GABRIELA DE FIGUEIREDO JACINTHO

CITOGENÉTICA E TAXONOMIA DE ESPÉCIES NEOTROPICAIS DE *STRUMIGENYS* SMITH (HYMENOPTERA: FORMICIDAE: MYRMICINAE)

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*.

Orientador: José Eduardo Serrão

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RESUMO

JACINTHO, Gabriela de Figueiredo, M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Citogenética e taxonomia de espécies Neotropicais de *Strumigenys* Smith (Hymenoptera: Formicidae: Myrmicinae)**. Orientador: José Eduardo Serrão. Coorientadoras: Denilce Meneses Lopes e Luísa Antônia Campos Barros

As formigas *Strumigenys* são globalmente distribuídas e hiperdiversas, sendo compostas por centenas de espécies diminutas que habitam principalmente a serapilheira de florestas tropicais. O gênero foi revisado em 2000, aumentando em quase 28% no número de espécies descritas apenas nos neotrópicos. Atualmente há 213 espécies neotropicais que estão divididas em 41 grupos de espécies. Dentre estas, os grupos *gundlachi* e *louisianae* abrigam as formigas mais comuns na fauna Neotropical do gênero. Embora os dados moleculares para *Strumigenys* sejam recentes e a alfa-taxonomia bastante avançada, variações continuam a se acumular em coleções mirmecológicas. O aumento de registros de distribuição de representantes dos grupos *gundlachi* e *louisianae*, evidenciou variações morfológicas e cromossômicas em determinadas populações, indicando possíveis questões taxonômicas. A citogenética é uma ferramenta útil na visualização grupos taxonômicos complexos em Formicidae, e pode contribuir com resoluções destes grupos de espécies de *Strumigenys.* Apesar de sua alta abundância, o gênero raramente tem sido abordado em estudos citogenéticos, e os dados para a região Neotropical estão restritos a três espécies. Um capítulo desta dissertação consiste no estudo morfológico e taxonômico do complexo-*crassicornis* (grupo-*gundlachi*), descrevendo novas espécies ao gênero. O segundo capítulo descreve e analisa os cariótipos de seis espécies neotropicais de *Strumigenys* dos grupos *gundalchi* e *louisianae*. O presente estudo faz uso combinado de morfologia e citogenética tanto para evidenciar questões taxonômicas dentro de *Strumigenys*, quanto para propor soluções.

Palavras-chave: Formigas. Morfologia. Cariótipo.

ABSTRACT

JACINTHO, Gabriela de Figueiredo, M.Sc., Universidade Federal de Viçosa, February, 2023. **Cytogenetics and taxonomy of Neotropical species of *Strumigenys* Smith (Hymenoptera: Formicidae: Myrmicinae)**. Advisor: José Eduardo Serrão. Co-advisors: Denilce Meneses Lopes and Luísa Antônia Campos Barros.

The ants *Strumigenys* are globally distributed and hyperdiverse, and are composed of hundreds of tiny species that mainly inhabit the litter of tropical forests. The genus was revised in 2000, increasing by almost 28% of species described in the Neotropics alone. Currently, 213 Neotropical species are classified into 41 species groups. Among these, the *gundlachi* and *louisianae* groups are home to the most common ants in the Neotropical fauna of the genus. Although molecular data for *Strumigenys* are recent and alpha-taxonomy is quite advanced, variations continue to accumulate in myrmecological collections. The increase in distribution records of representatives of the gundlachi and louisianae groups has shown morphological and chromosomal variations in certain populations, indicating possible taxonomic issues. Cytogenetics is a useful tool in visualizing complex taxonomic groups in Formicidae, and can contribute to resolutions of these *Strumigenys* group-species. Despite its high abundance, this genus has rarely been addressed in cytogenetic studies, and data for the Neotropical region are restricted to three species. One chapter of this dissertation consists of a morphological and taxonomic analysis of the *crassicornis*-complex (*gundlachi*-group), describing new species for the genus. The second chapter describes and analyzes the karyotypes of five neotropical species of *Strumigenys* from the two mentioned groups. The present study combined the use of morphology and cytogenetics both to highlight taxonomic issues within *Strumigenys* and to suggest solutions.

Keywords: Ants. Morphology. Karyotype.

SUMÁRIO

I. Introdução Geral

As formigas constituem Formicidae Latreille, 1809, sendo definidas por Bolton (1994) como himenópteros aculeatas com dimorfismo sexual, apresentando glândulas metapleurais, antenas geniculadas e forte constrição entre o segundo e o terceiro segmentos abdominais, o pecíolo. Atualmente há mais de 13 mil espécies válidas, sendo caracterizadas pela eusocialidade obrigatória ou por serem parasitas sociais (Bolton, 2003; Boudinot *et al.*, 2021). A família é ecologicamente dominante na maioria dos hábitats terrestres em termos de abundância e biomassa, distribuindo-se globalmente (Hölldobler and Wilson, 1990; Ward, 2007). Myrmicinae Lepeletier de Saint-Fargeau, 1835 constitui a maior subfamília dentre as 19 válidas para Formicidae, contando com mais de sete mil espécies distribuídas em 147 gêneros (Bolton, 2023).

Dentre os Myrmicinae, *Strumigenys* Smith, 1860, membro de Attini, é formado por mais de 850 espécies (Bolton, 2023). As formigas representantes do gênero são diminutas e cosmopolitas (Bolton, 1999). Apesar das diversas espécies encontradas em ecossistemas temperados, *Strumigenys* atinge o pico de sua diversidade em florestas tropicais (Bolton, 1998). O grupo compõe uma significativa parte da fauna de formigas destes ambientes, tanto em termos de riqueza quanto de abundância, habitando, predominantemente, a serapilheira e solo superficial, sendo as principais predadoras de colêmbolas e outros insetos de corpo mole (Bolton, 1999; Ward, 2000).

Em 2000, Bolton revisou integralmente o gênero, o qual teve sua diversidade quase dobrada. A partir de 2000, diversas descrições taxonômicas foram realizadas para *Strumigenys*, e apenas nos Neotrópicos o grupo cresceu quase 28%, agrupando atualmente 213 espécies (Longino, 2006; Sosa-Calvo *et al.*, 2006; Sosa-Calvo *et al.*, 2010; Rigato and Scupola, 2008; Baroni Urbani and De Andrade, 2007; Lattke and Aguirre, 2015; Booher *et al.*, 2019; Booher and Hoenle, 2021). Contudo, apesar de ter uma alfa-taxonomia bastante avançada, possíveis espécies novas ainda não descritas, continuam a se acumular em coleções mirmecológicas (J. Chaul, personal communication, November, 2022).

Em sua revisão, Bolton (2000) separou *Strumigenys* em pequenos grupos. Os grupos são considerados conjuntos informais de espécies provavelmente próximas e que normalmente estão restritas a apenas uma região biogeográfica. Seguindo a classificação de grupos de Bolton, *Strumigenys* se divide em 117 grupos de espécies, 41 desses Neotropicais (Bolton, 2000). Dentre os grupos Neotropicais, alguns se destacam por abrigarem espécies que compõem os táxons de formigas mais comuns da fauna neotropical do gênero, sendo eles *gundlachi* (Bolton, 2000) e *louisianae* (Bolton, 2000) (Bolton, 2000; Silva *et al.*, 2022).

O grupo *gundlachi* possui duas divisões internas, o complexo-*gundlachi* (22 spp.) e complexo-*crassicornis* (10 spp.). Algumas das espécies deste grupo figuram entre as mais comumente encontradas na serrapilheira de toda região, incluindo *Strumigenys denticulata* Mayr 1887, *Strumigenys eggersi* Emery, 1890 e *Strumigenys subedentata* Mayr, 1887 (complexo-*gundlachi*), e *Strumigenys crassicornis* Mayr, 1887 (complexo-*crassicornis*), por representarem espécies comuns no Neotrópico (Silva *et al.*, 2022).

O grupo *louisianae* é composto por quatro espécies, sendo *Strumigenys louisianae* Roger, 1863 uma das mais comumente encontradas no mundo, com uma distribuição que cobre toda a área do sudeste dos Estados Unidos ao nordeste da Argentina (Guénard *et al.*, 2017). Ao longo dessa distribuição ocorre uma alta variação morfológica quanto ao tamanho, esculturação do exoesqueleto, pilosidade e, coloração, o que pode indicar a presença de um complexo de espécies (Bolton, 2000). Atualmente, existem 13 espécies, subespécies e /ou variações sinonimizadas sob *S. louisianae* (Brown, 1961; Bolton, 2000), sendo elas: *Strumigenys bruchi* Forel, 1921, *Strumigenys clasmospongia* Brown, 1953, *Strumigenys costaricensis* Weber, 1934, *Strumigenys cubaensis* Mann, 1920, *Strumigenys fusca* Emery, 1894, *Strumigenys guatemalensis* Weber, 1894, *Strumigenys lacicephala* Smith, 1931, *Strumigenys longicornis* Emery,1894, *Strumigenys obscuriventris* Wheeler, 1908, *Strumigenys producta* Brown, 1953, *Strumigenys soledadensis* Weber, 1934, *Strumigenys unidentata* Mayr, 1887 *e Strumigenys unispinulosa* Emery, 1890.

Dado o aumento no registro de representantes dos grupos de espécies supracitados, uma consequente expansão na distribuição destas é notado (Janicki *et al.*, 2016; Antweb, 2023). Essa ampliação de dados disponíveis evidencia variações entre diferentes populações de uma mesma espécie, o que coloca em dúvida sua unidade taxonômica. No grupo-*gundlachi* a variação morfológica entre populações de algumas espécies parece indicar problemas similares (Silva *et al.*, 2022). Além disso, a variabilidade morfológica e cromossômica observada em *S. louisanae* colocam em cheque os seus limites (Barros *et al.*, 2021). Portanto, apesar da detalhada descrição morfológica, Bolton (2000) não esgotou as questões taxonômicas existentes em *Strumigenys*.

Ao longo dos últimos anos, ocorreram avanços em estudos filogenéticos de formigas com o uso de dados moleculares (Moreau *et al.*, 2006; Brady *et al.*, 2006; Ward *et al.*, 2010; Brady *et al.*, 2014; Ward *et al.*, 2015; Ward *et al.*, 2016). Estes trabalhos propõem hipóteses filogenéticas entre clados, na maioria das vezes, compostos por centenas de espécies. Porém, a alfa-taxonomia, geralmente, é excluída dos estudos moleculares, o que favorece uma desproporção no conhecimento entre grupos supraespecíficos (maior resolução) e o nível de espécies (menor resolução) (Chaul, 2018). A primeira filogenia molecular de *Strumigenys* foi recentemente publicada (Booher *et al.*, 2021), revelando a estruturação do gênero para a maioria de suas espécies. Contudo, inclui apenas um único representante para a maioria das espécies analisas, não detectando a diversidade críptica do gênero, sendo necessário o uso de outras técnicas.

A citogenética tem se mostrado uma ferramenta útil na resolução de outros grupos taxonômicos complexos em Formicidae, permitindo distingui-los em relação ao número, morfologia, padrão de bandas cromossômicas e/ou a distribuição das regiões de genes ribossomais (citogenética molecular) (Cristiano *et al.,* 2013; Correia *et al.,* 2016; Santos *et al.*, 2016; Aguiar *et al.,* 2017). Estes estudos em formigas tiveram seu início em 1960, levando a descrição do cariótipo de aproximadamente 800 morfoespécies de diversas regiões biogeográficas (Lorite and Palomeque, 2010; Shattuck and Lubertazzi, 2021). Apesar de sua alta abundância, *Strumigenys* é um gênero pouco abordado em estudos citogenéticos, com o número de cromossomos (2n) conhecido, na sua maioria, em espécies das regiões Indo-Malaia e Australiana (Brown, 1949; Crozier, 1975; Imai *et al.*, 1977; Goni *et al.*, 1982; Imai *et al.*, 1983; Imai *et al.*, 1985). Em contraste, os dados para a região Neotropical se restringem a três espécies (Alves-Silva *et al.*, 2014; Aguiar *et al.*, 2020; Barros *et al.*, 2021; Teixeira *et al.,* 2021).

Estudos em espécies Neotropicias de *Strumigenys* se destacam por representarem os primeiros a utilizarem técnicas de bandamentos e técnicas citogenéticas moleculares para o gênero (Alves-Silva *et al.*, 2014; Barros *et al.*, 2021; Teixeira *et al.,* 2021). Além disso, também mostraram a citogenética como uma alternativa durante delimitação de espécies altamente similares — crípticas — agrupadas sob o mesmo epíteto, *e.g. S. louisianae* (Barros *et al.*, 2021). Visto isso, percebe-se uma série de lacunas taxonômicas no gênero *Strumigenys*, mesmo quando se trata de espécies comuns, ficando evidente a necessidade de análises integrativas que auxiliem na delimitação destas.

O presente estudo, apresentado em dois capítulos, tem o objetivo de aprimorar a caracterização morfológica e cromossômica de espécies Neotropicais altamente similares de *Strumigenys*. Mais especificamente, o primeiro capítulo, além de apresentar novos caracteres morfológicos para o complexo-*crassicornis* (grupo-*gundlachi*), descreve duas novas espécies, atualizando a diagnose de espécies proximamente relacionadas. No segundo capítulo, a citogenética clássica (número, e morfologia cromossômica) e molecular [localização dos genes rDNA 18S, do motivo telomérico $(TTAGG)_6$ e do motivo microssatélite $(GA)_{15}$] foram analisadas em seis espécies de *Strumigenys*. Apesar de também apontar um complexo de espécies através de dados citogenéticos compilados, somados de dados morfológicos, o capítulo tem o principal foco de comparar os dados já obtidos para o gênero e detectar padrões cariotípicos para *Strumigenys* Neotropicais.

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III. CAPÍTULO I

NEW SPECIES AND DIAGNOSES OF SIMILAR *STRUMIGENYS* SMITH OF *GUNDLACHI*-GROUP BOLTON (HYMENOPTERA: FORMICIDAE: MYRMICINAE)

ABSTRACT

The global ant genus *Strumigenys* has currently 213 valid species only in the Neotropics. Among them, 32 similar ones are clustered in the *gundlachi*-group. Inside this group *S. auctidens*, *S. stenotes*, and *S. zeteki* are likely closely related due to high morphological similarity, and since their description, their distribution has greatly expanded. Here we reassessed the boundaries of these three species by studying part of the material of these new records. As a result, we describe two new Neotropical *Strumigenys*, *Strumigenys* ufv-110 **sp.nov.**, and *Strumigenys* ufv-111 **sp.nov.**, and provide new diagnoses and comments on their morphological variation.

Keywords: Taxonomy; Leaf-littter ants; High-similarity; Morphology.

1. Introduction

Strumigenys Smith, 1860 is a cosmopolitan ant genus belonging to Attini (Bolton, 2000; Ward *et al.*, 2015). It has more than 850 extant species (Bolton, 2023), mainly in the tropical or subtropical region, and frequently found in leaf litter, rotten wood, and topsoil, where they nest and forage (Bolton, 1999; Ward, 2000). In 2000, Bolton revised *Strumigenys*, a comprehensive global analysis that divides the genus into several species groups. These groups represent informal collections of species with high morphological similarity, which are probably related, and usually restricted geographically (Bolton, 2000).

During that review, 78 new species of *Strumigenys* were described for the Neotropics (*sensu* Fisher, 2009), totaling 189 species in that biogeographic region (Bolton, 2000). After several studies, 24 other species have been described in the Neotropics and 213 species are now known (Longino, 2006; Sosa-Calvo *et al.*, 2006; Sosa-Calvo *et al.*, 2010; Rigato and Scupola, 2008; Baroni Urbani and De Andrade, 2007; Lattke and Aguirre, 2015; Booher *et al.*, 2019; Booher and Hoenle, 2021). According to Bolton's classification, there are 41 species-groups of the genus in the Neotropics (Bolton, 2000), including the *gundlachi*-group, which has two internal divisions: the *gundlachi*-complex (22 species) and the *crassicornis*-complex (10 species) (Bolton, 2000).

Species of the *gundlachi*-complex, such as *Strumigenys gundlachi* (Roger, 1862), *Strumigenys denticulata* Mayr, 1887, *Strumigenys eggersi* Emery, 1890, and *Strumigenys subedentata* Mayr, 1887, were formely grouped in the genus (or subgenus of *Strumigenys*, depending on the year) *Pyramica* Roger, 1862. In contrast, species in the *crassicornis*-complex have a more complex taxonomic history. In 1948, Brown described the genus *Neostruma* to group two species that were considered closely related to the subgenus *Pyramica* but differed considerably in mandible characteristics (dentition and apical fork): *Strumigenys crassicornis* Mayr, 1887, the type of *Neostruma*, and *Strumigenys brevicornis* Mann, 1922. A decade later, three additional species were added to *Neostruma*: *S. metopia* (Brown 1959), *S. myllorhapha* (Brown 1959), and *S. zeteki* (Brown 1959). In Bolton's revision, those species belonging to *Neostruma*, along with some described in his work [*Strumigenys aethegenys* (Bolton, 2000), *Strumigenys auctidens* (Bolton, 2000), *Strumigenys crementa* (Bolton, 2000), *Strumigenys pasisops* (Bolton, 2000), and *Strumigenys stenotes* (Bolton, 2000)], were included in the *crassicornis*-complex.

After a long debate about the boundaries of *Strumigenys* and related genera, it seems that the propositions of *Strumigenys* as a cosmopolitan and megadiverse genus, of which all dozens of genera described in the past are synonyms, have been supported (Baroni Urbani and DeAndrade, 1994; Bolton, 1999; Baroni Urbani and DeAndrade, 2007; Booher *et al.*, 2021). So, all the species mentioned in this work moved over the years between the genera *Strumigenys*, *Neostruma*, and *Pyramica* (and the subgenus *Pyramica*), which are currently in *Strumigenys*.

The *crassicornis*-complex presents a set of morphological characters that easily differentiate it from the *gundlachi*-complex: the presence of denticles along the inner margin of the mandible, a submedian tooth or an enlarged tooth, long and slender labral lobes, the presence of trigger and short hairs at the apices of labral lobes — hairs subequal or shorter than labral lobes (Bolton, 2000). According to Bolton, there is another split within the *crassicornis*-complex (hereafter termed groups i and ii): i) the species related to *S. crassicornis*: *S. aethegenys*, *S. crassicornis*, and *S. metopia*, all with conspicuous, spatulate, and subdecumbend ground-pilosity on the head, and scape ventral margin with an angled base (Bolton, 2000); and ii) the species related to *S. brevicornis*: *S. auctidens*, *S. brevicornis*, *S. crementa*, *S. myllorhapha*, *S. pasisops*, *S. stenotes*, and *S. zeteki*, all having inconspicuous ground-pilosity on the head, and scape ventral margin evenly convex (Bolton, 2000). Group ii of the *crassicornis*-complex is the focus of this study.

In group ii, *S. auctidens*, *S. stenotes*, and *S. zeteki* are closely related; they have narrowed heads and flattened pronotum with blunt margins (Bolton, 2000). Since their description, their distribution has expanded. *Strumigenys auctidens*, originally described in French Guyana, is now found in Amapá, Brazil, Costa Rica, Guyana, and Suriname (Bolton, 2000; Antweb, 2023). *S. stenotes*, which has been described in Amazonas and Amapá from Brazil, currently has records for Bahia and Guyana (Bolton, 2000; Antweb, 2023). Initially described from Panama, *S. zeteki*, is now reported in Costa Rica, Colombia, Peru, and Brazil (Amazonas and Goiás) (Bolton, 2000; Antweb, 2023)

The increasing representation of these species in myrmecological collections, including specimens from places far from the type localities, has made possible to detect populations displaying a combination of characters that are not exactly consistent with any of the described species in group ii of the *crassicornis*-complex. This represents an opportunity to better explore morphological variability and test species boundaries (Silva *et al.*, 2022). Therefore, a taxonomic investigation of these species was conducted. Two new Neotropical *Strumigenys* species closely related to *S. auctidens*, *S. stenotes* and *S. zeteki*, are described. A revised diagnosis of the *crassicornis*-complex is provided, as well as diagnoses and comments on the morphological variation of each species in the group ii.

2. Materials and methods

2. 1. Repository institutions

CELC, Coleção Entomológica do Laboratório de Sistemática de Coleoptera da Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
CEPLAC, Comissão Executiva do Plano da Lavoura Cacaueira, Ilhéus, Bahia, Brazil
DZUP, Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.
INPA, Instituto de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.
MPEG, Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.
MZSP, Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil.

Unique specimen identifiers and image authors are given under the figures. Images without indication of the author were made by us, all of them available at the Antweb.org in full resolution (Antweb, 2023). In addition to the physical examination of individuals, we studied images of specimens from various institutions deposited at Antweb.org, including types (their unique specimen identifiers are always cited along the text).

Images were obtained with various microscopes, a Leica S8APO stereomicroscope with a Canon 1100D attached, a Leica M205 stereomicroscope with a Leica MC170 HD camera, a Zeiss V20 Discovery stereomicroscope with a Zeiss AxioCam 506 digital camera, an Olympus CX41 compound microscope with a Canon 1100D attached. Images were stacked in LAS, ZEN or Zerene softwares and enhancements were made in Adobe Photoshop 21.1.1. In addition, scanning electron microscopy (SEM), was used for the investigation of smaller characters. Images were taken using a TESCAN MIRA 4 with FEG Schoottky electron emission, using the high voltage mode (HV) at a voltage of 5 keV. When not labeled automatically, scales bars were added in ImageJ (Schneider *et al.*, 2012) using measurements taken from the specimens during image acquisition.

2. 2. Morphology

Abbreviations of measurements follow Brassard *et al.* (2020), except for A4TL, which was called ATL in their work, and ommatidia number which was not counted. Measurements are presented in millimeters in the text (also depicted in Table1, and Figure 1):
MandL – mandible length.
HL – head length.

HW – maximum head width.
SL – scape length.
EL – maximum compound eye length.
ON – ommatidia number.
PrW – pronotal width.
WL – Weber's length.
PetL – maximum petiole length.
DpetW – dorsal petiole width.
PostPet+ATL – length of postpetiole plus the fourth abdominal tergite, in dorsal view, measured as a single line from the anterior margin of the postpetiole to the posterior margin of first gaster tergite.
MtfmL –maximum metafemur length, excluding the trochanter.
MttbL – maximum metatibia length.
TL – total length. Sum of MandL + HL + WL + PetL + PostPet+A4TL.
Cephalic Index (CI) – HW/HL X 100.
Mandibular Index (MI) – MandL/HL X 100.
Scape Index (SI) – SL/HL X100.
Pronotum Index (PI) – PrW/HL X 100.
Ocular Index (OI) – EL/HL X 100.

Terminology follows Bolton (2000). The term chaeta is according to Richter et al. (2021).

We described in a formula the dentition on the inner margin of mandible of each species by giving the number of denticles proximal of and distal of the larger tooth (or teeth) on the mandible, which is submedian on the inner margin and is represented as "sm" in the formula. For example, if a species has a submedian tooth, three denticles proximal of it and four denticles distal of it, its dentition formula will be 3/sm/4. The dentition formula does not describe the apical fork shape.

3. Results

3. 1. Taxonomic accounts

The morphological characters observed in adult workers and their states are: **1. Dentition type:** (0) type A, 4-5 denticles of the same size; (1) type B, 3-4/sm/4; (2) type C: 2-3/sm/5; (3) type D, 3-4/sm/5-7; (4) type E: sm/0-3/sm/5; (5) type F, 2/sm/3; (6) type G, 4-

5/sm/1-2/sm/2-3; (7) same dentition formula as G, denticles as developed and robust as the submedian teeth. **2. Curvature of outer margin of mandible: (**0) straight, very slightly concave or very slightly convex; (1) conspicuously convex; (2) conspicuously concave. **3. Scape ventral margin:** (0) convex; (1) with a basal angled lobe. **4. Head ground pilosity: (**0) narrow spatulate or spoon-shaped; (1) simple, short, appressed and inconspicuous. **5. Head depth: (**0) not dorsoventrally flattened; (1) strongly flattened dorsoventrally. **6. Mesonotal pair of setae:** (0) conspicuous: long, filiform or flageliform, and erect; (1) inconspicuous: very small, spoon-shaped to remiform (or simple and curved sometimes), posteriorly oriented, suberect to decumbent and overhanging a pair of lateral cavities on the mesonotum. **7. Smooth metapleural patch:** (0) small; (1) broad; (2) absent; **8. Petiole ventral margin:** (0) Sinuous, but not forming a strong curvature posteriorly; (1) strongly sinuous, forming a strong posterior curve or lobe just before the petiolar foramen; (2) straight or slightly curved. **9. Post petiole size:** (0). flatenned or regular; (1) inflated (see Fig.2).

***crassicornis*-complex** (*aethegenys, auctidens, brevicornis, crassicornis, crementa, metopia, myllhorhapha, pasisops, stenotes, zeteki*). **Diagnosis**. Mandibles never exceed total length of head. Mandibles inner margin with one (rarely two) enlarged tooth toward its apical third (when obviously enlarged named as submedian tooth), and a number of denticles of different sizes are present proximal of and distal of the submedian tooth. Apical fork with 3–5 intercalary denticles between apicodorsal and apicoventral teeth. Labrum shield trapezoidal, labral lobes long and slender, labral lobe chaeta almost straight and smaller than the labral lobes and always much smaller than the shield (Fig.3c, d, e, f, g, h, and i). Pronotum broad and flattened, with an angular humeri. Mesosoma mostly reticulate-punctate, except for a mesopleural smooth patch – that can also be present on the metapleura.

Two clusters of closely related species can be distinguished in the complex:

group i. Species related to *crassicornis* (*aethegenys, crassicornis, metopia*). Head not dorsoventrally flattened, its dorsal outline in profile with a large vertexal bump. Ground-pilosity on the head conspicuous, narrow spatulate or spoon-shaped, and not appressed. Scape with a basal angled lobe, and after that tapering to the apex. Paired specialised, conspicuous and erect hairs in the head, mesosoma, petiole, postpetiole and gaster.

group ii. Species related to *zeteki* (*auctidens, crementa, myllorhapha, pasisops, stenotes, zeteki, Strumigenys* ufv-110, *Strumigenys* ufv-111). Head strongly flattened dorsoventrally, its dorsal outline in profile with a low vertexal bump. Ground-pilosity on the head simple, minute, appressed and inconspicuous. Scape ventral margin convex. Paired specialised setae are distributed as follows: one minute apicoscrobal pair laterally projected;

vertex with an erect, single pair; one pronotal humeral pair; one mesonotal pair (suberect to decumbent); petiole with a pair, postpetiole with two pairs and dorsum of gaster with several remiform, suberect setae.

Among the species related to *zeteki*, *S. auctidens*, *S. stenotes* and *S. zeteki* are the most morphologically similar (Bolton, 2000).

Table 1. Comparison of 9 characters, one index, and four measures among *Strumigenys* species of the *gundlachi*-group (see text for the description of the states for each character). Characters, indexes, and measures are: **HL**, Head length; **MandL**, Mandible length; **WL**, Weber's length; **TL**, Total length; **MI**, Mandibular index; **1**, Dentition type; **2**, Curvature of outer margin of mandible; **3**, Scape ventral margin; **4**, Head ground pilosity; **5**, Head depth; **6**, Mesonotal pair of setae; **7**, Smooth metapleural patch; **8**, Petiole ventral margin; **9**, Post petiole size. *A morphospecies equivalent to *Strumigenys* aff. *crassicornis*. Measurements in milimiters.

Species	**Locality**	**HL**	**MandL**	**WL**	**TL**	**MI**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Strumigenys gundlachi	Cuba	0.45–0.47	0.29–0.30	0.50–0.52	1.26–1.27	0.62–0,67	0	0	0	0	0	0	0	0	0
Strumigenys eggersi	U.S. (Virgin Islands)	0.41–0.42	0.26–0.27	0.44–0.45	1.11–0.14	0.62–0.64	0	0	0	1	0	0	2	0	0
Strumigenys subedentata	Brazil (SC)	0.50–0.52	0.27–0.28	0.50–0.55	1.26–1.36	0.54–0.55	0	2	0	0	0	0	2	2	0
Strumigenys ufv-111 pop.1	Brazil (MG, Viçosa)	0.51–0.61	0,19–0.20	0,49–0.61	1,19–0.42	0.33–0.39	1	0	0	1	1	1	1	0	0
Strumigenys ufv-111 pop.2	Brazil (MG, Mariana)	0.49–0.54	0.19–0.25	0.53–0.58	1.26–1.32	0.35–0.51	1	0	0	1	1	1	0	0	0
Strumigenys ufv-111 pop.3	Brazil (MT, Canarana)	0.54–0.55	0.19–0.20	0.53–0.54	1.26–1.29	0.35–0.36	1	0	0	1	1	1	1	0	0
Strumigenys stenotes	Brazil (AM, Manaus)	0.60–0.63	0.23–0.25	0.58–0.61	1.41–1.48	0.38–0.41	3–1	1	0	1	1	1	0	0	0
Strumigenys auctidens	Brazil (PA, Paragominas)	0.49–0.59	0.17–0.24	0.44–0.51	1.14–1.34	0.35–0.40	4	2	0	1	1	1	1	0	0
Strumigenys ufv-110	Brazil (AM, Manaus)	0.49–0.53	0.16–0.19	0.45–0,50	1.10–1.21	0.32–0.36	2	0	0	1	1	1	1	1	0
Strumigenys zeteki	Panama, Colombia, Costa Rica	0.49–0.57	0.20–0.22	0.50–0.55	1.19–1.32	0.36–0.41	3	2	0	1	1	1	0–2	2	0
Strumigenys crementa	Costa Rica	0.57–0.61	0.26–0.28	0.64–0.66	1.49–1.53	0.46	2	2	0	1	0	0	0	2	0
Strumigenys myllorhapha	Costa Rica	0,61	0,38	0.67–0.68	1.66–1.67	0.62	6	0	0	1	1	0	1	2	0
Strumigenys pasisops	Costa Rica	0.69–0.72	0.31–0.32	0.78–0.80	1.80–1.82	0.44–0.45	7	2	0	1	1	0	1	2	0
Strumigenys brevicornis	Panama, Costa Rica, Guatemala	0.49–0.52	0.20–0.24	0.47–0.57	1.19–1.32	0.39–0.45	1	0	0	1	0	0	2	2	?
Strumigenys ufv-18*	Brazil (MT, RO)	0.54–0.59	0.20–0.25	0.57–0.61	1.31–1.45	0.37–0.42	5	1	1	0	0	0	2	2	1
Strumigenys crassicornis	Brazil (SC, MG)	0.56–0.57	0.20–0.22	0.58–0.65	1.35–1.43	0.36–0.39	1	0	1	0	0	0	2	2	1
Strumigenys metopia	Peru, Brazil (RO, PA)	0.38	0.11–0.12	0.37–0.41	0.87–0.90	0.30–0.32	5	1	1	0	0	0	1	0	1
Strumigenys aethegenys	Costa Rica	0.49–0.50	0.16–0.17	0.49–0.51	1.15–1.17	0.32–0.35	5	0	1	0	0	-	0	2	0

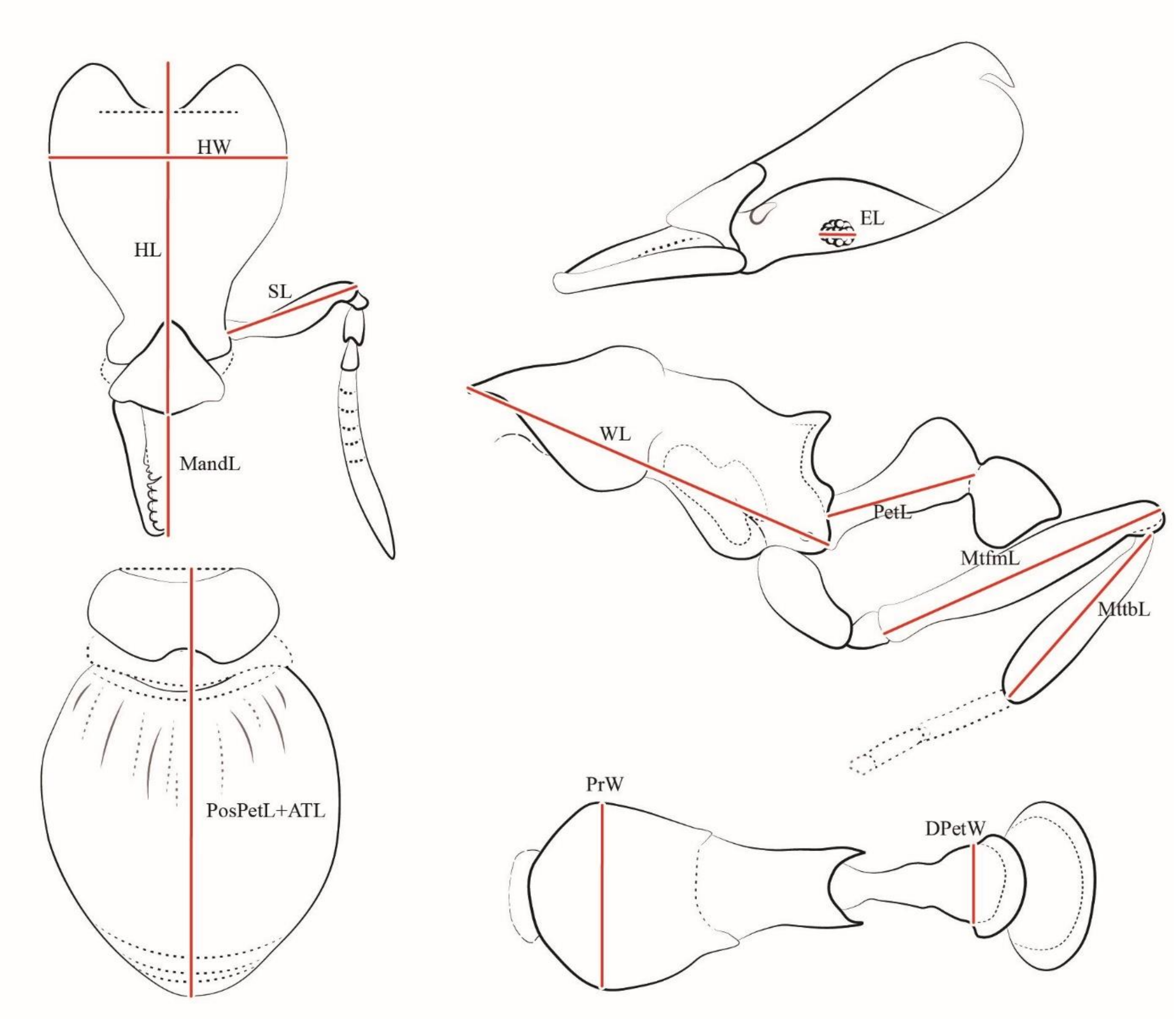

Fig.1. Glossary of measurements taken from *Strumigenys* workers. Abbreviations: **DPetH**, dorsal petiole high; **EL**, eye length; **HL**, head length; **HW**, head width; **MandL**, mandible length; **MttbL**, metatibial length; **MtfmL**, metafemur length; **PetL**, petiole length; **PetW**, petiole width; **PostPetL+ATL**, the length of postpetiole and abdominal tergum IV; **PrW**, pronoro width; **SL**, scape length; **WL**, Weber's length.

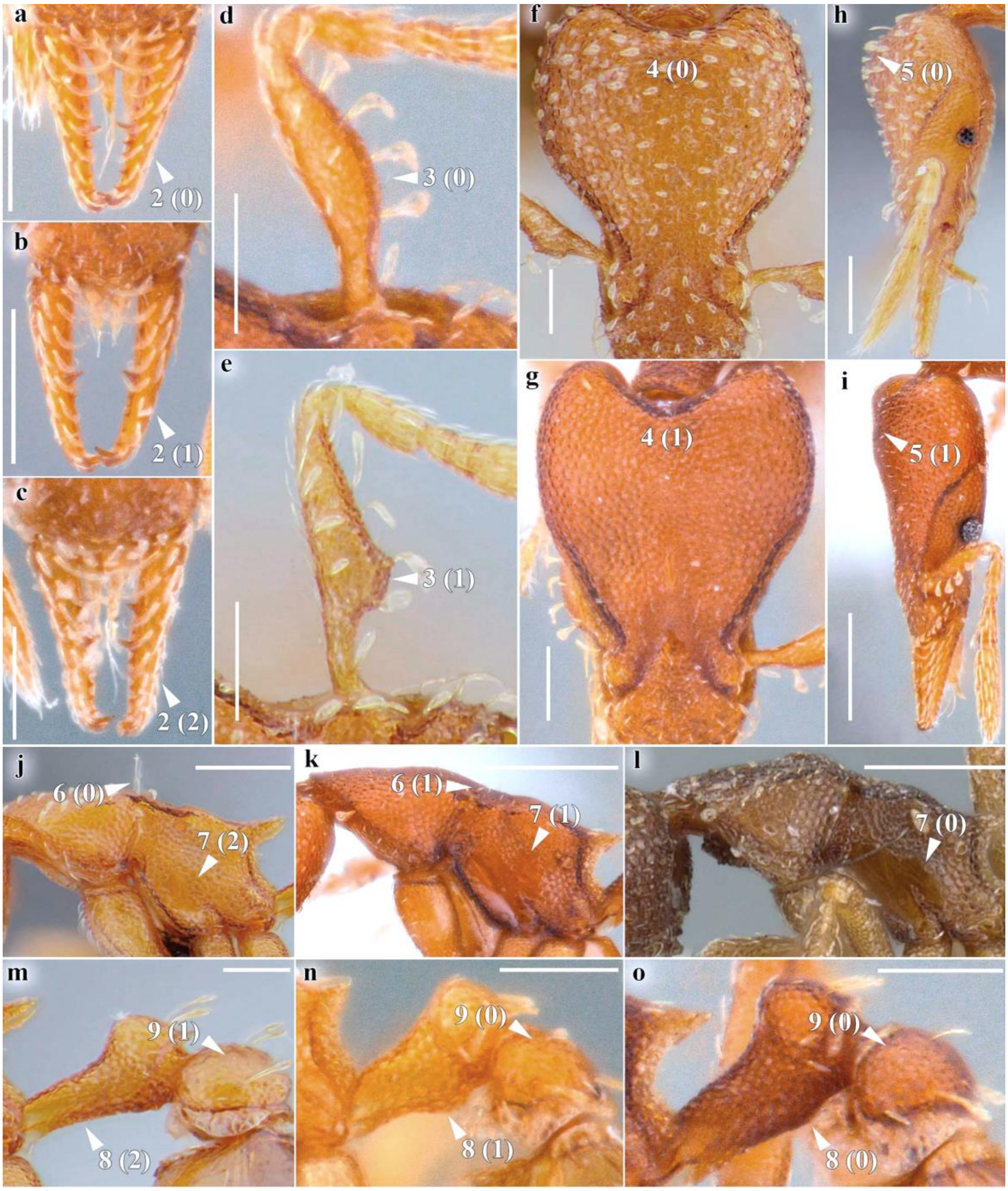

Fig. 2. Main morphological differences, and its character states, among workers of *Strumigenys* of the *gundlachi*-group. Outer margin of mandible, **a**, **b** and **c** (*Strumigenys* ufv-111, ANTWEB1047846; *S. stenotes* UFV-LABECOL-009419; *S. auctidens*, UFV-LABECOL-001676, respectively). Scape ventral margin, **d**, and **e** (*Strumigenys* ufv-111, ANTWEB1047846; *S. crassicornis*, UFV-LABECOL-001906, respectively). Head ground pilosity, **f**, and **g** (*S. crassicornis*, UFV-LABECOL-001906; *Strumigenys* ufv-111, ANTWEB1047846, respectively). Head depth, **h**, and **i** (*S. crassicornis*, UFV-LABECOL-001906; *Strumigenys* ufv-111, ANTWEB1047846, respectively). Mesonotal pair of setae and metapleural smooth patch, **j**, **k**, and **l** (*S. crassicornis*, UFV-LABECOL-001906; *Strumigenys* ufv-111, ANTWEB1047846; *S. aethegenys*, INBIOCRI001283198, image by Will Ericson, respectively). Petiole ventral margin, and postpetiole size, **m**, **n**, and **o** (*S. crassicornis*, UFV-LABECOL-001906; *Strumigenys* ufv-110, ANTWEB1038955; *Strumigenys* ufv-111, ANTWEB1047846, respectively). Scales bars are 0.1 mm in **c**, **d**, **e**, **f**, **g**, and **o**; 0.2 mm in **a**, **b**, **h**, **i**, **j**, **k**, **l**, **m**, and **n**.

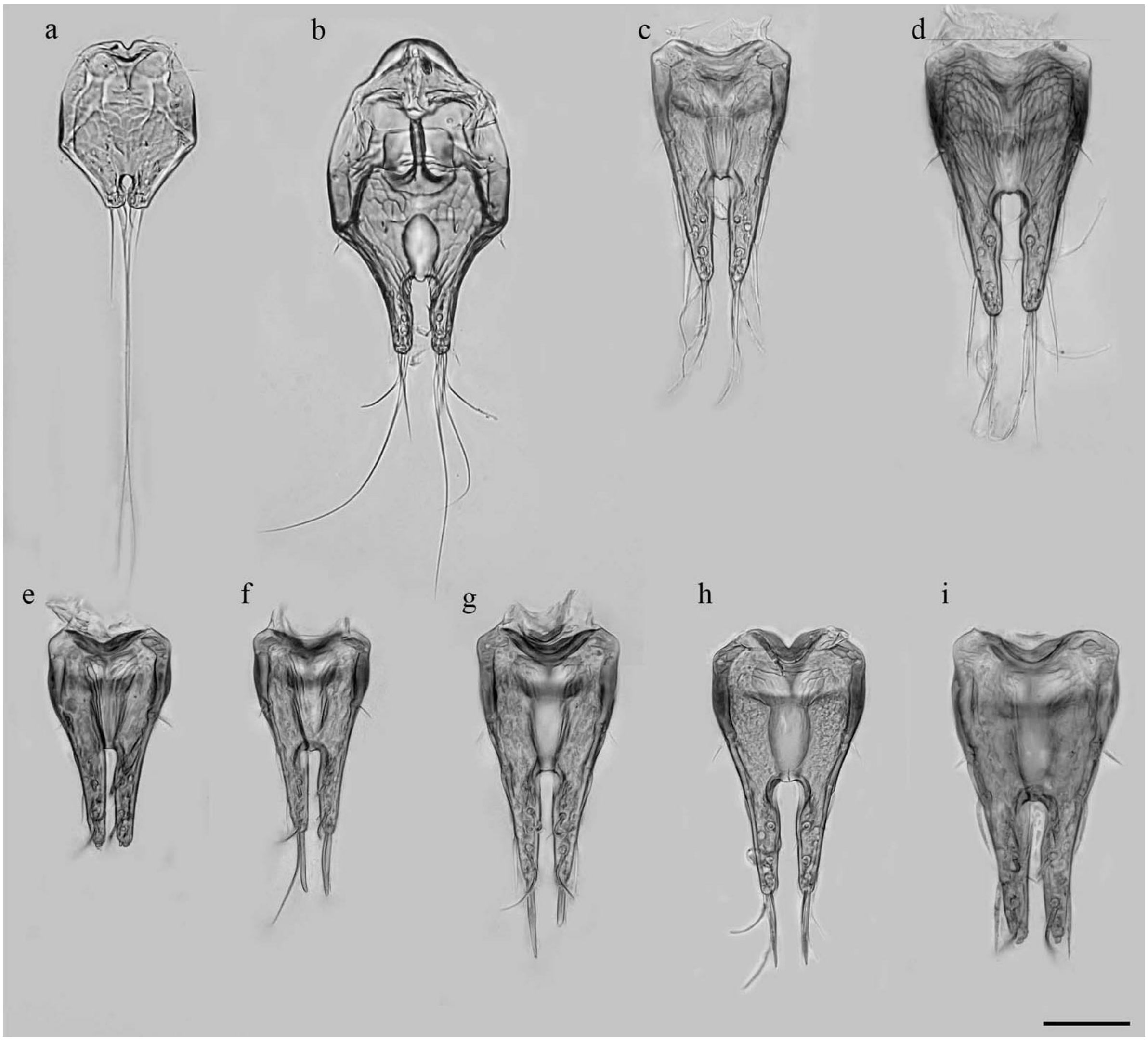

Fig. 3. Labra of various species of *gundlachi*-group. **a,** and **b** being species of *gundlachi*-complex; **c**, **d**, **e**, **f**, **g**, **h**, and **i** species of *crassicornis*-complex. **a.** *Strumigenys eggersi* (UFV-LABECOL-001788); **b.** *Strumigenys* subedentata (UFV-LABECOL-007326); **c.** *Strumigenys crassicornis* (ANTWEB1032002); **d.** *Strumigenys* ufv-18 (a morphospecies equivalent to *Strumigenys* aff. *crassicornis*, ANTWEB1032004); **e.** *Strumigenys auctidens* (UFV-LABECOL-001734); **f.** *Strumigenys ufv-110* (ANTWEB1038966,); **g.** *Strumigenys* ufv-111 (population 3, UFV-LABECOL-001848); **h**. *Strumigenys* ufv-111 (population 1, UFV-LABECOL-001493); **i.** *Strumigenys stenotes* (ANTWEB1047055). Scale bar: 0.05 mm. Labral lobes chaetae in **e** and **i** abraded.

Note: All species mentioned above in group ii as "species related to *S. zeteki*" were considered as "species related to *S. brevicornis"* by Bolton (2000). However, in our morphological analysis, *S. brevicornis* has a set of character states that makes it not completely fitting in group i or group ii, being more adequately treated as an isolated species in the *crassicornis*-complex. In line with group i, the head of *S. brevicornis* is not dorsoventrally flattened, having a large vertexal bump in the head dorsal outline in profile, and the mesonotum with a standing pair of setae. In line with group ii, on the other hand, *S. brevicornis* has the ground-pilosity on the head simple, minute, appressed and inconspicuous, and its scape does not have a basal angled lobe, its ventral margin being evenly curved.

3.1.1. Species Accounts

Strumigenys auctidens **(Bolton, 2000)**

Pyramica auctidens Bolton, 2000: 179, fig. 130. Holotype worker: French Guiana [NHMUK]. Combination in *Strumigenys*: Baroni Urbani & De Andrade, 2007: 116.

Geographic range. Brazil (Pará), Costa Rica, French Guiana, Guyana, Suriname.

Diagnosis. Mandible outer margin concave. Dentition formula: sm/0-3/sm/5. Apical fork with 4 intercalary denticles between apicodorsal and apicoventral teeth. Pronotal humeral pair of minute setae spoon-shaped. Mesonotal pair of setae spoon-shaped to remiform, inconspicuos, posteriorly oriented, subdecumbent. Mesopleural and metapleural smooth patch broad and continuous. Propodeal spine long, and medially fused to propodeal lamella, the latter developed along the entire propodeal descending margin, curved at the height of propodeal spiracle (less developed than propodeal spine). In lateral view, petiole shortened, ventral margin strongly sinuous, forming a strong posterior curve or lobe just before the petiolar foramen. Small species. Measurements for this species are HL 0.49–0.59, MandL0.17–0.24, MandL0.17–0.24, WL 0.44–0.51, TL 1.14–1.34, MI 0.35–0.40 (measurements taken from CELC specimens, and images of the holotype CASENT0900183).

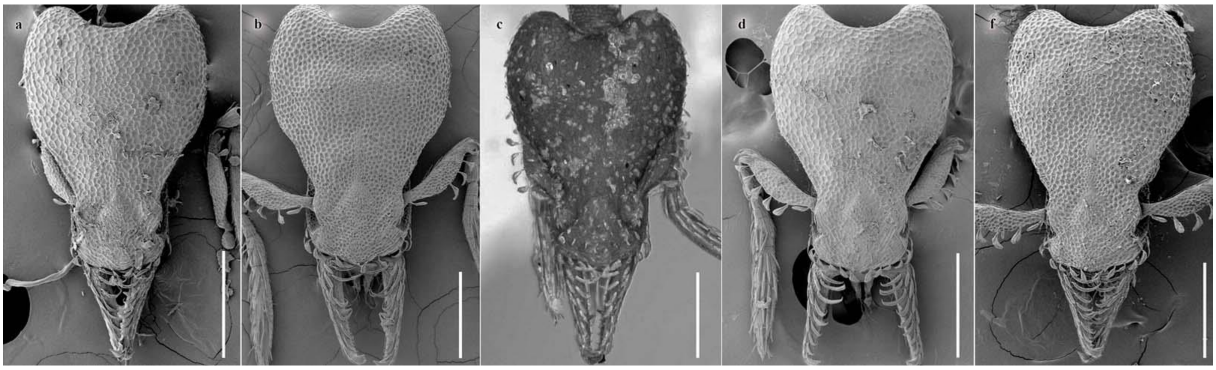

Fig. 4. Head of species related to *S. zeteki* in the *crassicornis*-complex of the *gundlachi*-group of *Strumigenys*. **a.** *Strumigenys auctidens* (UFV-LABECOL-001734); **b.** *Strumigenys stenotes* (ANTWEB1047055); **c.** *Strumigenys zeteki* (CASENT0915704, image by Harald Bruckner); **d.** *Strumigenys* ufv-110 (ANTWEB1038956); **e.** *Strumigenys* ufv-111 (pop.1, ANTWEB1047769). Scale bars: 0.2 mm.

Comments: Within the *crassicornis*-complex, *S. auctidens* is most morphologically similar to *S. stenotes*, *S. zeteki*, *Strumigenys* ufv-110, and *Strumigenys*-111, but can be differentiated from all of those by its unique dentition formula sm/0-3/sm/5 (Fig.6a). *S.*

auctidens shares with *Strumigenys* ufv-111 the strongly sinuous ventral margin of the petiole, similar head sculpturing (Fig.4a, and d), and the small overall body size.

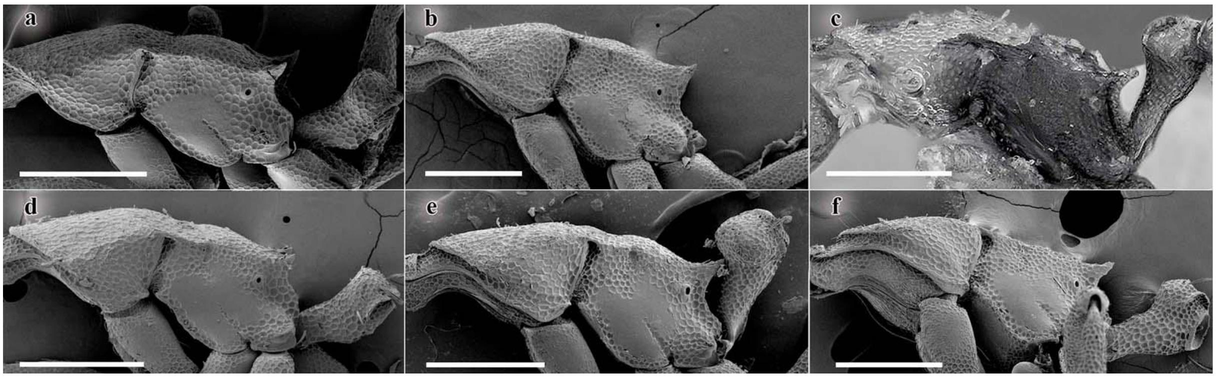

Fig. 5. Mesosoma and petiole lateral view of species related to *S. zeteki* in the *crassicornis*-complex of the *gundlachi*-group of *Strumigenys*. **a.** *Strumigenys auctidens* (UFV-LABECOL-001734); **b**. *Strumigenys stenotes* (ANTWEB1047055); **c.** *Strumigenys zeteki* (FMNHINS0000050835, image by Stephanie Ware); **d.** *Strumigenys* ufv-110 (ANTWEB1038956); **e.** *Strumigenys* ufv-111 (population 2, UFV-LABECOL-001830); **f**. *Strumigenys* ufv-111 (population 1, ANTWEB1047769). Scales bars: 0.2 mm.

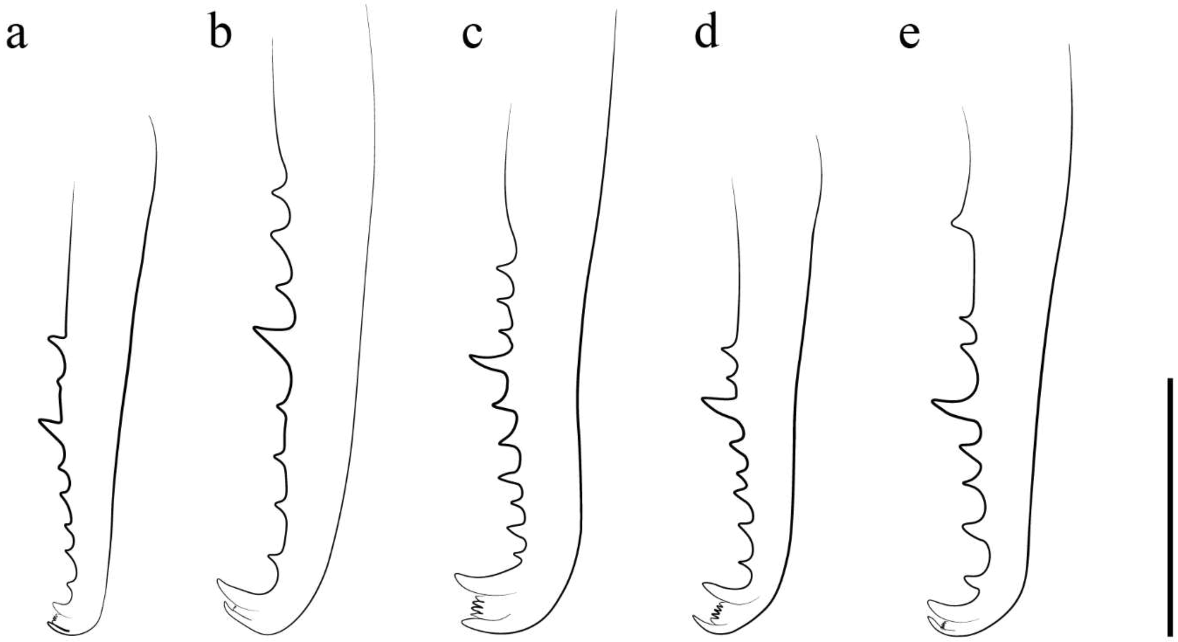

Fig. 6. Zoomed of mandibles in full-face view of species related to *S. zeteki* in the *crassicornis*-complex of the *gundlachi*-group of *Strumigenys*. **a.** *Strumigenys auctidens*; **b.** *Strumigenys stenotes*; **c.** *Strumigenys zeteki*; **d.** *Strumigenys* ufv-110; **e.** *Strumigenys* ufv-111 (pop.1). Scale bar: 0.1 mm.

Strumigenys crementa (Bolton, 2000)

Pyramica crementa Bolton, 2000: 182. Holotype worker: COSTA RICA. [NHMUK].

Combination in *Strumigenys*: Baroni Urbani & De Andrade: 2007: 118

Geographic range. Costa Rica, Panama.

Diagnosis. Mandibles outer margin conspicuously concave. Dentition formula 2-3/sm/5. Pronotal humeral pair of long and remiform setae. Mesonotal pair of conspicuous, long, remiform and erect setae. Mesopleural smooth patch broad, and metapleural smooth patch absend or small. Propodeal spine long, pointed and basally fused to the propodeal lamella Propodeal lamella developed along the entire descending propodeal margin, following its curvature (less developed than propodeal spine). In lateral view, petiole shortened, ventral margin straight. Measurements for this species are HL 0.61, MandL 0.28, WL 0.64, TL 1.53, MI 0.46 (measurements taken from the images of the holotype CASENT0900187).

Comments: *Strumigenys crementa* differs from *S. zeteki*, *S. auctidens* and *S. stenotes*, *Strumigenys* ufv-110, and *Strumigenys* ufv-111 by the larger overall size, and erect mesonotal pair of setae.

Strumigenys myllorhapha **(Brown, 1959)**

Neostruma myllorhapha Brown, 1959b: 12, fig. 4. Holotype worker: COSTA RICA [MZSP].
Combination in *Strumigenys*: Baroni Urbani & De Andrade, 2007: 124

Geographic range: Costa Rica, Panama.

Diagnosis. Species with the longest mandibles in the *crassicornis*-complex. Mandibles outer margin convex. Dentition formula 4-5/sm/1-2/sm/2-3, denticles varying in size and submedian teeth clearly more robust than these. Pronotal humeral pair of long and remiform setae. Mesopleural pair of conspicuous, long, filiform and erect setae. Mesopleural smooth patch broad, and metapleural smooth patch absend or small. Propodeal spine long, pointed and medially fused to the propodeal lamella. Propodeal lamella developed along the entire descending propodeal margin, following its curvature (less developed than propodeal spine). In lateral view, petiole shortened, ventral margin straight. Measurements for this species are HL 0.61, MandL 0.38, WL 0.68, TL 1.67, MI 0.62 (measurements taken from images of specimen CASENT0914603).

Comments: *S. myllorhapha* shares several character states with *S. pasisops*, both with overlapping distribution in Central America. *S. millorhapha* can be easily distinguished from *S. pasisops* through the mandible morphology. *S. myllorhapha* has a longer mandible, and, despite having the same dentition formula of *S. pasisops*, *S. myllorhapha* has denticles varying in size and submedian teeth clearly more robust than these while in *S. pasisops* the denticles are almost as robust as the submedian teeth. In addition, *S. pasisops* is slightly larger than *S. myllorhapha*, and has shorter mandibles.

***Strumigenys pasisops* (Bolton, 2000)**

Pyramica pasisops Bolton, 2000: 192. Holotype worker: COSTA RICA. [BMNH]

Combination in *Strumigenys*: Baroni Urbani & De Andrade, 2007: 125

Geographic range: Costa Rica.

Diagnosis. Largest species of *crassicornis*-complex. Mandibles outer margin conspicuously concave. Dentition formula 4-5/sm/1-2/sm/2-3, denticles as developed and robust as the submedian teeth. Pronotal humeral pair of large and spoon-shaped setae. Mesopleural pair of conspicuous, long, filiform and erect setae. Mesopleural and metapleural smooth patch broad and fused. Propodeal spine long, sharp and basally fused to the propodeal lamella. Propodeal lamella developed along the entire descending propodeal margin, following its curvature (less developed than propodeal spine). In lateral view, petiole shortened, ventral margin straight. Measurements for this species are HL 0.69–0.72, MandL 0.31–0.32, WL 0.78–0.80, TL 1.80–1.82, MI 0.44–0.45 (measurements performed through images available on Antweb.org from the holotype and paratype, CASENT0900185 and CASENT0914609 respectively)

Comments: See above under *S. myllorhapha.*

***Strumigenys stenotes* (Bolton, 2000)**

Pyramica stenotes Bolton, 2000: 192. Holotype worker: BRAZIL (Amazonas) [BMNH]

Combination in *Strumigenys*: Baroni Urbani & De Andrade, 2007: 128

Geographic range. Brazil (Amazonas, Amapa, Bahia), Guyana.

Diagnosis. Mandibles outer margin convex. Dentition formula 3–4/sm/4–7. Apical fork with 4-5 intercalary denticles between apicodorsal and apicoventral teeth. Pronotal humeral pair of minute setae spoon-shaped. Mesopleural smooth patch broad, and metapleural smooth patch small, both fused. Propodeal spine long, sharp and medially fused to propodeal lamella, the latter thick along the entire propodeal descending margin, forming a curve at the height of propodeal carina, below propodeal spiracle (less deloped than propodeal spine). In lateral view, petiole shortened, ventral margin sinuous, but not forming a strong posterior curve or lobe. Measurements for this species are HL 0.60–0.63, MandL 0.23–0.25, WL 0.58–0.61, TL 1.41–1.48, MI 0.38–0.41 (measurements taken from CELC specimens, and images of the holotype CASENT0900184).

Comments: *S. stenotes* is most similar to *Strumigenys* ufv-111 and *S. zeteki* and differs from both by having the mandible outer margin conspicuously convex, the other two with the margin very slightly concave or conspicuously concave, respectively (Fig.7b, f, and j). Besides, *S. stenotes* is the largest among these three species. *S. stenotes* can also be separated from *S. zeteki* by the shape of the pronotal pair of setae (smaller, spoon-shaped in *S. stenotes* and longer, remiform in *S. zeteki*) (Fig.7a, and e) and from *Strumigenys* ufv-111 by the size of metapleural smooth patch (small in *S. stenotes* and wide in *Strumigenys* ufv-111, Fig. 5B, and f; Fig.7g, and k; Fig.7h, and l, respectively).

Strumigenys zeteki **(Brown, 1959)**

Neostruma zeteki Brown, 1959b: 10: figs. 1, 5. Holotype worker: PANAMA (Barro Colorado I). [USNM]

Combination in *Strumigenys*: Baroni Urbani & De Andrade, 2007: 130

Geographic range. Brazil (Amazonas, Goiás), Colombia, Costa Rica, Panama, Peru.

Diagnosis: Mandibles outer margin convex. Dentition formula 3-4/sm/5-7. Apical fork with 3-4 intercalary denticles between apicodorsal and apicoventral teeth. Pronotal humeral pair of long setae spoon-shaped. Mesopleural smooth patch broad, and metapleural smooth patch small or absent. Propodeal spine long, sharp and fused to propodeal lamella, the latter developed along the entire propodeal descending margin, forming a curve at the height of propodeal spiracle, above the propodeal lobe (less developed than propodeal spine). In lateral view, petiole shortened, ventral margin sinuous, but not forming a strong posterior curve or lobe. Measurements for this species are HL 0.55–0.57, MandL 0.21–0.22, WL 0.50–0.55, TL 1.27–1.32, MI 0.36–0.40 (measurements taken from images of the paratype CASENT0915704, and the specimen CASENT 0914621).

Comments: Species closely related to *S. auctidens*, *S. stenotes*, and *Strumigenys* ufv-111. Characters separating these from *S. zeteki* are noted above under their diagnosis.

Since Bolton's work (Bolton, 2000) work, the number of records of *S. zeteki* has greatly increased. The geographic distribution considered here is the sum of the distribution given in the description of the species (Brown, 1959), the material examined by Bolton in his review (Bolton, 2000), and all localities which had specimens we could confirm by images to belong to *S. zeteki* (Antweb, 2023), the species occurs in Panama, Brazil (Amazonas e Goiás), Costa Rica, Colombia, and Peru. However, the distribution of *S. zeteki* through online specimen database Antmaps.org that compile literature records is much greater: in addition to the above-mentioned locations, the species is also recorded for Bolivia, Ecuador, Guyana, French Guiana, and the Brazilian states of Roraima, Acre, Rondonia, Mato Grosso, Minas Gerais, Para, and Maranhão (Janicki et al., 2016).

Given the high similarity between species closely related to *S. zeteki*, it is possible to question the large expansion in the distribution of this species. Part of the problem might be solved with the description of *Strumigenys* ufv-111 below, as many southern Brazilian records of *S. zeteki* might in fact represent *Strumigenys* ufv-111. In the Amazonian region, however, there remain unsolved issues. The case of a Guyanan specimen (USNMENT00413739) exemplifies this problem. The specimen is identified as *S. zeteki*, however it has the pair of humeral setae smaller than the type of *S. zeteki* and its mesonotal smooth patch is broader. Besides, the specimen also seems more robust than the type. In the present work we were not able to physically study any *S. zeteki* specimen, so we can not better delimitate species boundaries in *S. zeteki,* however it is likely that more diversity is found within this core of species very closely related *S. zeteki*.

***Strumigenys* ufv- 110, Jacintho & Chaul, sp. nov.**

(Figs. 2n; 3f; 4d; 5d; 6d; 8).

Type specimen. ***Holotype:*** One pinned worker. Original label: “BRA: AM, Manaus. Colosso camp. 12 – 21. viii.16. Boudinot, B.; Fernandes, I.; Chaul, J.” “*Martiallis Expedition*. Specif coordinates: -2.403723, -59.865735. Day: 10. Winkler sample code: W218” [INPA, ANTWEB1038955].

Paratypes: Two workers on the same pin. Original label: “BRA: AM, Manaus. Colosso camp. (-2.403723, -59. 865735) 12 – 21. viii.16. Boudinot, B.; Fernandes, I.; Chaul, J.” “Day 6, Plot 3” [CELC, ANTWEB1038966]; One pinned worker. Original label: “BRA: AM, Manaus. Colosso camp. 12–21. viii.2016. Boudinot, B.; Fernandes, I.; Chaul, J.” “*Martiallis Expedition.* Specifc coordinates: -2.389194, -59.854361. Day 3” [CELC, UFV-LABECOL-010502]; One pinned worker. Original label: “BRA: AM, Manaus. Colosso camp. 12–21. viii.2016. Boudinot, B.; Fernandes, I.; Chaul, J.” “*Martiallis Expedition.* Specifc coordinates: -2.40262, -59.86655. Day 8. Winkler sample code: 187” [DZUP, ANTWEB038956].

Measurements. ***Holotype worker***: MandL 0.16, HL 0.5, HW 0.34, SL 0.15, EL 0.04, ON 7–8, PrW 0.23, Dpet W 0.11, WL 0.47 PetL 0.18, PostPet+A4L 0.36, MtfmL 0.24, MttbL 0.19, TL1.49, CI 67.50, MI 32.50, SI 44.40, PI 33.30, OI 11,10.

Fig. 7. Main morphological differences among workers of *S. zeteki* (**a–d**), *S. stenotes* (**e–h**), and *Strumigenys* ufv-111(**i–l**). **a, e**, and **i,** mesosoma dorsal view, arrows indicate the umeral seta. **b, f,** and **j,** zoom of mandibles in full-face view, arrows indicate the curvature of mandible outer margin. **c, g,** and, **k**, mesosoma and petiole in lateral view. **d, h**, and **l,** dorsal view of postpetiole and abdominal tergum IV. Images all show the same specimens of *S. zeteki* (CASENT0915704, images by Harald Bruckner), *S. stenotes* (UFV-LABECOL-009419), and *Strumigenys* ufv-111 (ANTWEB1047846), save for image **c.**, that shows specimen FMNHINS0000050835, images by Stephanie Ware, of *S. zeteki*. Scales bars: 0.2 mm.

***Paratypes** (n=4 workers):* MandL 0.16–0.19, HL 0.49–0.53, HW 0.35–0.36, SL 0.15-0.19, EL 0.04, ON 7–8, PrW 0.24–0.26, DpetW 0.10–0.13, WL 0.49–0.5, PetL 0.18–0.19, PosPet+4ATL 0.34–0.39, MtfmL 0.25–0.26, MttbL 0.20–0.21, TL 1.55–1.44, CI 66.7–70, MI 33.30 –35.7, SI 45.50–57.10, PI 30.77–35.10, OI 11.54–12.50.

Geographic range. Brazil (Amazonas).

Diagnosis. Small species (TL 1.44-1.59). Mandibles outer margin very slightly concave. Dentition formula 2-3/sm/5. Apical fork with 5 intercalary denticles between apicodorsal and apicoventral teeth. Pronotal humeral pair of setae spoon-shaped. Mesopleural and metapleural smooth patch broad and continuous. Propodeal spine almost entirely fused to propodeal lamella, the latter uniformly curved and being well-developed along the entire propodeal descending margin (as developed as propodeal spine). In lateral view, petiole shortened, ventral margin strongly sinuous, forming a strong posterior curve or lobe just before the petiolar foramen.

Description. Worker. *Head.* In full-face view, head longer than wide (CI: 66.7-70), with its widest portion at the anterior end of the posterior third of the head. Head markedly flattened, its dorsal outline in profile with a low vertexal bump. Mandible outer margin very slightly concave; its inner margin straight to very slightly convex and having a single large, posteriorly sloped, submedian tooth, 2–3 minute denticles proximal of the submedian tooth and 5 minute denticles distal of it (dentition formula: 2–3/sm/5). Apical fork of mandible with 5 intercalary denticles between apicodorsal and apicoventral teeth. Trapezoid shield labrum, labral lobes long and slender, bulky labral chaeta, slightly straight and equivalent to more than half the length of the labral lobes. Anterior clypeal margin evenly convex, epistomal sulcus medially curved. Scape short, approximately a third of head length, with a convex ventral margin. In lateral view, compound eye with seven to nine ommatidia, located at the anterior level of the middle third of head. Posterolateral margins of head slightly convex. Median concavity of vertexal margin between vertexal lobes strongly concave. *Mesosoma.* In lateral view, promesonotum convex, metanotal impression area concave and propodeum convex. Propodeal spine almost entirely fused to propodeal lamella, the latter uniformly curved and being well-developed along the entire propodeal descending margin (as developed as propodeal spine). *Metasoma.* In lateral view, petiole shortened, ventral margin strongly sinuous, forming a strong posterior curve or lobe just before the petiolar foramen. Petioloar node, in dorsal view, much wider than long, its lateral margins mildly diverging posteriorly, node posterior margin slightlly convex. Postpetiole dorsoventrally flattened. In lateral view, spongiform tissue on postpetiole sternite triangular and relatively large, in dorsal view a collar along petiolar node posterior margin, anterior and posterior margins of postpetiole and first gaster tergite anterior margin. *Pilosity.* Head ground pilosity simple, short, appressed and inconspicuous. Apicoscrobal pair of setae spoon-shaped. Vertex with a single pair of erect, spoon-shaped setae. Pronotal humeral pair of setae spoon-shaped. Mesonotal pair of setae spoon-shaped to remiform, inconspicuos, posteriorly oriented, suberect to decumbent and overhanging a pair of lateral cavities on the mesonotum. Mesosoma dorsum ground pilosity simple, short, apressed and incospicuous. On dorsal view, petiole node with a posterolateral pair, postpetiole with two pairs, one posterolateral and other posteromedial, and dorsum of gaster with several remiform, suberect setae. *Sculpture.* Head entirely reticulate-punctate, punctae relatively large and with narrow borders. Mesosoma mostly reticulate-punctate, except for a large and fused mesopleural and metapleural smooth patch. Petiole and postpetiole reticulate-punctate. Gaster entirely smooth, except for basigastral costulae.

Comments. *Strumigenys* ufv-110 is the smallest species of the *crassicornis*-complex, it is closely related to *S. auctidens*, *S. stenotes*, *Strumigenys* ufv-111, and *Strumigenys zeteki*.

Strumigenys ufv-110 shares with *S. auctidens* a similar head reticulate pattern, composed of relatively large “cells” of the reticulation net and with narrow borders (Fig.4a, and d). They also share the a strongly sinuous petiole ventral margin, forming a strong posterior curve or lobe just before the petiolar foramen, and they also have a propodeal lamella almost as developed as the propodeal spine (Fig.5a, and d). The two can be easily differentiated though by the dentition formula, sm/0-3/sm/5 for *S. auctidens*, and 2–3/sm/5 for *Strumigenys ufv-110*.

Strumigenys ufv-110 clearly differs from *S. stenotes*, *S. zeteki* and *Strumigenys* ufv-111 on the morphology of ventral margin of petiole, which is sinuous but not lobed on *S. stenotes* and in *Strumigenys* ufv-111, and only slightly curved on *S. zeteki.* The pilosity pattern on the postpetiole is another difference with *S. stenotes* and *Strumigenys* ufv-110 — in *S. stenotes*: on dorsal view, postpetiole with two pairs, one posterolateral and other anteromedial, and in *Strumigenys* ufv-110: on dorsal view, postpetiole with two pairs, one posterolateral and other posteromedial (pattern of *Strumigenys* ufv-110 on Fig.7j, and of *S. stenotes* on Fig.7h).*Strumigenys* ufv-110 is known only by the holotype and paratype specimens. It is possible that additional specimens of *Strumigenys* ufv-110 await discovery in museum collections where they are identified as *S. stenotes* or *S. zeteki*.

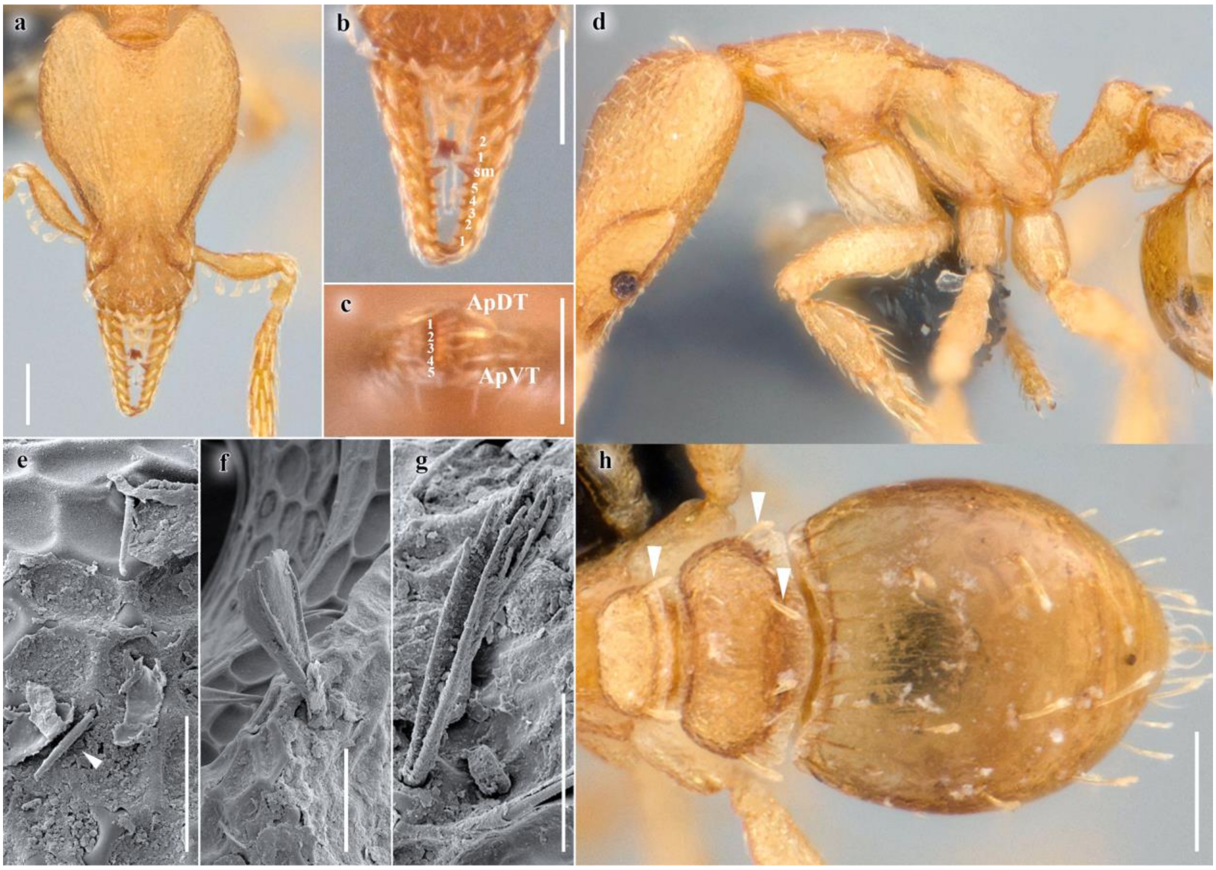

Fig. 8. *Strumigenys* ufv-110, holotype worker (ANTWEB1038955, images **a**, **b**, **c**, **d**, and **h**), paratype worker (ANTWEB1038956, images **e**, **f**, and **g**). **a.** full face view; **b.** zoom of mandible; **c.** apical fork; **d.** zoom of profile view; **e**. zoom of head ground pilosity; **f.** zoom of the umeral hair; **g.** zoom of petiolar setae; **h.** dorsal view of postpetiole and abdominal tergum IV. Abbreviations: **ApDT.** apicodorsal tooth; **ApVT**. Apicoventral tooth; **sm**. submandibular tooth. Scales bars: 0.1 mm in **a, b, d**, and **h**; 0.02 mm in **e, f,** and **g**; 0.05 mm in **c.**

Strumigenys ufv-111, Jacintho & Chaul, sp. nov.

(Figs. 2a, d, c, i, k, o; 3g, h; 4e; 5e, f; 6e; 7i–l; 9; 10).

Type specimen. ***Holotype:*** One pinned worker. Original label: "BRA: MG, Viçosa, Mata da Biologia, 20.754045 42.86143, 23..vii.21. Manual sampling in the litter/small logs. Jacintho, G.F.; Chaul, J." [CELC, ANTWEB1047846];

Paratypes: One pinned worker. Original label: "BRA: MG, Viçosa, Mata da Biologia, 20.754045 42.86143, 24.iii.22. Manual sampling in the litter/small logs. Jacintho, G.F.; Chaul, J." [CELC, ANTWEB1047844]; One pinned worker. Original label "BRA: MG, Viçosa, Mata da Biologia, 20.754045 42.86143, 24.iii.22. Manual sampling in the litter/small logs. Jacintho, G.F.; Chaul, J." [CEPLAC, ANTWEB1047845]; One pinned worker. Original label: "BRA: MG, Viçosa. Mata do Paraíso. 13.iii.98. Winkler. Soares, S." [CELC, UFV-LABECOL-001826]; Two pinned workers. Original label: "BRA: MG, Viçosa, UFV, Mata da Biologia, 01.i.2015.

Manual sampling. Chaul, J.” [CELC, UFV-LABECOL-000763]; Two pinned workers. Original label: “BRA: MG, Viçosa, UFV, Mata da Biologia, 01.i.2015, -20.757408 -42.863792. Chaul, J.” [INPA, UFV-LABECOL-001492]; One pinned worker. Original label: “BRA: MG, Viçosa, UFV, Mata da Biologia, -20.757408 -42.863792, 01.i.2015. Chaul, J.” [DZUP, UFV-LABECOL-001493]; One pinned worker. Original label: “BRA: MG, Viçosa, 16.iii.2018. Winkler extractor. Ferreira, F,” [MZSP, UFV-LABECOL-009447]; One pinned worker. Original label: “BRA: MG, Viçosa, Mata do Seu Nico, -20.798621 -42.847783, 13.iv.2012, Epigaeic pitfall. Schmidt, F. A.; Rezende, F.; Jesus, R.” [MPEG, UFV-LABECOL-001828]; One disarticulated and pinned worker. Original label: “BRA: Viçosa, MG, Mata da Biologia, -20.757408 -42.863792, 2022. Manual sampling in the litter/small logs. Jacintho, G.F.; Chaul, J.” [CELC, ANTWEB1047769]

Additional material examined: One pinned worker. Original label: “Brazil, Minas Gerais, Araponga, 2011. iv. Berlesate of colonized litter bags (previously sterilized). Muscardi, D.” [CELC, UFV-LABECOL-001827]; One pinned worker. Original label: “Brazil, Minas Gerais, Viçosa, 1994. ii. Baited pitfall. Sperber, C.; Louzada, J.; Lopes, P.” [CELC, UFV-LABECOL-001829]; One disarticulated and pinned worker. Original label: “BRA: MG, Viçosa, 01.i.2017. Chaul, J” [CELC, UFV-LABECOL-008984]; One pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p298. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001840]; One pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p12. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001847]; One pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p298. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001839]; One disarticulated and pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p326. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001825]; One pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p298. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001848]; One pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p386. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001834]; One dissected, coated for SEM and pinned worker. Original label: “BRA: MT. Próx. a Querência. Faz. Tanguru. vi.2013. -13.076001 -52.386339, winkler, p298. Bicalho, M. L.; Ribeiro, V.” [CELC, UFV-LABECOL-001844]; One dissected, coated for SEM, and pinned worker. Original label: “BRA: MG, Mariana. Samarco e entorno. -20.230555 -

43.449722, 20.ix.2012, baited pitfall. Paolucci, L." [CELC, UFV-LABECOL-001830]; One pinned worker. Original label: "BRA: MG, Mariana. Samarco e entorno. -20.230555 -43.449722, xiii.2014, baited pitfall. Paolucci, L." [CELC, UFV-LABECOL-001832].

Worker measurements. ***Holotype***: MandL 0.20, HL 0.59, HW 0.35, SL 0.24, EL 0.05, ON 13, PrW 0.29, DpetW 0.14, WL 0.58, PetL 0.24, PostPet+A4L 0.49, MtfmL 0.38, MttbL 0.26, TL 1.86, CI 59.57, MI 34.04, SI 67.86, PI 82.14, OI 14.29.

Paratypes (*n=10*): MandL 0.19–0.21, HL 0.49–0.61, HW 0.36–0.41, SL 0.20-0.24, EL 0.05–0.06, ON 12-16, PrW 0.26–0.30, DpetW 0.13–0.15, WL 0.53–0.61, PetL 0.19–0.27, PosPet+4ATL 0.43–0.54, MtfmL 0.30–0.39, MttbL 0.21–0.28, TL 1.36–1.86, CI 65.96–78.48, MI 33.33–39.02, SI 53.33–65.63, PI 68.18–74.19, OI 12.90–14.06.

Geographic range. Brazil (Mato Grosso, Minas Gerais).

Diagnosis. Mandibles outer margin slightly concave. Dentition formula 3-4/submandibular/4. Apical fork with with 5 intercalary denticles between apicodorsal and apicoventral teeth. Pronotal humeral pair of setae small, spoon-shaped. Mesopleural and metapleural smooth patch broad and continuous. Propodeal spine long, sharp and medially fused to propodeal lamella, the latter developed along the entire propodeal descending margin, forming a curve at the height of propodeal spiracle, above the propodeal lobe (less deloped than propodeal spine). In lateral view, petiole ventral margin sinuous, but not forming a strong posterior curve or lobe

Description. Worker. *Head.* In full-face view, head longer than wide (CI: 66–78.5), with its widest portion at the anterior end of the posterior third of head. Head markedly flattened, its dorsal outline in profile with a low vertexal bump. Mandible outer margin slightly concave; its inner margin slightly convex and having a single large, posteriorly sloped, submedian tooth, with 3–4 minute denticles proximal of the submedian tooth, 4 minute denticles distal of it (dentition formula: 3–4/sm/4). Trapezoid shield labrum, labral lobes long and slender, bulky labral chaetae, slightly straight, with tapering tips, chaetae more than half the length of lobes. Anterior clypeal margin evenly convex, epistomal sulcus medially curved. Scape relatively long (SI 67.86), approximately half of total head length, with a convex ventral margin. In lateral view, compound eye with 12 to 16 ommatidia, located at the anterior level of the middle third of head. Posterolateral margins of head slightly convex. Median concavity of vertexal margin between vertexal lobes concave. *Mesosoma.* In lateral view, promesonotum convex, with a feeble concavity delimiting pronotal and mesonotal area. Propodeal spine long, sharp and medially fused to propodeal lamella, the latter developed along the entire propodeal descending margin, forming a curve at the height of propodeal spiracle, above the propodeal lobe (less deloped than propodeal spine). *Metasoma.* In lateral view, petiole ventral margin sinuous, but not forming a strong posterior curve or lobe. Petiolar node in dorsal view, slightly wider than long, its lateral margins mildly diverging posteriorly, node posterior margin convex. Postpetiole dorsoventrally flattened, its posterior margin medially incised. In lateral view, spongiform tissue on ventral postpetiole triangular and relatively large, in dorsal view a collar across petiolar node posterior margin, anterior and posterior margins of postpetiole and first gaster tergite anterior margin. *Pilosity.* Head ground pilosity simple, short, appressed and inconspicuous. Apicoscrobal pair of setae spoon-shaped. Vertex with a single pair of erect, spoon-shaped setae. Pronotal humeral pair of setae small, spoon-shaped. Mesonotal pair of setae spoon-shaped to remiform, minute, posteriorly oriented, suberect to decumbent. Mesosoma dorsum ground pilosity simple, short, apressed and incospicuous, as in the head. On dorsal view, petiole node with a mediolateral pair of standing, remiform setae. PPostpetiole with two pairs of standing remiform suberect setae, one pair posterolateral and the other anteromedial on postpetiole disc. Dorsum of gaster with several stading, remiform, suberect setae. *Sculpture.* Head entirely reticulate-punctate, punctae relatively large and with broad margins. Mesosoma mostly reticulate-punctate, except for a large and fused mesopleural and metapleural smooth patch. Petiole and postpetiole reticulate-punctate. Gaster entirely smooth, save for basigastral costulae and fine straight striations between it.

Comments. *Strumigenys* ufv-111 is closely related to *S. stenotes*, and *Strumigenys zeteki*. *Strumigenys* ufv-111 and *S. zeteki*. Both have a well-developed propodeal spine and thin propodeal lamella, petiole ventral margin sinuous, but not forming a strong posterior curve or lobe. The two species can be differentiated by the long and remiform pronotal humeral setae in *S. zeteki* and small and spoon-shaped in *Strumigenys* ufv-111 (Fig.7a, and i). The standing pair of setae on the petiole node in *Strumigenys* ufv-111 is mediolateral, and in *S. stenotes* is anterolateral (Fig.9h and Fig.7h, respectively). Furthermore, *S. zeteki* outer margin of mandible is conspicuosly convex, while in *Strumigenys* ufv-111 it is straight to slightly concave (Fig.7b, and j), and their dentition formulae also differ, 3-4/sm/5-7 for *S. zeteki*, and 3-4/sm/4 for *Strumigenys* ufv-111.

Different series of this species show slight variation in the dentition formula, some measurements and indexes, and in the curvature of propodeal lamella, although maintaining the diagnostic traits for the species. Three populations with specific morphological patterns were identified, namely pop.1 (BRA: MG, Viçosa), pop.2 (BRA: MG, Mariana), and pop.3 (BRA: MT, Canarana). Pop.1, presents sharp denticles of same size, metapleural smooth patch equivalent to half the size of the mesopleural smooth patch (Fig.10a). Pop.2, presents poorly defined denticles proximal of the submedian, and those distal of the submedian with rounded tips and varying in size, metapleural smooth patch equivalent to a third the size of the mesopleural smooth patch (Fig.10b). Pop.3, presents well-defined denticles, with a wider base, rounded ends and varying in size, metapleural smooth patch equivalent to three-quarters the size of the mesopleural smooth patch (Fig.10c).

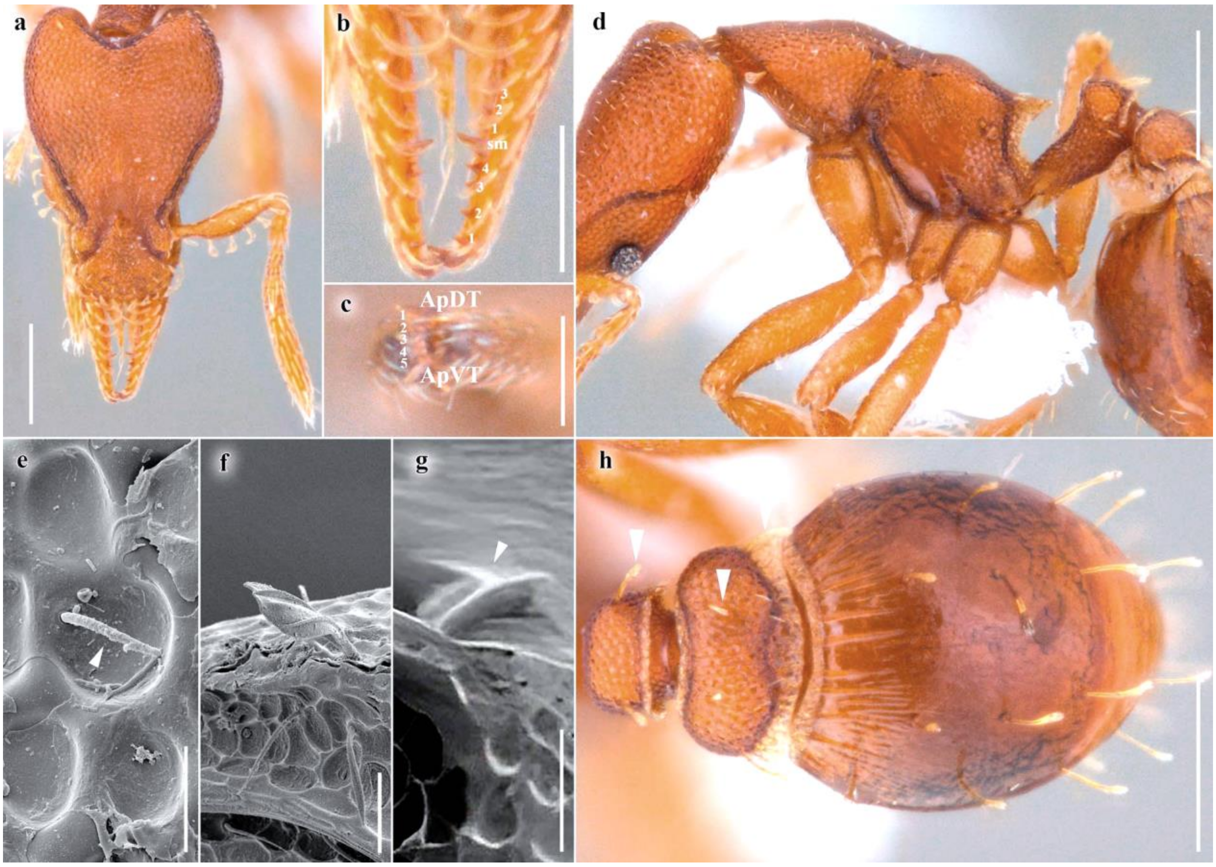

Fig. 9. *Strumigenys* ufv-111, holotype worker (ANTWEB1047846, images **a**, **b**, **c**, **d**, and **h**), paratype worker (ANTWEB1047769, images **e**, **f**, and **g**). **a.** full face view; **b.** zoom of mandible; **c.** apical fork; **d.** zoom of profile view; **e.** zoom of head ground pilosity; **f.** zoom of the humeral seta; **g.** zoom of petiolar setae; **h.** dorsal view of postpetiole and abdominal tergum IV. Abbreviations: **ApDT**. apicodorsal tooth; **ApVT**. Apicoventral tooth; **sm.** submandibular tooth. Scales bars: 0.1 mm in **b**; 0.02 mm in **a, d**, and **h;** 0.05 mm in **c;** 0.02 in **e** and, **f.**

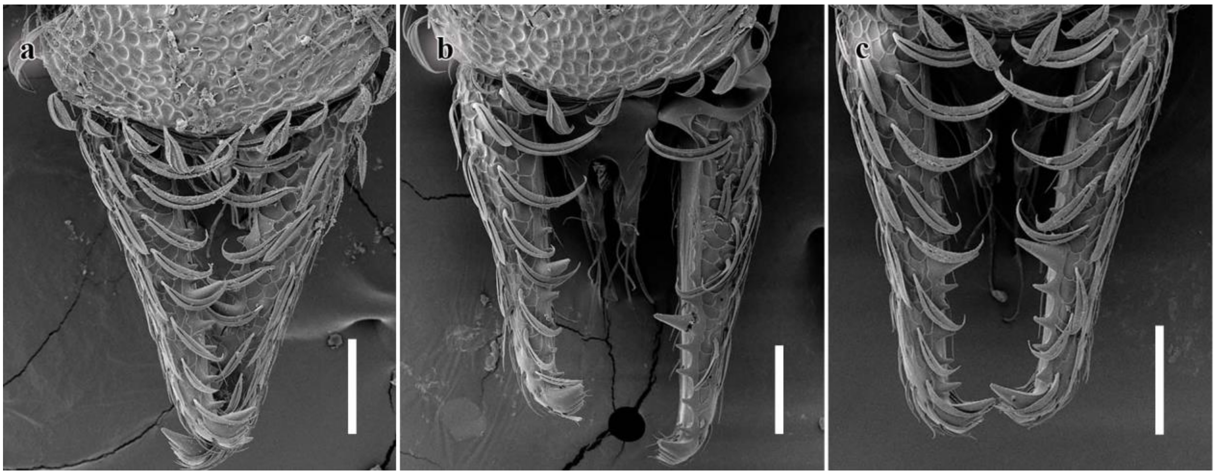

Fig. 10. Zoomed of mandibles in full-face view of the three populations of *Strumigenys* ufv-111 **a.** population 1 (BRA: MG, Viçosa, ANTWEB1047769); **b.** population 2 (BRA: MG, Mariana, UFV-LABECOL-001830); **c.** population 3 (BRA: MT, UFV-LABECOL-001844). Scales bars: 0.05 mm.

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IV. CAPÍTULO II

CYTOGENETICS OF *STRUMIGENYS* SMITH: AN COMPARATIVE ANALYSES BETWEEN *GUNDLACHI* AND *LOUISIANAE* GROUPS BOLTON (HYMENOPTERA: FORMICIDAE: MYRMICINAE)

ABSTRACT

Many *Strumigenys* ants in the Neotropics belong to the *gundlachi* and *louisianae* groups, and there are few cytogenetic data for the genus. In the Neotropical region, only three species were previously studied, *S. diabola* with 2n=40, *S. louisianae* from the Atlantic rainforest with 2n=4, and *S. louisianae* from Oiapoque with 2n=26, showing that synonyms may not reflect a taxonomic unit. Due to their cryptic nature, many species in the tropics remain unknown. Morphological analyses alone may not be enough to separate them, highlighting the need for for different approaches. So, following the integrative taxonomy proposal, we studied classical and molecular cytogenetics in five species of the Neotropical *Strumigenys*. The chromosome numbers and karyotype formulas found were: 2n=26 (20m+6sm) for *S. crassicornis*, 2n=16 (8m+8sm) for *Strumigenys* ufv-111, 2n=18 (16m+2sm) for *S. denticulata*, 2n=18 (18m) for *S. subedentata* representatives of *gundlachi*-group, and *S. louisianae* (*Strumigenys* ufv-06) 2n=20 (18m+2sm) from *louisianae*-group. A chromosomal heteromorphism was observed in *S. crassicornis* and *Strumigenys* ufv-06, possibly originated by translocations. All studied species showed a pair of 18S rDNA sites. The physical mapping of repetitive sequences $(TTAGG)_6$ and $(GA)_{15}$ on the chromosomes showed the fisrt for the genus. *S. crassicornis*, *S. subedentata*, and *Strumigenys* ufv-06 varied in the $(GA)_{15}$ distribution. Thus, cytogenetic techniques and morphological data combined were effective in delineating boundaries of *gundlachi* and *louisinae-group* cryptic species.

Keywords: Molecular cytogenetics; Karyotype; Myrmicine ants.

1. Introduction

Strumigenys Smith, 1860 is a globally distributed and hyperdiverse ant genus comprising more than 850 extant species (Bolton, 2022). In 2000, Bolton performed a complete revision of the genus, that divided it into several groups of species. The Neotropical region has 215 species arranged into 41 groups of *Strumigenys* (two of which have been described in the previous chapter) — and possibly there is more to be described (Silva and Feitosa, 2019; Bolton, 2023; Silva *et al.*, 2022). Some of these groups comprise the most common ant taxa of the Neotropical fauna, including species of the *gundlachi* and *louisianae* groups (Bolton, 2000; Silva *et al.*, 2022).

The *gundlachi*-group has two internal divisions: the *gundlachi*-complex (22 spp.) and *crassicornis*-complex (10 spp.). Some species in this group have had greatly expanded distribution data since their description (e.g., *Strumigenys auctidens* (Bolton, 2000), *Strumigenys crassicornis* Mayr, 1887; *Strumigenys denticulata* Mayr, 1887; *Strumigenys stenotes* (Bolton, 2000); *Strumigenys subedentata* Mayr, 1887; and *Strumigenys zeteki* (Brown, 1959) (Bolton, 2000; Silva *et al.*, 2022; Antweb, 2023). As *Strumigenys* species become more widely represented in myrmecological collections, there is growing documentation of the morphological variability originally described by Bolton (2000). Consequently, a reevaluation of the taxonomic boundaries between these especies and the observed has become necessary.

The *louisianae*-group is composed of four species, with *Strumigenys louisianae* Roger, 1863 being one of the most commonly found species of *Strumigenys* worldwide (Bolton, 2000; Booher, 2021). Its distribution covers the entire area from the southeastern United States to northeastern Argentina (Bolton, 2000; Guénard *et al.*, 2017; Booher, 2021). Throughout this distribution, there is high morphological variation in terms of size, sculpture, pilosity, and even color (Bolton, 2000). Currently, there are 13 species, subspecies, and/or variations synonymized under *S. louisianae* (Brown, 1961; Bolton, 2000), namely: *Strumigenys bruchi* Forel, 1921, *Strumigenys clasmospongia* Brown, 1953, *Strumigenys costaricensis* Weber, 1934, *Strumigenys cubaensis* Mann, 1920, *Strumigenys fusca* Emery, 1894, *Strumigenys guatemalensis* Weber, 1894, *Strumigenys laticephala* Smith, 1931, *Strumigenys longicornis* Emery, 1894, *Strumigenys obscurventris* Wheeler, 1908, *Strumigenys producta* Brown, 1953, *Strumigenys soledanensis* Weber, 1934, *Strumigenys unidentata* Mayr, 1887 and *Strumigenys unispinulosa* Emery, 1890.

In addition to morphological variation, chromosomal variation also exists between populations of *S. louisianae* (Alves-Silva *et al.*, 2014; Barros *et al.*, 2021). Thus, the wide distribution and cytogenetic differences, in addition to the morphological variation between localities, indicate that the synonyms of several taxa of *S. louisianae* may not reflect the taxonomic unit of the species. In the case of *S. louisianae*, despite its detailed morphological description, Bolton (2000) did not exhaust all taxonomic questions existing in the genus. Moreover, the large morphological variation between populations of some *gundlachi*-group species indicates similar problems (Silva *et al.*, 2022).

The *gundlachi* and *louisinae*-group species have become a problem, given the difficulty of separating them only through morphological analysis, which highlights the importance of using multiple characters during delimitation. Therefore, exploratory tools based on hypotheses of reproducible data have been presented as an ideal methodology for delimiting morphologically similar species (Seifert *et al.*, 2014). Cytogenetics is a useful tool in solving complex taxonomic groups in Formicidae (Cristiano *et al.* 2013, Correia *et al.* 2016, Santos *et al.* 2016, Aguiar *et al.* 2017).

Cytogenetics studies on ants began in 1960, leading to the description of the karyotype of approximately 800 morphospecies from different subfamilies and biogeographical regions (Lorite and Palomeque, 2010; Shattuck and Lubertazzi, 2021). However, this number is low compared to more than 13,000 described species (Bolton, 2023). Even with scarce information about the karyotype of Formicidae, there is high variation in the known data between different groups of ants, and even between morphologically similar species (Barros *et al.*, 2021; Mariano *et al.*, 2012).

Despite its high abundance, *Strumigenys* is a genus rarely studied with cytogenetic approches, with the chromosome number (2n) known mostly in species from the Indo-Malaysian and Australian regions: *Strumigenys doherty* Emey, 1987 (2n=24) (Imai *et al.*, 1983), *Strumigenys doriae* Emery, 1887 (2n=22) (Imai *et al.*, 1985), *Strumigenys friedae* Forel, 1915 (2n=24) (Imai *et al.*, 1977), *Strumigenys godeffroyi* Mayr, 1866 (2n=40-44) (Imai et al., 1983; Imai *et al.*, 1985), *Strumigenys mutica* (Brown, 1949) (2n=36) (Imai *et al.*, 1985) and *Strumigenys* spp. (2n=16, 2n=38 and n=13) (Goni *et al.*, 1982; Imai *et al.*, 1985; Crozier, 1975). All records for species from these regions show only the chromosome number, without data on chromosomal structure. In contrast, data for the Neotropical region are restricted to three species: *Strumigenys diabola* Bolton, 2000 (2n=40) from the Amazon region (Aguiar *et al.*, 2020); *S. louisianae* (2n=4) from the Atlantic Forest, which corresponds to the lowest chromosome number observed in Myrmicinae (Alves-Silva *et al.*, 2014); and *S. louisianae* (2n=26) from the Amazon region (Barros *et al.*, 2021).

Strumigenys has taxonomic gaps that require integrative analyses, including classical and molecular cytogenetics, to help delimit common species and resolve species complexes [*sensu* Bickford *et al.* (2007), not Bolton (2000)] (Barros *et al.*, 2021; Mariano *et al.*, 2015; Teixeira *et al.*, 2021). Therefore, aiming to understand patterns for better delimitation of the species, the present study evaluated the cytogenetic using classical and molecular techniques in five species of *Strumigenys*: *S. crassicornis* and *Strumigenys* ufv-111 from *crassicornis*-complex, *S. denticulata* and *S. subedentata* from *gundlachi*-complex, and *S. louisianae* from *louisianae*-group.

2. Materials and methods

Colonies of *Strumigenys* were collected from small logs buried in the soil below the litter in two fragments of the Atlantic Forest in the municipality of Viçosa, Minas Gerais, Brazil: Mata da Biologia (-20,757, -42,865) and Estação de Pesquisa, Treinamento e Educação Ambiental (EPTEA) Mata do Paraíso (-20,801, -42,866). Specimen collection was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) with authorization number 32459. Adult specimens were identified using the key to Neotropical and Nearctic *Strumigenys*, and the key to Neotropical *Strumigenys* (as *Pyramica*), both present in Bolton (2000). The identifications were confirmed by MSc. Júlio Chaul and later, the specimens were deposited in the Coleção Entomológica do Laboratório de Coleoptera (CELC, collection abbreviation follows Evenhuis, 2022) at the Universidade Federal de Viçosa (UFV), Brazil.

High-resolution images of at least one representative of each karyotyped were taken using a TESCAN MIRA 4 with FEG Schoottky electron emission, using the high voltage mode (HV) at a voltage of 5 keV. When not generated automatically, scales bars were added in ImageJ (Schneider *et al.*, 2012) using measurements taken from the specimens during image acquisition. Images taken in a stereomicroscope of the same karyotyped populations can be found in AntWeb under the codes UFV-LABECOL-001906 for *S. crassicornis*, UFV-LABECOL-00066 for *S. denticulata*, UFV-LABECOL-007326 for *S. subedentata,* UFV-LABECOL-000763 for *Strumigenys* ufv-111*,* ANTWEB1038277 for *Strumigenys* ufv-06, and UFV-LABECOL-001576 for *Strumigenys* ufv-02.

Mitotic metaphase chromosomes were obtained from the larval brain, after the elimination of the *meconium*, using hypotonic colchicine solution (0.005%) and fixatives, following the protocol proposed by Imai *et al.* (1988). The material obtained was submitted to conventional staining with 4% Giemsa to define the chromosome number and characterize metaphase. At least five scattered metaphases were analyzed, with similar degrees of chromosome condensation and evident centromeres for each colony, to determine the number and chromosomal morphology of the studied species. For subsequent analyses, at least 10 metaphases were analyzed for each species.

Chromosomes were measured, arranged in decreasing order of size, and classified as: m – metacentric (ratio between the length of long and short arms – r = 1–1.7), sm – submetacentric (r = 1.7–3), st – subtelocentric (r = 3–7) and a – acrocentric (r>7), following Levan *et al.* (1964). Chromosomes were organized using Adobe Photoshop® 21.1.1 software, measured in Image ProPlus®.

The microsatellite $(GA)_{15}$ and telomere motifs $(TTAGG)_6$ were used as probes directly labeled with Cyanine-3 (Cy3) at the 5' end (Sigma, St. Louis, MO, USA). Mapping of repetitive sequences was performed following the protocol proposed by Pinkel *et al.* (1986) with modifications proposed by Teixeira *et al.* (2022a).

The 18S rDNA probes were amplified by polymerase chain reaction (PCR) using the rDNA primers 18SF1 (5′GTC ATA GCT TTG TCT CAA AGA3′) and 18SR1.1 (5′CGC AAA TGA AAC TTT AAT CT3′) designed for the bee *Melipona quinquefasciata* Lepeletier, 1836 (Pereira, 2006). Genomic DNA from the ant *Camponotus rufipes* (Fabricius, 1775) was used as a template in the PCR reactions. Probes were labeled using an indirect method with digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany). FISH signals were detected using antibody against digoxigenin rhodamine conjugated (Roche Applied Science), following the manufacturer’s instructions.18S rDNA regions were detected by fluorescence *in situ* hybridization (FISH), following the protocol of Pinkel *et al.* (1986), with modifications proposed by Teixeira *et al.* (2021).

Metaphases were analysed and photographed using an Olympus BX53F epifluorescent microscope coupled to an Olympus MX10 camera, and images were captured using CellSens software. WU (330-385 nm) and WG (510-550 nm) filters were used to analyze DAPI and rhodamine and Cy3, respectively.

The phylogenetic relationship among *Strumigenys* species followed the previously published molecular phylogeny from Booher *et al.* (2021). The ideogram was generated using EasyIdio 1.0 software.

3. Results

Five ant species of *Strumigenys* were cytogenetically analyzed (Table 1). The karyotypes of four species were described for the first time: *S. crassicornis*, *S. denticulata*, *S.*

subedentata, and *Strumigenys* ufv-111. Karyotypic information for other population of *S. louisianae* from the southeast Atlantic rainforest was included. Telomeric and microsatellite motifs have been explored for the first time in this genus. The chromosomal data obtained for the *Strumigenys* studied are summarized in Figure 1, and Table 1.

In *crassicornis*-complex, data obtained from *S. crassicornis* showed a pattern of 2n=26 (2n=20m+6sm) chromosomes in females and n=13 (n=10m+3sm) chromosomes in males (Fig.2a; 3a, b; Table 2). And, *Strumigenys* ufv-111 revealed 2n=16 (2n= 8m+8sm) (Fig.2b; 3c; Table 3). In the sister group, *gundlachi*-complex, the chromosome number observed in *S. denticulata* was 2n=18 (2n= 16m+2sm) and *S. subedentata* revealed 2n=18 (2n= 18m) chromosomes (Fig.2c, d; Fig.3d, e; Table 4 and 5, respectively). In the louisianae-group, analysis of four colonies of *S. louisianae* showed variations in karyotypes among the populations studied. One colony, referred to from now on as *Strumigenys* ufv-02, revealed 2n=4 (2n=4m) chromosomes for females, while three other colonies, henceforth denoted as *Strumigenys* ufv-06, showed 2n=20 (2n=18m+2sm) (Fig.2e, f; Fig.3f, g; Table 6 and 7, respectively).

Concerning the ribosomal gene location, *S. crassicornis* 18S rDNA sites are located in the terminal region of the long arm and/or in the interstitial region of the third metacentric pair, the only block is labeled in the terminal region of the long arm of the same pair in males, revealing a heteromorphism (Fig.4a, b). *Strumigenys* ufv-111 18S rDNA genes were located interstitially in the long arm of the second metacentric pair (Fig.4c). Both species of *gundlachi*-complex showed the 18S rDNA blocks located in the pericentromeric region of the short arm, on the second metacentric pair of *S. denticulata*, and on the third metacentric pair of *S. subedentata* (Fig.4d, e). In the louisianae-group, *Strumigenys* ufv-02 showed 18S rDNA blocks located in the interstitial region of the short arm of the first chromosome pair while in *Strumigenys* ufv-06, 18S rDNA are located in the terminal and interstitial region of the long arm of the second metacentric chromosome pair, presenting a heteromorphism (Fig.4 f, g).

Table 1. Summary of cytogenetic data available in the literature of *Strumigenys* species. Groups according to Bolton (2000). **Abbreviations**: **BRA.** Brazil; **MG.** state of Minas Gerais; **AP.** state of Amapa; **GUF.** French Guiana; **MYS.** Malaysia; **IDN.** Indonesia. *Strumigenys* ufv-01, *Strumigenys* ufv-02, and *Strumigenys* ufv-06, are morphospecies from different studied populations that currently correspond to *S. louisianae* species.

Species	Locality	Group	2n	Karyotypic formula	References
Strumigenys crassicornis	BRA: Viçosa, MG	*gundlachi (crassicornis-*complex)	26	20m + 6sm	This study
Strumigenys denticulata	BRA: Viçosa, MG	*gundlachi (gundlachi-*complex)	18	16m + 2sm	This study
Strumigenys diabola	GUF: Sinnamary	*mandibularis*	40	4m + 10sm + 26a	Aguiar *et al.* (2020)
Strumigenys ufv-01	BRA: AP, Oiapoque	*louisianae*	26	12m + 12 sm + 2st	Barros *et al.* (2021)
Strumigenys ufv-06	BRA: Viçosa, MG	*louisianae*	20	18m + 2sm	This study
Strumigenys ufv-02	BRA: Viçosa, MG	*louisianae*	4	4m	Alves-Silva *et al.* (2014)
Strumigenys subedentata	BRA: Viçosa, MG	*gundlachi (gundlachi-*complex)	18	18m	This study
Strumigenys ufv-111	BRA: Viçosa, MG	*gundlachi (crassicornis-*complex)	16	8m + 8sm	This study
Strumigenys doherty	MYS: Kuala Lumpur	*capitata*	24	-	Imai *et al.*, (1983)
Strumigenys doriae	IDN: Chibodas	*doriae*	22	-	Imai *et al.*, (1985)
Strumigenys friedae	AUS: Queensland	*godeffroyi*	24	-	Imai *et al.*, (1977)
Strumigenys godeffroyi	MYS: Kuala Lumpur; IDN	*godeffroyi*	40-44	-	Imai *et al.*, (1983); Imai *et al.*, (1985)
Strumigenys mutica	IDN: Bogor	*mutica*	36	-	Imai *et al.*, (1985)
Strumigenys sp.	MYS	-	16	-	Goni *et al.*, (1982)
Strumigenys sp.	IDN: Chibodas	-	38	-	Imai *et al.*, (1985)
Strumigenys sp.	-	-	13	-	Crozier, (1975)

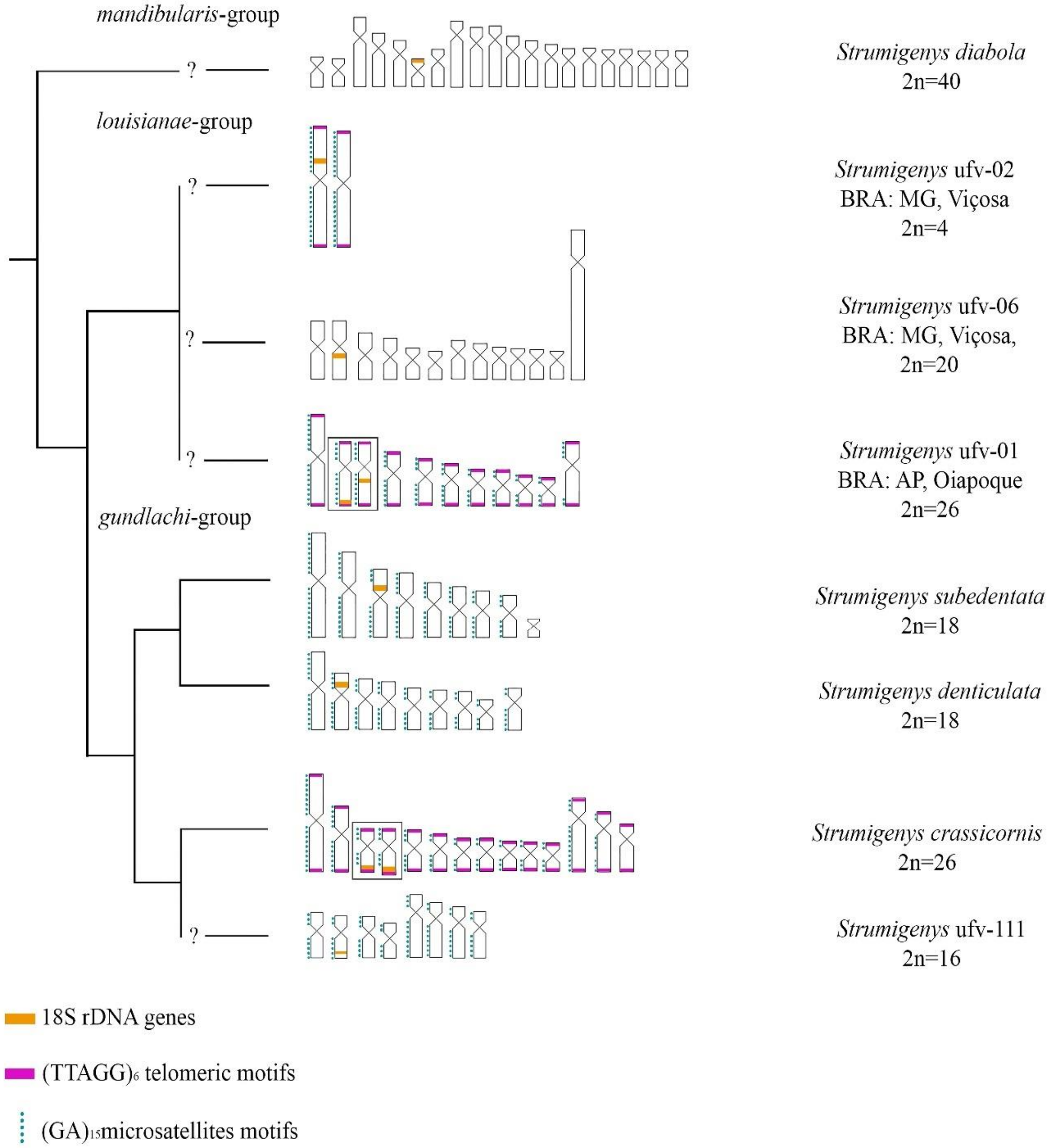

Fig. 1. Summary of available cytogenetic data (this study and literature) from Neotropic *Strumigenys* included in three species groups, namely *gundlachi*-group, *louisianae*-group, and *mandibularis*-group, defined in Bolton (2000). Ideograms show chromosome morphology, 18S rDNA clusters (orange), telomeres (pink), and microsatellite $(GA)_{15}$ (green dotted line) in the haploid complement. Boxes show heteromorphic pair present in *S. crassicornis* (pair 3) and in *Strumigenys* ufv-06 (pair 3) – only these pairs are duplicated in order to represent the variation between each chromosome unit. Phylogenetic relationships among the species are based on Booher *et al*. (2021). The sign "?" represents terminals of species that were not included in Booher's phylogeny, but that were included following Bolton's species-groups.

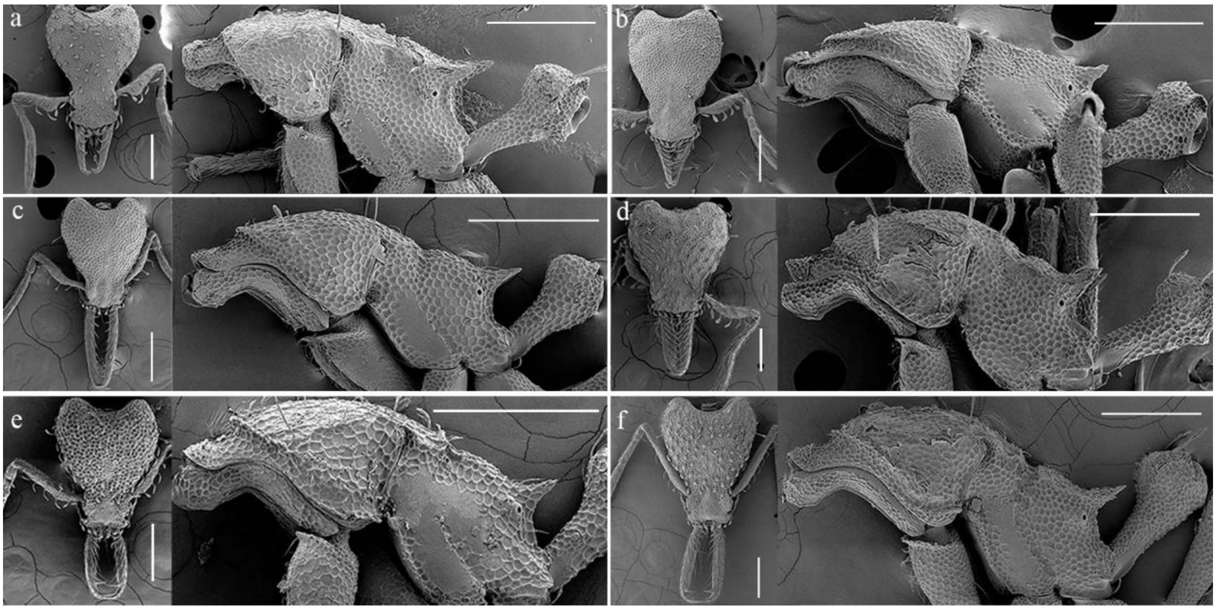

Fig. 2. Head and mesosome of females from karyotyped populations. **a**. *S. crassicornis*, **b**. *Strumigenys* ufv-111, **c.** *S. denticulata*, **d.** *S. subedentata*, **e.** *Strumigenys* ufv-02, **f.** *Strumigenys* ufv-06. Scales bars: 0.2mm.

In *S. crassicornis*, the telomeric motif (TTAGG)$_6$ is present in the telomeres of all the chromosomes (Fig.5a). *Strumigenys* ufv-111 signals of (TTAGG)$_6$ were scattered distributed, covering almost the entire length of chromosome, except for the small centromeric regions 9 (Fig.5b). In the *louisianae*-group, (TTAGG)$_6$, was present in the telomeres of all chromosomes of both populations (Fig.5c, d. Both *S. denticulata* and *S. subedentata* lacked signals for the telomeric motif (TTAGG)$_6$.

The *gundlachi*-group species had dispersed distributions of (GA)$_{15}$ chromosomes, with the exception of the centromeric region (Fig.6a–d). *S. crassicornis* and *S. subededentata* were the only species in the group that deviated from this distribution profile in some pairs, which have not been labeled in other regions (Fig.6a, and d, respectively). In the *louisianae*-group, (GA)$_{15}$ showed a dispersed pattern, except for the small centromeric regions and the long arm of the submetacentric pair are almost completely unlabeled in the *Strumigenys* ufv-06 (Fig.6e, f).

Fig. 3. *Giemsa* staining of **a.** female and **b.** male of *Strumigenys crassicornis*; **c.** *Strumigenys* ufv-111; **d.** *Strumigenys denticulata*; **e.** *Strumigenys subedentata*; **f.** *Strumigenys* ufv-02; and, **g.** *Strumigenys* ufv-06. Scales bars: 5 µm.

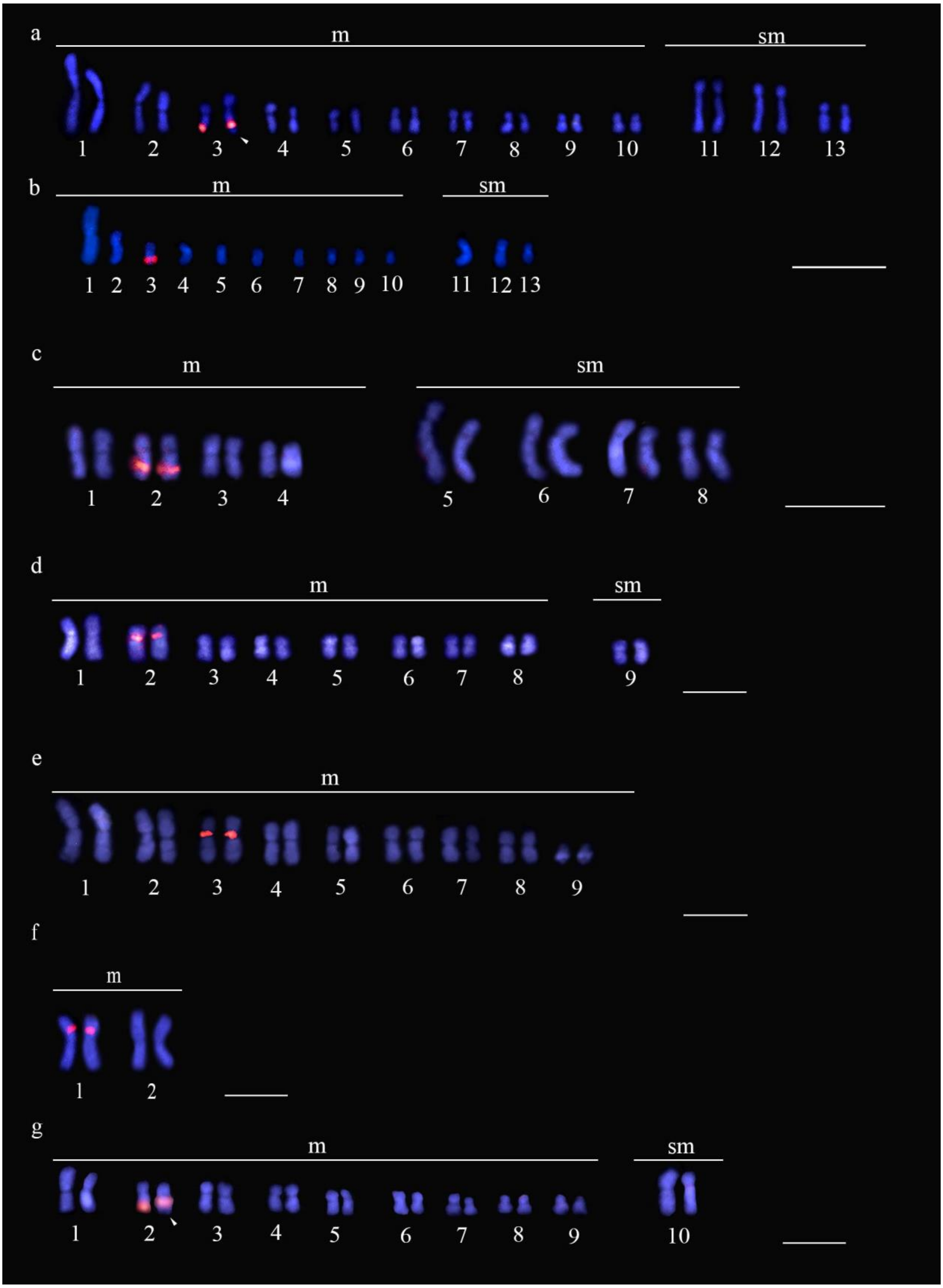

Fig. 4. Fluorescence *in situ* hybridization (FISH) with 18S rDNA probe (red blocks) of: **a.** female and **b.** male of *Strumigenys crassicornis*; **c.** *Strumigenys* ufv-111; **d.** *Strumigenys denticulata*; **e.** *Strumigenys subedentata*; **f.** *Strumigenys* ufv-02; and, **g.** *Strumigenys* ufv-06. Scales bars: 5 µm. Arrows in **a.** indicate the 18S rDNA chromosome carrier.

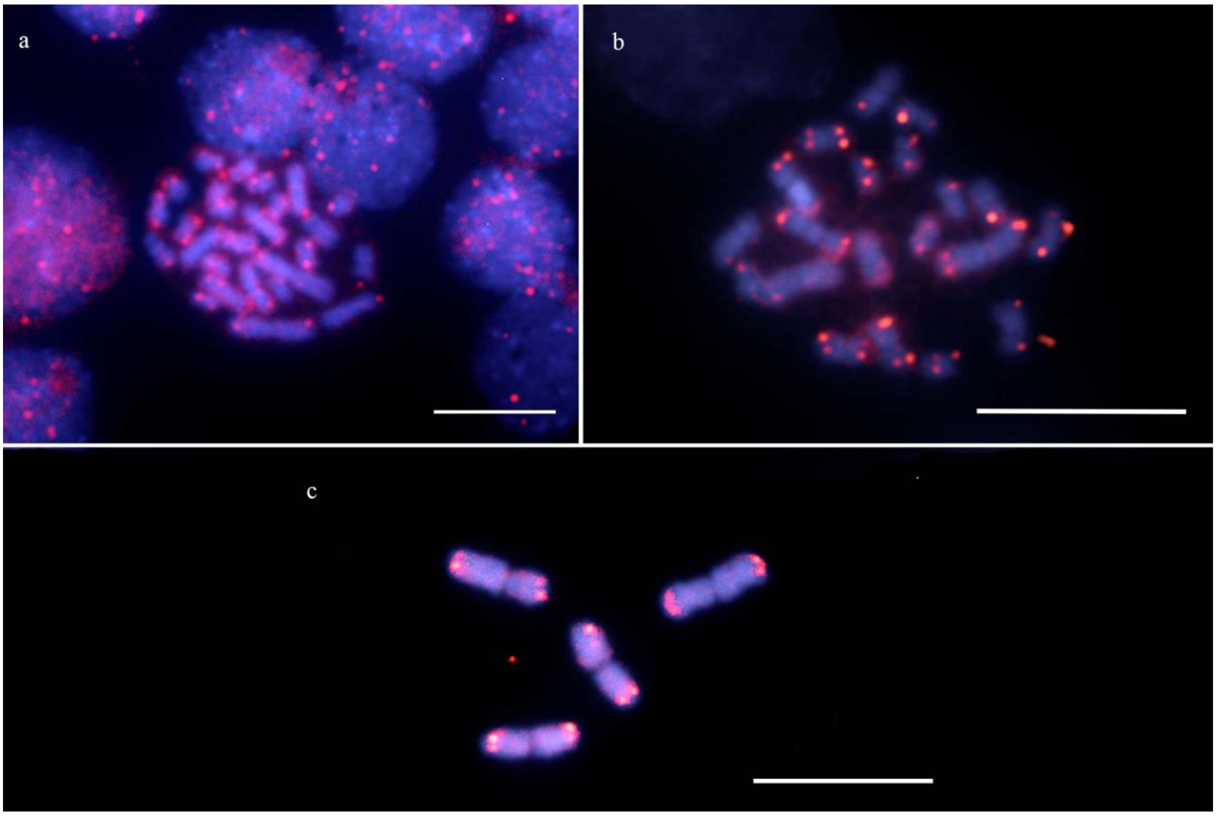

Fig. 5. Fluorescence *in situ* hybridization (FISH) with (TTAGG)$_6$ telomeric motif (red blocks) of: **a.** *Strumigenys crassicornis*; **b.** *Strumigenys* ufv-06; and, **d.** *Strumigenys* ufv-02. Scales bars: 5 µm.

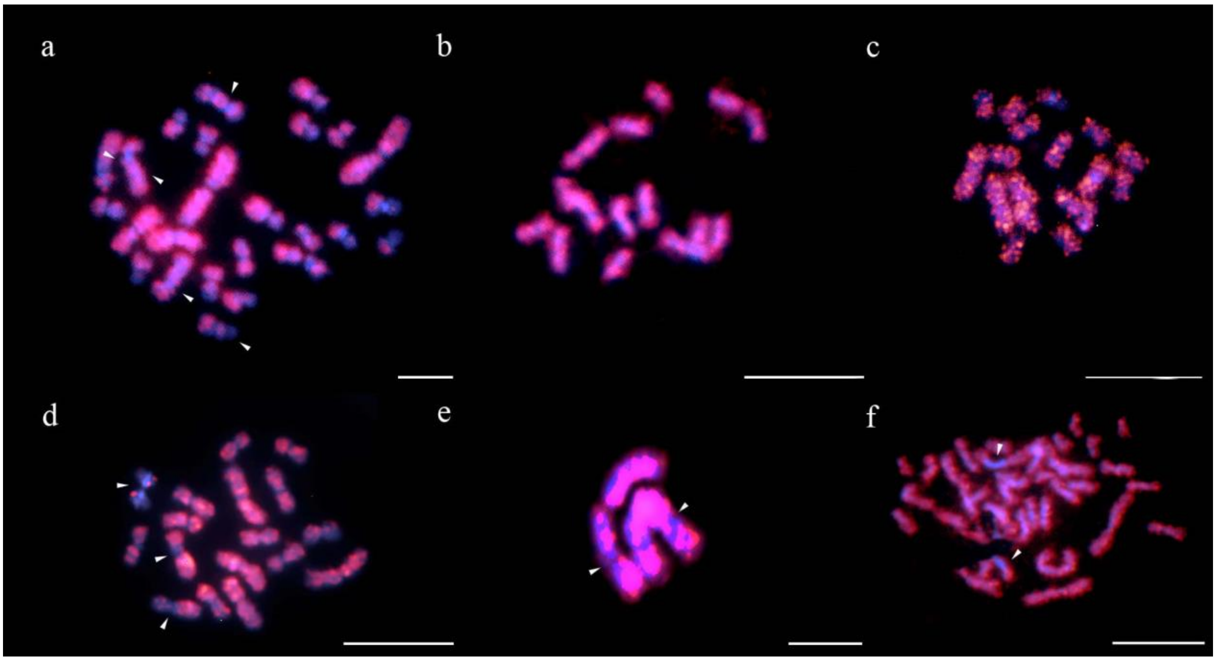

Fig. 6. Fluorescence *in situ* hybridization (FISH) with (GA)$_{15}$ microasstelit motif (red blocks) of: **a.** *Strumigenys crassicornis*; **b.** *Strumigenys* ufv-111; **c.** *Strumigenys denticulata*; d. *Strumigenys subedentata*; e. *Strumigenys* ufv-02; and, f. *Strumigenys* ufv-06. Arrows in **a, d, e,** and **f.** show negative regions for (GA)$_{15}$. Scales bars: 5 µm.

Table 2. Karyomorphometric analyses of the ant *Strumigenys crassicornis* 2n =26. Abreviations: **m**. metacentric; **sm.** submetacentric.

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	2.26 ± 0.67	2.37 ± 1.04	4.63 ± 1.68	7.98	1.03 ± 0.16	**m**
1	2.29 ± 0.98	2.5 ± 0.96	4.78 ± 1.93	8.24	1.11 ± 0.14	**m**
2	1.21 ± 0.44	1.78 ± 0.51	2.99 ± 0.84	5.15	1.58 ± 0.58	**m**
2	1.36 ± 0.51	1.69 ± 0.67	3.05 ± 1.16	5.25	1.25 ± 0.22	**m**
3	0.84 ± 0.26	1.32 ± 0.33	2.16 ± 0.58	3.72	1.61 ± 0.11	**m**
3	0.92 ± 0.26	1.14 ± 0.39	2.05 ± 0.61	3.53	1.25 ± 0.36	**m**
4	0.75 ± 0.22	1.02 ± 0.31	1.77 ± 0.53	3.05	1.37 ± 0.15	**m**
4	0.77 ± 0.31	1.1 ± 0.29	1.87 ± 0.59	3.22	1.5 ± 0.27	**m**
5	0.69 ± 0.2	1.1 ± 0.29	1.79 ± 0.47	3.08	1.63 ± 0.25	**m**
5	0.68 ± 0.15	1.02 ± 0.3	1.71 ± 0.43	2.94	1.51 ± 0.28	**m**
6	0.68 ± 0.16	0.92 ± 0.19	1.59 ± 0.34	2.74	1.38 ± 0.21	**m**
6	0.62 ± 0.16	0.92 ± 0.3	1.54 ± 0.43	2.65	1.49 ± 0.27	**m**
7	0.59 ± 0.14	0.84 ± 0.27	1.43 ± 0.41	2.46	1.4 ± 0.22	**m**
7	0.63 ± 0.13	0.87 ± 0.33	1.49 ± 0.44	2.56	1.36 ± 0.26	**m**
8	0.68 ± 0.26	0.79 ± 0.24	1.47 ± 0.43	2.53	1.3 ± 0.64	**m**
8	0.67 ± 0.17	0.9 ± 0.22	1.56 ± 0.37	2.68	1.36 ± 0.18	**m**
9	0.59 ± 0.2	0.77 ± 0.15	1.36 ± 0.32	2.34	1.34 ± 0.24	**m**
9	0.61 ± 0.22	0.8 ± 0.2	1.41 ± 0.41	2.43	1.36 ± 0.28	**m**
10	0.55 ± 0.17	0.8 ± 0.19	1.35 ± 0.33	2.32	1.52 ± 0.4	**m**
10	0.6 ± 0.29	0.8 ± 0.22	1.4 ± 0.46	2.41	1.67 ± 1.09	**m**
11	1.01 ± 0.28	2.33 ± 0.61	3.34 ± 0.86	5.75	2.34 ± 0.38	**sm**
11	0.92 ± 0.11	2.23 ± 0.78	3.15 ± 0.8	5.43	2.44 ± 0.77	**sm**
12	0.9 ± 0.15	1.95 ± 0.69	2.86 ± 0.79	4.93	2.16 ± 0.56	**sm**
12	0.99 ± 0.27	1.87 ± 0.69	2.86 ± 0.87	4.93	1.93 ± 0.58	**sm**
13	0.72 ± 0.18	1.5 ± 0.53	2.22 ± 0.68	3.82	2.08 ± 0.55	**sm**
13	0.74 ± 0.19	1.44 ± 0.54	2.18 ± 0.69	3.75	1.93 ± 0.43	**sm**
Total			58.01		100	

Table 3. Karyomorphometric analyses of the ant *Strumigenys* ufv-111 2n=16. Abreviations: **m**. metacentric; **sm.** submetacentric.

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	1.00 ± 0.22	1.46 ± 0.22	2.47 ± 0.37	8.31	1.51 ± 0.37	**m**
1	0.95 ± 0.21	1.45 ± 0.31	2.4 ± 0.46	8.07	1.55 ± 0.33	**m**
2	0.89 ± 0.14	1.38 ± 0.26	2.27 ± 0.3	7.64	1.58 ± 0.37	**m**
2	0.97 ±0.13	1.27 ± 0.19	2.25 ± 0.27	7.57	1.32 ± 0.2	**m**
3	0.91 ± 0.17	1.37 ± 0.5	2.28 ± 0.57	7.67	1.52 ± 0.49	**m**
3	0.88 ± 0.10	1.3 ± 0.22	2.18 ± 0.15	7.33	1.51 ± 0.4	**m**
4	0.84 ± 0.16	1.05 ± 0.19	1.9 ± 0.32	6.39	1.26 ± 0.19	**m**
4	0.80 ± 0.12	1.03 ± 0.08	1.82 ± 0.17	6.12	1.31 ± 0.16	**m**
5	0.95 ±0.20	2.43 ± 0.47	3.76 ± 0.85	12.65	2.58 ± 0.42	**sm**
5	0.92 ± 0.13	2.32 ± 0.41	3.25 ± 0.49	10.93	2.54 ± 0.4	**sm**
6	0.89 ± 0.18	2.12 ± 0.33	3.01 ± 0.46	10.12	2.45 ± 0.42	**sm**
6	0.84 ± 0.18	2.07 ± 0.32	2.92 ± 0.49	9.82	2.5 ± 0.29	**sm**
7	0.86 ± 0.09	1.93 ± 0.34	2.79 ± 0.39	9.38	2.26 ± 0.38	**sm**
7	0.86 ± 0.09	1.78 ± 0.16	2.65 ± 0.18	8.91	2.09 ± 0.34	**sm**
8	0.90 ± 0.18	1.67 ± 0.23	2.58 ± 0.23	8.91	1.94 ± 0.56	**sm**
8	0.94 ± 0.18	1.65 ± 0.11	2.59 ± 0.26	8.68	1.81 ± 0.35	**sm**
Total			29.73	100		

Table 4. Karyomorphometric analyses of the ant *Strumigenys denticulata* 2n=18. Abreviations: **m**. metacentric; **sm.** submetacentric.

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	1.72 ± 0.37	2.13 ± 0.36	3.95 ± 0.64	9.60	1.19 ±0.22	**m**
1	1.65 ± 0.43	1.9 ± 0.47	3.55 ± 0.88	8.63	1.17 ± 0.15	**m**
2	1.1 ± 0.2	1.73± 0.36	2.83 ± 0.42	6.88	1.63 ± 0.52	**m**
2	1.11 ± 0.27	1.61 ± 0.31	2.71 ± 0.48	6.59	1.50 ± 0.37	**m**
3	1.02 ± 0.22	1.47 ± 0.23	2.49± 0.33	6.05	1.51 ± 0.43	**m**
3	0.9 ± 0.15	1.34 ± 0.19	2.24 ± 0.24	5.45	1.54 ± 0.38	**m**
4	0.96 ± 0.21	1.37± 0.22	2.33 ± 0.38	5.66	1.47 ± 0.31	**m**
4	0.91 ± 0.17	1.28 ± 0.23	2.19 ± 0.31	5.32	1.43 ± 0.36	**m**
5	0.86 ± 0.12	1.19 ± 0.26	2.05 ± 0.32	4.98	1.40 ± 0.32	**m**
5	0.8 ± 0.13	1.16 ± 0.25	1.96 ± 0.27	4.77	1.48 ± 0.4	**m**
6	0.82 ± 0.19	1.13 ± 0.22	1.95 ± 0.36	4.74	1.42 ± 0.34	**m**
6	0.78 ± 0.15	1.12± 0.28	1.91± 0.36	4.64	1.46± 0.37	**m**
7	0.8 ± 0.18	1.06± 0.22	1.86± 0.37	4.52	1.33± 0.22	**m**
7	0.74± 0.18	1.04± 0.21	1.78± 0.36	4.33	1.45± 0.25	**m**
8	0.63± 0.17	0.91± 0.27	1.54± 0.41	3.74	1.45± 0.31	**m**
8	0.68± 0.24	0.87± 0.23	1.55± 0.44	3.77	1.35± 0.35	**m**
9	0.73± 0.21	1.38± 0.28	2.11± 0.33	5.13	2.01± 0.71	**sm**
9	0.79± 0.2	1.34± 0.21	2.13± 0.23	5.18	1.81± 0.61	**sm**
Total			41.13	100		

Table 5. Karyomorphometric analyses of the ant *Strumigenys subedentata* 2n=18. Abreviations: **m**. metacentric.

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	2.38±1.03	2.8±1.18	5.18±2.2	9.74	1.19±0.12	**m**
1	2.41±0.97	2.64±1.01	5.04±1.97	9.48	1.11±0.06	**m**
2	1.81±0.56	2.42±0.8	4.23±1.32	7.96	1.36±0.27	**m**
2	1.74±0.59	2.35±0.79	4.09±1.35	7.69	1.37±0.2	**m**
3	1.42±0.41	1.98±0.61	3.4±0.99	6.40	1.40±0.21	**m**
3	1.38±0.32	2.03±0.69	3.4±0.97	6.40	1.46±0.24	**m**
4	1.37±0.4	1.85±0.58	3.22±0.98	6.06	1.35±0.12	**m**
4	1.32±0.43	1.62±0.52	2.94±0.94	5.53	1.23±0.08	**m**
5	1.06±0.45	1.63±0.68	2.68±1.11	5.04	1.56±0.24	**m**
5	1.03±0.31	1.56±0.52	2.59±0.81	4.87	1.53±0.19	**m**
6	1.19±0.37	1.33±0.39	2.52±0.76	4.74	1.12±0.07	**m**
6	1.05±0.3	1.44±0.64	2.49±0.93	4.68	1.33±0.23	**m**
7	0.96±0.32	1.32±0.61	2.28±0.85	4.29	1.37±0.46	**m**
7	1.04±0.44	1.48±0.77	2.52±1.18	4.74	1.40±0.33	**m**
8	0.9±0.4	1.18±0.38	2.08±0.75	3.91	1.39±0.33	**m**
8	0.83±0.36	1.08±0.26	1.91±0.6	3.59	1.42±0.37	**m**
9	0.51±0.11	0.81±0.2	1.32±0.27	2.48	1.63±0.41	**m**
9	0.51±0.16	0.76±0.32	1.27±0.46	2.39	1.49±0.36	**m**
Total			53.16	100		

Table 6. Karyomorphometric analyses of the ant *Strumigenys* ufv-02 (*louisianae*-group) 2n=4. Abreviations: **m**. metacentric.

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	2.67 ±1.38	3.33±1.18	5.93±2.89	25.68	1.23±0.21	**m**
1	2.73±1.21	3.14±1.14	5.34±1.39	23.13	1.19±0.23	**m**
2	2.58±0.8	3.24±1.37	5.83±2.09	25.25	1.23±0.31	**m**
2	2.45±0.78	3.01±0.59	5.99±2.26	25.94	1.17±0.15	**m**
Total			23.09	100		

Table 7. Karyomorphometric analyses of the ant *Strumigenys* ufv-06 (*louisianae*-group) 2n=20. Abreviations: **m**. metacentric. **sm.** Submetacentric

Chromosome	Chromosome arm		Total length (p+q)	Relative length (%)	Arm ratio (q/p)	Chromosome morphology
	Short (p)	Long (q)				
1	2.07±0.31	2.45±0.33	4.61±0.6	9.60	1.14±0.11	**m**
1	1.95±0.3	2.15±0.28	4.11±0.53	8.56	1.11±0.12	**m**
2	1.41±0.16	2.2±0.29	3.61±0.38	7.52	1.57±0.23	**m**
2	1.04±0.19	1.72±0.3	2.76±0.41	5.75	1.69±0.39	**m**
3	1.07±0.25	1.63±0.18	2.69±0.4	5.60	1.57±0.27	**m**
3	0.98±0.14	1.53±0.11	2.52±0.19	5.25	1.58±0.25	**m**
4	0.92±0.07	1.43±0.18	2.34±0.18	4.88	1.57±0.25	**m**
4	0.92±0.14	1.4±0.18	2.32±0.24	4.83	1.56±0.25	**m**
5	0.8±0.14	1.29±0.14	2.09±0.26	4.35	1.64±0.21	**m**
5	0.79±0.15	1.22±0.18	2.01±0.25	4.19	1.59±0.41	**m**
6	0.73±0.14	1.21±0.17	1.94±0.25	4.04	1.70±0.42	**m**
6	0.73±0.13	1.17±0.14	1.9±0.2	3.96	1.66±0.38	**m**
7	0.77±0.17	1.08±0.18	1.85±0.31	3.85	1.45±0.35	**m**
7	0.72±0.13	1.03±0.19	1.75±0.28	3.65	1.45±0.27	**m**
8	0.63±0.08	0.95±0.12	1.58±0.18	3.29	1.51±0.18	**m**
8	0.61±0.1	0.9±0.2	1.51±0.27	3.15	1.49±0.3	**m**
9	0.62±0.08	0.83±0.14	1.45±0.2	3.02	1.33±0.18	**m**
9	0.5±0.1	0.82±0.17	1.32±0.21	2.75	1.69±0.39	**m**
10	1.15±0.2	2.04±0.29	3.19±0.43	6.65	1.79±0.28	**sm**
10	0.9±0.19	1.55±0.17	2.45±0.19	5.10	1.81±0.47	**sm**
Total			48	100		

4. Discussion

The five *Strumigenys* species studied show distinct chromosomal patterns, even in closely related species. This variation in chromosome number (2n=4 to 44) and morphology has been found in previously studied species from the Neotropics, Indo-Malaysian, and Australian regions (Crozier, 1975; Imai *et al.*, 1977; Goni *et al.*, 1982; Imai *et al.*, 1983; Imai *et al.*, 1985; Alves-Silva *et al.*, 2014; Aguiar *et al.*, 2020; Barros *et al.*, 2021). To date, only the Neotropical species *S. diabola*, *S. louisianae* from the Amazon region, and *S. louisanae* from the Atlantic rainforest had karyomorphometric analyses associated with their karyotypic descriptions (Alves-Silva *et al.*, 2014; Aguiar *et al.*, 2020; Barros *et al.*, 2021; Teixeira *et al.*, 2021). We presented karyomorphometric values associated with all descriptions, which provide new insights for comparisons between closely related species.

4. 1. *gundlachi*-group:

It is possible to notice a variation in the number and morphology of chromosomes of *gundlachi*-group species, despite they being considered quite close biologically (Bolton, 2000; Booher *et al.*, 2021). In contrast to it sister complex, the *crassicornis*-complex species exhibits a greater degree of variability in terms of karyotype count, along with significant variation in chromosome size and an increased frequency of submetacentric pairs. The *gundlachi*-complex karyotypes are characterized mainly by metacentric chromosomes with no significant differences in chromosome size among most of its pairs.

The numerical and morphological differences among these species can be attributed to pericentromeric inversions, as well as centric fissions or fusions. Such mechanisms are thought to be common in ant evolution (Imai *et al.*, 1977). The varying location of the 18S rDNA blocks in the two species complexes supports this hypothesis, given that they are located in the short arms of the *gundlachi*-complex species, and in the long arms of the *crassicornis*-complex species.

Regarding species, *S. crassicornis* shows a variation in the bearer chromosome pair of the 18S rDNA was observed between individuals from different *S. crassicornis* colonies. Females from two colonies had the third metacentric pair small and metacentric with telomeric labels. The other population presented a heterozygous condition, 18S rDNA genes also occur in the long arm of the third metacentric pair; however, one label was located in the interstitial and the other in the terminal region. It is also possible to notice a small difference in the size of the short arm between the chromosomes that constitutes the first metacentric pair, which is not observed in non-heteromorphic populations.

The structural chromosomal heteromorphism may be related to rearrangements such as non-reciprocal translocations. Nonreciprocal translocations occur when chromosomal fragments are broken and combined at another site, usually in a nonhomologous chromosome (Lorite and Palomeque, 2010). Imai *et al.* (1988) stated that these translocations play an important role in the chromosomal evolution of ants, and may be mechanisms that reduce chromosomal pairing or the chromosomal crossover rate in translocation heterozygotes

(Sherizen *et al.*, 2005). However, this pattern was not sufficiently observed to consistently confirm the existence of structural chromosomal dimorphism between colonies of *S. crassicornis*.

4. 2. ***louisianae*-group: *Strumigenys louisianae***

The results found for the first population of *S. louisianae* (2n=4) confirms the description made by Alves-Silva *et al.* (2014) as the species with the lowest chromosome number observed for the subfamily Myrmicinae, and for the Hymenoptera found in the Neotropical region. There are differences among the three karyotyped populations within the *louisianae*-group (Alves-Silva *et al.*, 2014; Barros *et al.*, 2021; the present study). This support the karyotype heterogeneity in the species *S. louisianae* and reinforce the argument that it is not a genuine species, as suggested by Bolton (2000). Despite the fact that it was not the objective of the present study to revise *S. louisianae*, it is possible to point out, we classified them into three distinct morphospecies: *Strumigenys* ufv-01 from Amazon population with 2n=26; *Strumigenys* ufv-02 from Atlantic Forest with 2n=4; and *Strumigenys*-ufv-06 from the second population of the Atlantic Forest with 2n=20.

Strumigenys ufv-01 has relatively large compound eyes, robust mandibles, ovoid and spoon-shaped head ground pilosity, and, in lateral view, a small smooth patch on mesosoma, restricted to the lower mesopleura. There is no information regarding the intercaste of alate queens in this morphospecies (Chaul *et al.*, in prep.). *Strumigenys* ufv-01 has a morphological pattern similar to that of populations in the southern USA, which is the type localityof *S. louisianae* (Barros *et al.* 2021; Chaul *et al.*, in prep.).

Strumigenys ufv-02 has relatively smaller compound eyes, slightly fragile mandibles, head ground pilosity made of thin spoon-shaped hairs, and, in lateral view, a larger smooth patch on mesosoma, covering the entire mesopleura. In addition to having smaller body sizes in workers, it also has an intercaste form, which probably represents the colony's only reproductive individuals since no alate queens were found. *Strumigenys* ufv-02 more closely resembles the syntype of the junior synonym *Strumigenys unidentata* Mayr, 1887 than the types of *S. louisianae* (Chaul *et al.* in prep.).

Strumigenys ufv-06 is the largest species, with relatively smaller compound eyes, longer mandibles, ovoid and spoon-shaped head ground pilosity, and, in lateral view, a broad smooth patch on mesosoma, covering the entire mesopleura. *Strumigenys* ufv-02 and *Strumigenys* ufv-06 may represents two of many different populations occurring in sympatry along the Brazilian Atlantic rainforest, which house the greatest morphological diversity of the *S. louisianae*-complex—*sensu* Bickford *et al.*, (2007), not Bolton, (2000) (Chaul *et al.* in prep.). The *louisianae*-complex karyotypic data highlight the importance of cytogenetic studies from distant populations, which is essential for understanding the evolution of chromosomes in widely distributed taxa, as has been observed in numerous other ant species (Mariano *et al.*, 2012; Micolino *et al.*, 2019a; Teixeira *et al.*, 2020).

Within the *lousianae* species complex, there is variation in the position of the 18S rDNA sites, *Strumigenys* ufv-02 has an interstitial rDNA site in the short arm, *Strumigenys* ufv-01 has a pericentromeric rDNA site in the long arm and *Strumigenys* ufv-06 has a heteromorphism in the pair of 18S rDNA—one chromosome showed the rDNA blocks in the terminal and other in the interstitial region, both in the long arm (see previous discussion of *S. crassicornis* heteromorphism in 4.1). It has been demonstrated that the rDNA is conserved within a species or natural population, and any intraspecific changes in these regions may be related to speciation processes (Raskina *et al.*, 2004; 2008). Therefore, the variation observed between the different morphospecies of the *louisianae*-complex suggests, once again, that they may be different species. However, this pattern was not sufficiently observed to consistently confirm the existence of that chromosomal dimorphism between colonies of *Strumigenys* ufv-06.

4. 3. Cytogenetic patterns of Neotropical *Strumigenys*

All *Strumigenys* species studied here and in previous works showed a single region labeled with 18S rDNA probe. Prior studies on these ants have revealed that GC-rich regions align with 18S rDNA sites, though we did not assess mapping techniques for such regions in our work (Alves-Silva *et al.*, 2014; Barros *et al.*, 2021). Nonetheless, it is plausible to infer that similar trends exist across related ant species based on our observations thus far. Moreover, most ants exhibit one pair of chromosomes containing both GC-rich and 18S rDNA sites as their predominant pattern (Teixeira *et al.,* 2021).

However, the results of our investigation reveal differences in cytogenetic banding configurations of 18S probe across distinct *Strumigenys* species, with variations in the positioning, chromosome pairs, arms, and regions labeled. *S. diabola*, a species from the *mandibularis*-group, has 18S rDNA blocks in the terminal region of the chromosome (Teixeira *et al.*, 2021). Both species of the *crassicornis*-complex have a subterminal and terminal rDNA site located on their chromosome long arm. In contrast, species of the *gundlachi*-complex have intrachromosomal rDNA sites in their short arms. Within the *lousianae* species complex, *Strumigenys* ufv-02 has an interstitial rDNA site, *Strumigenys* ufv-01 has a pericentromeric rDNA site in the long arm, and *Strumigenys* ufv-06 has a terminal and interstitial site. No pattern of arm-specific localization was observed within the *louisinae*-complex—*sensu* Bickford *et al.*, (2007).

The microsatellite $(GA)_{15}$, mapped for the first time for the genus, presented two distributions patterns: (1) dispersed distribution along the both arms of chromosomes, except for the centromeric region, and (2) additional clustering to the dispersed distribution in specic chromosome pair. Most of the *Strumigenys* species chromosomes's revealed the first pattern, similary to that of other ant genera (Teixeira *et al*., 2022b; Barros *et al*., 2018) and bees (Lopes *et al.*, 2020; Barbosa *et al*., 2021). *Strumigenys* ufv-06, *S. crassicornis,* and *S. subedentata* have chromosomes that exceptionally follow the second pattern of distribution. *Strumigenys* ufv-06 has the long arm of the only submetacentric pair almost completely lacking positive markings for $(GA)_{15}$. *Strumigenys crassicornis* showed $(GA)_{15}$ clusters in the second submetacentric pair, and *Strumigenys subedentata* lacks $(GA)_{15}$ signals in the 18S rDNA pericentromeric site and in its whole 9th metacentric pair. A similar clustered pattern in some chromosome pairs has also been observed in *Trachymyrmex holmgreni*, *Mycocepurs goeldii*, and in *Sericomyrmex* sp. (Micolino *et al.,* 2019b; Teixeira *et al*., 2022a).

In the majority of ants species that have been analyzed so far, the microsatellite $(GA)_{15}$ is frequently found in euchromatic regions (Teixeira *et al*., 2022a). This suggests that despite the absence of C-banding data, it's likely that similar distribution patterns are present across *Strumigenys* studied here. Various mechanisms, such as the slippage activity of DNA polymerase during replication or repair of DNA, uneven crossover events, and transposable elements may play a role in microsatellite expansion, elimination, generation, or propagation within the genome (Li *et al*., 2002; Elegren, 2004). As such processes could be involved in (GA)15 clustering in some regions of *Strumigenys* ufv-06, *S. crassicornis*, and *S. subedentata* karyotypes. However, it remains ambiguous as to whether the complete lack of markings of this microsatellite in the last metacentric pair of *S. subedentata* is a result of analogous mechanisms or solely due to its complete heterochromatic nature.

Concerning the telomeric motif, $(TTAGG)_6$, mapped for the first time for the genus, was detected in all telomeres of *S. crassicornis*, *Strumigenys* ufv-02, and *Strumigenys* ufv-06. The other species, *Strumigenys* ufv-111, *S. denticulate*, and *S. subedentata*, lack positive marks for the telomeric sequence. No occurrence of interstitial $(TTAGG)_6$ marking was detected among the examined species. The telemoric sequence $(TTAGG)_n$ is believed to be an ancestral motif of the telomere of insects, and likely evolved from the vertebrate canonical telomeric reapet, $(TTAGGG)_n$ (Kuznetsova *et al*., 2019). The $(TTAGG)_n$ sequence is consistly preserved in Old World and Attina ant species (Kuznetsova *et al.,* 2019; Teixeira *et al.*, 2022a). So, tests with $(TTAGG)_6$ must be performed again in *Strumigenys* ufv-111, *S. denticulata*, and *S. subedentata*. However, considering that insects have a wide variety of telomere structures, the presence of other repetitive sequences associated with the telomeres of that species should be investigated, such as the canonical telomeric sequence, $(TTAGGG)_n$ (Meyne *et al.* 1995; Wurm *et al.*, 2011; Kuznetsova *et al.,* 2020; Lukhtanov, 2022).

In the genus *Strumigenys*, only 11 valid species and five morphospecies have their karyotypes already described, leaving a gap equivalent to almost 99% of the species. Because there have been relatively few cytogenetic data already collected for such a broad genus, and even so, they show significant variation (2n=4 to 2n=40, only in the Neotropical region), karyotypic evolutionary hypotheses for this genus are unlikely to be consistent with the current data.

5. Conclusion

The present study showed that it is possible to differentiate between the studied *Strumigenys* species of *crassicornis* and the *gundlachi*-complex using the number, chromosomal morphology, and carrier pair of the 18S rDNA chromosome. In addition, the results of the chromosome analysis of another sympatric population, also identified as *S. louisianae*, indicated taxonomic problems with the species, suggesting the existence of a complex of cryptic species. Overall, the results show that the use of classical and molecular cytogenetics, along with morphology, is effective in delineating the boundaries between cryptic species.

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CONSIDERAÇÕES FINAIS

Os capítulos desta dissertação apresentaram a alfa-taxonomia, morfologia e citogenética de uma parte do gênero *Strumigenys* (Myrmicinae: Attini). A região Neotropical apresentou um aumento de duas espécies no gênero *Strumigenys* com a descrição de duas novas espécies para o grupo-*gundlachi*. Além dessa novidade, foram atualizadas as diagnoses de seis espécies próximas às descritas. O estudo citogenético acrescentou uma série de novidades cariológicas para o gênero: descrições cariotípicas para seis espécies, além de técnicas moleculares inéditas para *Strumigenys*, como mapeamento telomérico e do microssatélite $(GA)_{15}$. Varições cromossômicas encontradas neste estudo, somadas a dados extraídos da literatura, analisadas em conjunto com a variação morfológica das populações cariotipadas, evidenciam problemas em relação a unidade taxonômica da espécie *S. louisianae,* a qual passa a ser tratada como um complexo de espécies crípticas. O uso cruzado de diversas técnicas durante as análises e delineamento de espécies com grande similaridade morfológica se provou um método eficaz. Esse método revelou uma considerável diversidade não descrita no gênero, destacando a importância de explorá-lo em estudos futuros. O presente trabalho é uma contribuição no sentido de apontar, através do uso combinado de morfologia e citogenética, a existência de lacunas taxonômicas dentro do gênero *Strumigenys.*