas (Morellato & Haddad 2000) . Da cobertura presente à época da chegada dos Portugueses ao Brasil, restam apenas 22%, com diferentes estágios de regeneração, sendo que apenas 7% encontram-se efetivamente conservados (Ministério do Meio Ambiente 2012, Morellato & Haddad 2000).

Barbosa et al. (2005) apontaram os integrantes da ordem Orthoptera como um dos grupos mais abundantes em estudo conduzido em um fragmento de MA. Entretanto, dados sobre a diversidade, relações filogenéticas e evolução do grupo nos biomas brasileiros são escassos na literatura de um modo geral.

A maioria dos estudos filogenéticos já realizados em Orthoptera envolve grupos taxonômicos supragenéricos (Flook & Rowell 1997, Flook et al. 1999, Jost & Shaw 2006, Fenn et al. 2008, Ma et al. 2009, Legendre et al. 2010, Zhou et al. 2010), sendo raros os trabalhos em nível de gêneros e espécies. O monofiletismo de Ensifera foi inferido em diversos estudos baseados em filogenias moleculares e morfológicas (Jost & Shaw 2006, Fenn et al. 2008, Ma et al. 2009, Zhou et al. 2010), bem como o monofiletismo de Tettigoniidae (Flook et al. 1999, Fenn et al. 2008, Ma et al. 2009, Zhou et al. 2010, Legendre et al. 2010), apesar da escassez de estudos desta família baseados em caracteres moleculares (Naskrecki 2000, 2008).

Atualmente não há estudos de filogenia molecular com tetigonídeos brasileiros e nenhum trabalho publicado no mundo envolvendo as relações filogenéticas de representantes de Listroscelidinae, que também estão entre os tetigonídeos brasileiros menos conhecidos. A escassez de taxonomistas interessados em Listroscelidinae mantém o grupo marginalizado, apesar de sua importância ecológica como predadores e recurso alimentar de outros animais (Nickle 1992; Nickle & Heymann 1996; Naskrecki 2000, 2008). Somado a isso está o fato de serem difíceis de capturar, pois são mais ativos durante a noite (Nickle 1992). Ágeis, se camuflam bem na vegetação e não caem em armadilhas do tipo pitfall e nem são atraídos por armadilha luminosa como outros tetigonídeos (Bruner 1915, Nickle 1992, Naskrecki 2008). Outra dificuldade é a necessidade de coleta de indivíduos adultos, que apresentam todas as estruturas morfológicas usadas para diferenciação entre as espécies totalmente desenvolvidas, como as placas subgenital, supraanal, cercos, estilos e genitália. Em machos adultos, características da fileira estridulatória (presente em uma das asas coriáceas) são usadas adicionalmente como diagnóstico, uma vez que ortópteros utilizam o canto para atrair as fêmeas conspecíficas.

Diante deste tipo de problema, Hebert e colaboradores (2003ab) sugeriram a metodologia de identificação de espécies animais por “códigos-de-barra moleculares” (DNA Barcodes), baseados em um fragmento do gene codificador da subunidade I da enzima citocromo C oxidase (COI). A metodologia tem como pressupostos que cada organismo possui uma única cópia da sequência que codifica esta proteína em cada uma de suas mitocôndrias, que são todas descendentes das mitocôndrias presentes no óvulo fecundado. Além disso, espera-se que esta sequência não varie dentro do indivíduo e varie muito menos dentro (até 3% de variação) do que entre espécies (Hebert *et al.* 2003ab). Todos as sequências utilizadas como códigos de barras estão armazenadas em um banco mundial denominado *Barcode of Life Data Systems* (BOLD SYSTEMS) (http://www.boldsystems.org) (Ratnasingham & Hebert 2007).

Dos mais de 900 barcodes de Orthoptera disponíveis no BOLD SYSTEMS, apenas 226 são de espécies de tetigonídeos. Destas, nenhuma é brasileira e/ou corresponde à subfamília Listroscelidinae (dados obtidos em Junho/2012). Ainda, das 12 sequências nucleotídicas de Listroscelidinae depositadas GenBank (um banco de dados público, organizado pelo NIH - *National Institutes of Health*, que concentra todas as sequências já publicadas de quaisquer organismos em quaisquer revistas e jornais científicos), apenas cinco correspondem ao gene da subunidade ribossomal 18S (nuclear) e três ao da subunidade I da enzima citocromo C oxidase (mitocondrial), nenhuma proveniente do Brasil (dados obtidos em Junho/2012).

Diante das dificuldades apresentadas na coleta, descrição e identificação de espécies de Listroscelidinae, o objetivo principal deste trabalho foi estabelecer as relações filogenéticas entre os espécimes coletados na MA, utilizando dois segmentos gênicos como ferramentas principais: um gene nuclear (que é transcrito na subunidade 18S do RNA ribossomal nuclear) e um mitocondrial (a subunidade I da enzima citocromo oxidase), que também pode ser utilizado para identificação molecular (DNA Barcode). A partir de nossos resultados, pretendemos propor um protocolo padrão de estudos destas espécies que permita, com o auxílio de taxonomistas, que novas espécies sejam acrescentadas às hipóteses filogenéticas de forma rápida e padronizada. A partir disso, será possível construir um panorama mais completo da distribuição geográfica das diferentes espécies, além de fornecer subsídios para descrições de novas espécies.

MATERIAL E MÉTODOS

Coleta do Material

A amostragem foi conduzida em fragmentos de vegetação ao longo do bioma MA, no período de novembro de 2011 a janeiro de 2012. O procedimento de coleta foi de busca ativa no período noturno, entre 19h e 1h, com o auxílio de lanternas, durante pelo menos três noites consecutivas em cada Unidade de Conservação (UC) visitada. Os espécimes foram capturados com potes plásticos transparentes de 500 mL com tampa, para posterior processamento e montagem em coleção.

As UCs foram escolhidas de acordo com a disponibilidade de alojamento nas datas requisitadas. Os pontos amostrados foram marcados com o GPS Garmin E-TRex Vista H e um mapa foi gerado com estes pontos no programa ArcGis versão 9.3 (Figura 1).

Dos pontos amostrados, não foram obtidos espécimes de Listroscelidinae nos pontos 5, 6, 8, 12 e 14. A distribuição dos 99 espécimes coletados nas UCs amostradas para este trabalho consta na Tabela 1.

A presente dissertação não constitui publicação científica como definida nas normas do Código Internacional de Nomenclatura Zoológica. Assim, para evitar problemas nomenclaturais, os epítetos específicos foram substituídos por “sp. nov. #” (após o gênero), que quer dizer espécie nova daquele gênero; igualmente para o novo gênero “Gen. nov.” e para espécie do novo gênero “Gen. nov. sp. #”.

Processamento e montagem dos espécimes

Após serem fotografados, os espécimes foram mortos em câmara mortífera contendo acetato de etila e tiveram as vísceras removidas; a cavidade corporal foi preenchida com algodão e pó anti-fungo. Cada indivíduo foi então envolvido em papel higiênico com sua respectiva identificação com dados da coleta (número do espécime / subfamília / localidade) e acondicionado em pote com sílica gel para absorver a umidade e evitar o apodrecimento.

Testes de extração de DNA de Orthoptera com músculos do tórax e das pernas, realizados anteriormente (Apêndice A), mostraram bons resultados. Assim, a fim de preservar ao máximo as características morfológicas, logo após a morte, somente uma das pernas foi removida e colocada em criotubo estéril de polipropileno de 2,0 mL contendo álcool absoluto. Indivíduos muito pequenos foram colocados inteiros no criotubo. Posteriormente às coletas, as amostras destinadas aos procedimentos moleculares foram armazenadas a -20 °C no Laboratório de Bioinformática e Evolução (LBE) da UFV. Após secarem completamente, no Laboratório de Sistemática e Biologia de Coleoptera (LabCol) da UFV, os espécimes foram colocados em câmara úmida por aproximadamente dois dias e alfinetados em posição anatômica em caixas entomológicas para condução dos procedimentos para descrição e identificação das espécies, mencionados no Capítulo 1.

Análise molecular

Todos os procedimentos mencionados abaixo estão minuciosamente detalhados no Apêndice A dessa dissertação, desde sua fase inicial de testes até o protocolo padrão final. A padronização destes protocolos foi conduzida no Laboratório de Bioinformática e Evolução (LBE) da Universidade Federal de Viçosa (UFV).

Extrações de DNA

Extraímos o DNA total de todos os espécimes coletados a partir de tecidos da perna seguindo o protocolo modificado a partir do procedimento descrito em Waldschmidt e colaboradores (1997). O resultado de cada extração foi visualizado em géis de agarose 0,8% com tampão TBE 1X e marcador de quantidade lambda DNA (Amersham Biosciences).

Escolha dos genes e desenho dos *primers*

O COI e o 18S figuram entre os mais utilizados nos estudos de sistemática molecular de insetos desde que este tipo de estudo começou (Caterino et al. 2000). Entre os Orthoptera, boa parte das espécies representadas por sequências de DNA no GenBank pertencem a estes dois fragmentos (busca feita em Junho/2012). Além disso, dentre a sequências disponíveis no GenBank, as mais longas, e portanto as mais promissoras em termos filogenéticos, são destes dois fragmentos gênicos. Nós então desenhamos os *primers* para estas duas regiões gênicas a partir do alinhamento de sequências de espécies de outros Orthoptera com o auxílio do programa Oligo Explorer 1.4.

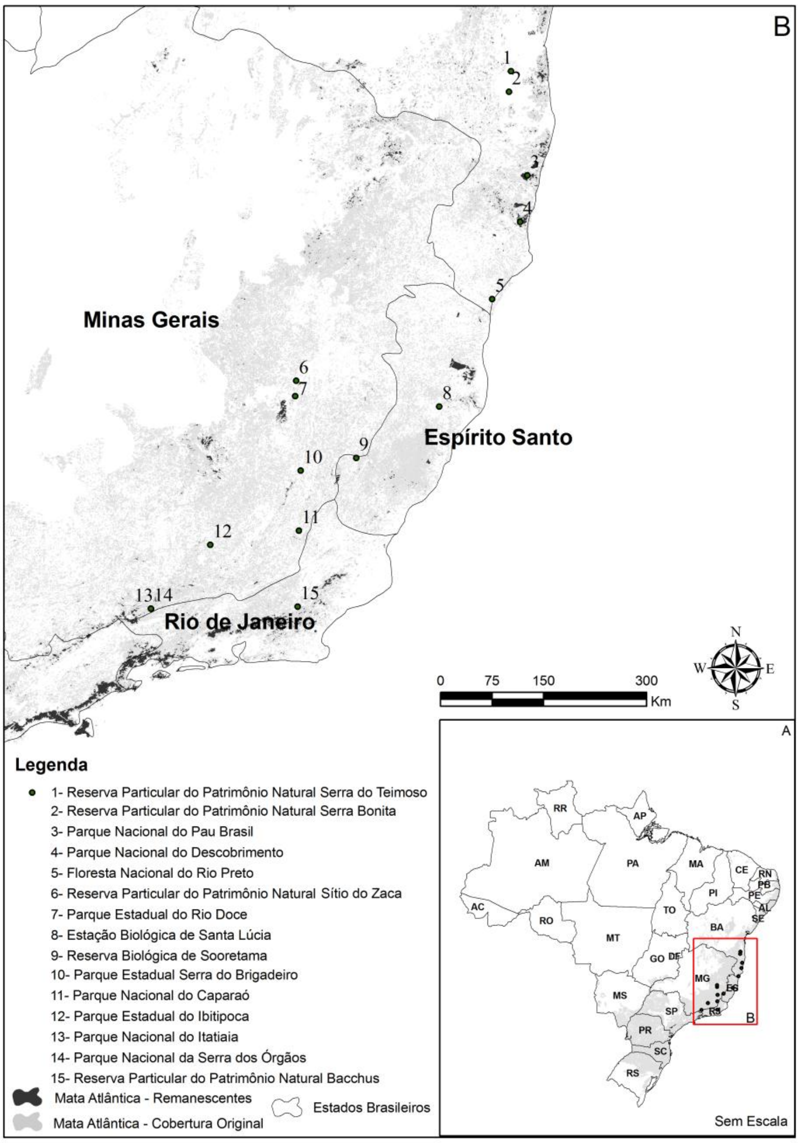

Figura 1: Mapa evidenciando todos os locais de coleta ao longo do bioma Mata Atlântica. As áreas cinza representam a MA: cinza escuro corresponde aos remanescentes e cinza claro à formação original no ano de 1500.

Amplificação

Nós amplificamos os fragmentos do COI e do 18S das amostras por PCR (*Polymerase Chain Reaction*) *touch down*, em termociclador BIOER com os pares (*forward* e *reverse*) de iniciadores COI-Orth-1F e COI-Orth-1R e 18S-Orth-1F e

18S-Orth-1R (Apêndice A). Para verificar se a reação de PCR foi bem sucedida e que apenas um fragmento tenha sido amplificado por amostra e par de *primers*, submetemos as amostras à eletroforese em gel de agarose 1,5% com tampão TBE 1X e marcador de tamanho 1 kb Plus DNA Ladder (Invitrogen).

Tabela 1: Número de indivíduos coletados (N) por espécie e por local de coleta (números referenciados na Figura 1) e número de sequências utilizadas nas análises filogenéticas dos genes COI e 18S.

Gênero	Espécie	Localidade	N	COI	18S
Cerberodon	*viridis*	15	4	4	3
	sp. nov. 1	11	2	2	1
Listroscelis	*carinata*	7	6	6	6
	sp. nov. 1	2	10	6 *	4
	sp. nov. 2	9	2	2	2
	sp. nov. 3	4	1	1 *	1
	sp. nov. 4	10	2	2	2
	sp. nov. 5	4	4	2	3
	sp. nov. 6	13	4	1	3
Monocerophora	*spinosa*	13	5	5	2
		9	5	3	4
	longispina	1	9	1	4
		2	6	3	1
		3	4	-	1
		4	3	1	3
Novo (Gen. nov.)	sp. nov. 1	4	12	8	4
	sp. nov. 2	3	1	-	1
		4	15	8	3
		TOTAL		48	48

* sequência(s) removida(s) da análise por apresentarem picos duplos, inserções e/ou *stop codons*.

Purificação e sequenciamento

Enviamos os produtos de PCR obtidos para purificação e sequenciamento ao laboratório da empresa Macrogen (http://dna.macrogen.com), localizada na Coréia, de acordo com as especificações sugeridas pelo laboratório (volume mínimo de 20 µl por amostra numa concentração de 100 ng/µl).

Alinhamento

Uma vez obtidas as sequências, avaliamos a qualidade das mesmas com o auxílio dos programas PhredPhrap (Ewing & Green 1998, Ewing *et al.* 1998) e *Consed* (Gordon et al. 1998), que permitem a obtenção de contigs (junção das sequências *Foward* e *Reverse*) para cada espécime. Por serem codificadoras de proteínas, sequências de COI devem ser primeiramente traduzidas para as sequências putativas de aminoácidos, alinhadas e posteriormente revertidas às sequências de nucleotídeos. Este procedimento garante a homologia dos sítios alinhados, uma vez que as sequências de aminoácidos são mais curtas (3 vezes menores) e mais conservadas que as de nucleotídeos. Utilizamos o algoritmo MUSCLE (Edgar 2004) para alinhar tanto o 18S (que foi alinhado diretamente) quanto o COI e todo o procedimento de alinhamento de ambos os genes foi feito com o auxílio do programa MEGA 5.0 (Tamura et al. 2011).

Na análise filogenética do gene COI, foi utilizada como *outgroup* a espécie *Oxya chinensis* (Acrididae: Orthoptera) (número de acesso no GenBank: NC010219.1). Já na árvore de 18S não foram encontrados *outgroups* apropriados, uma vez que as sequências disponíveis no GenBank ou são demasiado distantes do grupo, enviesando os resultados, ou acabavam figurando dentro do grupo, apesar de não pertencerem à Listroscelidinae.

Análise filogenética: Inferência Bayesiana

Fizemos inferências filogenéticas independentes para cada segmento gênico utilizando Inferência Bayesiana (IB) (Yang & Ranalla 1997), implementada no programa MrBayes versão 3.1.2 (Ronquist & Huelsenbeck 2003). Utilizamos o programa MrModelTest versão 2.3 (Nylander 2004) para delimitar quais parâmetros deveriam ser inferidos através da análise bayesiana para cada conjunto de sequências (o MrModelTest 2.3 avalia qual o melhor modelo de substituição de nucleotídeos para cada alinhamento).

A IB foi gerada a partir de 50 milhões de passos via Cadeia de Markov, que se iniciam com parâmetros aleatórios (topologia, tamanhos de ramos e parâmetros dos modelos de substituição de nucleotídeos) que vão sendo modificados passo a passo. A cada passo é calculada a verossimilhança da topologia, tamanhos de ramos e parâmetros dos modelos de substitução. A busca pela melhor hipótese filogenética foi feita através de duas corridas independentes, cada uma com quatro cadeias de Markov, sendo três quentes e uma fria, segundo o *default* do programa MrBayes (Ronquist & Huelsenbeck 2003). Após a corrida, eliminamos 10% das primeiras topologias, que apresentaram baixas verossimilhanças. A partir das topologias restantes, foi inferida a topologia consenso, com tamanhos de ramos médios. A probabilidade posterior de cada nó da árvore foi dada pela frequência de cada ramo no conjunto final de árvores.

DNA Barcodes

O alinhamento das sequências de COI foi avaliado em busca de uma região pouco variável dentro de uma mesma espécie (conforme avaliação morfológica, Capítulo 1) e mais variável entre espécies. Uma vez detectada esta região, foi construído um dendrograma baseado na distância de Kimura-2-Parâmetros (Kimura 1980) utilizando o método de Neighbor-Joining (Saitou & Nei 1987), que foi comparado com a árvore construída com as sequências completas do COI obtidas neste trabalho.

RESULTADOS

Extrações, amplificações e sequenciamento de Listroscelidinae da MA

O DNA total dos 99 espécimes de Listroscelidinae coletados foi extraído com sucesso numa média de 100ng/uL. Sequências parciais do 18S e do COI foram obtidas, conforme esperado, com aproximadamente 1600pb e 1300pb, respectivamente.

Em quatro das 99 amostras (três espécimes de *M. Longispina*, duas coletadas no ponto 1 e uma no ponto 2, Figura 1 e uma espécie de *M. Spinosa* coletada no ponto 13), não foi possível obter o produto de PCR com ambos os pares de *primers* utilizados, mesmo depois de três tentativas. Todas as demais amostras amplificaram apropriadamente, e nós enviamos todos os produtos de PCR do COI para sequenciamento. Como o gene 18S não apresentou variação intraespecífica em uma amostra das sequências, nós optamos por sequenciar um número menor de indivíduos por espécie.

Apesar de termos obtido 95 sequências do COI, optamos por incluir apenas 48 na árvore filogenética. Isso se justifica pelo fato de que o COI é uma sequência codificadora (deve ser transcrita e traduzida) e deve estar presente em cada indivíduo em cópia única. No entanto, ao avaliar os cromatogramas, nós encontramos picos duplos em algumas amostras. Além disso, quando tentamos traduzir estas sequências para as sequências putativas de aminoácidos, observamos alguns *indels* (inserções e deleções e um ou dois pares de bases) e/ou *stop codons* (códons que codificam o término da tradução) no meio das sequências. Estes problemas são indicativos da presença de *numts*, que serão melhor investigados em outro trabalho.

A Tabela 1 (Materiais e Métodos) mostra um resumo com os dados de todos os espécimes que tiveram suas sequências de 18S e COI utilizadas nas análises filogenéticas.

Análises Filogenéticas

Gene mitocondrial COI

O alinhamento completo das 48 sequências do gene COI resultou em 1290 sítios alinhados, dos quais 540 são variáveis. A Figura 2 mostra as relações filogenéticas inferidas entre os Listroscelidinae com o COI.

A hipótese filogenética mostrada na Figura 2 mostra uma subdivisão em Listroscelidinae que tem por um lado o gênero novo descrito no Capítulo 1 e por outro uma politomia contendo três grandes ramos, cada um correspondendo a um gênero, *Cerberodon*, *Monocerophora* e *Listroscelis*.

Dentro do gênero *Cerberodon*, há uma clara subdivisão em dois clados, o primeiro correspondendo a espécimes de *C. viridis* e o segundo a espécimes da espécie nova 1 de *Cerberodon*. No clado que contém o gênero *Monocerophora*, também aparecem duas subdivisões, a primeira contendo espécimes identificados como *M. spinosa* e a segunda espécimes identificados como *M. longispina*. A espécie *M. spinosa* parece estar subdividida em dois clados, o primeiro contendo espécimes da localidade 9 (ver Figura 1), em Sooretama, ES, e o segundo da localidade 13, no Parque Nacional do Itatiaia, RJ. O mesmo parece ter ocorrido com *M. longispina*, que apresenta um espécime, coletado na localidade 4 (Parque Nacional do Descobrimento, BA), filogeneticamente mais afastado de outros quatro espécimes, um da localidade 1 (Reserva Particular do Patrimônio Natural Serra do Teimoso) e outros três da localidade 2 (Reserva Particular do Patrimônio Natural Serra Bonita), ambos BA.

Listroscelis apresenta uma primeira divisão em dois grupos. No maior deles, espécimes identificadas como *L. carinata* aparecem juntos e têm como grupo irmão espécimes de *Listroscelis* sp. nov. 2. Estas duas espécies formam um grupo monofilético, irmão de outro grupo, que contém espécimes de *Listroscelis* sp. nov. 5 . O segundo grupo contém apenas duas espécies, *Listroscelis* sp. nov. 6, coletado na localidade 13, Parque Nacional do Itatilaia e *Listroscelis* sp. nov. 4, coletado na localidade 10, Serra do Brigadeiro em MG.

O grande clado contendo o gênero novo, é composto por duas espécies molecularmente bem diferenciadas, Gen. nov. sp. nov. 1 e Gen. nov. sp. nov. 2, ambos coletados na localidade 4, Parque Nacional do Descobrimento, BA.

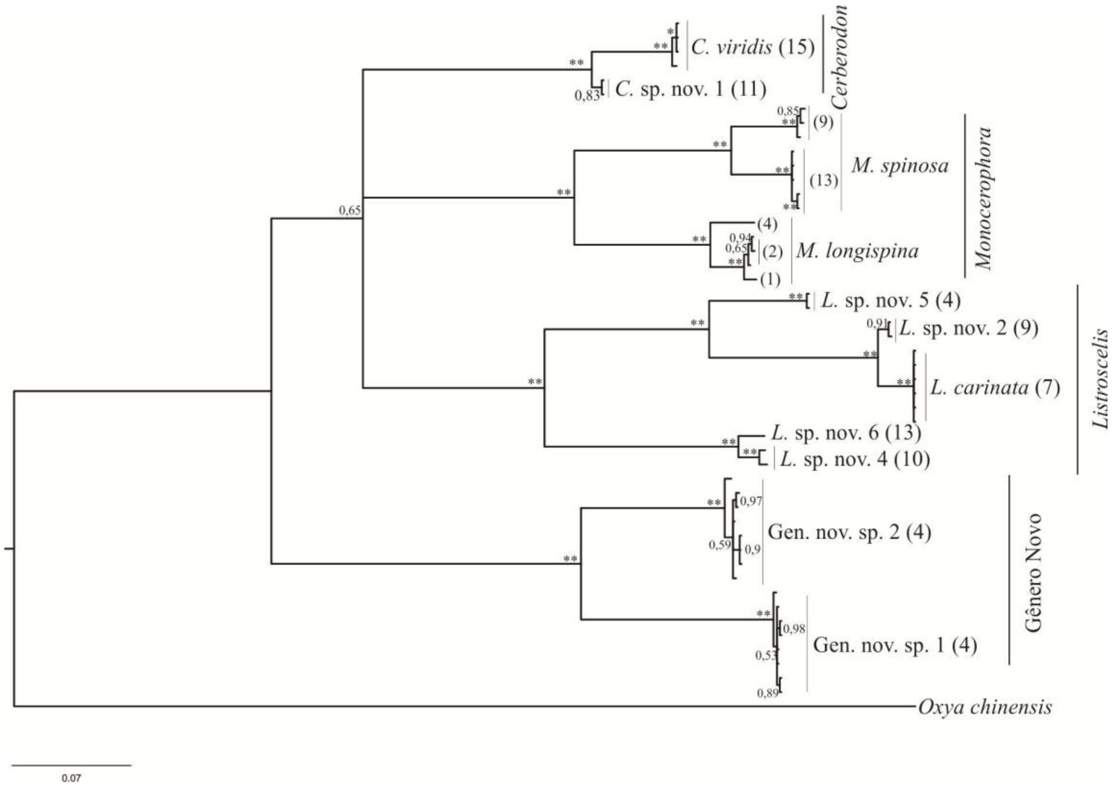

Figura 2: Árvore filogenética consenso do gene COI obtida por Inferência Bayesiana de espécies de Listroscelidinae coletadas ao longo do bioma Mata Atlântica. Os valores ao lado dos nós ancestrais da árvore correspondem à probabilidade posterior (PP). * simboliza PP >= 0,95 e ** PP = 1,0. Uma sequência de *Oxya chinensis* (GenBank: NC010219.1) foi utilizada como outgroup. As espécies e o gênero novos estão descritos no Capítulo 1. Os números entre parênteses correspondem aos locais de coleta, identificados na Figura 1. Barras verticais foram colocadas ao lado dos táxons terminais de cada ramo para indicar que se trata de vários espécimes de uma mesma espécie ou gênero.

Gene nuclear 18S

O alinhamento completo das 48 sequências de 18S resultou em 1615 sítios alinhados, dos quais apenas 11 são variáveis.

A Figura 3 mostra a hipótese filogenética obtida com as sequências de 18S. Assim como foi observado na árvore do COI (Figura 2), os Listroscelidinae foram divididos em dois grandes grupos, o primeiro contendo o gênero novo e o segundo contendo os gêneros *Listroscelis*, *Cerberodon* e *Monocerophora*. Ao longo de toda a árvore, todas as sequências pertencentes a espécimes identificados como sendo de mesmo gênero são idênticas.

O clado que contém *Listroscelis*, *Cerberodon* e *Monocerophora* foi subdivido em dois, o primeiro contendo todas as sequências de *Cerberodon* e o segundo contendo todas as sequências de *Monocerophora* e *Listroscelis*, que não apresentaram nenhuma variação. O segundo clado, que contém as sequências dos espécimes do gênero novo mostram uma separação muito marcada entre as espécies 1 e 2, consequência de quatro sítios variáveis, incluindo duas transições (sítios 102 e 606) e duas transversões (sítios 566 e 653) entre o Gen. nov. sp. nov. 1 e o Gen. nov. sp. nov. 2.

DNA Barcodes

Uma primeira análise mostrou que a porção final da sequência do COI apresentava mais variação interespecífica que intraespecífica. Com isso, delimitamos a região localizada entre os sítios 2394 e 2890 (Figura 4) do genoma mitocondrial completo de *Oxya chinensis* (GenBank NC010219.1). O dendrograma obtido com o método de Neighbor-Joining (Saitou & Nei 1987) utilizando a distância de Kimura-2-Parâmetros (Kimura 1980) foi praticamente idêntico ao obtido com a sequência completa (Figura 5).

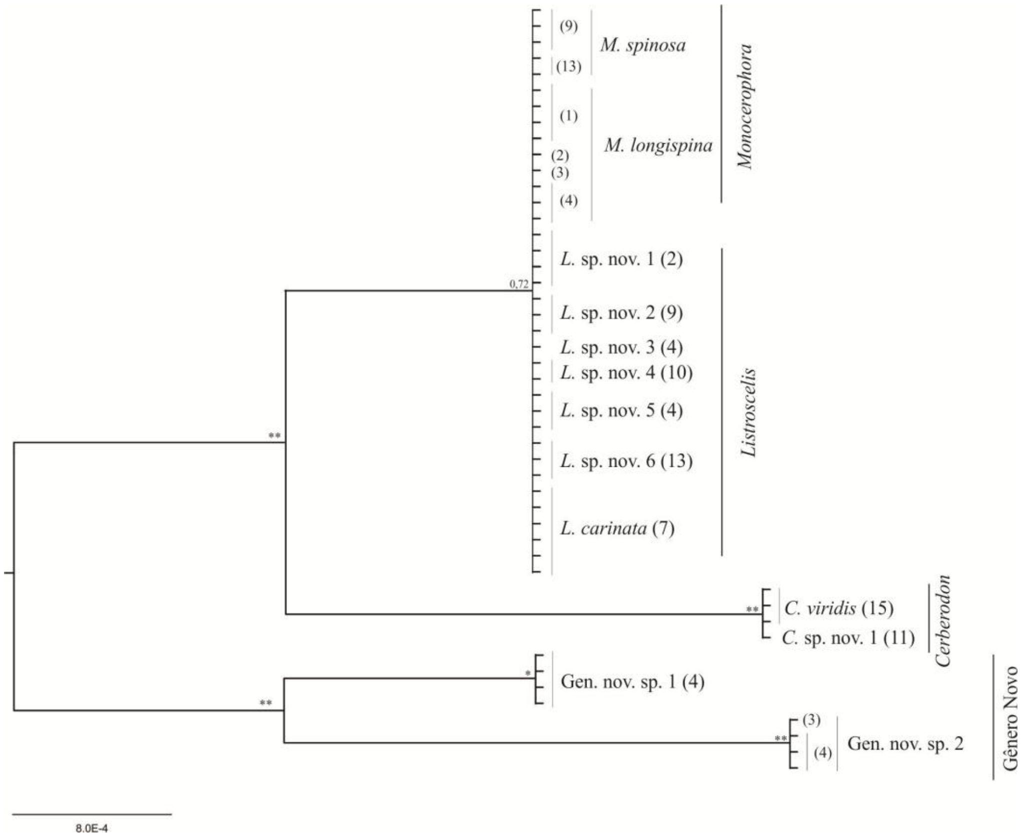

Figura 3: Árvore filogenética consenso do gene 18S obtida por Inferência Bayesiana de espécies da subfamília Listroscelidinae coletadas ao longo do bioma Mata Atlântica. Os valores ao lado dos nós ancestrais da árvore correspondem à probabilidade posterior (PP). * simboliza PP >= 0,95 e ** = PP = 1,0. As espécies e o gênero novos estão descritos no Capítulo 1. Os números entre parênteses correspondem aos locais de coleta, identificados na Figura 1. Barras verticais foram colocadas ao lado dos táxons terminais de cada ramo para indicar que se trata de vários espécimes de uma mesma espécie ou gênero.

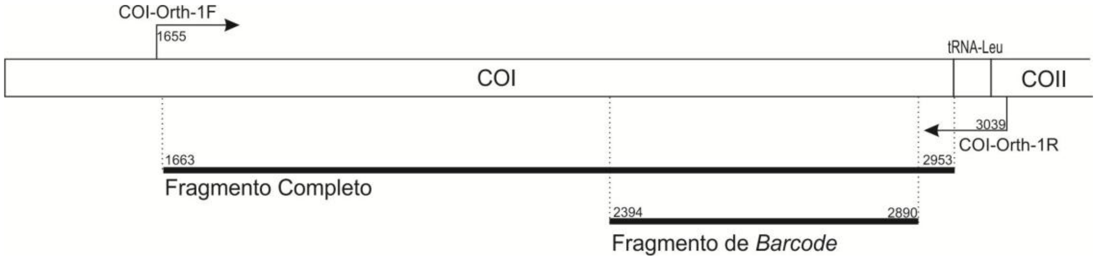

Figura 4: Representação esquemática dos *primers* utilizados para amplificar o COI, do fragmento efetivamente utilizado na Figura 2 e do fragmento proposto como DNA barcode para os Listroscelidinae. Os números indicados correspondem à posição no genoma mitocondrial completo da espécie *Oxya chinensis*, depositada no GenBank sob o número NC010219.

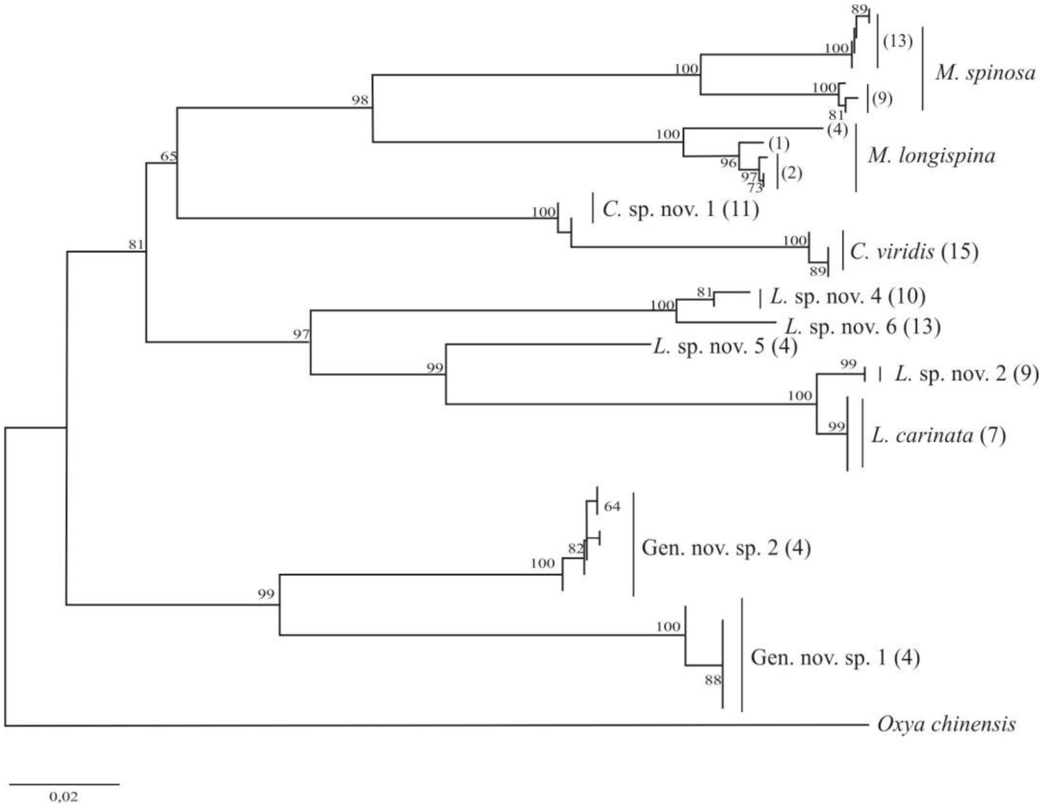

Figura 5: Dendrograma obtido com a região de *Barcodes* representada na Figura 4, utilizando o método de Neighbor-Joining (Saitou & Nei 1987) com distâncias de Kimura-2-Parâmetros (Kimura 1980). Os números ao lado dos nós são os valores de bootstrap, calculados com 1000 replicações. As espécies e o gênero novos estão descritos no Capítulo 1. Os números entre parênteses correspondem aos locais de coleta, identificados na Figura 1. Barras verticais foram colocadas ao lado dos táxons terminais de cada ramo para indicar que se trata de vários espécimes de uma mesma espécie ou gênero.

DISCUSSÃO

Numa análise filogenética, cada gene pode resultar em uma topologia diferente, uma vez que genes diferentes estão sob pressões seletivas diferentes, que permitem mais ou menos substituições, a taxas distintas em diferentes regiões das sequências, num mesmo intervalo de tempo. O genoma nucelar de eucariotos conta com poderosos mecanismos de reparo, ausentes no genoma mitocondrial, fazendo com que o DNA nuclear sofra menos substituições que o mitocondrial.

Com isso, genes nucleares são mais indicados para análises da diversidade e relacionamentos entre espécimes menos aparentados. Por outro lado, genes mitocondriais são bons marcadores para o estudo de grupos que divergiram recentemente, devido à sua alta taxa de substitução de nucleotídeos (Avise 1994). Assim, hipóteses filogenéticas baseadas em genes de genomas diferentes fornecem informações complementares, que permitem uma visão mais abrangente da evolução de um determinado grupo de organismos.

O objetivo do presente trabalho foi tentar estabelecer as relações filogenéticas entre espécies de Litrocelidinae coletadas ao longo do bioma Mata Atlântica. Os resultados das análises filogenéticas conduzidas com os genes COI e 18S foram complementares e condizentes com a descrição obtida a partir de dados morfológicos (Capítulo 1). Enquando o COI não foi apropriado para estabelecer as relações entre *Cerberodon*, *Monocerophora* e *Listroscelis*, estas relações foram esclarecidas pelo 18S, que não apresentou diferenças entre *Listroscelis* e *Monocerophora*, indicando que compartilham um ancestral comum mais recente que *Listroscelis, Monocerophora* e *Cerberodon.* Por outro lado, com o 18S não foi possível diferenciar espécies dentro destes três gêneros, enquanto que, com o COI, foi possível diferenciar molecularmente sete dos oito espécies descritas no Capítulo 1 pertencentes a estes três gêneros.

O par *Listroscelis* sp. nov. 4 e *Listroscelis* sp. nov. 6, diferenciados morfologicamente, foi o par de espécies que apresentou a menor diferenciação em termos moleculares (ramos curtos na Figura 2). Infelizmente não foi possível incluir mais sequências de *Listroscelis* sp. nov. 6, uma vez que estas apresentaram problemas no sequenciamento. Se mais sequências estivessem disponíveis, seria possível verificar se o COI de fato é uma ferramenta para diagnosticar estas espécies. É interessante ressaltar que os indivíduos de *Listroscelis* sp. nov. 4 foram coletados no Parque Estadual Serra do Brigadeiro, MG (ponto 10, Figura 1), enquanto os de *Listroscelis* sp. nov. 6 foram coletados no Parque Nacional do Itatiaia, RJ (ponto 13, Figura 1), estando, portanto, isolados geograficamente.

Por outro lado, os indivíduos identificados como *M. spinosa* foram divididos em dois clados, com diferenciação molecular compatível com duas espécies distintas na árvore do COI. Os três indivíduos presentes no clado coletados na Reserva Biológica de Sooretama, ES (ponto 9, Figura 1) foram classificados com base em registros de distribuição desta espécie no estado do Espírito Santo (Karny 1907). Como os três indivíduos são imaturos, não foi possível delimitar a que espécie pertencem com base apenas nos caracteres morfológicos. Em espécies hemimetábolas, como no caso dos Orthoptera, é comum que imaturos de espécies distintas sejam indistinguíveis, de modo que novas coletas em outras épocas do ano nesta localidade serão imprescindíveis para testarmos se temos ou não evidências de duas espécies distintas. O outro clado classificado como *M. spinosa* contém cinco indivíduos adultos coletados no Parque Nacional do Itatiaia, RJ (ponto 13, Figura 1). Embora sejam de diferentes localidades e estejam em ramos separados com alta probabilidade posterior (PP=1) e apesar do COI ser um gene bastante eficiente na diferenciação entre espécies próximas, não é possível estabelecer a existência de duas espécies distintas apenas com os resultados deste gene e sempre é recomendável ter caracteres diagnósticos que permitam a diferenciação de espécies. No entanto, Burns e colaboradores (2008) propuseram a existência e identificação de espécies crípticas de borboletas baseados apenas em caracteres moleculares, o que é uma prática cada vez mais comum hoje, mas que tem sido bastante debatida (Carvalho et al. 2007, 2008; Hamilton & Wheeler 2008).

Os espécimes classificados como *M. longispina* também parecem estar subdivididos em dois clados e possivelmente conter duas espécies diferentes. No entanto, não é possível afirmar que se trata de duas espécies pois o espécime mais afastado filogeneticamente provém da localidade 4 (Parque Nacional do Descobrimento, BA), de onde apenas fêmeas adultas foram coletadas. Como a maior parte dos caracteres morfológicos diagnósticos provém de machos adultos, o fato de termos só fêmeas desta localidade inviabiliza uma análise morfológica mais conclusiva.

Com relação às duas espécies descritas no gênero novo, ambos os genes mostraram grandes diferenças, compatíveis com linhagens separadas há muito tempo. Cabe ressaltar que todos os espécimes utilizados nas análises foram coletados na Bahia (todos os espécimes das espécies 1 e 2 foram coletados no Parque Nacional do Descobrimento, BA, ponto 4, Figura 1, exceto por um espécime da espécie 2, que foi coletado no Parque Nacional do Pau Brasil, BA, ponto 3, Figura 1). Os longos tamanhos de ramos da árvore do 18S, no entanto, sugerem uma diferenciação bastante acentuada entre as duas espécies. Considerando que os gêneros *Listroscelis* e *Monocerophora* sequer apresentaram diferenças nesta sequência, podemos levantar a hipótese de que estas duas espécies não sejam classificadas em um único gênero, e sim em dois gêneros distintos. Esta proposição deve ser melhor examinada em trabalhos futuros, após novas expedições de coleta, uma vez que, assim como no caso dos *M. spinosa* do Espírito Santo, não foram coletados indivíduos adultos, apenas imaturos no Gen. nov. sp. nov. 2.

Apesar dos esforços para concentrar nossas coletas em épocas com maior probabilidade de coletar indivíduos adultos, isso não foi possível em todas as espécies. Ainda, em algumas localidades, como o Parque Nacional do Descobrimento, foram coletados indivíduos adultos, mas apenas fêmeas da espécie identificada como *M. longispina*. Apesar disso, nossos resultados nos permitem sugerir que a abordagem molecular, conforme foi apresentada, pode ser uma boa ferramenta de apoio à taxonomia. Nossos resultados corroboraram a identificação baseada em dados morfológicos mostrada no Capítulo 1, além de apontar novas questões a serem resolvidas pelos taxonomistas, como a provável existência de duas espécies nos espécimes classificados como *M. spinosa*, a subdivisão de *M. longispina* em duas espécies e dois possíveis gêneros novos ao invés de um, nos espécimes classificados como gênero novo.

Quanto à abordagem de identificação molecular, nossos resultados nos permitiram sugerir o uso da porção final do COI. Esta porção pode ser obtida apenas com a amplificação do fragmento do COI utilizando os *primers* descritos neste trabalho e o sequenciamento de uma única fita, utilizando o primer COI-Orth-1R (Figura

4). Com isso, uma vez que as sequências estejam no BOLD SYSTEM, qualquer espécime de Listroscelidinae que seja coletado pode ter a porção final do COI amplificada e inserida num dendrograma, incluindo todas as sequências disponíveis para o grupo. Caso se trate de uma das espécies já descritas e inseridas no banco, será possível identificá-la de forma relativamente rápida. Caso contrário, será possível inferir a detecção de uma espécie nova (ou ainda não incluída no sistema), que deverá ser avaliada pelos taxonomistas, a fim de incluí-la no banco.

Acreditamos que a junção das abordagens morfológica e molecular possa alavancar os estudos envolvendo a taxonomia deste grupo de organismos, de modo que seja possível, em um intervalo relativamente curto de tempo, estudar a diversidade e distribuição das diversas espécies de Listroscelidinae, tanto na Mata Atlântica quanto em outros biomas brasileiros.

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CONSIDERAÇÕES FINAIS

A ordem Orthoptera, apesar de sua importância ecológica e econômica, ainda é pouco estudada no Brasil. Uma comprovação disso é o investimento de quase 1,8 milhão de reais do CNPq para as coletas e estudo dos espécimes da ordem em todos os biomas brasileiros, para ampliar significativamente o conhecimento sobre estes organismos.

Deste grande projeto, coube ao Laboratório de Bioinformática e Evolução - LBE/UFV desenvolver e utilizar ferramentas moleculares que permitam auxiliar a classificação dos Orthoptera bem como estudar a evolução do grupo. Neste contexto, fui incubida de buscar e testar protocolos de extração de DNA, escolher regiões gênicas com diferentes taxas de evolução (e consequentemente diferentes níveis de resolução) para serem estudadas e desenhar *primers* para a amplificação e sequenciamento destas regiões.

Igualmente foi necessário padronizar as coletas, de modo que além dos caracteres morfológicos, fosse preservado o DNA das organelas celulares. Para isso estabelecemos que de todas os espécimes coletados pelo menos uma perna de cada indivíduo seja conservada em álcool (acima de 92 °GL).

Testei também o material genético obtido a partir de espécimes mortos em álcool combustível, com o objetivo de reduzir o custo com soluções matadoras e descomplicar a logística das coletas feitas com armadilhas de queda (pitfall traps). Os resultados foram satisfatórios e a metodologia de coleta já foi estabelecida e está sendo utilizada nas campanhas de campo do projeto.

Esta dissertação marca, portanto, o início das atividades do LBE junto ao Projeto Biota de Orthoptera do Brasil, coordenado pelo Prof. Carlos Sperber, do Laboratório de Ortopterologia da UFV.

Considerações sobre a crise taxonômica

A classificação de organismos se baseia tradicionalmente em caracteres morfológicos diagnósticos. No entanto, a taxonomia clássica atravessa uma crise ocasionada pela escassez de profissionais especializados na área. Este problema se deve, principalmente, ao fato de que o esquema produtivista da ciência atual é incompatível com a taxonomia, que requer tempo para a descrição de novas espécies, uma vez que é necessário examinar, comparar e descrever vários indivíduos de maneira pormenorizada (Meier et al. 2006).

Além disso, os trabalhos de taxonomia são sempre publicados em revistas de circulação restrita aos especialistas de cada grupo, de modo que os fatores de impacto em geral são muito baixos, o que desestimula os estudantes a seguir nesta área (Samyn & Massin 2002, Will et al. 2005, Waegele et al. 2011). O resultado é que os poucos taxonomistas que existem estão normalmente muito atarefados, o que os torna relativamente inacessíveis à maioria dos outros pesquisadores, que não podem prescindir de seu trabalho (Meier 2008, Stoeckle 2003).

Na contramão dessa realidade, o processo acelerado de perda de diversidade (Bortolus 2008, Mora et al. 2011) torna os trabalhos em taxonomia ainda mais importantes e necessários, pois muitas espécies estão sendo extintas antes mesmo de serem registradas (Samways & Lockwood 1998).

O produto da taxonomia é a base dos estudos de ecologia, comportamento e evolução, dentre outros. Dessa forma, é de suma importância o aumento dos investimentos em inventários, sistemática e utilização de diferentes ferramentas, para produção e aumento do conhecimento da biodiversidade, de forma a sustentar em bases sólidas estudos posteriores nas mais diversas áreas do conhecimento.

O presente trabalho, bem como os trabalhos que vem sendo desenvolvidos no âmbito do Projeto SISBIOTA de Orthoptera, tornaram-se possíveis devido à colaboração entre pesquisadores envolvidos em equipes de diferentes áreas.

Graças à colaboração dos sistematas Prof. Cristiano Lopes Andrade e Juliana Chamorro Rengifo, do LabCol/UFV, pude aplicar a metodologia molecular ao estudo taxonômico de Listroscelidinae, de forma a validar a utilização dos caracteres moleculares em consonância com a taxonomia. Uma contribuição significativa foi dada ao conhecimento dos representantes brasileiros de Listroscelidinae, uma vez que acrescentamos pelo menos oito espécies e um gênero novos à subfamília. Os resultados obtidos com as metodologias moleculares ainda precisam ser revistos morfologicamente, para a possível descrição de duas novas espécies e um novo gênero. Esta subdivisão de táxons requer, no entanto, novas coletas em busca de machos adultos.

A metodologia de extração do DNA e obtenção dos fragmentos amplificados via PCR foi estabelecida da forma mais simples e barata possível, de modo que pode ser utilizada por qualquer pesquisador interessado em estudar qualquer espécie de Orthoptera com o mínimo de equipamentos e reagentes. Os *primers* descritos neste trabalho foram testados em diversos espécimes de esperanças, grilos e gafanhotos. Deste ponto em diante, será necessário apenas identificar as melhores regiões a serem utilizadas como DNA Barcodes para outros grupos de Orthoptera.

Resta ainda esclarecer porque o DNA de alguns espécimes não pode ser amplificado por nenhum dos pares de *primers* aqui sugeridos, e também verificar porque a sequência do COI de alguns espécimes apresentou picos duplos e indels e/ou stop codons quando traduzidos para a sequência putativa de aminoácidos. É possível que estejamos diante de cópias de sequências mitocondriais presentes no núcleo das células, fragmentos denominados *numts*, bastante comuns em insetos (Cristiano et al. 2012), e principalmente entre ortópteros (Bensasson et al. 2001, Moulton et al. 2010). Esta hipótese deve ser melhor estudada, o que vai ser feito em meu doutorado, que envolverá a clonagem dos fragmentos de COI amplificados nas espécies que apresentaram problemas para verificar quantas cópias estão presentes no núcleo e se é possível isolar apenas o fragmento mitocondrial.

APÊNDICES

Apêndice A:

Padronização dos protocolos para obtenção de sequências nucleotídicas

Espécimes coletados no campo para estudos genéticos requerem condições específicas, de modo a inibir a atividade das nucleases, principais responsáveis pela degradação do DNA. Em longas campanhas de coleta, o acesso ao freezer é inviabilizado, sendo necessária a utilização de alguma substância capaz de preservar o material biológico. Quando os espécimes são coletados em armadilhas do tipo pitfall, é essencial que a substância usada na armadilha também preserve as moléculas.

Para a escolha da solução mais apropriada para matar e manter os espécimes, deve-se considerar alguns pontos críticos: a logística do transporte, se é permitido ou não o transporte por lei; os custos, uma vez que a maioria das pesquisas são realizadas com verba pública; a toxicidade, para o manipulador da solução e para o ambiente; a eficiência na preservação do material biológico e os efeitos da interação da substância utilizada com as intepéries ambientais.

Neste contexto, uma série de testes foi iniciada em janeiro de 2011 para padronização dos procedimentos de preservação e obtenção de sequências nucleotídicas de espécimes de Orthoptera, para utilização em estudos que envolvam taxonomia morfológica e molecular, ecologia e evolução, com o menor custo, de forma menos agressiva possível ao ambiente e de modo a preservar o maior número de estruturas possível para identificação posterior dos espécimes.

Coletas e espécimes utilizados

Os espécimes utilizados nos testes foram coletados manualmente, com redes entomológicas (puçás) e com armadilhas de queda (pitfall-traps) na Estação Experimental de Treinamento e Educação Ambiental Mata do Paraíso e na Mata da Biologia da UFV, em Viçosa/MG; bem como no município de Teixeiras/MG.

Foram coletados grilos, gafanhotos e esperanças, identificados apenas no nível de família e separados conforme morfotipos. Para todos os testes conduzidos foram utilizados 10 indivíduos.

Teste do protocolo de extração

A partir dos protocolos descritos por Waldschmidt e colaboradores (1997) para extração de DNA de abelhas, um protocolo modificado foi testado com tecidos frescos de ortópteros sem a adição de RNAse, conforme metodologia abaixo:

Protocolo inicial: inicialmente o tecido (conteúdo do tórax e/ou de uma das pernas) de cada indivíduo foi pulverizado mecanicamente com pistilo, após congelamento por nitrogênio líquido, em microtubos de centrífuga de polipropileno 2,0 mL, devidamente identificados. Foram adicionados 1000 µL de tampão de extração: CTAB (Hexadecil Trimetil Brometo de Amônio) 2%; EDTA (Ácido Tetraacético Diamina Etileno) 20 mM (pH 8,0); NaCl 1,4 M; Tris-HCl 100 mM (pH 8,0); água ultra-pura e 100 µg⁄ml de protease. Posteriormente as amostras foram incubadas a 55 °C por 60 minutos. A desproteinização foi feita em duas etapas: com um volume de fenol: clorofórmio (1:1) e com um volume de clorofórmio: álcool isoamílico (24:1); antes e após a adição destas soluções, o macerado foi resfriado por 10 minutos em gelo, devido ao fato destes componentes serem muito voláteis. Após cada desproteinização, o macerado foi centrifugado a 13.000 rpm por 8 minutos, à temperatura ambiente. A fase superior foi transferida para um novo conjunto de microtubos identificados. A precipitação dos ácidos nucléicos foi feita com um volume de isopropanol PA, seguida de incubação a -20 °C por 24 horas. O material foi então submetido à centrifugação a 14.000 rpm por 30 minutos e o precipitado formado (*pellet*) foi lavado duas vezes com etanol 70 % e seco à temperatura ambiente. Os ácidos nucléicos foram ressuspendidos em 100 µL de TE (Tris-HCl 10mM, pH 8,0; EDTA 1 mM, pH 8,0) e armazenado a -20 °C.

Com este protocolo foi possível obter DNA íntegro de todas as amostras de tecido fresco, bem como de tecidos preservados em álcool absoluto a -20 °C, testados posteriormente.

Modificações no protocolo de extração incial

Para economia de tempo, reagentes e, consequentemente, dinheiro, algumas modificações foram conduzidas e testadas com tecido fresco.

A primeira modificação realizada foi a eliminação da primeira etapa de desproteinização, que requer o uso de fenol, um potente solvente orgânico, tóxico tanto para o manipulador quanto para o meio ambiente. A segunda, foi a redução do tempo nas etapas de precipitação do DNA, de 24 para 10 horas, sob a mesma temperatura. A terceira modificação foi feita no sentido de reduzir o volume de reagentes total da extração: a partir do volume inicial de 1 mL, foi testado o volume de 750 uL. Esta redução inicial leva à redução do volume em todos os passos subsequentes, minimizando os custos do procedimento e reduzindo ainda mais o volume de resíduos gerados.

Todas as modificações não afetaram negativamente a integridade, pureza e quantidade do material genético obtido nas extrações, permitindo economia de tempo e reagentes, diminuição dos riscos de intoxicação ao manipulador e produzindo menos poluentes ao meio ambiente.

Um teste posterior foi conduzido a fim de aumentar a pureza do DNA extraído, aumentando o tempo de incubação com Proteinase K a 55 °C para 2 horas. Os resultados mostraram melhora no grau de pureza do material genético, com menor quantidade de proteínas degradadas ao final do processo.

Quando os testes conduzidos utilizando tecidos frescos apresentaram resultados positivos, amostras de tecidos preservados em álcool absoluto a -20 °C também foram testadas. Para estes tecidos preservados os resultados foram igualmente satisfatórios.

O resultado de todos os testes de extração foi verificado (integridade, pureza e quantidade do DNA obtido) por eletroforese em gel de agarose 0,8% com tampão TBE 1X e 1 uL de marcador de quantidade *Lambda DNA* (Amersham Biosciences), utilizando o volume de 3 uL de DNA e GelRed^{TM} (conforme diluição abaixo), na proporção 1:1.

Diluição do GelRed^{TM}:

- 499 uL de tampão de amostra tipo IV (tampão sacarose com azul de bromofenol)
- 1 uL GelRed^{TM} 10.000X

Para visualização das bandas de interesse os géis eram colocados sobre o transiluminador. A luz UV emitida por este aparelho faz com o GelRed^{TM} emita fluorescência, permitindo a visualização e comparação das bandas com o marcador, a fim de certificar que o fragmento correto foi amplificado.

Definição do tecido para extração de DNA

Após definição do protocolo padrão final de extração de DNA, foram conduzidos testes para a escolha da parte anatômica de onde seria retirado o tecido para extração.

Músculos frescos do tórax e pernas foram testados e ambos resultaram em quantidade e qualidade semelhantes.

Assim, a perna posterior de um dos lados foi definida para utilização, de modo a preservar a maior quantidade possível de partes anatômicas para outros estudos, como futuras revisões taxonômicas, por exemplo. Este procedimento permite ainda a eventual repetição da extração caso surjam dúvidas quanto aos resultados obtidos em estudos de sistemática

Teste de extração de DNA de amostras de coleção

O protocolo padrão final foi testado em 10 amostras de coleção preservadas em álcool 80 % sob temperatura ambiente há mais de um ano. Em todas as amostras utilizadas não foi possível obter o material genético devido à degradação. Para estas mesmas amostras foi utilizado o Kit de extração *Wizard® Genomic DNA Purification Kit* (PROMEGA), com controles positivos (amostras preservadas em álcool absoluto a -20 °C há mais de um ano). Somente foi obtido DNA dos controles positivos.

Escolha dos genes

Os genes escolhidos para desenho dos pares de *primers* foram o citocromo C oxidase I (mitocondrial) e o rRNA 18S (nuclear), devido à maior representatividade em diferentes grupos de Orthoptera no GenBank (http://www.ncbi. nlm.nih.gov/).

A partir do alinhamento de sequências completas de diferentes famílias em Orthoptera, disponíveis no GenBank, foram desenhados dois dois conjuntos de *primers*, cada um com um *forward* e um *reverse* pelo programa Oligo Explorer (ref). As sequências dos *primers* obtidos segue abaixo:

- COI_Orth_1F: 5’- ATACCTATTATAATTGGAGG -3’
- COI_Orth_1R: 5’- TARRTTTRATCAKGTWGCCA -3’
- 18S_Orth_1F: 5’- CGCGAATGGCTCATTAAATC -3’
- 18S_Orth_1R: 5’- CCTCACTAAATCATTCAATCGG -3’

Os iniciadores (*primers*) foram testados quanto ao fragmento de DNA obtido na PCR e sua repetibilidade, os resultados esperados foram obtidos após os testes descritos na seção Amplificação.

Amplificação

A amplificação dos fragmentos de interesse foi feita em 25 µl, conforme especificado na Tabela 1. Para amplificação dos genes de interesse, com os *primers* desenhados, foram conduzidos testes com PCR gradiente, sob 12 diferentes temperaturas de *annealing* para detectar a temperatura adequada para obtenção das sequências em Orthoptera. Foram utilizadas diferentes espécies de grilos, gafanhotos e esperanças. Os resultados dos testes mostraram que a temperatura ideal variava entre as amostras: de 48°C a 55°C para o COI e de 50°C a 59°C para o 18S.

Com isso, optei por utilizar a PCR na modalidade *touch down*, que consiste em ciclos descrescentes de temperatura de *annealing*, a fim de obter o amplicon independente da variação da temperatura de *annealing* de cada espécie. As reações de PCR foram então realizadas conforme os passos detalhados abaixo, num total de 40 ciclos, usando temperaturas próximas às mínimas e máximas que amplificaram nos testes de gradiente.

Tabela 1: Concentrações e volumes (em µl) dos reagentes utilizados nas reações de amplificação do gene de interesse em diferentes ortópteros

Reagentes	Concentração	Volume
Tampão*	1X	5
dNTP	0,01mM	0,125
Primer Forward	0,2uM	1
Primer Reverse	0,2uM	1

$MgCl_2$	1,5mM	1,5
Taq DNA Polimerase**	1U/reação	0,2
DNA molde	20ng	1
água mili-Q	-	13,175
Total	-	25

* GoTaq® Flexi Buffer Promega (10mM Tris-HCl, pH 8,3; 50mM KCl);
** GoTaq® Flexi DNA Polymerase Promega.

Programa “COI_TD55_45” (para amplificação do gene COI):

- Desnaturação a 94°C por 5’.
- 10 ciclos onde a temperatura de *annealing* diminui 1°C a cada ciclo. Cada ciclo compreende: 1’ a 94°C; 1’:45’’ na temperatura de *annealing* a (55 - 45°C); 1’:45” de extensão a 72°C.
- 30 ciclos com 1’ de desnaturação a 94°C, 1’45’’ a 45°C, 1 ’45 ‘‘ a 72°C.
- 5’ a 72°C
- resfriamento a 4°C.

Programa “18S_TD60_45” (para amplificação do gene 18S):

- Desnaturação a 94°C por 5’.
- 15 ciclos onde a temperatura de *annealing* diminui 1°C a cada ciclo. Cada ciclo compreende: 1’ a 94°C; 1’:45’’ na temperatura de *annealing* a (60 - 45°C); 1’: 45” de extensão a 72°C.
- 25 ciclos com 1’ a 94°C, 1’: 45’’ a 45 °C e 1’: 45’’ a 72°C.
- 5’ a 72°C
- resfriamento a 4°C.

A amplificação dos genes COI e 18S das amostras por PCR foi realizada em termociclador de gradiente BIOER com os pares (*forward* e *reverse*) de iniciadores (*primers*) COI-Orth-1F e COI-Orth-1R e 18S-Orth-1F e 18S-Orth-1R.

O resultado das amplificações foi verificado por eletroforese em gel de agarose 1,5 % com tampão TBE 1 X e 1 uL de marcador de tamanho 1 kb Plus DNA Ladder (Invitrogen), utilizando o volume de 3 uL de DNA (agora *amplicons*) e GelRed™ (conforme diluição descrita anteriormente), na proporção 1:1.

Testes com soluções matadoras e preservadoras

Diferentes procedimentos de coleta e acondicionamento do material coletado foram testados quanto ao rendimento e qualidade das extrações de DNA.

Para simular a exposição a diferentes condições de campo e de transporte campo - laboratório, os espécimes coletados foram submetidos a diferentes tratamentos:

Tratamento 1

Etanol comercial (92,8° INPM): os espécimes foram coletados manualmente e com puçá, mortos em álcool comercial e mantidos sob temperatura ambiente por até 30 dias (para simular o período entre a coleta e a chegada ao laboratório). As extrações de DNA foram feitas após 1 dia e após 30 dias nestas condições.

Tratamento 2

Etanol combustível (96° INPM): os espécimes foram coletados manualmente e com puçá, mortos em álcool combustível e mantidos sob temperatura ambiente por até 30 dias. As extrações de DNA foram feitas após 1 dia e após 30 dias nestas condições.

Tratamento 3

Solução matadora detergente: os espécimes foram coletados com *pitfall traps* contendo solução matadora aquosa com 2,5% detergente (Sperber et al 2003) e mantidos por 24 e 48h no campo. Posteriormente, metade dos espécimes foi armazenada em recipientes contendo etanol comercial e a outra metade em etanol combustível, mantidos sob temperatura ambiente por até 30 dias. As extrações de DNA foram feitas após 1, 7, 14 e 30 dias nestas condições.

Tratamento 4

Solução matadora formaldeído: os espécimes foram coletados com *pitfall-traps* contendo solução matadora aquosa com formol (10 %), etanol (70 %) e glicerina (10 %) (Sperber et al 2003) e matidos por 24 e 48h no campo. Posteriormente, metade dos espécimes foi colocada em etanol comercial e a outra metade em etanol combustível, mantidos sob temperatura ambiente por até 14 dias. As extrações de DNA foram feitas após 1, 7, 14 e 30 dias nestas condições.

Tratamento 5

Acetato de etila: os espécimes foram coletados manualmente e com puçá e posteriormente sacrificados em câmara mortífera contendo acetato de etila (que preserva a coloração dos espécimes, importante na identificação de algumas espécies). Seis indivíduos tiveram o DNA extraído imediatamente, seis foram matidos em etanol comercial e seis em etanol combustível à temperatura ambiente por até 30 dias. A extração de DNA dos espécimes nas duas últimas condições foi feita após 1, 7, 14 e 30 dias.

Dos tratamentos testados, apenas as extrações do Tratamento 4 não funcionaram, devido à presença de formol, que causa danos à molécula de DNA.

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Ethanol fuel improves arthropod capture in pitfall traps and preserves DNA

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Abstract

We tested the value of ethanol fuel as a killing solution in terms of sampling efficiency (species richness and accumulated abundance) and DNA preservation of Ensifera ground-dwelling specimens. Sampling efficiency was evaluated comparing abundance and species richness of pitfall sampling using 100% ethanol fuel, with two alternative killing solutions. We evaluated the DNA preservation efficiency of the killing solutions and of alternative storage solutions. Ethanol fuel was the most efficient killing solution, and allowed successful DNA preservation. This solution is cheaper than other preserving liquids, and is easily acquired near field study sites since it is available at every fuel station in Brazil and at an increasing number of fuel stations in the U.S. We recommend the use of ethanol fuel as a killing and storage solution, because it is a cheap and efficient alternative for large-scale arthropod sampling, both logistically and for DNA preservation. For open habitat sampling with high day temperatures, we recommend doubling the solution volume to cope with high evaporation, increasing its efficacy over two days.

Keywords

Killing solutions, molecular tools, taxonomy, large-scale fieldwork, Brazil

Introduction

Several sampling techniques are used to assess biodiversity of different animal species (King and Porter 2005). All present advantages and disadvantages, so the choice is at the discretion of the researcher. Small organisms (e.g. arthropods) are frequently hand-sampled, which provides information on the organism's habits and behavior, but this method is of little use for ecological comparisons, because of collector interference (Krebs 1999, Southwood and Henderson 2000).

Pitfall traps are a good alternative for collecting ground-dwelling arthropods (Dahl 1896). This kind of trap is inexpensive and easy to handle, allowing both rich and abundant samples. It can be used for taxonomic (although some coloration characters may be lost), ecological, morphological and molecular studies (Gurdebeke and Maelfait 2002, Schoereder et al. 2004, Sperber et al. 2007, Mews et al. 2008, Pereira et al. 2010). One of the main challenges is deciding which killing solution to use in the pitfall traps, which depends on the objectives of each study. As far as sampling involves financial, environmental and researcher's effort costs, the ideal solution should minimize those costs and maximize the utility of the sampled material. The utility of the samples may extrapolate strictly ecological purposes, and should involve other scientific areas, such as morphology and molecular biology. Therefore an ideal should also preserve the specimens' tissues and DNA (Stevens et al. 2011).

Regarding methodological necessities in pitfall sampling, a good killing solution should minimize evaporation, as far as many pitfall trap regimes check traps every 2 weeks or more. A good solution should not be toxic to the researcher nor environmentally harmful. Regarding sampling efficiency, a good solution should kill quickly so as to reduce the escape of specimens. In addition, the trap solution cannot be prohibitively expensive, and must be readily available.

Finding a solution that meets all of these specifications is not easy. Many types of solutions have been used and tested, for example water and detergent, which is inexpensive but accelerates the decomposition of tissues and genetic material (Schmidt et al. 2006). Mixtures of formaldehyde and ethylene glycol (Barber 1931, Sperber et al. 2003b, Schmidt et al. 2006), are efficient in killing and preserving tissue, but are toxic and do not preserve DNA (Aristophanous 2010). Other solutions contain salt brines (Sasakawa 2007) and acetic acid (Gurdebeke and Maelfait 2002), which do not preserve tissues and can alter gonads, genitalia and eggs (Sasakawa 2007). An additional class of solutions contains different concentrations of commercial alcohol (Sperber et al. 2003a, Paquin 2008, Chen et al. 2011), which evaporates faster than the other solutions, but preserves the internal and external organs through tissue dehydration.

It has been shown that at concentrations higher than 95%, commercial alcohol preserves DNA (Nagy 2010), but the use of highly concentrated commercial alcohol as a killing solution may be prohibitively expensive when needed in large quantities, such as in large-scale biodiversity sampling. In Brazil, for example, it is illegal to carry large amounts of commercial alcohol on long journeys, which could hinder its use in extensive field expeditions. Here we propose the use of ethanol fuel as a cheaper and logistically feasible alternative.

In Brazil, ethanol fuel and commercial alcohol have some differences. While the alcoholic concentration (92.6 to 93.8%) and the amount of water (6.2 to 7.4%) varies in ethanol fuel, in commercial alcohol the alcoholic concentration (92.8%) and the amount of water (7.2%) is fixed. The largest difference is, however, the quantity of gasoline present in ethanol fuel (up to 30 milliliters per liter), that is absent in commercial alcohol (BR0029 2011). In the United States, the highest concentration of ethanol fuel includes 85% ethanol and 15% gasoline (Tatum 2010). Ethanol fuel is available throughout Brazil, at all fuel stations, and at an increasing number of fuel stations in the U.S. (Méjean and Hope 2010, Sorda et al. 2010) and is at least 50% cheaper than commercial alcohol.

In this study, we tested the value of ethanol fuel as a pitfall trap killing solution in terms of sampling efficiency (richness and abundance) and DNA preservation of Ensifera ground-dwelling specimens, comparing 100% ethanol fuel with two alternative killing solutions.

Material and methods

Sampling efficiency

Field sampling site

To evaluate sampling efficiency, we conducted field sampling in a primary Atlantic Forest reservoir, the Iguaçu National Park, in Foz do Iguaçu municipality (25°32'S, 54°35'W, 195m above sea level), Paraná State, in January 2010. The vegetation is mostly tropical semideciduous forest and Araucaria forest, within the Atlantic Forest biome (Rizzini 1997, Dias et al. 1998). The climate is mesothermal subtropical superhumid, with average annual temperatures between 18 and 20 °C and an average rainfall of 1600mm (Peel et al. 2007).

Sampling design

We compared the efficiency of 100% ethanol fuel pitfall killing solution (Solution 1) for ground-dwelling Orthoptera, against the conventional killing solution, comprised of 80% commercial alcohol (80°GL) + 10% glycerin (P.A) + 10% formaldehyde (P.A) (Sperber et al. 2003b) (Solution 2), and a solution of 90% commercial alcohol (80°GL) + 10% glycerin (P.A) (Solution 3). GL is the amount, in milliliters, of absolute alcohol contained in 100 milliliters of hydro-alcoholic solution. P.A., or 'Pro Analysis' means that the sample is of a very high purity, sufficient to be used in chemical analyses. Formaldehyde is recommended for better preservation; glycerin is used to prevent stiffening of the sampled specimens.

For this comparison, we designed the following field experiment. We established a transect of 5km, starting at a distance of 100m from the forest's edge. At the beginning of the transect a set of five pitfall traps, containing one of the three killing solutions chosen randomly, were placed perpendicularly to the transect, 2m apart from one another. After the next 30m on the transect, we placed the second set with a different, randomly chosen, killing solution. After another 30m along the transect, we placed the third set, with the third killing solution. After an additional forty meters we began the procedure again, and repeated it a total of 50 sampling stations. In summary each sampling station contained five pitfall traps with each of the three killing solutions, for a total sampling effort of 750 pitfall traps. Traps consisted of polyethylene vials, 20cm in diameter and 22cm deep, filled with 500ml of killing solution. After 48 hours, specimens were removed from the the traps, identified and stored in ethanol fuel, after gathering the data.

Data analysis

To evaluate sampling efficiency of ethanol fuel as a pitfall killing solution, we compared cricket species richness and accumulated abundance (= total number of individuals per pitfall set) among the three solutions. Each pitfall set was considered one sampling unit, rendering 150 replicates. We performed one-way analysis of variance (ANOVA), adjusting generalized linear models (GLMs) with Poisson error distribution, correcting for over- or under-dispersion using quasi-Poisson when necessary. We considered cricket species richness and accumulated abundance in each set of five pitfall traps as response variables (n = 150), and the type of killing solution as the explanatory factor. We used contrast analyses to evaluate effect differences among the kinds of solution, simplifying the complete models by amalgamating non-significantly different factor levels (Crawley 2007). We used Chi-square (χ^2) test for Poisson error distributions, and the *F* test in cases where there was a correction for over- or under-dispersion, as recommended by Zuur et al. (2009). We checked residuals for homoscedasticity. All analyses were undertaken within the R 2.15 environment (R Development Core Team 2012).

DNA preservation

Killing and storage

To test the DNA preservation properties of each pitfall killing solution, we placed each of 18 living cricket specimens of *Gryllus* sp. (not identified) into one of the three pitfall killing solutions, totaling six specimens per solution. As a control, we separately placed another six crickets into undiluted commercial alcohol (92.8°GL), which is considered a good preservative of DNA (Nagy 2010). Twenty-four hours later, we took one leg of each individual and extracted its DNA. Twenty-four hours later (*i.e.* 48 hours after immersion into the killing solution), we removed a second leg off the crickets to evaluate DNA preservation, analogous to in the field procedure collecting time of 48 hours, as recommended by Sperber et al. (2003a) for ground-dwelling Orthoptera sampling.

To evaluate the efficiency of ethanol fuel as a storage solution, we stored each cricket specimen, after 48 hours in the killing solution, in one of two storage solutions: undiluted commercial alcohol (92.8°GL) or undiluted ethanol fuel. To test the effect of time and type of storage solution on the DNA preservation efficiency, we removed a third leg off each cricket after 15 days, and a fourth leg after 30 days in the storage solution.

We evaluated efficiency of DNA preservation for the 24 crickets used in the above procedure. Each set of six individuals was submitted to one of four different killing solutions, and each individual provided two samples (= legs) for DNA extraction before storage (24 and 48 hours in the killing solution). Individuals from each killing solution were transferred to either commercial alcohol or ethanol fuel for storage, providing three replicates (individuals) per storage solution, and two further samples (= legs) per individual, 15 and 30 days in the storage solution. All specimens were maintained at room temperature for 30 days.

DNA extraction

Total DNA was isolated from each individual using the protocol described in Waldschmidt et al. (1997) but without the deproteinization step with phenol:chloroform (1:1). Preliminary analysis of fresh specimens killed by freezing showed that tissue extractions from the thorax or legs were equally effective. Therefore, we chose to use only the legs, allowing maximum preservation of anatomical parts for further studies, and repeated sampling of the same individuals with minimum tissue damage.

DNA extractions were verified via agarose gel (0.8%) electrophorese, prepared and run in 1X TBE Buffer, stained with ethidium bromide and viewed under UV light. The quality of the extractions was checked by comparison with the extract made from fresh material (specimens that were killed by freezing, with immediate DNA extraction). Extractions from fresh material presented two bands, the first clearly marked and bright, corresponding to genomic DNA and the second smaller, more opaque, corresponding to RNA. We considered DNA as properly preserved when we detected a well-defined single band of DNA without apparent trawlers.

Results

Sampling efficiency

We collected 3,528 individuals of 14 species from four different families of Orthoptera, following the classification of Desutter-Grandcolas (1987, 1988): Phalangopsidae (2,090 individuals of eight species), Trigonidiidae (835 individuals of two species), Gryllidae (394 individuals of two species) and Eneopteridae (209 individuals of two species). Species richness ($F_{2,147}$ = 177.09; $p < 0.001$) and abundance ($F_{2,147}$ = 104.64; $p < 0.001$) were significantly higher in pitfalls with ethanol fuel killing solution (Figure 1 A, B) than in those containing the other two solutions. Sampling efficiency was not different between killing solution 2 and 3 (richness: $F_{2,147}$ =0.34; $p = 0.55$; abundance: $F_{2,147}$ = 2.87; $p = 0.09$).

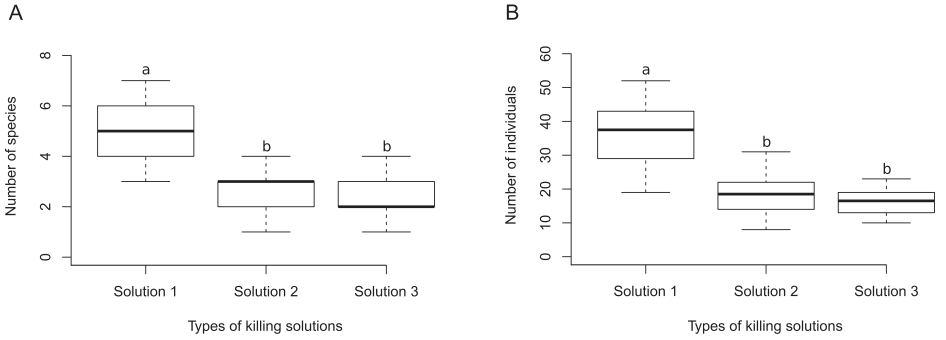

Figure 1. Boxplot showing sampling efficiency of different kinds of pitfall traps' killing solution. Traps with **Solution 1** (100% ethanol fuel) captured more species and individuals than **Solution 2** (80% commercial alcohol (80°GL) + 10% glycerin (P.A) + 10% formaldehyde (P.A)) and **Solution 3** (90% commercial alcohol (80°GL) + 10% glycerin (P.A)). **A** Total number of species per pitfalls' set. **B** Total number of individuals per pitfalls' set. Different lower case letters correspond to significant differences between killing solution levels, evaluated through contrast analyses.

DNA Preservation

Table 1 indicates that both solution 1 and solution 3 were efficient in preserving DNA and are appropriate for use as killing solutions in pitfall traps that must remain in the field for up to 48 hours, with no visible damage to DNA. In addition, these samples can be stored at room temperature for up to 30 days in either commercial alcohol or ethanol fuel. On the other hand, our results suggest that just 24 hours in solution 2 (commercial alcohol + glycerin + formaldehyde) are enough to destroy the DNA of the samples (Figure 2).

Table 1. Success (yes) or failure (no) of DNA extractions after different periods (Time in the solution) in Killing solution (Pitfall: 24h and 48h) and in storage solution (C.A. and E.F.: 15 and 30 days). C.A. = undiluted commercial alcohol (92.8°GL); E.F. = undiluted ethanol fuel; Solution 1 = E.F.; Solution 2 = 80% commercial alcohol (80°GL) + 10% glycerin (P.A.) + 10% formaldehyde (P.A.); Solution 3 = 90% commercial alcohol (80°GL) + 10% glycerin (P.A.). All material was maintained at room temperature. Asterisks mark the treatments shown in Figure 2.

Killing solutions	**Time in the solution**					
	Pitfall		C.A.		E.F.	
	24h	48h	15days	30days	15days	30days
C.A.	yes	yes	yes	yes*	yes	yes*
Solution 1	yes	yes	yes	yes*	yes	yes*
Solution 2	no*	-	-	-	-	-
Solution 3	yes	yes	yes	yes*	yes	yes*

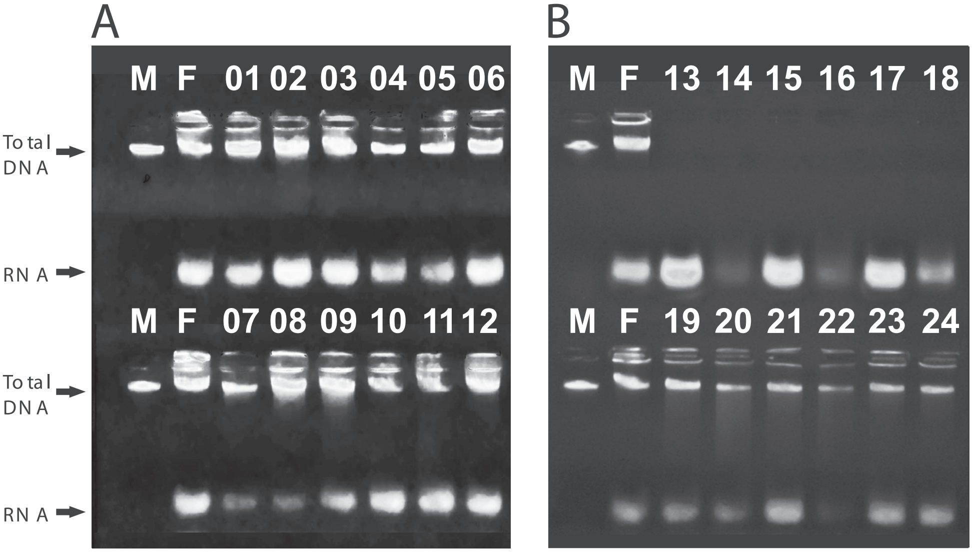

Figure 2. Electrophoresis of all 24 analyzed individuals. M represents the lambda DNA marker (100 ng/ul) and F represents the control extraction made using fresh tissue. A) Lanes 01 – 06, individuals killed in C.A. (undiluted commercial alcohol), maintained in the killing solution for 48 hours and then transferred to closed vials containing C.A. (01 – 03) and E.F. (03 – 06) and maintained in these storage solutions for 30 days. Lanes 07 – 12, individuals killed in Solution 1 (= E.F.), maintained in the killing solution for 48 hours and transferred to C.A. (07 – 09) and E.F. (10 – 12) and maintained in these storage solutions for 30 days. B) Lanes 13 – 18, individuals killed in the Solution 2 and maintained in this solution for 24 hours. Lanes 19 – 24, individuals killed in Solution 3, maintained in this solution for 48 hours, than transferred to C.A. (19 – 21) and E.F. (22 – 24) and maintained in these solutions for 30 days. All DNA extractions where successful, but those of crickets killed in solution 2 (lanes 13 – 18).

Discussion

In this study, we investigated the efficiency of ethanol fuel as a pitfall killing solution in terms sampling efficiency, as measured by species richness and accumulated abundance, and in terms of DNA preservation. Our results indicate increased sampling and preservation efficiency of ethanol fuel, compared to the commonly used alternatives. Below we discuss the advantages and disadvantages of using ethanol fuel as a pitfall killing and storage solution, with particular emphasis on large-scale field expeditions.

Financial costs

Of the solutions tested in our study, ethanol fuel is the least expensive option: 1 liter of ethanol fuel (US$ 1.25 on average) costs less than half the price of 1 liter of commercial alcohol (US$ 3.15), which does not include the other components, such as glycerin and formaldehyde, which cost around US$ 15.00 a liter (prices for Brazil).

Field logistics

The transportation of flammable or toxic liquids is dangerous and illegal under Brazilian and international law. This danger increases with the distance, and consequently time spent in transportation. Ethanol fuel presents a partial solution to this limitation: as it can be bought near the field study sites, at any fuel station in Brazil, the distance of transportation is diminished, decreasing the danger. Large field expeditions can use these facilities to reduce the distances of ethanol transportation, thus reducing the risks of accidents, and simplifying expedition logistics. Even so, for transportations and storage of collected material, we recommend using firm, pressure-resistant bottles, with sealed caps, fully filled with ethanol, so as to minimize oxygen within the bottle, reducing explosion risks. We used PET tubes, which have low costs and may be bought in large quantities.

Commercial alcohol has to be purchased in large shops when bought in large quantities, and is hardly available in the small towns that border most of the large conservation areas. Therefore it would require long-distance transportation and represent huge environmental and personal risks. The additional components of the tested killing solutions (glycerin and formaldehyde), are only available in specialized establishments, restricted to a few large cities in Brazil (Brazilian Federal Law n°10.357/2001).

Sampling efficiency

We showed that ethanol fuel presented higher sampling efficiency, both for species richness and accumulated abundance of ground-dwelling Orthoptera species, therefore maximizing the gains of the sampling effort. We hypothesize that this higher sampling efficiency is related to the lower density and surface tension of the solution 1 (density = 0.81 g/cm^3; surface tension = 21.55 mN/m^{-1}) than solution 2 (density = 0.92 g/cm^3; surface tension = 48.56 mN/m^{-1}) and solution 3 (density = 0.97 g/cm^3; surface tension = 55.34 mN/m^{-1}) (Adamson and Gast 1997), which could cause the crickets to sink and die faster in ethanol fuel, reducing their chances of escape from the trap.

One piece of evidence in favor of our hypothesis is that all winged cricket species captured in this study died exclusively within pitfalls that used ethanol fuel as the killing solution (94 individuals of *Eneoptera* sp. and 183 individuals of *Gryllus* sp.). These genera contain species of large body size, which are powerful jumpers as nymphs and powerful fliers as adults, and are rarely captured in conventional pitfall traps killing solution (N. Szinwelski, personal observation). Indeed, C.F. Sperber, in other field collections, has observed adults of *Eneoptera* sp. flying out of pitfalls with water + detergent killing solution. The alternative pitfall design used to prevent escape from traps, using an inverted funnel at the trap's top (Melbourne et al. 1997), may reduce sampling efficiency, especially for good jumpers and fliers.

DNA preservation efficiency

To obtain DNA samples, it is recommended that the sampled organisms be removed from the pitfall killing solution as soon as possible and placed in vials containing highly concentrated alcohol, preferably at low temperatures (Nagy 2010). Based on the results presented here, we suggest that sampled organisms may be safely stored in undiluted ethanol fuel at room temperature, without major damage to DNA quality, for up to 30 days.

Indeed, we were able to obtain sequences of mitochondrial DNA (COI) and nuclear (18S rRNA) of Orthoptera specimens kept for two weeks in ethanol fuel killing solution, before being sorted and stored in undiluted commercial ethanol (92.8°GL), where they remained at 38°C – 45°C room temperature for another 45 days (in Manaus – AM) and 70 days at similar temperature (in Cuiabá – MT).

Counterarguments

One of the main arguments against the use of ethanol fuel as a pitfall trap killing solution is that it evaporates faster than other solutions, making its use limited to high temperature areas. We were, however, able to use ethanol fuel pitfall traps successfully in Amazon forest sampling (38°C – 45°C), where the traps were kept for 48h in the field without significant volume reduction of the killing solution.

Solution evaporation is a limiting factor in open habitat with high temperatures as Brazilian "Campo Cerrado", for example. In such field conditions, we recommend increasing the killing solution volume by 100%, from 500ml to 1000ml, to maintain sufficient killing solution volume in the traps after 48h in the field.

Another problem with ethanol fuel is the fact that it can be denatured. In Brazil, that means that every liter of ethanol fuel can contain up to 30ml of gasoline. In the United States every liter of ethanol E85 contain 150ml of gasoline. This may represent an environmental problem if the pitfall is damaged and the solution is spread in the environment. Moreover, gasoline might hinder DNA preservation. For Brazilian ethanol fuel we showed that this did not occur. Even specimens collected in ethanol fuel, were successfully preserved and we were able to extract DNA and run PCR reactions obtaining sequences of mitochondrial COI and nuclear rRNA18S .

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