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Sistemática e ecologia de ácaros Erythraeoidea (Acari: Parasitengona)

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Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Zoologia, da Universidade Federal de Minas Gerais, como parte dos requisitos necessários para a obtenção do título de Mestre em Zoologia.

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Sistemática e ecologia de ácaros Erythraeoidea (Acari: Parasitengona)

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Esta dissertação foi apresentada em sessão pública e submetida a avaliação em 26 de março de 2019, pela Banca Examinadora composta pelos seguintes membros:

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DEDICATÓRIA

Aos meus amados pais, que sempre me deram apoio no caminho das ciências da natureza. A minha mãe que já no maternal preparava a merendeira cheia de amor e até hoje em meio a correria da pós-graduação uma marmita com sobremesa e lanchinhos que me lembram do apoio que vem de casa. A meu pai que tem me motivado desde a infância com os inúmeros acampamentos dos quais surgiram meu gosto pela natureza, até o apoio incondicional a minha dedicação a vida acadêmica, que é por muitos desvalorizada.

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Aos meus irmãos e inúmeros amigos pelo apoio ao longo da jornada de pós-graduação. Entre eles se destacam Sara, Baruc, Dante, Pedro, Samuel, Lucas, Luciana, Carlos, Higor, Gabriel, Franciele, especialmente a Qinghe por ter também me hospedado durante o intercambio e a minha querida Gabriela que tanto tem me apoiado nesta reta final.

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“Work hard, play hard” (Guetta, 2011)

SUMÁRIO

Costa SGS. Sistemática e ecologia de ácaros Erythraeoidea (Acari: Parasitengona) [dissertação]. Minas Gerais: Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; 2019.

RESUMO

Durante seu ciclo de vida, ácaros Parasitengona passam por um ovo, uma prelarva caliptostásica regressiva, uma larva ectoparasítica e heteromórfica, uma protoninfa caliptostática, uma deutoninfa predadora de vida livre, uma tritoninfa caliptostática e um adulto predador de vida livre. Para que seja feita uma descrição completa das fases larvars e pós larvais é necessário a criação ou associação molecular de instares heteromórficos. As dificuldades relacionadas a estes procedimentos de associação fezeram com que a maioria das espécies descritas até hoje sejam conhecidas apenas a partir de larvas ou de estágios pós-larvas. No primeiro capítulo deste estudo, duas novas espécies de Erythraeidae (Acari: Parasitengona) habitantes de cavernas são descritas, incluindo todos os estágios ativos e uma possivel espécie troglóbia. Além disso, uma descrição completa dos adultos e da deutoninfa de *Charletonia rocciai* Treat & Flechtmann, 1979, notas sobre a morfologia larval e o comportamento são fornecidas. A associação dos estágios foi feita através de experimentos de criação e análise molecular empregando o gene mitocondrial citocromo oxidase I (COI) e a implementação bayesiana do algoritimo *Generalized Mixed Yule Coalecent* (bGMYC) para delimitação de espécies. Também é demonstrado experimentalmente que as larvas de *Charletonia rocciai* não estão ligadas ao mesmo hospedeiro durante todo o estágio, sendo capazes de trocar de um inseto hospedeiro para outro. A presença de troglomorfismo e a distribuição dos espécimes no ambiente cavernícola ou epígeo também são discutidas. No segundo capítulo do presente estudo, uma investigação taxonômica sobre o gênero smaridídeo *Trichosmaris* Southcott, 1963, levou à descrição, pela primeira vez, de seu estágio larval. Larvas e pós-larvas foram associados com base na criação da espécie tipo *T. dispar* Southcott, 1963 e uma nova espécie de *Trichosmaris*. Eles são descritos juntamente com uma nova espécie do sudeste do Brasil, uma chave para gêneros de Smarididae e uma para espécies de *Trichosmaris* são fornecidas. As relações filogenéticas da família Smarididae são inferidas com base em quarenta e nove caracteres morfológicos de larvas e adultos, incluindo todas as espécies para as quais as larvas são conhecidas. A evolução de um gnatossoma altamente modificado para Smarididae larval sugerindo um possível comportamento predatório é discutida, além de sinonímias e novas combinações propostas.

Costa SGS. Systematics and ecology of Erythraeoidea (Acari: Parasitengona) mites [Dissertation]. Minas Gerais: Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; 2019.

ABSTRACT

During their life cycle Parasitengona go throught an egg, a regressive calyptostaic prelarva, a heteromorphic ectoparasitic larva, a calyptostatic protonymph, free-living predaceous deutonymph, a calyptostatic tritonymph and a free-living predaceous adult. This complex life cycle requires rearing or molecular barcoding association of heteromorphic instars. The necessity of using those technics and the difficults associated with them, made most of the described species known from either post larval instars or larva. In the first chapter of this study, two new cave dwelling Erythraeidae (Acari: Parasitengona) species are described including all active instars and a possibly troglomorphic sepecies. Additionally, a complete description of the adults and deutonymph of *Charletonia rocciai* Treat & Flechtmann, 1979 and notes on the larval morphology and behavior are provided. The instar association were made through rearing experiments and molecular analysis employing the mitochondrial cytochrome oxidase I (COI) gene and the Bayesian Generalized Mixed Yule Coalescent (bGMYC) algorithm for species delimitation. We also demonstrated experimentally that *C. rocciai* larvae are not attached to the same host during the entire stage, being able to swap from a host insect to another. The presence of troglomorphism and the distribution of the specimens in the cave or epigean environment are also discussed. In the second chapter of the present study a taxonomic investigation on the smaridid genus *Trichosmaris* Southcott, 1963 led to the description, for the first time, of its larval instar. Larvae and post-larval instars were associated by rearing to *T. dispar* Southcott, 1963 and a new *Trichosmaris* species. They are described along an additional new species from the southeast of Brazil and a key for Smarididae genera and for *Trichosmaris* species. Phylogenetic relationships among Smarididae are inferred based on forty-nine morphological characters from larvae and adults, including all species for which the larvae are known. The evolution of a highly modified gnathosoma for larval Smarididae and a possible predatorial behavior is discussed. In addition, synonymies and new combinations proposed.

LISTA DE ABREVIAÇÕES MORFOLOGICAS

1a – Seta esternal I

1b – Seta coxa I

2a – Seta esternal II

2b – Seta da coxa II

2c – Segunda seta da coxa II

3a – Seta intercoxal III

3b – Seta da coxa III

3c – Segunda seta da coxa III

AAS – Distância entre sensilas anteriores e primeiro par de setas comuns do escudo

AL – Seta comum anterolateral do escudo

AP – Distância entre as setas comuns anterolateral e posterolateral do escudo

ASBa - Distância entre as sensilas anteriores e a borda anterior do scutum.

Asens – Sensila anterior ou comprimento da sensila anterior

AW – Distância entre a base das setas normais anteriores do escudo

B – Seta barbada comum

BFe – Basifêmur

CX – Coxa

DS – Comprimento das setas dorsais

elcl– Micro seta da coxa I.

elcp– Micro seta da coxa do palpo.

eζ – Eupatídio do tarso do palpo da larva.

fn – Número de setas em seguimentos das várias pernas. Por exemplo: fnTi= 18-19-19 é o número de setas comuns na Tíbia 1-2-3 respectivamente.

Ga ou *cs* – Galeala (seta adoral)

Ge – Genu

GL – Comprimento do gnatossoma

GW – Largura do gnatossoma

hc, is, ds, gs, nc – Setas do palpo tarso das larvas de Erythraeoidea. Respectivamente da mais distal *hc* para a mais basal *nc*.

Hya ou *as* – Seta hipostomal anterior

Hyb ou *bs* – Seta hipostomal posterior

IL – Comprimento do idiossoma

IP – Soma do comprimento das pernas

ISD – Distância entre as bases da seta anterior sensilar e as da seta posterior sensilar

IW – Largura do idiossoma

L – Comprimento Máximo do escudo

LX – Distancia entre a seta antero lateral do scutum e a borda anterior.

legI/legII – Razão entre o comprimento da Perna 1 e da Perna 2

ML – Seta comum mediana do escudo

MW – Distância entre as bases das setas normais medianas do escudo

N - Seta lisa comum.

PaScFed – Seta dorsal do fêmur do palpo

PaScGed – Seta dorsal do genu do palpo

PL – Seta comum posterolateral do escudo

PSBp – Distância entre a sensila posterior e a borda posterior do scutum.

Psens – Sensila posterior ou comprimento da sensila posterior

PW – Distância entre as setas normais posteriores do escudo

S ou N – Seta lisa

Sba – Distância entre as bases das sensilas anteriores do escudo

Sbp – Distância entre as bases das sensilas posteriores do escudo

Ta (H) – Tarso, largura

Ta (L) – Tarso, comprimento

TaI/TiI – Razão entre o comprimento da Tarso I e da Tíbia I

TFe – Telofêmur

Ti – Tíbia

TiI/GeI – Razão entre o comprimento da Tarso I e do Genu I

TiI/TaI – Razão entre o comprimento da Tíbia I e do Tarso I

TiI/W – Razão entre o comprimento da Tíbia I e a largura do escudo.

TiIII/GeIII – Razão entre o comprimento da Tíbia III e do Genu III

Tr – Trocânter

tr ou *ta* – Seta tricobotrial com mais barbas na metade distal.

W – Largura Máxima do escudo

x – Seta acessória do buraco tricobotrial composto.

Z ou Cp – Seta companheira.

ε – Fâmulo (Uma pequena seta quimiorreceptora)

ζ – Eupatídio (Um quimiorreceptor com um poro terminal)

κ – Micro seta.

φ - Solenídio (Um quimiorreceptor com um poro terminal) da tíbia.

ω – Solenídio (Um quimiorreceptor com microporos distribuídos ao longo do seu comprimento) no tarso.

1 INTRODUÇÃO

1.1 Os ácaros Parasitengona classificação e ciclo de vida.

A subclasse Acari (ácaros e carrapatos) é um grupo difilético e altamente diverso de Chelicerata com mais de 55 mil espécies descritas, divididas entre duas ordens: Parasitiformes e Acariformes (Walter & Krantz, 2009; Pepato *et al.*, 2010). O corpo dos ácaros pode ser dividido em uma região compacta contendo sistema nervoso, digestivo e reprodutor chamada idiossoma e um aparelho bucal, o gnatossoma (Fig. 2B). O gnatossoma é composto por um par de queliceras e de apêndices denominados palpos, entre outras estruturas que podem variar entre as duas linhagens.

Entre os Acariformes, a subcoorte Parasitengona (Acariform, Prostigmata) é uma das linhagens mais diversas com mais de 9 mil espécies descritas (Wohltmann, 2000). Ácaros Parasitengona (Acariformes, Prostigmata) podem ser encontrados em todos os continentes exceto Antártica e possuem como principal característica o ciclo de vida (Fig. 1) composto por sete fases: Ovo, pré-larva caliptostática (imóvel), larva ectoparasita de artrópodes ou vertebrados, protoninfa caliptostática, deutoninfa predadora de vida livre, tritoninfa caliptostática e adultos predadores de vida livre (Krantz & Walter, 2009; Wohltmann, 2000). Como os estágios são muito distintos do ponto de vista morfológico, frequentemente não são imediatamente associados e a grande maioria das descrições são baseadas em um único estágio (Makol & Wohtmann, 2012).

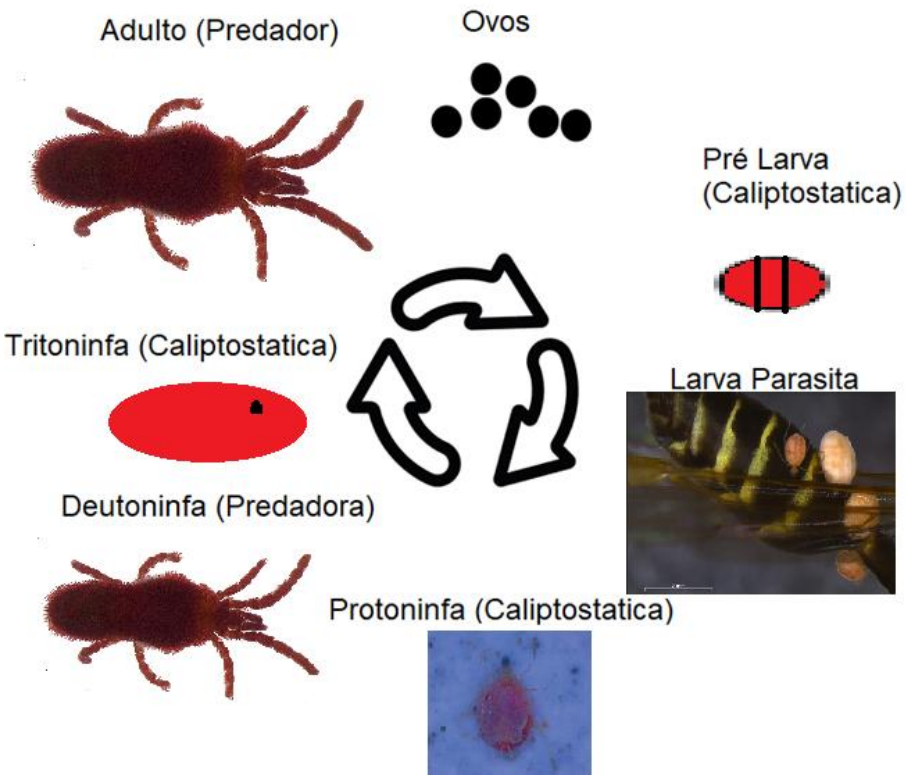

Figura 1 – Ciclo de vida dos Parasitengona.

1.2 Visão geral sobre os Erythraeoidea

Os Erythraeoidea (Acari, Parasitengona) são divididos em duas famílias: Smarididae Vitzthum, 1929 e Erythraeidae Robineau-Desvoidy, 1828. As principais características de Erythraeoidea na fase adulta são quelíceras longas e um gnatossoma que pode ser retraído parcial ou totalmente para o interior do idiossoma (Fig. 2A and B). Já as larvas podem ser diagnosticadas pela ausência de ânus e do poro epimeral ou urstigma.

No estágio de deutoninfa e adultos, os ácaros Smarididae podem ser diferenciados dos Erythraeidae pela sua habilidade de everter e retrair o gnatossoma inteiro para dentro do idiossoma (Fig. 2A), enquanto os Erythraeidae retraem apenas as quelíceras e não são capazes de everter o mesmo. Já no estágio larval, os Smarididae se diferenciam dos Erythraeidae pela presença de um órgão sensorial que consiste em um buraco com uma seta mecanorreceptora chamada tricobotria (ta) e uma ou mais, pequenas setas (x) que atuam em conjunto (Fig. 2B).

Em seu trabalho a respeito da filogenia dos ácaros de água doce (Hydrachnidia, Parasitengona), Dabert et al. (2016) recuperou a família Erythraeidae parafilética em relação à Smarididae. No entanto, a inferência da posição exata da família dependeria da amostragem de gêneros de várias subfamílias e está além do escopo do presente trabalho.

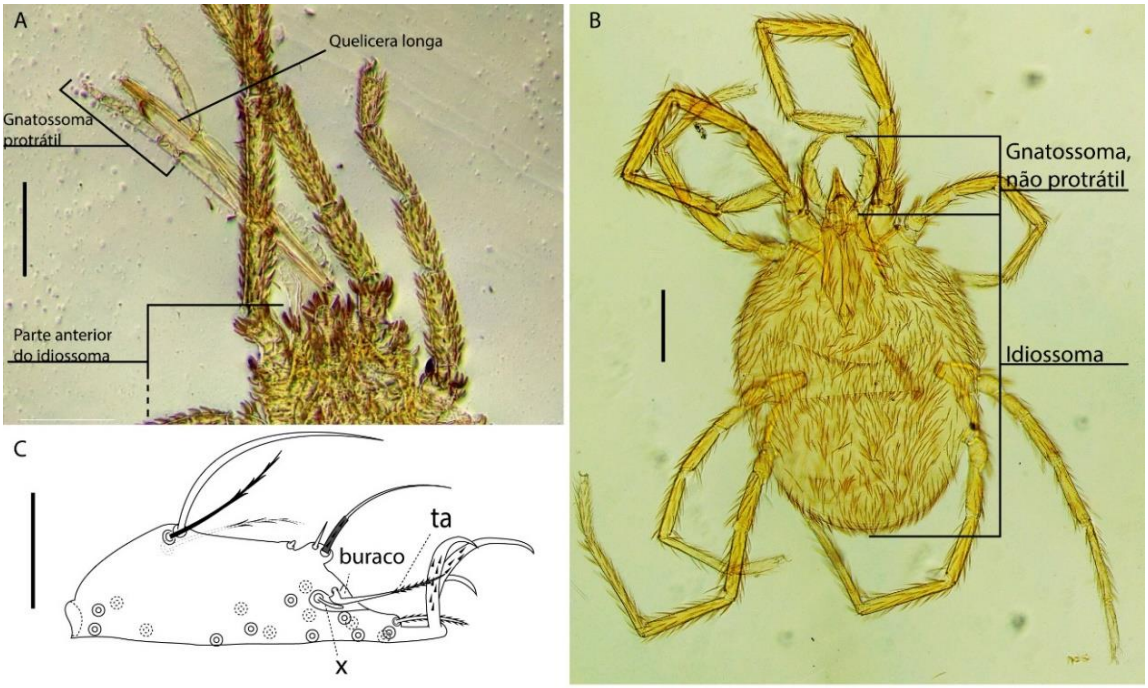

Figura 2 – Características gerais e diagnosticas dos Erythraeoidea. A: Parte anterior de um ácaro *Smaris* (Smarididae) ilustrando o gnatossoma eversivel com relação ao idiossoma; B: Vista geral de um ácaro *Leptus* (Erythraeidae), ilustrando o gnatossoma não eversivel e o plano corpóreo típico dos ácaros dividido em Idiossoma e gnatossoma; C: Tarso da primeira perna da larva de *Trichosmaris* (Smarididae), ilustrando o caractere diagnóstico da família: Um buraco trichobothrial composto contendo uma tricobotria (ta) e uma seta acessória (x). Escala: A e B= 200 µm, C= 25 µm.

1.3 Escopo e desafios

Devido ao complexo ciclo de vida com fases heteromórficas menos de 8% dos Parasitengona terrestres conhecidos tiveram seu instar larval e pós-larval descritos (Makol & Wolthmann, 2012). A associação de larvas e adultos requer a criação dos indivíduos da fase larval até a pós-larval. Uma outra opção é a manutenção em cultura de uma fêmea grávida até se obter ovos e larvas. Estes dois procedimentos podem ter baixa taxa de sucesso e são muito mais proveitosos quando utilizados de maneira integrada com técnicas moleculares. A associação de instares e descrição de espécies da família Erythraeidae coletados em cavernas de Minas Gerais é o foco do capítulo 1 desta dissertação, no qual também são apresentadas notas sobre a biologia de uma espécie conhecida.

A sistemática da família Smarididae é pouco desenvolvida e sofre com caracteres imprecisos para o diagnóstico de gêneros (e.g., "margem anterior do escudo arredondada"), além da falta de informação sobre os ciclos de vida. A família é pequena e agrupada em duas subfamílias: Hirstiosomatinae, com cinco gêneros (dois conhecidos por larvas e adultos, dois conhecidos apenas por adultos e um conhecido apenas por larvas) e Smaridinae, com quatro gêneros (dois conhecidos por larvas e adultos e dois apenas por adultos) (Mąkol & Wohltmann, 2012). Membros dessa família, larvas e adultos, são usualmente amostrados em meio à serapilheira e pouco se sabe quanto aos hospedeiros das larvas. No segundo capítulo desta dissertação é apresentada a primeira filogenia para

a família, sinonimías e novas combinações são propostas. Além disso, o até então desconhecido estágio larval do gênero *Trichosmaris* Southcott, 1963 e duas novas espécies são descritas.

1.4 Ilustrações dos caracteres morfológicos mais utilizados

Nesta sessão são apresentadas ilustrações dos caracteres morfológicos mais utilizados na taxonomia dos Erythraeoidea que se baseia majoritariamente no estágio larval. Eles estão indicados pelas siglas listadas na lista de abreviações e ilustrados por fotos ou desenhos de várias espécies de Erythraeoidea depositadas no CCT-UFMG.

Gnatossoma, larva:

O gnatossoma das larvas é composto por um par de palpos (Fig. 3A e D), além de um cone bucal composto pelo hipostômio e o *capitulum* (Fig. 3B). O palpo pode ser dividido em cinco segmentos: tarso, tíbia, genu, fêmur e trocânter (Fig. 3A). Normalmente esses segmentos são referidos com o prefixo "palpo" diferenciando-os dos segmentos homônimos presentes nas pernas. As estruturas do gnatossoma de maior relevância na taxonomia baseada em larva são indicadas na figura 3 (Ver lista de abreviações).

Idiosoma e Scutum larva:

O idiossoma das larvas de Erythraeoidea possuem na porção ventral três pares de coxas. Como previamente dito, o ânus e o poro epimeral são ausentes e as principais setas utilizadas na taxonomia são as inter coxais *1a*, *2a* e *3a* (Fig. 4D). Na parte dorsal o idiossoma possui uma placa esclerotizada chamada de scutum (Fig. 4C). O scutum possui dois pares de sensilas Asens e Psens, além das setas antero, médio e póstero laterais (AL, ML e PL, respectivamente). As distâncias entre os vários elementos do escudo são utilizadas na taxonomia do grupo e estão ilustradas na figura 4 A e B.

Pernas:

As pernas das larvas da maioria dos Erythraeoidea são divididas em sete segmentos que são chamados da parte distal para proximal: Tarso, Tíbia, Genu, Telofêmur, Basifêmur, Trocânter e Coxa. Cada um destes segmentos é dotado de uma série de setas receptoras de estímulos mecânicos ou químicos relevantes na taxonomia das larvas (Fig. 5).

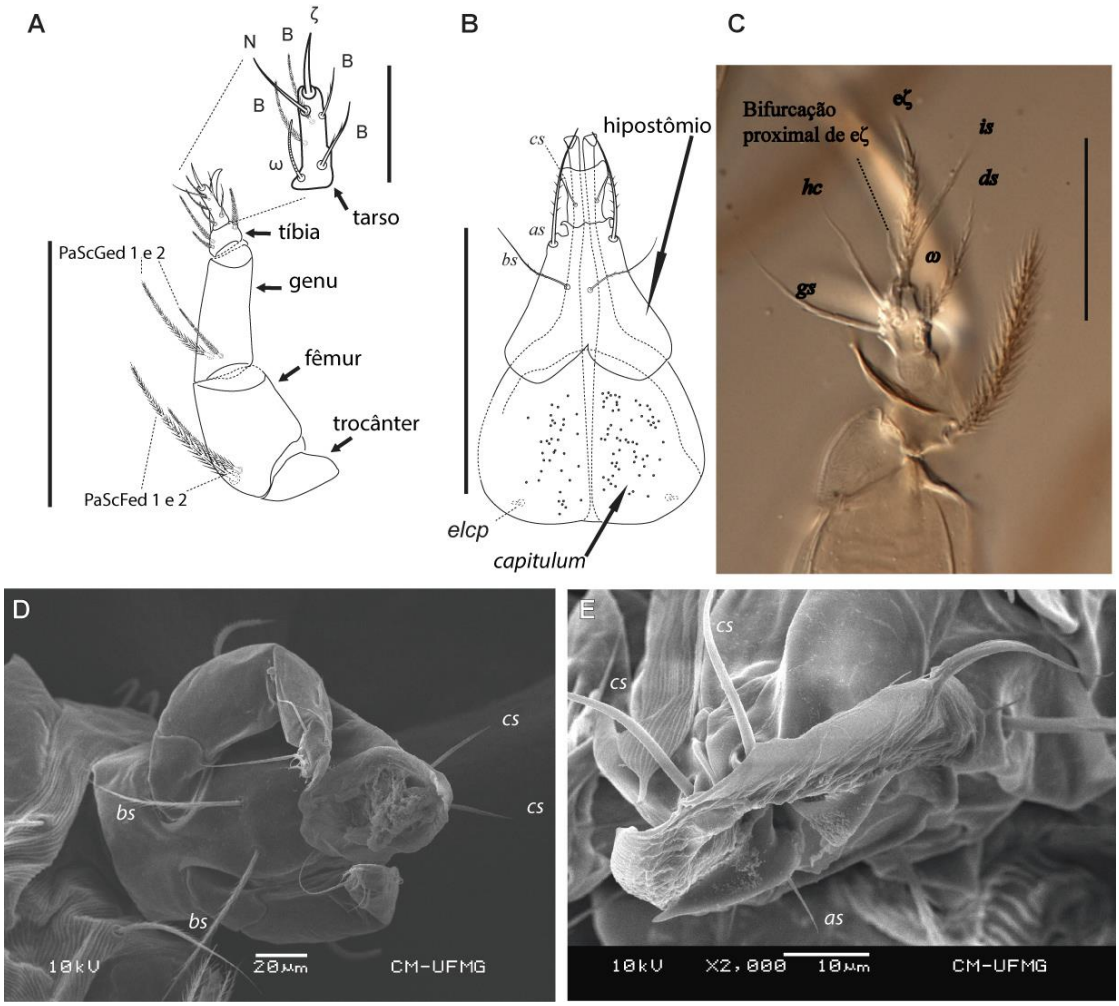

Figura 3 – Posição dos principais caracteres morfológicos citados na lista de abreviações para o gnatossoma de Erythraeoidea. A: Palpo mostrando as setas do fêmur e genu além dos segmentos; B: Cone bucal composto por um *capitulum*, envolto por um hipostômio; C: Palpotarsos mostrando as abreviações das principais setas; D: Vista ventral de um gnatossoma; E: Vista anterior do gnatossoma. D e E foram adaptadas de Costa et. al. (2017). Escala: A= 100 µm e 20 µm, B=25 µm.

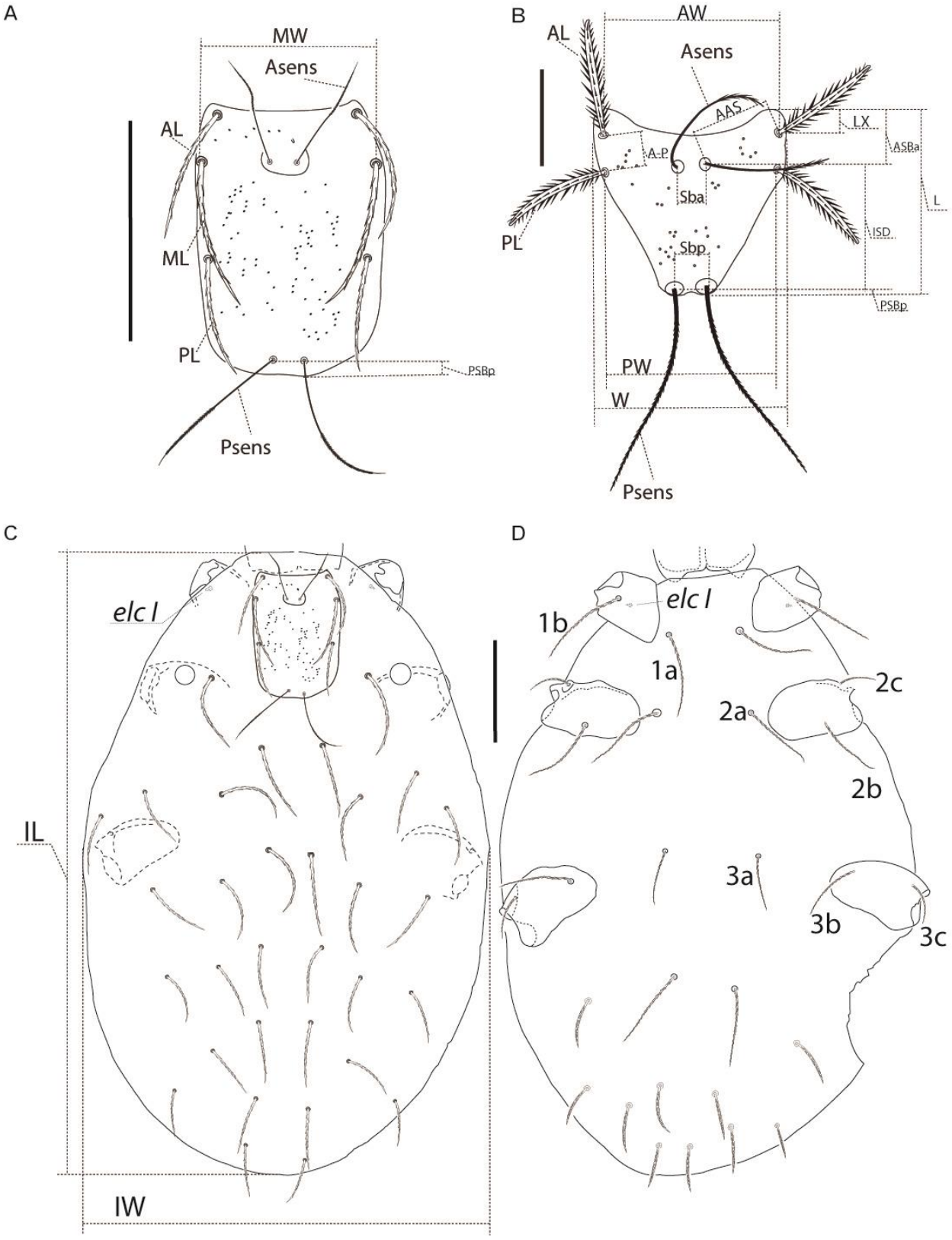

Figura 4 – Posição dos principais caracteres morfológicos citados na lista de abreviações para o idiosoma e scutum dos Erythraeoidea. *Callidosoma selmae* Costa et. al. (2017); A: Scutum com três pares de setas laterais AL, ML e PL. *Leptus* sp. nov; B: Scutum com dois pares de setas laterais AL e PL. *Callidosoma selmae;* C: Idiossoma dorso, D: Idiossoma ventre. Ver lista de abreviações. Itens A, D e E foram adaptadas de Costa et. al. (2017). Escala= 40 µm.

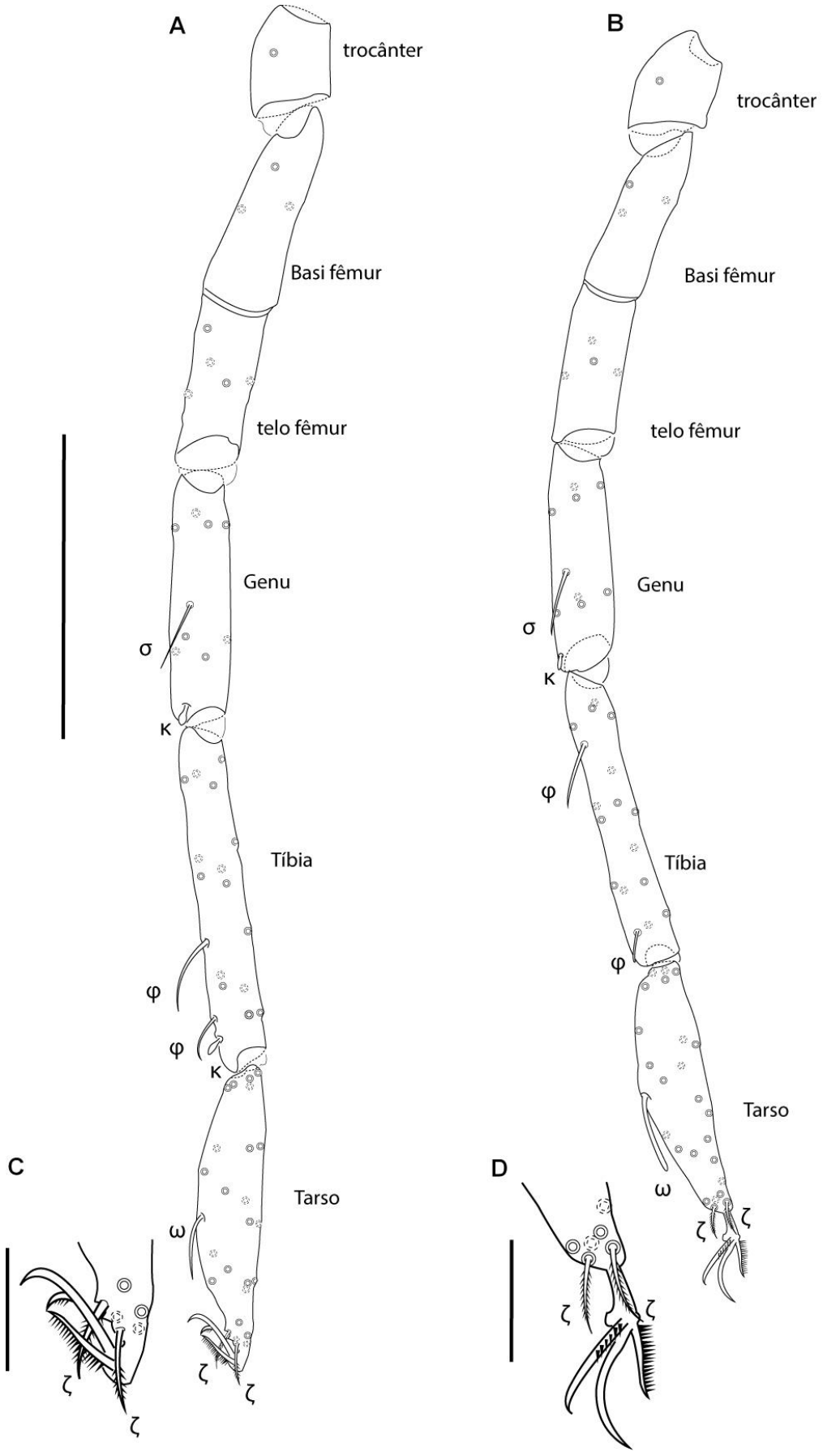
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Figura 5 – Posição dos principais caracteres morfológicos citados na lista de abreviações para as pernas dos Erythraeoidea. *Leptus* sp. nov; A: Perna I, B: Perna II. As setas comuns são representadas apenas por um circulo indicando onde elas se encaixam. C e D: ponta do tarso da perna I e II, respectivamente. Escala: A e B= 100; C e D= 20.

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Chapter 1 - Integrative taxonomy towards the association of heteromorphic specimens: The multi-instar descriptions of cave dwelling Erythraeidae (Acari: Parasitengona).

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Abstract

The Parasitengona life cycle includes major morphological changes precluding an instar association based only on the morphology. This makes rearing and/or molecular data necessary to associate the heteromorphic instars. Most of the described species are known from either post larval instars or larva. In the present study the instar association was made through an integrative approach including rearing trials and molecular analysis of the cytochrome oxidase I (COI) gene with the Bayesian Generalized Mixed Yule Coalescent (bGMYC) algorithm for species delimitation. This approach can be adapted for other taxa with heteromorphic instars, specimens or sex and the advantages and problems of each association method employed are discussed. Illustrating this approach, two new cave dwelling Erythraeidae (Acari: Parasitengona) species are described *Lasioerythraeus jessicae* sp. nov. and *Leptus sidorchukae* sp. nov. including all active instars. Additionally, a complete description of the unknown adults of *Charletonia rocciai* Treat & Flechtmann, 1979 are provided with notes about the larvae and deutonymph. We also demonstrated experimentally that *Ch. rocciai* larvae are not attached to the same host during the entire stage, being able to swap from a host insect to another and discuss the presence of troglomorphisms in *Leptus sidorchukae* sp. nov., as well the distribution of the specimens in the cave and epigean environment.

Key words: Troglodyte, Troglomorphism, Neotropical, Minas Gerais, Life cycle, Brazil.

Introduction

The taxonomical literature on invertebrate taxa with a heteromorphic life cycle often has one instar overrepresented. This may preclude accurate estimates of diversity in natural communities' due difficulties in identifying the other instars. For example, in the Psephenidae (Coleoptera: Byrrhoidea) diagnostic characters are mostly based on adult stages, even though larvae are more available, as they are long-lived and common in streams. Knowledge of all instars, made possible through associations of adults and immature instars, led to improved resolution in phylogenetic analyses (Lee et al. 2007).

Heteromorphy is also an issue in systematics of the mite family Erythraeidae. Similar to other Parasitengona, their life cycle includes an egg and six postembryonic stages: a regressive calyptostatic prelarva, an ectoparasitic larva, a calyptostatic protonymph, a free-living, predaceous deutonymph, a calyptostatic tritonymph, and a free-living, predaceous adult. Among active stages, larvae morphology departs from deutonymphs and adults, precluding easy association based on external characters (Wharton & Fuller 1952, Robaux 1974, Belozerov 2008). This and the fact that larvae and post-larval instars occupy different habitats with different ecological traits lead to different collecting probability and makes them often described as separate species or even genera (Zhang, 1998, Mąkol & Wohltmann 2012).

The genus *Charletonia* Oudemans, 1910 (Erythraeidae, Callidosomatinae) illustrates the kind of taxonomical issues raised by the lack of correct association of larvae and post-larval stages. Currently, the genus comprises more than 116 species, distributed over all continents except Antarctica, and its larvae parasite a wide range of arthropods, mostly insects, including Lepidoptera, Diptera, Hemiptera, Orthoptera, and Psocoptera (Mąkol & Wohltmann 2012). As a result, more than 90 species are currently known from the larva, but only six from both larval and post-larval instars, mostly deutonymphs (Mąkol & Wohltmann 2012). Until 1980, deutonymphs and adults were assigned to *Sphaerolophus* Berlese 1910. Only after rearing experiments by Rosa & Flechtmann (1980) *Sphaerolophus* species were moved to *Charletonia*.

Unfortunately, many parasitengones have proven difficult to rear, making description of all instars rare. Alone or combined with rearing, an integrative approach that includes molecular data provides an alternative method for associating heteromorphic instars (Stålstedt et al. 2016).

This study aims at employing molecular data from the mitochondrial Cytochrome Oxidase I (COI), a fast-evolving mitochondrial gene, in addition to rearing and morphological analysis to associate the larvae and post-larval instars of three Erythraeidae genera: *Lasioerythraeus* Welbourn & Young, 1987, *Leptus* Latreille, 1796, and *Charletonia*, including cave dwelling and epigean specimens from different localities of Brazil. The associations are followed by the descriptions of the new species *La. jessicae* sp. nov. and *Le. sidorchukae* sp. nov., based on both larvae and the associated post larval individuals

and the discussion of the possible troglobiont status of *Le. sidorchukae* sp. nov. We also provide re-descriptions of the larva and deutonymph, descriptions of the adults, notes on distribution and behavior, plus amendments to the holotype description of *Charletonia rocciai* Treat & Flechtmann, 1979.

Material and methods

Collecting, mounting, rearing and morphological description.

Abbreviations for collections: CCT-UFMG AC, acarological collection of the *Centro de Coleções Taxonômicas* at the *Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil; ESALQ, *Departamento de Entomologia e Acarologia,* ESALQ Universidade de São Paulo, Piracicaba, São Paulo, Brazil; OSAL, Ohio State University Acarology Collection, Columbus, Ohio, USA. Access to the genetic heritage from Brazilian mites was registered in SisGen (#PROVIDED UPON ACCEPTANCE).

Preservation and mounting. Mites were fixed in 70 to 90% ethanol on field and all were transferred to 95% ethanol upon arrival at the CCT-UFMG AC. Both, fresh specimens and the exoskeleton recovered from DNA extraction (see below) were mounted on microscope slides with Hoyer's medium (Walter & Krantz 2009).

Sampling. Aiming to associate instars by testing species boundaries, Erythraeid mites of various development stages and localities belonging to the same genus were chosen among those deposited at CCT-UFMG AC. Most specimens deposited at CCT-UFMG AC came from unnamed caves and the Table I summarizes details on sampling, collection and GenBank accession numbers.

Rearing of Ch. rocciai. To obtain the post larval instars of *Ch. rocciai*, infested psocopteran hosts were kept in cylindrical plastic containers with a diameter of 7.5 cm and height of 5.5 cm. The containers received a 1.5 cm thick layer of a mixture containing 50% plaster of Paris, 10% of the ground coal, and 40% of construction cement. The containers were kept in a shaded area at air temperature, which varied from 14° C to 35° C, average of 23° C (Source: Instituto Nacional de Meteorologia, Brazil). The moisture was kept by adding filtered water to the substrate after daily check. Psocopterans harboring a single mite were marked with water-based ink (gouache paint) and observed for changes in the number of mites due to host shifting. Nine of the Psocopteran hosts were deposited at CCT-UFMG under the identification numbers (IPS 1700000 to 1700009). A few larval specimens of *Ch. rocciai* were removed from the hosts and mounted on slides for morphological studies of the larval instar.

Drawings and measurements. Drawings were made with the aid of a *camera lucida*. Final artwork was prepared in the program Adobe Illustrator CC and Photoshop CC. Photos and measurements were taken in different microscopes: Nikon Eclipse 90i with a Nikon DS-fi1 camera, Zeiss Primo Star with an AxioCam ERC5s camera, and a Leica DM750 with an ICC50W camera. All measurements are

given in micrometers (µm). Maps were generated with the web application MapMakerApp.com and Google maps.

Terminology: Terminology and abbreviations used in the description are adapted from Southcott (1961, 1991) and Barr (1972), except for *cs*, *as*, *bs*, *elc*, *elcp* taken from Grandjean (1947). The idiosomal setae in the post larval instars have two shapes, varying in length and width (reflected in abbreviations). Text abbreviations for post-larval instars: ILCM, Overall length of idiosoma up to the tip of mouth cone; ACW, Anterior Crista Width around the anterior sensillary area; PCW, Posterior Crista Width around the posterior sensillary area; L, crista Length (including naso); ECO, Eye Cornea (see Fig. 18G); Eye Ring, diameter of the external eye ring around the cornea (see Fig. 18G); EC-EC, distance between the centers of the eyes; EC-ASE, distance between a line crossing the centers of both eyes to one crossing the anterior sensillae bases; LDS, Long Dorsal Setae; SDS, Short Dorsal Setae; LVS, Long Ventral Setae; SVS, Short Ventral Setae. When discussing caves and cave dwelling mites we employed the definitions of Trajano & Bichuette (2006) about caves zonations, Zacharda (1980) about cave dwelling mites and Sket (2008) about the classification of subterranean animals.

Molecular analyses

Sampling. Erythraeid mites morphologically identified as members of the same genus, including those meant as outgroups had their DNA sequenced and are reported in Table I. Additional outgroup sequences were originally obtained by Söller et al. (2001) (407 bp long, hence shorter than those obtained by us): *Charletonia cardinalis* (Koch, 1837), GenBank accession number (GbN): AJ238261; *Erythraeus* spp. GbN: AJ238264 and AJ238265; and *Leptus ignotus* Oudemans, 1903, GbN: AJ238262. One extra sequence from *Erythraeus* sp. by Dowling et al. (2010), 407 bp long (GbN: HQ423154) also was included.

DNA extraction: Genomic DNA was extracted from entire mites using the QIAamp® DNA Micro Kit (Qiagen) for larvae, and the Wizard® Genomic DNA Purification Kit (Promega) for adults, according to the manufacturer`s protocol, with the exception that the mite`s exoskeleton was recovered to be used as a voucher after the digestion process.

Amplification and sequencing: Amplification was carried out in a 20 µL volume containing 2.0 µL 10× PCR buffer, 1.4 µL of 50 mM MgCl2, 1.4 µL of dNTPs (10 mM each), 1.6 µL each of 5 pM primer, 0.12 µL Platinum®Taq Polymerase, and typically 3 µL of DNA template. The same primer pair was employed for amplification and sequencing: COI Forw: 5'-TGATTTTTTGGTCACCCAGAAG-3' (Söller et al. 2001) and Mite COI 2R 5'-GGRTARTCWGARTAWGGNCGWGGTAT-3' (Otto & Wilson, 2001). The PCRs were run on an "Eppendorf® Mastercycler® Pro Thermocycler vapo protect" thermocycler. The thermocycler program included an initial denaturing step of 4 min. at 94°C, 38 cycles of 30 s. of denaturation at 94°C, 35 seconds annealing at 45°C, 1 min. of extension at 72°C; final extension period 5 minutes at 72°C. The PCR products were tested for presence of bands with

the expected length of 570 pb in an agarose gel and then purified with Agencourt AMPure XP® (Beckman Coulter) following manufacturer's protocol. The DNA was quantified using a NanoDrop® (Thermo Fisher) detector and sequenced in a ABI3730 (Applied Biosystems) sequencer, using the POP7™ polymer (Applied Biosystems) and BigDye ™ Terminator v3.1 (Applied Biosystems) according to the manufacturer`s protocol.

The chromatograms were reviewed using the software SeqScape ® version 2.6, sequences were aligned using Muscle (Edgar, 2004) with the default values as implemented in Mega V 7.0.17 (Kumar et al. 2016), and haplotypes were identified using the software DnaSP V. 5.

All analyses were performed in CIPRES (Miller et al. 2010). The best partition scheme for the alignment was chosen using the greedy algorithm in Partition Finder 2 (Lanfear et al. 2016). Lowest Akaike's Information Criterion model calculated by JmodelTest 2 program (Darriba 2012) provided values for gamma shape and the proportion of invariable sites to inform the priors for substitution model in downstream Bayesian analysis in BEAST v.2.4.6 (Bouckaert et al. 2014) using the algorithm Reversable Jump (RJ) (Bouckaert et al. 2004).

The bGMYC (Reid & Carstens, 2012) was employed to delimitate putative species, associating the instars with different haplotypes as a single species. It consists in the Bayesian implementation of the GMYC (Pons et al. 2006), a method which uses the distinct expectations on branching patterns between interspecific divergence and intraspecific diversification to distinguish between species and population processes, assigning a threshold to the waiting times between successive branching events on an ultrametric tree. We chose the Bayesian implementation because it considers the uncertainty in tree estimation and parameters of the model, giving the result as the posterior probability of haplotypes be recovered as a single species. We regarded larval and post-larval specimens as a single species if their haplotypes were associated with more than 95% posterior probability. When necessary, specimens from the same instar were associated trough morphology.

We ran strict and relaxed lognormal clock analyses in Beast v. 2.4.6 (Bouckaert et al. 2014) for each genus. All analyses lasted for $3x10^8$ generations, sampled every $3x10^4$ generations, under the Yule tree model. Convergence check and clock model comparison using AICM (Baele et al. 2012) were performed in Tracer v.1.6.0 (Rambaut et al. 2014). Resulting trees are summarized using the software TreeAnnotator v1.8.4 (Drummond et al. 2012), with a 10% burnin, on the maximum clade credibility tree displaying the median heights for the tree nodes.

The resulting ultrametric trees from stationary phase of Markov chain had outgroups removed and submitted to a bGMYC analysis (Reid & Carstens, 2012) in R v. 3.5.1. From the 10 thousand trees sampled in the BEAST2 run, a sample with 100 trees was randomly picked for the bGMYC analysis following the bGMYC R package (Reid & Carstens, 2012) instructions. The priors and parameters

used in the analysis were chosen based on Reid & Carstens (2012) and are coded in the following command lines for the bGMYC R package:

Lasioerythraeus: bgmyc.multiphylo(amostralassout, mcmc=50000, burnin=40000, thinning=100, t1=1, t2=5,start=c(1,0.5,3), scale=c(1,40,0.5))->splblasout

Charletonia: bgmyc.multiphylo(amostracharsout, mcmc=50000, burnin=40000, thinning=100,t1=1, t2=8, start=c(1,0.5,3), scale=c(1,40,0.5))->splbcharout

Leptus: bgmyc.multiphylo(amostralep, mcmc=50000, burnin=40000, thinning=100,t1=1, t2=12, start=c(1,0.5,3), scale=c(1,40,0.5))->splblepout

The convergence of the analyses was checked graphically. The bGMYC results were plotted with the phylogenetic three edited using the software FigTree v1.3.1. The final figures were edited using Adobe Photoshop and Illustrator CC.

Table I – Detailed sampling data and GenBank accession numbers. All specimen identification numbers (ID) refer to the acarological collection of the *Centro de Coleções Taxonomicas da Universidade Federal de Minas Gerais*. Abreviations: Larva (L), deutonymph (DN), male (M), female (F), not applicable (N. A.).

Species	ID	Locality	Coordinates (Lat., Long)	GenBank Accession	Instar/ Sex	Date	Host or Habitat
Lasioerythraeus jessicae sp. nov.	161064	Conceição do Mato Dentro, Minas Gerais State (MG), Brazil	-19.096111, -43.356944	MK479908	DN	26th Jul. - 13th Oct. 2016	Cave in an iron ore terrain
	161067		-19.096111, -43.356944	MK455822	L		
	161068		-19.217778, -43.385000	MK455823	F		
	161069		-19.217778, -43.385000	MK479909	F		
	161070		-19.005278, -43.393333	MK479910	F		
	161072		-19.005278, -43.393333	MK455824	F		
	161061		-19.096111, -43.356944	MK455823	F		
	161062		-19.096111, -43.356944	MK479907	F		
La. sp. 1	160761		-19.005278, -43.393333	MK455821	F	Sep. 10th 2015	
La. sp. 2	150954	Pedro Leopoldo, MG, Brazil	-19.57111111, -44.01388889	MK455820	L	Jul. 22th – Aug. 18th 2015	Limestone cave
Leptus sp. 1	160023	Pains, MG, Brazil	-20.363056, -45.614167	MK455834	DN	Nov. 24th-27th 2015	Limestone cave
Le. sp. 2	1300429	PE Serra do Rola Moça, near to Belo Horizonte, MG, Brazil	-20.066850, -44.002417	MK455827	L	Mar. 1th - 2th 2013	Unidentified insects
Le. sp. 3	1300434		-20.066850, -44.002417	MK455828	L		
Le. sp. 4	1300425		-20.00221667, -43.97728333	MK479873	L	Apr. 2th 2013	
Le. sp. 4	1300581		-20.00221667, -43.97728333	MK455829	L		
Le. sp. 5	1300424		-20.002217, -43.977283	MK455826	L	Jun. 3th 2013	
Le. sp. 6	1300406		-20.053722, -44.001472	MK455825	M	Jun. 15th 2012	Leaf litter
Le. sp. 7	171002	Pedro Leopoldo, MG, Brazil	-20.09544, -43.68053	MK455835	F	Jul. 22th - Sep. 18th 2015	Cave in an iron ore terrain
Leptus sidorchukae sp. nov.	150701	Diamantina, MG, Brazil	-18.278611, -43.536111	MK479876	F	Jan. 5th-8th 2016	Limestone cave
	150693		-18.278611, -43.536111	MK455830	F	Dec. 3th- 10th 2015	Salitre's cavern
	150875	Pedro Leopoldo, MG, Brazil	-19.576944, -44.015278	N. A.	L	July 22th to August 18th 2015	Limestone cave
	150932		-19.576667, -44.020000	MK479894	M	Mar. 4th-20th 2015	
	151100		-19.576667, -44.020278	N. A.	L	Jul. 22th - Aug. 18th 2015	
	150851		-19.57583333, -44.01916667	MK479888	F	Mar. 4th-20th 2015	
	151510		-19.57638889, -44.01944444	MK455833	L	Jul. 22th - Aug. 18th, 2015	
	151098		-19.576667, -44.020278	N. A.	L		
	150705		-19.57083333, -44.01083333	MK479877	F		
	150707		-19.57083333, -44.01083333	MK479878	F		
	150715		-19.57055556, -44.01055556	MK479879	F		
	150716		-19.57055556, -44.01055556	MK479880	F		
	150722		-19.57694444, -44.01527778	MK479881	F		

Table I. Continuation.

Species	ID	Locality	Coordinates (Lat., Long)	GenBank Accession	Instar/ Sex	Date	Host or Habitat
Leptus sp. nov.	150725	Pedro Leopoldo, MG, Brazil	-19.57111111, -44.01472222	MK479882	M	Jul. 22th - Aug. 18th, 2015	Limestone cave
	150729		-19.570833, -44.013889	MK479883	DN		
	150730		-19.570833, -44.013889	MK479884	DN		
	150845		-19.58027778, -44.0183333	MK479885	F	Mar. 4th-20th, 2015	
	150862		-19.58027778, -44.01833333	MK479892	F		
	150863		-19.58027778, -44.01833333	MK479893	F		
	150846		-19.56388889, -44.01222222	MK479886	F		
	150847		-19.56388889, -44.01222222	MK479887	F		
	150853		-19.56388889, -44.01222222	MK479889	M		
	151108		-19.57083333, -44.01083333	MK455831	F		
	151180		-19.57944444, -44.01361111	MK479902	F		
	150855		-19.58027778, -44.01694444	MK479890	F		
	151107		-19.57277778, -44.01222222	MK479900	F		
	150859		-19.57055556, -44.01055556	MK479891	F		
	151033		-19.57194444, -44.01472222	MK479895	L		
	151101		-19.57194444, -44.01472222	MK455831	DN	Jul. 22th - Aug. 18th, 2015	
	151078		-19.57638889, -44.01944444	MK479896	L		
	151093		-19.576667, -44.020278	MK479898	L		
	151206		-19.57138889, -44.02083333	MK479903	DN	Jan. 5th-8th, 2016	
	151208		-19.57138889, -44.02083333	MK479904	DN		
	151233		-19.57138889, -44.02083333	MK479905	F		
	151215		-19.57111111, -44.02111111	MK479906	M		
	171238		-19.581541, -44.019683	MK479911	F	Nov. 21th- Dec. 2th, 2016	
	151245		-19.571389, -44.020833	MK455832	F	Jan. 5th-8th, 2016	
	150694	Diamantina, MG, Brazil	-18.278611, -43.536111	MK479874	F		
	150696			MK479875	F		
Charletonia rocciai	171474	Conceição do Mato Dentro, MG, Brazil	-19.2213, -43.3942	MG490384	DN	Jan. 16th - 26th, 2017	Cave in an iron ore terrain
	1301036	Lavras Federal University, Lavras, MG, Brazil	-21.2267, -44.9807	N. A.	L	Jul. 2013	Psocoptera
	1301034			N. A.	L		
	151437			MG490388	L	Sep. 2015	
	151442			MG490389	L	Sep. 2015	
	1300421			MG490385	F	Jul. 2013	Leaf litter
	1300612			N. A.	DN		Reared from larvae

Table I. Continuation.

Species	ID	Locality	Coordinates (Lat., Long)	GenBank Accession	Instar/ Sex	Date	Host or Habitat
Charletonia rocciai	1300612	Lavras Federal University, Lavras, MG, Brazil	-21.2267, -44.9807	N. A.	DN	Jul. 2013	Reared from larvae
	1300613			N. A.	DN		
	1301038			N. A.	DN		
	1300423	PE Serra do Rola Moça, near to Belo Horizonte, MG, Brazil	-20.0669, -44.0024	MG490385	L	Mar. 2nd, 2013	Unidentified insect
	1400806	Atuba Park, Curitiba, Paraná State, Brazil	-25.3817, -49.2033	MG490387	L	Feb. 12nd, 2013	*Auchmerina limbatipennis Enderlein, 1918*
	1301003	Serra do Japi, Jundiaí, São Paulo State, Brazil	-23.2419, -46.9347	MG490386	M	Aug. 2014	Leaf litter
Ch. sp.	160863	Jaguariaíva, Paraná, Brazil	-24.166975, -49.650667	MG490380	L	Jan. 15 th - 16 th, 2016	Unidentified Hemiptera
Callidosoma selmae Costa, S., Klompen, H., Santos, E., Favretto M. & Pepato R., 2017	1300435	RPPN-Cachoeira do Campo, Serra do Salitre, MG, Brazil	-19.1684, -46.5693	MG490381	L	Oct. 11th-15th, 2012	*Hamadryas epinome (C. Felder & R. Felder, 1867)*
Callidosoma welbourni Treat, 1985	150772	Pedro Leopoldo, MG, Brazil	-19.5711, -44.0139	MG490383	L	Jul. 22th – Aug. 18th, 2015	Limestone cave

Molecular analyses

Sampling. Erythraeid mites morphologically identified as members of the same genus, including those meant as outgroups had their DNA sequenced and are reported in Table I. Additional outgroup sequences were originally obtained by Söller et al. (2001) (407 bp long, hence shorter than those obtained by us): *Charletonia cardinalis* (Koch, 1837), GenBank accession number (GbN): AJ238261; *Erythraeus* spp. GbN: AJ238264 and AJ238265; and *Leptus ignotus* Oudemans, 1903, GbN: AJ238262. One extra sequence from *Erythraeus* sp. by Dowling et al. (2010), 407 bp long (GbN: HQ423154) also was included.

DNA extraction: Genomic DNA was extracted from entire mites using the QIAamp® DNA Micro Kit (Qiagen) for larvae, and the Wizard® Genomic DNA Purification Kit (Promega) for adults, according to the manufacturer`s protocol, with the exception that the mite`s exoskeleton was recovered to be used as a voucher after the digestion process.

Amplification and sequencing: Amplification was carried out in a 20 µL volume containing 2.0 µL 10× PCR buffer, 1.4 µL of 50 mM MgCl2, 1.4 µL of dNTPs (10 mM each), 1.6 µL each of 5 pM primer, 0.12 µL Platinum®Taq Polymerase, and typically 3 µL of DNA template. The same primer pair was employed for amplification and sequencing: COI Forw: 5'-TGATTTTTTGGTCACCCAGAAG-3' (Söller et al. 2001) and Mite COI 2R 5'-GGRTARTCWGARTAWGGNCGWGGTAT-3' (Otto & Wilson, 2001). The PCRs were run on an "Eppendorf® Mastercycler® Pro Thermocycler vapo protect" thermocycler. The thermocycler program included an initial denaturing step of 4 min. at 94°C, 38 cycles of 30 s. of denaturation at 94°C, 35 seconds annealing at 45°C, 1 min. of extension at 72°C; final extension period 5 minutes at 72°C. The PCR products were tested for presence of bands with the expected length of 570 pb in an agarose gel and then purified with Agencourt AMPure XP® (Beckman Coulter) following manufacturer's protocol. The DNA was quantified using a NanoDrop® (Thermo Fisher) detector and sequenced in a ABI3730 (Applied Biosystems) sequencer, using the POP7™ polymer (Applied Biosystems) and BigDye ™ Terminator v3.1 (Applied Biosystems) according to the manufacturer`s protocol.

The chromatograms were reviewed using the software SeqScape ® version 2.6, sequences were aligned using Muscle (Edgar, 2004) with the default values as implemented in Mega V 7.0.17 (Kumar et al. 2016), and haplotypes were identified using the software DnaSP V. 5.

All analyses were performed in CIPRES (Miller et al. 2010). The best partition scheme for the alignment was chosen using the greedy algorithm in Partition Finder 2 (Lanfear et al. 2016). Lowest Akaike's Information Criterion model calculated by JmodelTest 2 program (Darriba 2012) provided values for gamma shape and the proportion of invariable sites to inform the priors for substitution

model in downstream Bayesian analysis in BEAST v.2.4.6 (Bouckaert et al. 2014) using the algorithm Reversable Jump (RJ) (Bouckaert et al. 2004).

The bGMYC (Reid & Carstens, 2012) was employed to delimitate putative species, associating the instars with different haplotypes as a single species. It consists in the Bayesian implementation of the GMYC (Pons et al. 2006), a method which uses the distinct expectations on branching patterns between interspecific divergence and intraspecific diversification to distinguish between species and population processes, assigning a threshold to the waiting times between successive branching events on an ultrametric tree. We chose the Bayesian implementation because it considers the uncertainty in tree estimation and parameters of the model, giving the result as the posterior probability of haplotypes be recovered as a single species. We regarded larval and post-larval specimens as a single species if their haplotypes were associated with more than 95% posterior probability. When necessary, specimens from the same instar were associated trough morphology.

We ran strict and relaxed lognormal clock analyses in Beast v. 2.4.6 (Bouckaert et al. 2014) for each genus. All analyses lasted for $3x10^8$ generations, sampled every $3x10^4$ generations, under the Yule tree model. Convergence check and clock model comparison using AICM (Baele et al. 2012) were performed in Tracer v.1.6.0 (Rambaut et al. 2014). Resulting trees are summarized using the software TreeAnnotator v1.8.4 (Drummond et al. 2012), with a 10% burnin, on the maximum clade credibility tree displaying the median heights for the tree nodes.

The resulting ultrametric trees from stationary phase of Markov chain had outgroups removed and submitted to a bGMYC analysis (Reid & Carstens, 2012) in R v. 3.5.1. From the 10 thousand trees sampled in the BEAST2 run, a sample with 100 trees was randomly picked for the bGMYC analysis following the bGMYC R package (Reid & Carstens, 2012) instructions. The priors and parameters used in the analysis were chosen based on Reid & Carstens (2012) and are coded in the following command lines for the bGMYC R package:

Lasioerythraeus: bgmyc.multiphylo(amostralassout, mcmc=50000, burnin=40000, thinning=100, t1=1, t2=5,start=c(1,0.5,3), scale=c(1,40,0.5))->splblasout

Charletonia: bgmyc.multiphylo(amostracharsout, mcmc=50000, burnin=40000, thinning=100,t1=1, t2=8, start=c(1,0.5,3), scale=c(1,40,0.5))->splbcharout

Leptus: bgmyc.multiphylo(amostralep, mcmc=50000, burnin=40000, thinning=100,t1=1, t2=12, start=c(1,0.5,3), scale=c(1,40,0.5))->splblepout

The convergence of the analyses was checked graphically. The bGMYC results were plotted with the phylogenetic three edited using the software FigTree v1.3.1. The final figures were edited using Adobe Photoshop and Illustrator CC.

Results

Data set characteristics.

The *Charletonia* COI alignment was 521 nucleotides long, without gaps. Excluding outgroups, it presented 342 conserved positions and 65 parsimony informative nucleotides. The complete alignment base content was T= 32.8 %; C= 17.6 %; A= 34.9 %; G= 14.7 %. Among the 15 sequences, we obtained 12 haplotypes. The *Lasioerythraeuys* COI alignment was 557 nucleotides long, without gaps, and, excluding outgroups, presented 438 conserved positions and 15 parsimony informative nucleotides. The complete alignment base content was T= 31.7 %; C= 19.0 %; A= 32.4 %; G= 16.9 %. Among the 13 sequences, we obtained eight haplotypes. The *Leptus* COI alignment was 505 nucleotides long, without gaps, and, excluding outgroups, presented 279 conserved positions and 193 parsimony informative nucleotides. The complete alignment base content was T= 33.1 %; C= 19.2 %; A= 32.5 %; G= 15.2%. Among the 46 sequences, we obtained 12 haplotypes.

Data set preliminary analysis.

For all three alignments, the PartitionFinder 2 analysis pointed to a partition scheme in which each of the three codon positions evolved independently. The substitution models chosen were TrN+I, HKY+I, and HKY+G for *Charletonia*; TRN+I, GTR+G, GTR for *Lasioerythraeus*; and TRN+I, GTR and GTR+G for *Leptus*; for the first, second and third positions respectively, with the nucleotide frequency estimated during the MCMC.

For the first, second and third positions of the partition scheme, respectively, the values recovered by JmodelTest and used as priors to the RJ during the BEAST analysis were: I= 0.628; I= 0.689 and G= 1.358 for *Charletonia*; I= 0.628; I= 0.771 and no I or G on the third position for *Lasioerythraeus*; I= 0.536; no I or G on the second position and G= 2.525 for *Leptus*.

The BEAST2 log files were checked on tracer and in all cases, it was achieved high effective sample sizes, good convergence and the strict molecular clock model were chosen since it presented lower AICM values.

Species delimitation results.

The ultrametric trees obtained from BEAST v.2.4.6 are presented in Fig. 1, while Fig. 2 presents bGMYC results. Two females (CCT-UFMG AC 161061 and 161062), one larva (CCT-UFMG AC 161067), and one deutonymph (CCT-UFMG AC 161064) of *Lasioerythraeus jessicae* sp. nov. share the same haplotype 1, allowing a straightforward association. Moreover, three other females (CCT-UFMG AC 161069, 161070 and 161072) share the haplotype 2 and one female (CCT-UFMG AC 161068) representing the haplotype 3 (Fig. 1A) were associated by the bGMYC with PP>95% (Fig. 2B). Additional female (CCT-UFMG AC 160761) and larva (CCT-UFMG AC 150954) were identified

as belonging to two different and potentially new species of *Lasioerythraeus* and are considered for further description given additional material become available.

The four haplotypes of *Leptus sidorchukae* sp. nov. were associated by the bGMYC with PP>95% (Fig. 2B). The haplotypes 1 to 3 (Fig. 1B) came from mites collected in caves near Pedro Leopoldo municipality (see Table I for coordinates): Haplotype 1 from a single female (CCT-UFMG AC 151245); Haplotype 2 from three specimens, two females and one larvae (CCT-UFMG AC 150851, 150932 and 151510); Haplotype 3 was sequenced from 28 individuals, including four larvae, five deutonymphs, 17 females and three males (CCT-UFMG AC 150705, 150707, 150715, 150716, 150722, 150725, 150729, 150730, 150845, 150846, 150847, 150853, 150855, 150859, 150862, 150863, 151033, 151078, 151082, 151093, 151101, 151107, 151108, 151180, 151206, 151208, 151233, 151235 and 171238). Haplotype 4 was recovered in all four female specimens from Salitre`s cave, Diamantina municipality (CCT-UFMG AC 150693, 150694, 150696 and 150701).

Haplotypes of *Charletonia rocciai* are split into two clades (Fig. 1C) that could not be associated by the bGMYC with PP>95% (Fig. 2D). However, larval individuals associated by morphology and from the same population, found associated to Psocoptera feeding on Pau-ferro trees (*Libidibia ferrea* (Mart. ex Tul.) Queiroz, 2009) at Universidade de Lavras campus, could be associated with heteromorphic specimens from both clades obtained at different locations (PP>95%). It is the case for the haplotype 1 with 2; 3 with 4 and 5 with 3 (Fig. 1C and 2D). For the first time, a male (CCT-UFMG AC 1301003) could be associated (PP=99.43) with other active instars (larva CCT-UFMG AC 151437, and deutonymph 171474). Furthermore, a deutonymph, bigger than the reared specimens and found in a cave, could be associated (PP=99.03) with a larva (CCT-UFMG AC 151442). A larva (CCT-UFMG AC 1400806) from Paraná state in the south of Brazil, a female (CCT-UFMG AC 1300421) from Lavras municipality, in Minas Gerais state, and a larva (CCT-UFMG AC 1300423) from the P.E. da Serra do Rola Moça, in Minas Gerais state were associated due to the presence of the same haplotype 5.

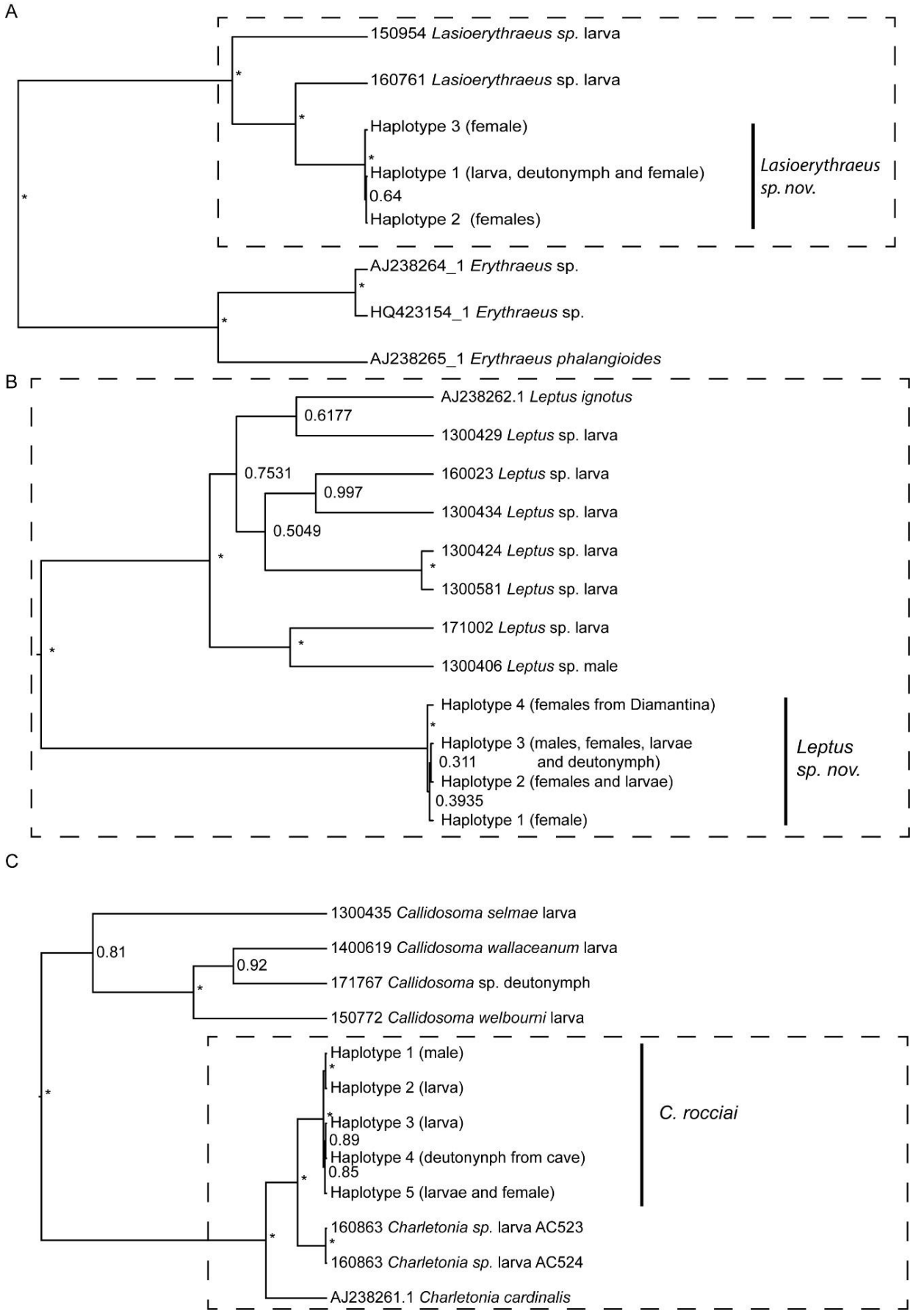
A
150954 Lasioerythraeus sp. larva
160761 Lasioerythraeus sp. larva
Haplotype 3 (female)
Haplotype 1 (larva, deutonymph and female)
0.64
Haplotype 2 (females)
Lasioerythraeus sp. nov.
AJ238264_1 Erythraeus sp.
HQ423154_1 Erythraeus sp.
AJ238265_1 Erythraeus phalangioides
B
AJ238262.1 Leptus ignotus
0.6177
1300429 Leptus sp. larva
0.7531
160023 Leptus sp. larva
0.997
1300434 Leptus sp. larva
0.5049
1300424 Leptus sp. larva
1300581 Leptus sp. larva
171002 Leptus sp. larva
1300406 Leptus sp. male
Haplotype 4 (females from Diamantina)
Haplotype 3 (males, females, larvae
0.311 and deutonymph)
Haplotype 2 (females and larvae)
0.3935
Haplotype 1 (female)
Leptus sp. nov.
C
1300435 Callidosoma selmae larva
0.81
1400619 Callidosoma wallaceanum larva
0.92
171767 Callidosoma sp. deutonymph
150772 Callidosoma welbourni larva
Haplotype 1 (male)
Haplotype 2 (larva)
Haplotype 3 (larva)
0.89
Haplotype 4 (deutonynph from cave)
0.85
Haplotype 5 (larvae and female)
C. rocciai
160863 Charletonia sp. larva AC523
160863 Charletonia sp. larva AC524
AJ238261.1 Charletonia cardinalis

Figure 1 – Ultrametric bayesian phylogenetic trees based on COI. A: *Lasioerythraeus*; B: *Leptus*; C: *Charletonia.* Numbers on the nodes indicates the posterior probability. Dashed squares highlight the in group submitted for the bGMYC analysis (Fig. 2). *100% posterior probability.

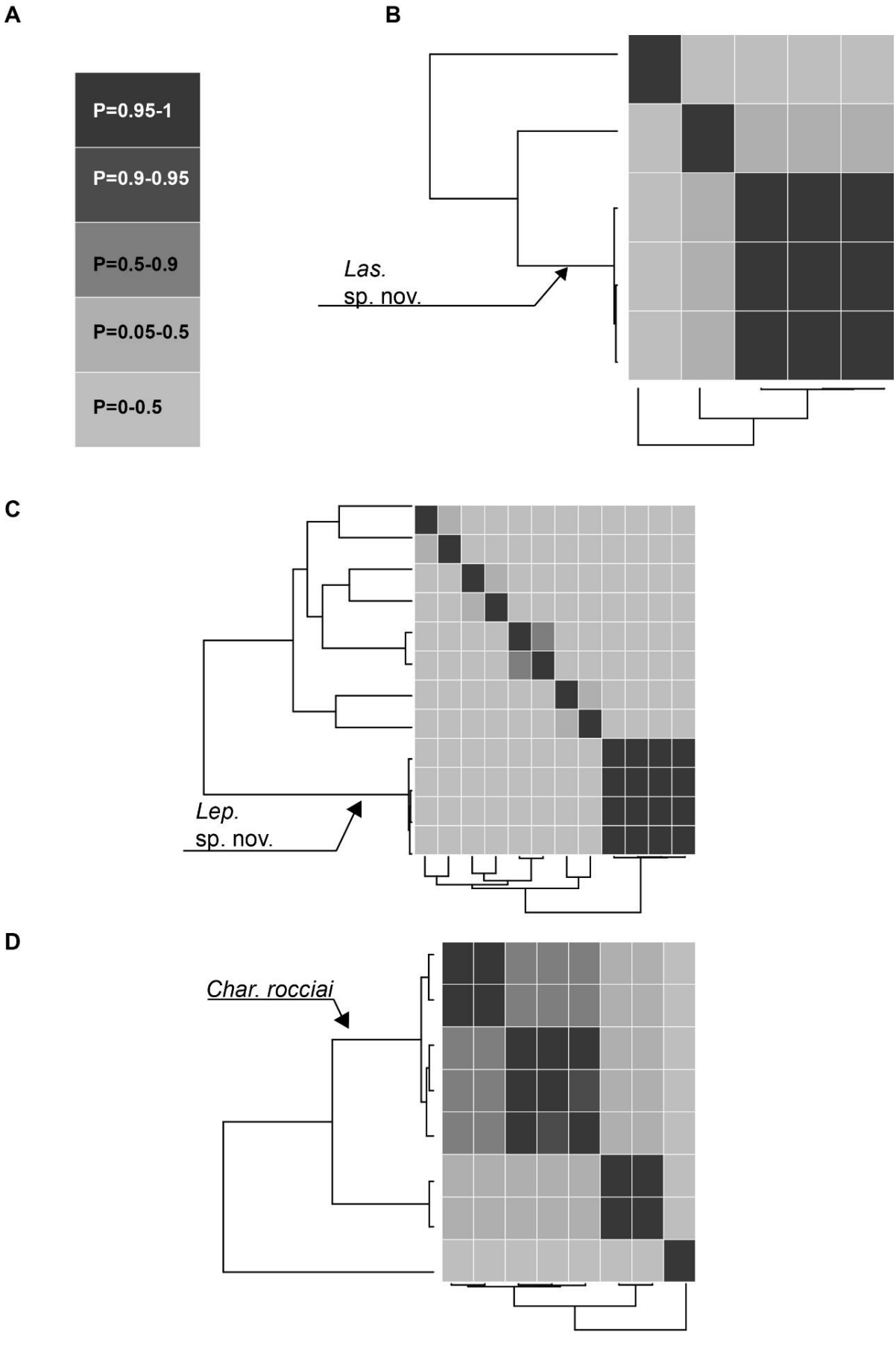
A
P=0.95-1
P=0.9-0.95
P=0.5-0.9
P=0.05-0.5
P=0-0.5
B
Las.
sp. nov.
C
Lep.
sp. nov.
D
Char. rocciai

Figure 2 – Matrix illustrating the posterior probability of recovering a terminal (line) and any of the other terminals (columns) as a single species according to the bGMYC. A: Colors scheme and its equivalent posterior probability; B: *Lasioerythaeus*; C: *Leptus*; D: *Charletonia.* The terminals identification and phylogenetic relationships are informed in the dashed squares of fig. 1.

Descriptions

Family Erythraeidae Robineau-Desvoidy, 1828

Family diagnosis according to Southcott (1961).

Genus *Lasioerythraeus* Welbourn & Young, 1987

Genus diagnosis according to Welbourn & Young (1987), with the exception that our post larval specimens have the prodorsal sclerite weakly developed or absent.

Lasioerythraeus sp. nov.

Diagnosis

Larvae: Dorsal setae heteromorphic with two shapes: flagelliform and blade-like (Fig. 3A and E); fn Ge 8-8-8; fn Ti 15-15-15; palp setal formula: B-B-BBB-1N5B1ζ1ω; long legs (IP≈2535); *Deutonymph*: Anal valves placed at the distal end of the idiosoma (black arrow in Fig. 5A) and bearing 4 barbed setae each (Fig. 5G); anterior sensillar area bearing three robust and weakly barbed setae; prodorsal sclerite around crista metopica weakly developed or absent, lacking well delimited borders (Fig. 5B); eye plate bearing two heteromorphic setae, one long and robust internal, one short and thin external (Fig. 5I); *Female*: Anal valves placed at the distal end of the idiosoma (black arrow in Fig. 6A); crista metopica bearing two pairs of filiform sensillae with faint setules and nine robust scutellae with distinct setules, 118-248 long; of those nine, two scutellae are placed between the two sensillar areas and five in the anterior sensillar area, three anterior, and two posterior, to the anterior sensilla (Fig. 7C); palp genu bearing three dorsal setae, two in the proximal half and one longer in the distal half (white arrow in Fig. 7A); palp tarsus with three to four spines (white arrow in Fig. 7A, B and F), palp tibia with smooth tibial claw (Fig. 7F).

Type Material. Holotype: Larva CCT-UFMG AC161067 collected at 19°05'46.0"S, 43°21'25.0"W, in a cave an iron ore terrain, Conceição do Mato Dentro municipality, Minas Gerais State, Brazil, between July 26 and October 13 of 2016. Paratype deutonymph: CCT-UFMG AC 161064. Paratype females: CCT-UFMG AC 161061, 161062, 161068, 161069 and 161070 (See Table I for detailed collecting data). The specimens were collected in the entrance and penumbra zone of unnamed caves.

Description

Larvae.

Measurements summarized in Table II. Idiosoma roughly oval with more than 50 dorsal barbed setae (precise number unknown due to damage), with two different shapes, flagelliform and blade like (Fig. 3A and E). Dorsal scutum punctate, wider than long, with two pairs of setae (scutellae) and two pairs of sensilla; anterior sensilla more barbed in distal half, while posterior homogeneously barbed (Fig. 3D). In addition, distance AP shorter than AW (AP<AW) (Fig. 3D). Two pairs of eyes on soft cuticle, without an evident eye plate. Anterior sensilla shorter than posterior (Asens<Psens). Idiosoma ventrally with two barbed setae between coxae I and II (1a), two between coxae II and III (2a) and two barbed setae posterior to coxae III (3a), followed by numerous setae with uncertain positions, due to damage (Fig. 3B). Coxae I, II, and III with one barbed seta each (1b, 2b and 3b), setae 1b much longer than 2b and 3b (Fig. 3B).

Gnathosoma bearing one pair of hypostomal setae (*as*), one pair of galeala (*cs*) (both smooth), and one pair of microsetae (*elcp*). Cheliceral blades short and curved (Fig. 3C).

Palp robust, with a bifid palptibial claw bearing spicules (Fig. 3F). Setal formula: B-B-BBB-1N5B1ζ1ω. Eupathidium barbed with one basal spine (Spn) (Fig. 3F and G).

Leg setae barbed. Legs III longer than legs I, which are longer than legs II. All tarsi with two identical branched claws and a claw-like empodium with a terminal hook (Fig. 4). Measurements in Table II.

Leg setal formula:

Leg I: Ta- 24B, 2 ζ, 1 ω, 1ε; Ti- 15B, 1κ, 2 φ; Ge - 8B, 1κ, 1 σ; Tf – 5B, Bf – 4B; Tr – 1B; Cx 1B.

Leg II: Ta- 21B, 1 ζ, 1 ω; Ti- 15B, 2 φ; Ge - 8B, 1κ; Tf – 5B; Bf – 4B; Tr – 1B; Cx 1B.

Leg III: Ta- 22B, 1 ζ; Ti- 15B,1 φ; Ge - 8B; Tf – 5B; Bf – 3B; Tr – 1B; Cx 1B.

Deutonymph.

Measurements summarized in Table III. Color in life unknown. Idiosoma roughly oval (Fig. 5A). Dorsal setae with weak setules and two shapes: long dorsal setae (LDS) and short dorsal setae (SDS) (Fig. 5B). Crista metopica bearing two pairs of filiform sensillar setae with faint setules and seven robust (125–160) setae with setules. Of those seven, four are placed between the two sensillar areas and three in the anterior sensillar area, anteriorly to the anterior sensilla (Fig. 5B). Prodorsal sclerite weakly developed or absent, without clearly delimited borders (Fig. 5B). Two pairs of eyes placed in an eye plate with two heteromorphic setae: one long and robust, on internal side, the other shorter and thin, on external side (circled in Fig. 5B). Anal valves placed at distal end of idiosoma (black arrow on Fig. 5A) and bearing four barbed setae on each valve (Fig. 5G). This unusual position of the anal

plates can be observed in fresh specimens, indicating that it is not an artifact of the slide mounting process. Pre-genital plate bearing three setae (Fig. 5H).

Gnathosoma conical bearing two long and stylet-like cheliceral blades and fringed lips (black arrow on Fig. 5C and D). Palps long, bearing weakly barbed setae (Fig. 5F). Palp tibia with two spines (black arrows on Fig. 5F), seven barbed setae, and an entire palp tibial claw; palp genu bearing 14 weakly barbed setae; palp femur with 30 weakly barbed setae (Fig. 5C and D).

Relative legs lengths: leg I>III>II. Leg scobalae numerous, pointed, with weak setules and two shapes: long and short (Fig. 5E). Tibia I bearing 20 solenidia on dorsal side, most in distal half. Genu I and tibia I bearing one long microseta each (k1 and k2, respectively); shape and position similar to microsetae in larva and female (Fig. 5E; Fig. 4A and Fig. 6B, C and D).

Female.

Measurements summarized in Table IV. Color in life unknown. Idiosoma roughly oval (Fig. 6A). Prodorsal sclerite weakly developed or absent, without clearly delimited borders (Fig. 7C). Crista metopica bearing two pairs of filiform sensillar setae with faint setules and 9–11 robust scutellae with setules (118–248). One paratype carries eleven scutellae, all others nine, two pairs positioned between the two sensillar areas and five to seven on the anterior sensillar area, three anterior, two to four posterior to anterior sensilla (Fig. 7C). The specimen with eleven scutellae has an assymetric distribution, apparently with one setae positioned posterior to the sensillae triplicated. Two pairs of eyes each on an eye plate with two heteromorphic setae: one long and robust, on internal side, the other shorter and thin, on external side (circled on Fig. 7C), however this character is less evident in some paratypes. Anal valves placed at the distal end of idiosoma (black arrow on Fig. 6A) and bearing 8–11 barbed setae each (Fig. 7G). Genital pore bearing two heteromorphic pairs of overlapping genital acetabula (G. ac), the anterior one is kidney-like and the posterior one pea-like (Fig. 7D and E).

Gnathosoma conical with long, stylet-like, and fringed lips (Fig. 7A and B). Palps long, bearing barbed setae. Palp femur punctate (arrowhead on Fig. 7A). Palp genu bearing three dorsal setae, two in the proximal half and one longer in the distal half (white arrow on Fig. 7A). Palp tarsus with three to four spines (white arrow on Fig. 7A, B and F) and an entire palp tibial claw (Fig. 7F).

Relative legs lengths: IV>I>III>II. Leg scobalae numerous, pointed with weak setules (Fig. 6A and B), palp tibia bearing numerous dorsal solenidia. Genu and tibia with one long micro seta each (k1 and k2 respectively) (Fig. 6B, C and D). Tibia I bearing numerous dorsal solenidia on the dorsal side, most in distal half (black arrowhead on Fig. 6C).

Remarks

Lasioerythraeus Welbourn & Young, 1987 is a small widespread genus with six described species: *La. shirleyanneae* (McDaniel & Bolen, 1981) from Texas, USA; *La. johnstoni* Welbourn & Young, 1987, from Mississippi, Washington (USA) and Dominican Republic; *La. whitcombi* (Smiley, 1964), from Arkansas (USA); *La. cardonensis* Haitlinger, 2008, from Margarita Island, Venezuela; *La. saboorii* Khanjani et al. 2011, from eastern Iran and *La. setarius* Kamran & Bashir, 2013 from Punjab, Pakistan (Kamran & Bashir, 2013). *Lasioerythraeus whitcombi* was described based on post-larval instars, *La. johnstoni* on both larval and post-larval, and all other solely on larvae. *Lasioerythraeus* larvae were collected from either herbaceous plants or as ectoparasites of Hemiptera (Kamran & Bashir, 2013).

Lasioerythraeus jessicae sp. nov. larvae differ from other *Lasioerythraeus* due the presence of dorsal setae with two shapes: flagelliform and blade-like (Fig. 3A and E). Additionally, they differ from *Lasioerythraeus cardonensis* by the presence of barbed setae (vs. nude in *La. cardonensis*) on the palp tibia, differ from *La. saboorii* and *La. setarius* due the presence of eight normal setae on genu I, II and III (vs. 8–9–9 in *La. saboorii* and 9–9–8 in *La. setarius*), from *La. shirleyanneae* and *La. johnstoni* due leg lengths (IP is 2535 in *La. jessicae* sp. nov. vs. 1903 and 1934–2187 in *La. shirleyanneae* and *La. johnstoni,* respectively).

Deutonymphs of *La. jessicae* sp. nov. differ from *La. whitcombi* and *La. johnstoni* by the presence of eight setae on each anal plate (vs. 12–14 in *La. johnstoni* and 12 in *La. whitcombi*). Both deutonymphs and females differ from other species by the poor development of the prodorsal sclerite around the crista metopica, lacking well delimited borders (Fig. 5B). Females differ from *La. whitcombi* and *La. johnstoni* in the number of scutellae in the anterior sensillar area (Fig. 7C): *La. jessicae* sp. nov. has three setae anterior to the anterior sensillary setae (vs. seven in *La. johnstoni* and five to nine in *La. whitcombi*) and two to five at the level of, or posterior to, those sensilla (vs. six in *La. johnstoni*).

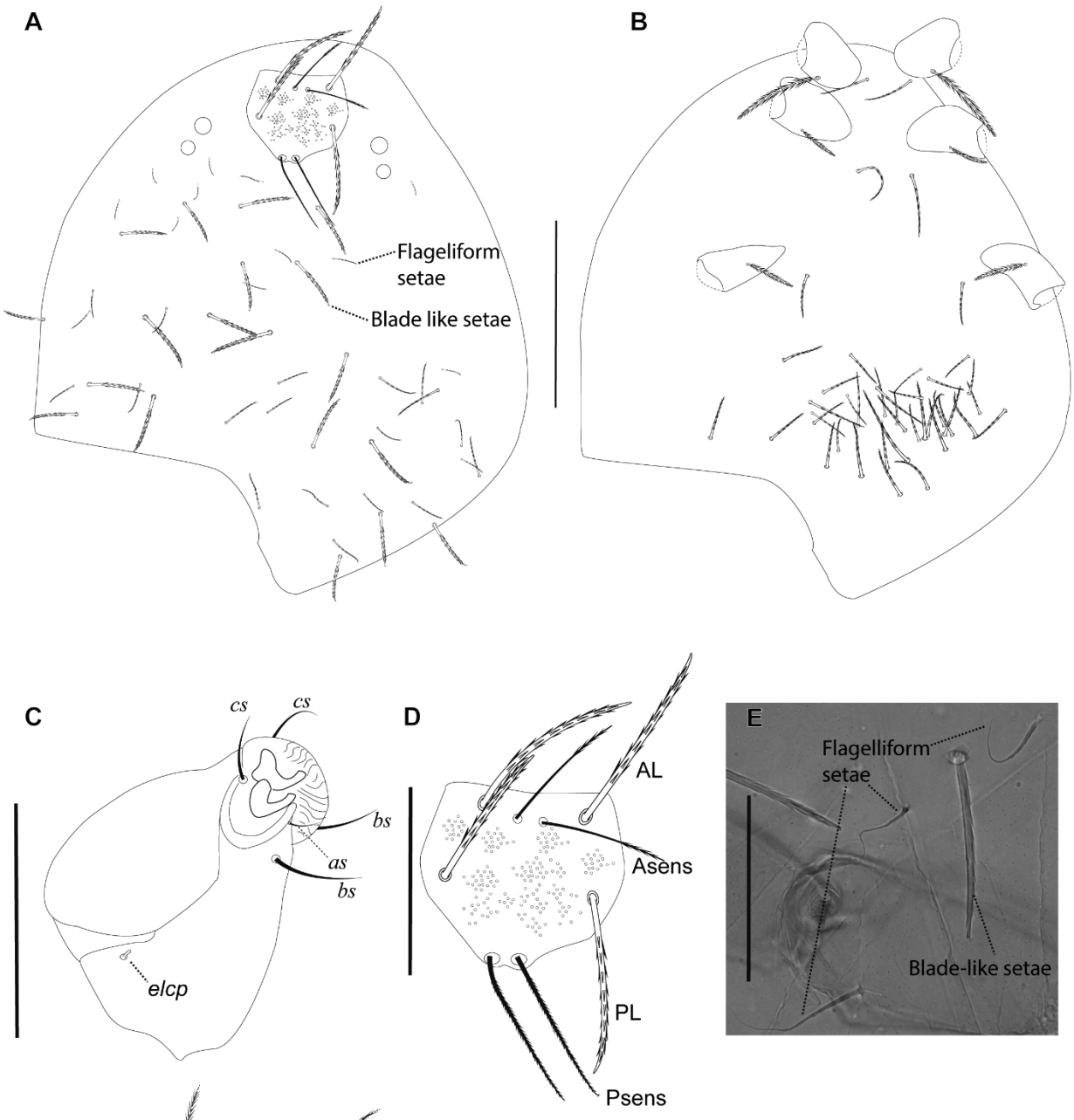
A
Flageliform setae
Blade like setae
B
C
cs
cs
bs
as
bs
elcp
D
AL
Asens
PL
Psens
E
Flagelliform setae
Blade-like setae
F
G
B
N
B
Spn.
B
ω
ζ
B
B

Figure 3 – *Lasioerythraeus* sp. nov. larva. A: Idiosoma, dorsal view; B: Idiosoma, ventral view; C: Gnathosoma, lateral view; D: Scutum; E: Dorsal setae: detail showing the blade-like and flagelliform setae; F: Palp; G: Palp tarsus. Scale: A, B, C, F= 200; D = 100; E, G = 50.


A
B
C
σ
κ
φ
φ
κ
ε
ω
κ
φ
φ
ω
φ
D
E
F
ζ
ζ
ζ
ζ
ζ

Figure 4 – *Lasioerythraeus* sp. nov. larva. A: Leg I; B: Leg II; C: Leg III; D: Distal portion of tarsus I; E: Distal portion of tarsus II; F: Distal portion of tarsus III. Scale: A, B, C= 200; D, E, F = 50.

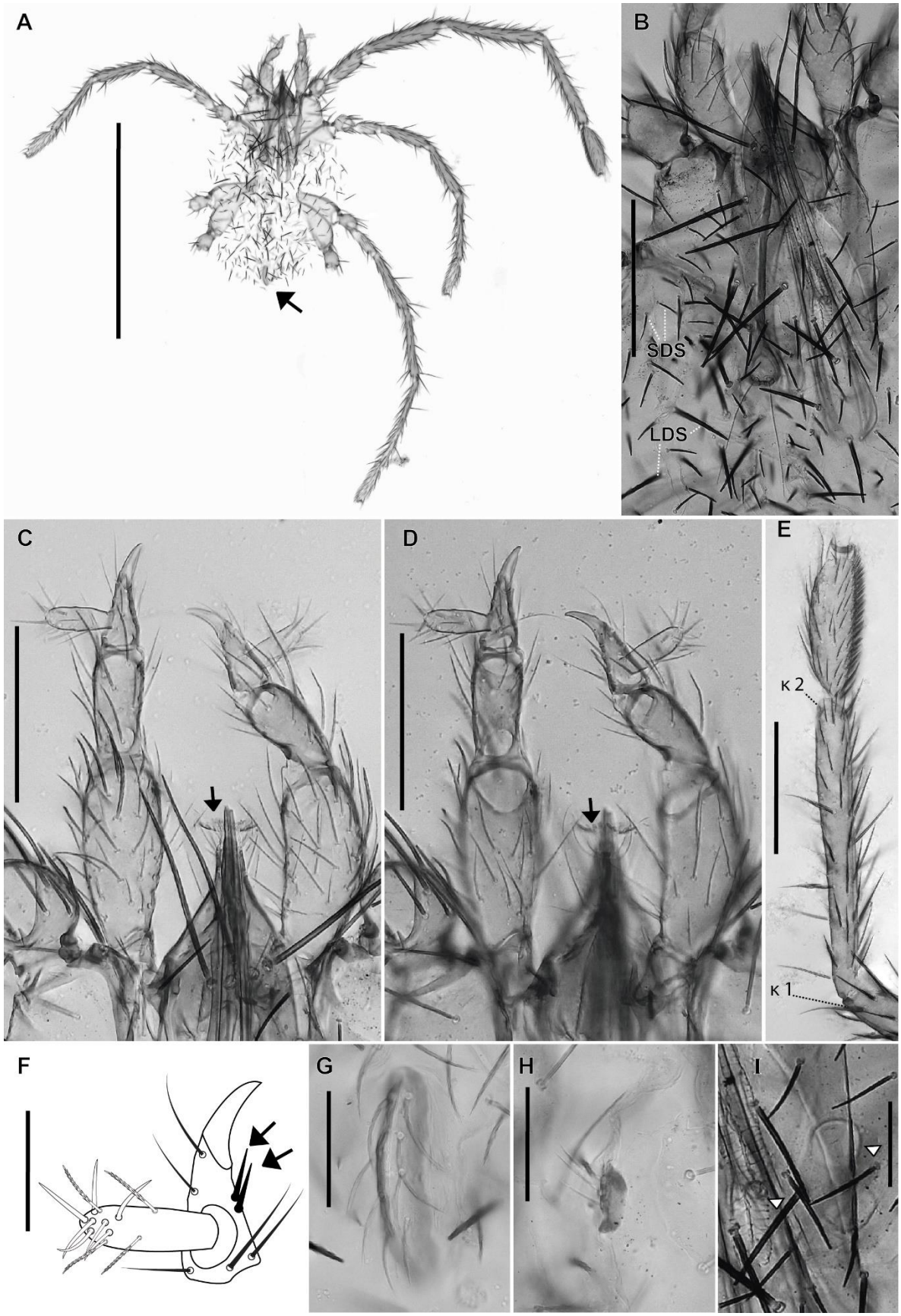

Figure 5 – *Lasioerythraeus* sp. nov. deutonymph. A: General view, black arrow pointing the anal pore; B: Crista metopica, eye plate indicated; C and D: Palp dorsal and ventral view, arrow pointing the gnathosoma fringe; E: Tarsus and tibia I; F: Palp tarsus and tibia, arrow pointing two spines; G: Anal pore; H: Undeveloped genital plate; I: Eye plate, arrow heads indicating a pair of heteromorphic setae. Scale: A= 1000; B and E= 200; C and D= 100; F, G, H and I = 50.

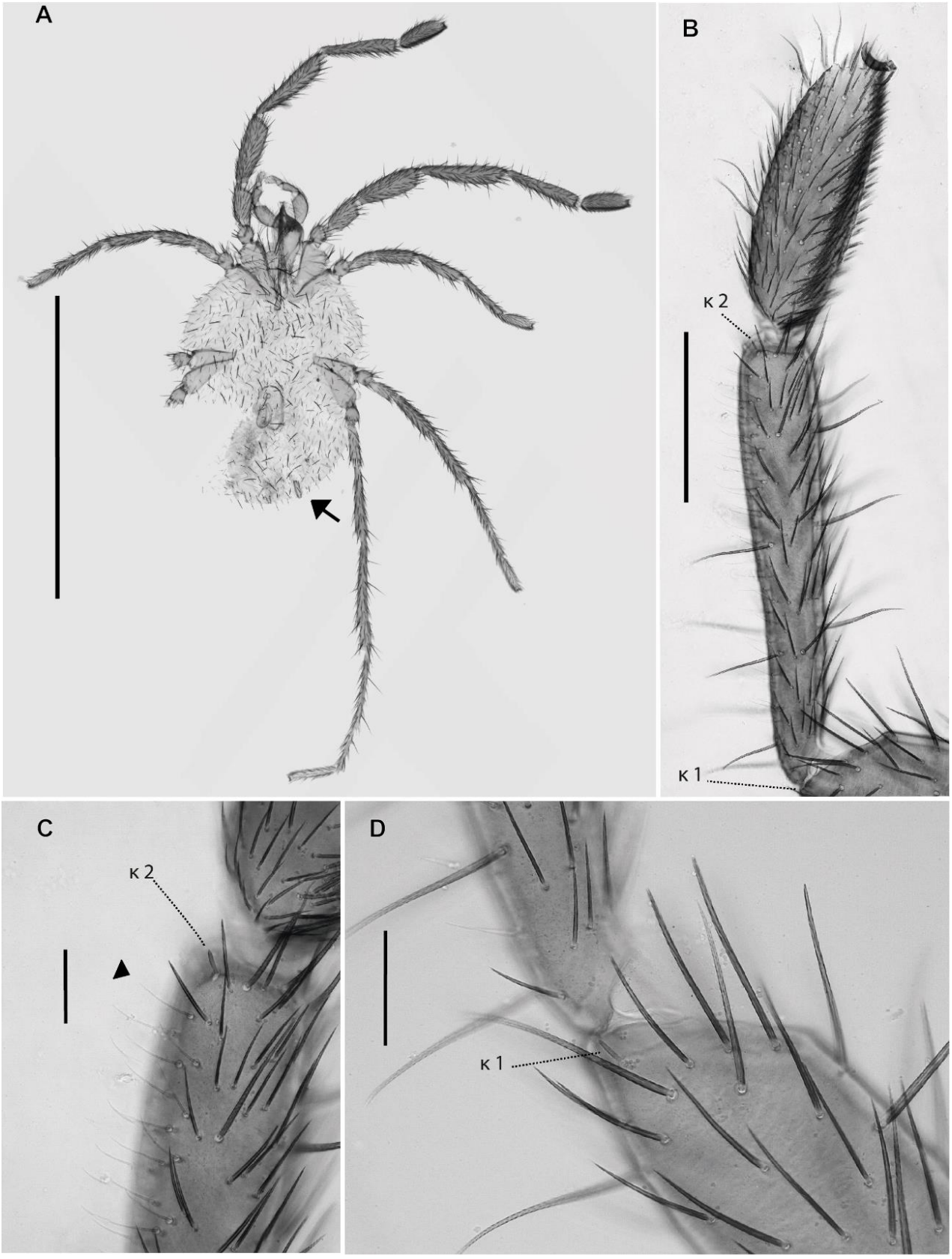

Figure 6 – *Lasioerythraeus* sp. nov. female. A: General view. B: Tibia and tarsus I. C: Joint between tibia and tarsus I, arrow head pointing one of the numerous dorsal solenidia. D: Joint between genu and tibia I. Scale: A= 2000; B= 200; C and D= 50.

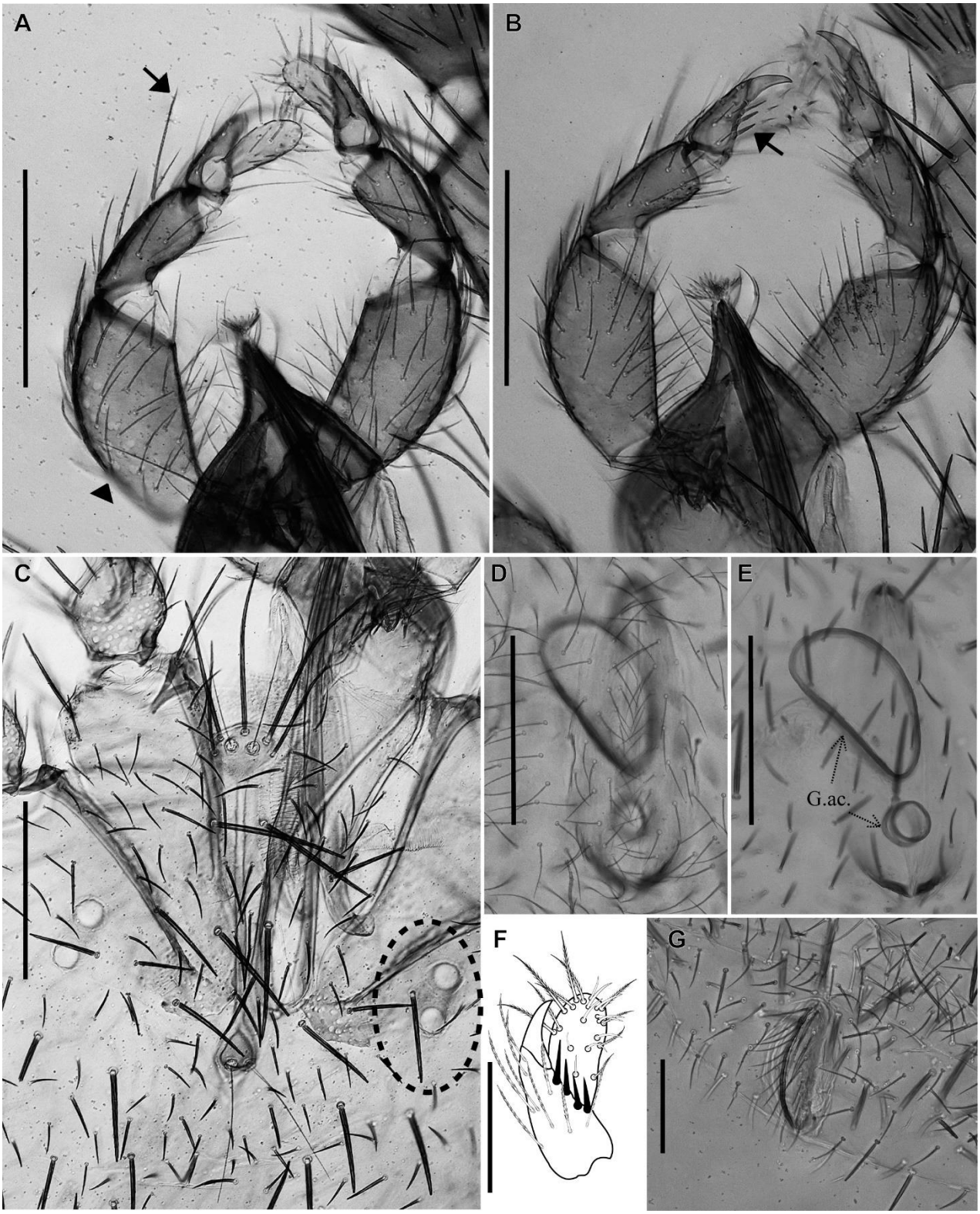

Figure 7 – *Lasioerythraeus* sp. nov. female. A: Gnathosoma ventral view, arrow pointing a dorsal robust seta, arrow head pointing to punctations on the palp-femur. B: Gnathosoma dorsal view, setae pointing the spines on palp-tibia. C: Crista

metopica, eye plate indicated. D: Genital pore superficial view. E: Genital pore internal structures showing the genital acetabula (G. ac.). F: Palp tiba and tarsus. G: Anal pore. Scale: A-E=200; F and G= 100.

Table II – Metric data for the larval specimens of *Leptus* sp. nov., *Lasioerythraeus* sp. nov., and five new specimens of *Charletonia rocciai* Treat & Flechtmann, 1979. Some of the metric characters are not applicable for all species and are marked by (NA).

Character	*Le.* sp. nov.		*La.* sp. nov.	*C. rocciai*	
	Mean (n=5)	Range	Value (n=1)	Mean (n=5)	Range
IL	427.6	359.0 - 507.0	557.0	601.0	410.0 - 806.0
IW	240.0	191.0 - 273.0	-	440.4	300.0 - 645.0
L	74.4	66.0 - 77.0	112.0	83.2	78.0 - 90.7
W	80.0	78.0 - 82.0	99.0	101.2	92.0 - 110.7
AW	71.6	70.0 - 73.0	59.7	64.1	57.0 - 70.0
MW	NA	NA - NA	NA	76.2	68.0 - 84.0
PW	69.4	65.0 - 74.0	88.7	93.6	85.0 - 101.0
ISD	49.2	48.0 - 51.0	82.7	54.9	50.0 - 59.0
AL	50.4	48.0 - 55.0	106.0	53.8	47.5 - 58.6
ML	NA	NA - NA	NA	53.9	49.0 - 61.0
PL	49.0	45.0 - 52.0	89.0	49.5	46.0 - 53.6
AP	15.4	14.0 - 16.0	46.0	42.8	38.0 - 49.0
ASE	48.6	43.0 - 52.0	74.0	44.8	42.0 - 49.0
PSE	86.3	83.0 - 90.0	88.0	65.7	56.0 - 72.1
Sba	12.0	11.0 - 14.0	17.0	9.7	8.8 - 10.7
SBp	12.2	11.0 - 13.0	17.0	18.2	18.0 - 18.6
GL	147.8	144.0 - 155.0	135.0	130.7	123.0 - 150.0
DS	40.6	30.0 - 50.0	50-69	43.1	36.0 - 51.0
Flag. DS	NA	NA - NA	28-37	NA	NA - NA
1a	27.3	25.0 - 29.0	54.0	36.7	33.0 - 39.3
2a	34.5	33.0 - 37.0	67.0	51.8	45.7 - 57.9
3a	37.7	32.0 - 40.0	56.0	48.8	46.4 - 50.7
3a'	NA	NA - NA	NA	43.5	40.7 - 45.7
1b	55.8	52.0 - 58.0	96.0	77.7	73.0 - 81.4
2b	32.0	30.0 - 35.0	43.0	64.4	59.0 - 70.0
2c	NA	NA - NA	NA	47.4	45.0 - 49.3
3b	39.0	36.0 - 42.0	54.0	55.7	49.3 - 60.0
3c	NA	NA - NA	NA	42.2	37.0 - 47.1
PascFed1	58.6	56.0 - 62.0	84.0	64.3	60.0 - 74.3
PascFed2	29.3	22.0 - 38.0	NA	NA	NA - NA
PascGed1	39.2	34.0 - 44.0	66.0	39.3	31.4 - 44.0
PascGed2	27.8	24.0 - 30.0	NA	NA	NA - NA
cs	13.7	11.0 - 16.0	24.0	28.1	25.0 - 31.0
bs	35.0	32.0 - 37.0	NA	31.4	29.0 - 34.0
as	41.4	40.0 - 46.0	38.0	12.3	11.6 - 12.9

Table II – Continuation.

Character	*Le.* sp. nov.		*La. sp. nov.*	*C. rocciai*	
	Mean (n=5)	Range	Value (n=1)	Mean (n=5)	Range
Ta I	102.6	97.0 - 111.0	152.0	127.5	123.0 - 136.0
Ti I	114.0	106.0 - 121.0	240.0	123.3	115.0 - 131.0
Ge I	85.2	81.0 - 93.0	181.0	102.7	93.0 - 111.0
Tf I	51.8	48.0 - 55.0	114.0	55.6	46.4 - 64.3
Bf I	66.6	61.0 - 72.0	105.0	65.5	60.0 - 75.0
Tr I	38.2	34.0 - 44.0	66.0	38.8	32.0 - 45.0
Cx I	52.4	48.0 - 56.0	79.0	58.3	50.0 - 64.0
Ta II	91.2	80.0 - 97.0	128.0	126.0	118.0 - 133.0
Ti II	99.0	92.0 - 106.0	196.0	116.5	110.0 - 152.0
Ge II	75.0	68.0 - 78.0	141.0	99.3	90.0 - 106.0
Tf II	48.6	47.0 - 51.0	94.0	58.3	55.7 - 60.7
Bf II	55.6	52.0 - 57.0	117.0	64.8	60.7 - 71.4
Tr II	38.0	36.0 - 40.0	66.0	40.4	35.7 - 49.3
Cx II	55.0	52.0 - 57.0	95.0	63.3	58.6 - 72.7
Ta III	96.5	93.0 - 101.0	166.0	131.6	123.0 - 154.0
Ti III	144.5	138.0 - 155.0	294.0	157.2	153.0 - 164.0
Ge III	90.3	83.0 - 97.0	158.0	106.0	98.0 - 113.0
Tf III	67.8	64.0 - 72.0	127.0	69.8	60.3 - 75.0
Bf III	56.2	53.0 - 59.0	121.0	66.0	57.0 - 77.1
Tr III	38.4	35.0 - 42.0	69.0	48.2	42.0 - 54.0
Cx III	54.4	53.0 - 56.0	96.0	61.6	57.0 - 68.0
Leg I	458.4	443.2 - 502.0	858.0	571.0	546.3 - 606.0
Leg II	407.4	385.0 - 424.0	742.0	564.8	549.5 - 587.0
Leg III	494.8	472.0 - 520.0	935.0	651.3	621.9 - 677.0
IP	1361.8	1305.8 - 1445.8	2535.0	1779.3	1731.0 - 1868.0

Table III - Metric data for the deutonymphs of *Leptus* sp. nov., *Lasioerythraeus* sp. nov., and five new specimens of *Charletonia rocciai* Treat & Flechtmann, 1979. Some os the metric characters are not applicable for all species and are marked by (NA). * Those characters are relative to the anterior eye for *Lasioerythraeus* which has two pairs of eyes.

Character	*Le.* sp. nov.		*La.* sp. nov.	*C. rocciai*	
	Mean (n=5)	Range	Value (n=1)	Mean (n=5)	Range
IL	1097.8	934 - 1411	901	620.5	534 - 697
ILCM	1243	1050 - 1688	1015	764.5	633 - 916
IW	804.7	653 - 900	629	500	427 - 602
Sba	16	15 - 17	20	18.5	15 - 22
Sbp	16.2	15 - 17	18	15.5	15 - 16
Asens	68.4	58 - 78	132	78	61 - 117
Psens	68.2	57 - 77	151	98.7	81 - 118
ISD	257.7	240 - 286	298	176.5	152 - 190
Scutalae	44	30 - 46	125-160	98.9	72 - 143
LDS	46.4	36 - 66	60-88	70.3	47 - 93
SDS	NA	NA - NA	31-47	34.4	28 - 42
LVS	37	24 - 53	50-72	103.3	77 - 127
SVS	NA	NA - NA	NA	44.1	34 - 54
ACW	39.6	38 - 42	67	57.5	53 - 62
PCW	32.7	32 - 34	45	33.3	30 - 37
L	307	293 - 335	324	259	215 - 290
Anterior ECO	22	22 - 22	28	26.3	24 - 30
Posterior ECO	NA	NA - NA	30	NA	NA - NA
Anterior Eye Ring	31	28 - 34	31	35	27 - 40
Posterior Eye Ring	NA	NA - NA	35	NA	NA - NA
EC-EC*	234	234 - 234	-	230.7	196 - 248
EC-Asens*	134	134 - 134	127	107	99 - 115
Cx I	108.2	94 - 129	281	141.4	124 - 154
Tr I	81.2	71 - 95	108	87.5	79 - 99
BFe I	184.6	168 - 206	378	177.8	144 - 208
TFe I	251.2	229 - 286	399	310.6	273 - 384
Ge I	278.4	255 - 313	454	300.4	265 - 371
Ti I	287.8	261 - 321	459	329.6	295 - 396
Ta I (L) (with claw)	232.4	223 - 243	247	222.4	192 - 258
Ta I (W)	56.4	48 - 63	68	90	85 - 95
Leg I (without Cx)	1315.6	1215 - 1463	2045	1408.6	1249 - 1702
Cx II	165.6	124 - 204	241	189	156 - 229
Tr II	73.4	63 - 85	88	87.5	59 - 119
Bfe II	110.2	88 - 130	234	104	81 - 147

Table III – Continuation.

Character	*Le.* sp. nov.		*La.* sp. nov.	*C. rocciai*	
	Mean (n=5)	Range	Value (n=1)	Mean (n=5)	Range
Tfe II	151.8	135 - 176	236	165	132 - 214
Ge II	188.2	165 - 219	262	169	150 - 203
Ti II	195.2	182 - 220	311	186	164 - 225
Ta II (L)	139.2	128 - 150	147	155.2	137 - 179
Ta II (W)	34.4	31 - 38	46	72	68 - 78
Leg II	858	790 - 963	1278	908.2	745 - 1372
Cx III	105.8	96 - 118	186	184.2	159 - 226
Tr III	72.4	61 - 83	89	77.2	62 - 93
Bfe III	102.8	80 - 134	249	109	91 - 129
Tfe III	163.8	145 - 196	304	220.7	193 - 272
Ge III	196	180 - 213	319	243.2	210 - 291
Ti III	228.2	211 - 258	430	254	228 - 300
Ta III (L)	154.8	131 - 177	174	154.2	138 - 183
Ta III (W)	30.4	25 - 35	37	70.2	67 - 75
Leg III	918	847 - 1028	1565	1110.7	928 - 1494
Cx IV	202.4	192 - 234	256	222.7	165 - 313
Tr IV	79.8	57 - 93	80	114.3	98 - 144
Bfe IV	146.2	132 - 179	-	160.7	135 - 207
Tfe IV	201.8	120 - 253	-	399.2	340 - 501
Ge IV	255.6	193 - 308	-	375	324 - 473
Ti IV	302.8	268 - 352	-	394.7	353 - 479
Ta IV (L)	207.6	155 - 305	-	160	140 - 194
Ta IV (W)	28	23 - 35	-	64.7	60 - 72
Leg IV	1193.8	1075 - 1322	-	1624.4	1393 - 2058
Palp Femur (L)	129.5	121 - 141	162	117.5	114 - 121
Palp Femur (w)	51.75	47 - 58	62	59. 7	54 - 69

Table IV - Metric data for the females of *Leptus* sp. nov., *Lasioerythraeus* sp. nov. and *Charletonia rocciai* Treat & Flechtmann, 1979. Some os the metric characters are not applicable for all species and are marked by (NA). * Those characters were measured relative to the anterior eye for *Lasioerythraeus* which has two pairs of eyes.

Character	*Le.* sp. nov.		*La.* sp. nov.		*C. rocciai*
	Mean (n=4)	Range	Mean (n=5)	Range	Value (n=1)
IL	1666.8	1335.0 - 2178.0	1624.4	1260.0 - 1799.0	2168.0
ILCM	1924.5	1606.0 - 2372.0	1877.6	1453.0 - 2093.0	2438.0
IW	1231.8	813.0 - 1587.0	1264.8	927.0 - 1486.0	1482.0
Sba	19.5	17.0 - 21.0	26.4	22.0 - 33.0	30.4
Sbp	24.5	21.0 - 28.0	26.2	23.0 - 29.0	24.4
Asens	94.5	85.0 - 107.0	164.0	140.0 - 185.0	-
Psens	103.3	88.0 - 115.0	200.0	189.0 - 218.0	-
ISD	381.8	360.0 - 403.0	443.0	366.0 - 499.0	438.1
Scutalae	50.4	40.0 - 70.0	186.9	118.0 - 248.0	116-194
LDS	34.8	19.0 - 47.0	100.9	55.0 - 192.0	57-130
SDS	NA	NA - NA	40.1	29.0 - 52.0	24-42
LVS	32.5	22.0 - 40.0	73.4	33.0 - 99.0	92-130
SVS	NA	NA - NA	NA	NA - NA	30-56
ACW	53.0	46.0 - 58.0	120.8	95.0 - 143.0	112.9
PCW	44.8	42.0 - 46.0	56.8	47.0 - 69.0	70.8
L	467.5	417.0 - 490.0	515.2	447.0 - 582.0	673.9
Anterior ECO	25.7	24.0 - 28.0	33.8	27.0 - 38.0	47.7
Posterior ECO	NA	NA - NA	31.2	26.0 - 38.0	NA
Anterior Eye Ring	33.3	30.0 - 37.0	47.2	44.0 - 52.0	87.7
Posterior Eye Ring	NA	NA - NA	51.0	45.0 - 60.0	NA
EC-EC*	433.3	369.0 - 535.0	390.2	286.0 - 438.0	585.0
EC-Asens*	230.7	210.0 - 246.0	271.4	218.0 - 306.0	380.0
Cx I	180.8	172.0 - 187.0	431.8	377.0 - 486.0	368.0
Tr I	114.8	101.0 - 124.0	173.6	151.0 - 189.0	-
BFe I	288.8	251.0 - 325.0	405.6	290.0 - 473.0	-
TFe I	364.8	338.0 - 386.0	549.2	474.0 - 613.0	-
Ge I	419.3	400.0 - 432.0	672.4	580.0 - 738.0	-
Ti I	418.8	394.0 - 437.0	581.2	522.0 - 629.0	-

Table IV – Continuation.

Character	*Le.* sp. nov.		*La.* sp. nov.		*C. rocciai*
	Mean (n=4)	Range	Mean (n=5)	Range	Value (n=1)
Ta I (L) (with claw)	353.8	341.0 - 363.0	400.8	344.0 - 457.0	-
Ta I (W)	79.0	73.0 - 84.0	129.6	113.0 - 150.0	-
Leg I (without Cx)	1960.0	1862.0 - 2030.0	2782.8	2394.0 - 3080.0	-
Cx II	247.5	212.0 - 293.0	377.8	347.0 - 398.0	433.0
Tr II	100.3	94.0 - 104.0	172.4	142.0 - 190.0	-
Bfe II	145.0	120.0 - 167.0	246.2	220.0 - 271.0	-
Tfe II	226.5	205.0 - 248.0	353.6	287.0 - 384.0	-
Ge II	283.0	266.0 - 299.0	390.2	340.0 - 427.0	-
Ti II	299.8	283.0 - 326.0	436.2	399.0 - 471.0	-
Ta II (L)	224.3	196.0 - 241.0	234.8	225.0 - 245.0	-
Ta II (W)	47.5	41.0 - 53.0	72.6	59.0 - 83.0	-
Leg II	1278.8	1170.0 - 1376.0	1833.4	1613.0 - 1961.0	-
Cx III	177.8	160.0 - 193.0	348.0	293.0 - 410.0	403.0
Tr III	116.5	99.0 - 135.0	170.0	147.0 - 190.0	-
Bfe III	153.8	139.0 - 161.0	280.5	237.0 - 314.0	-
Tfe III	237.8	224.0 - 250.0	419.3	366.0 - 469.0	-
Ge III	296.8	276.0 - 314.0	462.5	391.0 - 515.0	-
Ti III	336.8	328.0 - 358.0	557.3	396.0 - 630.0	-
Ta III (L)	226.5	209.0 - 250.0	264.0	226.0 - 288.0	-
Ta III (W)	44.8	41.0 - 48.0	59.5	48.0 - 65.0	-
Leg III	1368.0	1301.0 - 1437.0	2159.3	1790.0 - 2387.0	-
Cx IV	338.0	311.0 - 377.0	442.0	375.0 - 496.0	555.0
Tr IV	128.0	109.0 - 141.0	150.6	127.0 - 186.0	-
Bfe IV	225.5	203.0 - 249.0	371.0	330.0 - 412.0	-
Tfe IV	318.0	300.0 - 336.0	676.0	583.0 - 769.0	-
Ge IV	409.3	391.0 - 424.0	700.5	601.0 - 800.0	-
Ti IV	449.0	431.0 - 464.0	886.5	812.0 - 961.0	-
Ta IV (L)	300.0	285.0 - 310.0	333.5	303.0 - 364.0	-
Ta IV (W)	42.8	38.0 - 47.0	44.5	40.0 - 49.0	-
Leg IV	1829.8	1737.0 - 1889.0	3131.5	2771.0 - 3492.0	-
Palp Femur (L)	185.3	168.0 - 205.0	250.8	210.0 - 273.0	304.9
Palp Femur (w)	69.0	65.0 - 73.0	123.6	94.0 - 139.0	137.8

Genus *Leptus* Latreille, 1796

Generic diagnosis for the larva follows Southcott (1992) and for the post-larval instars Southcott (1961).

Leptus sp. nov.

Diferential diagnosis

Larvae: Idiosoma bearing 114-118 dorsal setae, ventral surface with a shorter pair of barbed setae between coxae I, four setae between coxae II and nine setae between coxae III. Palpal setal formula: 0-0-2B-2B-3B-4B,1N,1ζ,1ω. Gnathosoma conical, *as* barbed, long (40-46) and placed laterally; *bs* shorter (32-37) than *as*, thin and barbed; tibia and genu I each bearing one piriform micro seta (k1 and k2). *Deutonymph*: IL= 934-1411; ILCM= 1050-1688; IW= 653-900; ECO≈ 22; EC-EC≈ 234. Dorsal setae 36-66 long, barbed and homogeneously distributed. Eyes reduced IL/ECO≈44. Crista metopica short (293-335) restricted to the anterior half of the idiosoma. Sensillar area with 3-4 short (30-46) barbed setae, anterior to sensilla. Tibia and genu I each with k1 and k2, same shape and position as in larvae and adults. *Female*: IL=1335-2178; ILCM= 1606-2372; IW= 808-1587; ECO= 24-28; EC-EC= 369-535. Eyes reduced IL/ECO= 56-87. Short crista metopica (417-490) restricted to the anterior half of idiosoma. Anterior sensillar area with 7-8 short (40-70) barbed setae anterior to sensilla. Genital pore elongated (270-302) with two pairs of oval, small, and overlapping genital acetabula, placed posteriard to genital pore. Tibia and genu I each with k1 and k2, same shape and position as in other stadia. *Male*: IL= 1322-1540; ILCM= 1685-1865; IW= 793-1248; ECO= 26-29; EC-EC= 370-414. Eyes reduced (IL/ECO≈ 59). Similar to females in respect to most somatic characters. Genital pore elongated (198), bearing 25 setae on each side of the external border. Internally, operculum composed by two anteriorly articulated longitudinal sclerites of the ejaculatory complex with 11 eugenital setae on each side over protuberances.

Material examined. The specimens were collected by active search on caves in the entrance, penumbra and aphotic zone. Specimens are deposited in the CCT-UFMG AC.

Holotype: CCT-UFMG AC 151093 collected at 19°34'36.0" S, 44°01'13.0" W, in a cave in limestone terrain, Pedro Leopoldo municipality, MG, Brazil, between July 22nd and August 18th, 2015.

Larval paratypes: CCT-UFMG AC 150875, 151098, 151100 and 151510. Deutonymph paratypes: CCT-UFMG AC 151101, 151206, 150729, 151208 and 150730. Female paratypes: CCT-UFMG AC 151245, 151107, 150693 and 151108. Male paratypes: CCT-UFMG AC 150853, 150725 and 150932.

Description

Larvae.

Measurements are summarized in table II. Idiosoma oval. Dorsal scutum punctate, wider than long, with two pairs of scutellae and two pairs of sensilla; anterior sensilla more barbed in distal half,

posterior sensilla homogeneously barbed (Fig. 8 C). Idiosoma bearing 114-118 dorsal setae, ventral surface with a short pair of barbed setae between coxae I, four setae between coxae II and nine setae between coxae II and III, followed by 31-35 barbed setae (Fig. 8 D, E).

Palpal setal formula: 0-0-2B-2B-3B-4B,1N,1ζ,1ω. Gnathosoma conical, *as* barbed, long (40-46) and placed laterally; *bs* shorter (32-37) than *as*, thin and barbed. Short (16) and smooth galeala (*cs*) visible in only a few specimens. Palpal tibial claw entire (Fig. 8. A, B).

Legs with seven segments each, all normal setae barbed (Fig. 9). All tarsi bearing a pair of claws and a claw-like empodium; anterior claw with small spines, posterior claw lacking a hook element and bearing ventral setules (Fig. 9 D, E, F). Tibia and genu I each bearing one distally enlarged, piriform, distal microseta (Fig. 9 A).

Leg setal formula:

Leg I: Ta - 23B, 1Cp, 2 ζ, 1 ω; Ti - 15B, 1κ, 2 φ; Ge - 8B, 1κ, 1σ; TFe - 5B; BFe - 3B; Tr - 1B; Cx - 1.

Leg II: Ta - 22B, 1 ω, 2 ζ; Ti - 15B, 2 φ; Ge - 8B, 1κ, 1σ; TFe - 5B; BFe - 3B; Tr - 1B; Cx - 1B.

Leg III: Ta- 22B, 1 ζ; Ti- 15B,1 φ; Ge - 8B; TFe – 5B; BFe – 2B; Tr – 1B; Cx – 1B.

Deutonymph.

Measurements are summarized in table III. Color in life unknown. Idiosoma oval (Fig. 10A). Dorsal setae 36-66 long, barbed and homogeneously distributed (Fig. 10B). Short crista metopica restricted to anterior half of idiosoma, positioned between a pair of small eyes. To compare the eye development to other species, we introduced the idiosoma length by diameter of the eye cornea ratio (IL/ECO≈44). Crista with two pairs of filiform sensillary setae with faint setules and 3-4 short (30-46) barbed setae in anterior sensillar area, inserted anterior to sensilla (Fig. 10B). Anal pore ventral (Fig. 12B). Anal valves weakly developed, bearing 3-8 setae on each valve asymmetrically distributed (Fig. 10H).

Gnathosoma conical bearing two long and stylet-like chelicera (Fig. 10A). Palps long, bearing numerous barbed setae, palp tibia claw entire; length similar to that of cylindrical palp tarsus, which carries numerous distal eupathidia (Fig. 10D and E).

Relative legs lengths: IV>I> III>II. Leg scobalae, numerous, pointed and barbed; additional modified trichobothrial setae (tr) present on tarsus, tibia and genu of leg I (Fig. 10C and G). Tibia and genu I each bearing one piriform micro setae (k1 and k2); shape and position as in larvae and adults (Fig. 10F and E).

Female.

Measurements are summarized in table IV. Color in life unknown. Idiosoma oval (Fig. 11A). Dorsal setae barbed, homogeneously distributed (Fig. 11A and B). Short crista metopica (417-490) restricted to anterior half of idiosoma; positioned between a pair of small eyes, which are 369-535

apart. Crista carries two pairs of filiform sensillary setae with faint setules and 7-8 short (40-70) barbed setae on anterior sensillar area, anterior to sensilla (Fig. 11B). Anal pore placed ventrally near genital pore (Fig. 12B). Genital pore elongated, bearing two pairs of small and overlapping genital acetabula (G. ac.), both with the same oval shape and inserted in posterior half of genital pore (Fig. 12B).

Gnathosoma conical (Fig 11, A), bearing two stylet-like cheliceral blades. Palps bearing numerous barbed setae of the same shape; palp tibial claw entire, similar in length to the cylindrical palp tarsus (Fig. 11 C and D); palp tarsus with numerous distal eupathidia (Fig. 12C).

Relative legs lengths: I>IV>III>II. Leg scobalae, numerous, pointed and barbed, modified trichobothrial setae (tr) present on tarsus, tibia and genu I (Fig. 12A and D). Tibia and genu I each bearing one piriform micro setae (k1 and k2) (Fig. 12A and D).

Male.

Measurements are summarized in table V. Color in life unknown and most somatic characters similar to females (Fig. 13A). Dorsal setae barbed, homogeneously distributed (Fig. 13A and B). Short (474-560) crista metopica positioned between a pair of small eyes and restricted to anterior half of idiosoma. Crista with two pairs of filiform sensillary setae with faint setules and 6-8 short (40-70) and barbed setae on anterior sensillar area, inserted anterior to sensilla (Fig. 13B). Anal pore positioned ventrally near genital pore (Fig. 13C).

Genital pore elongated, bearing 25 setae on each side of the external border. Internally, the operculum is composed by two, anteriorly articulated, longitudinal sclerites of the ejaculatory complex each of which has 11 eugenital setae over protuberances on each side (black arrow on Fig. 13C). Tips of proximal arms of ejaculatory complex distinguishable and indicated on Fig. 13C.

Gnathosoma conical (Fig 13, E and F), bearing two stylet-like cheliceral blades. Palps bearing numerous barbed setae with the same shape, palp tibial claw entire; length similar to that of the cylindrical palp tarsus (Fig 13, E and F); tarsus with numerous distal eupathidia (Fig. 13H).

Relative legs lengths: I>IV>III>II. Leg scobalae, numerous, pointed and barbed; *tr* present on tarsus (Fig. 13G), tibia, and genu I. Tibia and genu I each bearing one piriform micro seta (k1 and k2) (Fig. 13D and G).

Remarks

Leptus Latreille, 1796 is a diverse and widespread genus that occurs on all continents except Antarctica. To date, twelve species have been described from Brazil, 31 species from the Neotropical region, and more than 270 worldwide (Šundić et al. 2017; Haitlinger et al. 2016, 2017; Mąkol & Wohltmann, 2012). Its larvae have been recorded as parasites of a vast number of terrestrial arthropods including eleven extant orders of Arachnida and numerous insect orders (Pereira et al. 2012). It is

classified in two subgenus *Leptus Leptus* Latreille, 1796 and *Leptus Amaroptus* Haitlinger, 2000, the species described herewith is classified in the subgenus *Leptus*.

Leptus sp. nov. may be set apart from all other *Leptus* (*Leptus*) by the presence of two palpfemorala, two palpgenuala and nine setae between coxae II and III. *Leptus sp. nov.* is similar to *Leptus maldonadoicus* Haitlinger, 2000, which shares the same palp chaetotaxy, but it differs of *Le. maldonadoicus* by the presence of nine setae between coxae II and III (vs. six in *Le. maldonadoicus*), by lacking a solenidion on genu III (vs. present in *Le. maldonadoicus*) and due to fn Ti 15-15-15 (vs. 12-12-14 in *Le. maldonadoicus*).

Concerning post-larval instars, *Le. sp. nov.* differs from *Leptus (L.) calvescens* (Berlese, 1888), the unique species know from post larval instars in South America, due to the crista restricted to the anterior half of the idiosoma and its length (IL/L= 2.75-4.21 vs. 1.92 in *Le. calvescens*). *Leptus sp. nov.* deutonymphs differ from other *Leptus* spp by its reduced eyes. Its idiosomal length by the eye cornea diameter ratio (IL/ECO) is 44, while data about the IL/ECO ratio made available by Southcott (1992, 1999) for *Leptus flindersi* Southcott, 1999, *Leptus calcar* Southcott, 1999, *Leptus charon* Southcott, 1992, *Leptus smithi* Southcott, 1999, *Leptus calix* Southcott 1992, and *Leptus ghiradellae* Southcott 1992 ranged from 22 to 29. The same is observed in adults (e.g. Figs. 13B, 11B), with *Le.* adults with IL/ECO ranging from 56 to 87. However, a numeric comparison with described species is precluded by the absence of these measurements in most descriptions.

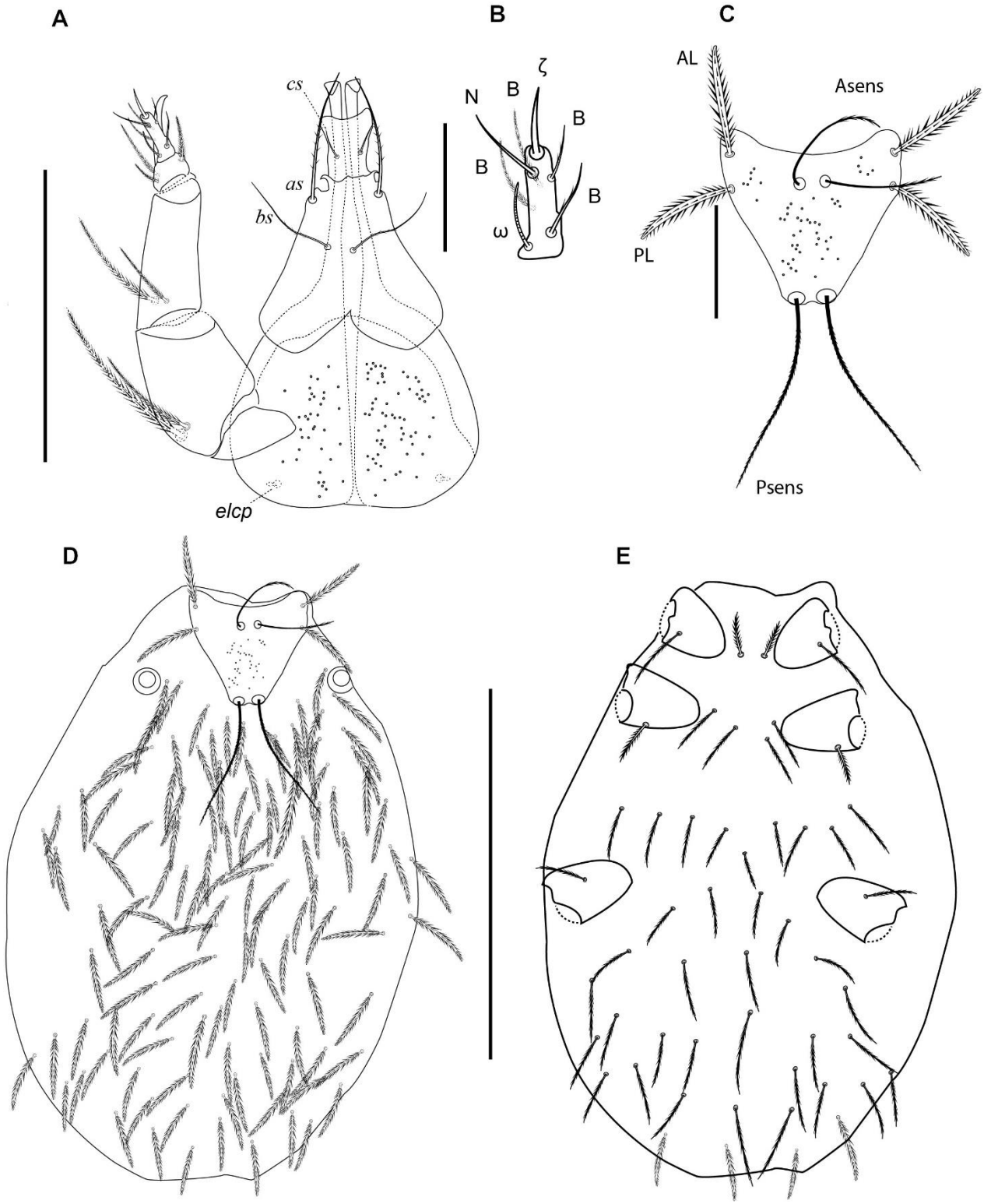

Figure 8 – *Leptus* sp. nov. larva. A: Gnathosoma ventral view; B: Palp-tarsus; C: Dorsal scutum; D: Idiosoma dorsal view; E: Idiosoma ventral view. Scale: A= 100, B= 20, C= 50; D and E= 200.

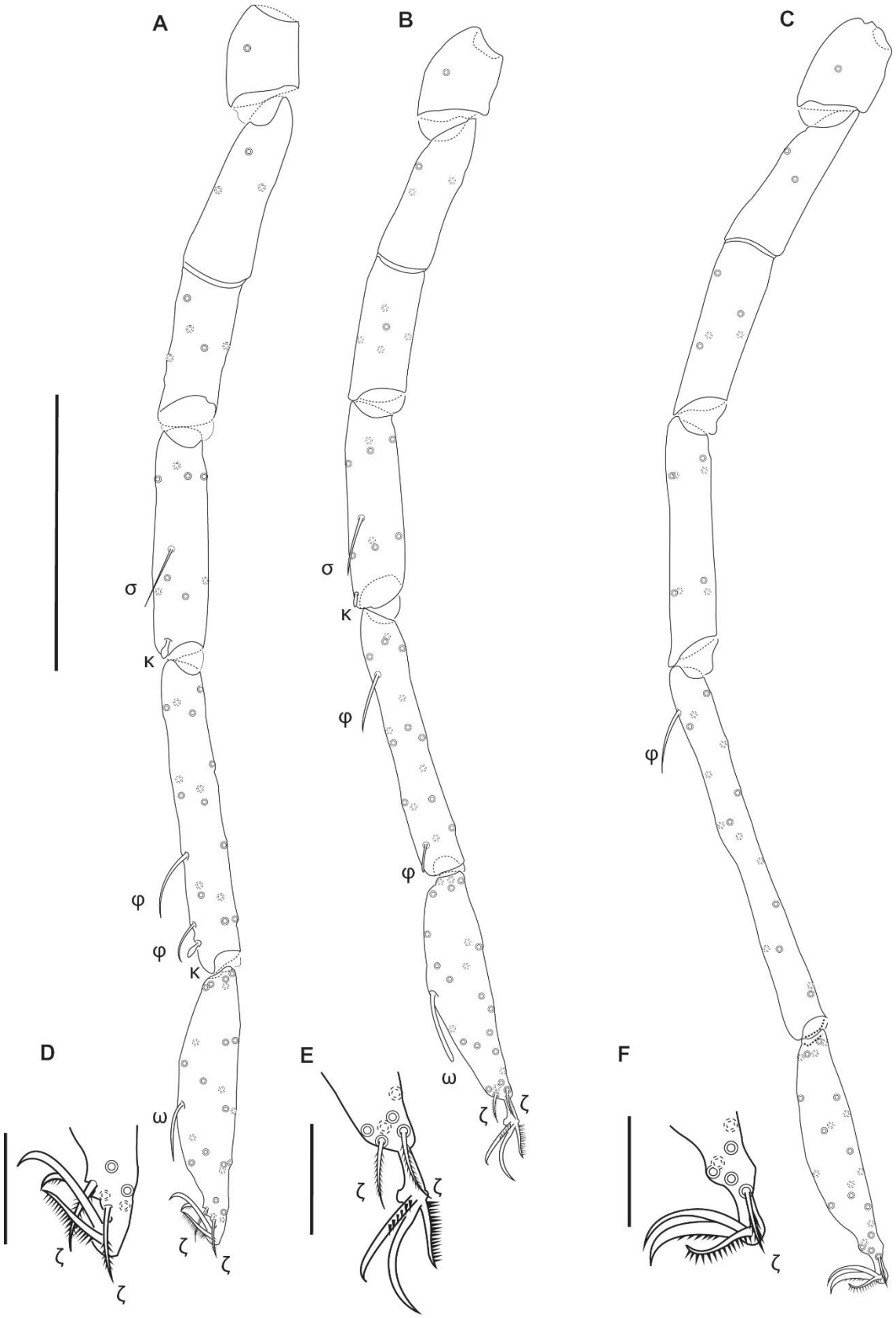

Figure 9 – *Leptus* sp. nov. larva. A: Leg I; B: Leg II; C: Leg III; D: Distal portion of tarsus I; E: Distal portion of tarsus II; F: Distal portion of tarsus III. Scale: A, B, C= 100; D, E and F = 20.

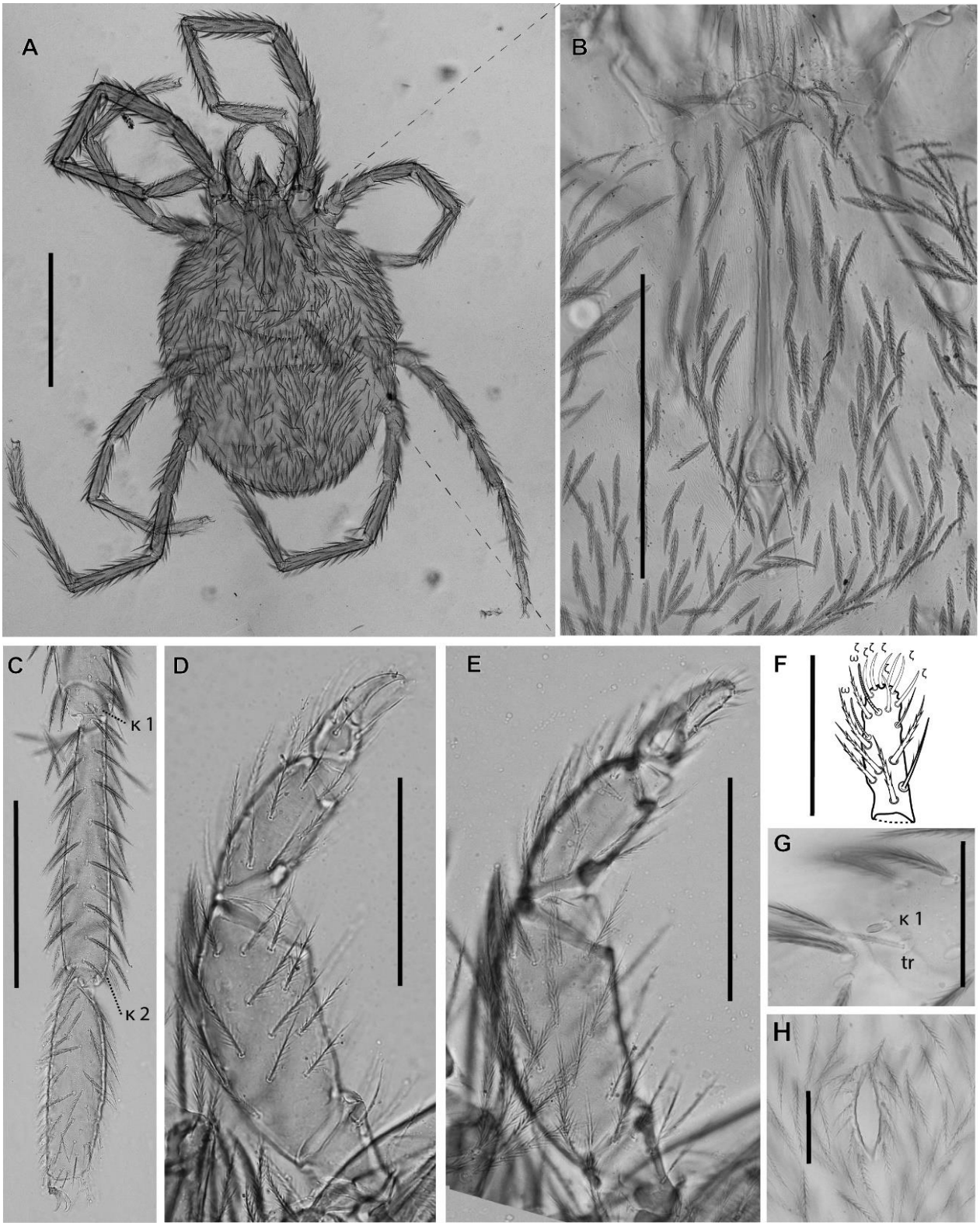

Figure 10 – *Leptus* sp. nov. deutonymph. A: General view; B: Crista metoptica, eyes and dorsal setae; C: Leg I, tibia and tarsus, relative position of micro setae k1 and k2 indicated; D: Palp dorsal view; E: Palp ventral view; F: Palp-tarsus; G: Piriform micro setae k1; H= Anal pore. Scale: A= 400, B and C= 200; D and E= 100; F, G and H= 50.

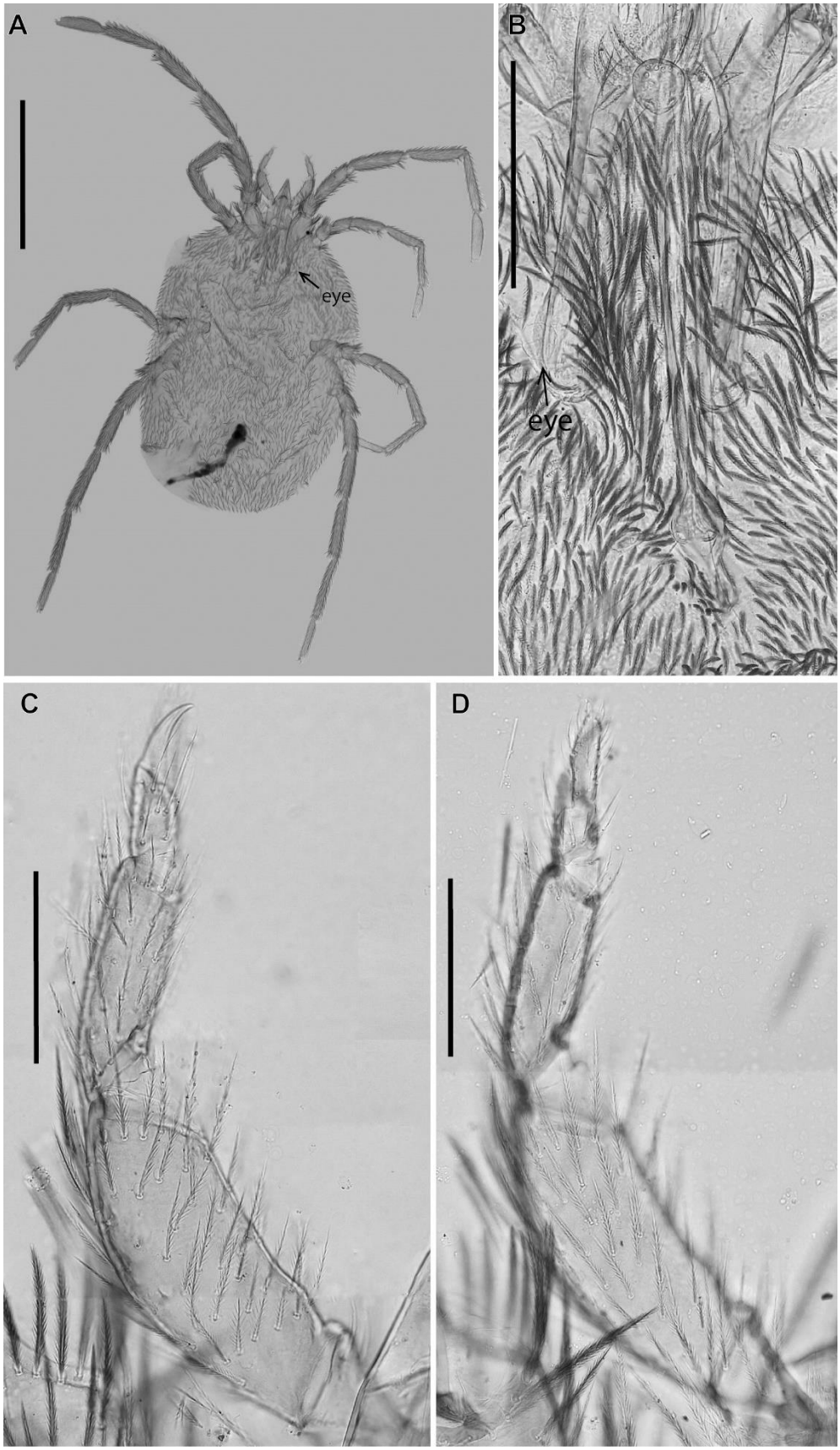

Figure 11 – *Leptus* sp. nov. female. A: General view; B: Crista metoptica, eyes and dorsal setae; C:Palp dorsal view; D: Palp ventral view. Scale: A= 1000, B= 200, C and D= 100.

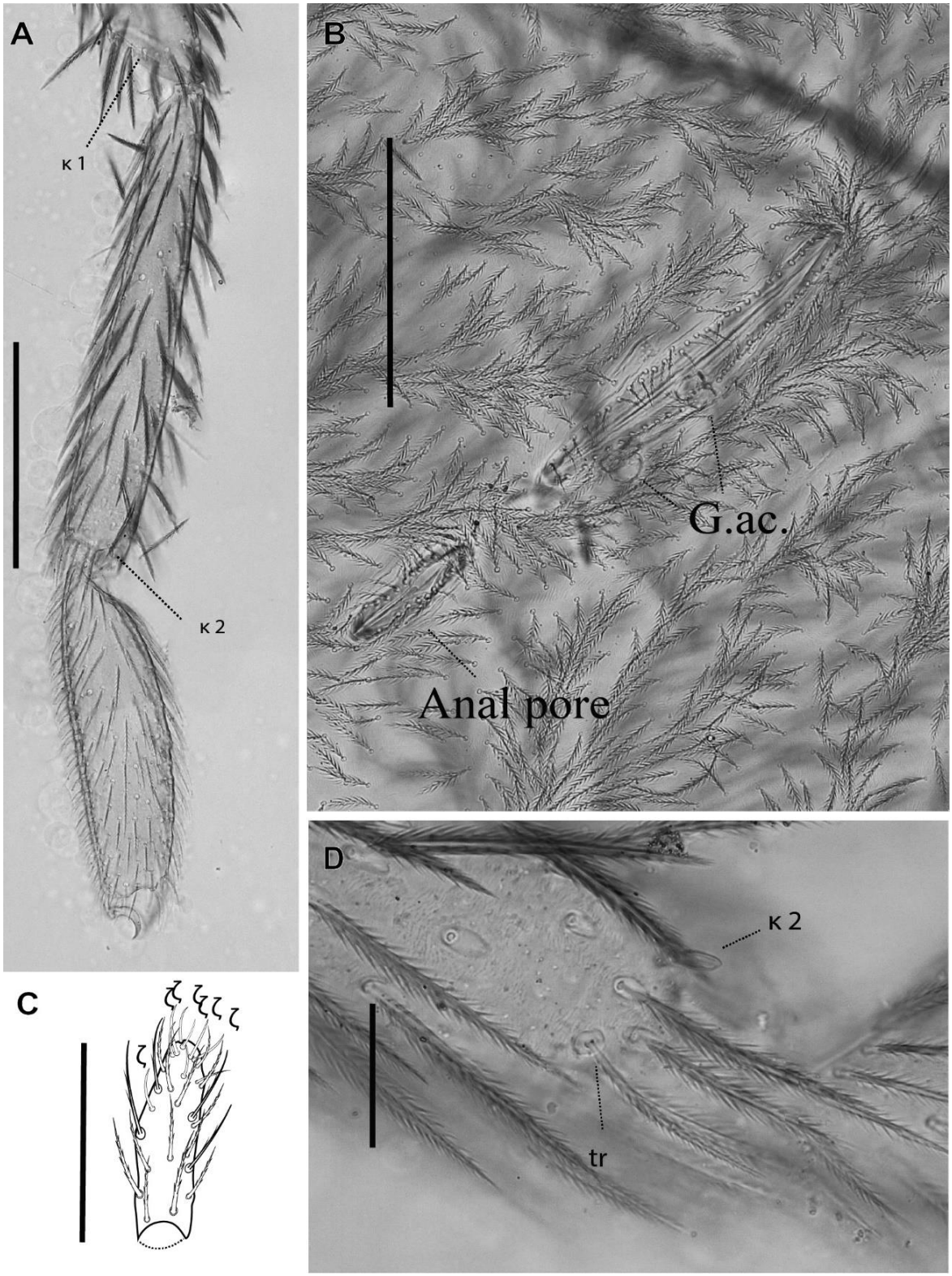

Figure 12 – *Leptus* sp. nov. female. A: tarsus and tibia I; B: Ventral view showing the anal and genital pore; : Palp tarsus dorsal view; D: Palp ventral view. Scale: A and B= 200, C= 50 and D= 20.

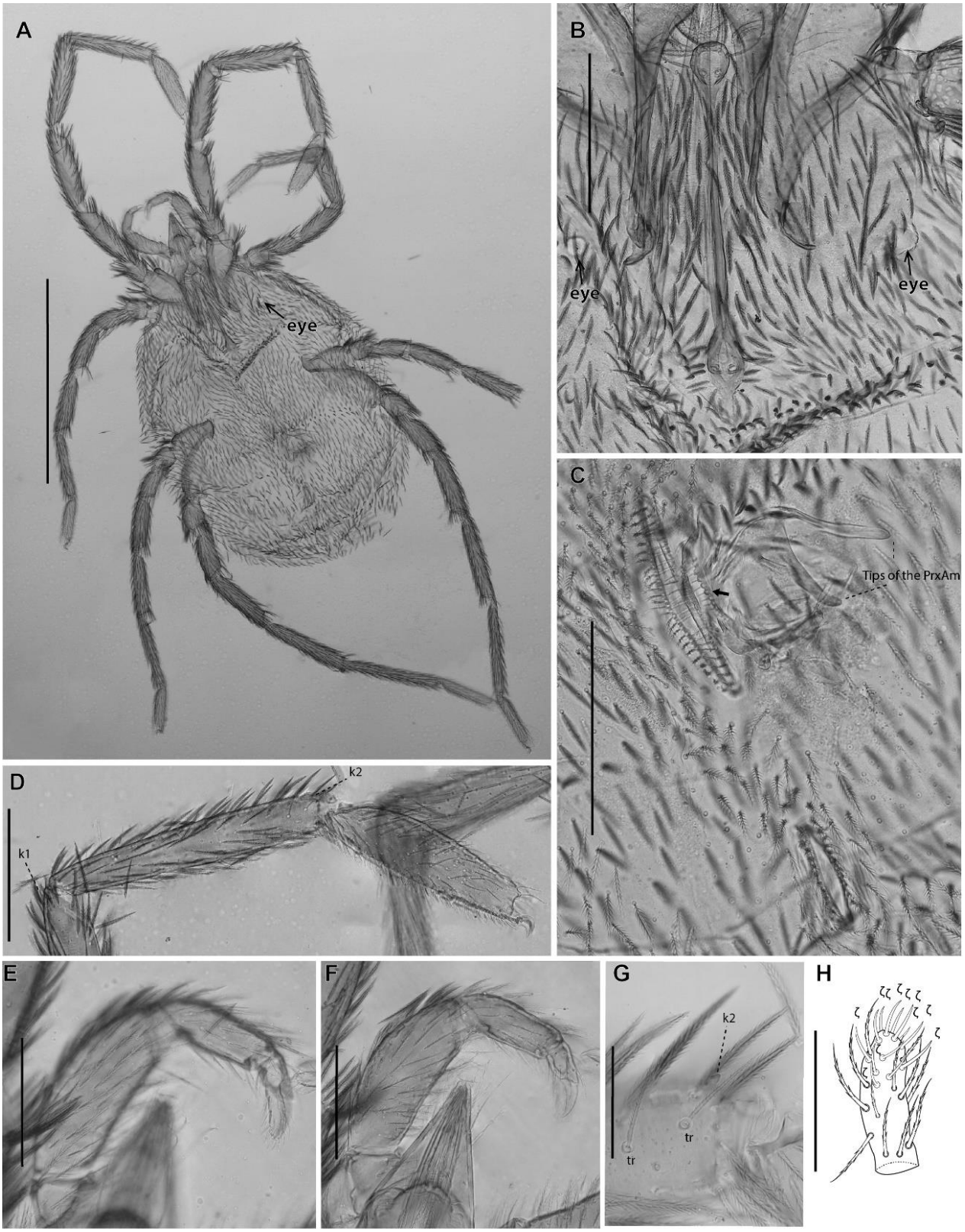

Figure 13 – *Leptus* sp. nov. male. A: General view; B: Crista metoptica, eyes and dorsal setae; C: Genital and anal pore, black arrow pointing internal setae of the ejaculatory comples; D: Leg I, tibia and tarsus, relative position of micro setae k1 and k2 indicated; E: Gnathosoma ventral view; F: Gnathosoma dorsal view; G: Piriform micro setae k2 and trichobothrial setae (tr); H= Palp tarsus. Scale: A= 400, B and C= 200; D and E= 100; F, G and H= 50.

Table V - Metric data for the males of *Leptus* sp. nov. and *Charletonia rocciai* Treat & Flechtmann, 1979. Some of the metric characters are not applicable for all species and are marked by (NA).

Character	*Le.* sp. nov.				*C. roccia*
	Mean (n=3)	Range			Value (n=1)
IL	1431.0	1322.0	-	1540.0	1845.0
ILCM	1775.0	1685.0	-	1865.0	2037.0
IW	1020.5	793.0	-	1248.0	1337.0
Sba	20.7	19.0	-	22.0	18.9
Sbp	24.0	22.0	-	25.0	19.1
Asens	110.5	109.0	-	112.0	85.0
Psens	127.0	97.0	-	157.0	117.0
ISD	402.7	370.0	-	427.0	375.6
Scutalae	55.3	47.0	-	74.0	76.7-161.4
LDS	43.7	34.0	-	54.0	54 -123
SDS	NA	NA	-	NA	28-46
LVS	39.0	25.0	-	45.0	89-146
SVS	NA	NA	-	NA	30-38
ACW	52.0	49.0	-	58.0	104.3
PCW	48.0	45.0	-	50.0	61.7
L	526.0	474.0	-	560.0	581.0
ECO	27.5	26.0	-	29.0	55.1
Eye Ring	38.0	38.0	-	38.0	76.4
EC-EC	392.0	370.0	-	414.0	489.7
EC-Asens	221.5	212.0	-	231.0	277.0
Cx I	198.3	188.0	-	211.0	403.0
Tr I	128.0	118.0	-	137.0	245.6
BFe I	354.0	305.0	-	411.0	454.0
TFe I	447.0	408.0	-	492.0	655.1
Ge I	484.3	451.0	-	537.0	574.6
Ti I	486.7	449.0	-	536.0	622.1
Ta I (L) (with claw)	383.0	366.0	-	414.0	464.2
Ta I (W)	87.3	82.0	-	95.0	193.1
Leg I (without Cx)	2283.0	2097.0	-	2519.0	3084.8
Cx II	286.3	265.0	-	302.0	407.7
Tr II	121.0	111.0	-	133.0	189.6
Bfe II	159.0	143.0	-	168.0	154.3

Table V – Continuation.

Character	*Le.* sp. nov.		*C. rocciai*
	Mean (n=3)	Range	Value (n=1)
Tfe II	234.7	249.0 - 296.0	329.5
Ge II	321.7	296.0 - 360.0	307.3
Ti II	345.0	321.0 - 379.0	334.6
Ta II (L)	217.7	209.0 - 232.0	269.6
Ta II (W)	49.7	46.0 - 52.0	136.9
Leg II	1399.0	1329.0 - 1554.0	1586.7
Cx III	194.7	171.0 - 226.0	359.7
Tr III	117.3	110.0 - 124.0	175.3
Bfe III	154.7	140.0 - 171.0	191.2
Tfe III	275.7	255.0 - 309.0	425.2
Ge III	342.7	318.0 - 378.0	412.6
Ti III	381.0	342.0 - 438.0	418.9
Ta III (L)	218.3	204.0 - 232.0	295.5
Ta III (W)	48.7	48.0 - 50.0	119.5
Leg III	1489.7	1369.0 - 1646.0	1934.5
Cx IV	365.3	348.0 - 388.0	506.0
Tr IV	156.0	127.0 - 174.0	207.6
Bfe IV	243.7	220.0 - 279.0	318.0
Tfe IV	374.3	341.0 - 407.0	767.0
Ge IV	469.7	438.0 - 513.0	646.9
Ti IV	516.7	479.0 - 579.0	640.6
Ta IV (L)	278.7	272.0 - 298.0	299.2
Ta IV (W)	44.0	43.0 - 45.0	110.8
Leg IV	2039.0	1877.0 - 2243.0	2897.0
Palp Femur (L)	206.3	195.0 - 217.0	245.7
Palp Femur (w)	82.0	75.0 - 92.0	88.5

Genus *Charletonia* Oudemans, 1910

Generic diagnosis following Southcott (1991).

***Charletonia rocciai* Treat & Flechtmann 1979**

Charletonia rocciai Treat & Flechtmann 1979: 117. *Charletonia rocciai*: Rosa & Flechtmann 1980: 215.

Rearing and behavior.

The collected Psocoptera specimens (Fig. 14A-D) were placed in humidified gypsum, coal and cement containers. In the first attempt, 38 insects, including six infected by 15 mites (prevalence 1-5 mites per insect) were kept for 10 days. In this occasion, only two mites developed to the deutonymphal instar.

In the second attempt, 26 Psocoptera specimens were collected, associated to 12 mites. In this occasion, insects were kept alive for 18 days and six mites reached the deutonymphal instar. Engorged larvae released from their hosts, seeking for shelter on cracks in the substrate and then remained motionless, becoming calyptostases, and remained as so for at least two days. After emerging, deutonymphs were preserved in Alcohol 100%.

Larvae swapped among hosts during the experiment. Three of the infested Psocoptera were marked with a white water-based ink (gouache paint) when bearing a single mite each and observed for five days. During this period, the number of mites per marked host counted from one to four mites in two, zero to three in the third specimen. The maximum number of mites per Psocopteran specimen observed, either on field or culture, was five mites per host.

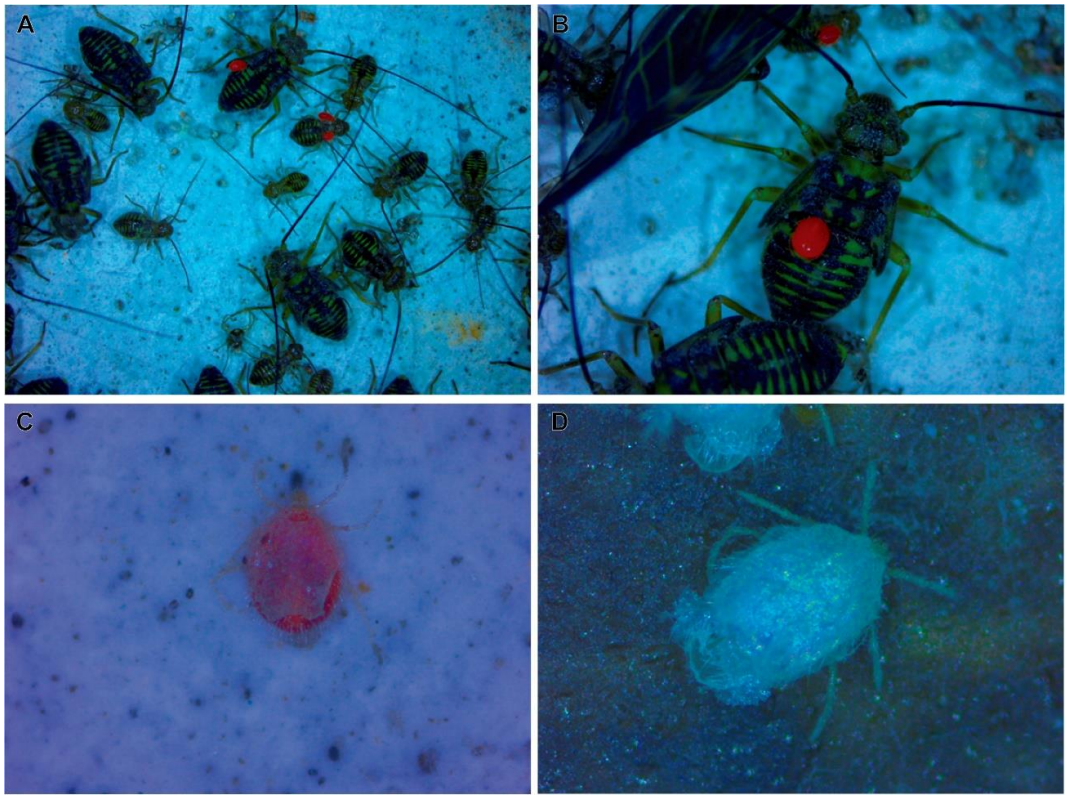

Figure 14 – *Charletonia rocciai* Treat & Flechtmann, 1979. A: Culture general view with Pscoptera host and ectoparasitic larvae; B: *C. rocciai* larvae attached to immature Psocoptera; C: Calyptostatic protonymph; D: Protonymph exoskeleton.

Description of new records.

In the present study *Charletonia rocciai* is recorded for five new localities, it is distributed across the southeast and south of Brazil as is illustrated in figure 15.

Figure 15 – Distribution of *Charletonia rocciai* Treat & Flechtmann, 1979 in the south east and south of Brazil, including five new records in addition to the type locality. Grey shades over the continental region indicate vegetation.

Diferential diagnosis.

Larvae: Four setae between coxae II and III; solenidia on distal half of Ge I; fnGe 12,12,12; leg I/leg II 0.98-1.06; fnTi 18,19,19; Ti III 153-164 long; two soledia on the Ti II; Ta I 123-136; Ge II with one micro setae (κ); Asens 42-49 µm; Psens 56-72 µm; PW/AW 1.4-1.5; Sba= 8.8-10.7; Sbp= 18-18,6. *Deutonymph*: Numerous dorsal setae with setules, abrupt terminal tapering end and 2 shapes (fine and short, long and blade-like); dorsal setae lenght (DS) 34-89; Leg IV>I>III>II; Vestigiala: GeI 1κ, TiI 3κ, TaI 1κ and 1ε, GeII and TiII 1κ (Fig. 17C, 18A-E); palp tibia with one long and lonely setae S (Fig.18F). *Adults:* Rounded tarsi palp; eyes positioned posteriorly to the middle of the crista, absence of concavity in the ventral border of palp femur; weakly barbed dorsal setae 24-130; absence of idiosomal hairy areola around the crista; crista metopica elongates beyond the posterior sensillar area (Fig. 19F); absence of cross impressions; dorsally punctate palp-femur with large points (Fig. 19D); palp tibia with one long and lonely setae S, as in the deutonymph (Fig. 18, F); relative length of the male's legs I>IV>III>II; male's GeI, TiII and GeII with 1κ, TiI with 3 κ; male's tarsi II more squarish (width/length= 0.51) than Ta I, III and IV (0.42, 0.40, 0.37, respectively).

Material examined: *Charletonia rocciai* specimens were obtained from five localities across southeastern and south Brazil. The CCT-UFMG AC 1301036, 1301034, 151437 and 151442 found over Psocoptera hosts, collected at 21°13'36.1" S, 44°58'50.5" W in *pau-ferro* trees (*Libidibia ferrea*

(Mart. ex Tul.) Queiroz 2009) on the campus of the University Federal de Lavras, Lavras, MG, in July 2013 and September 2015. In this same locallity a few more specimens were collected in July 2013: A female (CCT-UFMG AC 1300421) found on the leaf litter and other four larvae (CCT-UFMG AC 1300612, 1300613, 1301037 and 1301038) found over Psocoptera and reared up to deutonymph. Nine of the Psocopteran hosts are deposited in the *Centro de Coleções Taxonômicas* at the *Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais*, Brazil (CCT-UFMG) under the identification numbers (IPS 1700000 to 1700009). One deutonymph (CCT-UFMG AC 171474) was collected at 19°13'16.7" S, 43°23'39.1" W in a cave in an iron ore terrain between January 16th to 26th of 2017. One larva (CCT-UFMG AC 1400806), collected at 25°22'54.1" S, 49°12'11.9" W in the Atuba Park, Paraná state, Brazil, on February 12th, 2013 parasitizing *Auchmerina limbatipennis* Enderlein 1918 (Psyllidae), determined by D. Burckhardt and D. L. Queiroz but subsequently lost. One larvae parasiting an unidentified insect were collected at 20°04'00.8" S, 44°00'08.6" W in the Parque Estadual Serra do Rola Moça, near to Belo Horizonte, MG, Brazil. At last, one male (CCT-UFMG AC 1301003) were collected at 23°14'30.8"S 46°56'04.9"W in the Serra do Japi, Jundiaí municipality, São Paulo State, Brazil. In addition, a larval specimen and a deutonymph deposited at OSAL (OSAL 114444 and 114509, respectively) studied by Rosa & Flechtmann (1980) and the holotype deposited at ESALQ with no identification number were examinated.

Notes about the larval instar.

Measurements for our larvae in Table II. No significant morphological differences were observed between our specimen and the holotype. However, we could notice a few details unreported in the original description (Treat & Flechtmann, 1979). Larvae has one distal microseta present on genu II (Fig. 16), visible on only one side of the holotype and missing in the original description. Additionally, our specimens have fnTi: 18-19-19 (vs. 18-19-18 in the original description and 18-19-19 is observeable in only one side of the holotype). Complete illustrations of newly collected larvae are presented in Supplementary Material (Figs. S1-S3), as well additional images of the type material.

Deutonymph descripton.

Measurements summarized in Table III. Color in life red. Crista without a surrounding sclerite; two pairs of filiform sensillary setae with faint distal setules; 2-4, robust, scutelae with setules (100-143) at anterior border of crista, anteriorly to Asens (Fig. 17B). Dorsum with two types of setae, long (LDS) and short (SDS), often with setules. LDS setae with blunt tips, SDS with attenuate tips (Fig. 18G). Ventral setae (43-57) numerous, with very weak setules and few longer setae (104-111) (Fig. 18H).

Mouth cone bearing a few long setae distally in dorsal view and many setae in ventral view; hypostomal lip fimbriate. Cheliceral blades strong, straight, stylet like. Palpi robust, numerous palpal

scobalae with setules. Palp tibia with one long seta (Fig. 18F: S). Palp tibial claw strong, curved, pointed (Fig. 18F). Palp tarsus ovoid, with numerous setulose setae, and distally, many short setulous setae, brush like (Fig. 17D and E; Fig. 18F).

Relative legs lengths: Leg IV>I>III>II. Leg scobalae numerous, pointed, tapering, with slight indications of setules. Tarsi I with three distinct long setae named S1 and S2 in the proximal half and S3 in the distal half (Fig. 17, C). Similar setae are figured in drawings of *Charletonia oudemansi* (Southcott 1966) and are also present in *Charletonia cardinalis* (Koch 1837). Tarsal claws strong, smooth, falciform.

Vestigiala distribution on legs:

LegI: Cx 1κ; Ge 1κ (Fig. 18A); Ti 3κ (Fig. 18B); Ta 1κ 1ε (Fig. 18C).

LegII: Ge 1κ (Fig. 18D); Ti 1κ (Fig. 18E).

Female description.

Measurements in Table IV. Idiosoma oval, Crista without a surrounding sclerite; two pairs of sensilla; eight, robust, straight scutulae without distinct setules (116-194) in anterior region (Fig. 19F). Eyes positioned posterior to middle of crista (Fig. 19G). Numerous dorsal setae (DS), divided into two types: short, thin setae (SDS, 24-56), and robust, blade-like (LDS, 57 -130); both types with indistinct setules and rounded tips (Fig. 19G). Ventral setae (30-56) with numerous but weak setules, few longer setae (92-130). Genital pore bearing two similar pairs of genital acetabula (G.ac.) overlapping, kidney-shaped (Fig. 19B). Female anal plates lightly sclerotized, with six setae on each valve (Fig. 19B). The legs were broken and mixed in the slide during the extraction of the DNA, however it was possible to identify the tibia I and tarsus I by the vestigiala distribution (Fig. 19H). Gnathosoma and palp similar to the deutonymph (see Fig. 19E and F).

Male description.

Measurements in Table V. Idiosoma oval (Fig. 20A). Crista without a surrounding sclerite and two pairs of sensillar setae with almost imperceptible setules distally, bearing eight, robust, straight scutalae with weak setules (116-194) placed anteriard (Fig. 20C). Similar to the female regarding most characters, including idiosomal setae (Fig. 20C). There is evidence for sexual dimorphism regarding the ornamentation dorsal on palp femur, consisting in larger pits in males (black arrowhead on Fig. 20D), and faint dots in females (Fig. 19C-D). Male genital pore rounded, 441 long and 272 wide. Internally, operculum composed of two, anteriorly articulated, longitudinal sclerites of the ejaculatory complex with numerous eugenital setae over protuberances. Ejaculatory complex skeleton visible in light microscopy, distance between tips of proximal arms 331, between tips distal arms 385 (Fig. 20F).

Male anal plates lightly sclerotized, with nine setae on each valve (Fig. 20F). Anal plates with nine setae (vs six setae on females).

Leg I>IV>III>II. Leg setae numerous, pointed, tapering, with slight indications of setules. Tarsus II more squarish (width/length = 0.51) than Ta I, III and IV (width/length= 0.42, 0.40 and 0.37, respectively) (Fig. 20A and B). Tarsal claws strong, smooth, falciform.

Vestigiala distribution on legs:

LegI: Cx 1κ; Ge 1κ (Fig. 21C); Ti 3κ (Fig. 21A and B).

LegII: Ge 1κ; (Fig. 21E); Ti 1κ (Fig. 21D).

Remarks

Charletonia rocciai and *C. domawiti* Haitlinger 2004 are the only *Charletonia* species reported from Brazil. In 1974, *C. rocciai*`s type specimen was collected parasiting *Metagonistyluecm minense* Townsend, 1927 (Diptera. Tachinidae), a fly introduced as biological control in the southeast region of Brazil. Later, Rosa & Flechtmann (1980) collected numerous larvae of *C. rocciai* parasiting Acrididae (Insecta: Orthoptera), which were reared to deutonymphs identified as *Sphaerolophus* Berlese, 1910. Hence, Rosa & Flechtmann (1980) recognized *Sphaerolophus* as junior synonymous of *Charletonia* and provided a description of deutonymphs including drawings from palps, eyes, crista metopica and dorsal setae.

The results obtained from the rearing of these mites are similar to those reported by Rosa & Flechtmann (1980), except by observing for the first-time larvae swapping from a host to other. Most parasitengone larvae do not change host before turning into the calyptostatic protonymph, except for a short time after attachment, when larvae can release themselves and start searching for a new host, e.g. in case of host death or molting (Wohltmann, 2000). If the host detachment happens later, the larvae will search for shelter and undergo calyptostasis, even if they are not fully engorged (Wohltmann, 2000). In the present work we report spontaneous detachment of unengorged larvae from hosts and relatively frequent host-shifting by *Charletonia rocciai* Treat & Flechtmann, 1979 on their gregarious Psocopteran hosts.

A few differences were observed between studied larvae and the original descriptions. Mainly the number of setae on tibia III and the presence of micro setae on genu II. Those differences are likely due to minor mistakes in the arduous task of counting minute setae (Figs. S1-S3).

The deutonymphs obtained in this study by rearing or associating with DNA Barcoding match the description by Rosa & Flechtmann (1980). Compared to their congeners, *C. rocciai* deutonymphs differ from *C. cardinalis* (C. L. Koch, 1837) and *C. nishidai* (Southcott, 1991) by the presence of

dorsal idiosomal setae with setules, from *C. ishii* (Southcott, 1991) by the tarsus I/tibia I ratio (TaI/TiI: 0.65-0.75 vs. 0.48), and from *C. oudemansi* (Southcott, 1966) by the dorsal idiosomal setae with abrupt end, numerous small setules, and leg I < leg IV (vs. leg I > leg IV).

It is notable that one deutonymph collected in the entrance zone of an iron ore cave (UFMG-AC 171474) and associate by DNA barcoding is considerably bigger than those experimentally reared. Metric data per deutonymph specimen is available in the supplementary table I.

Charletonia includes 22 species for which the larval instar is unknown: *C. cavannae* (Berlese, 1885), *C. globinger* (Berlese, 1885), *C. sanctaehelenae* (Feider & Chioreanu 1977), *C. canadensis* (Smiley 1968), *C. cilissa* (Cooreman, 1955), *C. milloti* (André, 1946), *C. salti* (Evans, 1953), *C. similis* (Vitzthum, 1926), *C. cursor* (Vitzthum, 1926), *C. arborum* (Vitzthum, 1926), *C. politricha* (Berlese, 1910), *C. nuda* (Berlese, 1910), *C. goliath* (Berlese, 1910), *C. spinosa* (Berlese, 1910), *C. subnuda* (Berlese, 1910); *C. porcinus* (Hull, 1918), *C. westraliensis* (Womersley, 1934), *C. nova* (Oudemans, 1927), *C. gigas* (Khot, 1965), *C. delhiensis* (Khot, 1965), and *C. minuta* (Khot, 1965).

Adults of *C. rocciai* differ from all previously described species due to the combination of the following characters: dorsal setae length and shape; palp tarsus wider than long; eyes positioned posterior to the middle of the crista; absence of a concavity at the ventral margin of the palp femur; the absence of unusual setae patterns like the cross impressions in the dorsal side of the idiosoma described for *C. globinger* (Berlese, 1885) or the hairy areola described for *C. cursor* (Vitzthum, 1926); IW/IL ratio; presence of a prolongation of the crista metopica posteriorly to the posterior sensillar area and the leg II/ leg III ratio.

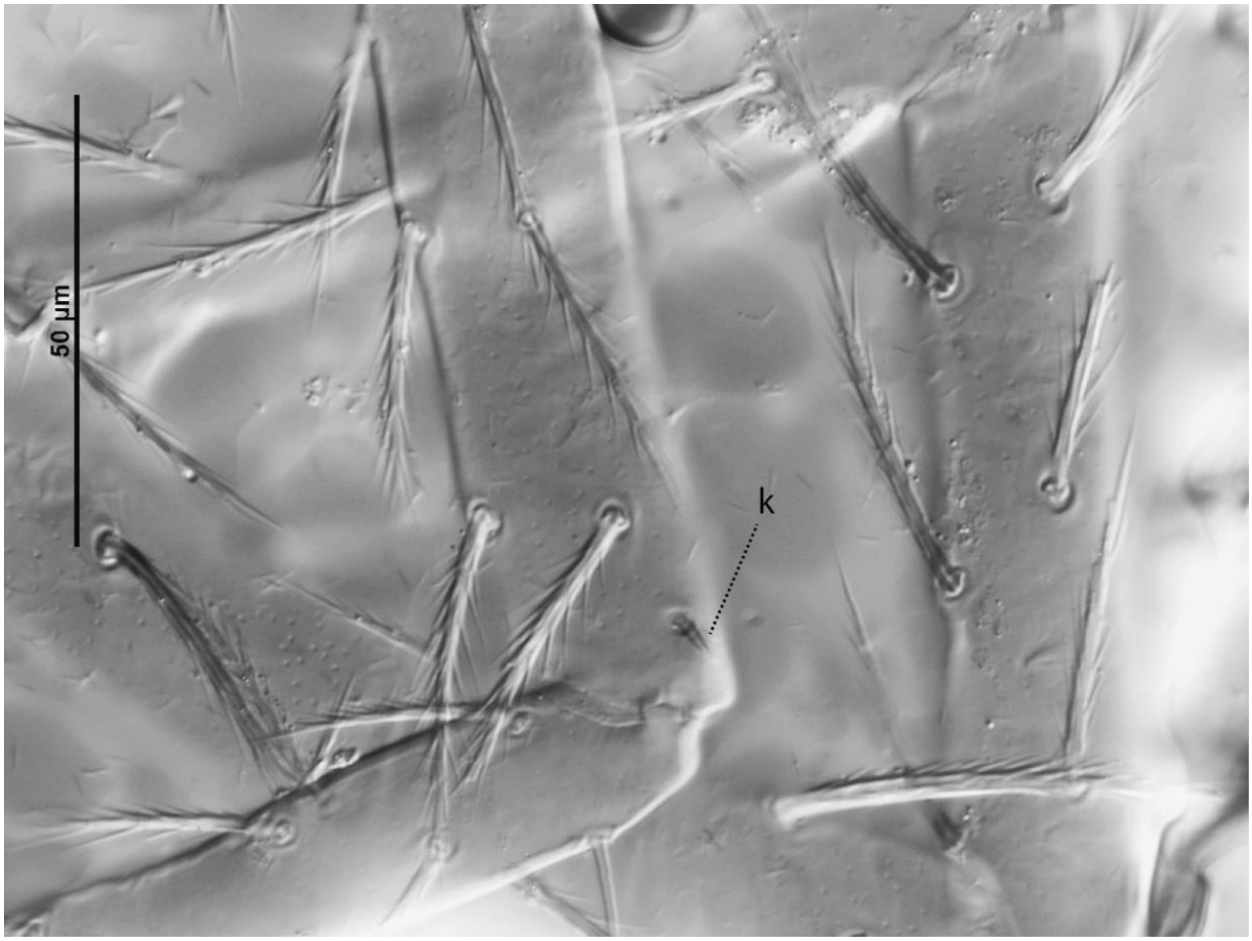

Figure 16 – *Charletonia rocciai* Treat & Flechtmann, 1979: joint between genu and tibia II, showing the presence of a micro setae on the holotype (larval instar).

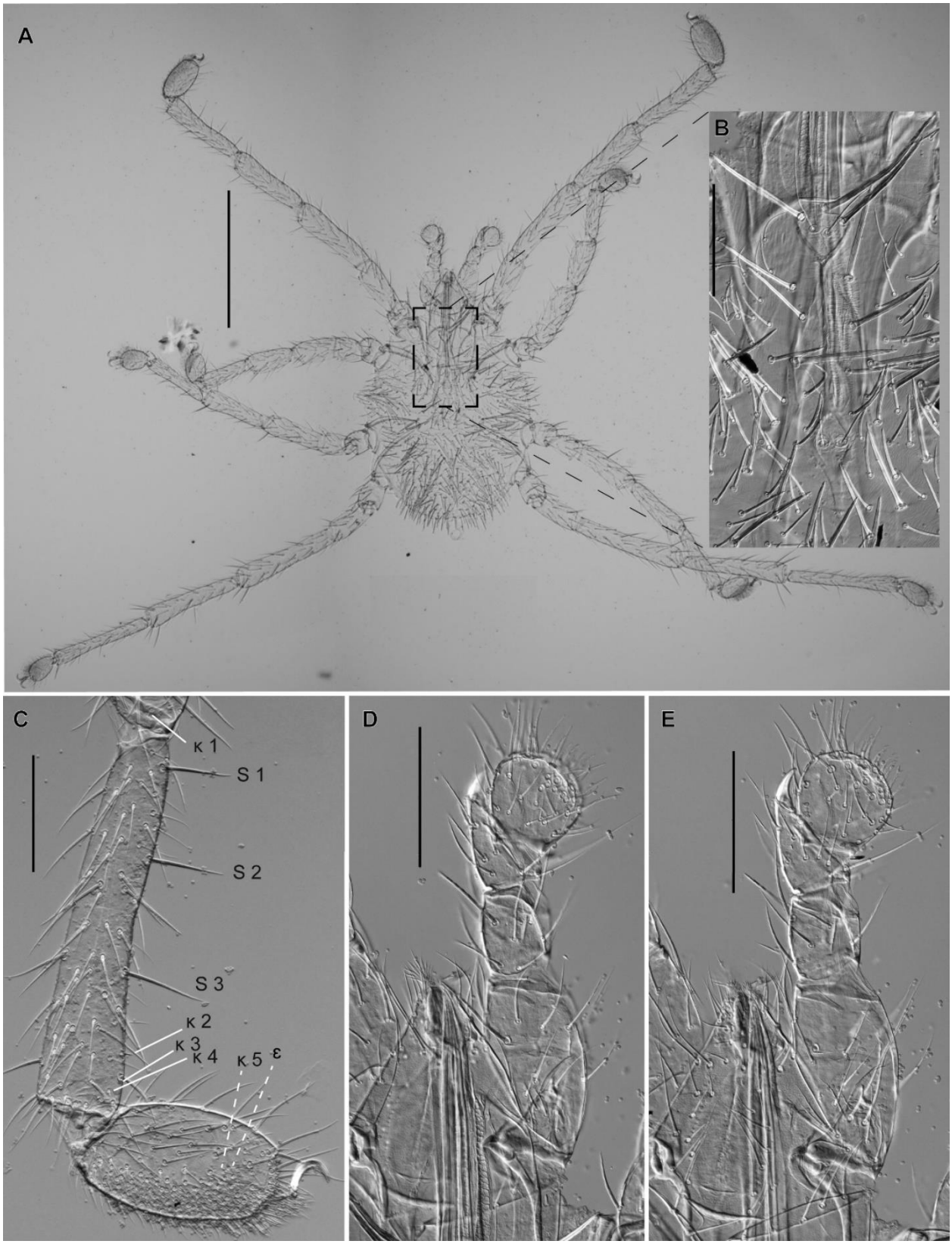

Figure 17 – *Charletonia rocciai* Treat & Flechtmann, 1979 deutonymph. A: General view. B: Detail from the dorsal part of the idiosoma showing the crista metoptica. C: Ti I and Ta I. D: Gnathosoma and palp dorsal view. E: Gnathosoma and palp ventral view. Scales: A=500; B-E= 100.

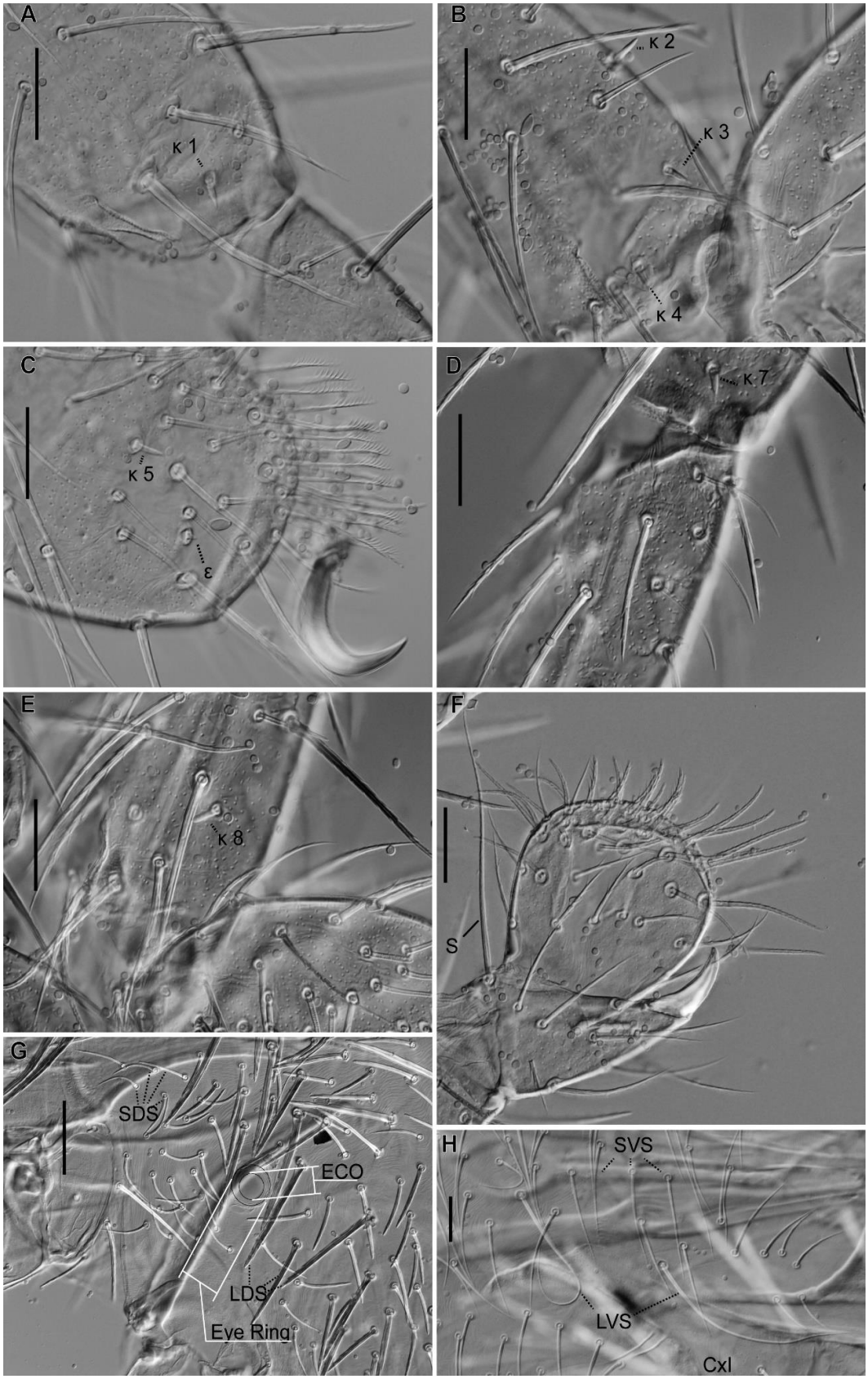

Figure 18 – *Charletonia rocciai* Treat & Flechtmann, 1979 deutonymph. A: Junction between Ge I and Ti I; B: Junction between Ti I and Ta I; C: Ta I; D: Junction between Ge II and Ti II; E: Junction between Ti II and Ta II; F: Tarsi-palp; G: Dorsal Setae; H: Ventral Setae. Scales: 25.

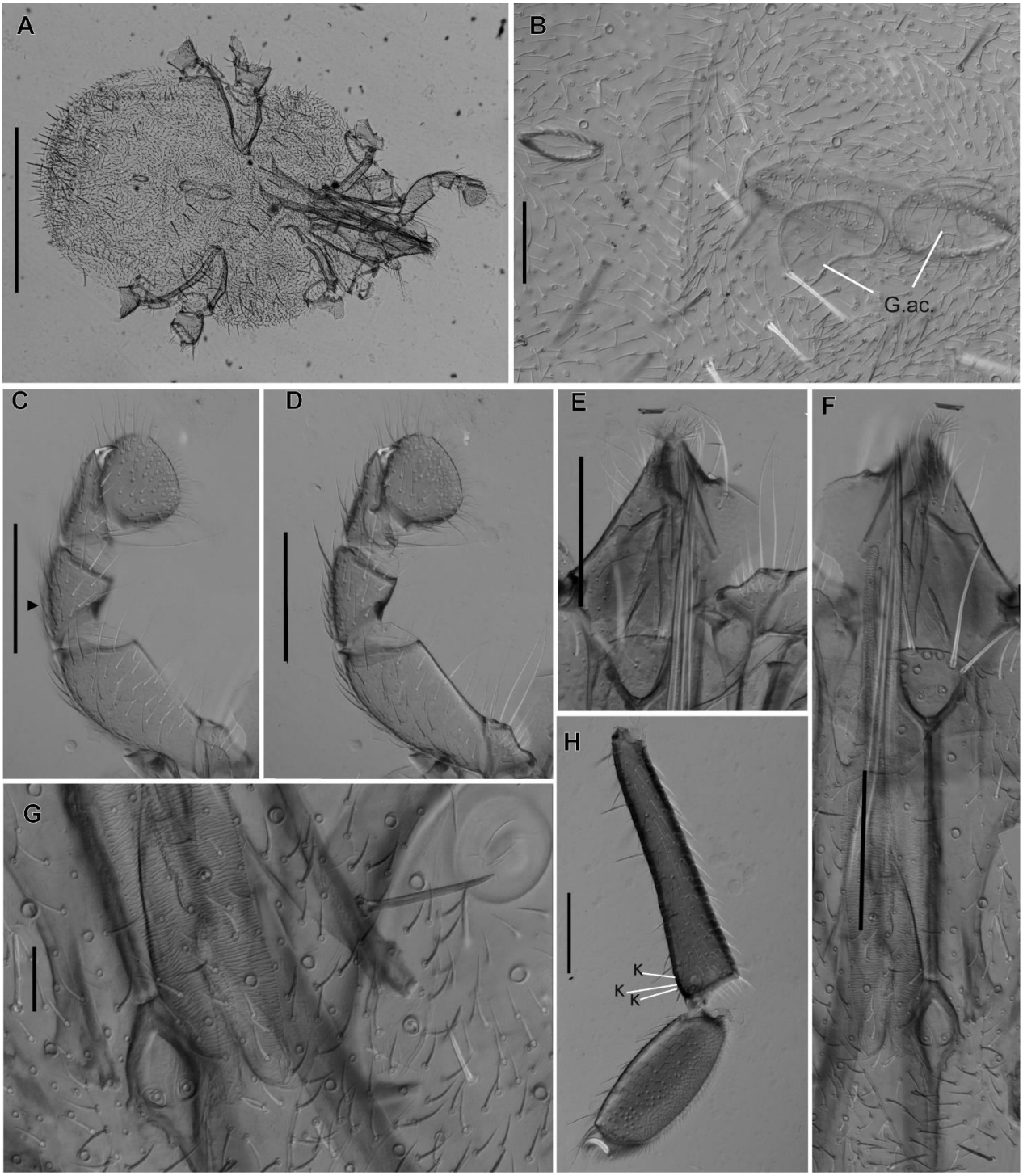

Figure 19 – *Charletonia rocciai* Treat & Flechtmann, 1979 female. A: Idiossoma general view; B: Female genital and anal pore; C: Palp dorsal view, arrow head pointing the palp genu with weak punctuations; D: Palp ventral view; E: Gnathosoma ventral view; F: Gnathosoma dorsal view and crista; G: Eye and dorsal setae; H: Ti I and Ta I, with white lines pointing the relative position of the vestigiala (k); Scale: A = 1000; B= 100; C,D,E, F and H = 200; G = 50.

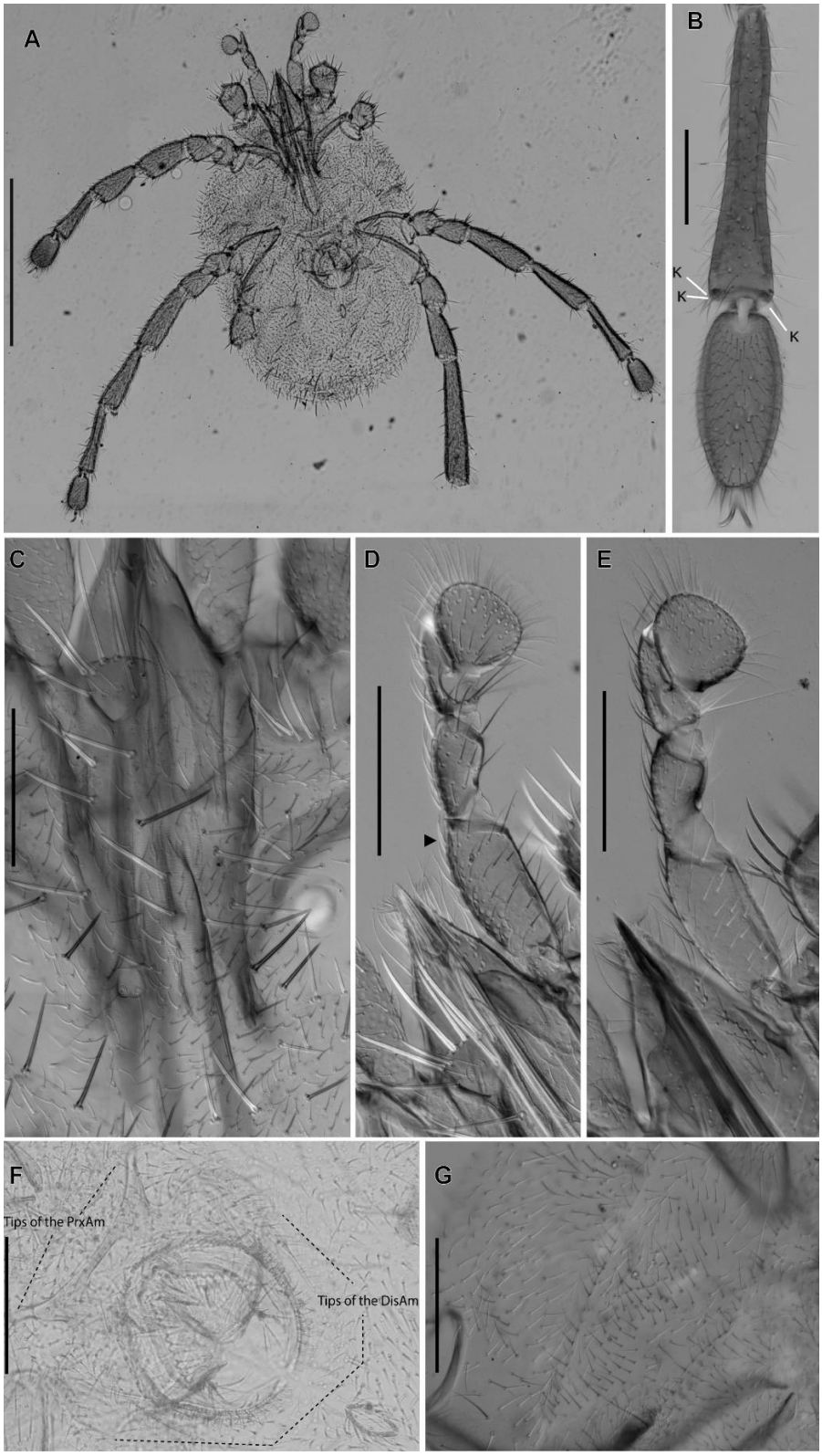

Figure 20 – *Charletonia rocciai* Treat & Flechtmann, 1979 female. A: General view; B: Ti I and Ta I, with lines pointing the relative position of the vestigiala (k), detailed in fig. 9 A and B; C: Crista metopica and eye; D: Palp and gnathosoma dorsal view, arrow head pointing the punctate palp femur; E: Palp and gnathosoma ventral view; F – Genital and anal pore, black lines indicating the tips of proximal and distal arms of the genital complex skeleton; G: Ventral setae. Scale: A = 1000; B-F = 200.

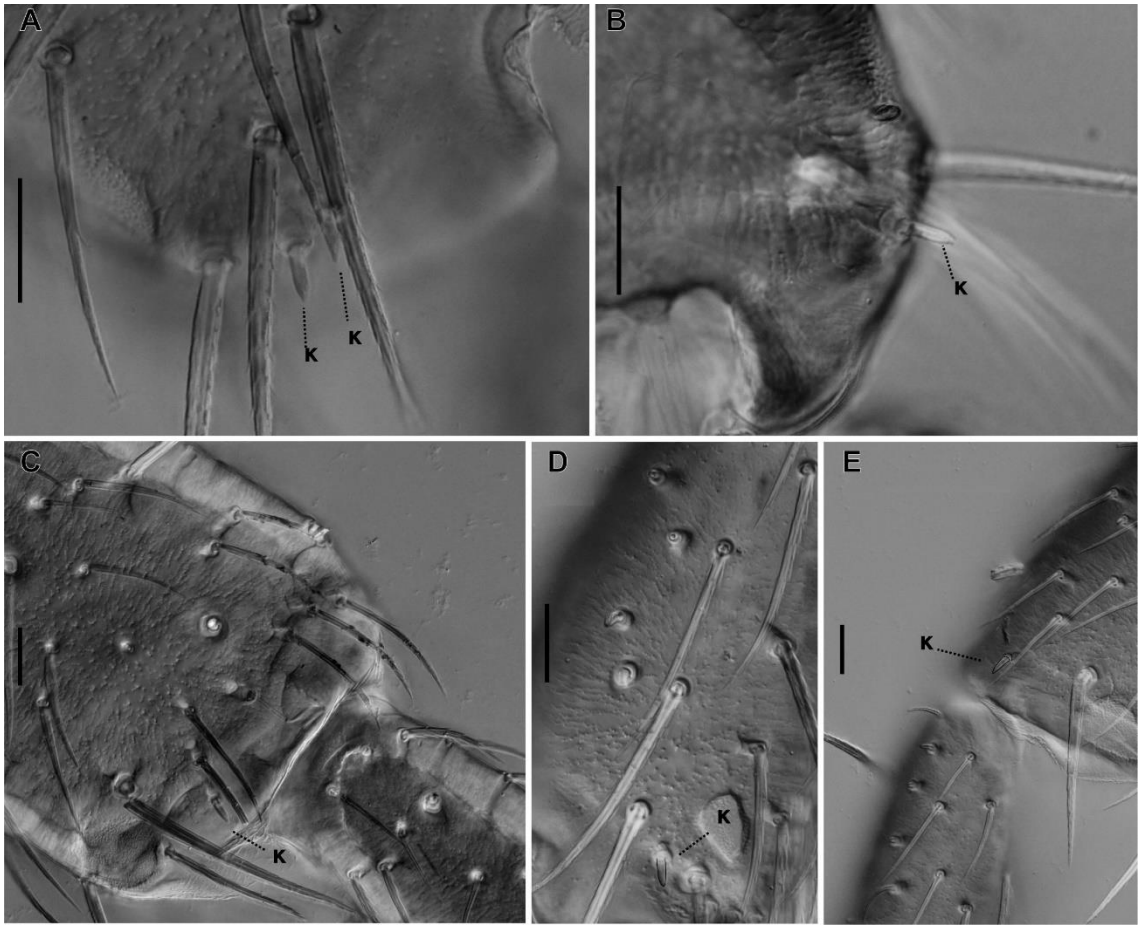

Figure 21 – *Charletonia rocciai* Treat & Flechtmann, 1979 male. A: Ti I distally side one; B: Ti I distally side two; C: Junction between Ge I and Ti I; D: Ti II distally; E: Junction between Ge II and Ti II. Scale: 10.

Discussion

Discussion

Charletonia rocciai Treat & Flechtmann, 1979 large deutonymph.

One deutonymph collected at the entrance zone of an iron ore cave (UFMG-AC 171474), and associated by bGMYC, is considerably bigger than those experimentally reared (Supplementary Table I). It has longer legs, crista, sensilla, and the anterior sensilla (Asens) is as long as the posterior sensilla (Psens) (vs. shorter than the posterior, in the reared specimens). However, due to the small sample size, we are not able to address whether these differences are correlated with the cave environment, populational or developmental traits. It is important to be aware of the possibility that the stress caused by lab conditions (e.g. temperature or host health) may have pushed incompletely fed larvae to undergo an early calyptostasis followed by the emergence of smaller deutonymphs (Wohltmann, 2000). For instance, studies on terrestrial Parasitengona have revealed that morphological abnormalities are more frequent in larvae that develop from eggs that are subject to extreme conditions (Makol, 2000 and Makol & Gabryś, 2002).

Parasitengona instar association.

Our instar association results have illustrated the value and problems of the three main ways to associate the heteromorphic instars of Parasitengona: geographic-temporal, molecular data, and rearing evidence. The first and weakest indicator consists of collecting instars at the same location and it is based on the '*methode de petit faunes*' of Grandjean (1947). For instance, sampling adults and non-engorged larvae walking freely on the leaf litter and identified as members of the same genus, constitutes a weak indication that they belong to the same species. This indication has been used before and led to correct associations of larval and postlarval instars (Grandjean, 1947), later confirmed by rearing experiments (Wohltmann, 2010). However, due to the possibility of sympatric species, association based on co-occurrence is not recommended and it has led to false associations (Wohltmann, 2010). We agree that geographic-temporal evidence by itself is inadequate for associating heteromorphic instars. However, in all associations made in the present study heteromorphic instars of the same species were collected at the same locations and at the same time. This weak indication that they belong to the same species were later confirmed by rearing and bGMYC. In conclusion, the geographic-temporal indication can be used to propose hypothesis of putative species to be tested with one or both of the following methods.

The second method is the evidence is provided by DNA barcoding (Hebert et al. 2003) and other more sophisticated DNA based species delimitation methods like the bGMYC employed in the present study. These are especially interesting due to their scalability. Given enough funds, all specimens of Parasitengona deposited in certain collections can have their DNA extracted by non-destructive methods and preserved for future instar correlation, as it is happening at CCT-UFMG AC. Alternatively, the hypothesis of correlation of heteromorphic specimens raised by geographic-temporal indication can be tested on a smaller and cheaper scale. The main limitations of this method are the cost of molecular technics and the scarcity of some instars, restricting collection and thus the option to extract DNA.

The third and strongest type of evidence is provided by rearing. It can be cheap and provides a precise association, also allowing the acquisition of rare instars. The main limitation to this technique is scalability and a low success rate for some taxa. Additionally, the stress caused by the laboratory conditions may lead to morphologically anomalous specimens (Makol, 2000 and Makol & Gabryś, 2002). Our results called our attention to this risk. The *Charletonia* deutonymph collected from nature had metric data distinct enough to suggest belonging to a species distinct from reared individuals. Hence, the description of abnormal (reared) specimens may mislead further identifications of normal specimens found in nature.

In conclusion, when working with heteromorphic instars of Parasitengona we advise special attention to the delimitation of species boundaries. Parasitengona species shouldn`t be synonymized or divided without considering their heteromorphic instars and employing adequate tests. DNA based methods or rearing can be employed alone, but they provide much better results when integrated. Special attention to geographic-temporal indication should be given in ecological studies. The heteromorphic life cycle may preclude a correct estimation of the species number and important taxa can be missed if one instar is ignored. At last, one must keep in mind that reared specimens may present morphological anomalies.

Host-shifting behavior.

Different attachment modes are correlated with the skill of Parasitengona larvae to shift between hosts before the end of the parasitic phase. The host shifting behavior observed in *Charletonia rocciai* suggests that this phenomenon may be more common that previously suspected and that gregarious habits of the Psocopteran hosts may facilitate the process of finding a new host. However, the trigger to the host shift behavior observed in our experiment wasn`t identifiable, since different instars of the host were present, the stress caused by the rearing conditions or another physiological aspect may be responsible. We speculate that the mild immunological reaction of the host to the parasitism of *Leptus* and the absence of a feeding tube (Åbro, 1987), may be present in other erythraeids and may facilitate the host-shifting behavior observed for *Charletonia*. Ultimately it may even help to explain the unusually wide range of hosts observed for the species of genera *Leptus* and *Charletonia* (Eg.: *Ch. rocciai* were found in four different insect orders). This idea arose from the fact that a feeding tube is created by the host`s immunological reaction to the water mites *Arrenurus* and *Hydrachna* infection and also in vertebrates parasited by Trombiculidae larvae (Åbro, 1979, 1984; Davids, 1973, Shatrov & Mirolubov, 2015, Pflügfelder, 1950) and the fact that losing their host while unfed lead to death in water mites (Wohltmann, 2000) making the host shift prohibitive.

The population of *Ch. rocciai* from the campus of Universidade Federal de Lavras have been observed in the same locality every year associated with Psocoptera. Our results suggest that this animal can be easily kept in culture, given its host range different hosts can be offered to the larvae and that they could be used for laboratory experiments to test hypotheses on parasite biology.

Caverniculous mites and potential troglomorphism.

Troglobiont mites, that is, mites restricted to the cave environment require special attention to be identified (Zacharda, 1980). As observed for most troglobiont vertebrate taxa (Rétaux & Casane, 2013), *Leptus sidorchukae* sp. nov. has normal eyes development in the larval instar and a strong reduction in the adults (Fig. 22A). This reduction is better illustrated considering the specimen's size, by comparing the idiosoma length with the eye cornea diameter using the ratio IL/ECO. The single deutonymph

where the eyes could be measured presented IL/ECO=44 vs. 22-29 in all six species which had the deutonymphs' eyes described by Southcott (1992, 1999), while in adults the IL/ECO ranges from 56-87. The small size of the eyes observed in adults couldn`t be numerically compared with the literature due to the lack of detailed description of the eyes, but they are considerable smaller when compared to the illustrations (Eg.: Shiba, 1976). For instance, even when compared with the eyes of a smaller (IL=1254) epigean male specimen (CCT-UFMG AC 1300406, Fig. 22B), it can be observed that *Leptus sidorchukae* sp. nov. (Fig. 22A) has considerably smaller eyes.

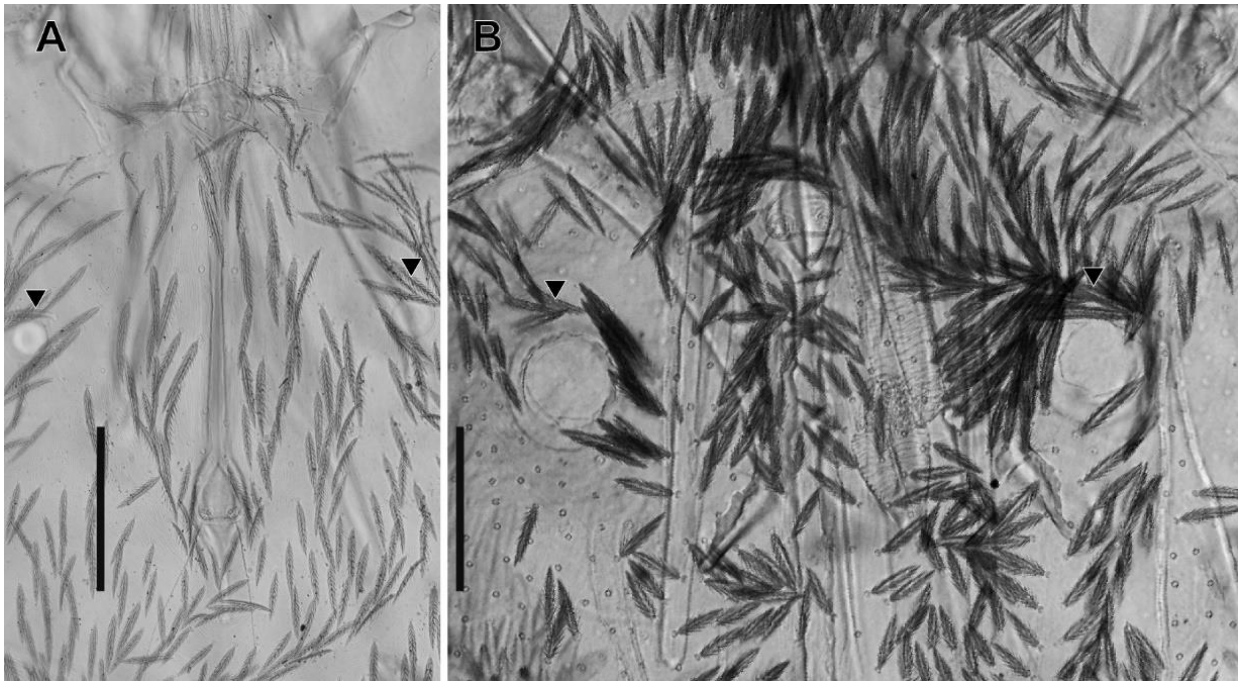

Figure 22 – *Leptus* eyes comparasion. A: *Leptus* sp. nov. male, arrow heads indicating the poorly developed eyes. B: A smaller *Leptus* sp. male CCT-UFMG AC 1300406, arrow heads pointing the bigger eyes with disernible eye cornea. Scale: A and B= 200.

Besides the eye reduction, a series of unusual characters for this genus may be correlated with the cave environment and interpreted as troglomorphisms according to Zacharda (1980). The shorter crista metopica, restricted to the anterior half of the idiosoma is one example, since less sclerotization is typical of troglomorphic mites (Zacharda, 1980). The larvae of this species have also an unusual characteristic of bearing two barbed setae on palp genu and femur; a condition shared only by a single congeneric species, *Leptus maldonadoicus*. Additionally, an enlarged micro seta is present in the distal part of the genu and the tibia of leg I, these setae are preserved during the molting process and are present in the post larval instars, with the same shape and position. It is also interesting to notice the presence of special mechanoreceptors, trichobothrial (tr) setae on the legs (Figs. 10G, 12D, 13G), but

due to the lack of detailed descriptions, this character couldn't be compared with the literature of epigean species.

Differently of *Charletonia rocciai* and *Lasioerythraeus jessicae* sp. nov., which were found only in the entrance and penumbra zones of the caves, *Leptus sidorchukae* sp. nov. including larvae, deutonymph and adults were also found in the aphotic zone. Given its wide host range (Pereira et al. 2012), *Leptus* species can found a suitable environment in a cave. There are numerous potential host species (e.g., opilionids and spiders) for the larvae and prey for the post larval instars (e.g., Colembola), allowing this species to complete the whole life cycle without leaving the cave.

Leptus sidorchukae sp. nov. was collected in caves of two distinct regions of Minas Gerais state, Brazil. Most of the samples came from numerous unnamed caves (Table I) in a limestone terrain in Pedro Leopoldo municipality and other four from Salitre`s cave, Diamantina municipality, Minas Gerais state. One may argue that the approximately 222 kilometers distance between the two municipalities where *Leptus sidorchukae* sp. nov. where collected (Table I) indicates that its distribution is not restricted to the caves, occurring in the epigean realm between these two localities. However, a wide distribution is already reported for troglobiont spiders (Snowman et al. 2010) which have a genetic structure observed between distinct cave populations like the observed in populations from different islands. The migrations of the cave-obligate spiders between distant caves or terrestrial species between distant islands, although rare still occur. In addition, it is known that the parasitic larvae of Parasitengona can act as a dispersal form by parasitizing flying insects (Wohltmann, 2001). Based on those previous studies, we speculate that the Parasitengona larvae dispersal skill also allow the eventual and lucky dispersion of *L. sidorchukae* sp. nov. to other suitable cave environments over trogloxene hosts.

Instead of a wide distribution, the isolation of the two populations from Diamantina and Pedro Leopoldo municipality is also supported by the absence of shared haplotypes (Fig. 1B) between the two locations, suggests that their dispersal is a rare event as expected for relatively isolated populations of cave-dwelling populations. This haplotype distribution contrasts with the one observed for the epigean and widely distributed *Charletonia rocciai*. Despite being apart to each other by more than 600 Km and having a much smaller sample size, a larva (CCT-UFMG AC 1400806) from Paraná state in the south Brazil, a female (CCT-UFMG AC 1300421) from Lavras municipality, Minas Gerais State, and a larva (CCT-UFMG AC 1300423) from the *P.E. da Serra do Rola Moça*, Minas Gerais State share the same haplotype. Additionally, despite numerous Brazilian *Leptus* deposited in our collection, including the seven different species used as out-groups, being examined, this species where never found in an

epigean environment. Contrasting with dozens collected during environmental impact studies on caves (Table I).

Considering the morphological data, distribution, genetic, and ecological data we conclude that *Leptus sidorchukae* sp. nov. is a candidate for a troglobiont (Sket, 2008) species and to the best of our knowledge, the first among *Leptus*.

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Supplementary material

Supplementary table I – *Charletonia rocciai* Treat & Flechtmann, 1979 measurements of the deutonymph per specimen. The number in first row is the identification number of the specimens. *Heteromorphic (bigger) specimen from cave.

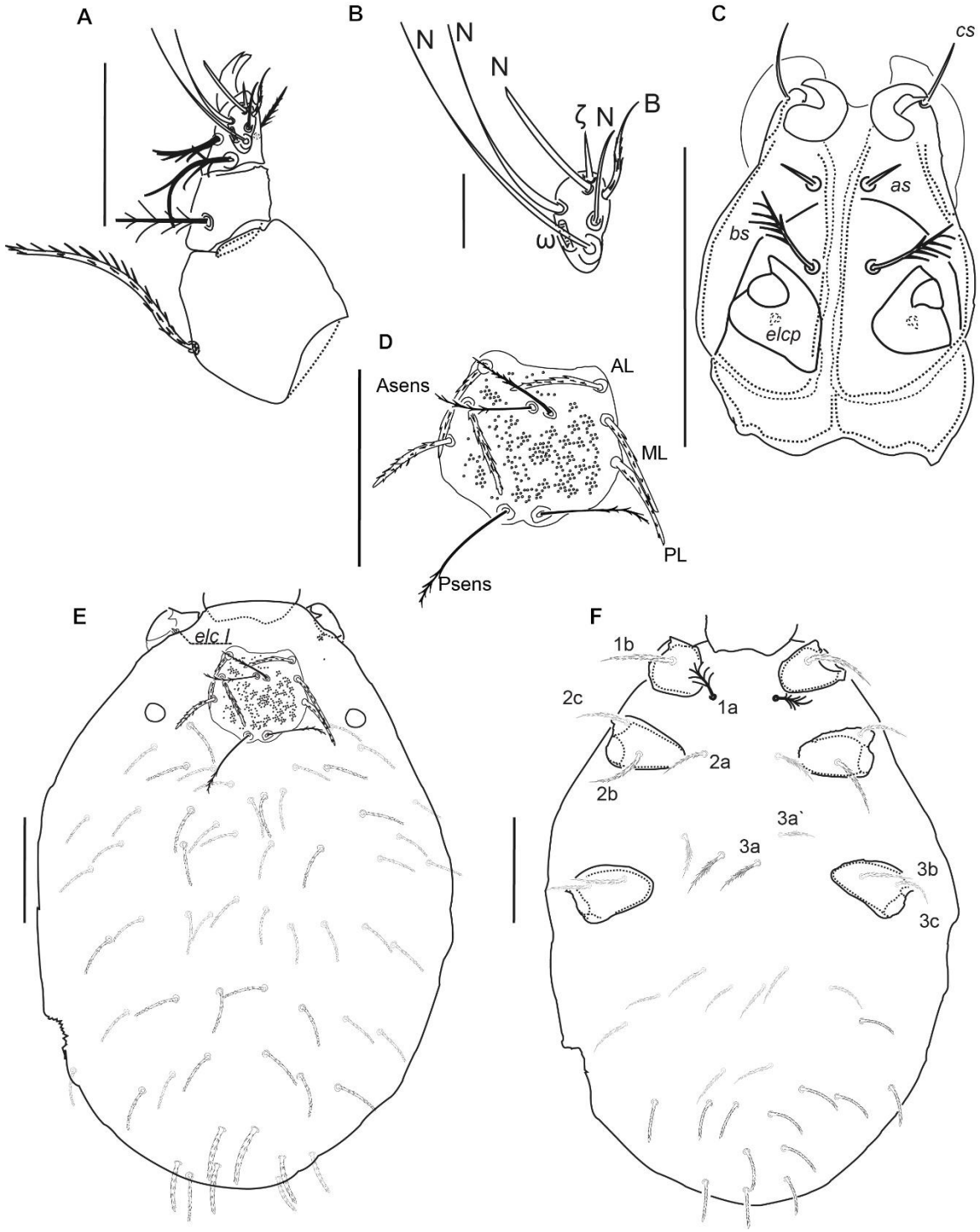
Character	CCT-UFMG AC					Range	
	1301037	1301038	1300612	1300613	171474*		
IL	668	697	583	534	834.00	534	- 697
ILCM	840	916	669	633	968.00	633	- 916
IW	542	602	427	429	652.63	427	- 602
Sba	14.78	13.4	15	22	16.95	15	- 22
Sbp	17.47	16.5	16	15	19.49	15	- 16
ASE	69	78	65	61	117	61	- 117
PSE	106.8	91	105	81	118	81	- 118
ISD	176	190	188	152	244.07	152	- 190
Scutalae	77-110	98-131	72-90	93-100	75 - 143	72	- 143
LDS	61-80	58-93	71-85	47-72	52-83.9	47	- 93
SDS	32-35	28-37	30-42	28-42	34-36.4	28	- 42
PDS	51-75	45-80	44-71	52-70	52-66	44	- 80
SVS	37-50	43-54	34-48	37-48	38-52	34	- 54
LVS	91-127	104-111	77-120	78-115	95-115	77	- 127
ACW	53.5	49.2	53	62	67.8	53	- 62
PCW	41.6	37	33	30	44.1	30	- 37
L	272	306.4	290	215	330.5	215	- 290
ECO	24	30	25	-	21.6	24	- 30
Eye Ring	40	38	35	27	42.4	27	- 40
EC-EC	248	258.9	248	196	280.5	196	- 248
EC-ASE	104.7	109.51	115	99	167.0	99	- 115
Cx I	153	130	154	146	124	124	- 154
Tr I	99	94.5	87	79	85	79	- 99
BFe I	199	177	161	144	208	144	- 208
TFe I	291	313	292	273	384	273	- 384
Ge I	278	311	277	265	371	265	- 371

Supplementary table I – Continuation

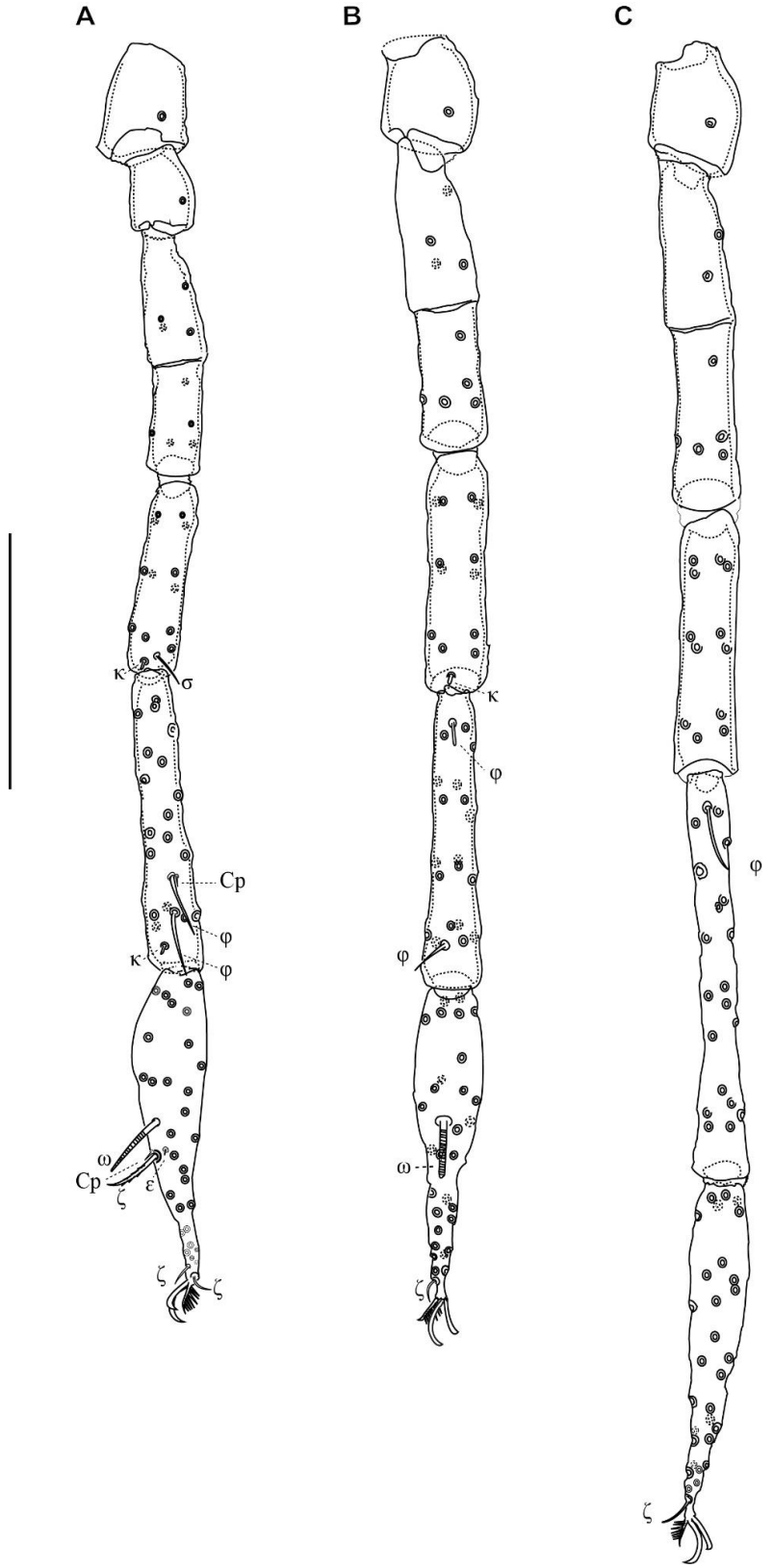
Character	CCT-UFMG AC					Range
	1301037	1301038	1300612	1300613	171474*	
Ti I	311	334	312	295	396	295 - 396
Ta I (L)	234	232	196	192	258	192 - 258
Ta I (W)	95	98.1	95	85	85	85 - 95
Leg I	1318	1450	1324	1249	1702	1249 - 1702
Cx II	193	209	158	156	229	156 - 229
Tr II	87	71.8	85	59	119	59 - 119
Bfe II	81	96.3	91	97	147	81 - 147
Tfe II	157	160.7	157	132	214	132 - 214
Ge II	167	179.5	156	150	203	150 - 203
Ti II	177.3	190.1	164	169	225	164 - 225
Ta II (L)	179	156.4	142	137	163	137 - 179
Ta II (W)	68	79.8	78	71	71	68 - 78
Leg II	774	854	796	745	1372	745 - 1372
Cx III	177	197.5	175	159	226	159 - 226
Tr III	91.2	79	75	62	93	62 - 93
Bfe III	107.1	107.6	107	91	129	91 - 129
Tfe III	211	223.8	207	193	272	193 - 272
Ge III	229.5	247	225	210	291	210 - 291
Ti III	253.5	237.2	228	234	300	228 - 300
Ta III (L)	156	154.4	140	138	183	138 - 183
Ta III (W)	67	73.5	71	68	75	67 - 75
Leg III	1039	1053.5	982	928	1494	928 - 1494
Cx IV	165	191.58	216	197	313	165 - 313
Tr IV	121.77	107.37	98	101	144	98 - 144
Bfe IV	154	185.38	147	135	207	135 - 207

Supplementary table I – Continuation

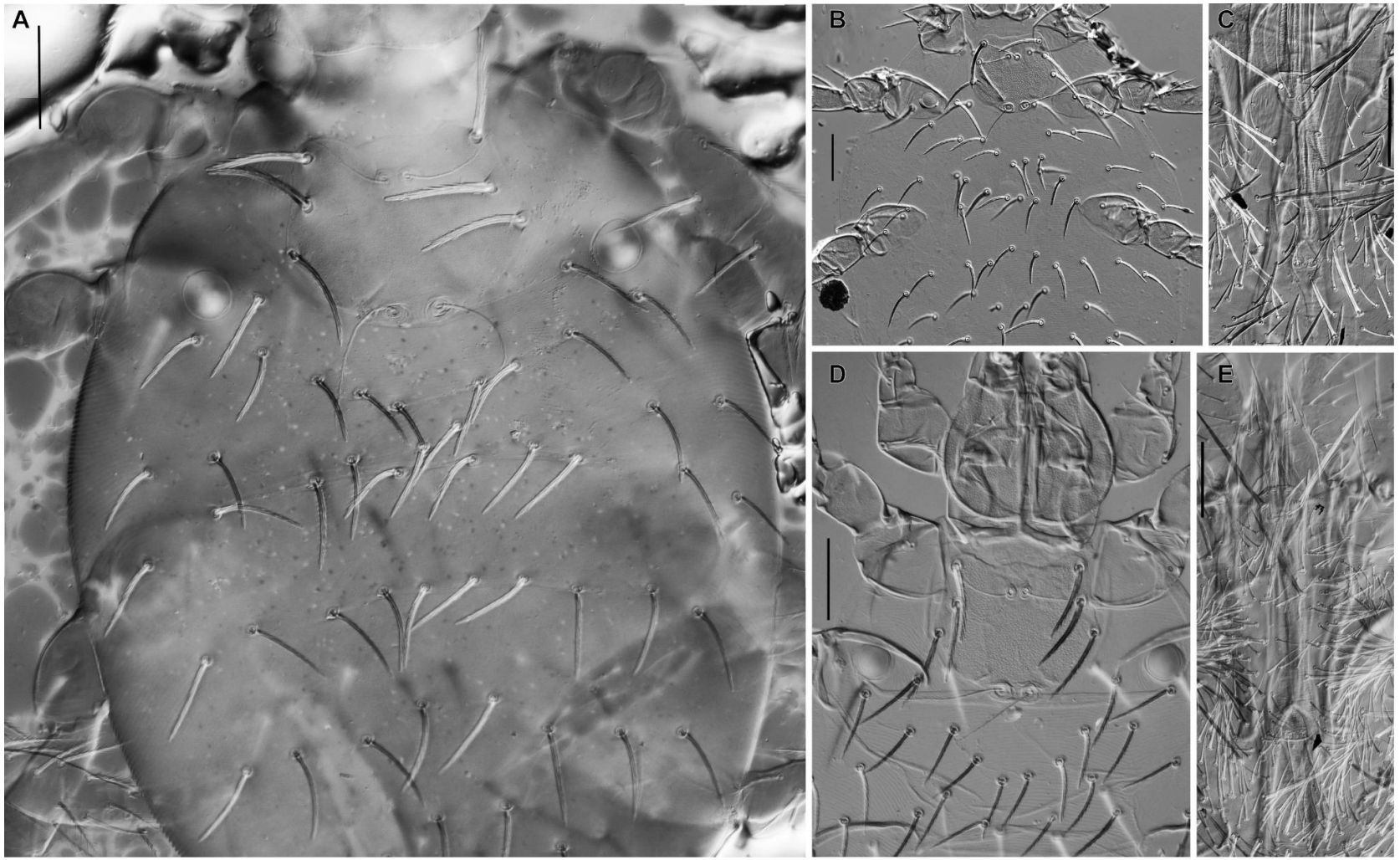
Character	CCT-UFMG AC					Range
	1301037	1301038	1300612	1300613	171474*	
Tfe IV	375	396.98	381	340	501	340 - 501
Ge IV	347	379.44	356	324	473	324 - 473
Ti IV	373	415.43	374	353	479	353 - 479
Ta IV (L)	153	172.47	153	140	194	140 - 194
Ta IV (W)	64	69.59	72	63	60	60 - 72
Leg IV	1505	1657	1509	1393	2058	1393 - 2058
Palp Femur (L)	134.14	152.5	114	121	168.6	114 - 121
Palp Femur (w)	52.7	73.31	56	54	69	54 - 69



Supplementary figure 1 - *Charletonia rocciai* Treat & Flechtmann, 1979 larva (CCT-UFMG AC 1300423): A – Palp; B –Palptarsi; C – Gnathosoma dorsal view; D – Scutum; E – Idiosoma dorsal view; F – Idiosoma ventral view. Scale: A = 50; B = 10; C, D, E - F= 100.



Supplementary figure 2 - *Charletonia rocciai* Treat & Flechtmann, 1979 larva (CCT-UFMG AC 1300423): A – Leg I; B – Leg II; C – Leg III. Scale: 100 μm.



Supplementary figure 3 - *Charletonia rocciai* Treat & Flechtmann, 1979: A- Holotype; B- Larva (CCT-UFMG AC 1300423):, from Lavras ; C- Reared deutonymph (CCT-UFMG AC 1301038), from Lavras, crista metopica. D- Larva. collected by Rosa & Flechtmann (1980) (OSAL 0114444); E- Deutonymph, reared by Rosa & Flechtmann (1980), crista metopica (OSAL 0114509). Scale: A, B and D= 50; C and E= 100.

Chapter 2 - Smarididae (Acari, Parasitengona) phylogeny and description of *Trichosmaris* larvae: enlightening the classification, two new species and the evolution of highly modified larval gnathosoma

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Abstract

Phylogenetic relationships of the family Smarididae are inferred based on fifty morphological characters from larvae and adults, including all species for which the larvae are known. This study also includes data gathered from the first description of the larval instar of *Trichosmaris*, presented hereupon. Larvae and post-larval instars of *Trichosmaris* were associated by rearing the type species *T. dispar* Southcott, 1963 and a new *Trichosmaris* species. They are described along an additional new species from the southeast of Brazil and keys for Smarididae genera and *Trichosmaris* species are provided. We discuss the evolution of a highly modified gnathosoma for smaridid larvae as well as propose a new classification, with synonymization of *Surasmaris* with *Trichosmaris* and new combinations for two *Sphaerotarsus* species.

Key words: Key to Genera – *Hirstiosoma* – Reversion of the parasitism – *Sphaerotarsus* – *Surasmaris*.

Introduction

As usual for Parasitengona mites (Acari, Prostigmata), Smarididae Vitzthum, 1929 have a life cycle composed of an egg and six postembryonic stages: a regressive calyptostatic prelarva, an ectoparasitic larva, a calyptostatic protonymph, a heteromorphic free-living, predaceous deutonymph, a calyptostatic tritonymph, and a free-living, predaceous adult (Wharton & Fuller 1952, Robaux 1974). Due to the heteromorphic post-larval instars and the relative low frequency in which larvae are found, usually in the forest litter, most descriptions are based solely on the post-larval instars. Those instars can't be associated with larvae based on morphology, requiring rearing or DNA based methods. Little is known about Smarididae ectoparasitic larvae's host, except by the record in Psocoptera hosts of larval *Smaris prominens* (Banks, 1916) (Womersley & Southcott, 1941). It contrasts with most Erythraeidae Robineau-Desvoidy, 1828 and other Parasitengona, for which descriptions are mainly based on larvae found attached to arthropods and vertebrates as ectoparasites (Mąkol & Wohltmann, 2012).

Regards the classification, Southcott (1946) divided Smarididae in four subfamilies based on adults: Smaridinae, Fessoniinae, Hirstiosominae and Phanolophinae. Later, Southcott (1961) moved Phanolophinae to Erythraeidae, and synonymized Fessoniinae with Smaridinae based on the following definition: "Adults and nymphs with eyes anterior to both sensillary areas. Sensillary areas well behind nasus. Two eyes each side." and renamed Hirstiosominae as Hirstiosomatinae. Southcott (1963) refers to *Calorema* Southcott, 1963 as an intermediary between *Smaris* Latreille, 1796 and *Fessonia* Heyden, 1826 because it shares the absence of a ventral scutum with *Fessonia*, and a dorsal enlarged scutum with *Smaris*, hence reinforcing the inclusion of former Fessoniinae in Smaridinae. The classification of those taxa was based only in similarity, since their phylogenetic relationships were never inferred.

Currently, Smarididae comprises two subfamilies. The first, Hirstiosomatinae Southcott, 1946 includes five genera: *Clavismaris* Southcott, 1963, *Hirstiosoma* Womersley, 1934 (9 spp), *Sphaerotarsus* Womersley, 1936 (8 spp), *Surasmaris* Southcott, 1995 (1 sp), and *Trichosmaris*

Southcott, 1963 (4 spp). Among them, *Hirstiosoma*, *Sphaerotarsus*, *Surasmaris* had the larvae described thus far. The second, Smaridinae Vitzthum, 1929 have four recent and one fossil genus: *Calorema* Southcott, 1963 (1 sp), *Fessonia* Heyden, 1826 (10 spp), *Kraussiana* Southcott, 1961 (2 spp), *Smaris* Latreille, 1796 (16 spp), and the fossil *Burfessonia* Konikiewicz & Makol, 2018 (1 sp). Among Smaridinae, only *Fessonia* and *Smaris* larvae are known (Mąkol & Wohltmann, 2012, Konikiewicz & Makol, 2018). In addition, *Immensmaris chewbaccei* Dunlop, Frahnert & Makol, 2018 remains as *incertae sedis*. It was described from Burmese amber (Cretaceous, ca. 100 Ma) and the scanty number of distinguishable characters precluded it to be assigned to one of the subfamilies. In the present study special attention is given to the larval instar since this have more identifiable morphological characters and issues with classification and identification.

The problem caused by the poor knowledge about the life cycle and the lack of phylogenetic studies to guide the classification is illustrated by *Sphaerotarsus*. This genus was originally described based on post larval instars and the larvae of the type species of this genus, *Sp. allmani* Womersley, 1936 remain unknown. Among others listed by Southcott (1961), the only diagnostic character exclusive of the post larval *Sphaerotarsus* is the male tarsi IV enlarged. The larvae of *Sp. leptopilus* Womersley & Southcott, 1941, associated by Southcott (1960) who reared male and female together until eggs and larvae were obtained, remains the only *Sphaerotarsus* with the heteromorfic larval and post larval instars described. Therefore, only *Sp. leptopilus* at same time has its larvae and post-larval instar known to be compared with the type species *Sp. allmani*. Other *Sphaerotarsus* known based only on larvae (*Sp. monticolus* Southcott, 1997; *Sp. quercus* Yazdanpanah, Saboori & Hakimitabar, 2016 and *Sp. baenai* Mayoral & Barranco, 2017) are classified based on their similarity to *Sp. Leptopilus* larvae. Due to their morphological differences the diagnosis for the larval instar of this genera become more and more inclusive to fit the new described larvae. These simplifications had led to very similar genera diagnosis as stated by Yazdanpanah et al. (2016) “The genera *Sphaerotarsus* and *Hirstiosoma* (syn.: *Clipeosoma* Southcott) are very similar morphologically…”.

Regards the new species of *Trichosmaris* Southcott, 1963. This genus occurs in Guatemala, Mexico, USA, and Papua New Guinea (Mąkol & Wohltmann, 2012), and is reported for the first time to Brazil. So far, four species were described: *T. dentella* Southcott, 1963, *T. dispar* Southcott, 1963, *T. jacoti* (Southcott, 1946), and *T. papuana* Beron, 2002. The larvae of this genus were never described.

The main diagnostic character of Smarididae deutonymphs and adults consists in the presence of an eversible gnathosoma. This highly modified compound structure was studied in detail by Witte (1998). However, few atention has been given to the larvae, which doesn`t had major changes in the gnathosoma described so far.

The present study aims to infer the phylogenetic relationships between Smarididae genera, considering the data from the unknown larvae of *Trichosmaris* Southcott, 1963 and discuss its implications to the classification and character evolution. In order to include data from *Trichosmaris* larvae to the analysis, the larval and post larval instars of one of two new species is described, along with the unpublished type species *T. dispar* Southcott, 1963 larvae. Identification keys to genera of larval Smarididae and to *Trichosmaris* species are also provided.

Material and methods

Sampling

One larva (CCT-UFMG AC 150559) of ***Trichosmaris sp. nov. 1*** was collected in an unidentified cave, 5 km east to Pains city, Minas Gerais, Brazil (20◦ 21' 59" S, 45◦ 37' 22" W) on April 23th to 30 th, 2015. This specimen was compared with museum specimens including larvae obtained from a female of *T. dispar* collected under rocks in the Lincoln National Forest, Cottonwood Lookout, Otero Co., New Mexico, U.S.A. (32.8°N, 105.6338° W) in July 31st, 1981 by Welbourn, W. C., who reared it until eggs and two larvae were obtained. Female and larvae are deposited in the Museum of Biological Diversity of the Ohio State University (OSAL 0119167 (Female), OSAL 0119168 (larva), OSAL 0119169 (larva).

Females of ***Trichosmaris sp. nov. 2*** were collected in a Cerrado (Savanna) fragment in an urbanized area, Americana municipality, São Paulo State (22°43`23.52" S, 47°17`20.04" W) on April 14th, 2018. Each female was individually placed alive in 50 ml falcon tubes with a humidified mixture of 50% gypsum and 50% coal until larvae were obtained. The substrate was distributed in an angle of approximately 45 degrees to maximize the surface available (Fig. 4A).

Descriptions

Photos were taken using a Nikon Eclipse 90i compound microscope equipped with Nikon DS-fi1 imaging system. Drawings were made with the aid of a *camera lucida* and final Artwork was done in Adobe Illustrator CC 2015 and Adobe Photoshop CC 2015. All measurements are given in micrometers (μm). Photos of *Surasmaris longirostris* Southcott, 1995 paratypes were kindly taken by Dra. Marla Schwarzfeld at the Canadian National Collection of Insects, Arachnids and Nematodes.

Phylogenic analyses

We included all Smarididae species for which the larval instar is known, comprising six genera (*Smaris*, *Fessonia*, *Hirstiosoma*, *Sphaerotarsus*, *Surasmaris*, and *Trichosmaris*) and 24 species including three out groups. The following species were included through data gathered from their original descriptions: *Sm. maraghehiensis* Saboori & Bagheri, 2011; *Sm. arenicola* Southcott, 1997; *Sm. prominens* (Banks, 1916); *F. papillosa* (André, 1924); *Sp. leptopilus* Womersley & Southcott, 1941; *Sp. monticolus* Southcott, 1997; *Sp. quercus* Yazdanpanah, Saboori & Hakimitabar, 2016; *Sp. baenai* Mayoral & Barranco, 2017; *Su. longirostris* Southcott, 1995; *H. ampulligera* (Berlese, 1887); *H. amfilohijei* Haitlinger & Šundić, 2017; *H. copiolarum* (Southcott, 1948); *H. latreillei* (Grandjean, 1947) and *H. furtadoi* (Shiba, 1976). In addition to descriptions, we included two *Trichosmaris* species described herewith, and four undescribed larval specimens from Barry M. Oconnor's acarological collection at University of Michigan (BMOC) - *Fessonia* sp. (BMOC 76 0419 2), two *Trichosmaris* sp. (BMOC 82 0718 5 and 86 0306 1), and a *Hirstiosoma* sp. (BMOC 83 1222 2). Original descriptions were supplemented by pictures from the type material of *Su. longirostris*, and data from Wohltmann

(2010) and Southcott (1961). In addition, we included three Erythraeidae species as outgroups: *Phanolophus oedipodarum* (Frauenfeld, 1868) was chosen because it has once been part of Smarididae; *Charletonia rocciai* Treat & Flechtmann, 1979 and *Lasioerythraeus johnstoni* Welbourn & Young, 1987 as outgroups based on previous molecular phylogenies that recovered Erythraeoidea monophyletic (Söller *et. al.* 2001, Dabert et. al. 2016).

Character matrix was built in Mesquite (Maddison & Maddison, 2016), treating all characters as unordered. From the fifty unordered characters, 41 were based on larvae and nine on post-larval stages, with a single character exclusive for males. The characters status related with shape and form (Eg.: long, short, weekly convex, very convex) are accompanied by numbers or ratios to allow an easy distinction between them. Some of those traits are continuous by nature (Eg.: Char. 14, 17 and 23), but their status can easily be differentiated by a morphological comparison and clear gaps can also be observed in the distribution of the metric data used to define them. Although parsimony analysis can deal with continuous characters, we opt to treat them as discrete since it is necessary to explore the same matrix under parsimony and in the Bayesian analysis.

Terminology and abbreviations are adapted from Southcott (1961), except by *cs, as, bs, elc, elcp* from Grandjean (1947) and a few terms introduced herewith: PSBp equals to the distance between bases of the posterior sensilla and the posterior border of scutum; similarly, ALBa equals to the distance between AL and the anterior border of the scutum (Fig. 8D and 9A).

Phylogenetic trees were inferred employing parsimony as optimality criterion and Bayesian analyses. Parsimony trees were inferred by heuristic search, starting with a Wagner tree and employing 100 thousand replicates of the Tree Bisection and Reconnection (TBR) algorithm saving 10 trees per replicate, followed by Subtree Pruning and Regrafting (SPR) of the saved trees as implemented in TNT V. 1.5 (Goloboff et al. 2008). Unweighted and implied weights analysis with default k value (K=3) (Goloboff et al.1993) were also employed. The stability of the recovered tree under different k

values was tested by increasing or decreasing it in intervals of 1 until a different topology is recovered or the k value became one. Doing so the stability of the three is given by the range of k values in which the same topology is recovered. For the consensus tree of the unweight analysis, 1000 replicates using a heuristic search (100 replicates of TBR and holding 10 trees per replicate) were done to calculate the bootstrap and jackknife support of its clades. Additionally, for the same unweighted trees Bremer support are provided and it was calculated in two rounds. At first the Bremer support was calculated based on suboptimal trees, longer up to the maximum branch length observed in the optimal trees. Those suboptimal trees were retained during TBR of the optimal trees recovered in the unweight analysis. In a second round, the highest support found in the first round was used as the difference in length to be retained for the suboptimal trees and the process repeated starting again with the optimal trees followed by a round of TBR. For the consensus tree of the implied weights analyses, support was estimated by symmetric resampling based on 1000 replicates and using a heuristic search (100 replicates of TBR and holding 10 trees per replicate) using the default change probability of 33%. In the main text, the strict consensus of all trees recovered under unweighted parsimony is presented (Fig. 5) along a Navajo hug illustrating the support of each clade which was also recovered in the tree inferred employing implied weight (K= 1–9) (S. Fig. 3) and in the Bayesian analysis (S. Fig. 4).

Bayesian analyses were run employing standard discrete (morphology) model (Lewis, 2001) in MrBayes v3.2.6 (parallel version; Ronquist et al. 2012). The parameters values were adapted from Wang et al. (2018) with the ascertainment (coding) bias set to variable (i.e. characters constant in all taxa cannot be observed). Two runs, each with four chains, one cold and three incrementally heated (temperatures 1.0, 0.91, 0.83 and 0.77, respectively) of $5x10^6$ generations, sampling every 500. To test the best evolutionary model for the data set, one of the two runs employed a gamma-distributed rate model while the other one employing equal rates, the logs of the two runs were then compared through AICM (Baele et al. 2012) criterion in Tracer v1.6.0 (Rambaut et al. 2014). The same software was also employed to check the convergence. Trees were summarized after discarding 25% as Burnin.

The strict consensus of the trees inferred under unweight parsimony are illustrated in the main text with unambiguous changes only (Fig. 5). Additionally, optimizations by accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) are presented in supplementary material S. Fig. 1 and 2, respectively. Optimizations and trees were built on the software WinClada 1.00.08 and the final artwork was done using Adobe Photoshop and Illustrator CC.

Table I – Smarididae larvae studied at BMOC collection.

Species	**ID (BMOC)**	**Locality**	**Habitat**	**Date**	**Collector**
Fessonia sp.	76-0419-002	Stanly Co., Morrow Mt. St. Park, NC, USA	bark and litter at base of *Pinus echinata*	22 Aug 1969	W. L. Brown
Trichosmaris sp.	82-0718-005	Berrien Co., Warren Woods State Park, MI, USA	On fungi *Coriolus versicolor*	18 Jul 1982	B.M. OConnor
Hirstiosoma sp.	83-1222-002	Pasoh Forest Reserve, Negri Sembilan, Malaysia	Collected with malaise trap.	22 Feb 1979	M.Wong & P.Becker
Trichosmaris sp.	86-0306-001	Pasoh Forest Reserve, Negri Sembilan, Malaysia	Collected with malaise trap.	29-30Jn1979	M.Wong & P.Becker

Character Statements

Retention index (RI) and Consistency index (CI) were calculated based on the strict consensus of the trees inferred under unweight parsimony.

Adults

0. *Dorsal idiosomal setae* (0= Common and barbed; 1= Modified bearing a pedicellus and scobillum; 2= Leaf-like). CI= 1, RI= 1.

   All post-larval Smaridídae sampled in this study have large modified dorsal setae (Fig. 1A, C and D), differing of the usually barbed setae of Erythraeidae (eg.: Fig. 1B). See Southcott (1963) for a detailed description of the Smaridídae setae. *Phanolophus oedipodarum* have leaf-like dorsal setae.

1. *Posterior sensilla shape* (0= Smooth; 1= Barbed; 2= Smooth on its distal portion). CI= 0.667, RI= 0.667.

   Posterior sensillae (Psens) are indicated by an arrowhead in Fig. 1A, C and D. It is barbed along its entire length (e.g. Fig. 1A) in all genera except by *Hirstiosoma*, where it is smooth (Fig. 1C) and in *Trichosmaris*, where it is basally barbed with a distal whip-like smooth region in (Fig. 1D).

2. *Eversible gnathosoma* (0= Absent; 1= Present). CI= 1, RI= 1.

   Smaridídae differs from Erythraidae in its eversible gnathosoma (Fig. 1E) and the presence of armilla. See Witte (1998) for a detailed description.

3. *Posterior sensillae shape* (0= Filiform 1= Clavate). CI= 1, RI= 0.

   All Smaridídae genera have filiform tapering *Psens* except by *Clavismaris* and *Sphaerotarsus*, with clavate sensillae.

4. *Ventral shield between Cx I and II* (0= Absent; 1= Present). CI= 1, RI= 0.

   This shield (Fig. 1F) is a diagnostic character of *Smaris* Latreille, 1796, but is absent from other Smaridinae like *Calorema* Southcott, 1963 or *Kraussiana*.

5. *Male tarsi IV* (0= Not enlarged; 1= Enlarged). CI= 1, RI= 0.

   Enlarged tarsi IV in males is a diagnostic character of *Sphaerotarsus*. See Wormersley & Southcott (1941) for a detailed description.

6. *Crista metopica* (0= Not enlarged; 1= Enlarged, forming a scutum). CI= 0.5, RI= 0.

   The crista metopica is a sclerotized area between the eyes, present in most Parasitengona (e.g. Fig. 13 B). In some cases, this structure is enlarged forming a prodorsal plate or a scutum, eg.: *Smaris* (Fig. 2A).

7. *Enlarged scutum* (0= Does not cover the eyes; 1= Covers the eyes). CI= 1, RI= 0.

   For some genera (e.g. *Smaris*, Fig. 2A) the dorsal scutum covers the eyes.

8. *Placement of anterior sensilla* (0= Anterior to eyes; 1= Posterior to eyes). CI= 0.5, RI= 0.

   Most Erythraeoidea has anterior sensilla placed anteriorly to eyes. In Smaridinae, however, it is placed posteriard to eyes (as in *Smaris*, Fig. 2A).

Larvae

9. *Number of scutellae* (0= 6; 1= 4). CI= 1, RI= 0.

   The scutal setae, excluding the sensilla, ranges from two to three pairs.

10. *Shape of scutum* (0= Transverse, much wider than long; 1= Partially reduced with a kite like shape, distinct by the striae distribution; 2= Trapezoidal; 3= Totally reduced without clear borders, since all borders are covered by idiosomal striae; 4= Oval). CI= 1, RI= 1.

Phanolophus oedipodarum and *Smaris* have a wide oval and transversal scutum, see Southcott (1997) and Daniel & Samsinak (1955) for illustrations. *Fessonia* (Fig. 2C) and Hirstiosomatinae (e.g. *Hirstiosoma* sp., Fig. 2B) have it trapezoidal. In *Surasmaris* and some *Trichosmaris* have a partially reduced scutum with a kite like shape on the striae distribution is observed (Fig. 8D and F), whereas other *Trichosmaris* the scutum is totally reduced, without a well-defined shape due to all borders being cover by idiosomal striae (Fig. 9A).

11. *Striated cuticle covering the scutum at least between Psens and PL* (0= Absent; 1= Present). CI= 1, RI= 1.

    In *Trichosmaris* and *Surasmaris* the scutum is partially or totally covered by the idiosomal striae, being always present between the posterior sensilla (Psens) and the postero-lateral scutella (PL) (Fig. 8D and F; 9A and G; 11D).

12. *Anterior margin of Scutum* (0= Convex; 1= Concave; 2= Straight). CI= 1, RI= 1.

    Outgroups *Lasioerythraeus, Phanolophus oedipodarum, Charletonia rocciai,* and *Smaris* have an anterior border of the scutum concave, while *Fessonia* (Fig. 2C), *Hirstiosoma* sp. (Fig. 2B) and *Trichosmaris* (Fig. 8A and G; Fig. 11D) have this border convex.

13. *Posterior border shape of Scutum* (0= Homogeneously concave; 1= Sharply pointed in the middle). CI= 0.33, RI= 0.6.

    Some species of *Hirstiosoma* have a sharply pointed posterior sensillar area trespassing the posterior border of the scutum (Fig. 2B).

14. *Posterior border convexity* (0= Weekly convex "PW/ distance between the posterior border and a line between PL > 5"; 1= Very convex "PW/ distance between the posterior border and a line between PL < 4"). CI= 1, RI= 1.

This character is based on the ratio between PW and the distance from the line uniting PL bases to the posterior border of the scutum. It aims at describing how concave is the posterior border of scutum. It was coded as missing data to *Trichosmaris sp. nov. 1* since its posterior border of the scutum is not well defined (Fig. 9G).

15. *Ending of antero-lateral scutellae* (0= Acute; 1= Blunt). CI= 0.2, RI= 0.2.

    In most species, AL tappers, ending in an acute extremity as in *Trichosmaris* (Fig. 11D). While *Fessonia* (Fig. 2C) and some *Hirstiosoma* species have it blunt.

16. *Antero-lateral scutellae length relative to posterior-lateral scutellae* (0= Shorter; 1= Longer; 2= Same length). CI= 0.22, RI= 0.3.

17. *Posterior sensillae position* (0= Near to the posterior border of scutum, SBp / PSBp > 2; 1= Far from the posterior border of scutum, SBp / PSBp < 1). CI= 1, RI= 1.

    In some species, as in *Fessonia* (Fig. 2C), *Smaris* and the outgroups the Psens is placed near to the posterior border of scutum, while in Hirtiosomatinae scutum extends beyond the Psens. Aiming to quantifying this, we employed the ratio between SBp by the distance from the line uniting PL bases to the posterior border of the scutum.

18. *Anterior sensilla relative to antero-lateral scutellae* (0= Posterior; 1= Anterior; 2= Almost at the same level). CI= 0.667, RI= 0.8.

    The state 0 (Asens posterior to PL) is observed in *Charletonia*, *Lasioerythraeus*, and *Smaris*. In *Phanolophus* and *Hirstiosoma ampulligera*, Asens lies almost at same level as AL. Finally, all other Hirstiosomatinae and *Fessonia* have Asens anterior to AL.

19. *Chelicera digit shape* (0= Short, S or corkscrew shaped and pointing sideward; 1= Slender, straight or weekly curved and pointing frontward). CI= 1, RI= 1.

The chelicera digits are remarkedly curved or even with a corkscrew shape in the outgroups (state 0) (Eg.: *Charletonia rocciai* Treat and Treat & Flechtmann, 1979), in *Smaris, Fessonia* and also in the in illustrations of *Sphaerotarsus monticulus* Southcott, 1997 the same state is observed. The illustrations of this state can be found the original descriptions Treat & Flechtmann (1979) and Southcott (1997). On the other hand, a relatively straight cheliceral digit, pointing frontwards is observed in the description of *Sphaerotarsus leptopilus* larvae and is illustrated for *Trichosmaris* and *Hirstiosoma* (Fig. 3B and 2D).

20. *Hypostomal setae* (0= Heteromorphic in respect to length and shape, with at least one of them much shorter, smooth or with poorly developed setules; 1= With a similar shape, both long and with setules). CI= 1, RI= 1.

    The hypostomal setae *as* and *bs* differ from each other by their length and shape in *Trichosmaris* (Fig. 8B and 9B), *Spaherotarsus monticulus*, *S. leptopilus* and in *Fessonia* (Fig. 2F). However, these setae are radically modified in *Hirstiosoma* (Fig. 2D), *S. quercus* and *S.baenai* which have long, similar and barbed anterior and posterior hypostomal setae (*as* and *bs*).

21. *Posterior hypostomal setae bs* (0= Barbed; 1= Smooth). CI= 0.5, RI= 0.889.

    The posterior hypostomal setae is smooth in most Smarididae and Erythraeidae included in the analysis, with exception of the outgroup *Charletonia*; the *Hirstiosoma* and three species of *Spaherotarsus, S. quercus, S.baenai* and *S. leptopilus.*

22. *Number of eyes in adults or larvae* (0=One pair; 1=Two pairs). CI= 0.5, RI= 0.83.

    The number of eyes does not change during ontogeny in all described Smarididae, hence it is codded as a single character. Smaridinae have two pairs of eyes, while Hirstiosomatinae have one pair. The character state is variable in the outgroup.

23. *Leg III lenght* (0= Short "<490"; 1= Long ">620", excluding coxae and claw). CI= 0.5, RI= 0.8.

The legs are short in *Fessonia*, *Smaris* and *Sphaerotarsus monticulus* (LegIII< 490). The out groups and the other Hirstiosomatinae have longs legs (>620) being longer than 1000 micrometers in some species.

24. *Number of normal setae on basifemur III* (0= 3; 1= 1; 2= 2). CI= 0.667, RI= 0.667.

The Smaridinae larvae have a single seta on basifemur III, while *Lasioerythraeus* has three. All other Smarididae and most Erythraeidae have two.

25. *Setae 2a* (0= Absent; 1= Present). CI= 0.33, RI= 0.5.

The setae *2a* (Fig. 2E) is absent in most Smarididae species.

26. *Setae 3a length* (0= Shorter than *1a*; 1= Same length than *1a*; 2= Longer than *1a*). CI= 0.286, RI= 0.545.

Most Smarididae and the out groups have setae *3a* longer or with the same length than *1a*, with exception of *Smaris maraghehiensis, Sphaerotarsus quercus, Sph. baenai* and most of the *Hirstiosoma*, which have setae *3a* shorter than *1a*.

27. *Distal compound trichobothria in tarsi I* (0= Absent; 1= Present). CI= 1, RI= 1.

The presence of a compound trichobothrial pit in the distal half of tarsi I is a diagnostic character of Smarididae. This consists in a trichobothria (*ta*) which articulates in the base with a modified setae (*x*) which is placed inside a pit (Fig. 6D, 10G and 12D). In some species, other micro setae of unknown nature can be observed inside the compound trichobothrial pit. *Phanolophus* has a pit which may be homologous to the one observed in Smarididae, see Southcott (1961) for details. However, due to the absence of *ta* or *x* in *Phanolophus* its pit on tarsi I was not considered homologous to the one in Smarididae.

28. *Pair of parallel trichobothrium in the proximal half of Tarsi I* (0= Absent; 1= Present). CI= 1, RI= 1.

    Hirstiosomatinae and *Fessonia* have a pair of parallel trichobothrium (tr) in the proximal half of Tarsi I is placed on each side of solenidion (ω) (Fig. 3A, 6D, 10G and 12D).

29. *Number of trichobothrium on tibia I* (0= Absent; 1= 1; 2= 7; 3= 3; 4= 4; 5= 5; 6= 6). CI= 0.83, RI= 0.89.

    In Hirstiosomatinae and *Fessonia*, trichobothrium are present not only in the tarsi I, but also in the tibia I (Fig. 3A, 6B, C, 10B and 12A). *Fessonia* has a single trichobothrium (tr) with a distinctly large base on tibia I (Fig. 3A).

30. *Number of trichobothrium on genu I* (0= Absent; 1= Numerous "4–6"; 2=2). CI= 0.5, RI= 0.8.

    Hirstiosomatinae has at least two trichobothria on genu I.

31. *Vestigiala of genu I* (0= Absent; 1= Present). CI= 0.33, RI= 0.66.

    All *Trichosmaris* and *Surasmaris* species, one *Fessonia* and a *Hirstiosoma* species, lack vestigiala on genu I.

32. *Number of solenidion on Tibia I* (0=2; 1=3; 2=4). CI= 0.33, RI= 0.

    Most Erythraeidae have two solenidia on tibia I (φ), while in Smarididae three and four solenidia may occur (e.g.: Fig. 6A).

33. *Distal solenidia in tibia II* (0= Absent; 1= Present; 2= Parallel). CI= 1, RI= 1.

    In *Fessonia*, the two solenidia on tibia II are placed side by side on the distal half, coded here as "parallel". Most Erythraeoidea have one solenidion placed on the proximal and other on the distal half of tibia II. See Wohltmann (2010) for illustrations.

34. *Number of Solenidia on genu I* (0= Three or more; 1= One). CI= 1, RI= 1.

Most Erythraeioidea and Smaridinae have a single solenidion on the genu I. On the other hand, Hirstiosomatinae counts numerous solenidia on genu I (σ) (Fig. 6B and C). Intraspecific variation and asymmetrical counts of the number of σ led us to restrict the coding of this character in just two states.

35. *Vestigiala on genu II* (0= Absent; 1= Present). CI= 0.25, RI= 0.57.

The vestigiala on genu II is absent in most Smarididae.

36. *Solenidion on genu II* (0= Absent; 1= Present). CI= 0.5, RI= 0.667.

A solenidion in the genu II is present in all *Smaris* and in *Fessonia* sp. (BMOC 76 0419 2).

37. *Solenidion on genu III* (0= Absent; 1= Present). CI= 0.5, RI= 0.5.

A solenidion in the genu III is also present in all *Smaris* and in *Fessonia* sp. (BMOC 76 0419 2). Although it may be correlated with Char. 38, we do not have enough evidence to exclude this character.

38. *One pair of dots between coxae II* (0= Absent; 1= Present). CI= 0.5, RI= 0.667.

Some *Smaris* and the *Fessonia* sp. (BMOC 76 0419 2) have one pair of dots present between coxae I and II (Fig. 2E). The nature of these dots is unknown. We speculate that it may consist in muscular insertions.

39. *Pretarsal lateral claws* (0=homomorphic, with terminal hook; 1=homomorphic, puvilliform without terminal hook; 2=heteromorphic, one with and other without a terminal hook). CI= 1, RI= 1.

Hirstiosomatinae has lateral claws with a terminal hook as observed in Fig. 6D and G. *Lasioerythraeus*, and the Smaridinae have a puvilliform or branched claw without a terminal hook (Fig. 3A). Finally, *Charletonia* and *Phanolophus* have a heteromorphic pair of claws, one with a terminal hook and another without hook.

40. *Palp segments relative to aboral setae cs* (0=the palp genu does not extend beyond *cs*; 1=only palp genu extend beyond *cs*; 2=palp genu and femur extend beyond *cs*). CI= 1, RI= 1.

Smaridinae and *Sphaerotarsus monticulus* have short palps, which genu does not extend beyond *cs* (Fig. 2F). In some *Sph. leptopilus*, *Surasmaris*, and all *Trichosmaris* (Fig. 3B) a long and slim palp has the genu ending beyond *cs*. *Hirstiosoma* (Fig. 3C), *S. quercus* and *S. baenai* have longer and stouter palps with femora extending beyond *cs*.

41. *Palp genu in relation to palp femur* (0= Short "shorter than palp femur"; 1= Long "longer than palp femur"; 2= Same length with the palp femur).

The palp genu is shorter than the palp femur (State 0) in the out groups, Smaridinae like *Fessonia* (Fig. 2F) and the Hirstiosomatinae *Hirstiosoma* and in *Sphaerotarsus monticulus*. In *Trichosmaris* (Fig. 3B) and *Surasmaris* it has the same length (State 2) or it is longer than the palp femur (State 1). A longer palp genu (State 2) is also observed in *S. quercus*.

42. *Palp tibia claw* (0= Short, similar length or shorter than the rest of the palp tibia; 1= Long, more than two times longer than the rest of palp tibia).

A long palp tibia claw (State 1) is observed in *Hirstiosoma* (Fig. 3C and E) and *Sphaerotarsus leptiopilus*. For those specimens, when measuring from the dorsal side, the length from the tip of the palp tibia claw to its base is more than two thirds of the palp tibia total length (State 0). While in the other Smarididae and the outgroups the palp tibia claw represents half or less than the total length the palp tibia (Fig. 2F and 8A, C).

43. *Conical and basal tooth in the tibial claw* (0=without a basal tooth; 1= Presenting a basal tooth or lump with blunt end). CI= 1, RI= 1.

    Hirstiosomatinae mites have a basal tooth with rounded end on basal palp tibia claw (Fig. 3E), regarded as homologous to the basal lump observed in some *Trichosmaris* (Fig. 9E) and *Fessonia.* The short and basal lump (Fig. 9E) was also illustrated for *F. papillosa* by Wohltmann, (2010) and Grandjean (1947). The out groups present the palp tibia claw with two prongs with or without setules and without a basal conical tooth. No evidence of a basal tooth was found for *Smaris* larvae.

44. *Length of basal tooth* (0=Same length or shorter than palp tarsi; 1=longer than palp tarsi; 2=lump-like structure). CI= 0.333, RI= 0.333.

    Hirstiosoma and some *Sphaerotarsus* species have a long distinct conical and basal tooth which is longer than the palp tarsi (Fig. 3E), while *Fessonia* and *Trichosmaris* species this tooth is a short or even reduced to a lump-like structure (Fig. 9E). Inapplicable to species without tooth (Character 45= 0).

45. *Pointed lateral terminal or subterminal tooth* (0= Absent; 1=one; 2=two). CI= 0.667, RI= 0.667.

    The outgroups have a main and a parallel lateral tooth with similar length. *Hirstiosoma* has a short lateral tooth (Fig. 3E) and *Smaris* has two lateral teeth making its palp tibia claw trifid, see Southcott (1997) for illustrations. The lateral tooth may appears in a more proximal position (Fig. 9E), in a medial position (Fig. 3E) or in a distal position leading to a short terminal split (Fig. 8C and 11C). These observations made us conclude that the short and terminal split present in some *Sphaerotarsus* species is homologous to the lateral tooth of *Hirstiosoma* and *Trichosmaris*.

46. *Lateral tooth origins* (0= long medial split originating tooth with similar length; 1= short medial split originating a short lateral subequal tooth; 2= short terminal split originating short two teeth with a similarly short length; 3= Dorsomedial split originating two dorsolateral teeth). CI= 0.5, RI= 0.75.

    As stated above a lateral tooth can originate of a short and medial split of the palp tibia claw, which originates a much shorter tooth in most *Hirstiosoma* (Fig. 3E). The split can also be observed in a terminal position which can be interpreted as a short terminal split of the main tooth in some *Sphaerotarsus* and *Trichosmaris* (Fig. 7C and 11C) or it can be a medial split giving origin to two similar and long teeth as observed in some *Trichosmaris* (Fig. 9E) and *Fessonia*. See illustrations of Grandjean (1947) and Wohltmann (2010). The two dorsolateral teeth present in *Smaris* were consider originated from splits in a dorsolateral position being at least one of them homologous to what is observed in other Erythraeoidea, see Southcott (1997) for illustrations. Inapplicable to species without a lateral tooth (Char. 45= 0).

47. *Number of smooth setae on palptibia* (0= 0; 1= 1; 2=2). CI= 0.4, RI= 0.4.

    All species included as outgroups lack smooth setae on palptibia, *Smaris* has a single nude seta, and *Fessonia* and *Sphaerotarsus monticulus* have two of such setae. *Trichosmaris* (Fig. 7A and C) and *Hirstiosoma* are variable in this concern, having one or none smooth setae on palptibia. *Surasmaris* have one smooth setae.

48. Setae *hc* in tarsi palp (0= Absent; 1= Present). CI= 1, RI= 1.

    This short distal setae on palp tarsi is absent in some *Smaris* leading to a total of six setae on palp tarsi. Names for paptarsal chaetotaxy after Grandjean (1947).

49. Setae *nc* on palptarsus (0= Absent; 1= Present). CI= 1, RI= 1.

The setae *nc* is a long basal seta on the palp tarsi (Grandjean, 1947, e.g. Fig. 2F) present in all Erythraeidae and Smaridinae. It is lacking from Hirstiosomatinae except by *Sphaerotarsus monticulus*.

Matrix:

Character number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Charletonia rocciai	0	0	0	0	0	0	0	-	0	0	2	0	1	0	1	0	2	0	0	0	0	0	0	1	2	1	2	0	0	0	0	1	0	1	1	1	0	0	0	2	0	0	0	0	-	1	0	0	1	1
Lasioerythraeus johnstoni	0	1	0	0	0	0	1	0	0	1	2	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	2	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	0	-	1	0	0	1	1
Phanolophus oedipodarum	2	1	0	0	0	0	0	-	0	1	4	0	1	0	1	0	1	0	2	0	0	1	1	1	-	1	-	0	0	0	0	-	1	1	-	-	-	-	0	2	0	0	0	0	-	1	0	-	-	-
Smaris maraghehiensis	-	-	-	-	-	-	-	-	-	1	0	0	2	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	-	2	3	1	0	1
S. arenicola	-	-	-	-	-	-	-	-	-	1	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	0	2	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	-	2	3	1	1	1
S. prominens	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	-	0	2	1	0	0	0	-	2	-	1	-	-	-	1	1	0	0	0	0	-	2	3	1	0	1
Fessonia sp BMOC 76 0419 2	-	-	-	-	-	-	-	-	-	1	2	0	0	0	1	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	0	0	1	2	1	0	1	1	1	1	0	0	0	1	2	1	0	2	1	1
F. papillosa	1	1	1	0	0	0	0	-	1	1	2	0	0	0	1	1	0	0	1	0	0	1	1	0	1	1	2	1	1	1	0	1	1	2	1	1	1	0	0	1	0	0	0	1	2	1	0	2	1	1
Sphaerotarsus leptopilus	1	1	1	1	0	1	0	-	0	1	2	0	0	0	1	0	0	1	1	1	0	0	0	1	2	0	2	1	1	5	2	1	2	1	0	1	0	0	0	0	1	0	1	1	0	1	2	-	1	0
Sp. monticolus	-	-	-	-	-	-	-	-	-	1	2	0	0	0	1	0	1	1	1	0	0	1	0	0	2	0	1	1	1	4	2	1	2	1	0	1	0	0	0	0	0	0	0	1	0	0	-	2	1	1
Surasmaris longirostris	-	-	-	-	-	-	-	-	-	1	1	1	0	0	1	0	1	1	1	1	0	1	0	1	2	0	2	1	1	5	1	0	1	1	0	0	0	0	0	0	1	2	0	1	0	0	-	1	1	0
Trichosmaris sp BMOC 82 0718 5	-	-	-	-	-	-	-	-	-	1	1	1	0	0	1	0	1	1	1	1	0	1	0	1	2	0	1	1	1	5	1	0	1	1	0	0	0	0	0	0	1	-	0	1	0	1	2	1	1	0
T. sp BMOC 86 0306 1	-	-	-	-	-	-	-	-	-	1	3	1	0	0	-	0	1	1	1	1	0	1	0	1	2	0	2	1	1	5	2	0	1	1	0	0	0	0	0	0	1	-	0	1	0	1	2	0	1	0
T. sp. nov. 1	-	-	-	-	-	-	-	-	-	1	3	1	0	0	-	0	1	1	1	1	0	1	0	1	2	0	1	1	1	5	2	0	1	1	0	0	0	0	0	0	1	1	0	1	2	1	0	1	1	0
T. sp. nov. 2	1	2	1	0	0	-	0	-	0	1	1	1	0	0	1	0	0	1	1	1	0	1	0	1	2	0	2	1	1	5	2	0	1	1	0	0	0	0	0	0	1	2	0	1	0	1	2	1	1	0
T. dispar	1	2	1	0	0	0	0	-	0	1	1	1	0	0	1	0	1	1	1	1	0	1	0	1	2	0	2	1	1	5	2	1	1	1	0	0	0	0	0	0	1	2	0	1	0	1	2	1	1	0
Sp. quercus	-	-	-	-	-	-	-	-	-	1	2	0	0	1	1	0	2	1	1	1	1	0	0	1	2	0	0	1	1	3	2	1	1	1	0	1	0	0	0	0	2	1	1	1	0	1	2	1	1	0
Hirstiosoma furtadoi	-	-	-	-	-	-	-	-	-	1	2	0	0	1	1	1	2	1	1	1	1	0	0	1	-	0	0	1	1	-	-	1	1	1	0	-	-	-	0	0	2	0	1	1	1	1	1	1	1	0
H. amfilohijei	-	-	-	-	-	-	-	-	-	1	2	0	0	0	1	0	1	1	1	1	1	0	0	1	2	0	0	1	1	2/ 5/6	1	0	1	1	0	0	0	0	0	0	2	0	1	1	1	1	1	1	1	0
H copiolarum	-	-	-	-	-	-	-	-	-	1	2	0	0	0	1	1	0	1	1	1	1	0	0	1	2	0	1	1	1	4	2	1	2	1	0	-	0	0	0	0	2	0	1	1	0	1	1	1	1	0
H ampulligera	1	0	1	0	0	-	0	-	0	1	2	0	0	1	1	0	2	1	2	1	1	0	0	1	2	0	0	1	1	4	2	1	1	1	0	0	0	0	0	0	2	0	1	1	1	1	1	1	1	0
H latreillei	1	0	1	0	0	0	0	-	0	1	2	0	0	1	1	0	2	1	1	1	1	0	0	1	2	0	0	1	1	4	2	1	1	1	0	0	0	0	0	0	2	0	1	1	1	1	1	1	1	0
H. sp BMOC 83 1222 2	-	-	-	-	-	-	-	-	-	1	2	0	0	1	1	1	1	1	1	1	1	0	0	1	2	0	0	1	1	4	2	1	1	1	0	0	0	0	0	0	2	0	1	1	1	1	1	0	1	0
Sp. baenai	-	-	-	-	-	-	-	-	-	1	2	0	0	1	1	0	2	1	1	1	1	0	0	1	2	0	0	1	1	6	1	1	1	1	0	0	0	0	0	0	2	0	1	1	0	1	2	1	1	0

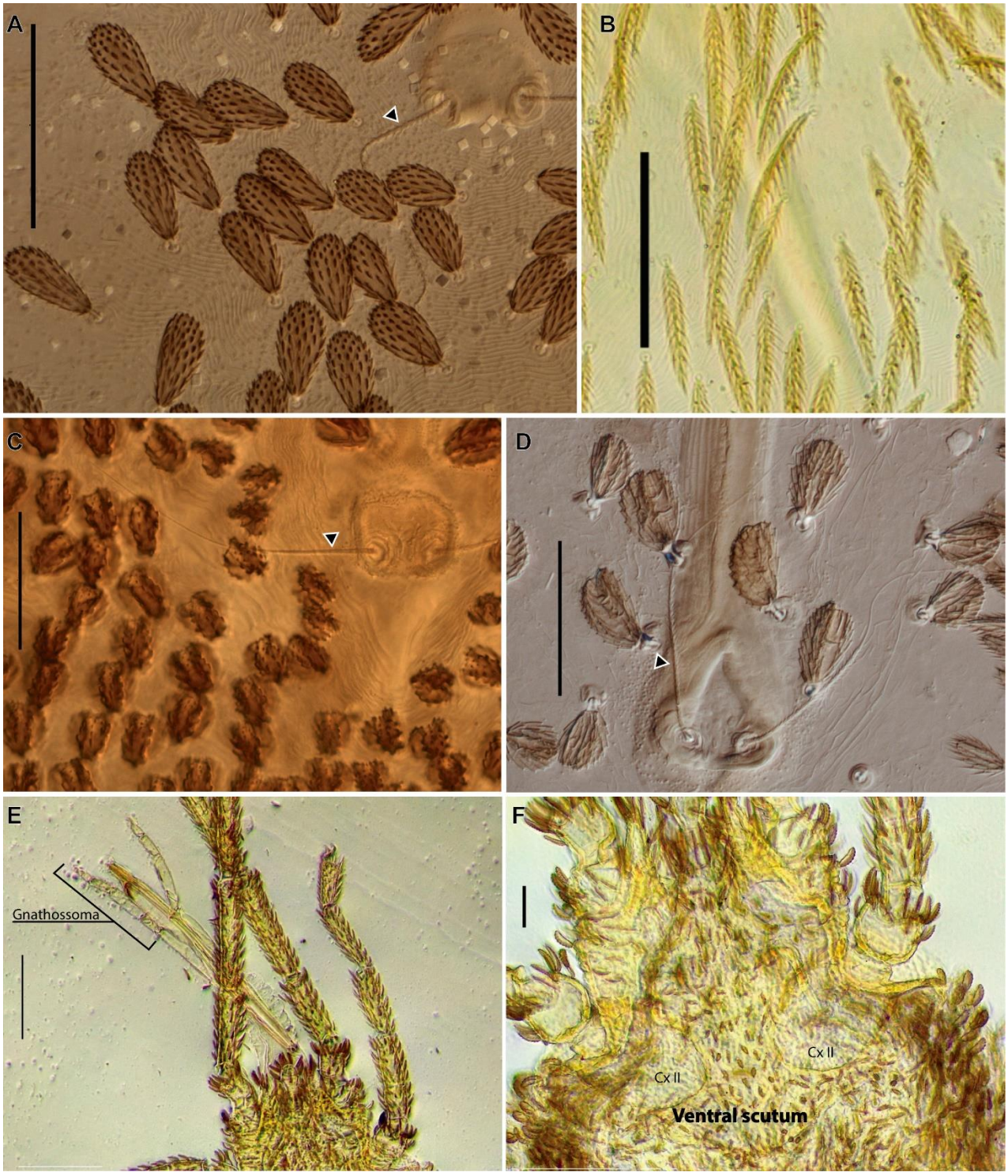

Figure 1 – Postlarval Erythraeioidea characters. *Fessonia* sp. (Adult, BMOC 76-0419-2) A: Posterior sensillar area and dorsal setae, arrowhead points to barbed sensilla; *Leptus* sp. (Adult, CCT-UFMG AC 151101) B: dorsal setae; *Hirstiosoma* sp. (Adult, BMOC 75-0306-2m) C: Posterior sensillar area and dorsal setae, arrowhead points to the smooth sensilla; *Trichosmaris* sp. (Adult, OSAL 0119167) D: Posterior sensillar area and dorsal setae, arrowhead points to the heteromorphic sensilla; *Smaris* sp. (Adult, CCT-UFMG AC 171413) E: Eversible gnathosoma F: Ventral scutum between coxae II. Scale: A-D and F=50 and E=200.

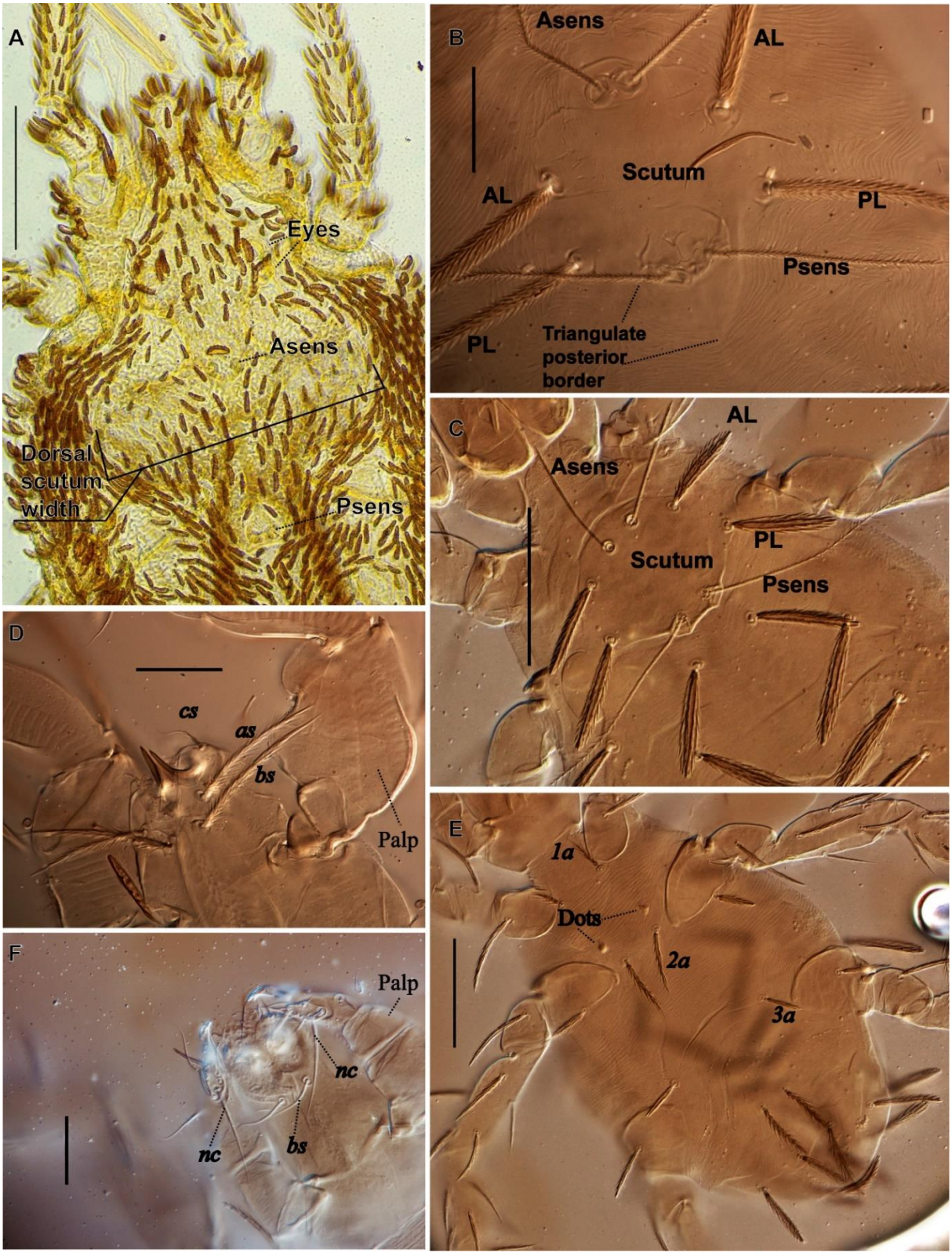

Figure 2 – Post larval and larval Smarididae characters. *Smaris* sp. (Adult, CCT-UFMG AC 171413) A: Enlarged dorsal scutum; *Hirstiosoma* sp. (Larva, BMOC 83-1222-2) B: dorsal scutum with a rounded anterior border; *Fessonia* sp. (Larva, BMOC 76-0419-2) C: dorsal scutum; *Hirstiosoma* sp. (Larva, BMOC 83-1222-2) D: gnathossoma tip; *Fessonia* sp. (Larva, BMOC 76-0419-2) E: ventral view of the idiosoma F: ventral view of the gnathosoma. Scale: A= 200; B and D= 25; C, E and F=50.

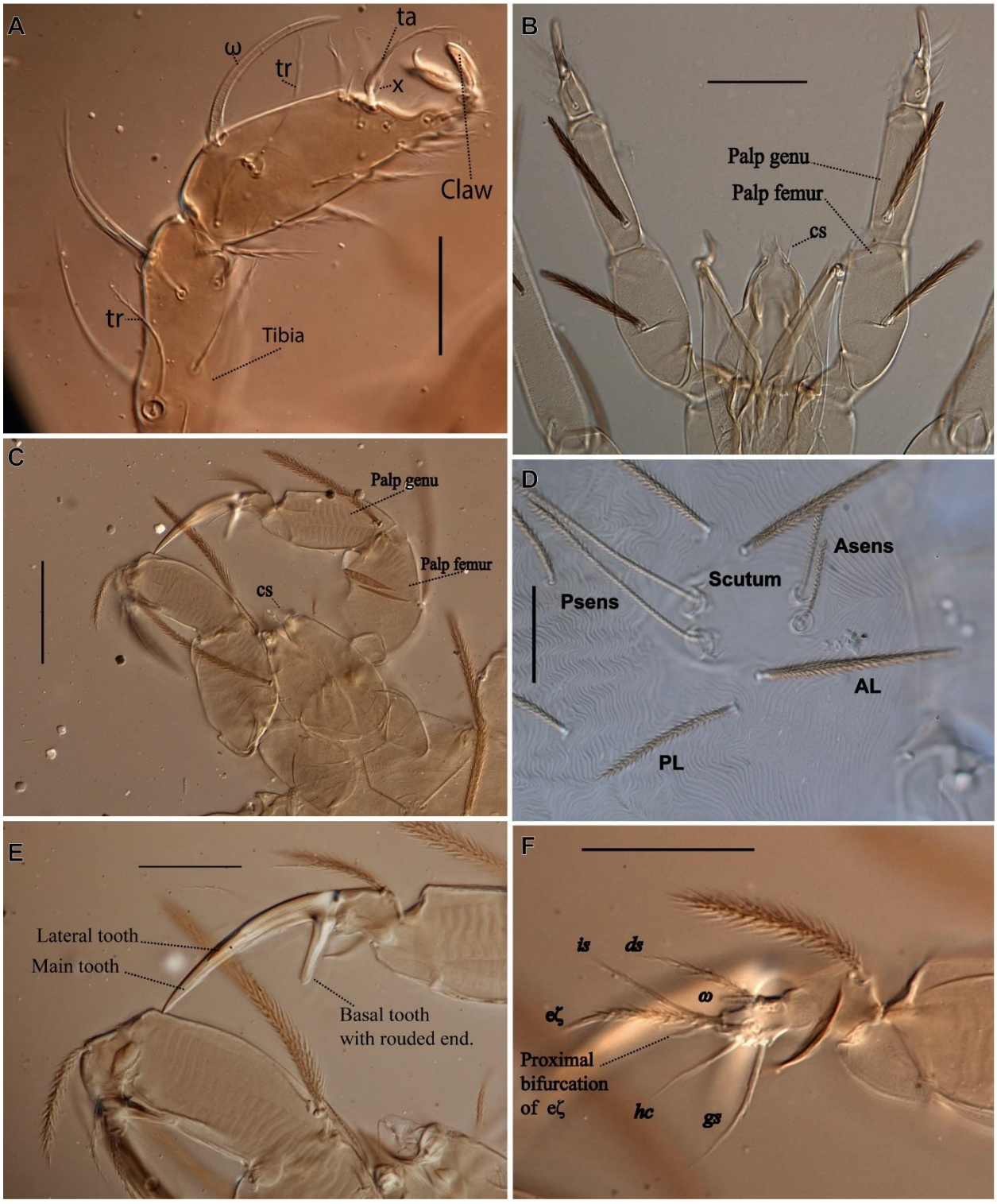

Figure 3 – Larval Smarididae characters. *Fessonia* sp. (BMOC 76-0419-2) A: tarsi and tibia I; *Trichosmaris dispar* (OSAL 0119168) B: Gnathosoma showing the elongated and thin palps; *Hirstiosoma* sp. (BMOC 83-1222-2) C: Gnathosoma showing the elongated and robust palps; E: Palp tibia claw showing the three distinct tooth and their shape; F: Palp tarsi, showing the six different setae present; *Trichosmaris longirostris* comb. nov. (Southcott, 1995) (ACA2631D) D: Scutum, showing the idiosomal striae covering its borders. Scale: A, D, E and F= 25; B and C= 50.

Results

Rearing experiments

After 42 days in culture five larvae (CCT-UFMG AC 180022, 180032–180035) were observed in the same vial of the female CCT-UFMG AC 180018. In the day 43, other 3 larvae (Including the CCT-UFMG AC 180039) were observed in a second vial with the female CCT-UFMG AC 180037. Small soil insects like Psocopteran were offered to those three larvae as potential hosts. No attachment were observed and during the process two larval specimens were lost. The specimen CCT-UFMG AC 180034 were chosen as holotype. The larvae reared from the female CCT-UFMG AC 180018 and the one reared from the female CCT-UFMG AC 180034 does not differ from each other and all specimens were considered as members of the same new species. Other adult *Trichosmaris* specimens placed in other cultures were lost due to Fungi contamination. The culture vial and the recently emerged *Trichosmaris sp. nov. 2* can be observed in Figure 4 A and B respectively.

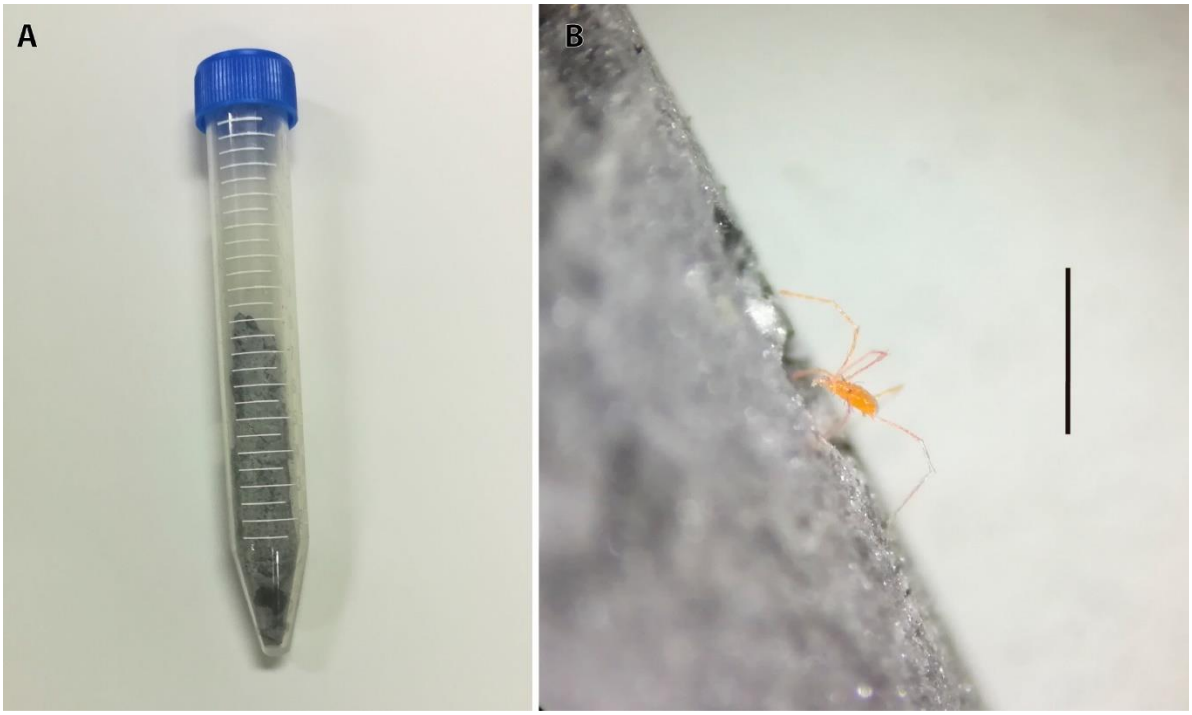

Figure 4 – *Trichosmaris sp. nov. 2* . A: Culture vial. B: Reared larvae. Scale: B= 1000.

Smarididae phylogenetic relationships

Figure 5 summarizes results from parsimony (unweighted and implied weights) and Bayesian analyses. It was recovered 8 most parsimonious trees with 122 steps in the unweighted analysis from which the strict consensus is presented (Fig. 5). For the implied weights analysis presented in the supplementary figure (S. Fig. 3), with the default K=3 employed, the same topology was recovered for K values ranging from 2 to 9. The tree inferred by the Bayesian analysis, for which the support is presented in (Fig. 5) for the common clades, are available in the supplementary material S. Fig. 3 and 4, respectively. In both cases, only unambiguous changes were illustrated. It is important to notice that some autapomorphies illustrated in the strict consensus (Fig. 5) are artefacts of optimizing characters in a consensus tree, being the single tree recovered in the analysis with implied weights more appropriated to observe autapomorphies (S. Fig. 3), bearing in mind the low support for some clades.

The parsimony trees without weighting (Fig. 5), with implied weights (S. Fig. 3) and the tree inferred with the Bayesian approach (S. Fig. 4), recovered a similar topology with most clades from Fig. 5 present.

In the parsimony-based analysis *Lasioerythraeus* were recovered as sister group of Smarididae due to the absence of inter coxal setae *2a* (Char. 25= 0) and the presence of two homomorphic claws without a well-defined terminal hook (Char. 39= 1) on larvae. Which supports the exclusion of *Phanolophus* of Smarididae made by Southcott, 1961. The precise location of the sister group of Smarididae and test their monophyly is beyond the scope of this study.

The family Smarididae was recovered in all analysis. It is supported by four unambiguous synapomorphies: presence of modified dorsal setae (Char. 0= 1) and gnathosoma eversible (Char. 2= 1) in adults; short legs (Char. 23= 0) and a distal compound trichobothrial pit in the tarsi I present (Char. 27= 1) in larvae.

In all analyses, Smaridinae is paraphyletic (Fig. 5, S. Fig 3 and 4), since *Fessonia* forms a monophyletic group with Hirstiosomatinae excluding *Smaris*. Although with low support (Fig. 5), this clade has four unambiguous synapomorphies based on the larval instar: the anterior margin the scutum in *Fessonia* and Hirstiosomatinae is convex (Char. 12= 0); the anterior sensilla is placed anteriorly to the antero-lateral scutella (Char. 18= 1); a pair of parallel trichobothrium is present in the proximal half of the tarsi I (Char. 28= 1) and a conical and basal tooth is present, even if just as a lump-like structure (Fig. 9E) (Char. 43= 1).

Hirstiosomatinae was recovered in all analyses, with *Sphaerotarsus monticulus* as sister group of all other Hirstiosomatinae (Fig. 5, S. Fig 3 and 4). The clade is well supported (Fig. 5) and have four unambiguous synapomorphies based on the larval instar: The posterior sensilla is placed far from the posterior border of the scutum (Char. 17= 1); presence of two trichobothria on genu I (Char. 30=2); presence of three or more solenidia on genu I (Char. 34= 0) and presence of homomorphic pre-tarsal claws with a well-defined terminal hook (Char. 39= 0). In addition, the homoplastic unambiguous loss of the second pair of eyes (Char. 22= 0).

Sphaerotarsus Womersley, 1936 was not recovered as a monophyletic group in any of the three analysis (Fig. 5, S. Fig. 3 and 4). Constraining *Sphaerotarsus* monophyletism in an unweighted analysis led to a tree 130 steps long (Vs. 122 without constrains). Constraining the monophyletism of *Sphaerotarsus baenai, Sp. quercus* and *Sp. leptopilus* led to a tree 125 steps long and constraining the monophyletism of *Sp. monticulus* and *Sp. leptopilus* led to a tree 126 steps long.

In the unweighted analysis (Fig. 5) and in the Bayesian analysis (S. Fig. 4). All Hirstiosomatinae with exception of *Sp. monticulus* formed a big and well supported clade (Fig. 5). This clade has three unambiguous synapomorphy: The chelicera blades are slender, straight or weekly curved and pointing frontwards (Char. 19= 1), the palps are long, with palp genu extending beyond setae *cs* (Char. 40= 1) and there are only six setae on the palp tarsi due to the loss of setae *nc* (Char.

49= 0). In addition, this clade has one unambiguous, homoplastic change: the legs are long (longer than 620 micrometers and more than 1000 micrometers in some specimens), as observed in some Erythraeidas (Char. 23= 1).

Sphaerotarsus leptopilus was recovered as the sister group of *Hirstiosoma* including *Sp. quercus* and *Sp. Baenai*. This clade is supported by one unambiguous synapomorphy, the presence of a long palp tibia claw, which represents more than 2/3 the total length of the palp tibia (Char. 42= 1). In addition, it is also supported by the homoplastic presence of a posterior hypostomal setae barbed (Char. 21=0), which also occurs in the out group *Charletonia*.

Hirstiosoma, Sp. quercus, and *Sp. baenai* clade is stable across analyses (Fig. 5, S. Fig 3 and 4), despite changes in relationships among species, with a good support (Fig. 5). The clade has five unambiguous synapomorphy in the unweighted analysis (Fig. 5): The posterior border shape of the larvae scutum is sharply pointed (Fig. 2B) (Char. 13= 1); the hypostomal setae *as* and *bs* have a similar shape and length being both long and barbed (Fig. 2D) (Char. 20= 1); the palps are very long with palp femur able to extend beyond the hypostomal setae *cs* (Char. 40= 2); a long basal tooth is present (Char. 44= 1) and a short and medial split originating a short lateral tooth is present (Char. 46= 1). In the fully resolved tree inferred under implied weights (S. Fig. 3) only Char. 20= 1, Char. 40=2 and Char. 46= 1 are recovered as unambiguous synapomorphy for the whole clade.

The results above support the inclusion of two *Sphaerotarsus* species in *Hirstiosoma* resulting two new combinations: *H. baenai* comb. nov. (Mayoral & Barranco, 2017) and *H. quercus* Yazdanpanah, Saboori & Hakimitabar, 2016. It partially agrees with Wohltmann, 2010 diagnosis of *Hirstiosoma* in respect to the palptibia claw apically bifurcate (Char. 46=2 or 1) and homomorphic hypostomal setae (Char. 20= 1). In addition, those two taxa do not fit in the genera definition of Southcott (1961) for larval *Sphaerotarsus* which was based in the only *Sphaerotarsus* species which had larvae and adults described and therefore the only which can be compared to the type species of

the genera, known only from adults. Among others, the point of discordance with the definition of Southcott (1961) is in that *S. baenai* and *S. quercus* have long and barbed hypostomal setae *as* and *bs* (Vs. a short and smooth *as* and a long barbed *bs* in the definition).

Despite internal relationships could not be fully resolved, *Surasmaris longirostris* Southcott, 1995 was recovered in a clade along *Trichosmaris* in all analyses (Fig. 5, S. Fig. 3 and 4) with a good support (Fig. 5). The *Trichosmaris/Surasmaris* clade have four unambiguous synapomorphies: The posterior sensilla in the adults has a barbed proximal part and a filiform distal part (Char. 1= 2), the larvae have a kite-like scutum (Char. 10= 1) and presence of idiosomal striae over the scutum borders including between Psens and AL (Char. 11= 1) and the presence of a with the same length of the palp femur (Char. 41=2).

The results of the phylogenetic studies and the similarity of the reared *Trichosmaris* and *Surasmaris* Southcott, 1995 supports synonymizing those two genera, regarding *Surasmaris* as junior synonymous of *Trichosmaris* Southcott, 1963 leading to the new combination *Trichosmaris longirostris* comb. nov. (Southcott, 1995) henceforward.

The evolution of highly modified gnathosoma, long legs and sensorial structures in Smarididae larvae

The scanty data on Smarididae biology makes the interpretation of morphological change along their phylogeny especially hard. Contrasting to erythraeid larvae, in most cases found parasitizing their insect host, all smaridid larvae were found free in the litter or vegetation with exception of *Smaris prominens* who were found with his host by Womersley & Southcott (1941). This and the path for mouthparts, locomotory appendages and sensorial organs evolution unveiled by this study made us speculate on Smarididae larvae diet.

As in most Erythraeidae, the out groups, the basal Smarididae *Smaris, Fessonia* and *Sphaerotarsus monticulus* have a simple and compact gnathosoma. In those specimens the gnathosoma

has short and compact palps (Char. 40= 0, Fig. 2F), the palp tarsi bears the long basal setae *nc*, usually counting seven setae (Char. 48= 1, Fig. 2F), the hypostomal setae differ by length and shape (Char. 20= 0 and 21= 1, e. g. Figs 8B, 9B, and 2F), the chelicera digits are short, S or corkscrew shaped and pointing sideward (Char. 19= 0), the palp tibia claw is short in relation with the rest of the palp tibia and its basal conical tooth is absent for Erythraeidae and *Smaris* (Char. 42= 0 and 43= 0, respectively).

The basal tooth on palptibia (Char. 43= 1) apparently evolved in the common ancestor of *Fessonia* and Hirstiosomatinae, since in *Fessonia* a short basal lump is already present (Char. 43= 2) in the base of the palp tibia claw, like the one observed in some *Trichosmaris* (Fig. 9E). The basal tooth is easily noticed in *Sphaerotarsus monticulus*, which is the most basal Hirstiosomatinae and still have some plesiomorphic states like the presence of the long basal setae *nc* in the palp tarsi and the , usually counting seven setae (Char. 48= 1, Fig. 2F) and the chelicera digits are short, S or corkscrew shaped and pointing sideward (Char. 19= 0).

The most interesting changes appears in the next branch of the tree, which leads to the clade formed by *Trichsomaris*, *Hirstiosoma* and *Sp. Lepitopilus* (Fig. 5). This clade is supported by the loss (Char. 49= 0) of the palp tarsi setae *nc* (Fig. 3F), a change in the chelicera digit shape, which become slender, straight or weekly curved and pointing frontward (Char. 19= 1), long legs (Char. 23= 1) and longer palps, with at least palp genu extending beyond the tip of the month cone (Char. 40= 1, Fig. 3B).

The elongation of the palps is a trend in the Hirstiosomtinae lineage, in *Hirstiosoma* (Fig. 2D and Fig. 3C, E and F) even longer and more robust palps are present, with the distal palp femur surpassing setae *cs* (Char. 40= 2, Fig. 3C). However, the changes which lead to this overall elongation of the palps were a slightly different in the *Trichosmaris* lineage when compared with *Hirstiosoma*. In the *Trichosmaris* lineage the gain in length of the palps were most due to the elongation of the palp genu, which become as long as the palp femur (Char. 41= 2). While in the *Hirstiosoma* lineage,

including the sister group *Sphareotarsus leptopilus*, the elongation was especially notable in the palp tibia claw which become two or more times longer than the rest of the palp tibia (Char. 42= 1). It is also notable that the accessory basal tooth of the palp tibia become robust and longer than the palp tarsi in some *Hirstiosoma* species (Char. 44= 1, Fig. 3E and F).

In parallel to the development of the long palps (Fig. 3 B and C) and long legs (Fig. 10 and 12), the Hirstiosomatinae lineage seems to have increased its sensorial skills due to sensorial setae on legs multiplied. While the sister group *Fessonia* have the trichobothria (special mechano-receptors) restricted to tarsi and tibia I, Hirstiosomatinae have at least two of them also present in the genu I (Fig. 6C) (Char. 30= 2), in some *Hirstiosoma* and *Trichosmaris* four to six are present in genu I (Char. 30= 1). Besides the multiplication of those modified mechanoreceptors, the number of solenidia (chemo-receptors) on genu I spiked from one in the most basal Smarididae lineages and the outgroups to numerous (3–16) (Fig. 6C) in Hirstiosomatinae (Char. 34= 0), with already three in the most basal *Sphaerotarsus monticulus* and up to 16 in some *Hirstiosoma* species.

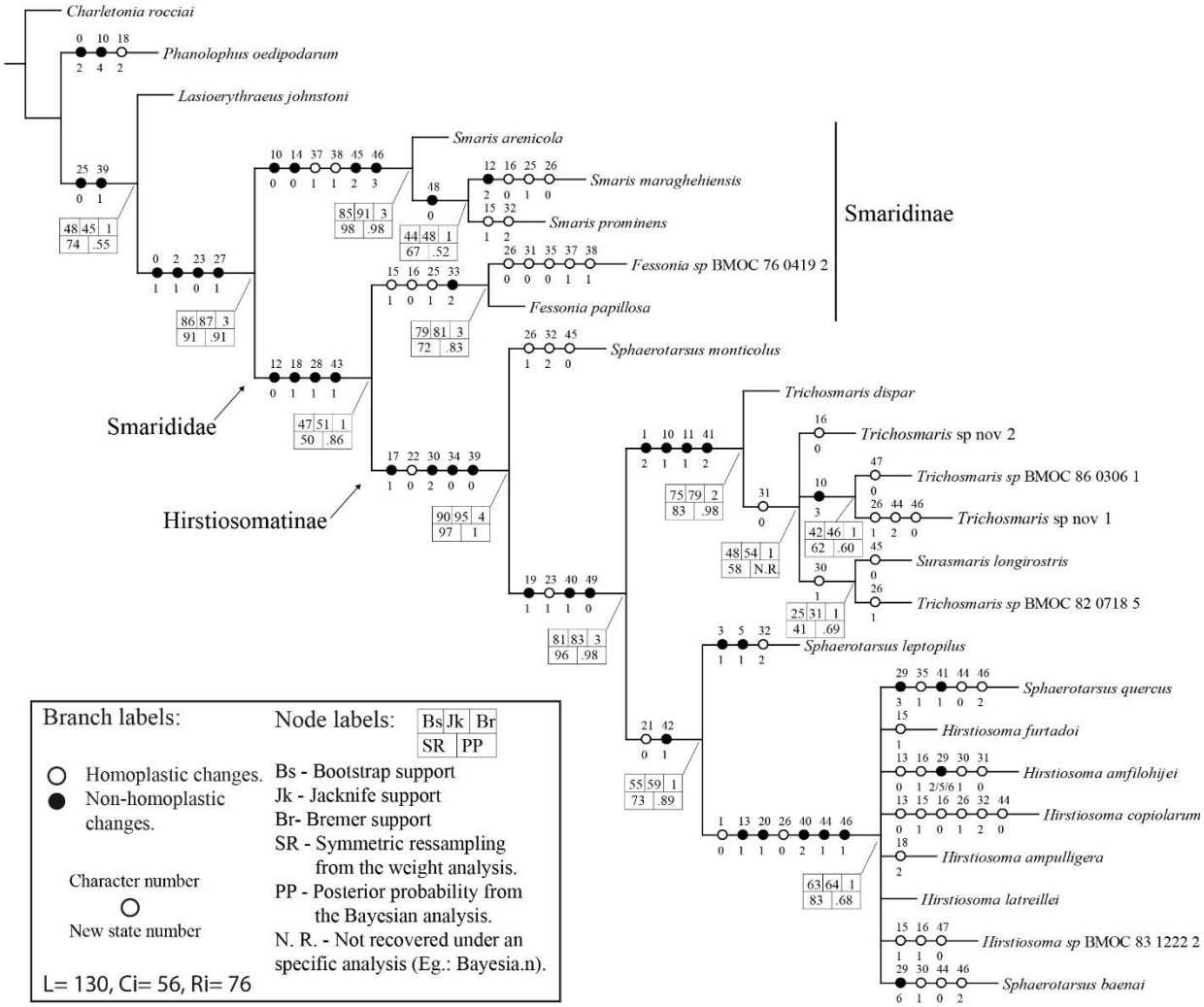

Figure 5 – Smaridídae phylogeny based on species with known larvae. The tree is the strict consensus of 8 trees with length of 122, which were inferred by 100 thousand Tree Bisection and Reconnection (TBR) replicates saving 10 trees per replicate, followed by SPR of the saved trees. The same data set was analyzed with the same procedure employing implied weights (K=3) and Bayesian analysis (Supplementary figures 3 and 4, respectively). The support values of the clades found in common in those two analyses are presented as node values. Symmetric resampling (P=33) is presented for clades which were also recovered in the weight analysis. Posterior probability is presented for clades recovered in the Bayesian analysis. All support values for resampling methods were calculated with 1000 replicates of heuristic search using TBR algorithm with 100 replicates and saving 10 trees per replicate. Characters optimization showing only unambiguous changes, see supplementary figure 1 and 2 for respectively accelerated and delayed transformation of the ambiguous changes.

Trichosmaris descriptions

Systematics

Family Smarididae Kramer, 1878

Family diagnosis according to Southcott, 1961.

Genus *Trichosmaris* Southcott, 1963

Genus diagnosis for post-larval instars according to Southcott, 1963.

Genus diagnosis for *larvae:* Compound trichobothrial pit present on the distal tarsi I (Char. 27= 1, Fig. 3A, 6D, 10G, and 12D), a single pair of eyes (Char. 22= 0), and absence of the long and basal setae *nc* (Char. 49= 0, Fig. 2F), hence counting six setae on palp tarsi (Fig. 3F). Scutum with borders and the area between Psens and PL covered by idiosomal striae (Char. 11= 1); shape of scutum either undefined due the idissomal striae over borders (Fig. 9 A and G, Char. 10= 3) or with a lozenge or kite-like shape (Figs 3D, 8D and F), identifiable by the striae distribution (Char. 10= 1); the legs are long, with leg III more than 920 (Char. 23= 1); the palps are slim and long, with palp genu extending beyond setae *cs* (Fig. 3B, Char. 40= 1). The oral setae (*as*) is short and smooth, while the hypostomal (*bs*) is long and smooth (Char. 20= 0 and 21= 1).

Trichosmaris dispar Southcott, 1963

Diferential diagnosis:

Larvae: Six solenidia on Ge I, five trichobothria on tibia I, Psens bases between ML and PL, eupathidium on tarsi placed over a lump, tarsi II with two bifid eupathidium, palpal formula is 0-0-B-B-BBN-2B,2N,1ζ 1 ω with the barbed setae in the palp genu arising from a recess.

Female: See (Southcott, 1963). With the exception that the number of common setae in the anterior sensillar area ranges from 18–21 (considering the 18 described in the original description).

Studied material. One female (OSAL 0119167) collected under rocks in the Lincoln National Forest, Cottonwood Lookout, Otero Co., New Mexico, U.S.A. (32,8°N, 105,6338° W) in July 31st 1981 and two reared larvae (OSAL 0119168 and 0119169).

Description

Female: Table II compares the metric data from Southcott (1963)'s original description to the female studied here and illustrated in Fig. 7C, D and E. It has no significative differences concerning qualitative characters if compared to the original description, except by the presence of 21 setae on the anterior sensillar area (Vs. 18 in the original description).

Table II - Comparison of metric data from the original description of *Trichosmaris dispar* Southcott 1963 (Orig.) and our specimen (AL 2534 OSAL 119168), both specimens are females.

	T. dispar (AL 2534)	*T. dispar* (Orig.)		*T. dispar* (AL 2534)	*T. dispar* (Orig.)
Ta I	242	210	Ti IV / Ge IV	1.05	0.96
Ti I	365	295	Asens	47	45
Ge I	491	382	Pars clavata	54	57
Ta IV	180	142	Flagellum	54	55
Ti IV	447	320	Clav./flag.	1	1.04
Ge IV	426	332	Sbp	18	21
Ta I /TiI	0.66	0.71	Sba	14	14
Ti I/ Ge I	0.74	0.77	ISD	549	402
Ta IV /Ti IV	0.40	0.44			

*Larvae***.**

Measurements summarized in Table III. Idiossoma oval, dorsal scutum kite-like, without clear borders since those are covered by idiosomal striae (Fig. 8 D and E). Two pairs of scutalae, idiosomal striation present between PL and the posterior sensillar area, posterior border convex, two pair of sensillae barbed (Fig. 8 D and F). Idiosoma ventrally with a shorter pair of barbed setae *1a* and a longer setae *3a* (Fig. 8 H and E). Setae 2a absent (Fig. 8. H).

Palpal setal formula: 0-0-B-B-BBN-2B2N,1ζ 1ω. Anterior hypostomal setae (*as*) smooth, short and pointed; posterior hypostomal setae (*bs*) longer than *as* and also smooth. Palp tibial claw trifurcate including a basal, a lateral and a main tooth (Fig. 8. C).

The legs are long, with all normal setae barbed (Fig. 6 and 7). All tarsi with a pair of lateral claws and a claw-like empodium barbed. Ta I with a compound trichobothrial pit and a long solenidion, (Fig. 6 D).

Leg setal formula:

Leg I: Ta- 18 B, 1 Cp, 1 ε, 2 ζ, 1 ω, 2 tr, 1 ta; Ti- 8 B, 5 tr, 1 κ, 3 φ, 1 Cp; Ge – 7 B, 2 tr, 6 σ, 1 κ; TFe- 5 B; BFe- 2B; Tr- 1B; Cx- 1 B.

Leg II: Ta- 20 B, 1 ω, 2 ζ; Ti- 15 B, 2 φ; Ge- 8 B; TFe- 5 B; BFe- 2 B; Tr- 1 B; Cx- 1 B.

Leg III: Ta- 18B, 1 ζ; Ti- 15 B,1 φ; Ge- 8 B; TFe- 5 B; BFe- 2 B; Tr- 1 B; Cx- 1 B.

Remarks

Trichosmaris dispar larvae differ from *T. longirostris* comb. nov. (Southcott, 1995) due to the presence of two trichobothria in Ge I (Vs. 5 in *T. longirostris*) and longer PW, Asens and A-P (77–78, 36–40 and 22–23, respectively Vs. 43–49, 25–26 and 9–11 in *T. longirostris*).

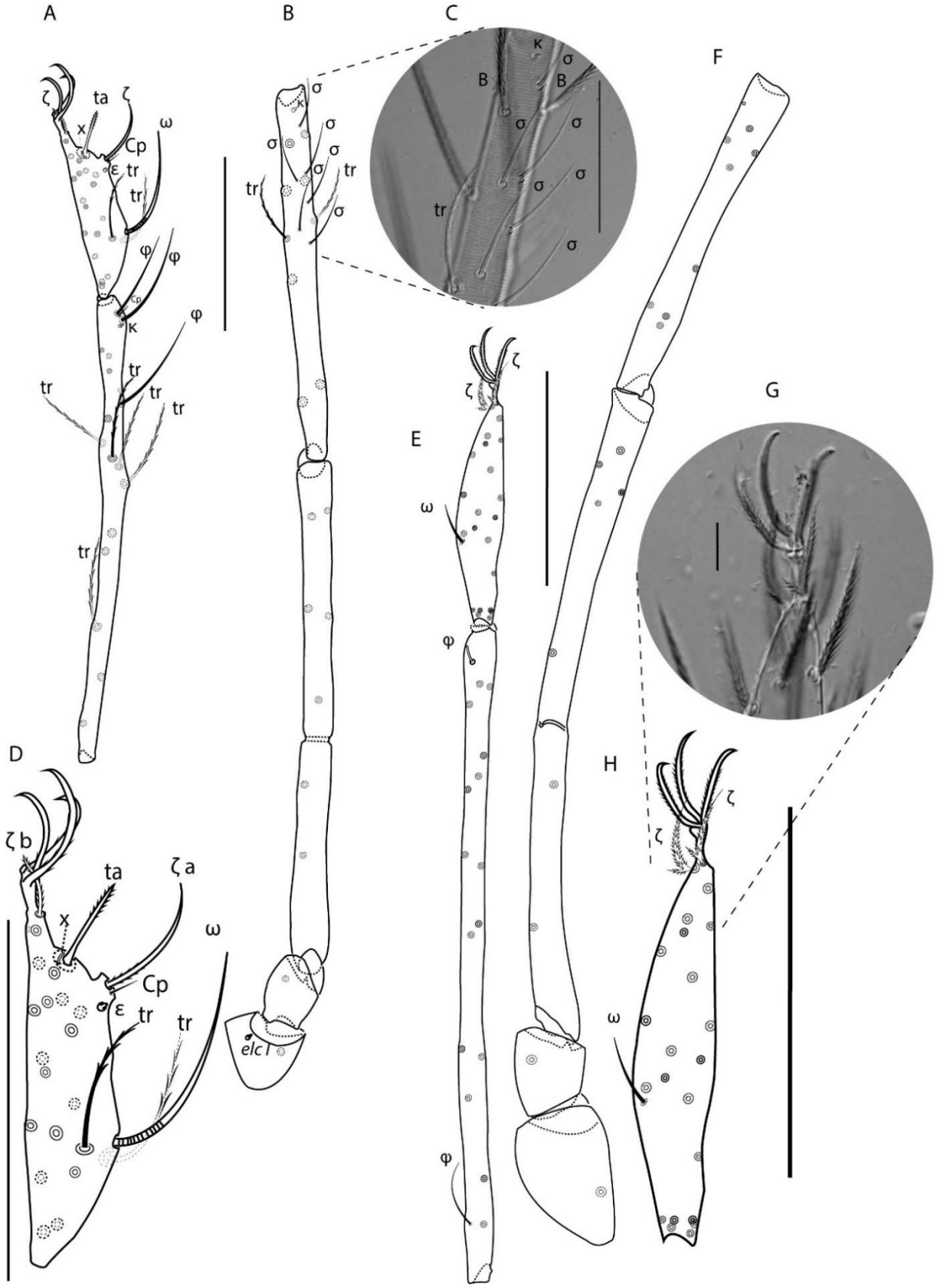

Figure 6 – *Trichosmaris dispar* larvae. A and B. Leg I; C: Tibia I setae; D: Tarsi I; E and F: Leg II; H: Tarsi II; G: Tarsal claw I. Scale: A, B, D, E, F and H= 100; C=50 and H= 10.

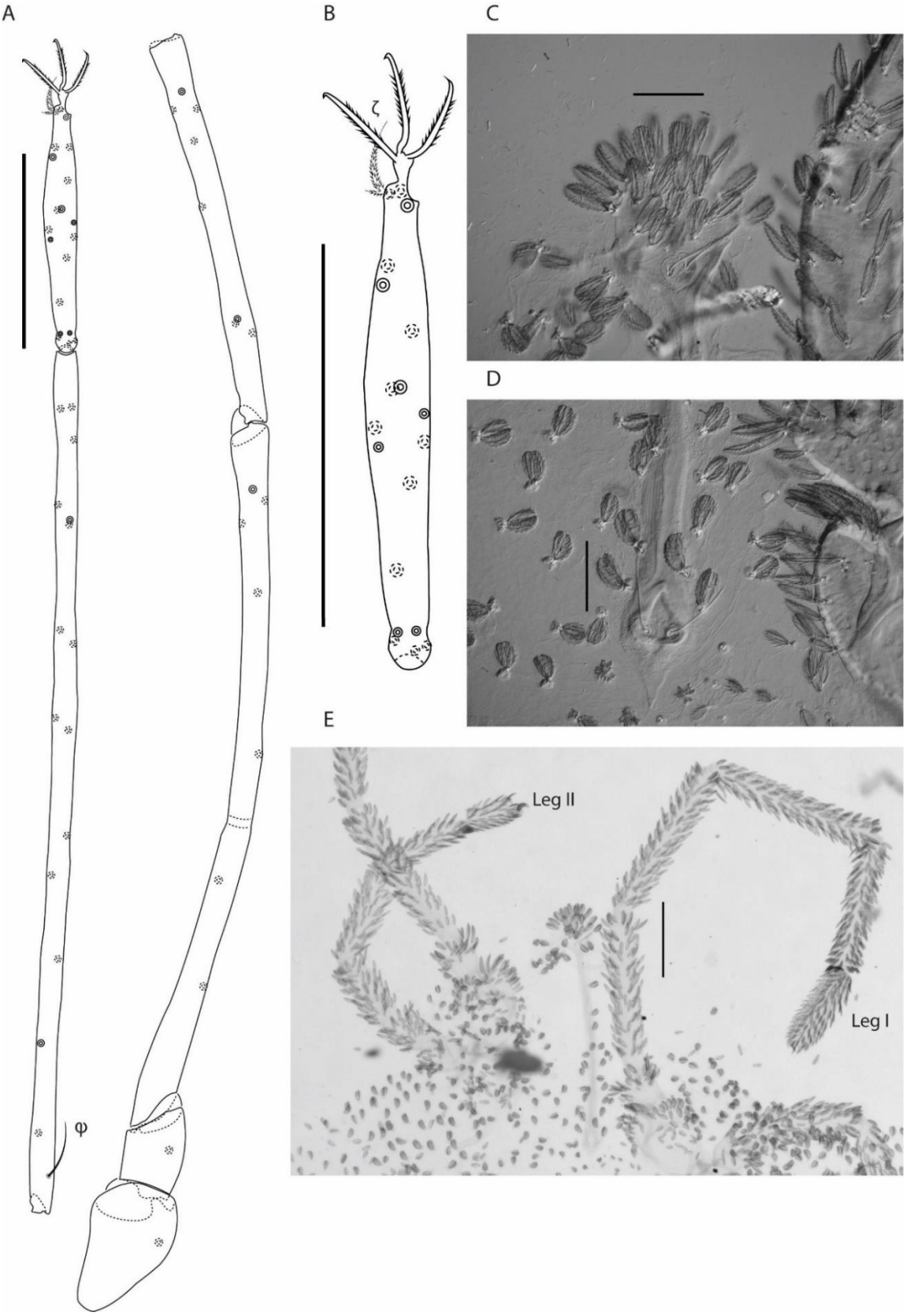

Figure 7 – *Trichosmaris dispar* larvae. A and B: Leg III; Female: C: Anterior sensillar area; D: Posterior sensillar area; E: General view of crista and Leg I and II. Scale: A and B= 100; C and D =50 and E= 200.

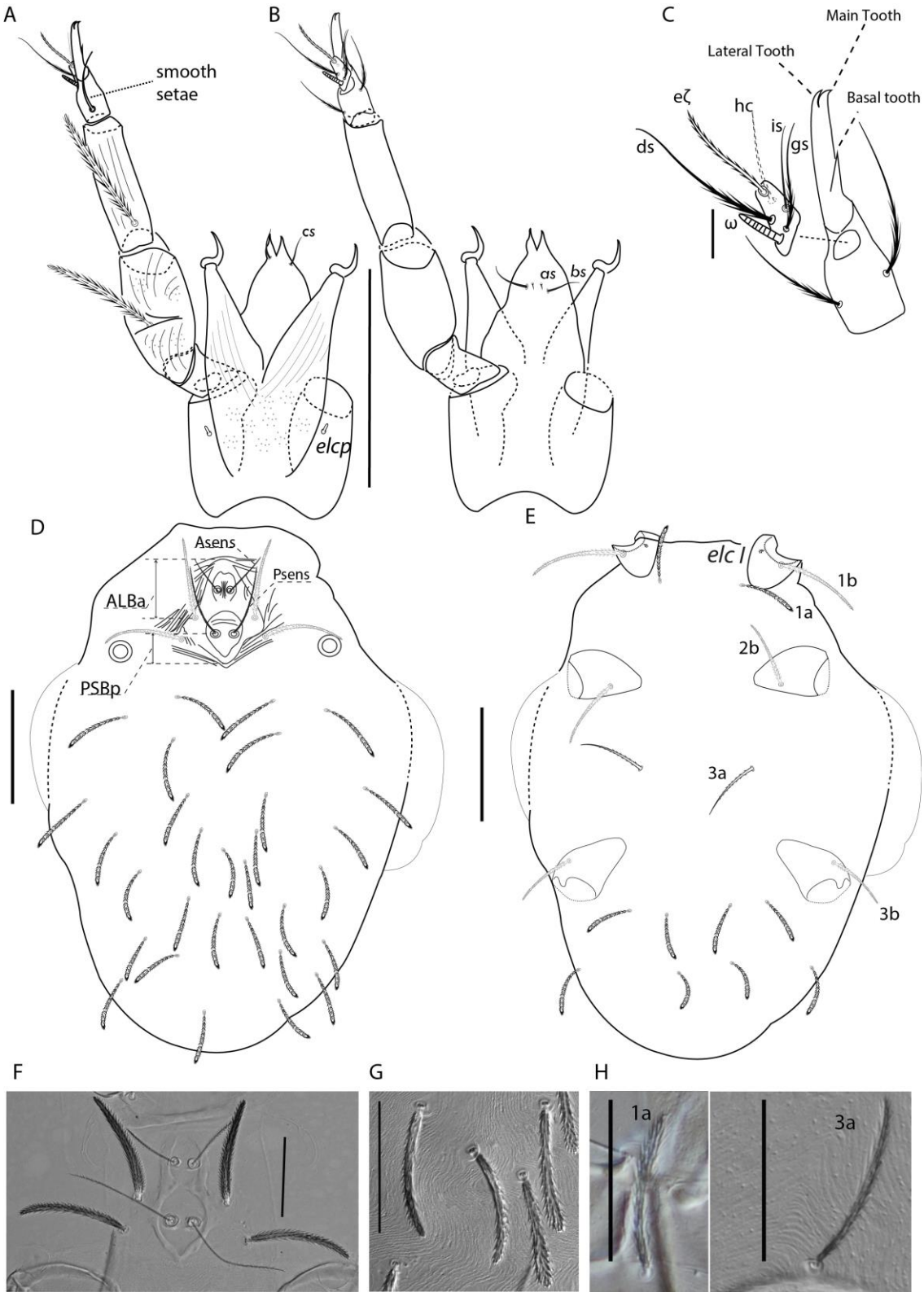

Figure 8 – *Trichosmaris dispar* larvae. A: Palp and gnathosoma dorsal view; B: Palp and gnathosoma ventral view; C: Palp tarsus and tibia ventral view; D: Idiossoma dorsal view; E: Idiossoma Ventral View; F: Scutum; G: dorsal setae; H: Ventral setae *1a* and *3a*. Scale: A, B, D, E= 100; C= 10; F, G and H= 50.

Trichosmaris sp. nov. 1

*Differential diagnosis***.**

Larvae: Eight to nine solenidia on Ge I, seven trichobothria on tibia I, Asens bases anterior to AL bases, Psens bases at the same level than PL, long legs (I, II and III, ≈1392, ≈1277, ≈1492 respectively and excluding Cx and tarsal claw), solenidion on TaI long (more than 2x the length of tarsal barbed setae) and palpal formula is 0-0-B-B-BBN-3B1N,1ζ 1 ω.

Holotype. CCT-UFMG AC 150559, at 20°21'59.0" S, 45°37'22.0" W, in an unnamed calcareous cave, 5 km east to Pains municipality, Minas Gerais.

Description.

Larvae.

Measurements in Table III. Idiossoma oval (Fig. 9F.), Dorsal scutum reduced, only sensillar area present with the two pairs of scutalae off scutum, idiosomal striation present between PL and the posterior sensillar area as well over the undefined anterior border of scutum, posterior border convex; two pair of sensillae (trichobothria) barbed, Asens close of the anterior and Psens close of the posterior (poorly defined due to striae) borders of scutum (Fig. 9A and G). Idiossoma ventrally with a two pairs of barbed setae *1a* (51.2) and *3a* (52) with similar length and shape. Setae 2a absent (Fig. 9F).

Palpal setal formula: 0-0-B-B-BBN-3B1N,1ζ,1ω. Anterior hypostomal setae (*as*) smooth, short, curved and pointed; posterior hypostomal setae (*bs*) longer than Hya and also smooth. Palpal tibial claw bifurcate, with a lateral tooth and proximal lump (Fig. 9. B, C, D and E).

Legs long, with all normal setae barbed (Fig. 10). All tarsi with a pair of claws and a claw-like empodium barbed. Ta I with a compound trichobothrial pit and a long solenidion, more than twice longer than the normal barbed setae (Fig. 10G).

Leg setal formula:

Leg I: Ta- 17B, 1Cp, 1ε, 2 ζ, 1 ω, 3tr; Ti- 5B, 5tr, 1κ, 3 φ, 1Cp; Ge - 5B, 2tr, 8–9σ; TFe – 5B; BFe – 2B; Tr – 1B; Cx 1.

Leg II: Ta- 18B, 1 ω, 1 ζ; Ti- 15B, 2 φ; Ge - 8B; TFe –5B; BFe – 2B; Tr – 1B; Cx 1B.

Leg III: Ta- 20B, 1 ζ; Ti- 15B,1 φ; Ge - 7B; TFe – 5B; BFe – 2B; Tr – 1B; Cx – 1B.

Table III - Metric data of *Trichosmaris* larval instar.

Character	*T. dispar*		*T. sp. nov. 1*	*T. sp. nov. 2*	
	Mean (n=2)	Range	Value (n= 1)	Mean (n= 6)	Range
IL	479.5	470 – 489	431.9	317.2	268 – 381
IW	286	286 – 286	-	202.6	169 – 244
AW	56.5	54 – 59	37.2	42.4	40 – 44
PW	77.5	77 – 78	65.3	60.5	53 – 65
Sba	11.3	10.7 – 11.9	10.4	8.7	8 – 10
Sbp	15.7	14.8 – 16.6	12	11.9	11 – 14
A-P	22.4	22.3 – 22.5	22.8	16.6	15 – 18
AL	74.3	73.5 – 75	55.5	68.4	63 – 72
PL	71.4	71 – 71.8	45.6	70.1	66 – 75
Asens	38.2	36.9 – 39.5	38.7	36.2	33 – 38
PSE	70.2	66.5 – 73.9	72.9	63.4	60 – 66
Scutum_L	91.6	91 – 92.1	47.1	67.7	64 – 74
Scutum_W	39.5	37 – 42	27.5	68.8	64 – 72
ISD	38.1	37.9 – 38.4	28.6	37.6	35 – 39
PS-PB	27	26 – 28	14	19.4	16 – 23
1a	56.5	53 – 60	51.3	42.1	40 – 44
3a	57	56 – 58	51.5	65.0	61 – 68
Ta I (L)	104.1	103 – 105	121.7	84.8	81 – 87
Ta I (H)	29.9	29 – 30.7	20.8	25.7	24 – 28
Ti I	279.5	278 – 281	364.9	259.2	250 – 274
Ge I	243.2	243 – 243	333.4	229.3	224 – 237
TFe I	181.9	182 – 182	262	165.0	156.9 – 175
BFe I	146	136 – 156	234.7	140.1	133.2 – 149
Tr I	44.3	44 – 45	48.2	41.1	36.23 – 43.51
CX I	47	43 – 51	60.6	40.4	37.1 – 46.75
Leg I	1036	1030 – 1042	1163.5	919.6	897.5 – 961.7
Ta II	119.3	119 – 119	144.8	107.3	103.9 – 111.1
Ti II	307	303 – 311	358.1	260.6	253.39 – 277.3
Ge II	163.2	161 – 165	215.5	141.7	139.52 – 147.6
TFe II	155.8	150 – 162	216.4	143.7	141.35 – 150.1
BFe II	143.5	143 – 144	197.4	124.4	117 – 134.9
Tr II	42.5	40 – 45	54.3	35.5	28 – 39.32
Cx II	62.3	62 – 63	65.4	55.9	49 – 67
Leg II	976.5	962 – 991	1251.8	813.2	787.8 – 853.5
Ta III	133.9	133 – 135	127.5	122.1	117.2 – 126.6
Ti III	448	447 – 449	500.2	394.0	381.83 – 417
Ge III	208.5	208 – 209	262.9	187.5	182 – 193.4
TFe III	206.5	204 – 209	259.7	188.4	181.59 – 197.8
Bfe III	157.4	150 – 165	190.1	139.8	128.9 – 147.06
Tr III	44	42 – 46	46.3	39.6	36.2 – 44.69
Cx III	65.4	63 – 68	60.6	53.1	50.2 – 57.2
LeG III	1246	1238 – 1254	1447.2	1071.5	1047.5 – 1118.1

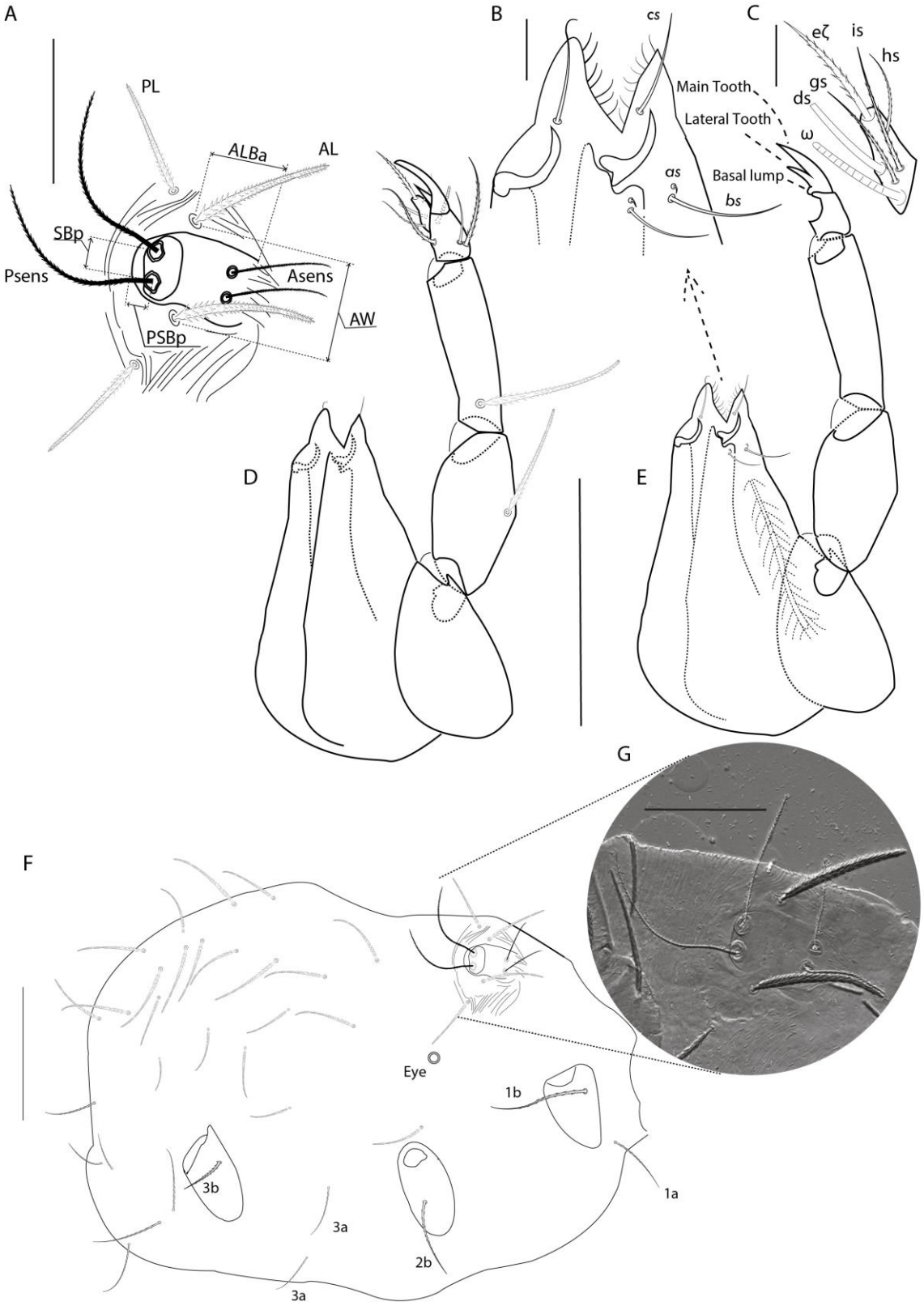

Figure 9 – *Trichosmaris sp. nov. 1* . A: Scutum; B: Tip of the gnathosoma, lateral view; C: palp tarsus ventral view; D and E: Gnathosoma, lateral view F: Idiossoma, lateral view. Scale: A=50; B and C= 10; D, E and F= 100.

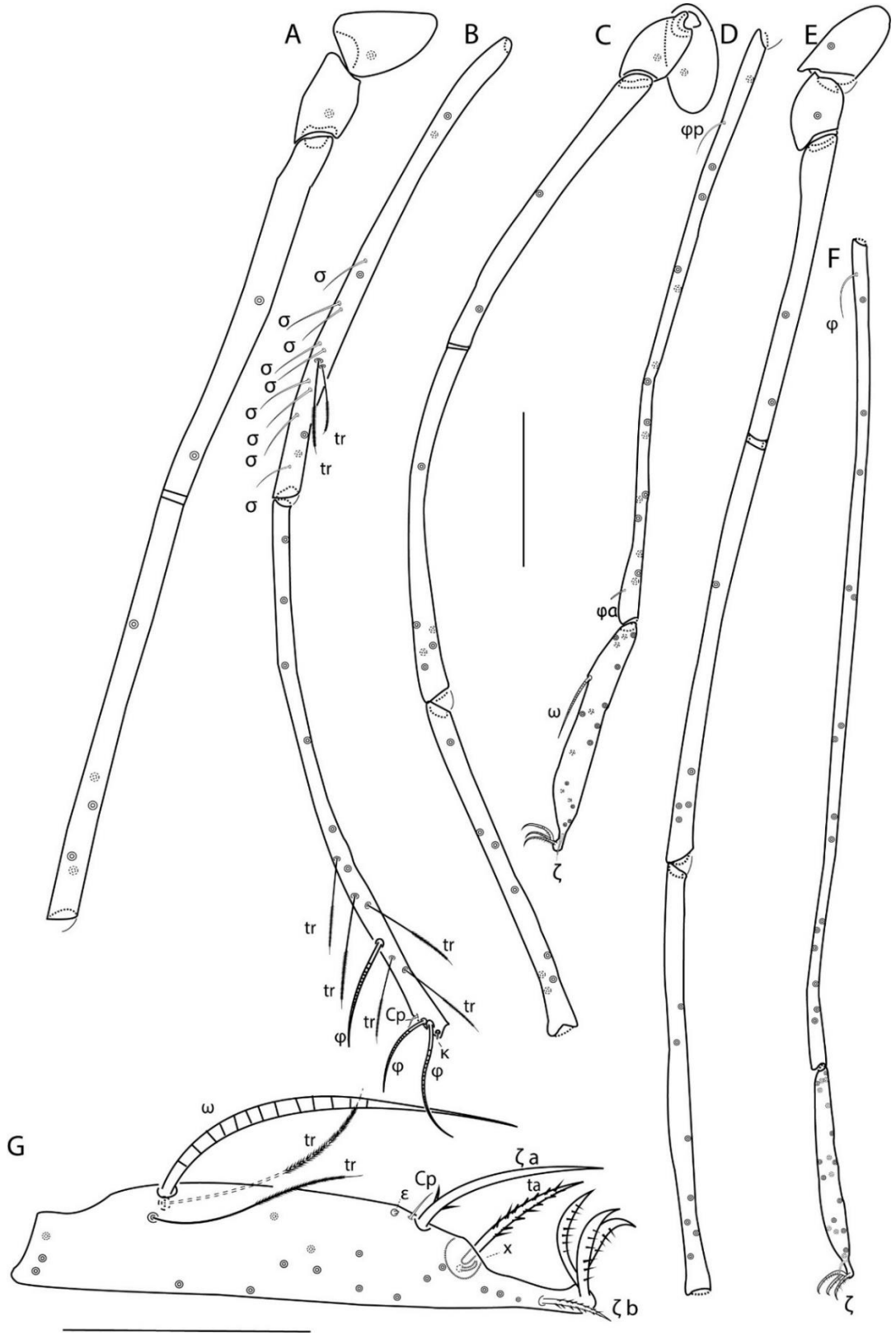

Figure 10 – *Trichosmaris sp. nov. 1* . A and B: Leg I; C and D: Leg II; E and F Leg III; G: Tarsi I. Scale: A-F= 100 and G=50.

Remarks

Trichosmaris sp. nov. 1 differ from the larval instar of *Trichosmaris dispar* and *Trichosmaris sp. nov. 2* due to the absence of a lump where the medial eupathidium setae arises on tarsi I, lack of bifid eupathidium on tarsi II, and number of solenidia on genu I (8–9 Vs. 6 in *T. dispar*). *Trichosmaris sp. nov. 1* also differs from *T. longirostris* comb. nov. (Southcott, 1995) due to presence of two trichobothria in Ge I (Vs. 5 in *T. longirostris*).

Trichosmaris sp. nov. 2

Diferential diagnosis.

Larvae with five trichobothria present on tibia I. Asens bases placed anteriorly to AL bases and Psens bases placed anteriorly to PL bases; AW= 40– 44, PW= 53–65 and ISD= 35–39. One eupathidium (ζ a) with an enlarged and barbed base present over a lump on tarsi I. One bifid distal seta present on tarsi II. Idiosomal striae covering the scutum borders.

Type Material. Holotype (CCT-UFMG AC 180034), larva reared from paratype female (CCT-UFMG AC 180018) collected in a small and dry fragment of Cerrado biome surrounded by an urbanized area of Americana municipality, São Paulo State, Brazil (22°43`23.52” S, 47°17`20.04” W). **Paratypes**. Five larvae (CCT-UFMG AC 180033, 180032, 180035, 180039 and 180022) reared from the females (CCT-UFMG AC 180018 and 180037). Collection data as holotype.

Description.

Larvae.

Measurements in Table III. Idiossoma oval (Fig. 11D and E.). Dorsal scutum reduced, only sensillar area present with the two pairs of scutelae, idiosomal striation present between PL and the sensillar area. The scutum’s posterior border is convex; there are two pair of barbed sensillae (trichobothria). The Asens is close of the anterior and Psens close of the posterior border of scutum

(Fig. 11D). Idiosoma ventrally with a short pair of barbed setae *1a* (40–44) and a longer pair with similar in shape of setae *3a* (61–68). Setae 2a absent (Fig. 11E).

Palpal setal formula: elcp-0-B-B-BBN-3B1N,1ζ,1ω. Anterior hypostomal setae (*as*) smooth, short, curved and pointed; posterior hypostomal setae (*bs*) longer than *as* and also smooth. Palpal tibial with a short distal bifurcation and a basal tooth (Fig. 11. A, B and C).

Legs long, with all normal setae barbed (Fig. 12). All tarsi with a pair of claws and a claw-like empodium, both with setules (Fig. 12D, E and F). Tarsi I with a compound trichobothrial pit with a trichobothria (*ta*) and an accessory setae (*x*), which is in contact with the base of *ta*. A modified eupathidium with a large and barbed base and a flagelliform and smooth distal part (ζ a) and a companion short setae (Cp) are observed over a lump, followed by a famulus (ε). A long solenidion (ω) is placed between a pair of trichobothria (tr) more than two times longer than the normal barbed setae (Fig. 12D).

Leg setal formula:

Leg I: Ta- 17B, 1Cp, 1ε, 2 ζ, 1 ω, 2tr, 1 ta; Ti- 9B, 5tr, 1κ, 3 φ, 1Cp; Ge - 7B, 2tr, 5–7σ; TFe – 5B; BFe – 2B; Tr – 1B; Cx 1B.

Leg II: Ta- 18B, 1 ω, 2 ζ; Ti- 15B, 2 φ; Ge - 8B; TFe –5B; BFe – 2B; Tr – 1B; Cx 1B.

Leg III: Ta- 17B, 2 ζ; Ti- 15B,1 φ; Ge - 8B; TFe – 5B; BFe – 2B; Tr – 1B; Cx – 1B.

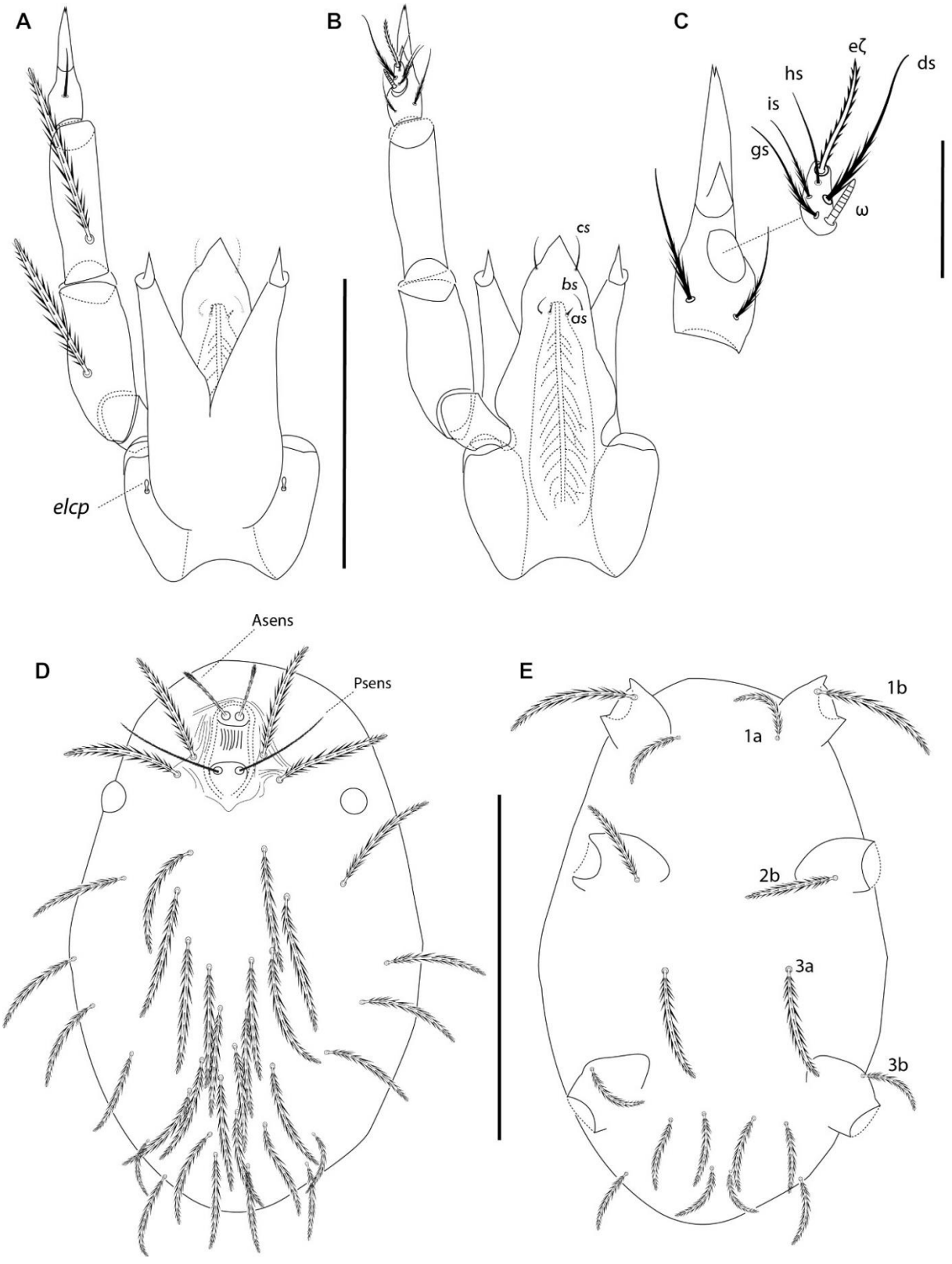

Figure 11 – *Trichosmaris sp. nov. 2* larvae. A: Gnathosoma dorsal view; B: Gnathosoma ventral view; C: palp tarsus ventral view; D: Idiossoma dorsal view and E: Idiossoma ventral. Scale: A and B= 100; C=20; D and E= 200.

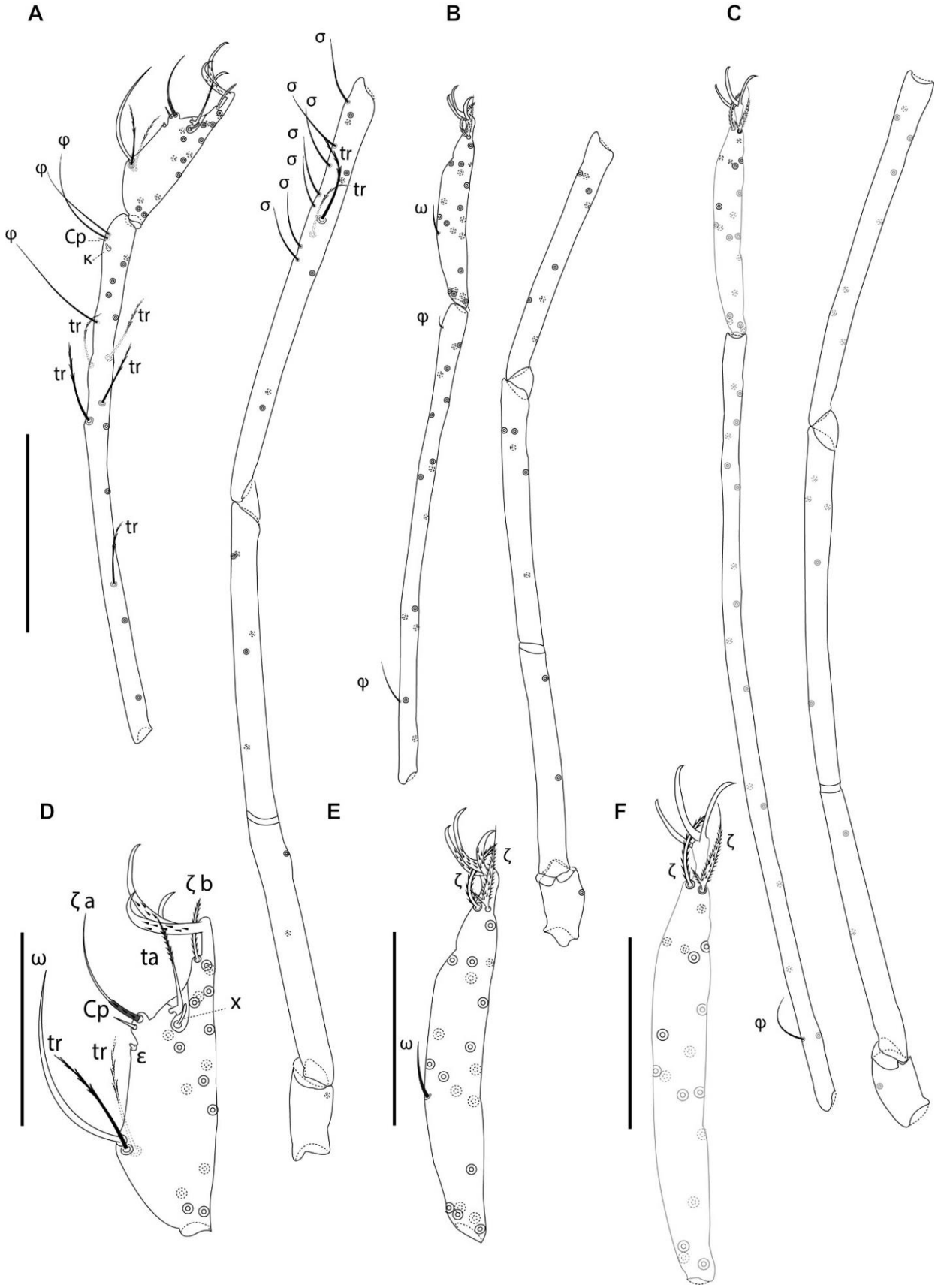

Figure 12 – *Trichosmaris sp. nov. 2* larvae. A: Leg I; B: Leg II; C: Leg III; G: Tarsi I, E: Tarsi II and F= Tarsi III. Scale: A–C= 100 and D–F=50.

Female.

Measurements summarized in Table IV. Color in life red. Idiossoma roughly oval (Fig. 13A). Crista metopica bearing two pairs of filiform sensillar setae and a *naso* bearing 10 short (24–40) scutellae with setules (Fig. 13B and D). One paratype (CCT-UFMG AC 180018) does not have the posterior sensillar area or Psens, which is apparently an abnormality. Asens clavate (Fig. 13B and D). Psens indicated by an arrowhead in Fig. 13B and G, with a long (≈64.4) barbed proximal region (*pars clavata*) and a short (≈ 39.4) distal smooth portion (*flagellum*). One pair of eyes placed between Asens and Psens, eye cornea diameter 27.9–28.5 (Fig. 13B). Anal and genital pores are not observable in both specimens due to crystals of guanine. Dorsal setae short (20–32) with rows of setules with up to 12 setules each in the dorsal side. Those setae are illustrated on Fig. 13H by stacking photos of the dorsal (on the left) and ventral side (on the right) of the same setae.

Gnathosoma conical, eversible, with long, stylet-like chelicera (Fig. 13 D and E). Palps bearing simple barbed setae (Fig. 13 D and E) that differ from those on dorsal idiosoma (Fig. 13H). Palptarsus with seven setae, including one basal solenidion (ω), two common barbed setae (B), and four barbed eupathidium (ζ). Palp tibial claw entire (Fig. 13F).

Relative legs lengths: I>IV>III>II. Leg scobalae numerous, pointed with setules (Fig. 13C and H), palp genu bearing numerous extremely thin dorsal setae of unknown nature (arrowhead, Fig. 13H).

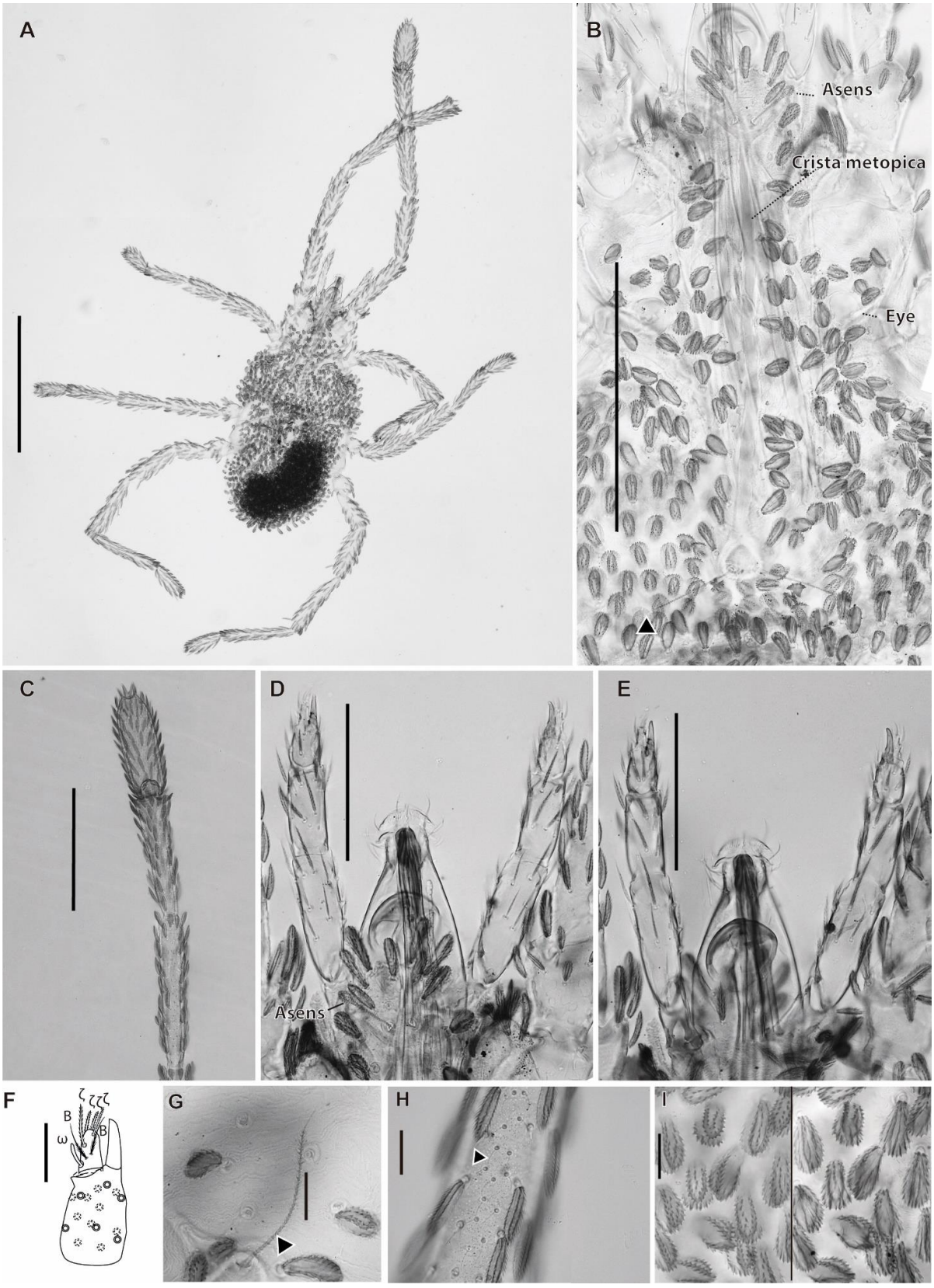

Figure 13 – *Trichosmaris sp. nov. 2* female. A: General view; B: Crista metopica, arrow head pointing to the posterior sensilla; C: Leg I distal part; D: Gnathosoma dorsal view; E: Leg III: Gnathosoma ventral view; F: Palpi tibia and Tarsi; G: Posterior sensilla pointed by an arrow head; H: Thin setae on genu I of unknow nature pointed by an arrow head; I: Dorsal setae, the figure is divided in the middle with focus at the dorsal (on the left) and ventral (on the right) sides of the same setae. Tarsi I, E: Tarsi II and F= Tarsi III. Scale: A–C= 100 and D–F=50.

Table IV - Metric data of *Trichosmaris sp. nov. 2* females.

Character	CCT-UFMG AC		Character	CCT-UFMG AC	
	180037	180018		180037	180018
IL	877	779	Leg I (without Cx)	1207.8	1166.1
IW	458	360	Cx II	130.4	112.6
Sba	13.7	12.9	Tr II	64.5	37
Sbp	17.6	-	Bfe II	85.8	94
Asens	41.3	48	Tfe II	126.3	111
Psens	103.8	-	Ge II	151.4	150.7
Pars clavata	64.4	-	Ti II	170.5	160.3
Flagellum	39.4	-	Ta II (L)	120.3	138.4
ISD	340	-	Leg II	718.8	691.5
Scutalae	24.8–35.4	30.7–40	Cx III	110.3	106.7
DS	20–29	20–32	Tr III	64.9	57.5
VS	14.4–19.2	15.2–19.5	Bfe III	85.5	73.06
ACW	62.3	56.5	Tfe III	135.9	124.3
PCW	36	-	Ge III	175.3	171.4
L	366.3	-	Ti III	196	183.3
ECO	28.5	27.9	Ta III (L)	136.7	129.2
Eye Ring	36	32.7	Leg III	794.3	738.7
EC-EC	143	127	Cx IV	182.2	173.2
EC-Asens	147	132.7	Tr IV	86.8	87.9
Cx I	121.7	144.5	Bfe IV	146.1	152.4
Tr I	66.3	66.2	Tfe IV	210.5	182.0
BFe I	234	213.1	Ge IV	258.5	242.2
TFe I	245.5	226.2	Ti IV	292.8	271.4
Ge I	263.8	252.6	Ta IV (L)	159.2	165.4
Ti I	203.9	210.8	Leg IV	1153.9	1101.4
Ta I (with claw)	194.3	197.2			

Remarks

Trichosmaris sp. nov. 2 larvae differ from the larval instar of *Trichosmaris dispar* Southcott, 1963, described in the present work, due to the presence of a single bifid setae on tarsi II (Vs. 2 *T. dispar*), shorter AW and PW (40–44 and 53–65, respectively) while *T. dispar* have 54–59 and 77–78, respectively. In addition, there are 9 barbed setae on Ti I and 18 on tarsi II (Vs. 8 on Ti I and 20 Ta II for *T. dispar*). It also differs from *T. sp. nov. 1* and *T. longirostris* comb. nov. (Southcott, 1995) due to the presence of a eupathidium and a companion seta over a lump on Ta I (Vs. the absence of any lump

on the Ta I of *T. sp. nov. 1* . Furthermore, *Trichosmaris sp. nov. 2* differs from *T. longirostris* comb. nov. (Southcott, 1995) due to due to the presence of two trichobothria in Ge I (Vs. 5 in *T. longirostris*), and longer PW, Asens and A-P (53–65, 33–38 and 15–18, respectively Vs. 43–49, 25–26 and 9–11 in *T. longirostris*).

The adult *Trichosmaris sp. nov. 2* differs of *T. dispar* Southcott, 1963 due to a shorter ISD (340 Vs. 402–577 in *T. dispar*). It also differs from *T. dispar* and *T. papuana* Beron, 2002 due to the presence of 10 scutella in the anterior sensillar area (Vs. 18 in *T. dispar* and 5 in *T. Papuana*). In addition, *T. sp. nov. 2* differs of *T. Papuana* due to enlogated scutella in the anterior sensillar area, having those a ratio length/width= 3 – 4.3 (Vs. 1.27–1.35 in *T. Papuana*). At last, *T. sp. nov. 2* differs of *T. jacoti* (Southcott, 1946) in the posterior sensilla Pars clavata length (64.4 Vs. 45 in *T. jacoti*), Sba (12,9–13,7 Vs. 16 in *T. jacoti*), GeI length (252–263 Vs. 341–377 in *T. jacoti*) and by having a Ti IV longer than the Ge IV, Ti IV/Ge IV= 1.12–1.13 (Vs. shorter, Ti IV/ Ge IV= 0.94 in *T. jacoti*).

Taxonomical key to larval Smaridídae genera

1 – Anterior border of the scutum concave or straight (Char. 12= 1 or 2), anterior sensilla posterior to the anterolateral sucutella (AL) (Char. 18= 0); no trichobothria in the proximal half of the palp tarsi (Char. 28= 0) and the basal tooth with rounded end is absent (Char. 43= 0).---------- ---------- *Smaris* Latreille, 1796.

– Anterior border of scutum convex (Char. 12= 0, Fig. 2C); anterior sensilla anterior to the anterolateral scutella (AL) (Char. 18= 1); a pair of parallel trichobothrium (Fig. 3A, 6D, 10G and 12D) present in the proximal half of palp tarsi (Char. 28= 1) and a basal tooth with a rounded end (Fig. 3E) is present, even if reduced to a small lump (Fig. 9E) (Char. 43= 1).---------- 2

2 – Posterior sensilla near the posterior border of the scutum "SBp/PSBp>2" (Char. 17= 0, Fig. 2C); two pairs of eyes (Char. 22= 1); no trichobothrium on genu I (Char. 30= 0); one solenidium on genu I (Char. 34= 1) and pretarsal claws without a terminal hook (Char. 39= 1, Fig. 3A). ---------- ---------- *Fessonia* Heyden, 1826.

- Posterior sensilla far from the posterior border of the scutum " SBp/PSBp<1" (Char. 17= 1, Fig. 8D); one pair of eyes (Char. 22= 0, Fig 8D); two or more trichobothrium (tr) on genu I (Char. 30= 1 or 2, Fig. 6C); three or more solenidium on genu I (Char. 34= 0, Fig. 6C) and pretarsal claws with a terminal hook (Char. 39= 0, Fig. 6G and H). ---------- 3

3 -Chelicera digit short, S or corkscrew shaped and pointing sideward (Char. 19= 0), short legs, leg III, excluding claw and coxae, with less than 500 micrometers (Char. 23= 0); palps short, with genu not able to extend beyond setae *cs* (Char. 40= 0, Fig. 2F) and seven setae on the palp tarsi due to setae *nc* present (Char. 49= 1, Fig. 2F).---------- *Sphaerotarsus monticulus** Southcott, 1997.

*As explained in the discussion, we suspect that this species is not a *Sphaerotarsus*, but a new genus or the undescribed larvae of *Clavismaris* Southcott, 1963.

- Chelicera digit slender, straight or weekly curved and pointing frontward (Char. 19=1), long legs, leg III, excluding claw and coxae, with more than 600 micrometers (Char. 23= 1); palps long, with at least palp genu extending beyond setae *cs* (Char. 40= 1 or 2, Fig. 3B and C) and six setae on the palp tarsi due to setae *nc* absent (Char. 49= 0, Fig. 3F).---------- 4

4 - Scutum reduced with idiosomal striae covering its borders and always present between Psens and PL (Char. 11= 1, Fig. 3D), Scutum shape poorly defined, kite-like or totally reduced (Char. 10= 1 or 3, Fig 3D and 9F and G); posterior hypostomal setae *bs* smooth (Char. 21= 1, Fig. 9B), palp tibia claw short, with a similar length or shorter than the rest of the palp tibia (Char. 41= 0). ---------- ---------- *Trichosmaris* Southcott, 1963.

- Scutum not reduced or covered by idiosomal striae (Char. 11= 0) and with a trapezoidal shape (Char. 10= 2, Fig. 2B); posterior hypostomal setae *bs* barbed (Char. 21= 1, Fig. 2D), palp tibia claw long, more than two times longer than the rest of the palp tibia (Char. 41= 0). ---------- 5

5 - Anterior hypostomal setae (*as*) short and smooth, much shorter than the posterior (*bs*) (Char. 20= 0, Fig. 9B); the palps are moderately long with only palp genu able to extend beyond setae *cs* (Char. 40= 1, Fig. 3B). ---------- *Sphaerotarsus* Womersley, 1936 (Strict to *Sp. leptopilus*).

- Anterior hypostomal setae (*as*) long, barbed and like the posterior (*bs*) in shape (Char. 20= 1, Fig. 2D); palps very long and robust with palp genu and femur able to extend beyond setae *cs* (Char. 40= 2, Fig. 3C). ---------- *Hirstiosoma* Womersley, 1934.

Artificial key for larval and adult Trichosmaris *species*

1 - Adult ---------- 2

- Larvae ---------- 6

2- Pars clavata= 42–45, ISD<380 ---------- 3

- Pars clavata= 54–76, ISD= 402–577 ---------- 5

3- Anterior sensilar area with 5 short and large setae (Lenght/Width= 1.27–1.35), like those observed in the idiossoma ---------- *Trichosmaris papuana* Beron, 2002* from Papua New Guinea.

- Sensilar area with 10 setae (24–40) long (Lenght/Width= 2.48–4), longer and more parallel-sided than those observed in the idiossoma. ---------- 4

4- GeI length= 252–263, Ti IV longer than Ge IV—*Trichosmaris sp. nov. 2* from Brazil.

-Ge I length= 341–377, Ti IV shorter than Ge IV ---------- *Trichosmaris Jacoti* (Southcott, 1946)* from USA.

5 - Pars clavata= 54–57, Pars clavata/Flagellum= 1.00–1.03, dorsal setae with tectum comparatively shorter, serrations and spicules less proeminent, including the serrations of the carinal flange. ---------- *Trichosmaris dispar* Southcott, 1963 from USA, Mexico and Guatemala.

- Pars clavata≈ 76, Pars clavata/Flagellum= 1.26, dorsal setae with tectum comparatively longer, serrations and spicules more proeminent, including the serrations of the carinal flange. ---------- *Trichosmaris dentella* Southcott, 1963** from Guatemala.

6- PW= 53–78, Asens= 33–40, A-P 15–23 ---------- 7

- PW= 43–49, Asens= 25–26, A-P 9–12 ---------- *Trichosmaris longirostris* comb. nov. (Southcott, 1995) from Costa Rica.

7 - Eupathidium (ζ a) arising from lump on tarsi I (Fig. 6D), bifid eupathidium on tarsi II (Fig. 6G and H) ---------- 8

- Eupathidium (ζ a) arising from from the same level of other setae on tarsi I (Fig. 10 G), without bifid eupathidium on tarsi II (Fig. 10 D). ---------- *Trichosmaris sp. nov. 1* from Brazil.

8- AW= 54–59, PW= 77–78. ---------- *Trichosmaris dispar* Southcott, 1963 from USA, Mexico and Guatemala.

- AW= 40–44, PW= 53–65. ----------*Trichosmaris sp. nov. 2* from Brazil.

*Based only on male.

** Based only on female.

Discussion

New generic definitions for Hirstiosoma *and* Trichosmaris *larvae*

The discovery of *Trichosmaris* larvae and the phylogenetic analyses motivated by them led us to propose a definition to the larvae of this genus and *Hirstiosoma*. A definition for *Hirstiosoma* Womersley, 1934 based on the characters included in this study is as follow: compound trichobothrial pit present in the distal part of tarsi I (Char. 27= 1) including setae *ta* and *x* (Fig. 3A, 6D, 10G, and 12D); single eye pair (Char. 22= 0); absence of the long and basal setae *nc* on palp tarsi (Char. 49= 0, Fig. 2F) leading to palp tarsi with only six setae; Hypostomal setae *as* and *bs* homomorphic and setulose (Char. 20 and 21= 0, Fig. 2D); palps robust and long, with palp femur extending beyond the aboral setae (*cs*) (Char. 40= 2, Fig. 3C). Now, the genus includes the nine species originally described as *Hirstiosoma* and two new combination *Hirstiosoma baenai* comb. nov. (Mayoral & Barranco, 2017) and *H. quercus* comb. nov. (Yazdanpanah, Saboori & Hakimitabar, 2016).

Concerning the newly discovered *Trichosmaris* Southcott, 1963 larvae, they share with *Hirstiosoma* and *Sphaerotarsus leptopilus* the compound trichobothrial pit on distal tarsi I (Char. 27=

1, Fig. 3A, 6D, 10G, and 12D), single pair of eyes (Char. 22= 0), and absence of the long and basal setae *nc* (Char. 49= 0, Fig. 2F), hence counting six setae on palp tarsi (Fig. 3F). In addition, exclusive to *Trichosmaris*: scutum with borders and the area between Psens and PL covered by idiosomal striae (Char. 11= 1); shape of scutum either undefined due the idissomal striae over borders (Fig. 9 A and G, or partially reduced with a lozenge or kite-like shape (Char. 10= 3 or 1, respectively; Figs 3D, 8D and F); the legs are long, with leg III more than 920 (Char. 23= 1 ">620"); palp slim and long, with palp genu, but not femur, extending beyond setae *cs* (Fig. 3B, Char. 40= 1). It is important to note that the plesiomorphic state (Char. 20= 1) of setae the oral setae (*as*) short and smooth, while the hypostomal (*bs*) is long and smooth (Char. 21= 1) are still present (Fig. 8B and 9B). Differing the *Trichosmaris* larvae from the most derivate Hirstiosomatinae (Fig. 5). The genus now includes six species originally described as *Trichosmaris* and a new combination *Trichosmaris longirostris* comb. nov. (Southcott, 1995).

Are the Hirstiosomatinae larvae really parasites?

The trends observed in the gnathosomal evolution and reinforced by the sensorial and locomotory organs (Fig. 5) apparently doesn't fit to a parasitic life style: mainly, the long palps and palp tibia claw and its basal tooth seems to be better suited to predation, something to be verified by running experiments while rearing these mites, offering potential preys. It could reveal a rare case of reversal from a parasitic to free living larvae in Parasitengona, which would explain why those larvae are rarely found attached to their host despite decades of studies.

The polyphyletic Sphaerotarsus

The polyphyletic distribution of *Sphaerotarsus* along the Smarididae phylogeny lead us to propose some modifications in their classification. For instance, *Sphaerotarsus monticolus* and *Sp. leptopilus* were not recovered monophyletic. *Sp. monticulus* phylogenetic position (Fig. 5, S. Fig. 3 and 4) and its morphological characters lead us to conclude that it consists in an undescribed Smarididae genus. Alternatively, it may also consist in the larval instar of *Clavismaris,* the only

Hirstiosomatinae genus known based only on adults after the present work. *Sphaerotarsus monticulus* radically differs from all other larval Hirstiosomatinae by the presence of seven setae on palp tarsi due to setae *nc* present (Char. 49= 1) (Vs. absent), short legs with leg III with 490 micrometers or less (Char. 23= 0) (Vs. longer than 620) and short palps with palp genu not extending beyond the setae *cs* (Char. 40= 0) (Vs. genu and/or femur extending beyond *cs*). In addition, *Sp. monticulus* differs from the paraphyletic Smaridinae due to a single pair of eyes (Char. 22= 0) and the other numerous synapomorphies of Hirstiosomatinae (Fig. 5). *Sphaerotarsus monticulus* also differs from *Sp. leptopilus* due to the posterior hypostomal setae smooth (Vs. barbed in *Sp. leptopilus*) (Char. 21= 1).

Although Southcott (1961) provided a long definition for the *Sphaerotarsus* larvae based on *Sp. leptopilus*, the diagnosis for larval *Sphaerotarsus* became reduced and more inclusive to fit the newly discovered larval species. For instance, the diagnosis of Southcott (1997) was much shorter and did not have characteristics like "Anterolateral hypostomala simple; posteromedial hypostomala stronger, pointed, ciliated" (Southcott,1961) that is not observed in any of the recently described species. Moreover, *Sphaerotarsus allmani*, the type species of *Sphaerotarsus* is known based only on adults and can only be compared with *Sp. leptopilus* making this species the most accurate representant of the *Sphareaotarsus* larvae.

In conclusion, in the present work the new combinations of *H. quercus* comb. nov. and *H. baenai* was proposed. However, to solve the classification problem caused by the paraphyly of *Sp. monticulus* and *Sp. leptopilus* (Fig. 5) molecular data or rearing experiments associating the instars of *Sp. monticulus* and/or *Sp. allmani* are necessary. Until such study is done, we recommend that Southcott`s (1961) definition for larval *Sphaerotarsus* should be used to identify and describe new *Sphaerotarsus* larvae.

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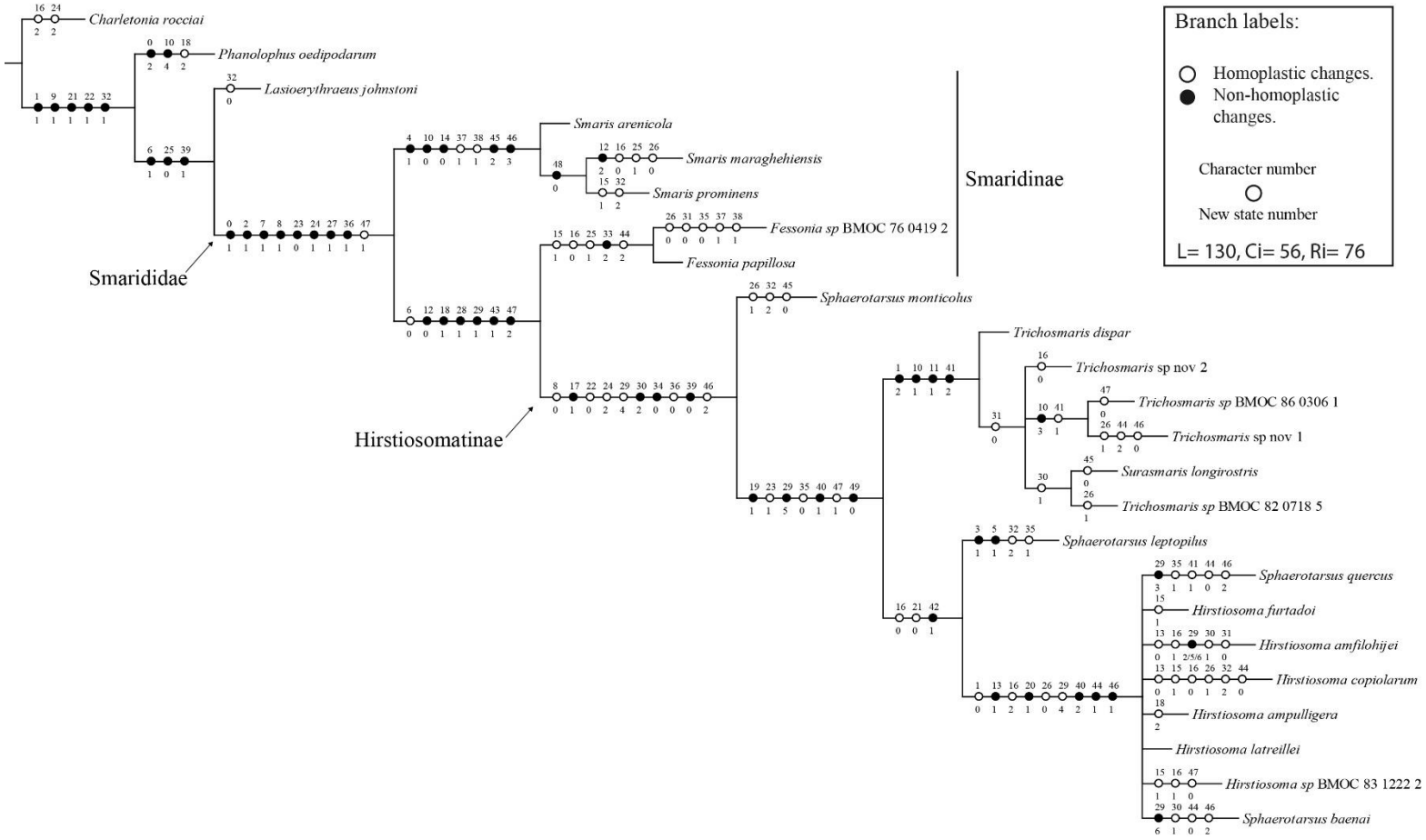
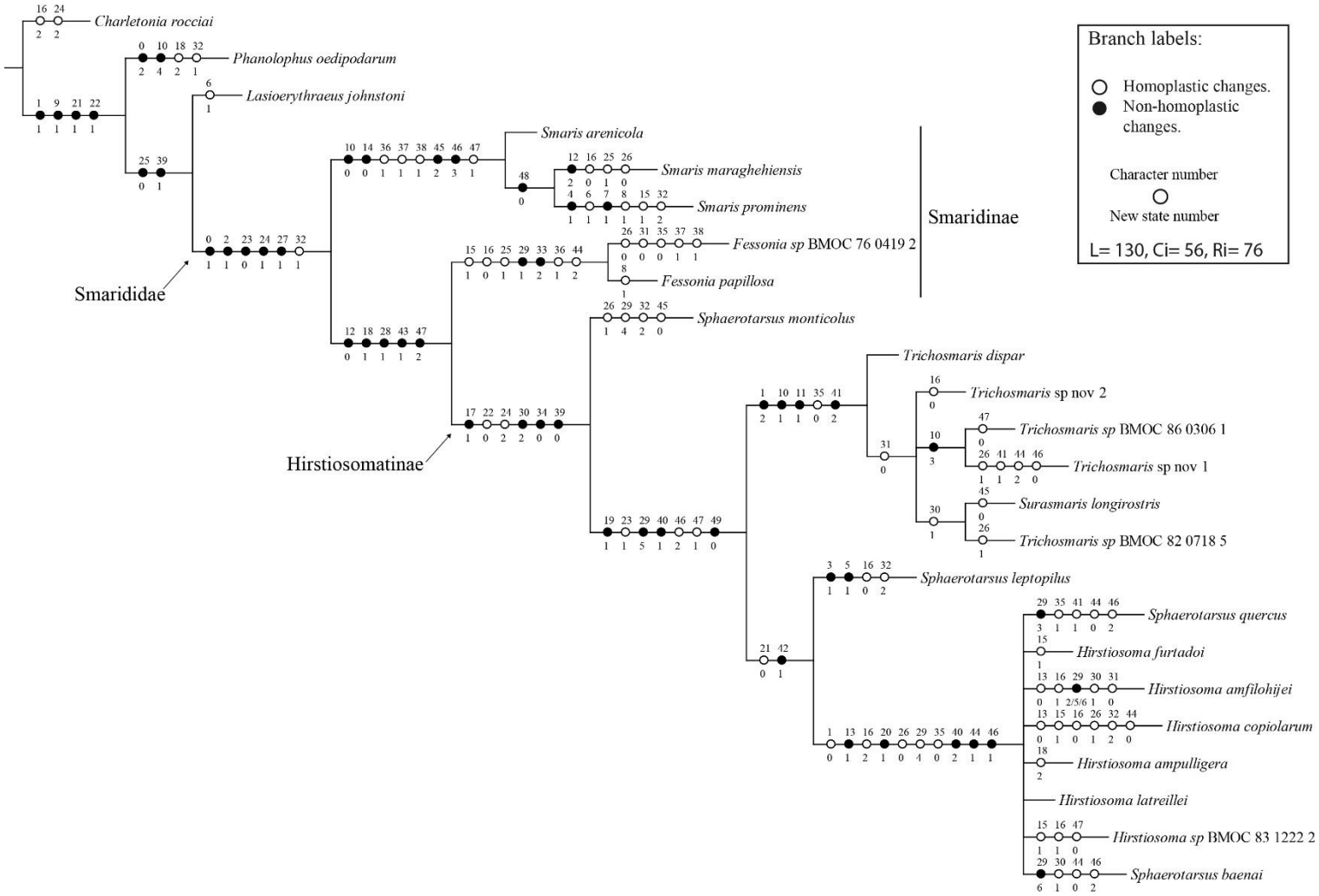
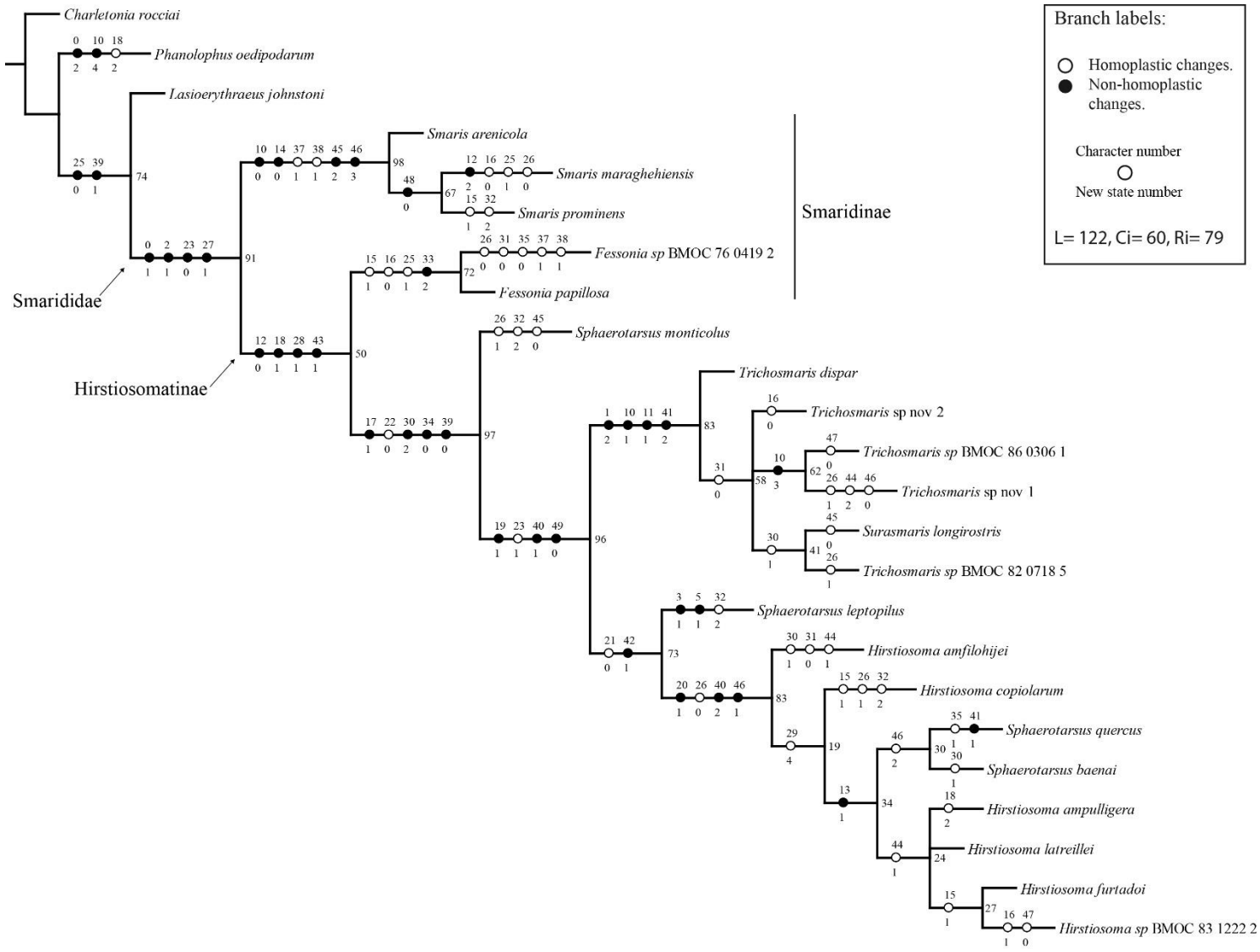
Supplementary Material



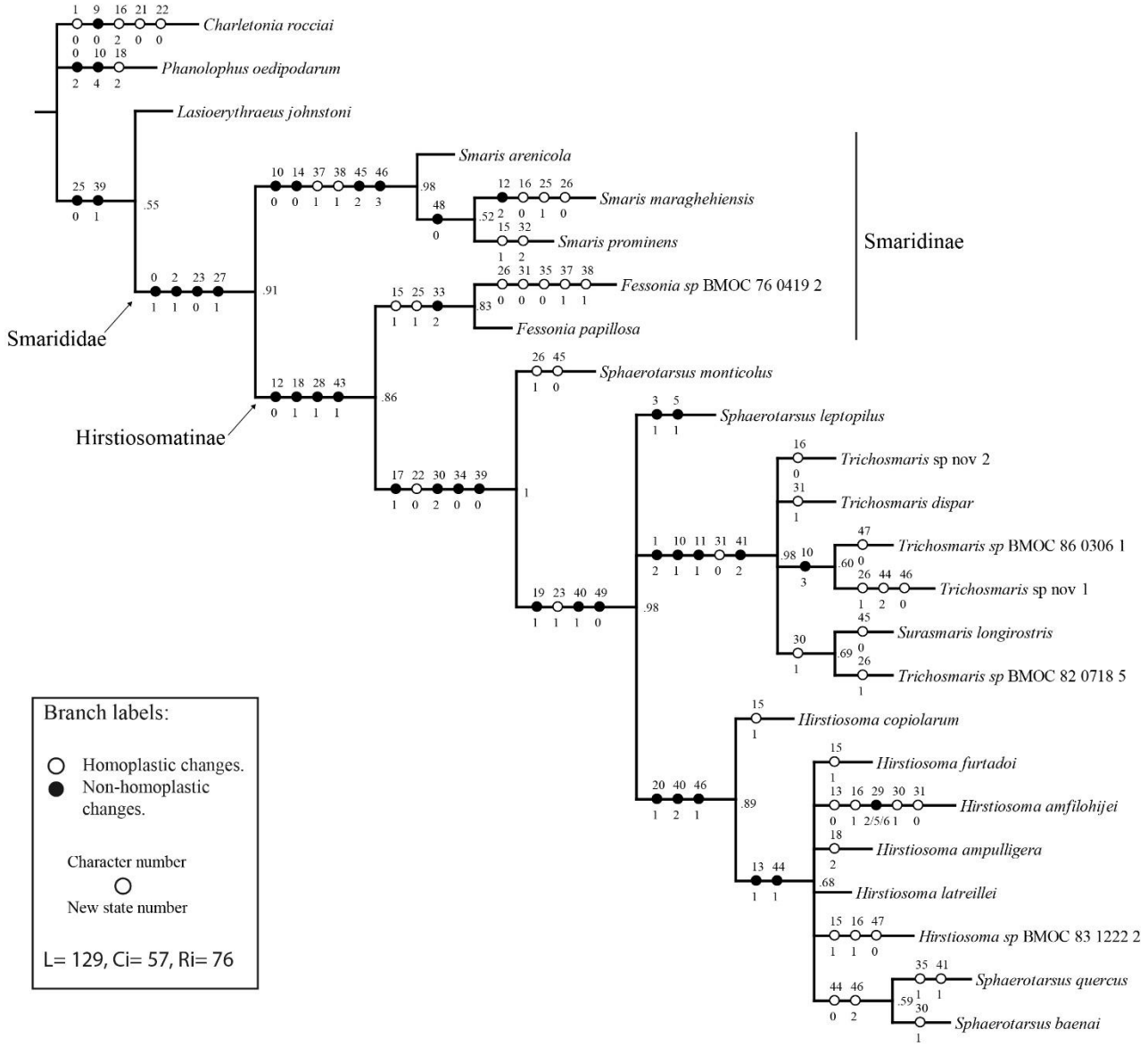
Supplementary figure I – Smaridididae phylogeny based on species with known larvae. The tree is the strict consensus of 8 trees with length of 12 steps, which were inferred by 100 thousand Tree Bisection and Reconnection (TBR) replicates saving 10 trees per replicate, followed by SPR of the saved trees. The characters optimization is showing only unambiguous changes. See Figure 5 for support values.



Supplementary figure II – Smarididae phylogenetic tree based on species with known larvae. The tree is the strict consensus of 8 trees with length of 122 steps, which were inferred by 100 thousand Tree Bisection and Reconnection (TBR) replicates saving 10 trees per replicate, followed by SPR of the saved trees. The characters optimization is showing only unambiguous changes. See figure 5 for support values.



Supplementary figure III – Smarididae phylogenetic tree based on species with known larvae. A single tree was inferred based on 100 thousand Tree Bisection and Reconnection (TBR) replicates saving 10 trees per replicate, followed by SPR of the saved trees and employing implied weights. The characters optimization is showing only unambiguous changes. Support values were calculated by symmetric resampling (P=33) with one thousand replicates, each with 100 replicates of TBR saving 10 trees per replicate.



Supplementary figure IV – Smarididae phylogenetic tree based on species with known larvae. The tree was inferred employing discrete (morphology) model (Lewis, 2001). The support values are based on the posterior probability. The characters optimization is showing only unambiguous changes.