UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Zoologia

Samuel Geremias dos Santos Costa

FILOGENIA E TAXONOMIA INTEGRATIVA DE ÁCAROS PARASITENGONA (ACARI: TROMBIDIFORMES) TERRESTRES

Belo Horizonte – Minas Gerais

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Samuel Geremias dos Santos Costa

FILOGENIA E TAXONOMIA INTEGRATIVA DE ÁCAROS PARASITENGONA (ACARI: TROMBIDIFORMES) TERRESTRES

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Orientador: Dr. Almir Rogério Pepato

Coorientador: Dr. Pavel Klimov

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ATA DE DEFESA DE DISSERTAÇÃO / TESE

Às quatorze horas do dia Dezoito de Agosto de dois mil e vinte e três reais, utilizando-se a plataforma Teams, realizou-se a sessão pública para a defesa da tese intitulada: **Filogenia e taxonomia integrativa de ácaros Parasitengona (Acari: Trombidiformes) terrestres** de **Samuel Geremias dos Santos Costa.** A presidência da sessão coube ao Dr. **Almir Rogério Pepato**, orientador. Inicialmente, o presidente fez a apresentação da Comissão Examinadora assim constituída: **Dra Liana Johann** (Universidade do Vale do Taquari), **Dr. Luiz Gustavo Almeida Pedroso** (Universidade Estadual Paulista), **Dr. Fernando Araujo Perini** (Universidade Federal de Minas Gerais) e Dr. **Kirstern Lica Follmann Haseyama** (Universidade Federal de Minas Gerais). Em seguida, o candidato fez a apresentação do trabalho que constitui sua **Tese de Doutorado.** Seguiu-se a arguição pelos examinadores e, logo após, a Comissão reuniu-se, sem a presença do candidato e do público, e decidiu considerar **aprovada** a **Tese de Doutorado**. O resultado final foi comunicado publicamente ao candidato pelo presidente da Comissão. Nada mais havendo a tratar, o presidente encerrou a sessão e lavrou a presente ata que, depois de lida, se aprovada, será assinada pela Comissão Examinadora.

Belo Horizonte, 21 de agosto de 2023.

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Aos meus pais, por mostrarem o caminho das Ciências Naturais.

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***So many mites, so little time*!**

Barry M. O’Connor

RESUMO

Os ácaros Parasitengona são um grupo mega diverso com mais de 11.000 espécies descritas, que podem representar uma pequena fração das linhagens existentes. Os Parasitengona são encontrados em todos os continentes, exceto Antártica, sua fauna é particularmente desconhecida na região Neotropical, África e Ásia devido à concentração histórica de especialistas na Europa, América do Norte e Austrália. O reduzido número de especialistas é um fator limitante para taxonomia dos Parasitengona, especialmente quanto a paleontologia, onde apenas 4 autores publicaram mais que um artigo entre 2000 e 2020. Os Parasitengona Possuem como principal característica o ciclo de vida, que consiste em ovos, uma pré larva imóvel, uma larva parasita, protoninfa imóvel, deutoninfas predadora de vida livre, tritofinfa imóvel e adultos predadores de vida livre. Com exceção de uma família (Allotanaupodidae), cuja larva morfologicamente semelhante aos adultos é descrita pela primeira vez no presente trabalho, uma drástica mudança morfológica ocorre entre as larvas e deutoninfas. O que torna sua associação desafiadora e faz com que muitas sejam descritas com base apenas na larva ou nas fases pós larvais. Para remediar esse problema utilizamos a criação e sequências de DNA para associar espécimes hetermórficos. A classificação dos Parasitengona é controversa, com diferentes autores considerando de 3 até 9 superfamílias e de 18 a 20 famílias. Isso se deve à falta de uma filogenia abrangente que permita que as propostas de classificação convirjam no sentido de refletir as relações evolutivas. Um problema que se destaca nas linhagens terrestres, uma vez que o estudo mais abrangente publicado até então focou nas linhagens aquaticas. O presente estudo visa descrever parte da biodiversidade dos Parasitengona recentes e um fóssil, incluindo dados morfológicos, ecológicos, ontogenéticos e moleculares. Em seguida, são inferidas suas relações filogenéticas e um novo sistema de classificação é proposto.

Palavras-chave: Filogenia, Taxonomia Integrativa, Erythraeidae, Johnstonianidae, Smarididae

ABSTRACT

Parasitengona mites are a mega diverse group with more than 11,000 described species, which may represent a small fraction of existing lineages. Parasitengona are found on all continents except Antarctica. The Parasitengona mite fauna is particularly unknown in the Neotropics, Africa and Asia due to the historical concentration of specialists in Europe, North America and Australia. The reduced number of specialists is a limiting factor for the taxonomy of Parasitengona, especially regarding paleontology, where I estimate that there are less than 10 specialists currently working. The Parasitengona have as their main characteristic the life cycle. This consists of eggs, an immobile prelarva, a parasitic larva, immobile protonymph, free-living predatory deutonymphs, immobile tritonymph, and free-living predatory adults. With the exception of Allotanaupodidae, whose larva morphologically similar to adults is described for the first time in the present work, a drastic morphological change occurs between larvae and deutonymphs. That makes their association challenging and causes many to be described based only on the larva or the adults. To remedy this problem, we use DNA creation and sequencing to associate heteromorphic specimens. The classification of Parasitengona is controversial. With different authors considering from 3 to 9 superfamilies and from 18 to 20 families. This is due to the lack of a comprehensive phylogeny that would allow classification proposals to converge towards reflecting evolutionary relationships. A problem that stands out in terrestrial lineages, since the most comprehensive study published so far focused on aquatic lineages. The present study aims to describe part of the biodiversity of recent Parasitengona and a fossil, including morphological, ecological, ontogenetic and molecular data. Then their phylogenetic relationships are inferred and a new classification system is proposed.

Key words: Phylogeny, Itegrative Taxonomy, Erythraeidae, Johnstonianidae, Smarididae

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1c – Segunda seta da 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

ACW – Largura da área sensillar anterior.

AP – Distância entre as setas comuns anterolateral e posterolateral do scutum.

as – Seta hipostomal anterior

AW – Distância entre a base das setas normais anteriores do scutum.

B – Seta barbada comum

BFe – Basifêmur

bs – Seta hipostomal posterior

Cp – Seta companheira

cs – Galeala (seta adoral)

CX – Coxa

DS – Comprimento das setas dorsais

EC-EC – Distância entre o centro dos olhos.

EC-*Vi* – Distância entre uma linha ligando o centro dos olhos e uma linha ligando as bases das sensilas anterior.

ECO – Lente do olho

elcl – Micro seta da coxa I

elcp – Micro seta da coxa do palpo

Flagellum – Porsão distal lisa da sensilla posterior de *Trichosmaris*.

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

Ge – Genu

IL – Comprimento do idiossoma

IP – Soma do comprimento das pernas

ISD – Distância entre as bases das setas sensilares anteriores e posteriores

IW – Largura do idiossoma

L – Comprimento Máximo do scutum

legI/legII – Razão entre o comprimento da Perna I e da Perna II. Esse formato também se aplica a outros segmentos ou apêndices. Ex.: Ti I/ Ti II.

ML – Seta comum mediana do scutum

MW – Distância entre as bases das setas normais medianas do scutum.

N – Seta lisa comum.

Pars clavata – Porção proximal com barbulas da sensilla posterior de *Trichosmaris*.

PW – Distância entre as setas normais posteriores do scutum

PCW – Largura da área sensillar posterior.

Sba – Distância entre as bases das sensilas anteriores do scutum

Sbp – Distância entre as bases das sensilas posteriores do scutum

se ou PL – Seta comum posterolateral do scutum

si ou PSE – Sensila posterior ou comprimento da sensila posterior

Ta – Tarso, comprimento

TFe – Telofêmur

Ti – Tíbia

Tr – Trocânter

ve ou AL – Seta comum anterolateral do scutum

vi ou ASE – Sensila anterior ou comprimento da sensila anterior

W – Largura Máxima do scutum

ε – Fâmulo (Uma pequena seta quimiorreceptora)

ζ – Eupatídio (Um quimiorreceptor com um poro terminal)

ζ – Eupatídio.

κ – Micro seta.

σ – Solenídio no genu.

φ – Solenídio (Um quimiorreceptor com poros ao longo de sua extensão) da tíbia.

ω – Solenídio no tarso.

Sumário

1 INTRODUÇÃO

1.1 Introdução geral

Os ácaros Parasitengona são um grupo mega diverso, com relações filogenéticas pouco conhecidas e que constantemente nos surpreende com novas famílias, características morfológicas inesperadas, características ecológicas e até mesmo ontogenéticas inéditas (ex.: Allotanaupodidae capítulo 1.4), principalmente ao explorarmos regiões pouco estudadas como o Neotrópico. Devido a isso, a presente tese teve como objetivo inicial explorar a biodiversidade dos ácaros Parasitengona recentes e fósseis com uma abordagem taxonômica que empregasse diversas fontes de dados úteis disponíveis. Entre estes dados, temos dados moleculares, morfológicos, ecológicos, filogenéticos, estratigráficos, entre outros. Portanto, a exploração se deu início sem que se soubesse quais táxons ou que novidades seriam encontradas em regiões não mapeadas no universo dos ácaros Parasitengona.

A classificação dos Parasitengona é controversa, com diferentes autores considerando de 3 até 9 superfamílias e de 18 a 20 famílias. Isso ocorre, entre outras razões, devido à falta de um estudo filogenético abrangente que sirva de base para classificação. Portanto, a inferência das relações filogenéticas entre os Parasitengona terrestres foi proposta neste trabalho, pois essa permite classificar nossos achados taxonômicos em um sistema que reflita nosso conhecimento sobre a evolução. Isso é importante, pois se diferentes grupos de pesquisa seguirem com suas propostas de classificações buscando refletir a história evolutiva das linhagens, elas tendem a convergir em uma única, amplamente aceita e com sentido biológico. Além disso, cada táxon fóssil é um retrato do passado, que nos permite entender melhor a evolução e a diversidade que observamos hoje.

À medida que amostras foram triadas, coletadas, recebidas do exterior e identificadas, foram encontradas novas espécies e características morfológicas incomuns. Algumas características tão pouco usuais que provocaram debates sobre sua interpretação, como a presença de um escudo dorsal no fóssil Erythraeinae gen. et sp. nov. (Cap. 1.2.1), táxons que a princípio não se enquadravam bem em nenhum gênero conhecido como *Rainbowia* sp. nov. (Cap. 1.2.2), além de dados moleculares inéditos, que após analisados nos levaram a propor uma grande mudança na classificação das superfamílias (Cap. 2). Como resultado do estudo exploratório inicial, hipóteses foram formuladas, sumarizadas na seção de objetivos e testadas ao longo da tese. A liberdade exploratória empregada no desenvolvimento deste trabalho é um

requisito fundamental para lidar com um táxon tão diverso, onde cada pá de serapilheira abre uma nova janela em um mundo pouco explorado.

A tese encontra-se dividida em três partições: no capítulo um são descritos novos táxons;no capítulo dois é apresentada a filogenias dos ácaros Parasitengona com foco nos terrestres; na conclusão, a classificação dos táxons do capítulo 1 famílias e superfamílias é reavaliada.

1.2 Classificação corrente dos ácaros e hipóteses a respeito da sua filogenia.

Tradicionalmente, os acarólogos consideram Acari uma subclasse composta por duas superordens: Parasitiformes e Acariformes, que juntas incluem mais de 55 mil espécies descritas (Krantz & Walter, 2009). Os dois grupos são facilmente diagnosticados, o primeiro compartilha a presença de estigmas (poros respiratórios) localizados posteriormente a coxa II (coxas móveis), o tarso da perna I possui um agrupamento de setas quimiorreceptoras subdistal e o tarso do palpo com apotele (garra). Enquanto o segundo não possui estigmas em posição posterior a coxa II, suas coxas são fundidas ao corpo, o tarso da perna I possui as setas quimiorreceptoras sensoriais não agrupadas e são destituídos de apotele no tarso do palpo (Krantz & Walter, 2009).

Se a distinção entre os dois grandes grupos de ácaros baseia-se em um grande número de características, o monofiletismo de Acari é apoiado apenas (1) pela divisão do corpo em um gnatossoma (pseudotagma que inclui a quelícera, palpo com as coxas fundidas, boca e faringe) e um idiossoma (formado pelo restante das estruturas dos segmentos que portam quelícera e palpos e os demais segmentos do corpo, completamente fundidos ou com apenas vestígios da segmentação ancestral); e (2) larvas hexápodas (Lindquist 1984).

Ainda que trabalhos morfológicos anteriores, como o de Van der Hammen (1989), já tenham apontado para o não monofiletismo de Acari, foi com a entrada em cena dos dados moleculares que o monofiletismo dos ácaros se tornou especialmente contencioso, com trabalhos recuperando o clado (por exemplo, Lozano-Fernandez *et al.* 2019) e outros que o rejeitam, recuperando cada superordem como irmã de diferentes ordens de aracnídeos (exemplos: Ballesteros & Sharma, 2019 e Pepato & Klimov, 2015).

Historicamente, várias ordens de aracnídeos foram propostas como irmãs dos Acariformes, além é claro dos ácaros Parasitiformes: Pseudoscorpiones (Arribas *et al.*, 2019),

Solifugae (Pepato *et al.*, 2010; Dabert *et al.*, 2010), Palpigradi (van der Hammen, 1982) e Ricinulei (Shultz,2007).

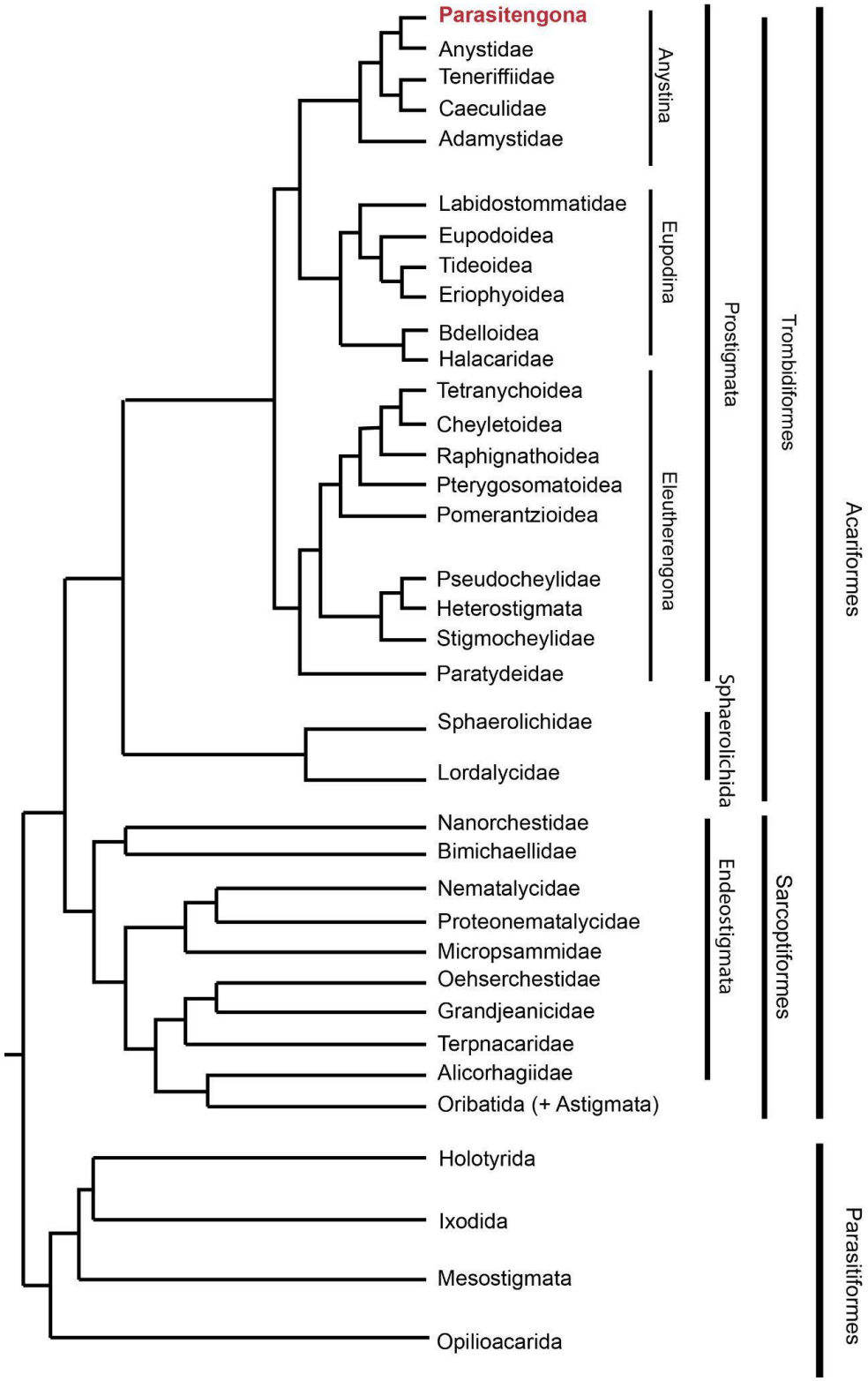

Figura 1. Classificação e relações filogenéticas de Acari tradicionalmente aceitas. Parasitengona indicado em vermelho. Modificado de Norton *et al.* (1993).

A figura 1 ilustra uma tendência de retratar a filogenia dos Acariformes com uma divergência basal dando origem a duas linhagens principais. A primeira, Trombidiformes, incluí o clado Prostigmata e Sphaerolichida. Já a segunda, Sarcoptiformes, incluindo todas as linhagens basais conhecidas coletivamente como Endeostigmata, os Oribatida. Entre os Oribatida encontramos ácaros de vida livre de solo incluindo a diversificada linhagem dos Astigmatas, que compreende ácaros da poeira doméstica, de ninhos e parasitas de diferentes grupos de animais (O'Connor, 1984).

Quanto aos Trombidiformes, temos Sphaerolichida como ramo basal, e três linhagens principais entre os Prostigmata: Anystina, incluindo Parasitengona e famílias como Anystidae, Teneriffidae e Caeculidae; Eupodina, incluindo Eriophyoidea, Bdelloidea, Halacaroidea, Labidostomatidae e Eupodoidea; Eleutheringona, incluindo Raphignathoidea, Cheyletoidea, Tetranychoidea e Heterostigmata. Pode ou não incluir Paratydeidae, Pseudocheylidae, Pomerantzoidea e Stigmocheylidae, que caso contrário são incluídos em Anystina (Norton *et al.*, 1993; Lindquist, 1996).

Há duas hipóteses sobre as relações entre as principais linhagens das árvores. A expressa por Lindquist (1996) assume estados de caráter, como a presença de um complexo garra dedão no palpo e redução do dedo fixo das quelíceras, como apomorfias unindo Anystina e Eleutheringona. A alternativa, proposta por Kethley e mencionada em Norton *et al.* (1993), aponta para essa característica como homoplástica ainda que não apresente uma discussão completa dos caracteres que sustentam essa suposição e nem uma análise filogenética formal.

Trabalhos recentes utilizando dados moleculares subverteram completamente o quadro tradicional expresso acima, com a exceção a maior parte das relações filogenéticas entre as linhagens de Sarcoptiformes, recuperada com uma topologia muito semelhante à de O'Connor (1984).

No que diz respeito às divergências basais da árvore Acariformes, Dabert *et al.* (2010), empregando apenas os genes 18S e COI, recuperaram a divisão em Trombidiformes e Sarcoptiformes, incluindo Endeostigmata neste último. Já Pepato & Klimov (2015), em análises empregando as subunidades pequena e grande quase completas de RNA ribossômico, recuperaram uma fração de Endeostigmata, compreendendo Alycidae, Nanorchestidae e Nematalycidae, basal em relação a todos os outros Acariformes. Em 2017, Xue e colegas, não incluíram Endeostigmata ao analisar mitogenomas de ácaros acariformes, mas também

recuperaram duas linhagens basais, com Eriophyoidea como Sarcoptiformes basais, ao invés de Trombidiformes. A exclusão ou inclusão dessa diversificada superfamília de ácaros fitófagos, que inclui alguns formadores de galhas, é debate que segue até hoje, ainda que exista crescente evidência molecular e morfológica para a considerá-los como grupo-irmão dos também vermiformes Nematalycidae (Bolton *et. al.* 2023)

A provável exclusão de Eriophyoidea não foi o único golpe dos dados moleculares no conceito de Eupodina. Pepato & Klimov (2015), empregando sequências dos genes nucleares ribossomais, apontaram pela primeira vez os ácaros Halacaroidea (principalmente marinhos) como grupo-irmão dos Parasitengona, resultado também recuperado por Dabert e colaboradores no ano seguinte (Dabert *et al.*, 2016). Além disso, Labidostomatidae tem sido sistematicamente recuperado como basal em relação às demais linhagens de Trombidiformes, enquanto as linhagens restantes de Eupodina (Eupodoidea + Tydeoidea) e Bdelloidea, não são recuperados em um grupo monofilético, ainda que as suas afinidades não recebam um suporte robusto.

O conceito de Anystina também não é suportado pelos trabalhos moleculares. As famílias tradicionalmente incluídas neste grupo são recuperadas em um grande clado compreendendo Halacaroidea, Parasitengona e todas as famílias tradicionalmente incluídas em Eleutherengona. Isso sugere que torna a redução do dígito fixo e a presença do complexo polegar-garra surgiu uma única vez, embora essa característica tenha se perdido em algumas linhagens, conforme sugerido por Lindquist (1996). De qualquer forma, há crescente evidência para um clado formado por Halacaroidea, Parasitengona com a família Anystidae como seu grupo irmão (Pepato *et al.* 2022). Isso facilita os trabalhos que abordam as relações filogenéticas entre os Parasitengona, pois os coloca em uma posição bem suportada dentre os Trombidiformes. Na figura 2, a classificação dos ácaros a luz dos dados moleculares mais recentes é resumida.

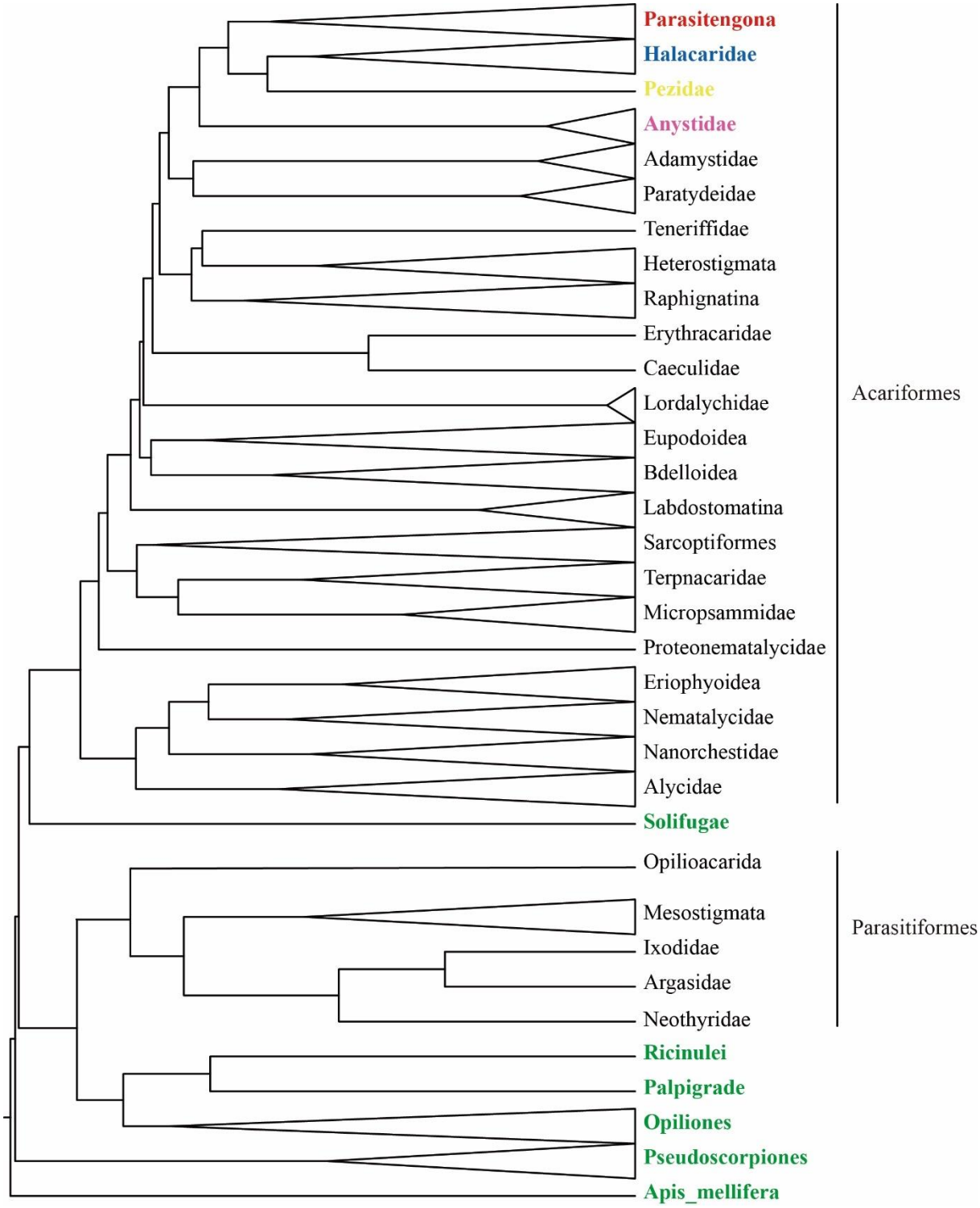

Figura 2. Classificação e relações filogenéticas de Acari. Grupos externos indicados em verde. Anystidae, Pezidae Halacaridae e Parasitengona indicados em rosa, amarelo, azul e vermelho, respectivamente. Adaptada de Pepato *et al*. (2022).

2.2. Os Parasitengona: composição e relações filogenéticas

A subcoorte Parasitengona (Acariformes, Prostigmata) é particularmente diversificada, com mais de 11 mil espécies descritas distribuídas globalmente (Konikiewicz *et al.* 2016). Os ácaros Parasitengona (Acariformes, Prostigmata) são encontrados em todos os continentes, exceto Antártica, e são comuns no solo, tanto na superfície quanto em cavernas, na água doce e com uma linhagem (Pontarachnidae) frequente no infralitoral marinho e águas salobras estuarinas. Sua característica principal é o ciclo de vida composto por sete fases distintas (Fig. 3): ovo, pré-larva caliptostática (imóvel), larva ectoparasita de artrópodes ou vertebrados (com algumas exceções predadoras), protoninfa caliptostática, deutoninfa predadora de vida livre, tritoninfa caliptostática e adultos predadores de vida livre (Krantz & Walter, 2009; Wohltmann, 2000). Devido à grande diferença morfológica entre o estágio larval e os pós-larvais, sua associação se torna um desafio que resultou na maioria das descrições terem sido baseadas em apenas um estágio. A manutenção de culturas, o sequenciamento e análise de sequências de DNA têm sido aplicados para associar os estágios heteromórficos, espécimes com dimorfismo sexual, entre outros polimorfismos (Ex.: Costa *et al.* 2019, 2021 e o presente estudo).

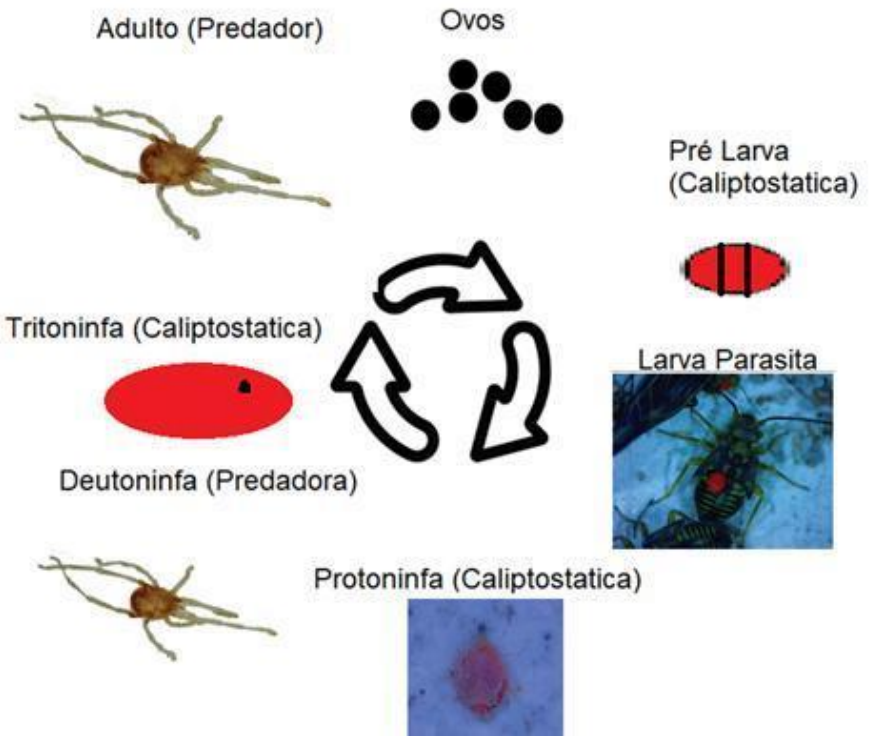

Figura 3. Ciclo de vida de *Charletonia rocciai* Treat & Flechtmann, 1979

Apesar de sua diversidade, a taxonomia dos Parasitengona é limitada pelo pequeno número de especialistas que historicamente se concentram na Europa, América do Norte e Austrália e, devido a isso, a maioria das espécies descritas são dessas regiões (Mąkol & Wohtmann 2012). Um fenômeno que é especialmente notável na paleontologia de Parasitengona, tópico no qual apenas 4 autores (Konikiewicz, Wohltmann, Mąkol e Dunlop) publicaram mais que um artigo entre 2000 e 2023, algumas vezes como um único grupo de pesquisa (Dunlop *et al.* 2023, Bartel *et al.* 2015).

A falta de conhecimento sobre a biodiversidade e representatividade das faunas de outras regiões dificulta a classificação de táxons morfologicamente distintos e limita a inferência filogenética entre os grandes grupos devido a subamostragem.

2.3 Famílias de Parasitengona: Uma Visão Geral das Linhagens Terrestres

Não há consenso quanto ao número exato de famílias e a classificação em geral dos ácaros Parasitengona. Mąkol & Wohtmann (2012), Nielsen *et al.* (2021) e Smit (2020) somados, listam 20 famílias terrestres, três das quais monotípicas. Ou 18 famílias se considerarmos Eutrombidiinae Thor, 1935 e Leeuwenhoekiinae Womersley, 1944 como subfamílias de Microtrombidiidae e Trombiculidae, respectivamente, ao invés de famílias independentes, como defendido por Mąkol *et al.* 2021 e Kudryashova (1998). Além disso, são conhecidas aproximadamente 11 famílias aquaticas, segundo Smit (2020).

A classificação das famílias terrestres em superfamílias é motivo de debate com diferentes autores considerando mais ou menos grupos. Por exemplo, em adição aos ácaros parasitas de vertebrados (Trombiculoidea), Zhang (1998) e Zhang & Fan (2007) propuseram sete superfamílias terrestres. Enquanto Söller *et al.* 2001 considerou apenas três (Calyptostomatoidea, Erythraeoidea e Trombidioidea). No presente estudo consideramos 18 famílias terrestres conforme ilustrado na Tabela I, onde também são comparadas as propostas de Söller *et al.* 2001, Zhang (1998) and Zhang & Fan (2007).

Em nosso estudo filogenético apresentado no capítulo 2, foram amostradas 14 das 15 famílias não monotípicas de Parasitengona terrestres, o que significa a adição de 6 famílias aos trabalhos mais abrangentes já publicados (Dabert *et al.* 2016 e Pepato *et al.* 2022). As famílias e sua distribuição geográfica são listadas na tabela I, criada com base em dados de Söller *et al.* 2001, Nielsen *et al.* (2021) e Smit (2020).

Graças a parcerias com diversos pesquisadores internacionais foi possível amostrar dados morfológicos e moleculares de Parasitengona coletados em todos os continentes onde ocorrem, exceto África. Na figura 4, é ilustrada a distribuição e o número de táxons coletados em cada localidade e que estão depositados no Centro de Coleções Taxonômicas da UFMG.

Tabela I – Famílias de Parasitengona terrestres. Dois sistemas de classificação alternativos são apresentados na coluna 1 e 2. As 14 famílias amostradas na filogenia molecular apresentada no capítulo 2 são indicadas na coluna 6. Distribuição geográfica global se refere a todos os continentes exceto a Antártica. A classificação das famílias considerou Eutrombidiinae Thor, 1935 (parte de Microtrombidiidae) e Leeuwenhoekiinae Womersley, 1944 como subfamilia de Trombiculidae em concordância com Kudryashova (1998).

Classificação das superfamílias segundo:		**Família**	**Autor**	**Distribuição Geográfica**	**Amostrada**	**Subfamílias amostradas / existentes**
Söller *et al.* 2001	Zhang (1998) e Zhang & Fan (2007)					
Calyptostomatoidea	Calyptostomatoidea	Calyptostomatidae	Oudemans, 1923	Global	Sim	1/1
Erythraeoidea	Erythraeoidea	Erythraeidae	Robineau-Desvoidy, 1828	Global	Sim	5/7
		Smarididae	Vitzthum, 1929	Global	Sim	2/2
Trombidioidea	Chyzeroidea	Chyzeriidae	Womersley, 1954	Global, exceto América do Norte.	Sim	2/3
	Trombidioidea	Microtrombidiidae*	Thor, 1935	Global	Sim	3/3
		Neothrombiidae	Feider, 1959	Global	Sim	1/1
		Trombidiidae	Leach, 1815	Global	Sim	1/4
	-	Podothrombiidae	Thor, 1935	Hemisfério norte com uma espécie na Austrália.	Sim	1/1
	-	Achaemenothrombiidae	Saboori, Wohltmann & Hakimit	Irã	Sim	1/1

			abar (2010)			
	Amphothrombioidea	Amphotrombiidae (monotípica)	Zhang, 1998	EUA	Não	0/1
	Allotanaupodoidea	Allotanaupodidae	Zhang & Fan, 2007	Nova Zelândia e Argentina	Sim	1/2
	Tanaupodoidea	Tanaupodidae	Thor, 1935	Hemisfério norte	Não	0/1
	Yurebilloidea	Yurebillidae (monotípica)	Southcott, 1996	Australia	Não	0/1
	Trombiculoidea	Audyanidae (monotípica)	Southcott, 1987	Malásia	Não	0/1
		Johnstonianidae	Thor, 1935	Global	Sim	1/2
		Trombellidae	Thor, 1935	Global	Sim	1/5
		Neotrombidiidae	Feider, 1955	Global	Sim	1/1
		Trombiculidae (após Kudryashova 1998)**	Ewing, 1944	Global	Sim	2/5

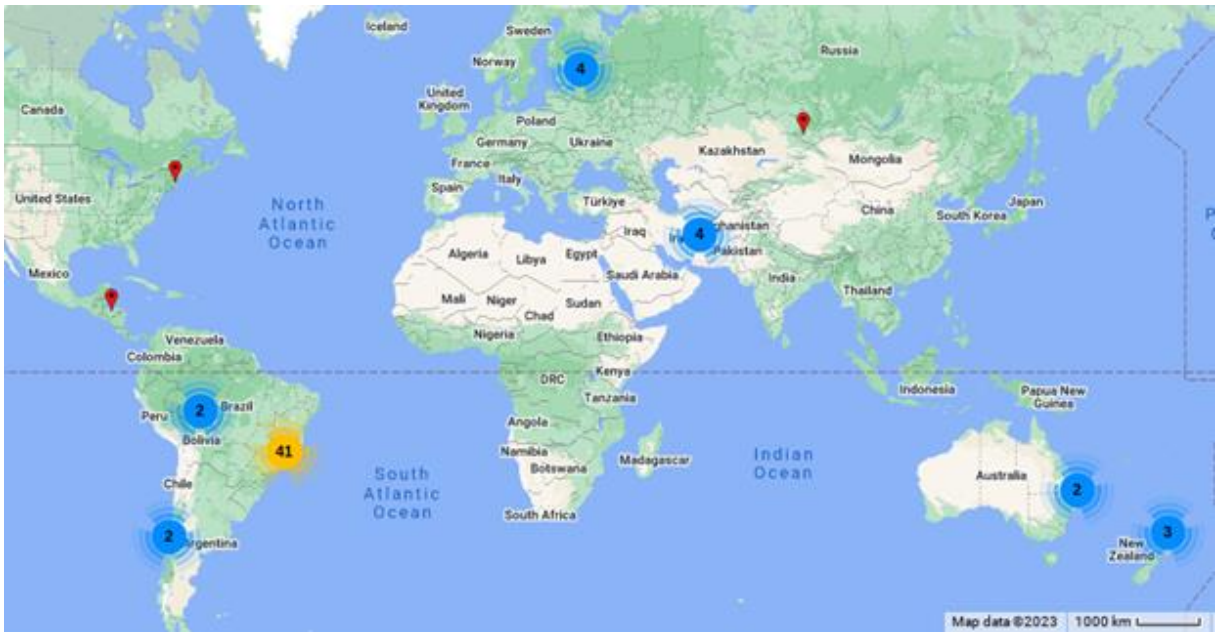

Figura 4. Distribuição geográfica dos ácaros Parasitengona depositados no Centro de Coleções Taxonômicas da UFMG e inclusos na inferência filogenética (capítulo 2). Pinos vermelhos indicam um único táxon, enquanto clusters indicam vários táxons coletados em uma região. Fonte: Google maps e Map Maker (maps.co).

2.3.1 Calyptostomatoidea

Calyptostomatoidea é composta por uma única família, Calyptostomatidae Oudemans, 1923, que possui distribuição global. Preferem ambientes úmidos e dependem de uma umidade relativa do ar de 100% para se reproduzir (Wolthmann, 2000). Esses ácaros são uma exceção para o típico ciclo de vida heteromórfico dos ácaros Parasitengona por possuírem larvas relativamente parecidas com os adultos. Nenhum dos estágios ativos possui scutum dorsal ou crista metópica, mas apenas com um par de sensilas, cujas bases podem ser um pouco esclerotizadas. Entretanto, no estágio pós larval os Calyptostomatoidea possuem um gnatossoma modificado com longas quelíceras em forma de estilete que se retraem dentro do idiossoma e tem função perfurante similar ao que se vê em Erythraeoidea. Apesar da forma semelhante, as quelíceras de Calyptostomatidae diferem daquelas observadas em Erythraeoidea devido a presença de um plesiomórfico dígito móvel terminal (Witte, 1998).

2.2.2 Erythraeoidea

Entre outras características, os Erythraeoidea diferem dos demais Parasitengona devido à ausência de ânus e do poro epimeral (urstigma) nas larvas, quelíceras em forma de estilete

sem dígito móvel nas fases pós larvais, além de possuírem dois pares de sensilla (vs. 1 em Calyptostostoma). Os Erythraeoidea e os Calyptostomatoidea foram considerados grupos irmãos pela filogenia morfológica de Soller (2001) por compartilharem a presença das quelíceras em forma de estilete e uma série de estruturas internas a elas associadas. Vale notar que Soller (2001) também apresenta uma filogenia molecular com base apenas no gene Citocromo Oxidase I, porém esse gene com rápida taxa de substituição não apresentou um sinal filogenético que permitisse esclarecer as relações evolutivas entre as linhagens antigas.

A superfamília é composta pelas famílias Smarididae Vitzthum, 1929 e Erythraeidae Robineau-Desvoidy, 1828. Nas fases pós larvais, os Smarididae diferem dos Erythraeidae devido a presença de um gnatossoma protrátil que se estende com a ajuda de uma membrana de aparência sanfonada chamada armilla. Já as larvas diferem devido a presença de setas mecanorreceptoras com bases modificadas (tricobótrias), ao menos nos tarsos das pernas de Smarididae e que estão totalmente ausentes em Erythraeidae (Southcott, 1961).

Os Erythraeidae são encontrados nos mais diversos habitats, da serrapilheira úmida a regiões costeiras e até sobre o concreto quente em cidades. Possuindo diversas adaptações ao ambiente seco como a perda dos poros epimerais, além de secreções lipídicas e proteicas que evitam a perda de água (Wolthmann, 2000). No capítulo 1, descrevemos duas novas espécies de Erythraeidae, incluindo uma espécie recente sexualmente dimórfica e um novo gênero fóssil do Eoceno. A posição filogenética da espécie recente é inferida no capítulo 2 e foi utilizada para melhor classificá-la.

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. Neste trabalho testamos essa hipótese amostrando sete das nove subfamílias de Erythraeoidea (Capítulo 2).

2.3.3 Trombidioidea

Na fase pós larval, os ácaros Trombidioidea (diferem dos Erythraeoidea e Calyptostomatoidea devido a suas quelíceras compostas por um segmento proximal (eixo queliceral) curto e uma garra móvel (dígito móvel) com capacidade limitada de retração ou extensão (vs. longas quelíceras retráteis em forma de estilete). Sendo essa considerada a condição plesiomórfica para os Parasitengona e observada em grupos próximos como Anystidae Oudemans, 1936 e Halacaridae Murray, 1877 (Soller 2001). Considerando a

classificação de Mąkol & Wohtmann (2012) e Söller *et al.* (2001) Trombidioidea possui 17 famílias que serão brevemente descritas abaixo.

Achaemenothrombiidae Saboori, Wohltmann & Hakimitabar, 2010 é uma família conhecida apenas pelas larvas e um único gênero *Achaemenothrombium* Saboori, Wohltmann & Hakimitabar, 2010 que só foi encontrado no Irã. Essas larvas possuem três escleritos dorsais no idiossoma, sem adornos laterais em forma de retalhos. Na região ventral, não são observados escleritos anais. Já o fêmur e o genu do palpo possuem apenas uma seta cada. As setas supracoxais estão presentes nas coxas da perna I e o número de setas nas coxas I – III é 2, 2 e 1, respectivamente. Os tarsos I e II possuem duas garras falciformes e um empódio tipo garra, já o tarso III possui a garra interna curvada para fora, mais curta e ligeiramente engrossada (Saboori *et al.* 2010). Até o presente estudo, essa família nunca havia sido incluída em análises filogenéticas e por isso suas relações evolutivas são incertas.

Allotanaupodidae é uma família que ocorre apenas na Austrália e no sul da Argentina. Essa família difere de todas as outras por não possuir sensilas, por possuir os tarsos do palpo em uma posição ventral em relação a garra da tíbia do palpo, placas dorsais com duas ou mais setas, além de um amplo scutum dorsal (Zhang & Fan 2007). A falta de sensilas é uma característica rara entre os Parasitengona, observada apenas nos ácaros aquaticos. Até o presente trabalho, Allotanaupodidae era conhecido apenas pelos estágios pós larvais, veja capítulo 1 para mais detalhes sobre a família, ilustrações e a descrição do estágio larval associado utilizando sequências de DNA.

Amphotrombiidae é uma família monotípica com uma única espécie conhecida apenas pela larva encontrada nos EUA. Ela apresenta uma série de características semelhante às dos ácaros aquaticos como a presença de duas setas no genu do palpo, uma condição rara entre os Trombidioidea que é observada também em Stygothrombiidae e na larva de Allotanaupodidae descrita pela primeira vez no presente estudo. Amphotrombiidae difere dos ácaros aquaticos por possuir duas placas anais flanqueando o ânus (vs. uma única placa com o ânus no meio). Infelizmente não foi possível amostrar essa rara família nesse estudo.

Audyanidae é outra família monotípica, representada apenas por *Audyana thompsoni* Womersley, 1954, até o momento restrita à Malásia, e que é conhecida tanto pela larva, como pelo adulto. Suas larvas possuem um esclerito prodorsal (scutum) triangular semelhante ao de Trombellidae, com um par de sensilas e três pares de setas comuns, porém, destes três pares, os

dois anteriores são curtos e grossos em forma de pêra. As larvas são parasitas de escorpião, já os adultos possuem o idiossoma coberto por grandes papilas, cada uma delas abrigando várias setas lisas, curtas, sobre projeções cônicas da cutícula. Setas sobre projeções cônicas também são observadas em Allotanaupodidae e Johnstonianidae (Womersley 1954, Costa & Pepato 2023).

Chyzeriidae são ácaros trombidioidea grandes, incluindo o notável gênero *Chyzeria* Canestrini, 1897 encontrados no sul da Africa, Austrália e América do Sul, além da Nova Zelândia e que possuem longas papilas dorsais em forma de dedos. Chyzeriidae é composta por três subfamílias, Chyzeriinae, Pteridopodinae e Ralphaudyninae. Assim como ocorre em Johnstonianidae, Chyzeriidae apresenta um ou dois pares de sensilas, uma diferença marcante considerando que o número de sensilas é uma característica fixa dentro dos Erythraeoidea e Calyptostomatoidea. No capítulo 1 descrevemos uma nova espécie de Pteridopodinae cuja posição filogenética é inferida no capítulo 2.

Johnstonianidae são ácaros semiaquaticos que dependem de umidade relativa do ar muito alta para sobreviver (Wohltmann 2000). Eles possuem um scutum dorsal aproximadamente triangular, podendo apresentar um ou dois pares de sensilas. A classificação de Johnstonianidae tem sido tema de debates, com diferentes propostas para a delimitação da família e de suas subfamílias. Southcott (1987) a classificou em sete subfamílias (Johnstonianinae Thor, 1935, Lasseniinae Newell, 1957, Charadracarinae Newell, 1960, Polydisciinae Vercammen-Grandjean, 1972, Tetrathrombiinae Southcott, 1987, Pteridopodinae Southcott, 1987, Ralphaudyninae Southcott, 1987). Johnstonianidae incluia os gêneros *Lassenia* Newell, 1957, *Polydiscia* Methlagl, 1928, *Pteridopus* Newell & Vercammen-Grandjean, 1964 e *Parawenhoekia* Paoli, 1937. Entretanto, com base em um estudo filogenético Welbourn (1991) concluiu que se tratava de um grupo parafilético unido por características plesiomórficas. Ele então transferiu *Lassenia* e *Polydiscia* para Tanaupodidae; *Pteridopus* e *Parawenhoekia* para Chyzeriidae (Pteridopodinae), além de reconhecer que os Tetrathrombiinae eram na verdade ácaros aquaticos. Tendo restado apenas Johnstonianinae e Charadracarinae. Por fim, Wohltmann *et al.* (2004) questionou a validade de Charadracarinae como membro da família e sugeriu limitá-la a Johnstonianinae. Entre outros fatores que o levaram a essa decisão, Wohltmann *et al.* (2004) questiona a homologia entre o pequeno espinho ventro-medial na tíbia do palpo (basidonte) que é diagnóstico de Johnstonianidae e o espinho relativamente mais distal observado em Charadracarinae.

Trombiculidae (segundo Kudryashova 1998) são um grupo muito diverso cujas larvas são parasitas de vertebrados e que possui mais de 3000 espécies descritas, algumas delas carecendo de detalhes e a vasta maioria conhecida apenas pelo estágio larval (Nielsen *et al.* 2021). Esses são ácaros relevantes para saúde humana e animal por suas larvas serem agentes transmissores de rickettsioses como a potencialmente letal febre tsutsugamushi da Ásia, que é causada pelo patógeno *Orientia tsutsugamushi* (Low *et al.* 2019). Esse diverso grupo possui uma classificação controversa, como defendido por Jacinavicius *et al.* (2018), eles podem ser divididos em três famílias: Trombiculidae sensu stricto Ewing 1944, e Leeuwenhoekiidae Womersley 1944, and Walchiidae Ewing 1946. Ou como uma única família Trombiculidae e quatro subfamílias: Leeuwenhoekiinae, Apoloniinae, Trombiculinae e Gahrliepiinae (Kudryashova 1998 e Shatrov & Kudryashova 2008). Classificação defendida por autores como Shatrov & Kudryashova (2008). Ou ainda como apenas duas famílias, Trombiculidae sensu stricto Ewing 1944, e Leeuwenhoekiidae Womersley 1944 como defendido por Nielsen *et al.* (2021). Isso ocorre devido à falta de uma filogenia que permita refletir as suas histórias evolutivas em sua classificação. Optamos inicialmente por adotar a classificação defendida Kudryashova 1998 e Shatrov & Kudryashova (2008) considerando Trombiculidae Ewing, 1929 com quatro subfamílias: Leeuwenhoekiinae Womersley, 1944, Apoloniinae Wharton, 1947, Trombiculinae Ewing, 1929 e Gahrliepiinae Womersley, 1952.

Os Microtrombidiidae são uma família comumente encontrada na serapilheira em todo mundo. As fases pós larvais desses ácaros podem ser encontradas no subsolo e possuem uma característica fileira de espinhos em forma de pente na tíbia dos palpos, o que pode estar relacionado a sua capacidade de escavar mesmo em solo duro (Wohltmann *et al.* 2007). Esses ácaros possuem larvas parasitas de artrópodes e são altamente especializados para isso. Sendo que seu gnatossoma apresenta diversas modificações que possivelmente favorecem a fixação ao hospedeiro, como a presença de uma abertura oral em forma de ventosa. As formas adultas variam em tamanho podendo ter até um centímetro (observação pessoal). No presente estudo amostramos todas as três subfamílias de Microtrombidiidae: Euthrombidiinae Thor, 1935, Microtrombidiinae Thor, 1935 e Valgothrombiinae Gabryś, 1999.

Os Neothrombiidae são uma família pouco diversa, mas de distribuição global, cujas fases pós larvais também podem ser encontradas no ambiente edáfico. Segundo a literatura, suas larvas são conhecidas por parasitar Orthoptera, porém espécimes depositados na Coleção Acarológica do Centro de Coleções Taxonômicas da UFMG foram também observados

parasitando vespas (Krantz & Walter 2009). A família é frequentemente encontrada em cavernas de Minas Gerais e as formas adultas muitas vezes não possuem olhos.

Os ácaros Neotrombidiidae possuem distribuição global e tem como principal características setas dorsais trifurcadas nas fases pós larvais além de coxas adornadas com um padrão reticulado na fase larval. Assim como os Neothrombiidae, esses ácaros são frequentemente encontrados em cavernas de Minas Gerais e fazem parte da comunidade de artrópodes do guano de morcegos (Krantz & Walter 2009). Além dos adultos serem encontrados no guano, as larvas de *Monunguis streblida* Wharton, 1938 cavernícolas possuem uma interessante relação com os morcegos. Elas são encontradas com frequência parasitando moscas parasitas de morcegos em cavernas de Minas Gerais e uma caverna no México, em uma relação conhecida como hiperparasitismo (Reis *et al.* 2019). A não usual distribuição de *Monunguis streblida* dispersa por mais de 5000 km sugere a necessidade de uma investigação taxonômica mais profunda empregando dados moleculares.

Os Podothrombiidae são encontrados no hemisfério norte e incluem dois gêneros: *Podothrombium* e *Kurilothrombium* com 49 e duas espécies respectivamente (Cline 2021). Em contraste com os Microtrombidiidae de pernas curtas cuja fases pós larvais são encontradas no ambiente edáfico e os muitas vezes cegos Neothrombiidae, os Podothrombiidae são rotineiramente encontrados forrageando na superfície do solo, possuem pernas longas e dois grandes pares de olhos pedunculados (Cline 2021). O que sugere uma dependência da visão para caçar.

Tanaupodidae são encontrados em ambientes úmidos do hemisfério norte (Krantz & Walter 2009). Eles possuem setas lisas e curtas individualmente distribuídas sobre pequenos escleritos, porém sem grandes placas como aquelas observadas em Allotanaupodidae (Wohltmann *et al.* 2007). Suas larvas são parasitas de Diptera, Colembola e Hemiptera. A família consiste em nove gêneros, sendo dois fósseis e sete recentes (Mąkol & Featherstone 2021). Infelizmente não foi possível amostrar Tanaupodidae no presente estudo.

Os Trombellidae possuem distribuição global e algumas espécies compartilham com Johnstonianidae uma série de características consideradas plesiomórficas, como a divisão das pernas em sete segmentos (sem segmentos fundidos) em várias espécies e sensibilidade à dessecação (Southcott, 1987). As Larvas de Trombellidae possuem as coxas I e II bem separadas e costumam apresentar mais setas quimiorreceptoras (solenidium) do que outros

Parasitengona, podendo esses ser encontrados dos tarsos até o fêmur (Southcott, 1987). Apesar de ser raramente observada pela equipe do Laboratório de Acarologia da UFMG, essa família pôde ser encontrada no Brasil, na serapilheira, como larvas parasitando Culicídeos no sul do Paraná ou no solo de cavernas.

Trombidiidae são um dos grupos mais diversos de Trombidioidea com mais de 150 espécies, perdendo apenas para os ácaros parasitas de vertebrados. São ácaros relativamente grandes, densamente cobertos de setas que dão a eles uma aparência aveludada. Os Trombidiidae possuem distribuição global e costumam habitar o subsolo, assim como várias linhagens de Microthrombidiidae. *Dinothrombium* Oudemans, 1910 é um dos Trombidiidae mais estudados devido a sua abundância e tamanho. Como demonstrado por Schmidt & Schmidt (2022) em uma série de experimentos, *Dinothrombium* são raramente predados, por possuírem como defesa tegumento flexível resistente a perfuração, cor vermelha chamativa, odor e sabor desagradável. Extratos de *Dinothrombium* são utilizados na medicina tradicional da Índia onde podem ser comprados por até US$20/kg para o tratamento de uma série de doenças, e experimentos demonstraram que o mesmo apresenta atividade antifúngica (George *et al.* 2011). Os *Dinothrombium* são também predadores vorazes capazes de comer diversos tipos de invertebrados e emergem do subsolo em grandes números para se reproduzir após as chuvas em algumas regiões de clima seco. Trombidiidae são frequentemente encontrados em amostras de serrapilheira e cavernas do Brasil, porém na maioria dos casos os espécimes pertencem ao mesmo gênero *Allothrombium* Berlese, 1903 (Observação pessoal).

Yurebillidae é uma família monotípica que ocorre no sul da Austrália. Sendo conhecida apenas pela larva de *Yurebilla gracilis* Southcott, 1996. Essa larva se assemelha a Podothrombiidae com um scutum triangular abrigando um par de sensilas e três pares de setas comuns. Entretanto, Yurebillidae não possui um scutelum (pequeno sclerito dorsal localizado posteriormente ao scutum) e seu hospedeiro é desconhecido (Southcott, 1996). No presente estudo não foi possível amostrar essa família.

3 OBJETIVO GERAL

Descrever parte da diversidade de ácaros Parasitengona recentes e fósseis, classificando-os com base em um estudo filogenético das famílias terrestres recentes.

3.1 Objetivos específicos

1. Amostrar a maior diversidade de ácaros Parasitengona possível nas diversas regiões do planeta, triar, identificar e escolher táxons a serem priorizados para estudo.
2. Associar espécimes heteromórficos com base em dados moleculares.
3. Descrever as espécies novas de parte dos ácaros Parasitengona recentes e fósseis amostradas.
4. Inferir as relações filogenéticas entre os ácaros Parasitengona amostrados utilizando dados moleculares.
5. Testar o monofiletismo de Erythraeidae em relação a Smarididae.
6. Inferir a posição filogenética dos supostamente basais Allotanaupodidae.
7. Estimar como ocorreram as transições entre habitats aquaticos e terrestres ao longo da evolução dos Parasitengona.
8. Sugerir mudanças na classificação dos Parasitengona que melhor reflitam a história evolutiva.

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Capítulo 1 – Taxonomia integrativa de ácaros Parasitengona

Neste capítulo são apresentados uma série de achados taxonômicos utilizando de forma integrada ou isolada dados morfológicos, moleculares, ecológicos e paleontológicos das espécies estudadas. O capítulo inclui a descrição de sete espécies novas pertencentes às famílias Chyzeriidae, Erythraeidae, Johnstonianidae e Smarididae. Sendo seis espécies recentes e uma fóssil do Eoceno. Além disso, é apresentada a descrição inédita da fase larval de Allotanaupodidae. Dos oito táxons aqui abordados, seis foram incluídos como terminais na análise filogenética apresentada no capítulo 2. Sua classificação é posteriormente discutida na conclusão dessa tese. O capítulo está organizado em quatro Capítulos escritos em inglês e formatados de forma a serem publicados em revistas da área. Ele incluí *Smaris hajiqanbari* Costa, Gomes-Almeida & Pepato, 2022, *Newellia xakriaba* Costa, Klimov & Pepato, 2023 e *Centrotrombidium krenak* Costa, Klimov & Pepato, 2023 que já foram publicados.

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Capítulo 1.1 – Smarididae

Neste Capítulo é apresentada a descrição morfológica de duas novas espécies de Smaridiidae. *Trichosmaris* sp. nov. que é representada no estudo filogenético apresentado no capítulo 2 pelo espécime UFMGAC160011 e *Smaris hajiqanbari* para o qual não foi possível obter dados moleculares.

Capítulo 1.1.1: A new larval species of the genus *Smaris* (Smarididae, Parasitengona) from a Brazilian cave

SAMUEL GEREMIAS DOS SANTOS COSTA* [1, 2], BRENDA GOMES ALMEIDA[1] & ALMIR ROGÉRIO PEPATO [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 10 Semakova Str., 625003 Tyumen, Russia.*

* *Corresponding author. E-mail:* estoupa.bob@gmail.com

Abstract

Here, we describe a new smaridid species, *Smaris hajiqanbari* sp. nov., based on the larva. This is the first description of a *Smaris* species from Brazil and the fourth larval *Smaris* ever described. An identification key to larval *Smaris* is also provided.

Key words: Key to species, Parasitengona, Neotropical

Introduction

Smarididae Kramer, 1878 is a widely distributed family of Parasitengona (Mąkol & Wohltmann 2012), found in forest litter and caves in tropical and temperate regions. Parasitengona usually have life cycles with three active instars: ectoparasitic larvae, and free living, predaceous deutonymphs and adults (Wharton & Fuller 1952; Johnston & Wacker 1967; Robaux 1974; Wohltmann 2000). Due to the major ecological and morphological differences between larval and postlarval instars, most terrestrial Parasitengona are known only from either larvae or deutonymphs/adults (Costa *et al.* 2019; Mąkol & Wohltmann 2012).

Currently, *Smaris* has 16 described species, two of them (*Smaris maraghehiensis* Saboori & Bagheri, 2011, and *Smaris arenicola* Southcott, 1997) based on larvae, 13 species are known from postlarval stages, and a single species, *Smaris prominens* (Banks, 1916), is known from both larval and postlarval stages (Mąkol & Wohltmann 2012). The hosts of smarididids are poorly known, with the single record of larval *Smaris prominens* found on Psocoptera (Womersley & Southcott, 1941).

Notably, adults of another smaridid mites of the genus *Calorema* Southcott, 1963 also can be found in the leaf litter and caves. Both taxa have the post larval instars morphologically similar, differing by the presence of a ventral scutum between coxae I and II in *Smaris* (vs. absent in *Calorema*) and the width of the dorsal scutum, broader in *Smaris*, including the eyes (vs. scutum narrow and not reaching the eyes in *Calorema*). The larvae of *Calorema* have never been described, hence cannot be compared to *Smaris.*

Here, we describe a new larval smaridid species, *Smaris hajiqanbari* sp. nov. collected in the soil of a cave. This is the first description of a *Smaris* from Brazil, and the fourth larval species of *Smaris* ever described. Identification key to larval *Smaris* is also provided. It must be kept in mind, however, that assignment to genera among smaridid may change as new associations between larvae and post-larval stages are acknowledged. As illustrated by a recent study where genera were synonymized, and new combinations proposed (Costa *et al.* 2021).

Material and methods

A single specimen of the new species was found free on the cave floor, by a naked-eye search, collected using a brush, and placed in a vial with 70% ethanol. The specimen was mounted on microscope slides using Hoyer's medium (Walter & Krantz 2009), identified to the family level

and donated to us by Dr. Lepoldo Bernadi (*Universidade Federal de Lavras* and *Ativo Ambiental Co.*, Lavras, Minas Gerais, Brazil). The type was kept at the Acarological Collection at the C*entro de Coleções Taxonômicas da Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil (UFMG AC).

Photos were taken using a Leica DM750 compound microscope with a Leica ICC50W camera. Drawings were made in the software On Top Replica v. 3.5.1. Final artwork was done in Adobe Illustrator CC 2015 and Adobe Photoshop CC 2015. All measurements are given in micrometers (µm). Terminology and abbreviations were adapted from Grandjean (1947), Goff *et al.* (1982) and Southcott (1961, 1992). For clarity, setae on the legs are represented only by their bases in the drawings.

Our nomenclatorial act was registered in ZooBank: http://zoobank.org/urn:lsid:zoobank.org:pub:88EB4FAB-DBA6-423C-BEB8-C9B176E4F3FA

Systematics

***Smaris hajiqanbari* sp. nov.**

(Figs 1-3)

Diagnosis (larva)

Gnathosoma dorsally striated. Two solenidia on genu I and one on genu II and III (Fig. 2A). Setae *2a* present (Fig. 1B). Relative lengths of ventral idiosomal setae: *3a=2a>1a*. Eight ventral barbed setae situated posterior to coxae III in two rows of four setae each. Palpal setal formula: fPp=0-0-B-B-BBN-3N,1B,1ζ,1ω, with one branched seta with long barbs (B) on the palp tarsus (Fig. 1D). Setation of leg tibiae: fnTi= 16-14-14. Idiosoma with 32 dorsal setae. Post larval stages unknown.

Holotype. Larva, *Smaris hajiqanbari* sp. nov. (UFMG AC 210357), Brazil, Minas Gerais, Itabirito municipality, cave MP-29, 20°13’21”S, 43°50’56”W. Col. *Ativo Ambiental* Co. May 6th 2021.

Description

Larva: Idiosoma nearly oval, bearing 32 dorsal barbed setae arranged in irregular rows (Fig. 1A). Scutum wider than long, with two pairs of scutella and two pairs of sensilla; both with barbs on their whole extension (Fig. 1A). Two pairs of eyes situated on a weakly sclerotized eye plate (Fig. 3A). Idiosoma ventrally with three pairs of intercoxala (*1a*, *2a* and *3a*, with relative lengths: *3a=2a>1a*, Fig. 1B), and eight barbed setae situated posterior to coxae III in two rows of four setae each (Fig. 1B). A pair of "dots" present between coxae II and I (Fig. 3B).

Gnathosoma dorsally striated, bearing two pairs of smooth ventral hypostomal setae (*as* and *bs*) and one dorsal pair of smooth galealae (*cs*); seta *bs* much longer than *as*. Odontus with four prongs. Cheliceral blades short, screw-shaped and pointed sideways (Fig. 1C and D). Palps relatively long and curved. Palp tibia extending beyond gnathosomal tip (Fig. 1C and D). Palp tibia with one ventral, one dorsal barbed seta, and one poorly visible internal smooth seta. Palp genu and femur each with single, much wider and barbed setae (Fig. 1C). Palpal setal formula: fPp=0-0-B-B-BBN-3N,1B,1ζ,1ω (Fig. 1C and D).

Legs short. Each tarsus bears a claw-like empodium and pair of enlarged claws without a terminal hook (Fig. 2). Coxae I to III with one barbed seta each, seta on coxa II near its posterior margin, seta on coxa III near its anterior margin (Fig. 1B). Legs covered by thick barbed setae (Fig. 3A and B), except by dorsal side of tibia I and tarsus I with few thin, distally barbed setae in addition to thick barbed setae (Fig. 2A). Tarsi I with a Trichobothridial pit bearing large trichobothria, short seta "*x*" and companion seta (Cp), one long solenidion (ω) close to trichobothridial pit's margin and one distal euphatidium (ζ) (Fig. 2A). Three solenidia on tibia I, two on genu I and one on genu II and III (Fig. 2). Leg III > leg I > leg II. Measurements summarized in Table I.

Leg chaetotaxy:

Leg I: Ta– 25B, 1*Cp*, 1ζ, 1ω, 1ta, 1*x*; Ti– 14B, 1*Cp*, 1k, 3φ; Ge– 8B, 2σ; TFe– 5B; BFe– 2B; Tr– 1B; Cx– 1B.

Leg II: Ta– 20-21B, 1ω, 1ζ; Ti– 14B, 2φ; Ge– 8B, 1σ; TFe– 5B; BFe– 2B; Tr– 1B; Cx– 1B.

Leg III: Ta– 22B, 1ζ; Ti– 14B, 1φ; Ge– 8B, 1σ; TFe– 5B; BFe– 1B; Tr– 1B; Cx– 1B.

Remarks

Smaris hajiqanbari sp. nov. differs from *Smaris prominens* (Banks, 1916), *Smaris arenicola* Southcott, 1997 and *Smaris maraghehiensis* Saboori & Bagheri, 2011 in the presence of two solenidia on genu I (vs. one). It also differs from *S. prominens* and *S. arenicola* by the presence of setae *2a* (vs. absent).

Etymology

The new species is dedicated to Dr. Hamidreza Hajiqanbar (University of Tehran, Iran), for his great contribution to systematic acarology.

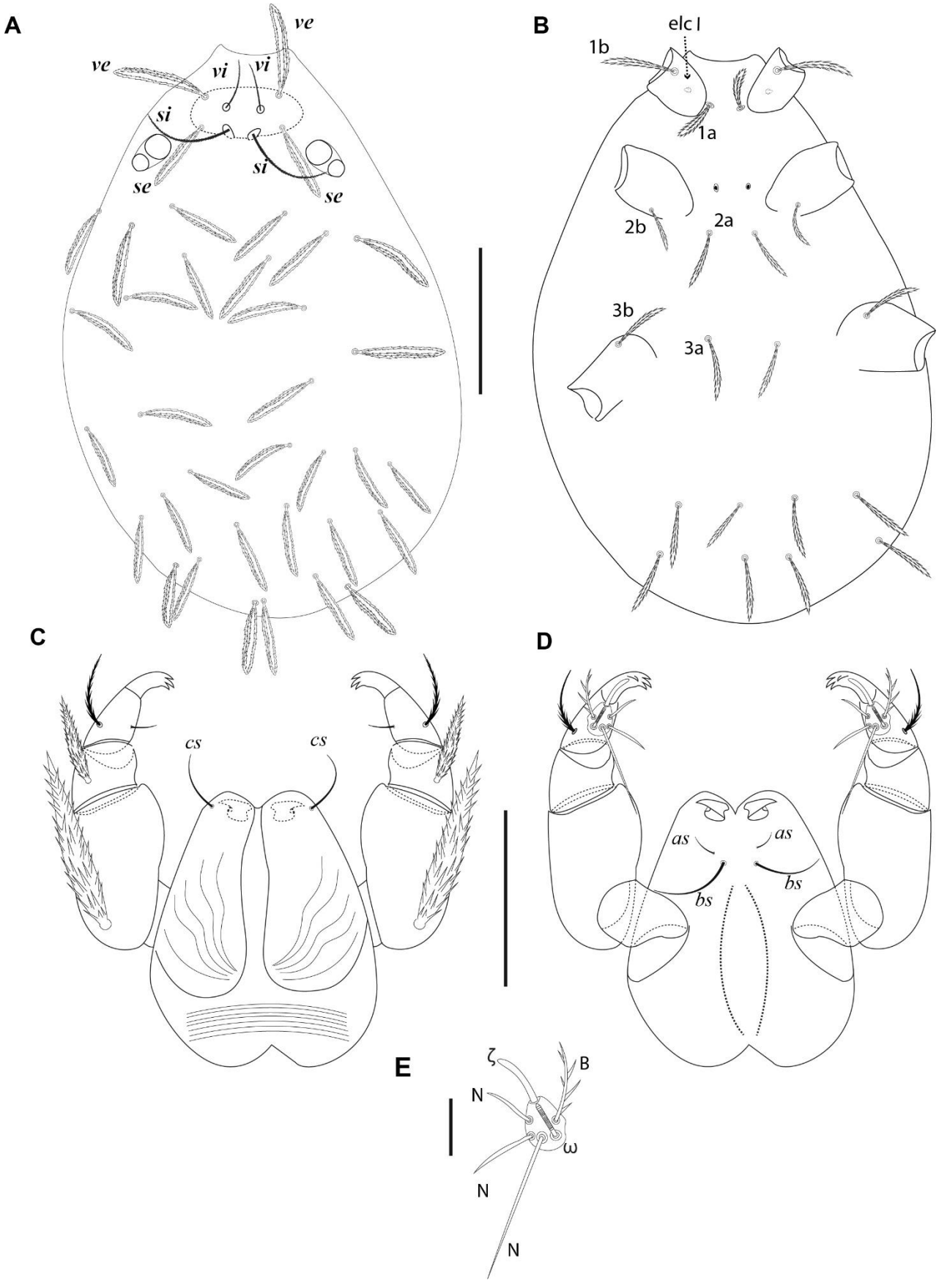
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Figure 1. *Smaris hajiqanbari sp. nov.* A: Dorsal view. B: Ventral view. C: Dorsal view of gnathosoma. D: Ventral view of gnathosoma. E: Palp tarsus. Scale: A-D = 50 µm, E= 10 µm.

Table I – Measurements of *Smaris hajiqanbari* sp. nov. UFMG AC 210357 (Holotype).

Measurement	*S. hajiqanbari*	Measurement	*S. hajiqanbari*
IL	365	TFe I	45.7
IW	267	BFe I	33
AW	50.5	Tr I	30.5
PW	54	CX I	45
Sba	23	Leg I	315.1
Sbp	11.3	Ta II	62
A-P	18.5	Ti II	76.3
AL	62	Ge II	57.8
PL	52	TFe II	36
ASE	31.5	BFe II	37
PSE	67	Tr II	34
Scutum_L	81.5	Cx II	61
Scutum_W	41.5	Leg II	303.1
ISD	13	Ta III	75
1a	30	Ti III	89
2a	38	Ge III	79
3a	39	TFe III	49
Ta I (L)	68.1	Bfe III	30
Ta I (H)	29.3	Tr III	39
Ti I	73.3	Cx III	55
Ge I	64.5	Leg III	361

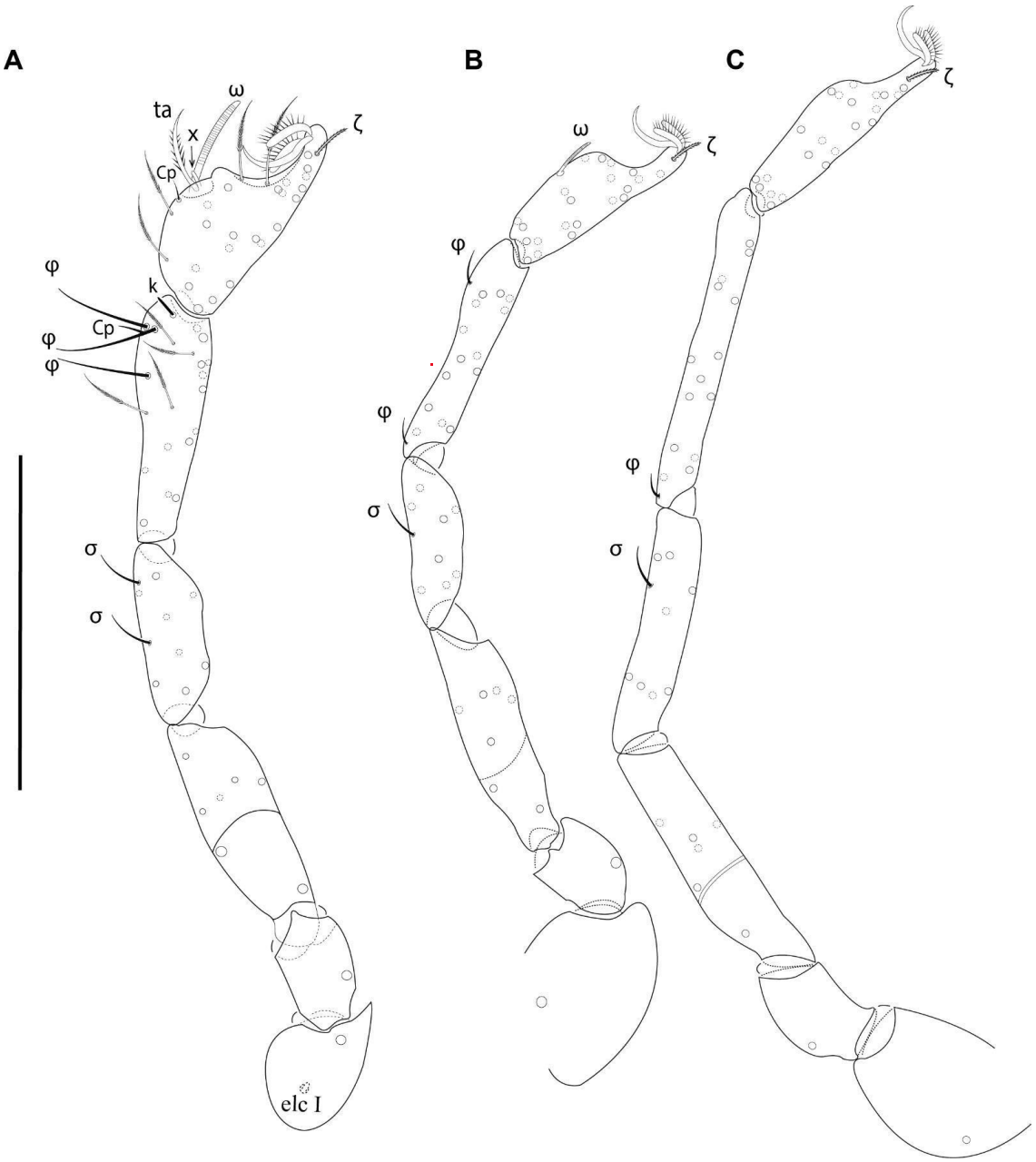

Figure 2. *Smaris hajiqanbari sp. nov.* A: Leg I. B: Leg II. C: Leg III. Scale: 50µm.

Key to *Smaris* species (larvae)

1 – Setae *2a* present (Fig. 1B) … 2

– Setae *2a* absent … 3

2 – Two solenidia present on genu I. Brazil. … *Smaris hajiqanbari* sp. nov.

– One solenidion present on genu I. Iran. … *Smaris maraghehiensis* Saboori & Bagheri, 2011

3 – Setae 1a and 3a with different shapes (1a with longer setules than observed in 3a), scutelae AL blunt-ended. Australia. … *S. prominens* (Banks, 1916)

– Setae 1a and 3a with similar shape, scutelae AL pointed. Australia. … *S. arenicola* Southcott, 1997.

Modified from Southcott (1997).

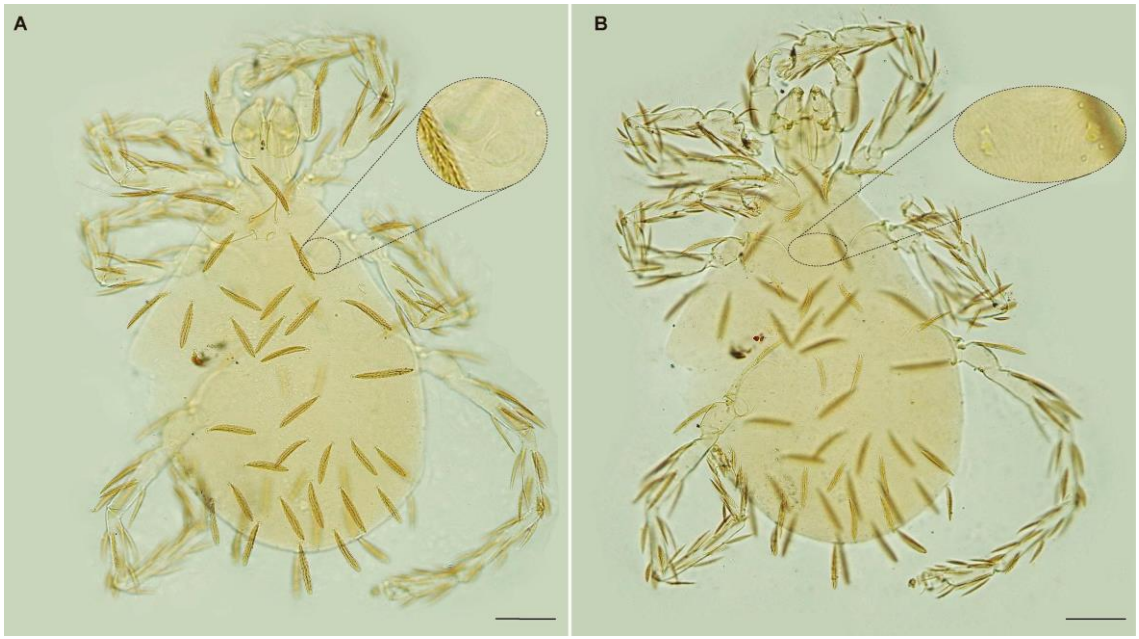

Figure 3. *Smaris hajiqanbari sp. nov.* A: Dorsal view, detail showing the eyes. B: Ventral view, detail showing two ventral dots of unknown nature. Scale: 50µm.

Acknowledgements

We thank the company *Ativo Ambiental* for collecting and Dr. Leopoldo Bernardi from *Universidade Federal de Lavras* for depositing the specimen at UFMG AC, Dr. Alexander Khaustov (Tyumen State University, and the X-Bio institute) for receiving SGSC as a visiting Scholar funded by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES – Print, Finance Code 001), *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* FAPEMIG in partnership with the company *Vale Sociedade Anônima* for funding (FAPEMIG-VALE Edital Nº 007/2018), and Dr. Pavel B. Klimov (University of Purdue) for assistance concerning grammar and style. SGSC and BGA acknowledge the *Programa de Pós Graduação em Zoologia da* UFMG and FAPEMIG – Graduate Support Program PAPG and FAPEMIG-VALE Edital Nº 007/2018, respectively, for their scholarships.

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Capítulo 1.1.2 – A new cave dwelling *Trichosmaris* (Acari, Smarididae) species

SAMUEL GEREMIAS DOS SANTOS COSTA* [1, 2], ALEXANDR ALEXANDROVICH KHAUSTOV & ALMIR ROGÉRIO PEPATO [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 10 Semakova Str., 625003 Tyumen, Russia.*

* *Corresponding author. E-mail: estoupa.bob@gmail.com*

Abstract

Smarididae mites are a globally distributed Parasitengona that can be found in the forest litter, caves, and dry areas of cerrado biome of Brazil. *Trichosmaris* belongs to the Hirstiosomatinae subfamily and have species described from Oceania; South, Central and North America. In the present study we describe a new *Trichosmaris* species associated with Brazilian caves, based on females. To allow further association of other instars or heteromorphic specimens, sequences of the mitochondrial gene cytochrome oxidase subtunit I and the ribosomal gene 28S, are provided. An updated identification key to *Trichosmaris* is also provided.

Key words: Key to species, Parasitengona, Brasil, Hirstiosomatinae, Neotropical.

Introduction

Smarididae mites are a globally distributed Parasitengona found on soil, in epigenous and cave litter, both in dry and rainy areas biomes. As most Parasitengona, its life cycle consists of an egg and six postembryonic instars: a regressive (calyptostatic) prelarva; an often ectoparasitic larva; calyptostatic protonymph; heteromorphic, free-living, predaceous deutonymph; calyptostatic tritonymph, and a free-living, predaceous adult (Wharton & Fuller 1952; Johnston & Wacker 1967; Robaux 1974).

Trichosmaris Southcott, 1963 has seven described species: *T. calcarensis* Costa *et al.,* 2021, known only by its larva (L.) found in Brazil. *T. dentella* Southcott, 1963, known only by post larval instars (P.) from Guatemala. *T. dispar* Southcott, 1963, known by P. and L. from Guatemala, Mexico, and USA. *T. jacoti* (Southcott, 1946), P. from USA. *T. longirostris* (Southcott, 1995), L. from Costa Rica. *T. papuana* Beron, 2002, P. from Papua New Guinea and *T. paulensis* Costa *et al.* 2021, P. and L. from Brazil.

Here we describe a new *Trichosmaris* species associated with Brazilian caves. Sequences of its cytochrome oxidase subunit I mitochondrial gene and the ribosomal gene 28S are provided. Allowing further association of heteromorphic specimens and tests of the species boundaries. An updated identification key to *Trichosmaris* adults is also provided.

Material and methods

Abbreviations for collections

UFMG AC, Acarological Collection at *Centro de Coleções Taxonômicas* at the *Instituto de Ciências Biológicas*, *Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil.

Studied Material

Trichosmaris sp. nov. **Holotype** (UFMG AC 160024), female, Brazil, Minas Gerais, Pains municipality, in the entry zone of a cave named ICPA 858, 20° 22' 28” S, 45° 36' 37” W, collected by staff of a private company *Carste Ciência e Meio Ambiente*, November 24 to 27 of 2015. Deposited in UFMG AC. **Paratypes** (UFMG AC 160002, 160009, 160011 and 160018), four females collected in the same region (see table I).

Table I – Sampling data and Gen Bank accession numbers “Provided upon acceptance” of *Trichosmaris* sp. nov. specimens. Not available (-).

UFMG AC	Cave	Cave zone	Lat. – Long. °	Date	Gen Bank	
					COI	28S
160002	ICPA 891	Entrance	45° 36' 45" W, 20° 21' 47" S	Oct./ 20 – 21, 2015	xxxx	-
160009	ICPA 894	Penumbra	45° 36' 45" W, 20° 21' 47" S	Oct./ 20 – 21/ 2015	xxxx	-
160011	ICPA 863	Entrance	45° 36' 14" W, 20° 22' 08" S	Oct./ 24 – 27/ 2015	OM401563	OM641862
160018	ICPA 895	Entrance	45° 36' 45" W, 20° 21' 47" S	Oct./ 20 – 21/ 2015	xxxx	-
160024	ICPA 858	Entrance	45° 36' 37" W, 20° 22' 28" S	Oct./ 24 – 27/ 2015	xxxx	-

Molecular data

DNA extraction, amplification and sequencing were performed as described by Costa *et al.* (2019) for the cytochrome oxidase I. For the 28S gene amplification and sequencing were performed as described by Pepato *et al.* 2018. The resulted DNA sequences are provided in Table I allowing further comparison.

Descriptions

Photos were taken using a Leica DM750 compound microscope with a Leica ICC50W with a camera and Zeizz Primo Star compound microscope and a ZZCAT SX08.U2.5000K.0806 camera. Drawings were made with the aid of simulated *camera lucida* using the cameras and the software On Top Replica. Final artwork was done in Adobe Illustrator CC 2015 and Adobe Photoshop CC 2015. All measurements are given in micrometers (µm). Terminology and abbreviations were adapted from Southcott (1961), Goff *et al.* (1982); and Southcott (1992).

Data Register

Nomenclature acts were registered on ZooBank and are available at: "Provided upon acceptance".

Systematics

Family Smarididae Kramer, 1878

Diagnosis: See Southcott, 1961.

Genus *Trichosmaris* Southcott, 1963

Diagnosis: See Costa *et al.* (2021) for larvae and Southcott (1963) for post larval instars.

Trichosmaris sp. nov.

(Figs 1-3)

Diagnosis.

Female with 14 relatively short setae (25–37, length/width= 2 – 2.95) in the anterior sensillar area (naso). Distal end of the gnathosoma with 14 long smooth ventral setae placed distally to the palp trochanter and 2 dorsal, over the hypostomal lips (Fig. 3A). Palp femur smooth, without protuberances or papillae and covered by smooth setae (Fig. 1A and 3A). Palp tarsus with two barbed normal seta (B), five smooth and one barbed eupathidia (ζ) and one solenidion (ω) (Fig. 3B). Long and slim palps with palp genu length/width= 2.59 – 3.09. Ta I/Ti I= 0.72 – 0.88, Ti I/Ge I= 0.72 – 0.80, Ti III/Ge III= 1.06 – 1.13, Ti IV/Ge IV= 1.05 – 1.14. Long feather-like sensorial setae placed on the distal end of genu and telofemur I to IV and in the distal end of tarsi II to IV (Fig. 1A). Anal valves asymmetric, with 4 and 5 setae each (Fig. 2F).

Holotype. UFMG AC 160024, female, Brazil, Minas Gerais, Pains municipality, in the entry zone of a cave named ICPA 858, coordinates: 20° 22' 28" S, 45° 36' 37" W, collected by staff of a private company *Carste Ciência e Meio Ambiente*, Novemeber 24 to 27 of 2015.

Description.

Female.

Idiosoma roughly oval (Fig. 1A). Crista metopica bearing two pairs of filiform trichobothria (*vi* and *si*) and a naso with 14 long, setulate setae (25–39), longer than wide (length/width= 2 – 2.95) (Fig. Fig. 2A and B). Anterior trichobothria (*vi*) barbed (Fig. Fig. 2B), posterior trichobothria (*si*) (Fig. 2C) with a long (66–79) barbed proximal region (= *pars clavata* Southcott 1963) and a long (30–55) distal smooth portion (= *flagellum* Southcott 1963). One pair of eyes situated between the trichobothria (*vi* and *si*), eye cornea diameter 25–35 (Fig. 1A).

Dorsal setae 19–27 long (Fig. 2A, C, D and E; Fig. 3C and D). Each dorsal seta placed on a chitinized papilla thicker on one side (expanded amphora) (Fig. 3C and D). Tectum setae with two pairs of well-defined columns, separated by a longitudinal reentrance, columns with approximately 13 serrate spicules (Fig. 2D and E; Fig. 3C and D). Setal carina expanded and wider than tectum. In addition, the carina has irregular columns with serrate spicules (Fig. 3D).

Genital pore (partially damaged in all specimens) bearing two heteromorphic pairs of genital acetabula that differ in size and shape (Fig. 1A, 3E). Genital valves with short smooth setae in most of its extension, with a few short, barbed setae in the anterior half. Being those setae thinner than the normal ventral idiosomal setae. Asymmetric anal valves with 5 and 4 barbed setae each (Fig. 3F). This asymmetry is observed in all specimens. Ventral setae with a poorly defined rows of spines in the tectum and carina with acute spicules (Fig. 1B).

Gnathosoma conical, eversible, with long, stylet-like chelicera (Fig. 1A and 3C). Elongated mouth cone with 18 long smooth setae situated anteriorly to the palp trochanter bases. 16 ventral and distributed in rows of 2 and two dorsal over the hypostomal lips (Fig. 3C). Long and slim palps with palp genu length/width= 2.59 – 3.09. Simple smooth setae present in the palp trochanter, femur, and proximal half of the genu. Weakly barbed setae present on palp tibia and in the distal end of palp genu (Fig. 3C). Measurements in Table II.

Palp chaetotaxy: Ta– 2B, 6 ζ, ω; Ti– 15 to 19B; Ge– 13B; Fe– 16 to 17B; Tr– 3B (Fig. 3C).

Relative leg lengths: I> IV> III> II. Legs with numerous barbed setae. Similar to dorsal idiosomal setae but elongated and with poorly defined carina and tectum (Fig. 1A and B). Distal end of tibia and genu I to IV, with a terminal long, barbed, feather-like seta (Fig. 1A (arrow)).

Remarks

Females of *Trichosmaris* sp. nov. differ from those of *T. dispar, T. papuana* and *T. paulensis* due to anterior sensillar common setae number or shape. *T.* has 14 relatively long (length/width ratio= 2 – 2.95) normal setae in the anterior sensillar area, while *T. dispar* has 18–21 relatively long (length/width= 1.97 – 3), *T. papuana* has 5 short and large (length/width=1.27–1.35) and *T. paulensis* and 10 very long setae (3 – 4.3). *T.* also differs from *T. papuana* and *T. paulensis* due to the presence of only thin, smooth, or weakly barbed setae on palp genu (vs. barbed setae with an expanded tectum in *T. papuana* and barbed setae over protuberances in the palp genu of *T. paulensis*). *T.* sp. nov. differs from *T. jacoti* by the length of the barbed portion of the posterior sensilla (66 – 79 vs. 45 in *T. jacoti*), and by having tibia IV longer than the genu IV (vs. shorter in *T. jacoti*). *T.* sp. nov. also differs from *T. dentella* due to tibia III longer than genu III and tibia IV longer than genu IV (vs. shorter in *T. dentella*).

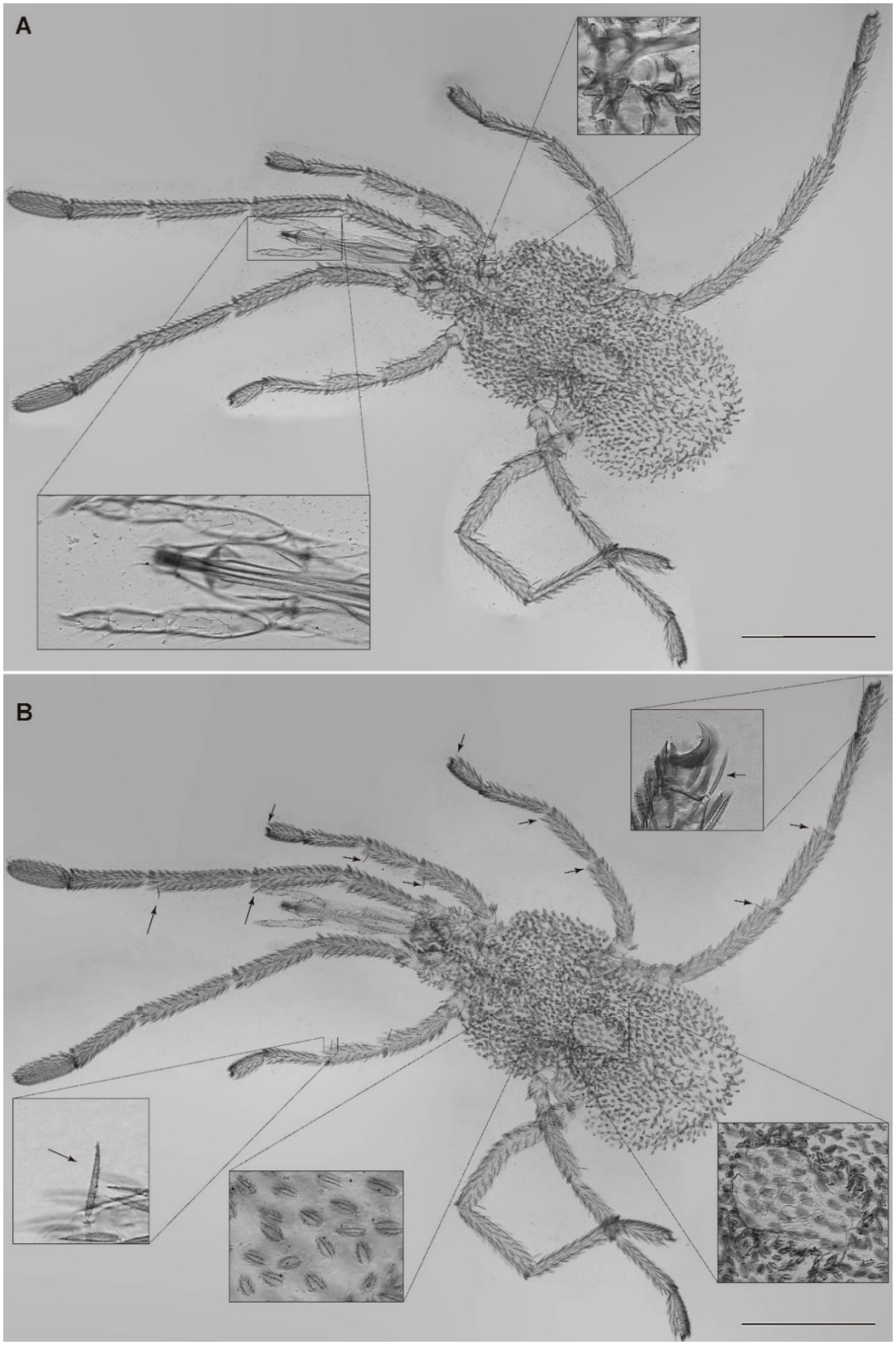

Figure 1. *Trichosmaris sp. nov.* A: Dorsal view, details showing gnathosoma and eye. B: Ventral view, details showing feather-like setae on genu II and Tarsus IV, ventral common setae, damaged genital plates. Black arrows pointing to the distribution of feather-like setae. Scale: A and B= 500 µm.

Discussion

Trichosmaris sp. nov. was collected in a region near to *T. calcarensis* Costa *et al.* 2021 (known based only on larvae). However, without molecular data of *T. calcarensis* or living specimens for rearing experiments, it was impossible to test the species boundaries between the two taxa. Two new expeditions were made to the region in December 2017 and February 2021 to collect more specimens and test species boundaries. However, co-specific specimens were not found. Rather than retaining our data indefinitely to complete this puzzle, we opt to share it with the scientific community. Allowing others to employ it for further integrative taxonomy and systematics studies.

Etymology

To be defined when publishing.

Table II – Metric data for *Trichosmaris sp. nov.* females.

UFMG AC	160002	160009	H – 160011	160018	160024	Range
IL	1650	1143	-	-	1441	1143 – 1650
IW	868	497	822	-	700	497 – 868
Sba	14	-	12	15.6	14.3	12 – 15.6
Sbp	11	17.8	17.3	20.6	10.6	10.6 – 20.6
Vi	52.1	41	48	49	51.6	41 – 52.1
Si	112.5	100	108	132	121	100 – 132
Pars clavata	72	70	67	79	66	66 – 79
Flagellum	40.5	30	41	53.21	55	30 – 55
ISD	400	400	391	453	430	391 – 453
DS	23 – 27	21 – 27	22 – 27	19 – 27	20 – 25	19 – 27
VS	20 – 25	15 – 24	14 – 18	20 – 25	19 – 26	14 – 26
ACW	60	-	60	69	67	60 – 69
PCW	37	37	38.5	42	38	37 – 42
L	524	473	499	586	550	473 – 586
ECO	-	-	-	35	25	25 – 35
Eye Ring	34.6	-	36	42	36	34.6 – 42
EC-EC	300	-	204	324	180	180 – 324
EC-*Vi*	250	-	167	232	216	167 – 250
Cx I	191	136	165	198	175	136 – 198
Tr I	97.5	91	114	113	101	91 – 114
BFe I	311	294	308	325	340	294 – 340
TFe I	372	329	342	366	370	329 – 372
Ge I	423	405	386	438	415	386 – 438
Ti I	323	316	307	317	312	307 – 323
Ta I	250	258	222	247	275	222 – 275

Table II – Continuation.

UFMG AC	1600023	1600094	H – 1600115	1600186	1600247	Range
Leg I	1776.5	1693	1679	1806	1813	1679 – 1813
Cx II	150	158	144	127	150	127 – 158
Tr II	76.8	70	89	64	84	64 – 89
Bfe II	138.7	109	99	105	139	99 – 139
Tfe II	188.7	180	174	195	213	174 – 213
Ge II	229.9	197	208	232	231	197 – 232
Ti II	265.3	230	241	231	241	230 – 265.3
Ta II (L)	153.3	152	146	146	170	146 – 170
Leg II	1052.7	938	957	973	1078	938 – 1078
Cx III	155	143	152	181	158	143 – 181
Tr III	83.1	96	86	94	90	83.1 – 96
Bfe III	148	218	127	128	156	127 – 218
Tfe III	220	226	180	211	210	180 – 226
Ge III	281	250	251	278	275	250 – 281
Ti III	307.3	265	270	314	297	265 – 314
Ta III (L)	166.2	176	155	136	175	136 – 176
Leg III	1205.6	1231	1069	1161	1203	1069 – 1231
Cx IV	200	193	217	183	242	183 – 242
Tr IV	116.5	103	103	109	119	103 – 119
Bfe IV	181	198	193	197	178.5	178.5 – 198
Tfe IV	309.5	289	280	290	322.5	280 – 322.5
Ge IV	378.8	334	325	380	371	325 – 380
Ti IV	408	371	370	399	392	370 – 408
Ta IV (L)	200	187	188	192	215.5	187 – 215.5
Leg IV	1593.8	1482	1459	1567	1598.5	1459 – 1598.5

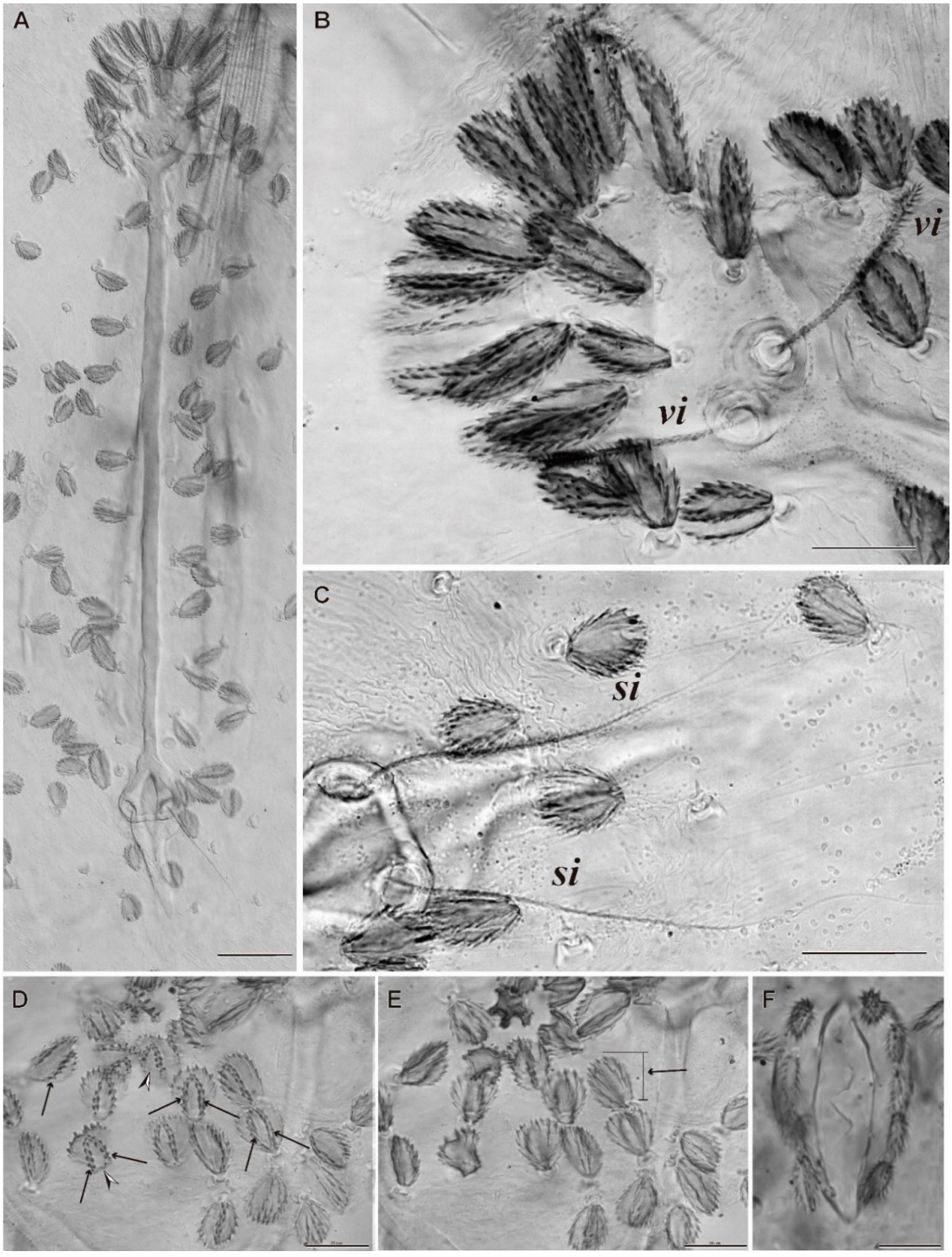

Figure 2. *Trichosmaris sp. nov.* A: Crista metopica. B: Anterior sensillar area. C: Posterior sensillar area. D: Dorsal setae showing tectum, black arrow pointing two pairs of spicules rows and arrowhead pointing a reentrance in the middle of tectum. E: The same setae focused on the carina, black arrow indicating the carina. F: Anal valves. Scale: A=50 µm, B to F= 20 µm.

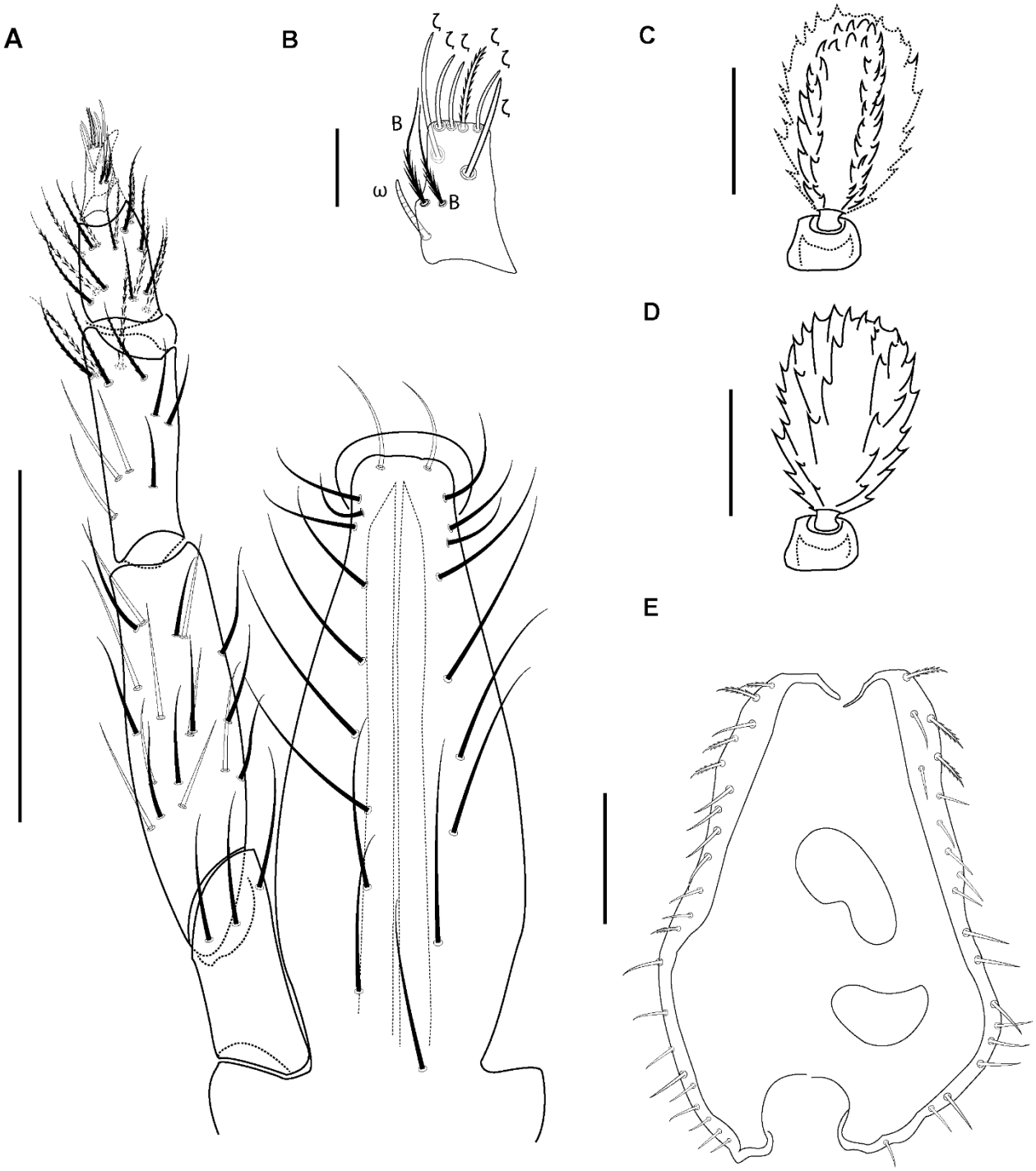

Figure 3. *Trichosmaris sp. nov.* A: Gnathosoma ventral view. B: Palp tarsi. C: Dorsal setae showing the tectum. D: Dorsal setae showing the carina. Scale: A= 100 µm, B to D = 10 µm, E = 50 µm.

Key to *Trichosmaris* species (larvae and adults). Updated from Costa *et al.* (2021)

1 – Eight legs, well developed genital pore present, adult. … 2

– Six legs, genital pore absent, larva. … 7

2 – Barbed portion of setae *si* = 42–45, ISD<380. … 3

– Barbed portion of setae *si* = 54–79, ISD= 390–577. … 5

3 – Anterior sensillar area with 5 short and large setae (setal length/width ratio= 1.27–1.35), similar in appearance to other idiosomal setae; Papua New Guinea. … *Trichosmaris papuana* Beron, 2002

– Anterior sensillar area with 10 long setae (setal lenght/width ratio= 2.48–4), longer and more parallel-sided than those observed on the idiosoma (Fig. 15B). … 4

4 – Genu I length= 252–263, tibia IV longer than genu IV; Brazil. … *Trichosmaris paulensis* Costa *et al.*, 2021

– Genu I length= 341–377, tibia IV shorter than genu IV; USA. … *Trichosmaris jacoti* (Southcott, 1946)

5 – Tibia III longer than genu III, Tibia IV longer than genu IV; Brazil. … *Trichosmaris sp. nov.*

Tibia III shorter than genu III, Tibia IV shorter than genu IV … 6

6 – Barbed portion of setae *si* (*pars clavata*) = 54–57; ratio barbed/smooth portion of setae *si*= 1.00–1.03; dorsal setae with relatively shorter tectum, serrations (including those of carinal flange) and spicules less conspicuous (Fig. 8A and F); USA, Mexico. … *Trichosmaris dispar* Southcott, 1963

– Barbed portion of stae *si* (*pars clavata*)≈ 76, ratio barbed/smooth portion of the *si* = 1.26, dorsal setae tectum with relatively longer serrations (including those of carinal flange) and spicules conspicuous; Guatemala. … *Trichosmaris dentella* Southcott, 1963.

7- PW= 53–78, *Vi*= 33–40, A-P 15–23. … 8

– PW= 43–49, *Vi*= 25–26, A-P 9–12; Costa Rica. … *Trichosmaris longirostris* (Southcott, 1995).

8 – Eupathid (ζ a) and companion seta (Cp) on tarsi I situated on a protuberance, at least one eupathid on tarsi II bifid. … 9

– Eupathid (ζ a) and companion seta (Cp) on tarsi I not on a protuberance, eupathidium on tarsi II simple; Brazil. … *Trichosmaris calcarensis* Costa *et al.* 2021.

9- Barbed setae on palp femur situated in a fold, AW= 54–59, PW= 77–78; USA, Mexico. … *Trichosmaris dispar* Southcott, 1963

– Barbed setae on palp femur situated on the surface, fold absent, AW= 40–44, PW= 53–65; Brazil. … *Trichosmaris paulensis* Costa *et al.* 2021.

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Capítulo 1.2 – Erythraeidae

Neste Capítulo são apresentadas as descrições morfológicas de dois novos táxons de Erythraeinae (Erythraeidae). Um novo gênero fóssil do Eoceno e uma nova espécie sexualmente dimórfica do Chile. A nova espécie Chilena foi incluída na filogenia apresentada no capítulo 2 pelo espécime UFMG AC 170770.

Capítulo 1.2.1: Erythraeinae, gen. et sp. nov. (Acariformes: Parasitengona: Erythraeidae) from Eocene Baltic amber

Samuel Geremias Dos Santos Costa* [1, 2], Alexandr Alexandrovich Khaustov [2], Pavel B. Klimov[2,3] & Almir Rogério Pepato [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 6 Volodarskogo Str., 625003 Tyumen, Russia.*

[3] *Purdue University, Lilly Hall of Life Sciences, G-226, 915 W State St, West Lafayette, IN 47907.*

** Corresponding author. E-mail: estoupa.bob@gmail.com*

Abstract

Here, we describe a new genus and species of a post larval erythraeid mite from Baltic amber, Erythraeinae gen. *et* sp. nov. (Parasitengona: Erythraeidae: Erythraeinae). The new genus differs from all other genera of Erythraeidae by the combination of the following characters: absence of modified spine-like setae on the palp tibia, simple dorsal setae, elongated leg I, presence of a large dorsal shield and two pairs of eyes. Erythraeid mites in amber are briefly reviewed and some of those described by Anton Menge are treated here as *nomina dubia*.

Key words:

Erythraeinae review, Measurements in amber, Invertebrates paleontology.

Introduction

Parasitengona is a diverse and widely distributed mite lineage known from all continents except Antarctica (Mąkol & Wohltmann 2012). They live in a variety of habitats, such as fresh water (Hydrachnidae), soil (some Trombidioidea), and hot and dry surfaces (e.g.: *Balaustium* von Heyden, 1826) (Wohltmann 2000). The life cycle has three active stages: parasitic larva, the deutonymph, and adult. The postlarval stages are predatory and morphologically heteromorphic in comparison to larvae (Wharton & Fuller 1952), rarely larvae may be predatory too (e.g.: *Balaustium*). Due to the profound morphological differences, morphologies of both larval and post larval stages are used in parallel in systematics, although not every taxon is known from both. Among the terrestrial Parasitengona, the family Erythraeidae is mainly distinct by the long, styliform chelicerae that may be retracted into the idiosoma on the post larval instars, absence of Claparède's organs (urstigmata) in all instars and the absence of the ânus in larvae (Welbourn 1991). The family is subdivided into seven subfamilies: Abrolophinae Witte, 1995, Balaustiinae Grandjean, 1947, Callidosomatinae Southcott, 1957, Erythraeinae Robineau-Desvoidy, 1828, Leptinae Billberg, 1820, Myrmicotrombiinae Southcott, 1957 and Phanolophinae Southcott, 1946a. The main diagnostic traits of the subfamily Erythraeinae are the presence of two pairs of eyes in all active instars (Southcott 1961), which may be a homoplasy (Pepato *et al.*, 2022) combined with the lack of prominent anterolateral projections at the idiosoma of post larval specimens' "shoulders" (vs. present in Phanolophinae). Larval Erythraeinae also differ from Phanolophinae due to telofemur of leg I without solenidia and contiguous prodorsal eyes (vs. solenidia present and prodorsal eyes well separated).

The earliest known fossil of Parasitengona is an undescribed taxon (reported from Lebanese amber dated from the Cretaceous, lower Barremian (~ 129 my (Azar, 2007, Maksoud *et al.* 2016, Maksoud & Azar 2020). The oldest described Parasitengona fossil, *Pararainbowia martilli* Dunlop, 2007, from the limestone in the Crato formation, Cretaceous, Aptian (at least 112 my), is a large erythraeid mite that couldn't be assigned to a subfamily due to the lack of detail (Dunlop, 2007).

Various fossil erythraeids were also found in Cretaceous amber: *Leptus* Latreille, 1796 from the Early Cretaceous (upper Albian) in Spain (Arillo *et al.*, 2018); *Burerythrites* Konikiewicz & Mąkol, 2018 and *Burphanolophus* Konikiewicz & Mąkol, 2018 from Cretaceous Burmese amber.

The following Erythraeidae were described from 40-50 million old Late Eocene Baltic amber (Dunlop *et al.* 2020; Weitschat & Wichard 2002; Bogri *et al.* 2018): *Arytaena* Menge *in* Koch & Berendt, 1854, *Balaustium illustris* (C. L. Koch & Berendt, 1854), *Eatoniana crinita* Sidorchuk *et al.* 2019, *Erythraeus longipes* (C. L. Koch & Berendt, 1854), *E. foveolatus* (C. L. Koch & Berendt, 1854), *E. bifrons* (Menge *in* C. L. Koch & Berendt, 1854), *E. hirsutus* (Menge *in* C. L. Koch & Berendt, 1854), *E. lagopus* (Menge *in* C. L. Koch & Berendt), *E. proavus* (Menge *in* C. L. Koch & Berendt, 1854), *E. procerus* (Menge *in* C. L. Koch & Berendt, 1854), *E. raripilus* (Menge *in* C. L. Koch & Berendt, 1854), *E. rostratus* (Menge *in* C. L. Koch & Berendt, 1854), *E. saccatus* (C. L. Koch & Berendt, 1854), and *Leptus incertus* (C. L. Koch & Berendt, 1854).

Here we describe a new fossil genus and species of the subfamily Erythraeinae from Baltic amber. The new species has greatly elongated first pair of legs (Fig. 1C, 4A) and simple weakly barbed dorsal setae (Fig. 1A).

Material and methods

Material Studied

One adult or deutonymph (the specific instar could not be confirmed) from Baltic amber, Yantarnii mine, Kaliningrad region, Russia. The amber piece was certified by the International Amber Association, code JDC-8676. The specimen is preserved in a water/thymol solution and deposited at the Acarological Collection at *Centro de Coleções Taxonômicas* at the *Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil (UFMG AC), code: UFMG AC 220974. The amber piece was processed as described in Sidorchuk & Vorontsov (2018) by D. Vorontsov and observed in saturated fructose solution (Khaustov *et al.* 2021). New names were registered in ZooBank as follows: “Will be provided when the ms is accepted for publication”.

Description

Microphotographs were taken using a Leica DM 2500 compound microscope with a Leica DFC 450 camera and later Zeiss Imager.A2 compound microscope with an Axiocam 506 camera (Fig. 6). Drawings were made using the camera and the OnTopReplica v.3.5.1.0 software. Consecutive photos taken in different focal distances were stacked and Artwork was done in Adobe Photoshop CC 2015 and Adobe Illustrator CC 2015.

Most setae have a non-sclerotized core, giving them a hollow appearance. In some of these setae the core is filled by air giving a dark appearance, while in other translucid fossilized tissue may be present. Those setae are illustrated by dashed lines inside the setae, as illustrated on fig. 1, 2 and 3 and are referred to as “hollow setae” in the description.

Artifacts were tracked by employing reflective and transmitted light in different angles and exploring different configurations of the microscopes listed above. The sample was later submerged in water inside a syringe and vacuum was applied on it for 4 days to reduce air bubbles.

Terminology and abbreviations were adapted from Southcott (1961), Grandjean (1947). Welbourn and Young (1987) compared the two systems and discusses the terms L1 and L2.

Measurements

All measurements are given in micrometers (µm). The fossil specimen is tridimensional (Fig. 4), instead of being flattened as in a usual mite slide preparation. This may make traditional measurements imprecise. Because any two points in the mite body may be separated by a considerable vertical distance (Δ*y*), in addition to the traditionally measured horizontal distance (Δ*x*). Therefore, the real distance between two points can be represented as the hypotenuse of a triangle.

$$Real\ lenght = \sqrt{\Delta x^2 + \Delta y^2}$$

The observed vertical distance between any given pair of focal points (d’) was measured using the scale in the fine focus adjustment of Leica DM 2500. The real vertical distance (Δ*y*) was then estimated considering the refractive indexes of the dry objective (n0 = 1), standard slide coverslip (n1= 1.52), the saturated fructose medium used between coverslip (n2= 1.49 at 25°), and the Baltic amber (n3= 1.54) (Penney 2016, Ke & Imai 2014, Khaustov *et al.* 2021).

This estimation was done by employing the formula provided by Leica DM2000 Operating Manual item 10.2 leading to:

$$\Delta y = d' * \frac{n1}{n0} * \frac{n2}{n1} * \frac{n3}{n2}$$

$$\Delta y = d' * \frac{1.52}{1} * \frac{1.49}{1.52} * \frac{1.54}{1.49}$$

$$\Delta y = d' * 1.54$$

The measurements of the estimated *Real length*, the measured vertical distance *d'*, the estimated vertical distance (Δy) and the traditionally measured horizontal distance (Δx), are presented in table 1.

Results

Systematics

Family Erythraeidae Robineau-Desvoidy, 1828

Diagnosis:

Adults and deutonymphs with long needle-like cheliceral blades that may be retracted into the idiosoma, gnathosoma without any extensible collar (armilla) enabling its forward projection. Larvae without trichobothria on legs, ânus or excretory pore. Claparède organs absent (adapted from Southcott, 1961).

Subfamily Erythraeinae Robineau-Desvoidy, 1828

Diagnosis:

Two pairs of eyes in larval and post larval instars. Post larval instars lacking prominent anterolateral projections at the idiosoma ("shoulders"). Larva lacking solenidia on telofemur of leg I and with contiguous prodorsal eyes (modified from Southcott, 1961).

Genus Erythraeinae gen. nov.

Type species: Erythraeinae gen. et sp. nov.

Diagnosis:

Adult with the diagnostic character states of Erythraeidae and Erythraeinae. Dorsal idiosoma covered by simple weakly barbed setae (Fig. 1A). Legs with seven segments; two pairs of homomorphic claws with terminal hook and medial empodium lacking on all legs; crista metopica well developed connecting anterior and posterior sensillar areas; eyes between the two sensillar areas (Figs. 1A, and 6). Palp tibial claw entire, with a short basal prong; palp tibia and genu without conalae or spine-like setae (Fig. 2C); legs I greatly elongated 2.5 times longer than the idiosoma; coxae I modified, with long and short antero-lateral setae pointing forward (Figs. 1A and C, 4 A and B).

Erythraeinae gen. et sp. nov.

Diagnosis:

Adult: Tibia, genu and telofemur I, tibiae II, III and IV with thin dorsal solenidia (Figs. 2A, 3 and 5C); leg and palpal setae with a poorly sclerotized core or hollow, i.e., as evidenced by dark core and air bubbles (Figs. 1D, 5A, B and D). All setae on palps smooth or weakly barbed (Figs. 2B, 5D); distal end of tibia bearing thick barbed and hollow setae (Fig. 5B). Two pairs of distinct long setae along crista metopica (L1 and L2, Fig. 1A). Tarsi of leg I with four elongated distal hollow setae, representing possibly eupathidia (ζ) or solenidia (ω). Coxae I, II, III and IV with long thin setae (Fig. 1A).

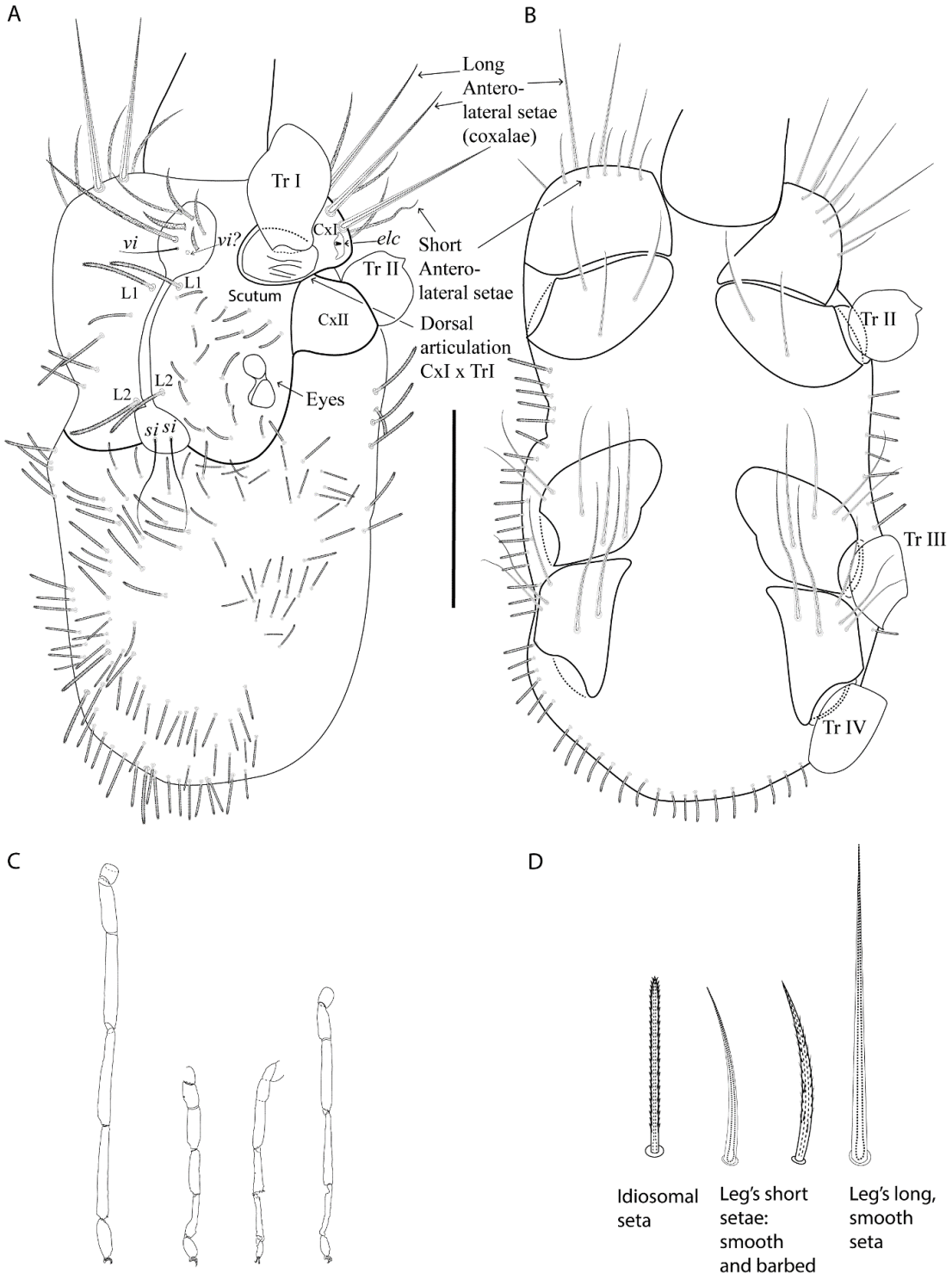

Figure 1. Erythraeinae gen. *et* sp. nov. A: Dorsal view, L1 and L2 indicate two pairs of distinctly long dorsal setae along the crista metopica, one seta *vi* is missing. B: Ventral view, trochanters (Tr) are shown. C: Relative leg lengths, see table I. D: Main types of hollow setae, hollow setae are indicated by dashed lines. Scale bar: A and B= 200 µm.

Description

Idiosoma nearly rectangular (Fig. 1A). Two pairs of eyes. Crista metopica well developed connecting the anterior and posterior sensillar areas bearing two pairs of filiform trichobothria (*vi* and *si*) (Figs. 1A, 4A, 6A). Anterior sensillar area with six long and weakly barbed setae. Anterior portion of idiosoma covered by a large scutum (see discussion bellow, Fig. 1A, 4A and 6). Two pairs of distinctly long, weakly barbed setae situated along crista metopica like the ones observed in *Lasioerythraeus* (L1 and L2, Fig. 1A). Anterior dorsal idiosoma covered by short, hollow, and weakly barbed setae (Fig. 1D). Genital and anal valves not observed.

Gnathosoma conical, without armilla and apparently non-extendible. Palps long and robust, covered by thin, smooth, or weakly barbed hollow setae. Palp tibial claw entire, with a short basal prong. Palp tibia and genu without conalae or spine-like setae (Figs. 2B, 5D).

Legs 7-segmented. Tarsi with one pair of finely barbed claws, median empodium absent (Figs. 2A and 3). Legs covered by typical hollow setae, some curved and short (barbed or smooth), other straight and long (Figs. 1D, 5A and B). Some of these setae are filled with air and look dark (Fig. 5B). Leg I antenniform, 2.8 times longer than idiosoma, 2 times longer than leg II, 1.84 x leg III and 1.5 x leg IV. Coxae I, II, III and IV with long setae pointing frontwards in our specimen, in addition, coxae I with a second morphotype consisting in antero-lateral short and thin setae. Joint between the coxa and trochanter I well sclerotized and observed in a dorsal position. This joint allows leg I to extend vertically more than 90˚ relative to idiosoma (Figs. 1A, C, 4A, 6). Tarsi of leg I with four distinctly long distal hollow setae, possibly eupathidion (ζ) or solenidion (ω). Tibia I and genu III with a distal microseta (Fig. 2A, 3B and 5A). Genu III with a distinct medial, thin, barbed seta (Fig. 3B). Tibiae I, II, III and IV, genua I and IV and telofemur I with thin dorsal solenidia (Fig. 2A, 3 and 5C). Coxa I sclerotized, punctuated and with supracoxala (*elc*) located inside a less sclerotized oval depression (Fig. 6D).

See table 1 for measurements.

Table I – Measurements of Erythraeinae gen. et sp. nov. Values are given as follows: distance between two points (Δx), vertical distance between the focus of the two points (d'), estimated vertical distance between the two points (Δy) (d' corrected by considering the influence of refraction), estimated real length.

Character	Δx	d'	Δy	**Estimated Real length**	**Character**	Δx	d'	Δy	**Estimated Real length**
IL	661	36	55.44	**663**	Ti II	202	0	0	**202**
IW	310	100	154	**346**	Ta II (L)	113	0	0	**113**
ISD	192	0	0	**192**	Leg II	-	-	-	**899**
L	260	0	0	**260**	Tr III	108	0	0	**108**
Scutum width	265	68	104.72	**285**	Bfe III	117	0	0	**117**
Distance beteween anterior eye's	187	67	103.18	**214**	Tfe III	208	0	0	**208**
Tr I	82	0	0	**82**	Ge III	217	46	70.84	**228**
BFe I	243	28	43.12	**247**	Ti III	214	99	152.46	**263**
TFe I	425	0	0	**425**	Ta III (L)	92	0	0	**92**
Ge I	433	21	32.34	**434**	Leg III	-	-	-	**1016**
Ti I	492	0	0	**492**	Tr IV	78	0	0	**78**
Ta I	195	0	0	**195**	Bfe IV	13	0	0	**13**
Leg I	-	-	-	**1875**	Tfe IV	318	104	160.16	**356**
Tr II	112	0	0	**112**	Ge IV	368	95	146.3	**396**
Bfe II	93	0	0	**93**	Ti IV	222	119	183.26	**288**
Tfe II	187	0	0	**187**	Ta IV (L)	118	0	0	**118**
Ge II	192	0	0	**192**	Leg IV	-	-	-	**1249**

Differential diagnosis

Erythraeinae gen. nov. belongs to the erythraeine genus group that lacks conalae on the palp tibia and palp genu in the post larval instars. The group consists of the following genera († indicates a fossil taxon): *Abalakeus* Southcott, 1994, † *Burphanolophus* Konikiewicz & Mąkol, 2018 (Cretaceous Burmese amber), † *Burerythrites* Konikiewicz & Mąkol, 2018 (Cretaceous Burmese amber), *Curteria* Southcott, 1961, *Eatoniana* Cambridge, 1898, *Eryhtraxus* Southcott, 1961, *Erythrellus* Southcott, 1946b, *Erythroides* Southcott, 1946b, *Kamertonia* Gabrys, 2000, *Neosmaris* Hirst, 1926, *Paraphanolophus* Smiley, 1968, *Rainbowia* Southcott, 1961. Although the fossil † *Eatoniana crinita* Sidorchuk *et al.* 2019 (Late Eocene Baltic amber) has three strong spines on the palp tibia, they do not seem homologous to conalae. Erythraeinae gen. nov. differs from all the genera listed above by the presence of a large scutum that includes the eyes and its greatly elongated leg I (2.8 times longer than the idiosoma, twice as long as leg II, 1.84 x leg III and 1.5 x Leg IV; vs. not distinctly elongated in other genera).

In addition, Erythraeinae gen. nov. differs from *Abalakeus* Southcott, 1994, † *Burphanolophus* Konikiewicz & Mąkol, 2018, *Eryhtraxus* Southcott, 1961, *Erythrellus* Southcott, 1946b, and *Kamertonia* Gabrys 2000 by the presence of a single type of simple-barbed dorsal setae (vs. dorsal setae either leaf-like and expanded, represented by two distinct morphotypes, scale-like or bifid in the five listed genera). It also differs from *Neosmaris* by the well-developed crista metopica (vs. reduced to sensillar areas in *Neosmaris*); from *Rainbowia* by the long palp tarsus (vs. short and rounded in *Rainbowia*); from *Erythroides* and *Paraphanolophus* by the absence of modified serrate setae (serratalae) on the legs (vs. present in *Erythroides* and *Paraphanolophus*); from *Curteria* by the typical position of crista metopica (vs. distinctly posterior to the rostrum in *Curteria*). Erythraeinae gen. nov. also differs from *Eatoniana* Cambridge, 1898 by legs IV being shorter than leg I and lacking plumes (vs. longer than leg I and with plumes in *Eatoniana*).

C. L. Koch & Berendt (1854) described several fossil post larval *Erythraeus* Latreille, 1806. Although Erythraeinae gen. nov. differs from *Erythraeus* by the absence of conalae (vs. present), the descriptions included in C. L. Koch & Berendt (1854) – *Erythraeus longipes, E. foveolatus,* and *E. saccatus* – were not detailed enough to indicate if conalae were present or not.

Erythraeinae gen. nov. differs from *E. longipes* and *E. foveolatus* due to leg I almost two times longer than leg IV (vs. subequal). Erythraeinae gen. nov. differs from *E. saccatus* C. L. Koch & Berendt, 1854 by legs II to IV much longer than the idiosoma (vs. shorter in *E. saccatus*).

In the same work (Koch & Berendt 1854), Menge introduced several other fossil species as footnotes: *Arytaena troguloides* Menge *in* C. L. Koch & Berendt, 1854, *Erythraeus bifrons* (Menge *in* C. L. Koch & Berendt, 1854), *E. hirsutus* (Menge *in* C. L. Koch & Berendt, 1854), *E. lagopus* (Menge *in* C. L. Koch & Berendt), *E. proavus* (Menge *in* C. L. Koch & Berendt, 1854), *E. procerus* (Menge *in* C. L. Koch & Berendt, 1854), *E. raripilus* (Menge *in* C. L. Koch & Berendt, 1854), *E. rostratus* (Menge *in* C. L. Koch & Berendt, 1854). Except by *A. troguloides*, these descriptions were short and lacking details or illustrations. Menge's types were not found in the Westpreußische Provinzialmuseum, Danzig (now Gdańsk) in Poland, (Dr. J. Dunlop, pers. comm.). Therefore, we consider all *Erythraeus* species cited in this paragraph *nomina dubia*. The larval species *A. tronguloides* Menge *in* C. L. Koch & Berendt, 1854 was described with more details, but can’t be compared to our adult specimen due to heteromorphic life cycle.

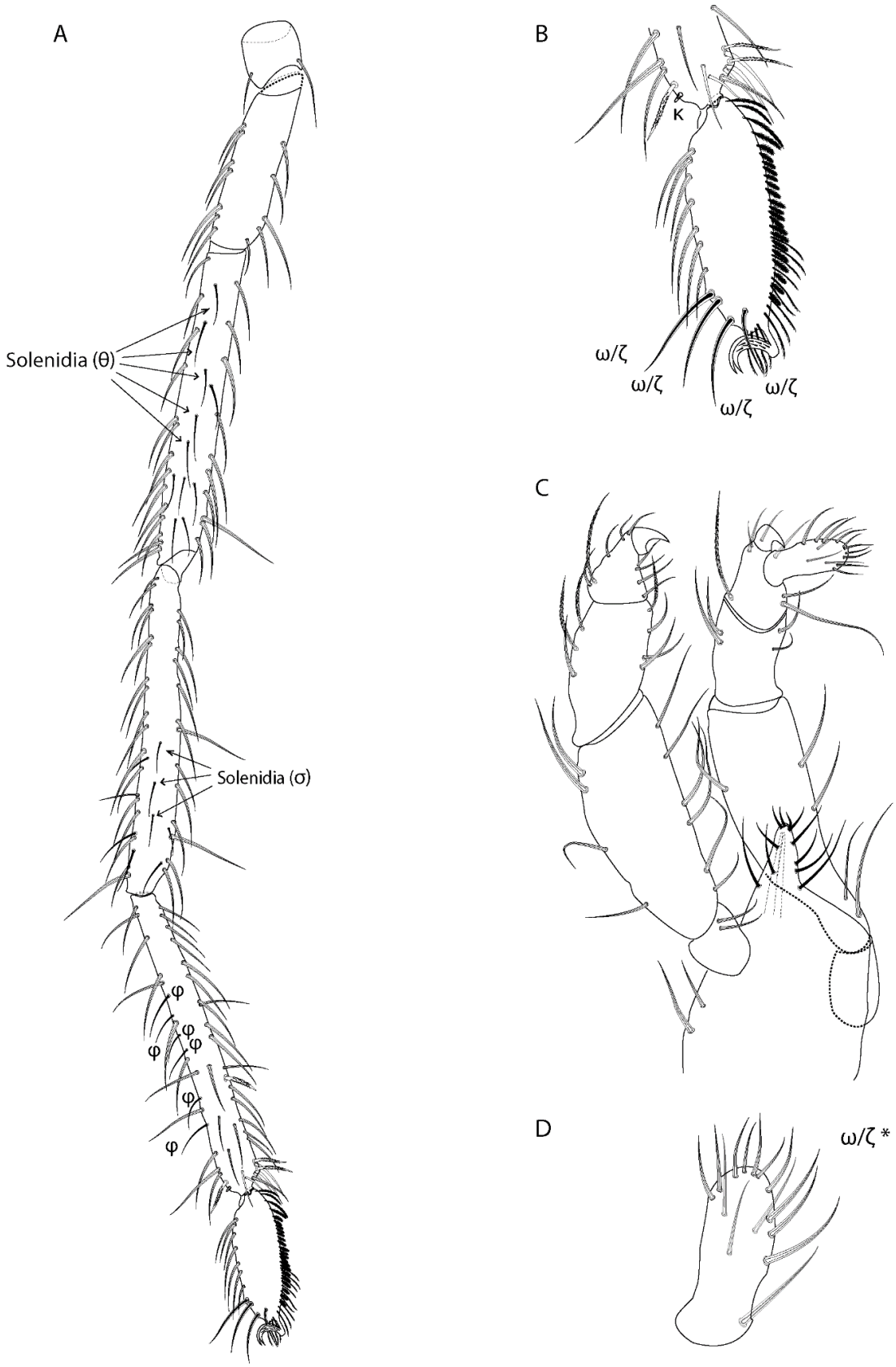

Figure 2. Erythraeinae gen. et sp. nov. A: Leg I, B: Tarsus I and distal portion of tibia I with micro seta (k). C: Gnathosoma, lateral. D: Palp tarsus, lateral. *Setae on the palp tarsi possibly are eupathidia (ζ) or solenidia (ω).

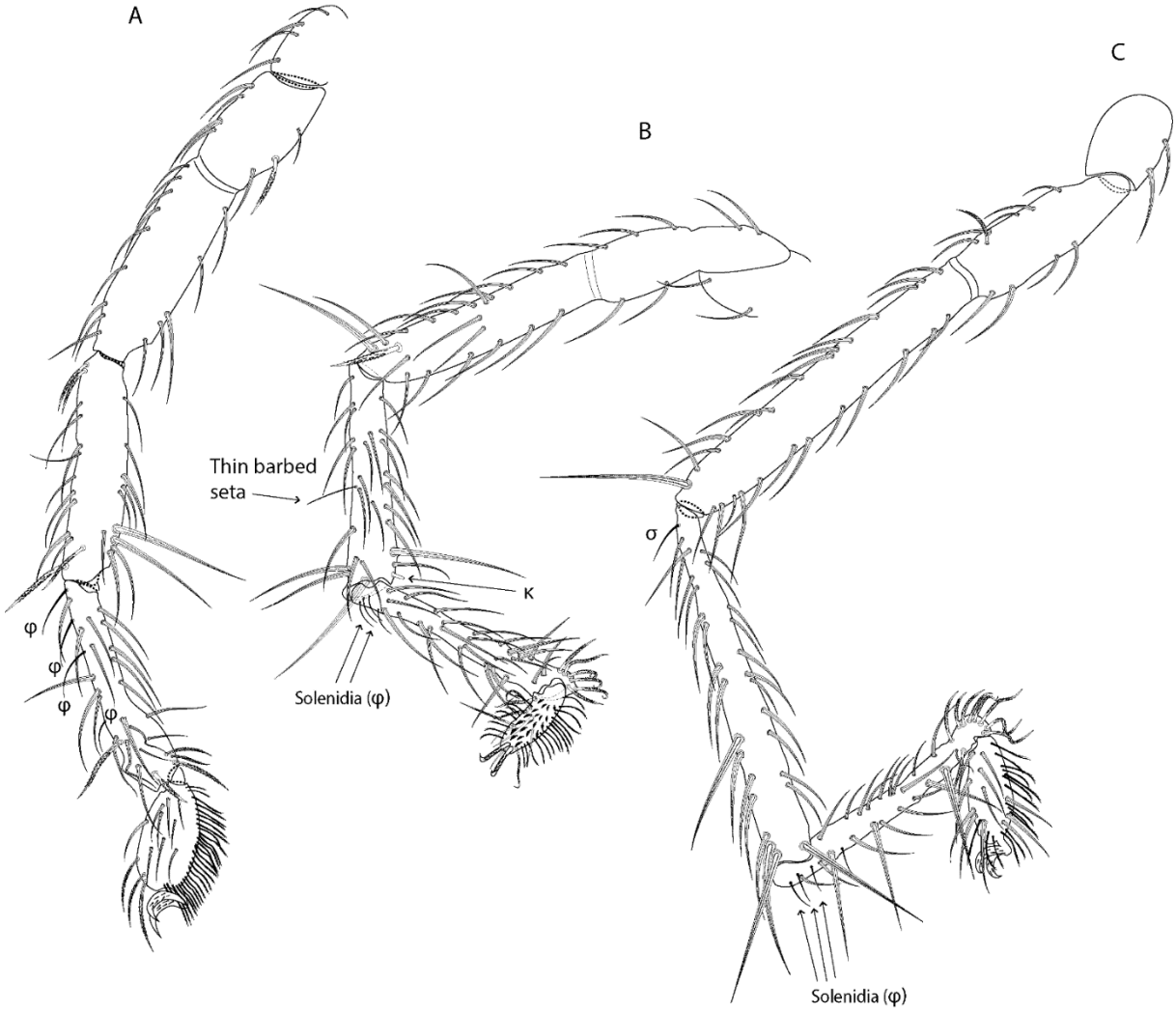

Figure 3. Erythraeinae gen. *et* sp. nov. A: Leg II. B: Leg III. C: Leg IV. Note: Not all leg's setae were visible due to optical limitations. Leg segment shapes may be distorted due to their orientation.

Etymology

To be defined when publishing.

The large scutum

On figure 6 the presence of a large dorsal scutum was investigated. The scutum is indicated on Fig. 1A, 4A and 6 and its borders are highlighted by black arrow heads in Fig. 6A and white ones in Fig. 6B. Trying to differentiate this scutum from an artifact (AF), photos were taken before and after vacuum treatment. In both cases, stacked photos were first with reflected light showing objects located over the idiosma's cuticle (Fig. 6A) and transmitted light that better illustrates objects below the surface (Fig. 6B, C and D).

Using transmitted light (Fig. 6B), a big air bubble (AF1) is observed under the cuticle of the scutum, this bubble looks dark under transmitted light (Fig. 6B and D), and its borders are marked by blue arrowheads (Fig. 6A, B and C). After applying vacuum for 4 days in the sample submersed in water the air bubble (AF1) was reduced in volume or changed position (Fig. 6C).

The borders of AF1 do not coincide with all borders of the scutum indicated by black arrow heads on Fig. 6A and white on Fig. 6B. Under reflected light (Fig. 6A) the borders of the scutum look light brown, the same color observed in the sclerotized crista metopica and can be distinguished from the white (due to reflection) limits of the air bubble (AF1), especially in the posterior left border. While in Fig. 6C the antero-lateral (right side) border of the scutum can be seen without the interference of AF1 (reduced by vacuum).

Therefore we could discard the possibility of the scutum being an artefact caused by AF1. The specimen deposited at the acarological collection from UFMG remains available for further analysis with nondestructive imaging methods.

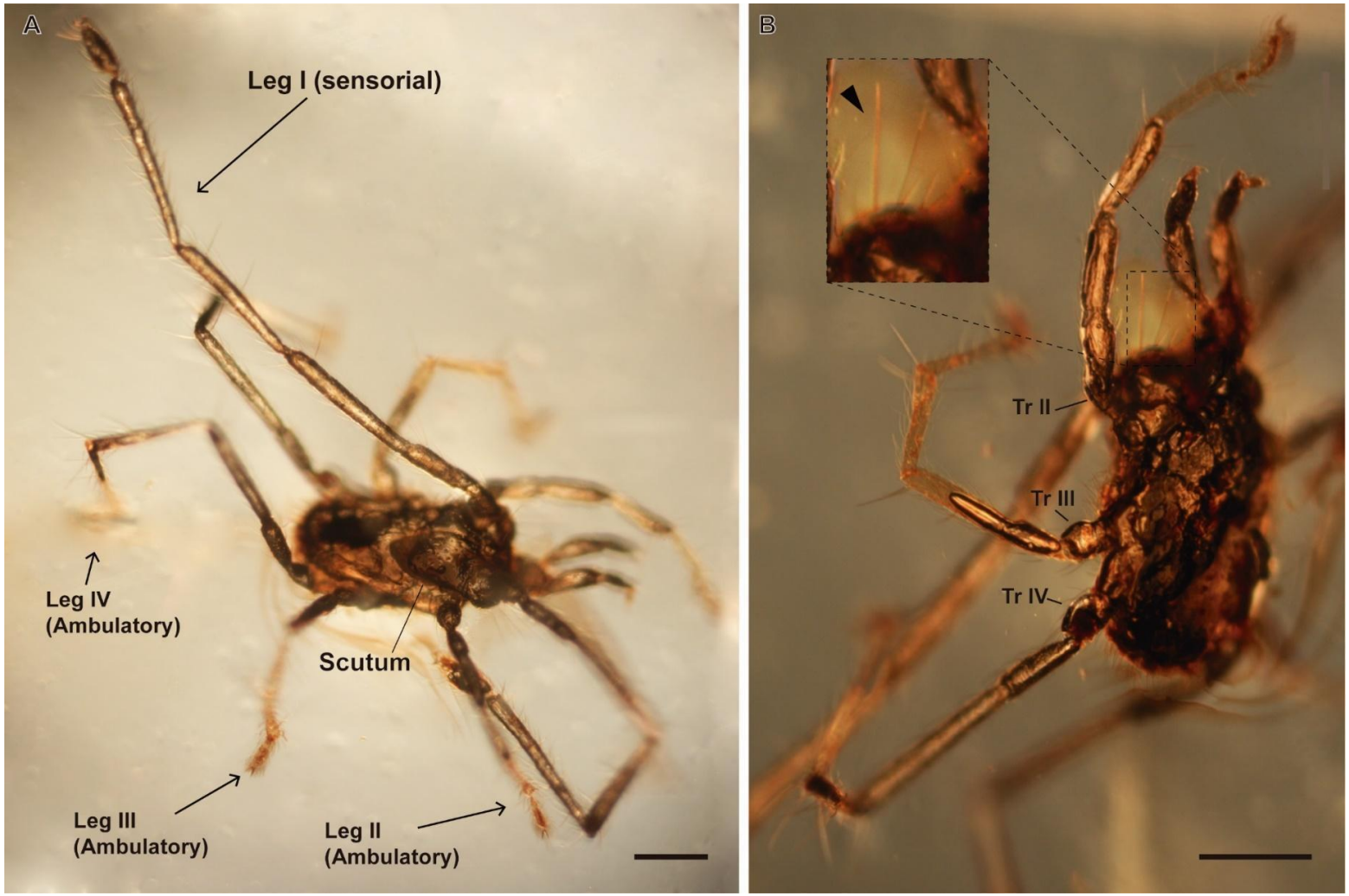

Figure 4. Erythraeinae gen. et sp. nov. A: Dorsal view. B: Ventral view, the arrowhead indicates long antero-lateral coxalae. Scale bars: A and B= 200 µm.

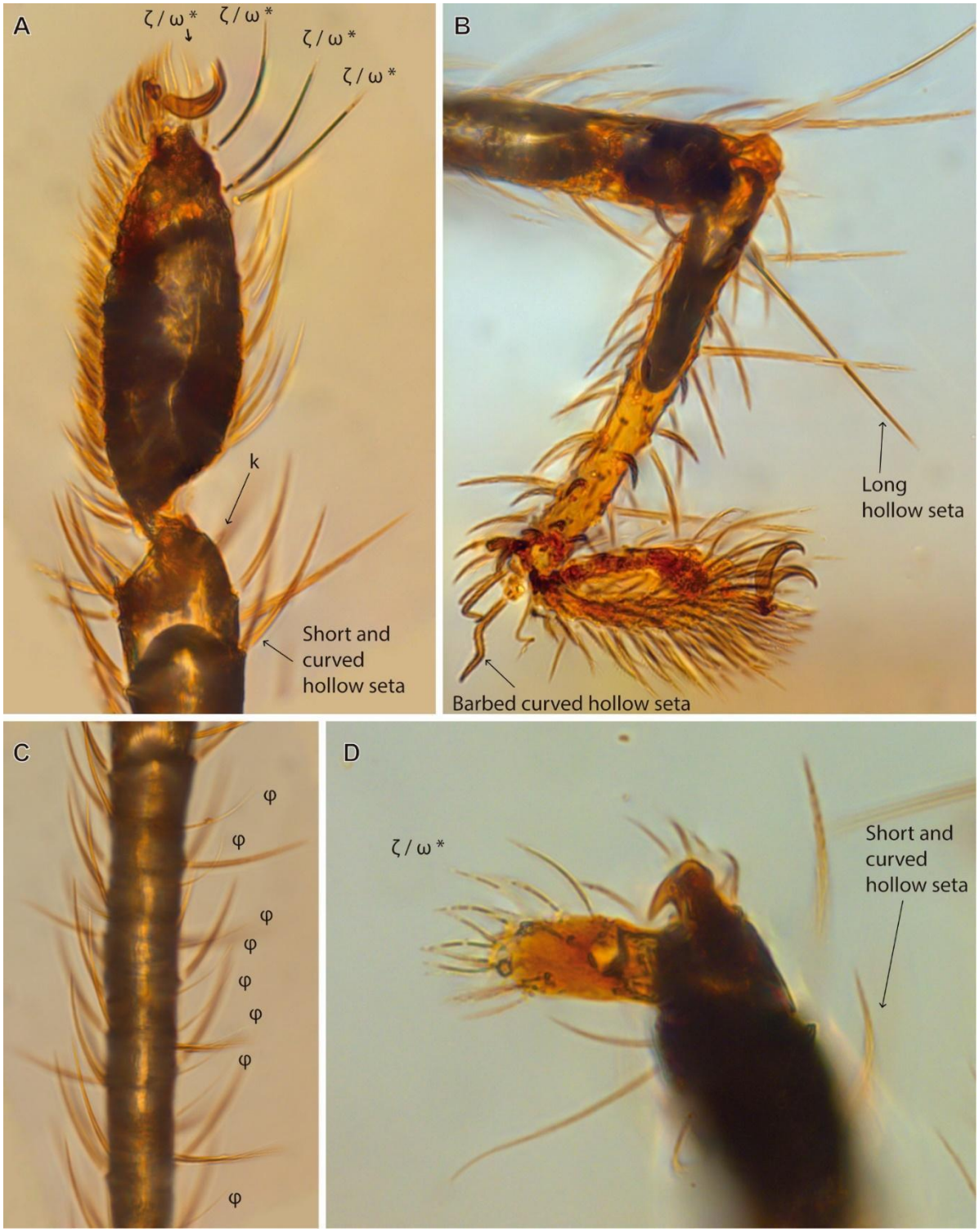

Figure 5. Erythraeinae gen. et sp. nov. A: Tarsi I. B: Leg IV. C: Tibia I. D: Distal portion of the palp. *All setae on the palp tarsi and indicated terminal setae on the tarsi I are eupathidia (ζ) or solenidia (ω).

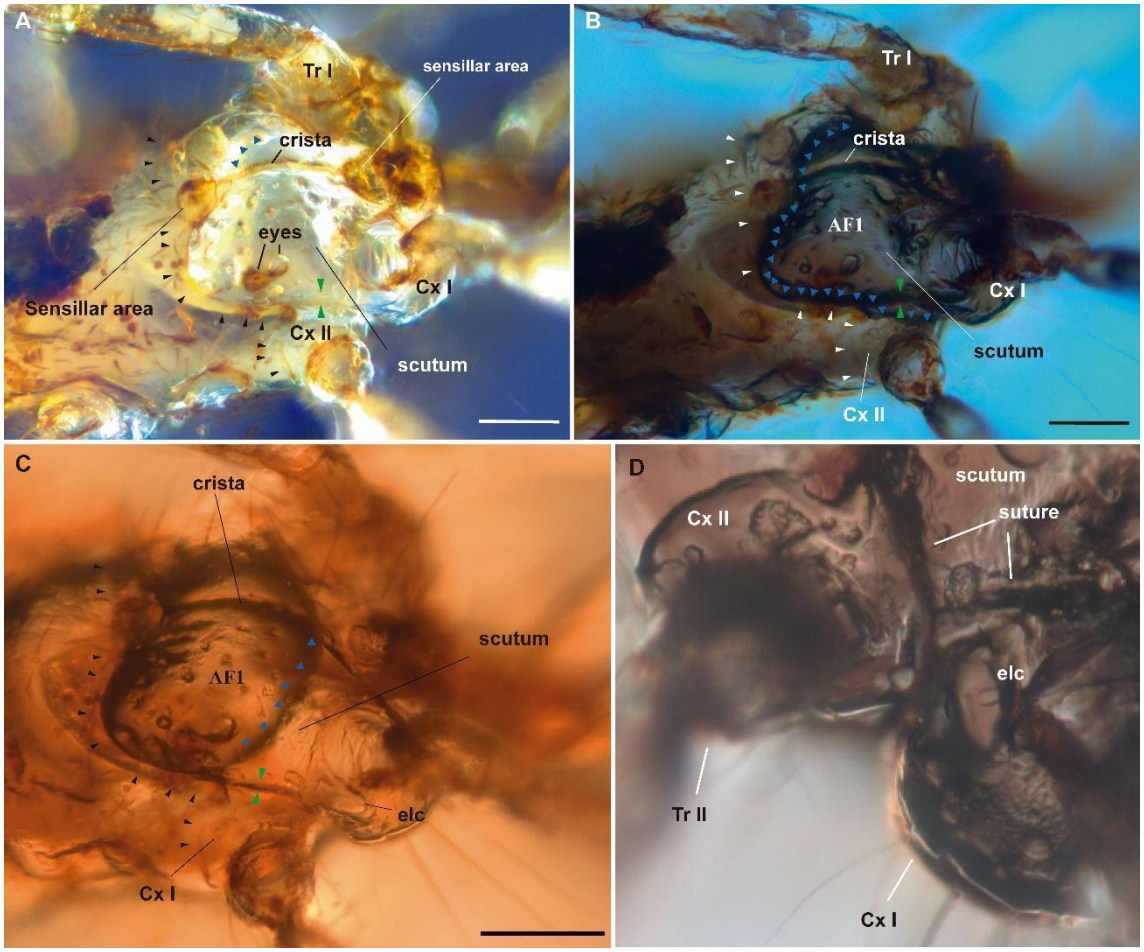

Figure 6. Erythraeinae gen. *et* sp. nov. A: Scutum observed under reflected light, B: Scutum observed under transmitted light. C: Scutum observed under transmitted light after vacuum treatment. D: Detail showing Cx I, II and seta *elc*. AF1 refers to an artifact caused by an air bubble. Black (Fig 6A) and white (Fig. 6B) arrowheads indicate the border of the scutum, green arrowheads indicate the limit between scutum and CxII, blue arrowheads indicate the limits of AF1. Scale bars: 100 µm.

Discussion

Although it can't be easily tested, the setae with non-sclerotized core observed on legs do not appear to be a taphonomic artifact. Although their central core may be degraded during the fossilization, similar setae covering legs and palps were observed in Recent undescribed Chilean conalae-less Erythraeinae, deposited at UFMG AC (e.g.: UFMG AC 170770). The content of this non sclerotized core in living specimens is unknown.

Due to its disproportionately long first pair of legs I, a unique condition among Erythraeinae, we suggest that Erythraeinae gen. nov. used the three posterior legs for locomotion (ambulatory) while the first pair of legs was primarily sensorial (Fig. 4A and B), as observed in some recent mite taxa, such as *Podocinum* Berlese, 1882.

In post larval Parasitengona, the scutum is an important site for muscle attachment and is reduced to the crista metopica in most Erythreaidae (Newell 1958). A few exceptions are *Lasioerythraeus johnstoni* Welbourn & Young 1987, *Balaustium*, *Abrolophus sigma* (Mihelčič, 1964) and *Erythraeus (E.) rupestris* (Linnaeus, 1758) have a thin dorsal shield represented only by a longitudinal band bearing. This condition differs from the large scutum of Erythraeinae gen. nov. that includes the eyes (Fig. 1A, 4A and 6).

Acknowledgements

We thank Dr. Dmitry Vorontsov for preparing the amber sample, to Dr. Jason Dunlop advice and the anonymous reviewers for their valuable contribution to improve this work. SGSC thanks *Programa de Pós Graduação em Zoologia da UFMG* and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* for his scholarship (CAPES – Print) for supporting this study, Tyumen State University and the X-Bio institute for providing facilities. PBK and AAK were supported by the cooperative agreement No. FEWZ-2021-0004 from the Russian Ministry of Science and Higher Education.

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Capítulo 1.2.2: A new sexually dimorphic Chilenean *Rainbowia* (Prostigmata, Parasitengona) and its remarkable resemblance with *Burerythrites* from Burmense amber

SAMUEL GEREMIAS DOS SANTOS COSTA* [1,2] , MARK S. HARVEY [3,4] & ALMIR ROGÉRIO PEPATO [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 10 Semakova Str., 625003 Tyumen, Russia.*

[3]*Collections & Research, Western Australian Museum, Welshpool, Western Australia 6106, Australia.*

[4] *School of Biological Sciences, University of Western Australia, Crawley, Western Australia 6009, Australia.*

** Corresponding author. E-mail: estoupa.bob@gmail.com*

Abstract

Rainbowia Hirst, 1928 (Parasitengona, Erythraeidae) are large Parasitengona mites often found under tree bark in South Australia. The genus has two described species: *Rainbowia celeripes* (Rainbow, 1906) and *Rainbowia imperator* (Hirst, 1928). *Rainbowia* females have long and short idiosomal setae, while males have only short setae, as described by Southcott (1961). Here we describe a new heteromorphic Chilean *Rainbowia* species that are the remarkable similarity to fossil *Burerythrites* Konikiewicz & Mąkol, 2018 from Burmense amber. Both sexes were associated by DNA sequences of the mitochondrial gene cytochrome oxidase I using bGMYC. Considering the new morphological data of the described species, we propose the new synonymy *Rainbowia pankowskii* syn. nov. (Konikiewicz & Mąkol, 2018) from *Burerythrites*.

Key words: Australia, Biodiversity, Erythraeidae, DNA barcoding.

Introduction

Parasitengona are large mites, often with intense red color, that can be found worldwide, except by Antarctica. These mites have a heteromorphic life cycle that includes three active instars: Larva, deutonymphs and adults. The adults and deutonymphs are free living predators and they drastically differ from the parasitic larvae (with rare exceptions). This causes most species to be described solely by larva or by the deutonymph. Researchers have been using molecular and rearing to associate the heteromorphic instars, but the association of sexually dimorphic specimens usually is not required, since most of Parasitengona species have males and females similar in most non-genital morphological characters (Costa *et al.* 2019, Mąkol & Wolthmann 2012).

Rainbowia are large Australian Erythraeinae (Erythraeidae, Parasitengona) mites, comprising only two described species, *Rainbowia celeripes* (Rainbow, 1906) and *Rainbowia imperator* (Hirst, 1928), both from south Australia (Enfield, New South Wales), and Lucindale (South Australia)). *Burerythrites* Konikiewicz & Mąkol, 2018 is a fossil genus from Burmense amber (Hukawng Valley, Myanmar middle Cretaceous c. 97–110 Ma) that only differs from the Australian *Rainbowia* due to palp tibia and tarsus relatively long and cylindrical vs. palp tibia in the shape of an equilateral cone and palp tarsus hemispherical or almost globular (Konikiewicz & Mąkol, 2018).

The present study describes a new sexually dimorphic *Rainbowia* species collected in the Parque Nacional Nahuelbuta (PNN) in Chile with specimens associated through molecular data. This species presents a notable intermediary condition between the Australian *Rainbowia imperator* (Hirst, 1928) and the fossil *Burerythrites* from Burmese amber.

Material and methods

Abbreviations for collections

UFMG AC, Acarological Collection at *Centro de Coleções Taxonômicas* at the *Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais*, Belo Horizonte, Minas Gerais, Brazil.

Studied Material

Rainboiwia sp. nov. Holotype (UFMG AC 170770), female collected by hand on leaf litter, between December 25th and 27th of 2016. Location: Chile, Araucánia, Angol municipality,

Parque Nacional Nahuelbuta, 37° 49' 44” S, 73° 0' 26” W. Collected by P. H. Martins and A. Anker. Deposited in UFMG AC. Paratype (UFMG AC 170769), male, same collecting data.

Rainbowia imperator (Hirst, 1928) (UFMG AC 221524), female collected by hand under *Eucalyptus rudis* tree bark, near Moore River, Regans Ford, Western Australia, 30°59'14.0"S, 115°42'10.0"E. Collected by M. S. Harvey e M. E. Blosfelds and deposited at UFMG AC.

Terminology and abbreviations were adapted from Barr 1972, Grandjean (1947) and Southcott (1961). When measuring legs, we summed the lengths of each segment from trochanters to tarsi, excluding tarsi claws and coxae.

Photos were taken using a Leica DM750 compound microscope with a Leica ICC50W camera. Drawings were made with the aid of a simulated *camera lucida* using the software On Top Replica V. 3.5.1 and the final artwork was done in Adobe Illustrator CC 2015 and Adobe Photoshop CC 2015. All measurements are given in micrometers (µm).

Nomenclature acts were registered on ZooBank and are available at: Provided upon acceptance.

Molecular data

DNA extraction, and Cytochrome Oxidase subunit I (COI) amplification and sequencing as described by Costa *et al.* (2019). The substitution model and best partition scheme, for the three different codon positions, were chosen with ModelFinder (Kalyaanamoorthy *et al.*, 2017) using the Bayesian Information Criterion (BIC) in IQ-Tree (Nguyen *et al.*, 2015). Phylogenetic trees were inferred in Beast 2 and bGMYC analysis (Reid & Carstens 2012) was performed as in Costa *et al.* (2019).

GenBank accession numbers of the taxa studied are provided in table I.

Table I – Studied material: GenBank access numbers.

Taxa	GenBank access number
Erythraeus sp. AD-2011	HQ423154.1
Erythraeus sp.	AJ238264.1
Lasioerythraeus jessicae UFMGAC 161067	MK455822.1
Lasioerythraeus jessicae UFMGAC 161068	MK455823.1
Lasioerythraeus jessicae UFMGAC 161072	MK455824.1
Lasioerythraeus jessicae UFMGAC 161062	MK479907.1
Lasioerythraeus jessicae UFMGAC 161069	MK479909.1
Lasioerythraeus jessicae UFMGAC161064	MK479908.1
Lasioerythraeus jessicae UFMGAC 161070	MK479910.1
Lasioerythraeus jessicae UFMGAC 171238	MK479911.1
Lasioerythraeus jessicae UFMGAC 161062	OM401569.1
Lasioerythraeus sp. UFMGAC 160761	MK455821.1
Rainbowia sp. nov. female UFMGAC 170770	Provided upon acceptance
Rainbowia sp. nov. male UFMGAC 170769	Provided upon acceptance
Rainbowia imperator UFMGAC 221524	Provided upon acceptance

Results

Species delimitation

The IQtree model choice analysis resulted in two partitions, one containing the first and second codon position and the other containing the third codon position. The substitution models chosen were HKY+F+G4 and TPM2u+F, respectively. The phylogenetic analysis recovered *Rainbowia* sp. nov. as sister group of *Rainbowia imperator* (Hirst, 1928). Male and female of *Rainbowia* sp. nov. were recovered by bGMYC as a single species (pp.: >98%) as illustrated in Figure 1.

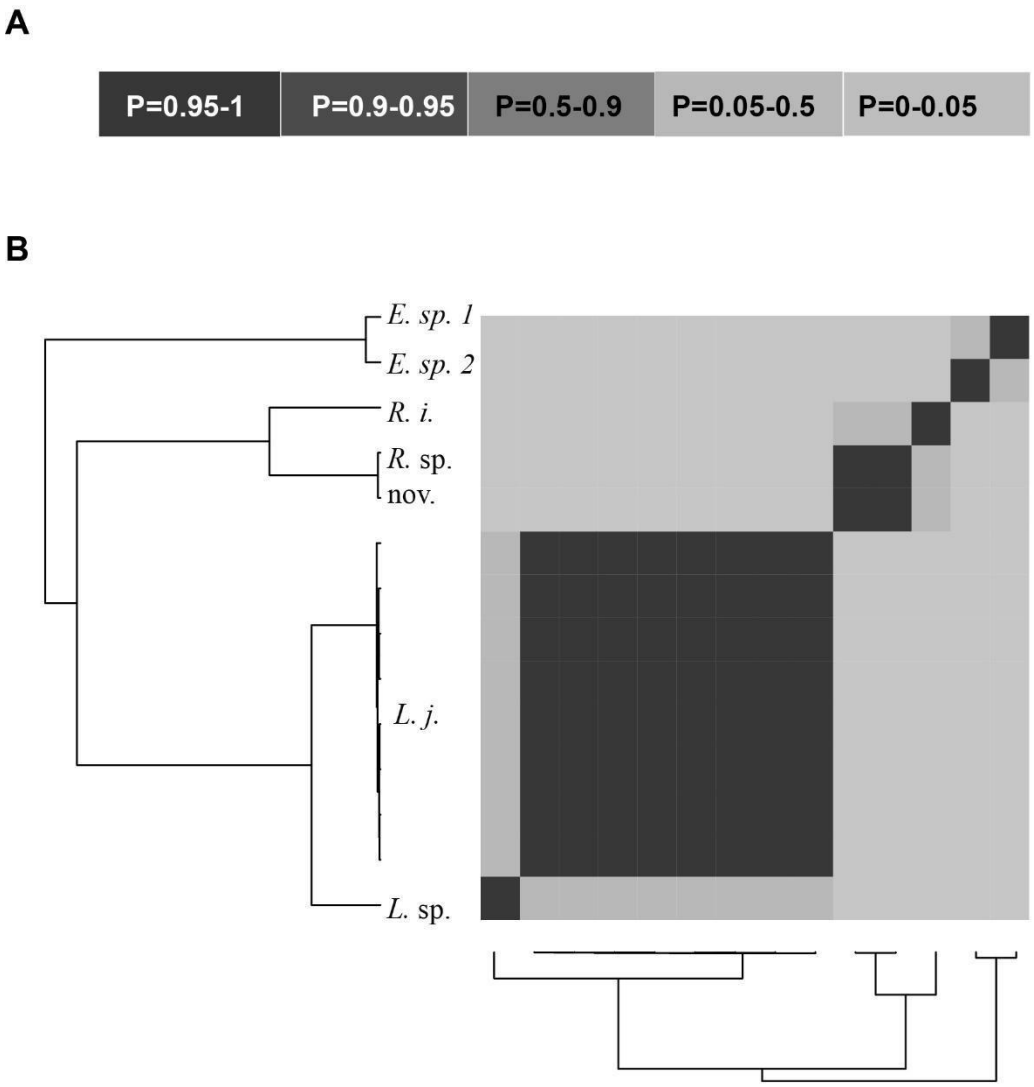

Figure 1. bGMYC results. A: Gray scale indicating the posterior probability of recovering two specimens as a single species. B: Heat map and phylogenetic tree displaying the posterior probability results. The acronym E. refers to *Erythraeus, R.* to *Rainbowia* and *R. i.* to *R. imperator, L. j.* to *Lasioerythraeus jessicae*.

Systematics

Family Erythraeidae Robineau-Desvoidy, 1828

Diagnosis: See Southcott, 1961.

Genus *Rainbowia* Southcott, 1961

Type species *Rainbowia imperator* (Hirst, 1928) by original designation.

Synonym of *Leptus imperator* Hirst, 1928.

Diagnosis:

Adults: Big erythraeid mites with two pairs of eyes located between *si* and *vi*, crista metopica well defined with two sensillary areas and a projection posterior to the posterior sensillary area. The anterior sensillary area forms a conical pointed nasus at the anterior pole of the dorsum of the idiosoma. In the adult male, the dorsal idiosomal setae are roughly uniform in size and shape, and the setae cannot be divided into distinct groups. In the adult female the dorsal idiosomalae are longer and tend to be divided into two distinct classes: (1) a shorter group which are somewhat longer and thinner than the standard of the male setae, and (2) interspersed freely among the first class are longer stronger setae, about 2-3 times as long as those of the first class (modified from Southcott, 1961). Palp of adult and nymph without any ventral conical spines (conalae), or elsewhere on palp.

Remarks: The genus comprises three extant species: *Rainbowia celeripes* (Rainbow, 1906) and *Rainbowia* sp. nov. known from post larval instars only and *Rainbowia imperator* (Hirst, 1928) known from larval and post larval instars. *Rainbowia celeripes* and *R. imperator* are distributed over southern Australia, while *Rainbowia* sp. nov. specimens were collected in the temperate rainforest of Chile. In addition, we propose that *Burerythrites* as a junior synonym *Rainbowia* leading to *Rainbowia pankowskii* syn. nov. (Konikiewicz & Mąkol, 2018) from Cretaceous Burmense amber.

***Rainbowia* sp. nov.**

(Figs 2-9)

Holotype. Female (UFMG AC 170770), collected by hand on leaf litter, between December 25th and 27th of 2016. Location: Chile, Araucánia, Angol municipality, Parque Nacional Nahuelbuta, 37° 49' 44" S, 73° 0' 26" W. Collected by P. H. Martins and A. Anker. Deposited in UFMG AC. Paratype male (UFMG AC 170769), same collecting data.

Diagnosis.

Adults.

Two pairs of eyes. Body surface in three colors: reddish legs, palps and anterolateral portions of the idiosoma; yellow medial and longitudinal stripe surrounded by a black cuticle (Fig. 2A, B, C and D). Palp tibia with numerous solenidia (Fig. 3C, D and 4D). Palp tarsi covered exclusively by striated solenidia (Fig. 4A). Telo-femur IV with the same length of genu IV (Tfe IV/Ge IV=1,0). Relative leg lengths: IV>I> III> II (Fig. 5). Crista metopica with a projection posterior to *vi* (Fig. 4E). Tibia I with zero, II with one, tibia III and IV with three, ventro-distal spines (Fig. 6A, B and C). Micro seta present on tibia I and genu II (Fig. 7A and B). Tarsi IV with only two solenidia (Fig. 7D).

Female.

Idiosoma's three colors are covered by a white velvet setae layer compound by two types of setae: thin, almost translucid and short setae; thick, white, long and apparently hollow setae (Fig. 2B, D and F). Anterior genital acetabula (G.ac.) oval, posterior genital acetabula long and curved (Fig. 4C).

Male.

Idiosoma without a white layer, covered only by thin, almost translucid and short setae (Fig. 2B, D and F). Genital pore surrounded by numerous short and thin setae, without a well-defined external genital plate (Fig. 6D). Internally, ejaculatory complex's anterior arms (AntAm) with 9-10 smooth eugenital setae each, located over protuberances (Fig. 6E). Lateral wall sclerite (LWScl) well developed and piriform (Fig. 6F). Anterior margin sclerite (AMScl) with a short pair of proximal horns (PrxHn) (Fig. 6G).

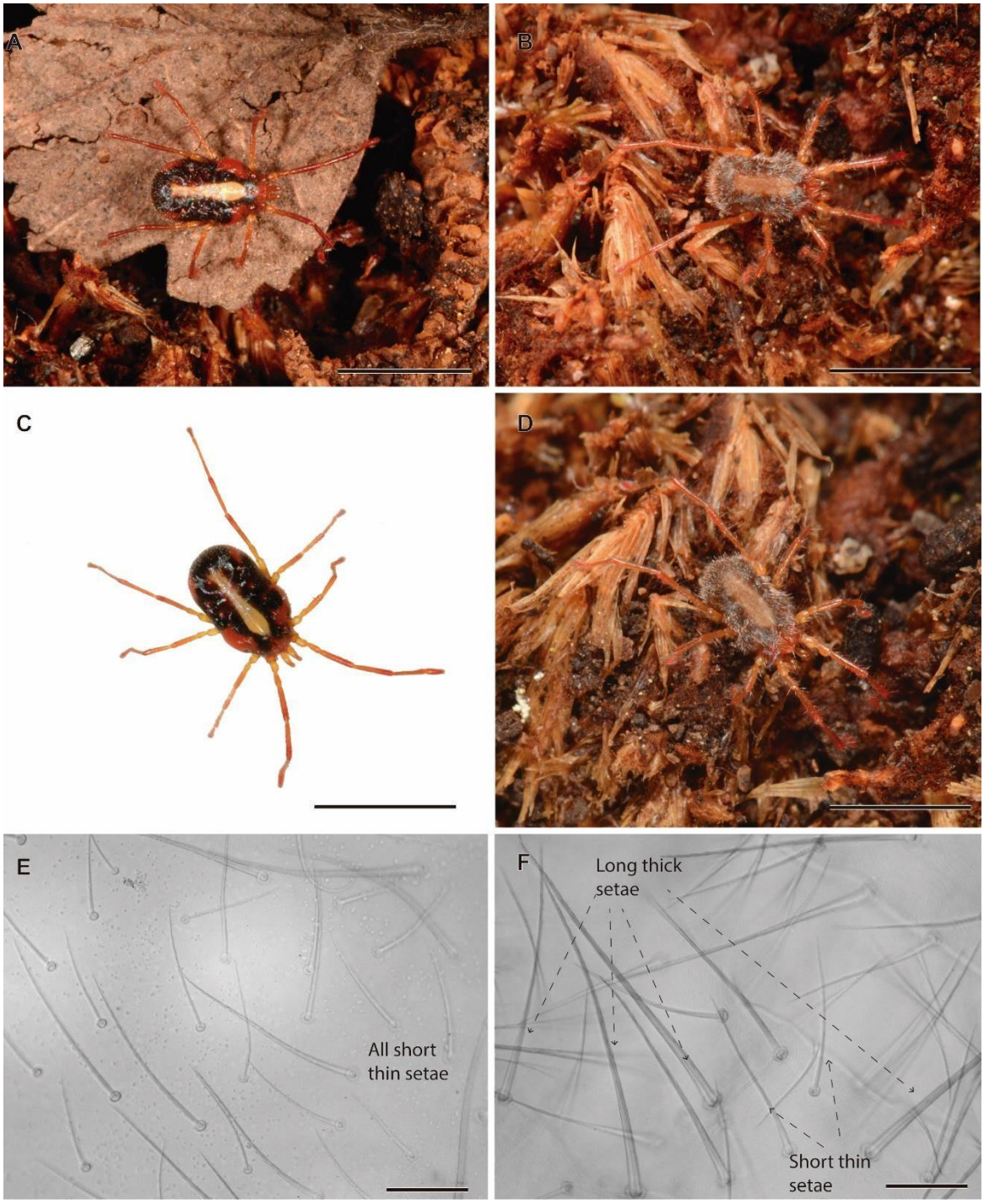

Figure 2. Adult *Rainbowia* sp. nov. . A and C: Live male (UFMG AC 170769); B and D: Live female (UFMG AC 170770); E: Male dorsal setae; F: Female dorsal setae. Scale: A to D= 3000 µm; E and F= 50 µm.

Description.

Female.

Idiosoma oval, covered by a white velvet setae layer compound by two types of setae: thin, almost translucent and short setae; thick, white, long and apparently hollow setae (Fig. 2B, D and F). Colors: reddish legs, palps and anterolateral portions of the Idiosoma; a yellow medial and longitudinal stripe; black cuticle surrounding the yellow stripe (Fig 2 B and D). Two pairs of well separated eyes, a small anterior and a large posterior placed on an ocular plate (Fig. 4E). Anterior sensillary area (naso) triangular, pointed, with six common setae and two sensilla (*si*) (Fig. 4E). Posterior end of crista metopica with two distally barbed sensilla (*vi*) and a posterior projection, apparently located under cuticle (Fig. 4E). Anterior and posterior sensilla (*si* and *vi*) weakly barbed in the distal end and with distinct X-shaped bases (Fig. 4E). Anal valves with eight weakly barbed setae each (Fig. 4B). Genital valves poorly sclerotized, with numerous weakly barbed setae, anterior genital acetabula (G.ac.) oval, posterior genital acetabula long and curved (Fig. 4C).

Gnathosoma with numerous smooth ventral setae in the conic mouth (Fig. 3B, same in the male 3E), cheliceral blades long and needle-like. Palps robust, palp tibia triangulate, covered by weakly barbed setae and solenidia (Fig. 3A, B, C and D; 4D). Including a distinct short, dorsal and distal solenidion (Fig. 4D), that is not observed in the male. Palp tarsi oval, all setae on the palp tarsi are striated solenidia (Fig. 4A). Palp tibia claw short, entire, with a main and a short basal prong (Fig. 3C and 4D).

Long and robust legs, covered by thin, weakly barbed setae (Fig. 5A and B). Tibia I with zero, II with one, tibia III and IV with three, ventro-distal spines (Fig. 6A, B and C). Telo-femur IV with the same length of genu IV (Tfe IV/Ge IV=1,0). Tarsus I to IV with two robust rook-like claws (Fig. 5A and B). Relative leg lengths: IV>I> III> II. Micro seta present on tibia I and genu II (Fig. 7A and B). Tarsi IV with only two solenidia (Fig. 6D).

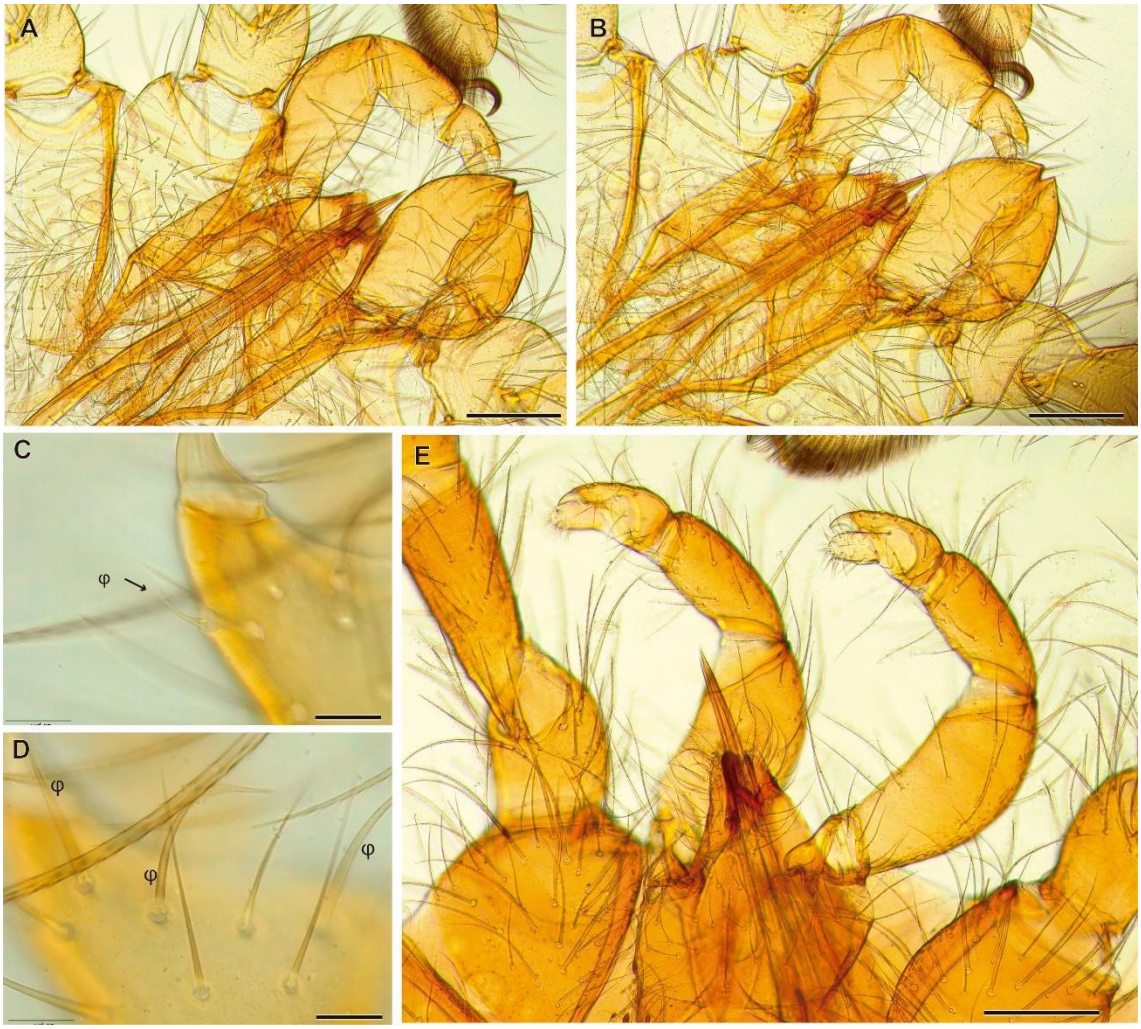

Figure 3. Adult *Rainbowia sp. nov.*. A: Female gnathosoma dorsal view; B: Female gnathosoma ventral view; C and D: Female palp tibia; E: Male gnathosoma ventral view. Scale: A, B and E= 200 µm; C and D= 20 µm.

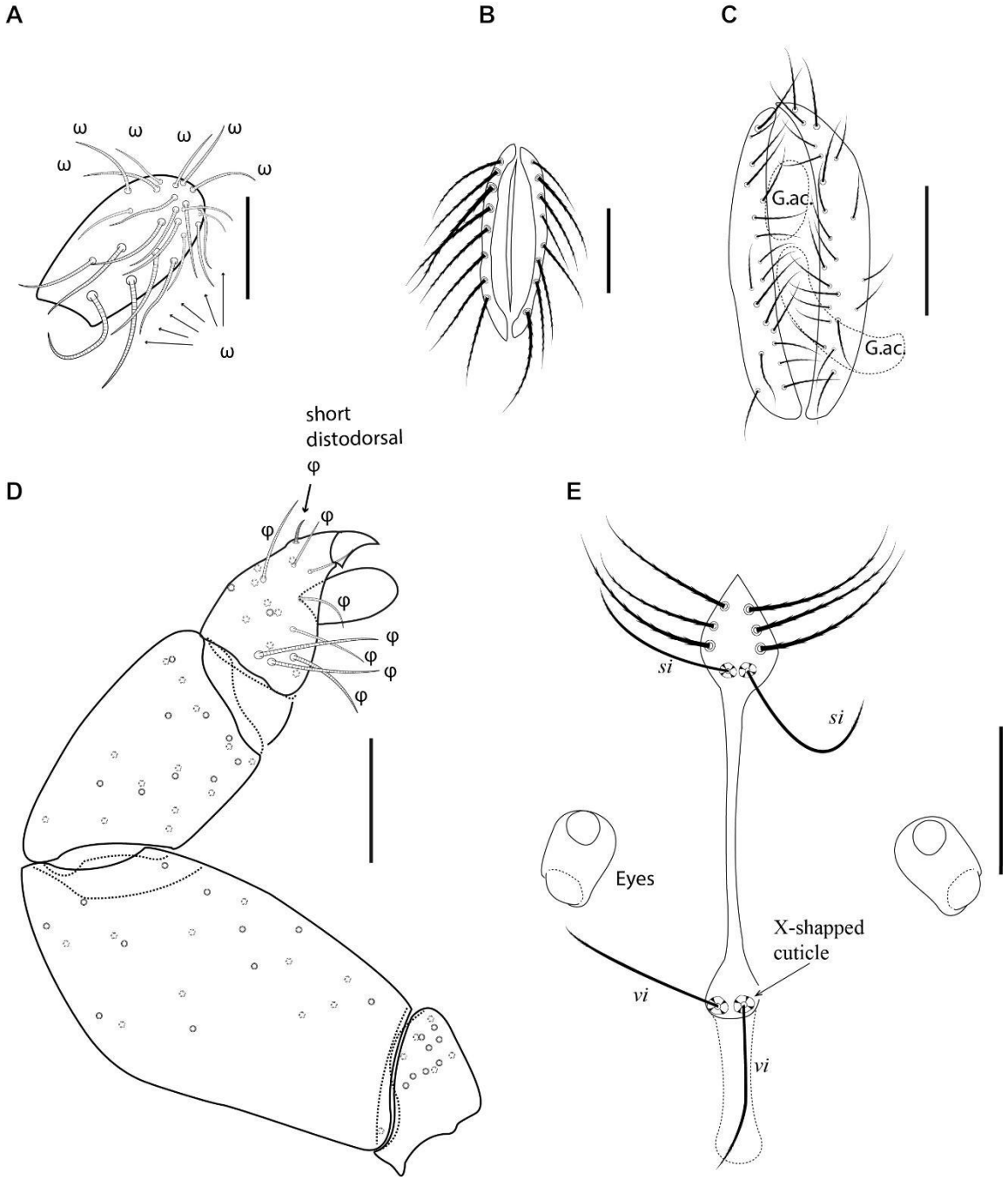

Figure 4. Female *Rainbowia sp. nov.*. A: Palp tarsus; B: Anal valves; C: Genital pore; D: Palp dorsal view; E: Crista metopica and eyes. Scale: A and B= 50 µm; C and E= 200 µm and D= 100 µm.

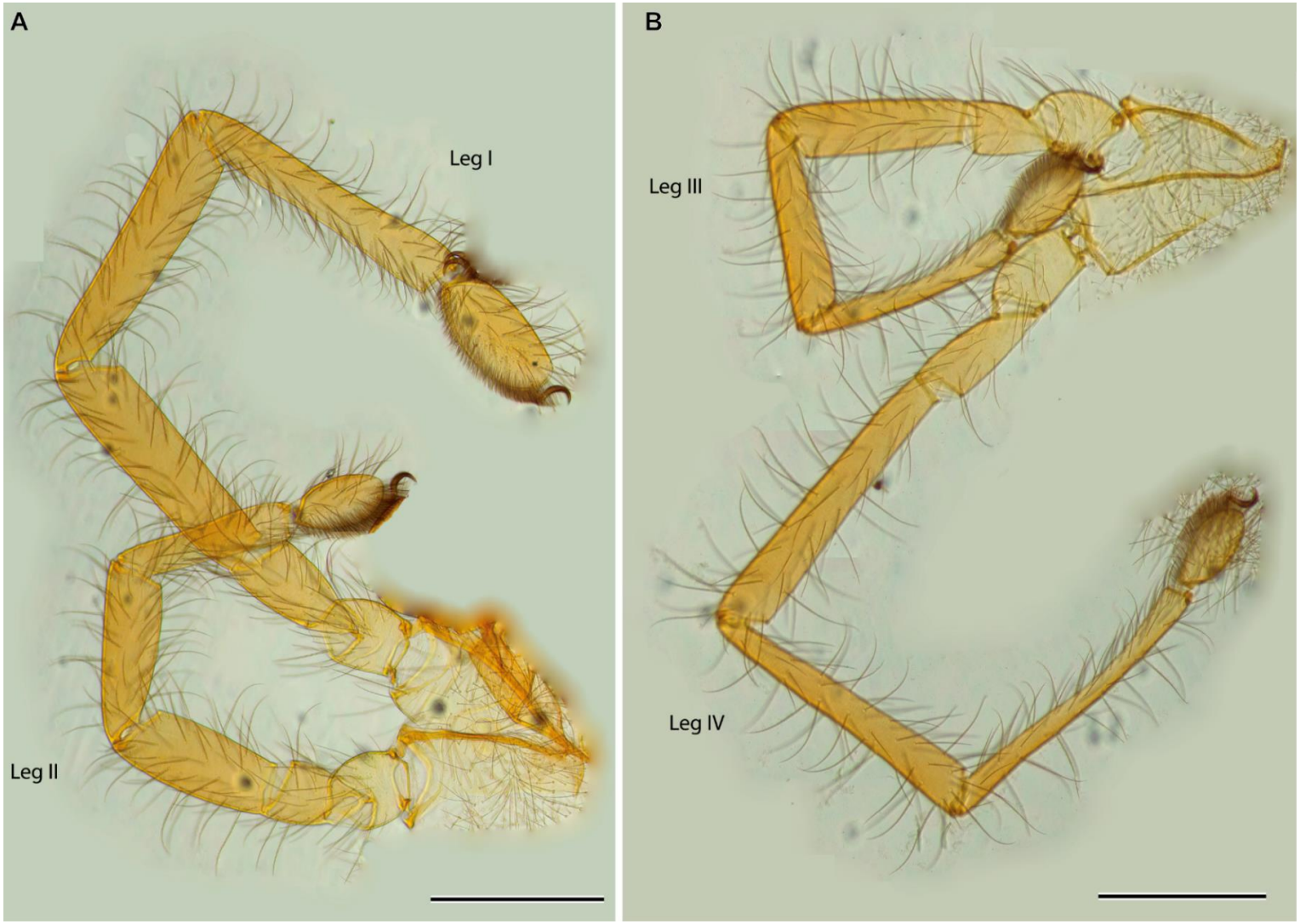

Figure 5. Female *Rainbowia* legs dorsal view. A: Leg I and II; B: Leg II and IV. Scale: 500 µm.

Male.

Colors: reddish legs, palps and anterolateral portions of the idiosoma; yellow medial and longitudinal stripe surrounded by a black cuticle and covered only by thin, almost translucent and short setae (Fig. 2A, C and E).

Palps more delicate (Fig. 3E) than in the female (Fig. 3A and B). Palp femur 2.31 times longer than wide (vs. 2 times in the female, Fig. 7), palp tibia triangulate, but more elongate than in the female (Fig. 3E and 7D), twice as long including the claw (vs. 1.51 times in the female, Fig. 3A and B; Fig. 7C). The palp tibia also lacks the distinct short, dorsal and distal solenidion observed in the female (Fig. 3D). All solenidia of the palp tibia similar in shape.

Genital pore surrounded by numerous short and thin setae, without a well-defined external genital plate (Fig. 6D). Internally, ejaculatory complex's anterior arms (AntAm) with 9-10 smooth eugenital setae on each side, on protuberances (Fig. 6E). Lateral wall sclerite (LWScl) well developed and piriform (Fig. 6F). Anterior margin sclerite (AMScl) with a short pair of proximal horns (PrxHn) (Fig. 6G). Male anal valves lacking, likely which probably is an artifact due to damage during the specimen preparation. Other characters as described for the female.

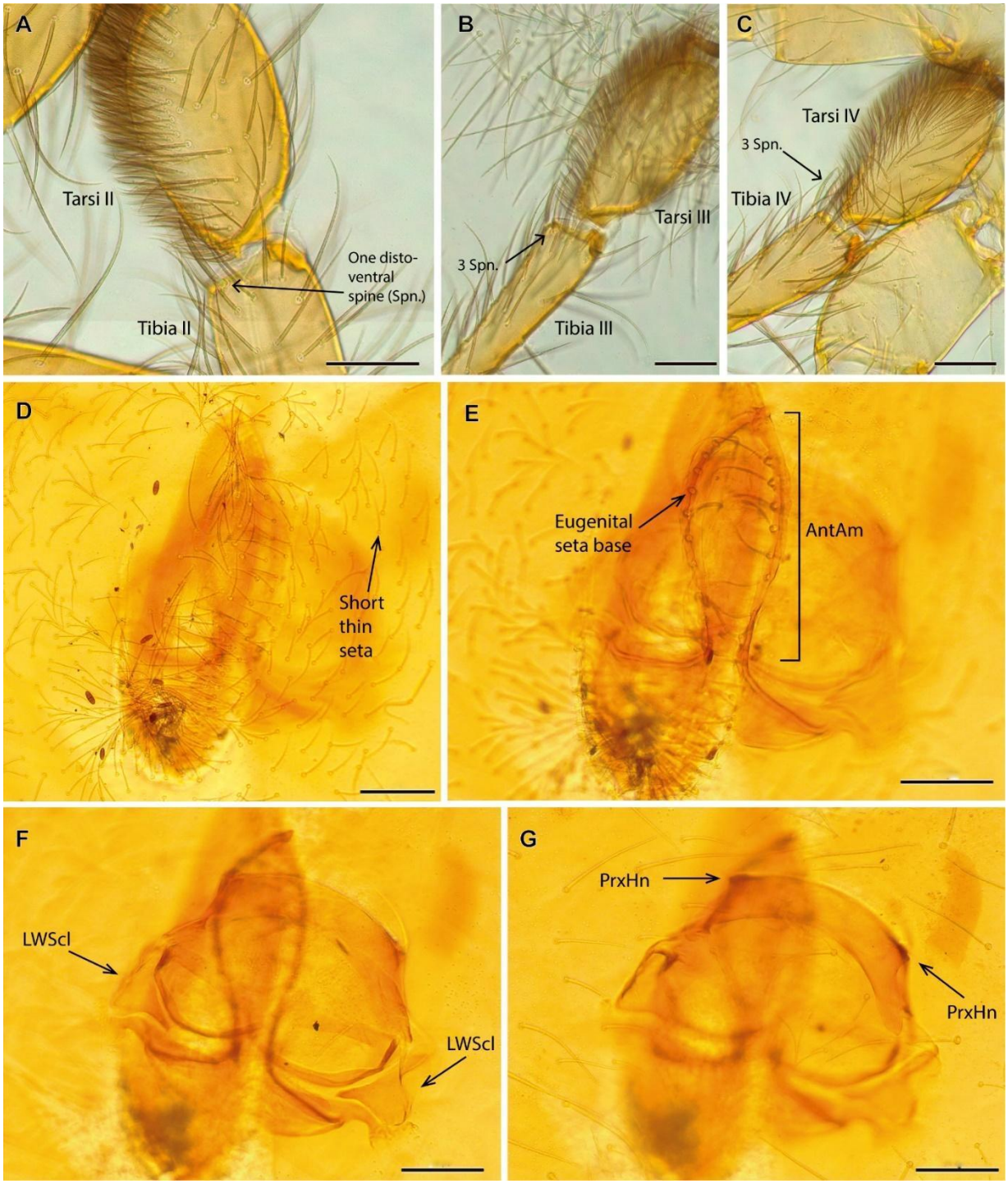

Figure 6. Adult *Rainbowia* sp. nov. A: Joint between tarsus and tibia II; B: Joint between tarsus and tibia III; C: Joint between tarsus and tibia IV; D: Male genital pore external view; E: Male genital pore internal view, first layer; F: Male genital pore internal view, second layer; F: Male genital pore internal view, third layer. Scale: 100 µm.

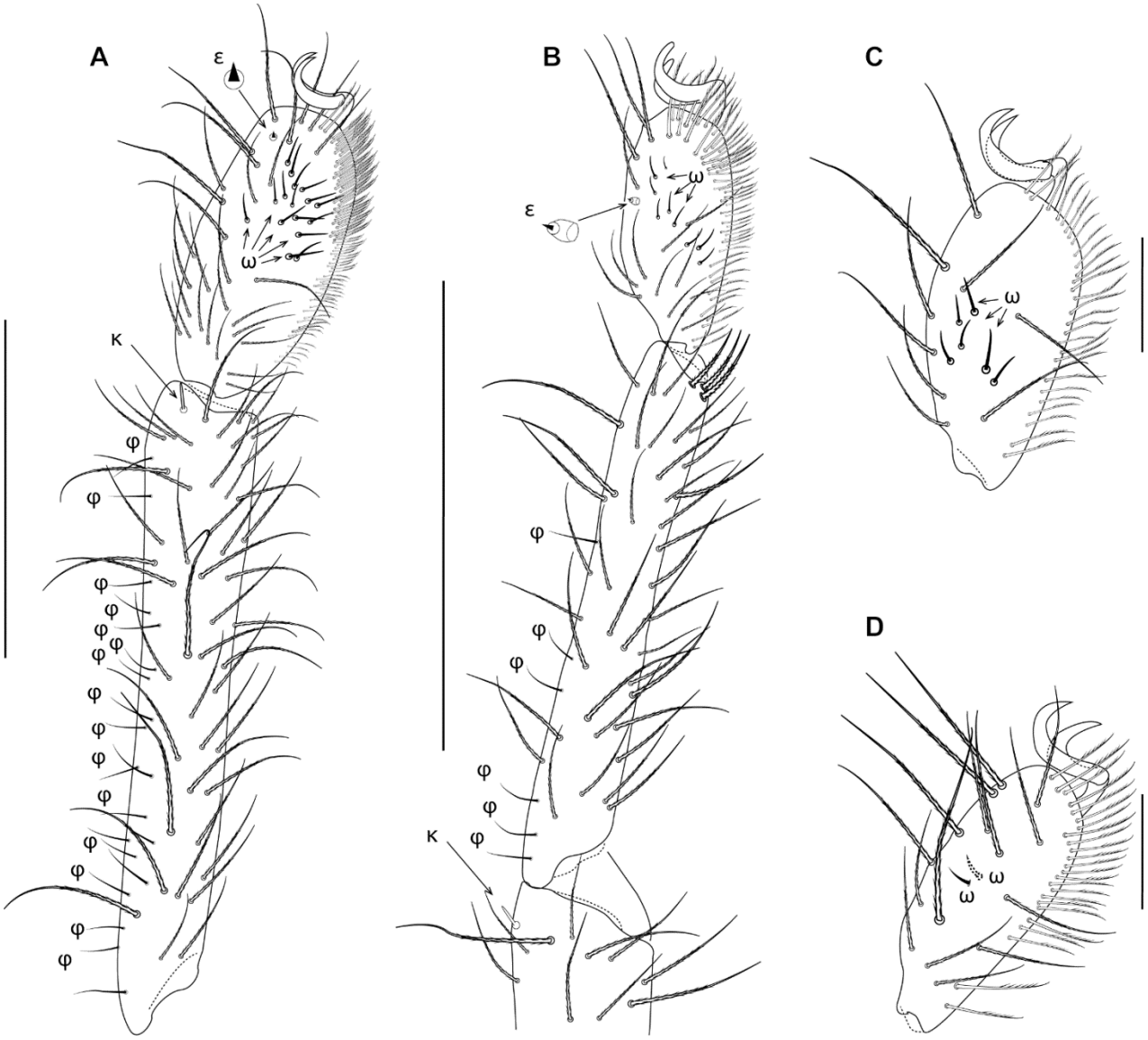

Figure 7. *Rainbowia* sp. nov. legs. A: Tarsi and Tibia I; B: Tarsi, tibia and distal portion of genu II; C: Tarsi III; D: Tarso IV. Scale: A and B= 500, C and D= 100.

Remarks

Rainbowia sp. nov. differ from the two recent species due to the colorful body divided in three major colors (black, yellow and red) vs. reddish in the other species. It differs from *Rainbowia pankowskii* syn. nov. (Konikiewicz & Mąkol, 2018) due to the tarsi I oval (vs. rectangular in *R. pankowskii*) and palp tibia claw short (Fig. 8C and D) (vs. long in *R. pankowskii*, Fig. 8E and F).

Table II – Measurements of *Rainbowia* sp. nov.

Character	UFMG AC		Character	UFMG AC	
	Male 170769	Female 170770		Male 170769	Female 170770
IL	2833	2503	Tfe II	479	546
IW	1618	1588	Ge II	531	590
Sba	31	29	Ti II	602	599
Sbp	32	30	Ta II	286	338
Vi	194.7	220	Leg II	2343	2531
Si	209.5	239	Cx III	487	574
ISD	409	468	Tr III	255	293
Crista metopica`s common setae	147–220	214–272	Bfe III	183	206
DS	70–160	82–252	Tfe III	602	646
VS	57–167	66–213	Ge III	656	709
ACW	97	118	Ti III	743	774
PCW	93	80	Ta III (L)	297	366
L	675	798	Leg III	2736	2994
Anterior eye	40–42	48–50	Cx IV	567	663
Posterior eye	59–60	61–65	Tr IV	348	327
Cx I	624	494	Bfe IV	321	345
Tr I	219	285	Tfe IV	975	1075
BFe I	388	323	Ge IV	949	1044
TFe I	825	881	Ti IV	1082	1109
Ge I	903	967	Ta IV (L)	288	380
Ti I	976	981	Leg IV	3963	4280
Ta I	428	491			
Leg I (without Cx)	3739	3928			
Cx II	599	591			
Tr II	140	160			

Bfe II	285	298			

Is *Burerythrites* a junior synonym of *Rainbowia*?

Rainbowia sp. nov. perfectly fits the *Burerythrites* Konikiewicz & Mąkol, 2018 diagnosis. Conalae absent; two pairs of eyes; crista metopica with well-defined sensillary areas; anterior sensillary area reaching anterior margin of aspidosoma; dorsal opisthosomal setae uniform in shape (observed in *Rainbowia* males, including *R. sp. nov.*), relatively long, covered with indistinct setules, with acuminate tip; legs longer than the idiosoma, similar to each other in robustness; tarsus I with half the length of tibia I (Konikiewicz & Mąkol, 2018).

Burerythrites differs from the two Australian *Rainbowia* species due to palp tibia and palp tarsus relatively long (vs. relatively short with palp tibia in the shape of an equilateral cone and palp tarsus hemispherical or almost globular, Fig. 8 A and B) (Konikiewicz & Mąkol, 2018). However, *Rainbowia* sp. nov. shows an intermediary condition between *Burerythrites* and the Australian *Rainbowia imperator* (Hirst, 1928).

Rainbowia males and females are sexually dimorphic concerning the palp tibia and tarsus shape. The female has a palp tibia and tarsus that reminds the Australian *Rainbowia* species (Fig. 8C). It has a similar shape, approximately 1.5 times longer than wide (vs. 1.45 in *Rainbowia imperator* (Hirst, 1928); Fig. 8A and B). While the male has a palp tibia two times longer than wide (Fig. 8D), that reminds *Burerythrites* (Fig. 8E and F). It is also notable that the *R.* female's palp tarsus is wider and shorter than what is observed in the male (length/width= 1,7 vs. 1.9 in the male). Reminding *R. imperator* (Fig. 8A and B), although smaller.

The palp tarsus and tibia of *Rainbowia imperator* (Hirst, 1928) (Fig. 8A and B), *R.* sp. nov. (Fig. 8C and D) and *Burerythrites* (Fig. 8E and F) seems to have a continuous distribution of its shape (Fig. 8). When observed side by side (Fig. 8), an objective threshold between one shape to the other cannot be established. In addition, male (Fig. 8D) and female (Fig. 8E) of the same species (*R.* sp. nov.) may differ in this shape (Fig. 8C and D). Which suggests that this character cannot be used to distinguish between genera. Therefore, we suggest a new synonym *Rainbowia pankowskii* syn. nov. (Konikiewicz & Mąkol, 2018) for *Burerythrites*.

Differences in the palp tibia and genu shape can be noticed when comparing *Rainbowia* sp. nov. male (Fig. 8D) with *Rainbowia imperator* (Hirst, 1928) (Fig. 8A and B) illustrated by

Southcott (1961). In addition, R. *pankowskii* syn. nov. (Konikiewicz & Mąkol, 2018) has dorsal opisthosoma setae uniform in shape as observed in *Rainbowia* males (vs. heteromorphic in females) and the palp tibia and tarsus elongated as observed only in males of *R. sp. nov.*. Suggesting that the fossil specimen may be a *Rainbowia* male. Micro tomography may be required to address this question.

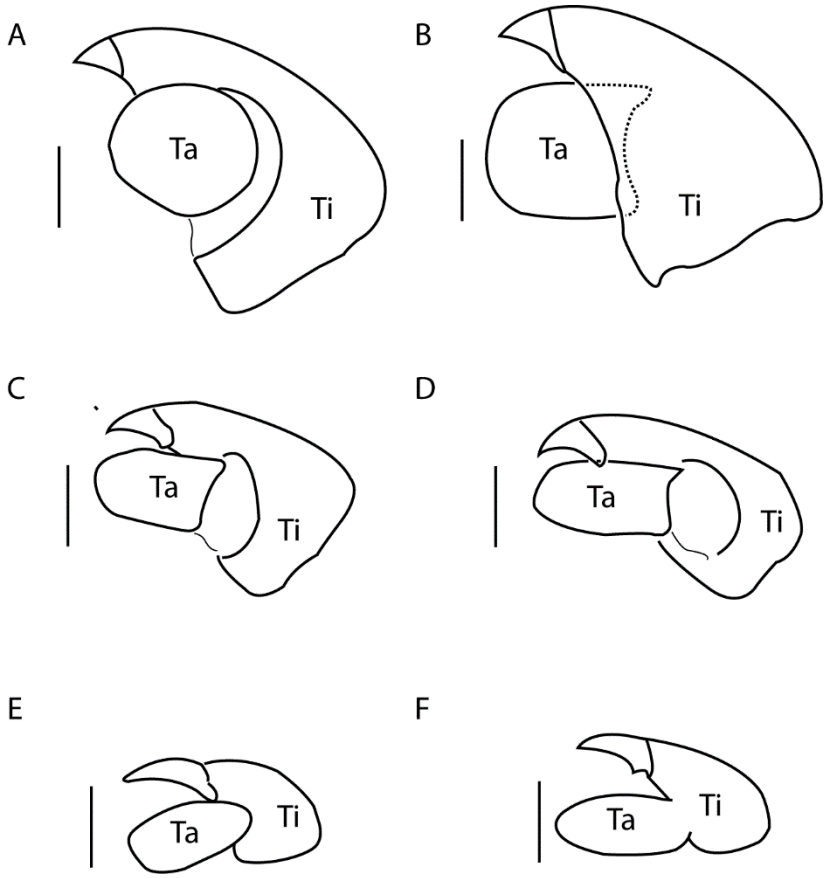

Figure 8. *Rainbowia* palp tibia and tarsus. A: *Rainbowia imperator* ventral view; B: *Rainbowia imperator* dorsal view; adapted from Southcott, 1961. C: *Rainbowia* sp. nov. female; D: *Rainbowia* sp. nov. male. E and F: *Rainbowia pankowskii* syn. nov., drawings adapted from Konikiewicz & Mąkol, (2018). Scale: 50µm.

Etymology

To be defined when publishing. .

Acknowledgements

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Capítulo 1.3 – Chyzeriidae

Nesse Capítulo é descrita uma nova espécie de *Perumaropta* (Chyzeriidae) coletada em uma caverna de Minas Gerais. A espécie foi incluída em nossa análise filogenética apresentada no capítulo 2.

Capítulo 1.3.1: A new larval species of the genus *Perumaropta* (Chyzeriidae, Parasitengona) from a Brazilian cave supplemented by DNA barcode.

SAMUEL GEREMIAS DOS SANTOS COSTA* [1, 2] & ALMIR ROGÉRIO PEPATO [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 10 Semakova Str., 625003 Tyumen, Russia.*

* *Corresponding author. E-mail:* estoupa.bob@gmail.com

Abstract

Perumaropta is a monotypic Chyzeriidae genus known based solely on larva from Peru. In the present study we describe a new species of *Perumaropta* from Minas Gerais state. We also provide DNA sequences from the mitochondrial gene cytochrome oxidase subunit I and the ribosomal gene 28S, allowing further association of the undescribed post larval specimens employing molecular species delimitation methods in the future.

Key words: Parasitengona, DNA barcoding, cave fauna

Introduction

Chyzeriidae Womersley, 1954 is a family of Parasitengona distributed worldwide, with its most symbolic genus *Chyzeria* Canestrini, 1897, with their long dorsal fingerlike projections in post larval instars. *Perumaropta* Haitlinger, 1999 is a poorly studied monotypic genus known based solely on the larval instar of *P. mirsadi* Haitlinger, 1999 from Peru. It belongs to the subfamily Pteridopodinae Southcott 1987. In the present study we describe a second *Perumaropta* species from a Brazilian cave. The description is supplemented by molecular data aiming further association of co-specific heteromorphic specimens (Costa *et al.* 2019).

Material and methods

The single specimen of *Perumaropta* was collected by B. Gomes-Almeida, S. Costa, and R. Torres within the soft soil of the aphotic zone of a cave named *Gruta da Viola*, Santana do Riacho municipality, Minas Gerais state, Brazil (19°17'44.2"S, 43°36'59.0"W) on 17th February of 2021 (raining season). Notably, a second visit to this cave in July of 2021 (dry season) was aborted because the entrance previously used had collapsed.

To sort microarthropods from cave soil the following procedure was done: (1)Approximately 2kg of soft cave soil was sampled, added to a bucket with water and vigorously stirred up, suspending organic particles. (2) The supernatant was filtered through a 50-micrometer sieve and fixed with 70% ethanol. (3) Sub samples of 10 ml were transferred to 50 ml PET tubes, and 5 ml of kerosene was added (kerosene floats on 70% alcohol, leading to a ~1 cm layer on top). (4) The tube was slowly inverted several times, making the sample pass through the kerosene. (5) As the chitin sticks to kerosene, a coat of small arthropods and nematodes appeared between the 70% alcohol and kerosene. (6) Finally, the coat was filtered in the sieve, washed with 99% ethanol and mites were visually sorted under a stereoscopic microscope.

We aim at providing DNA sequences of this mite available to allow further association of heteromorphic specimens. The DNA extraction, microscope slide preparation and the sequencing of the mitochondrial gene cytochrome oxidase subunit I gene (COI) was performed as described by Costa *et. al* (2019). For the 28S gene amplification and sequencing were performed as described by Pepato *et al.* (2018). Illustrations were made with the aid of Leica DM2500 compound microscope with a Mshiwi 38MP HDMI Microscope HD camera. Drawings were made with the aid of a simulated camera lucida using the software On Top Replica v3.5.1 and Adobe Illustrator CC 2015. Final artwork was done using Adobe Photoshop

CC 2015. All measurements are given in micrometers (µm). Terminology and abbreviations were adapted from Southcott (1961) and Grandjean (1947).

The COI and 28S sequences were deposited in GenBank database, access number:

Provided upon acceptance.

Our nomenclatoral act was registered in ZooBank:

Provided upon acceptance.

Systematics

Family Chyzeriidae Womersley, 1954

Diagnosis: See Mayoral *et al.* 2018

Subfamily Pteridopodinae Southcott, 1987

Diagnosis: See Mayoral *et al.* 2018

Genus *Perumaropta* Haitlinger 1999

Diagnosis: See Haitlinger 1999

***Perumaropta* sp. nov.**

(Figs 1 – 3)

Diagnosis (larva):

Idiosoma with 28 dorsal setae placed on large sclerites and 50 ventral setae placed on small, sclerotized bases. Dorsal setae length= 80 – 101. Ventral setae length= 40 – 61. Tfe III with four setae of similar length (Fig. 3B). Palpal setal formula: 0-0-B-N-2B,1N-9B,1ζ. Palp tarsus with seven weakly barbed setae and two barbed setae with long setules (Fig.1E and F). Leg chaetotaxy including Ti I: 8B, 3φ; Ge I: 4B, 2σ; Ti II: 8B, 2φ; Ge II: 4B, 2σ; Ti III: 8B, 1φ; Ge III: 4B, 1σ. Claws of tarsi I, II and III with two thin and L-shaped claws and a thick, sinuous empodium with a medial lump (Fig. 2D). Scutum wider than long (W/L= 1.4), scutum antero-lateral setae (ve) slightly expanded (Fig. 1A and 3C). Relative lengths: PW/AW= 1.7, Ti I/ Ge I= 1.07, Ti III/ Ge III= 1.2.

Holotype: Larva, *Perumaropta* sp. nov. (UFMG AC 210375), Brazil, Minas Gerais, Santana do Riacho municipality, cave *Gruta da Viola*, 19°17'44.2"S, 43°36'59.0"W. Collected by B. Gomes-Almeida *et al.* on 17th February of 2021.

Description

Larva: Idiosoma small and squarish (IL= 289, IW= 252), covered by 28 dorsal setae on rounded sclerites. Two pairs of eyes connected by a weakly sclerotized ocular plate (Fig. 1, 3C). Dorsal setae 80 – 101 long. Scutum triangulate, wider than long (W/L= 1.4), punctuated, bearing an antero-medial naso, two pairs sensilla (trichobothria *vi* and *si*) and two pairs of lateral setae *ve* (=AL) and *se* (=PL) with similar shape (Fig. 1A and 3C). Anterior sensilla (*vi*) weakly barbed in the distal half and anteriorly placed relative to the anterolateral setae (*ve*). Posterior sensilla (*si*) smooth, located between *ve* and *se* (Fig. 1A). Ventral idiosoma l with 50 distally barbed setae. Ventral setae 40 – 61 long. Well-developed Claparède organs between coxa I and II. Each anal sclerite with one long weakly barbed seta (Fig. 1).

Palps short and robust with bifid odontus and long setae on the palp tarsi. Cheliceral blades straight with several serrate teeth. Palpal setal formula: 0-0-B-N-2B,1N-9B,1ζ. Palp tarsi with seven weakly barbed setae and two barbed setae with long setules (Fig.1E and F). Adoral setae (*cs*) and hypostomal setae (*bs*) long and barbed. Hypostomal setae (*as*) absent (Fig. 1C – F, 3A).

Short legs (495 – 592), with seven segments each. Tarsi I, II and III elongated, curved and apparently flexible (Fig. 2). Claws of tarsi I, II and III with two thin and L-shaped claws and a thick, sinuous empodium with a medial lump (Fig. 2D). Coxae I with two pairs of setae (*1b* and *1c*), coxae II and III with one seta each (*2b* and *3b*) (Fig. 1B). See table I for measurements.

Leg chaetotaxy:

Leg I: Ta- 47B*, 1*Cp*, 1ε, 1ζ, 1ω; Ti- 8B, 3φ; Ge – 4B, 2σ; TFe- 5B; BFe- 1B; Tr- 1B; Cx- 2B.

Leg II: Ta- 45B*, 1 ω; Ti- 8B, 2φ; Ge – 4B, 2σ; TFe- 4B; BFe- 2B; Tr- 1B; Cx- 1B.

Leg III: Ta- 43B*; Ti- 8B, 1φ; Ge- 4B, 1σ; TFe- 4B; BFe- 2B; Tr- 1B; Cx- 1B.

* A precise estimation of setae in the Leg I tarsi is precluded by mounting conditions.

Remarks

Perumaropta sp. nov. larva differs from those of *P. mirsadi* by having all four setae on telofemur III with similar length, instead of a distinctly long seta on *P. mirsadi* telofemur III. See figure 3D. *Perumaropta sp*. nov. also differs from *P. mirsadi* due to shorter legs (Leg I, I and III= 576, 495 and 592 vs. 792–804, 678–700 and 834 in *P. mirsadi*).

Etymology

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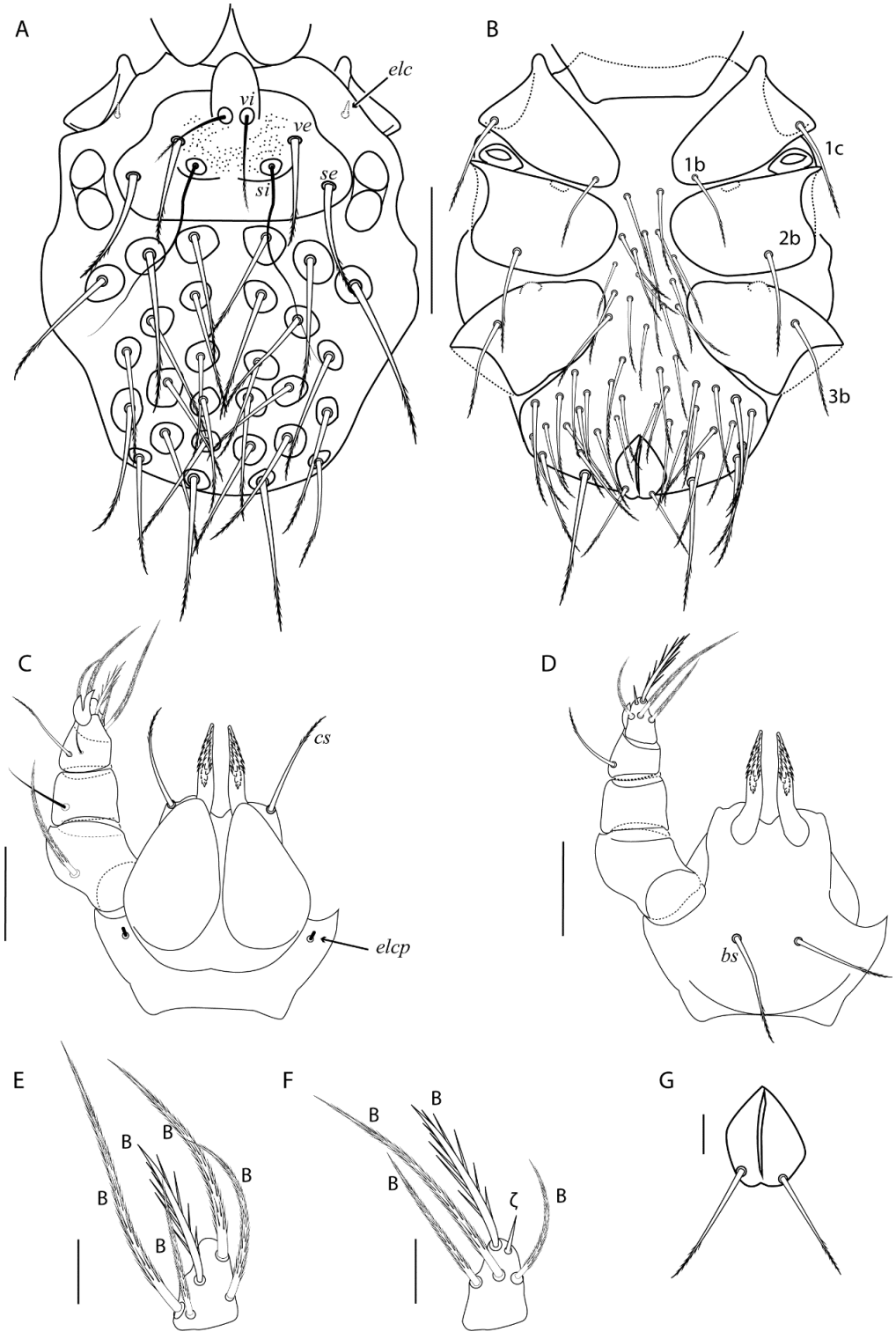

Figure 1. *Perumaropta* sp. nov. A: Idiosoma dorsal view. B: Idiosoma ventral view. C: Dorsal view of gnathosoma. D: Ventral view of gnathosoma. E: Palp tarsus dorsal view. F: Palp tarsus ventral view. G: Anal plates. Scale: A – B= 100 µm, C and D= 50 µm, E – G= 20 µm.

Table I – Measurements of *Perumaropta* sp. nov. UFMG AC 210375 (Holotype).

Character	Measurement	Character	Measurement
IL	289	Ta I	229,3
IW	252	Ti I	85,3
L	112	Ge I	79,5
W	158	Tfe I	65,4
AW	78.4	Bfe I	58
PW	135.4	Tr I	59
Sba	15.8	Cx I	101.63
Sbp	52.6	Leg I	576.5
AP	39.7	Ta II	197
ve (=AL)	65	Ti II	69.3
se (=PL)	77.6	Ge II	57.55
vi	68.5	Tfe II	57.15
si	149	Bfe II	55.8
ISD	35.4	Tr II	59
Dorsal setae	80 – 101	Cx II	92
Ventral setae	40 – 61	Leg II	495.8
Anterior eye width	16.5	Ta III	235.6
Posterior eye width	18.6	Ti III	86.7
cs	55.31	Ge III	65.1
bs	56.4	Tfe III	61.79
1b	43	Bfe III	78.9
1c	58	Tr III	64.1
1c	78.4	Cx III	104
2b	69	Leg III	592.19
3b	64.7		

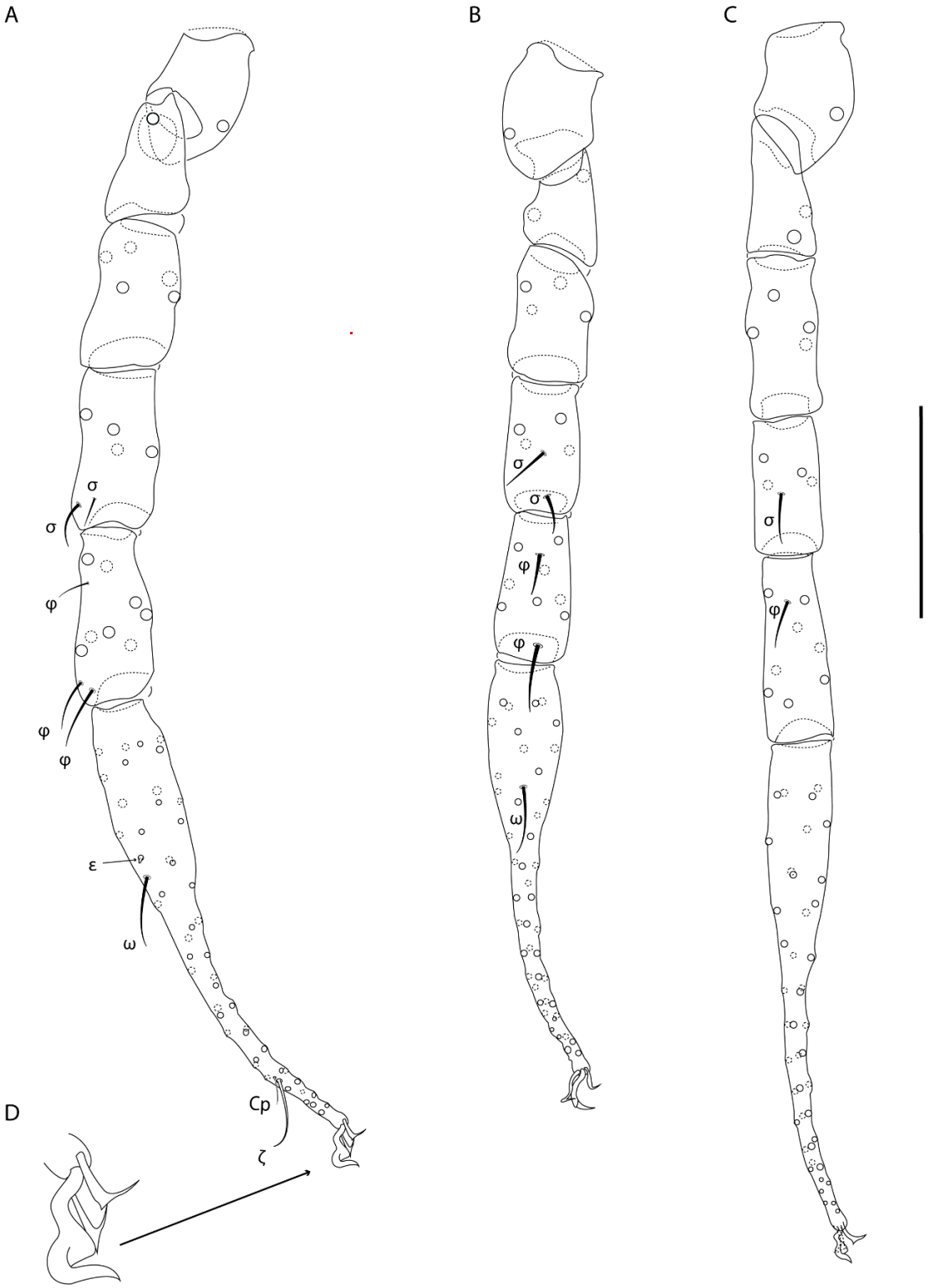

Figure 2. *Perumaropta* sp. nov. A: Leg I. B: Leg II. C: Leg III. D: Tarsus I's claw. Scale: 100µm.

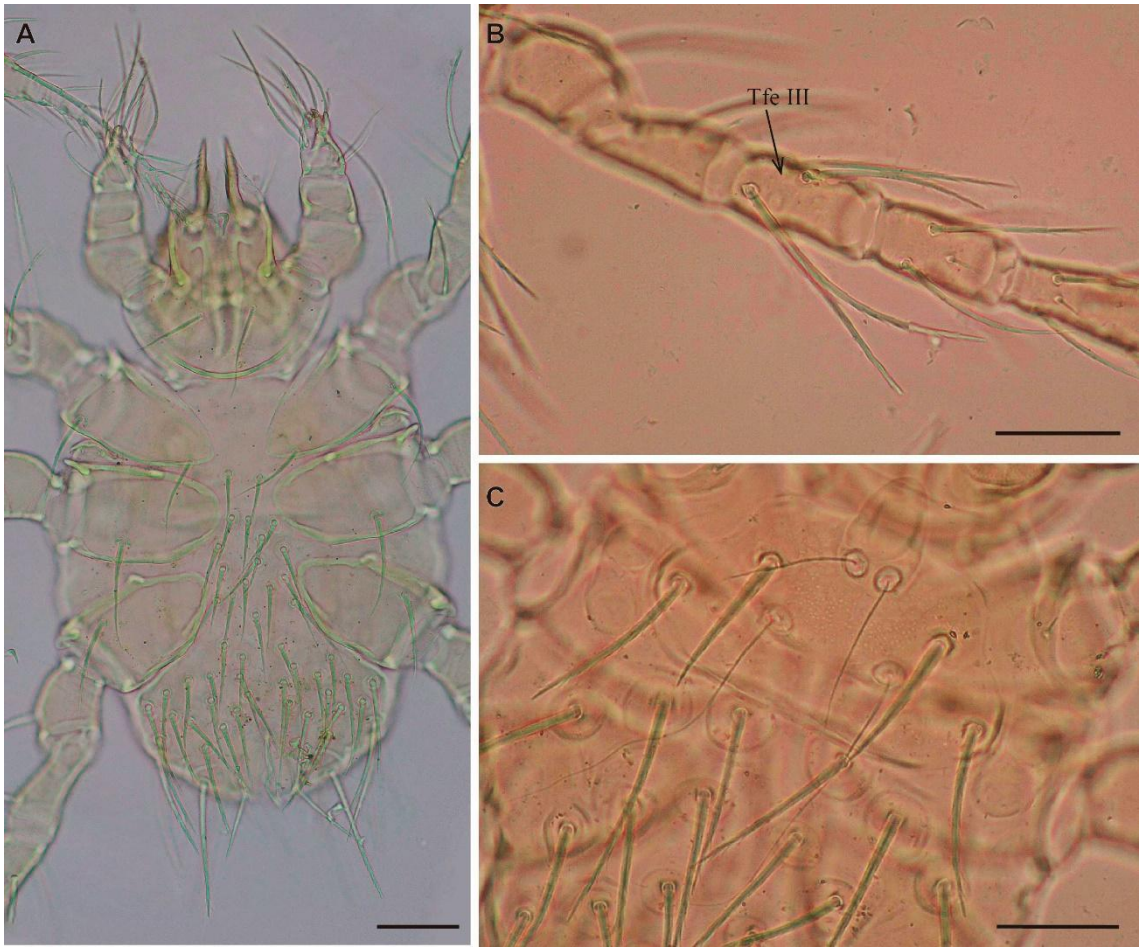

Figure 3. *Perumaropta* sp. nov. A: Ventral view. B: Leg III highlighting the setae's shape and lenght on Tfe III. C Scutum highlighting the dorsal setae shape. Scale: 50 µm.

Acknowledgements

Thanks due to Dr. Alexandre Khaustov, Tyumen State University, and the X-Bio institute for receiving SGSC as a visiting Scholar funded by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES – Print, Finance Code 001); to *Carste Ciência e Meio Ambiente* for collecting and depositing specimens at UFMG AC; to *Programa de Pós Graduação em Zoologia da* UFMG, the graduate program where SGSC is student; to *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* FAPEMIG for SGSC's scholarships (FAPEMIG – Graduate Support Program PAPG); to ADESITA (contract number 04/2016 – Agência de Desenvolvimento Econômico e Social de Itabirito) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) in partnership with the company Vale S.A. (Edital Nº 007/2018) for funding.

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Capítulo 1.4 – Johnstonianidae

Neste capítulo duas novas espécies de Johnstonianidae coletadas em cavernas brasileiras são descritas. Elas são posteriormente incluídas na filogenia apresentada no capítulo 2. Essa seção foi publicada no artigo:

Costa, S., Klimov, P. & Pepato, A. R. (2023) Two new species of Brazilian Johnstonianidae (Trombidiformes: Parasitengona). *Systematic & Applied Acarology*, 28(4), 680–694.

Capítulo 1.4.1: Two new species of Brazilian Johnstonianidae (Trombidiformes: Parasitengona)

SAMUEL GEREMIAS DOS SANTOS COSTA* 1, 2, PAVEL B. KLIMOV2,3 & ALMIR ROGÉRIO PEPATO 1,2

1 Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de

Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP:

31270-901.

2 Tyumen State University, 6 Volodarskogo Str., 625003 Tyumen, Russia.

3 Purdue University, Lilly Hall of Life Sciences, G-226, 915 W State St, West Lafayette, IN 47907.

** Corresponding author. E-mail: apepato@gmail.com*

Abstract

Two new species, *Newellia xakriaba* sp. nov. and *Centrotrombidium krenak* sp. nov. (Trombidiformes: Parasitengona: Johnstonianidae) from Brazilian caves, are described based on vouchering material for which sequences from multiple genes are available. This article expands the geographical range of the genus *Newellia* André, 1962 known previously only from Angola.

Key words: Johnstonianidae, *Newellia*, *Centrotrombidium*, new species, Brazil, caves

Introduction

Parasitengona (Acariformes: Prostigmata) is one of the most diverse mite lineages, with 11,000 extant species, distributed worldwide, and inhabiting many terrestrial and aquatic (mainly freshwater) habitats. Parasitengona has a distinct life cycle, including three active stages,

parasitic larva and predaceous deutonymph and adult. As drastic morphological changes occur between larvae and deutonymphs, all active mite stages can be associated with each other only by rearing or DNA sequencing (Wharton & Fuller 1952; Johnston & Wacker 1967; Robaux 1974; Wohltmann 2000; Konikiewicz *et al.* 2016; Costa *et al.* 2019). The descriptions of most Parasitengona species are based only on either larval or post larval instars, but not both (Mąkol & Wohltmann 2012). The family Johnstonianidae Thor, 1935 is semi-aquatic, requiring 100% relative humidity to complete their life cycle (Wohltmann 2000). In Brazil, the family Johnstonianidae has not been recorded, except for our recent molecular phylogenetic study that included two terminals from the country (Pepato *et al.*, 2022). Here we describe them. The specimens were collected from Brazilian caves as part of a mandatory biodiversity survey that precedes cave's suppression by mining activities. Some of these caves have already been mined or are inaccessible for further sampling, justifying the monotypic description.

Material and Methods

Mites were obtained by eye-naked browsing on iron ore caves floor conducted by the private company *Carste Ciência e Meio Ambiente* and deposited at the Acarological Collection (UFMG AC).

of the Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Pampulha, Belo Horizonte – MG, Brazil (CCT-UFMG). Microphotographs were taken using a Zeiss Imager.A2 compound microscope with an Axiocam 506 camera. Drawings were made based on photos using Adobe Illustrator CC 2015 and Adobe Photoshop CC 2015. Legs are densely covered by setae (Fig. 5A), therefore not all of them were illustrated and some are represented only by their protruding conical bases for clarity. The terminology and abbreviations were adapted from Grandjean (1947), Southcott (1961). All measurements are given in micrometers (µm).

The sclerotized projections of the ejaculatory complex were not labeled because homology with previous descriptions couldn't be established with certainty.

Systematics

Family Johnstonianidae Thor, 1935

Diagnosis

Adult. Cheliceral blades short and curved, not elongated or needle-like. Two pairs of genital sclerites (epivalve and centrovalve) without a pregenital tubercle. Idiosomal cuticle almost smooth without large, sclerotized areas. Posterior dorsal idiosomal setae smooth or weakly barbed. Prodorsal shield with a projecting naso. Palp tibia without ctenidium, but with a prominent spine (basidont) ventral to the odontus (modified from Wohltmann *et al.* 2004). See Wohltmann *et al.* 2004 for other instars.

Genus *Newellia* André, 1962

Diagnosis

Adults. With two pairs of filiform sensilla on a triangular dorsal shield bearing 2 to 4 normal setae situated between the anterior and posterior sensilla; crista metopica formed by a chitinized median band; basidont unidentate; palp tibia and tibia I without cuticular "shark fin"-shaped extensions (modified from Wohltmann *et al.* 2004).

***Newellia xakriaba* sp. nov.**

Diagnosis

Male. Two pairs of eyes situated on an eye plate (Fig. 1A). Idiosoma covered by short and smooth setae on small, sclerotized bases (Figs 1A, 5B). Prodorsal sclerite (scutum) triangular, punctate, with two pairs of sensilla (*vi* and *si*) and two pairs of normal setae (*ve* and se) all smooth, with relative lengths: *vi* > *si* > *ve* > *se* (Fig. 1C). Basidont unidentate (Fig. 2B). Genital pore with epivalve distinctly wider than the central valve and covered by short smooth setae, arranged irregularly, without well-defined rows (Fig. 1E). There are three pairs of dorsal idiosomal glandularlike striated projections. First pair enlarged distally, situated between coxae II and III; two other pairs posterior to coxae IV (Figs 1A, B, 5B–E). Palps long (557), fPp=0-0-6N-7N-9N-8N,3ζ,1ω, palp tarsus with finger-like projections ending in three claw-like setae (regarded as eupathidia ζ) (Figs 2A–C, 5F). Gnathosoma with a long mouth cone, twice as long as wide. Cheliceral blades elongated, more than four times longer than height (Fig. 2A). Legs long, leg IV 1.8 times longer than idiosoma (Fig. 5F), relative leg length=IV>I>III>II; most setae from legs and palps on cone-shaped projections, including some solenidia (Figs 2B, 3, 4, 5A). All leg claws with transversal striation, possibly due to minute setules (Figs 3, 4).

Holotype: Male (UFMG AC 170317), entrance zone of iron ore cave SPT-0060, 19° 10' 00" S, 43° 16' 23" W, Conceição do Mato Dentro municipality, Minas Gerais State, Brazil, Jan. 30–Feb. 13, 2017. Coll.: *Carste Ciência e Meio Ambiente*.

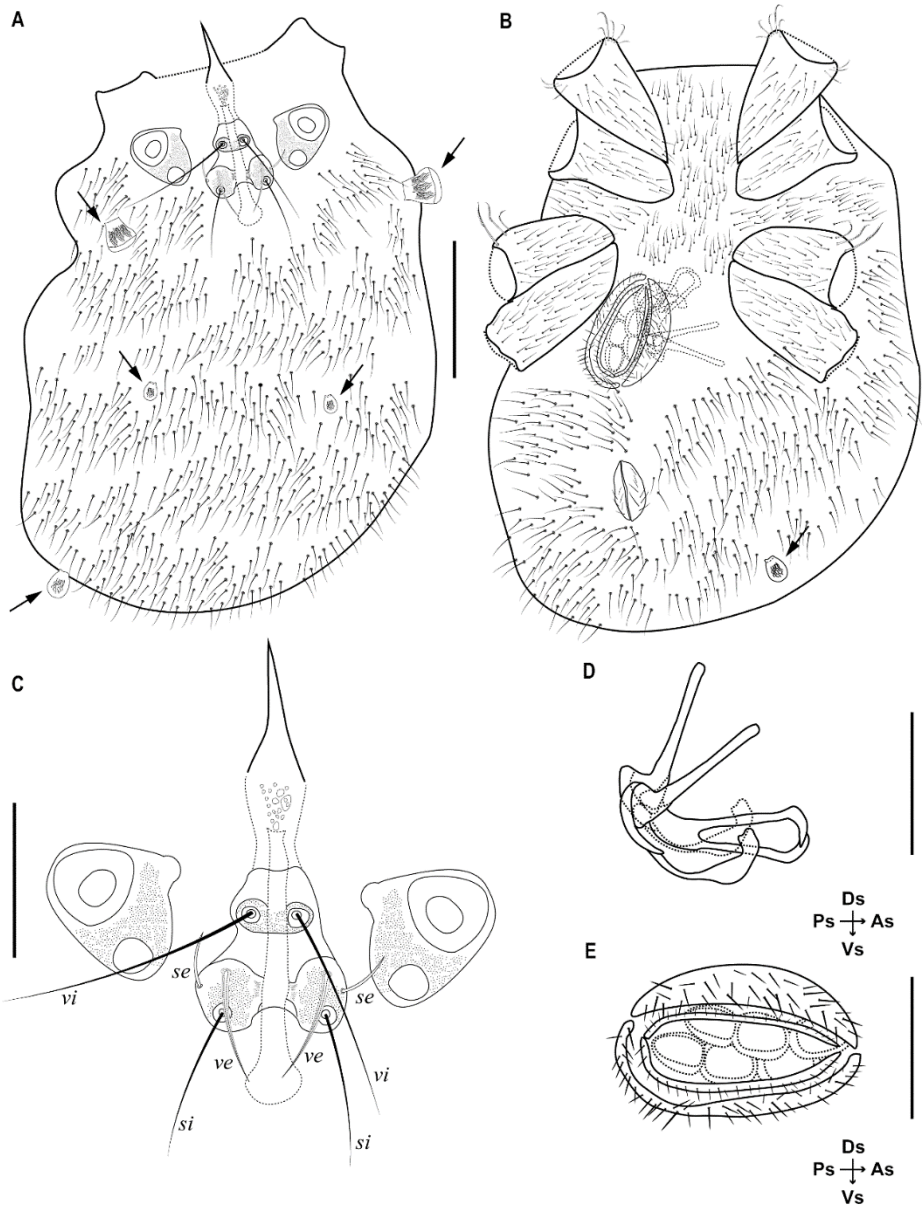

Figure 1. *Newellia xakriaba* sp. nov. male. A: Dorsal view, arrows highlighting the presence of glands. B: Ventral view. C: Prodorsal sclerite and eyes. D: Genital apparatus. E: Genital sclerites and genital acetabula. Spatial orientation indicated by arrows: dorsal (Ds), ventral (Vs), posterior (Ps) and anterior (As). Scale bars: A, B= 200 µm, C, D and E= 100.

Description

Male. Measurements summarized in table II.

Idiosoma nearly oval (Fig. 1A). Two pairs of eyes on punctate eye plate weakly raised above the idiosomal cuticle. Prodorsal sclerite (scutum) triangular and punctate, bearing two pairs of filiform smooth trichobothria (*vi* and *si*), two pairs of smooth common setae (*ve* and *se*) and a triangular naso. Scutal setae lengths: *vi* > *si* > *ve* > *se* (Fig. 1C). Dorsal setae on small, sclerotized bases (Figs 1A, 5B–E). Three pairs of dorsal jug-shaped structures, protruding on striated cuticle; one distally enlarged pair situated between coxae II and III and two pairs posterior to coxae IV. We interpreted these structures as glands, in accordance with André (1962) (Fig. 5 B–E).

Genital pore with epivalve distinctly wider than the central valve and covered by short smooth setae not arranged in rows, central valve with a single row of setae (Fig. 1E). Ejaculatory complex as illustrated (Figs 1D and 5G). The individual studied unsuitable for observing eugenital setae number or presence. Anal valve with thin smooth setae (Fig. 3C). Ventral setae similar to dorsal ones (Fig. 1B).

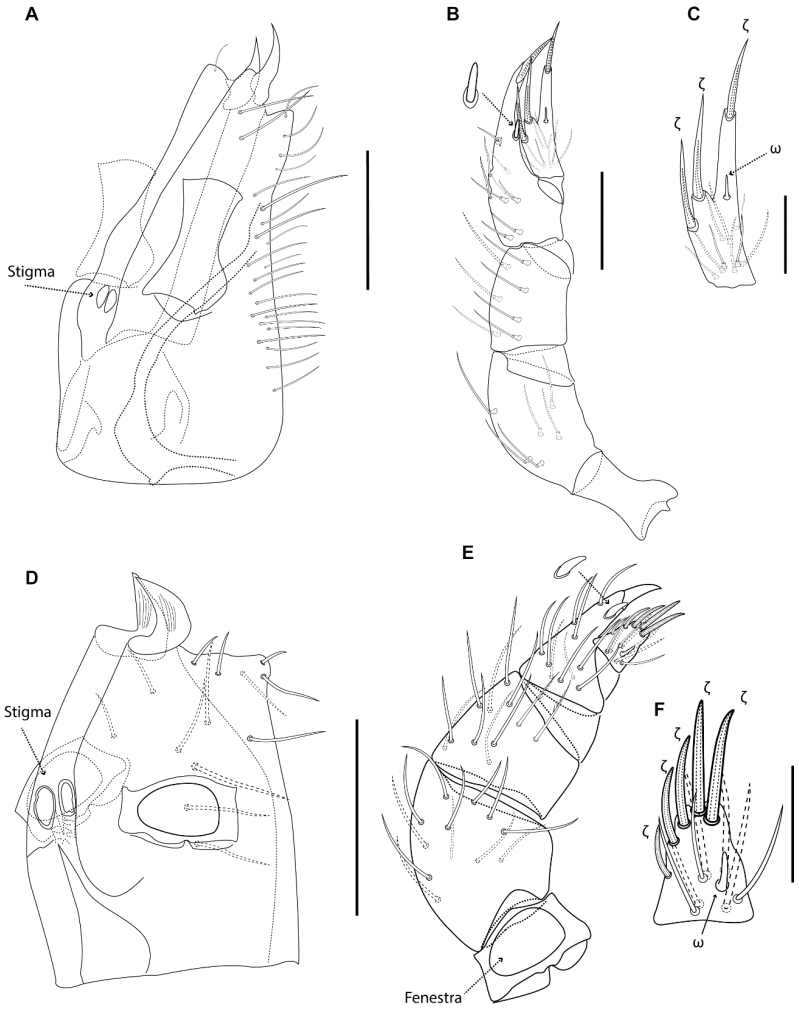

Figure 2. *Newellia xakriaba* sp. nov. male. A: Gnathosoma, lateral. B: Palp. C: Palp tarsi. *Centrotrombidium krenak* sp. nov. male. A: Gnathosoma, lateral. B: Palp. C: Palp tarsi. Scale bars: A, B, D and E= 100 µm, C and F= 50 µm.

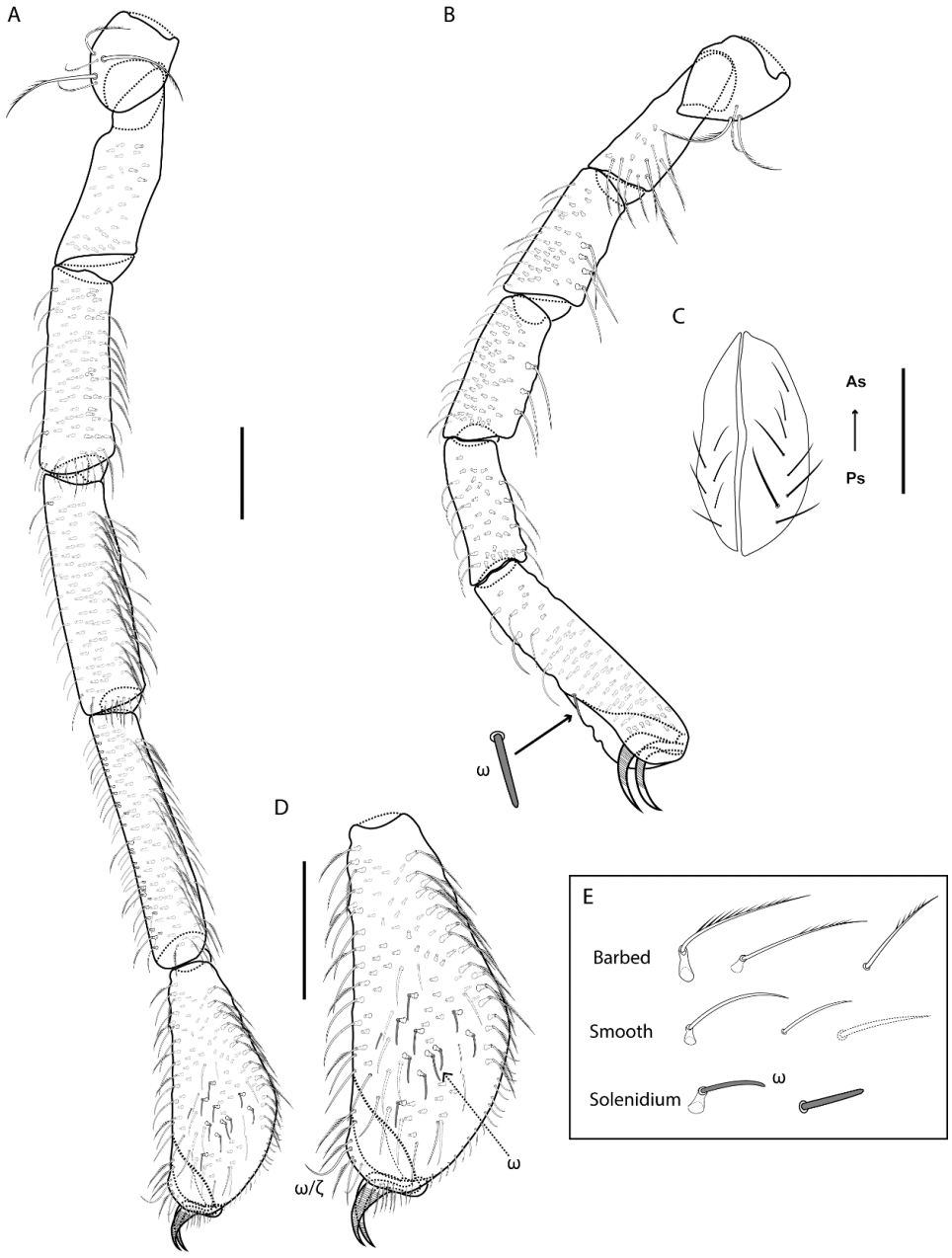

Figure 3. *Newellia xakriaba* sp. nov. male. A: Leg I. B: Leg II. C: Anal valves. D: Tarsi I. E: Setal types. Scale bars: A, B and C= 200 µm, D= 100. Spatial orientation of the anal valve indicated by arrows: Posterior (Ps) and anterior (As).

Gnathosoma with long mouth cone, twice as long as wide. Cheliceral blades more than four times longer than height. Palps long (557) covered by smooth setae on conical projections,

fPp=0-0- 6N-7N-9N-8N,3ζ,1ω. Palp tarsus with finger-like projections ending in three claw-like setae (interpreted as eupathidia ζ) (Figs 2 A–C, 5F).

Relative leg lengths: IV>I>III>II (Fig. 5F). Legs densely covered by smooth and weakly barbed setae (Figs 3, 4, 5A). Most of leg setae on conical papillae, setal morphologies illustrated in Fig. 3E. All tarsi with a distal claw fossae (Figs 3, 4, 5A, F). Tarsi I with solenidia concentrated in central region of anterior side (Fig. 3D), tarsi II with a single solenidion at proximal end of claw fossae. All claws with transversal striation, but without long setules (Figs 3 and 4).

Remarks

Newellia André, 1962 included two species from Angola, *N. glandulosa* André, 1962 and *N. longipes* André, 1962. *N. glandulosa* was sampled in gallery forest's leaf-litter in two locations, near Ngungu River, left sub-tributary of Cuango-Muqué and in Cuilo springs forest. André (1962): "*riv. Ngungu, sous-affl. gauche Cuango-Muqué*" and "*forestière des sources du Cuilo*". *Newellia longipes* was described based on a single specimen sampled near Dundundo Springs Forest, Dundo. André (1962): "détritus végétaux recouvrant le sol aux environs de Dundo, forêt des sources de la Dundundo".

Newellia xakriaba sp. nov. differs from *N. glandulosa* by the presence of four setae (*ve* and *se*, fig. 1C), between the anterior and posterior sensillae (vs. a single pair in *N. glandulosa*), and a single pair of glands between coxae I and II (vs. two pairs in *N. glandulosa*). *Newellia xakriaba* sp. nov. differs from *N. longipes* by the presence of dorsal glandules (vs. absent) and by distinctly longer legs, leg I= 1381, II=1097, III=1138, IV=1587 (vs. I=695; II=545; III=555; IV=840).

Newellia xakriaba sp. nov. combines traits of the two previously described *Newellia* species. So far, the remarkable presence of glands was exclusive of *N. glandulosa,* while long legs and four setae on the scutum was exclusive to *N. longipes*. The phylogenetic significance of these glands should be further investigated. They may be a plesiomorphic condition homologous to those observed in Trombellinae Thor, 1935 (Trombellidae): *Trombella* Berlese, 1887 (e. g., *Trombella glandulosa* Berlese, 1887) and *Durenia* Vercammen-Grandjean, 1955 (e. g., *Durenia glandulosa* Robaux, 1968).

Newellia xakriaba sp. nov. had been previously misidentified as *Johnstoniana* George, 1909 (Pepato *et al.* 2022). In the present study we corrected this in GenBank; sequence access IDs for *Newellia xakriaba* sp. nov. and *Centrotrombidium krenak* sp. nov. are provided in Table I.

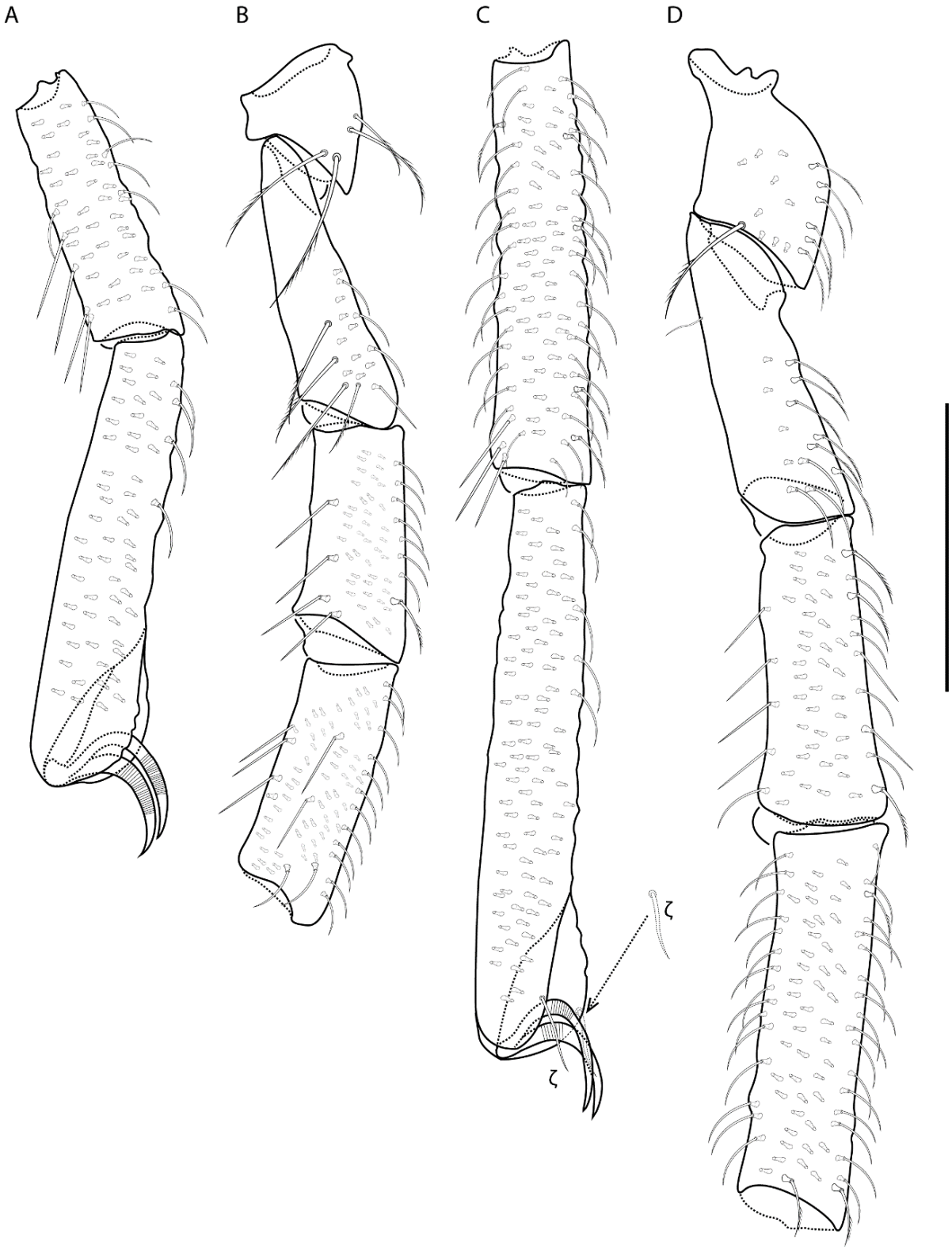

Figure 4. *Newellia xakriaba* sp. nov. male. A and B: Leg III. C and D: Leg IV. See figure 3 for setal types. Scale bar: 200 µm.

Etymology

The new species is named after the Xakriabá, an indigenous people of Brazil.

Figure 5. *Newellia xakriaba* sp. nov. male. A: Tarsi and tibia I. B: Anterior gland surface. C: Anterior gland, internal focus. D: Posterior gland surface. E: Posterior gland, internal focus. F: General view showing relative appendage lengths. G: Genital apparatus, lateral view. Spatial orientation indicated by arrows: dorsal (Ds), ventral (Vs), posterior (Ps) and anterior (As). Scale bars: A= 100, B – E and G= 20 µm, F= 500.

Genus *Centrotrombidium* Kramer, 1896

Diagnosis

Adults. With one pair of globular sensilla (*si*) on an elongated dorsal shied, which bears one pair normal setae (*ve*); basidont unidentate; palp tibia and tibia I without cuticular triangular extensions, previously referred as “shark fin shape” (Wohltman *et al.* 2004).

***Centrotrombidium krenak* sp. nov.**

Diagnosis

Male. Sensilla twice as long as wide, with globose ends. Dorsal setae thin and smooth, on rounded sclerites (Fig. 9D). Tarsi I with 2 or 3 club-shaped (short and wide) solenidia (2 observed on the left and 3 on the right leg of our specimen), tarsi II with a club-shaped solenidia and famulus ε (Figs 7B, 9A, B). Ventral setae long, especially in areas of Cx I and II (48–86). *Ve* barbed and relatively short (47 µm, *Ve/Si*=1.38, *Ve*/Sbp=1.14). Palp genu shorter than palp tibia (pGe/pTi=0.74). Tarsi I longer than wide (length/width= 1.72). Palp tibia with only two spine-like setae (odontus and paradont).

Holotype: Male (UFMG AC 171520), entrance zone of iron ore cave SPT-0385, 19°13'05.1"S 43°23'07.3"W, Morro do Pilar municipality, Minas Gerais State, Brazil, Jan. 10–15, 2017. Coll.: *Carste Ciência e Meio Ambiente* team.

Table 1. GenBank accession numbers. COI sequences could not be obtained for *Centrotrombidium krenak* sp. nov.

Voucher	**18S**	**28S**	**SRP54**	**HSP70**	**COI**
Newellia xakriaba sp. nov. UFMG AC 170317	OM386730	OM641871	OM471520	OM432061	OM401571
Centrotrombidium krenak sp. nov. UFMG AC 171520	OM386735	OM641880	OM471527	OM432069	NA

Description

Male. M**e**asurements summarized in table II.

Idiosoma nearly oval (Fig. 6A). Two pairs of eyes, situated over stalked eye plates. Posterior eye poorly developed, without well delimited eye cornea (Fig. 6C). Prodorsal sclerite elongated (Fig. 1C). With one pair of short smooth trichobothria "*si*" with globose end (*si* length / *si* globe width = 2). On the base of *si*, six sclerotized bands connecting *si* to an external sclerotized ring (represented in gray, Fig. 6C). In addition, prodorsal sclerite with a pair of distally barbed common seta *ve* (Fig. 6C). Idiosoma dorsal setae smooth and short (11–22), situated near border of rounded sclerotized plates (Figs 6A, 9C).

Genital pore with epivalve and central valve with similar width. Epivalve bearing 12 and central valve six, smooth long setae arranged in a single row. Ejaculatory complex with ten smooth eugenital setae, arranged in two rows of four and one isolated centro/distal pair (Fig. 6E, D). Anal plate with five smooth setae each, setae arranged in a single row (Fig. 7C).

Gnathosoma with a short mouth cone, covered by smooth setae. Cheliceral blades striated longitudinally, short, and curved (Fig. 2). Palps short and massive, relative segments length: Palp (Pa) femur > PaTi > PaGe > PaTr. Palp chaetotaxy: fPp=0-0-12N-9N,2B-7N,5ζ,1ω. Palp tibia with spiniform paradont and odontus with a single prong; palp trochanter with a weakly sclerotized rounded area (fenestra) (Fig. 2E, F).

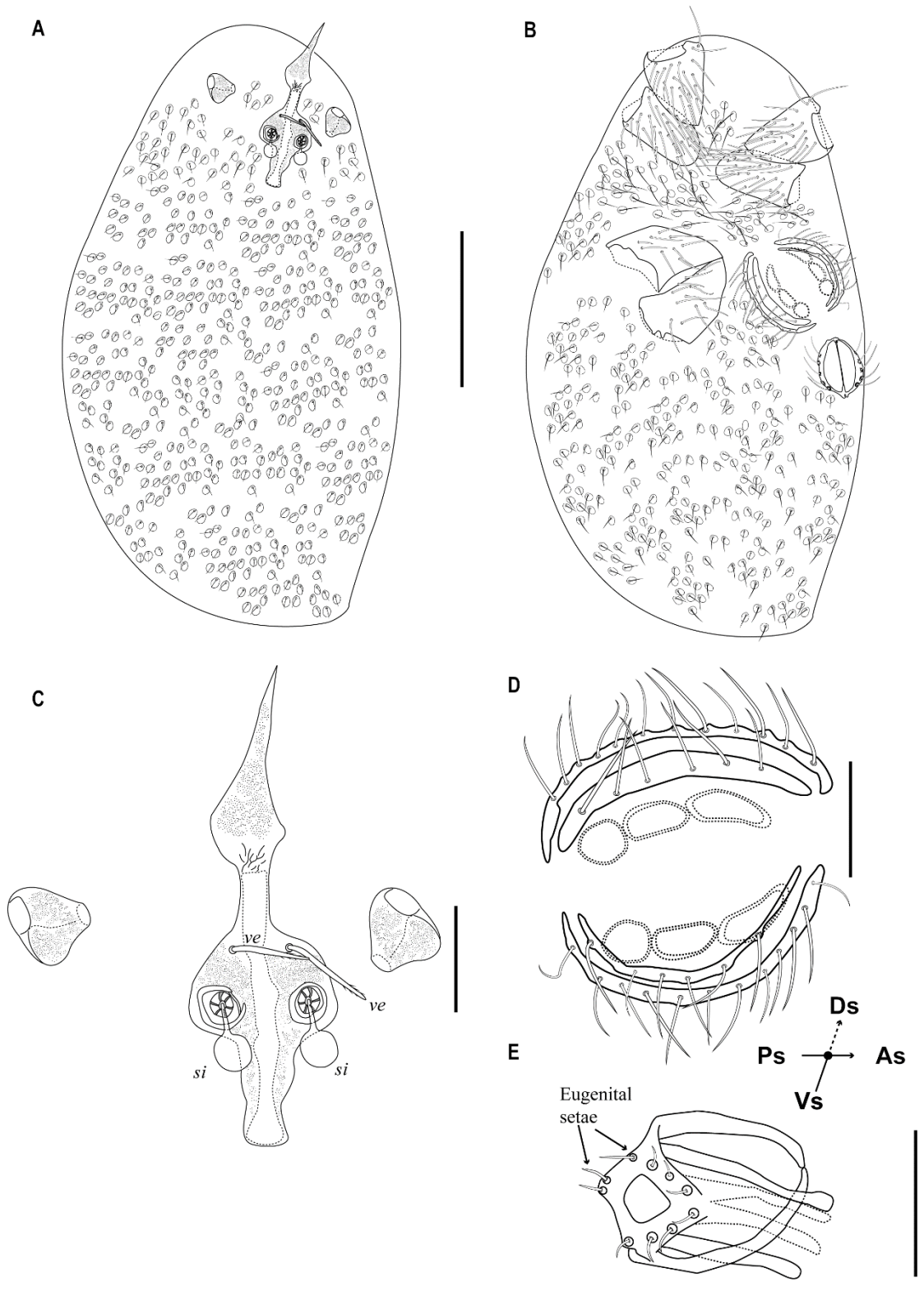

Figure 6. *Centrotrombidium krenak* sp. nov. male. A: Dorsal view. B: Ventral view. C: Prodorsal sclerite and eyes. D: Genital sclerites and genital acetabula. E: Genital apparatus. Spatial orientation is indicated by arrows: dorsal (Ds), ventral (Vs), posterior (Ps) and anterior (As). Scale bars: A, B= 100 µm, C, D and E= 50.

Table 2. Metric data for *Newellia xakriaba* sp. nov. (UFMG AC 170317) and *Centrotrombidium krenak* sp. nov. (UFMG AC 171520).

Characters	***Newellia xakriaba***	***Centrotrombidium krenak***	**Characters**	***Newellia xakriaba***	***Centrotrombidium krenak***
IL	843	794	Ti I	294	88
IW	632	450	Ta I	282	166
Sba	32	NA	Leg I	1381	541
Sbp	71	41	Cx II	178	122
Vi	154	NA	Tr II	101	62
Si	221	34	Bfe II	186	61
Se	38	NA	Tfe II	176	60
Ve	77	47	Ge II	175	66
ISD	66	NA	Ti II	164	71
DS	20 – 45	11 – 22	Ta II	295	139
VS	15 – 43	48 – 86	Leg II	1097	459
L	307	230	Cx III	149	118
Anterior ECO	23.5	-	Tr III	91	62
Anterior eye Ring	54	14	Bfe III	168	73
Posterior ECO	-	-	TfeIII	171	58
Posterior eye Ring	43	-	Ge III	181	63

Distance between anterior eyes	247	189	Ti III	201	73
Distance between posterior eyes	187	-	Ta III	326	134
Distance between *ve*	67	28	Leg II	1138	463
Distance between *se*	93	-	Cx IV	236	145
Anal valve lenght	99	86	Tr IV	163	94
Genital valve length	195	130	Bfe IV	177	74
Cx I	199	112	Tfe IV	224	69
Tr I	119	59	Ge IV	296	91
BFe I	181	64	Ti IV	322	118
TFe I	233	80	Ta IV	405	152
Ge I	272	84	Leg IV	1587	598

Relative leg length: IV>I>III=II (Fig. 9C). Legs covered by three main types of setae: long setae weakly barbed at distal ends; smooth setae, including eupathidia and solenidia (highlighted in grey on our drawings); few distinct club-shaped striated solenidia. Intermediary types of setae that couldn't be distinguished between solenidia and eupathidia present on legs (ω/ζ, Fig. 9A). Tarsi I with two to three club-shaped solenidia and tarsi II with a single club-shaped solenidion and famulus ε (Figs 7B, 9A, B). All claws without striae or setules. Most setae placed directly on cuticle, a few on conical projections on tibia and genu I.

Remarks

Centrotrombidium Kramer, 1896 comprises 13 species know from larvae (L), post larval instars (P) or both (P, L): *C. approximatum* Newell, 1957 (P, USA); *C. australasiae* Womersley, 1942 (P, Australia); *C. blackwellae* Baker, 1999 (L, Scotland, Great Britain), *C. culicoides* (VercammenGrandjean, 1957) (L, Great Britain), *C. delamarei* André & Lelièvre-Farjon, 1961 (P, Argentina), *C. dichotomicoxala* VercammenGrandjean & Cochrane, 1974 (L, USA), *C. distans* Newell, 1957 (P, L, USA), *C. hadroseta* Newell, 1957 (P, USA), *C. misellum* (Berlese, 1918) (P, Mexico), *C. motasi* (Feider, 1945) (P, L, Romania), *C. olgierdi* Haitlinger, 2005 (L, Switzerland), *C. romaniense* Vercammen-Grandjean & Feider, 1973 (L, Romania), *C. schneideri* Kramer, 1896 (P, L, Austria, Belgium, Czech Republic, France, Germany, Norway, Switzerland) (Mąkol & Wohltmann, 2012).

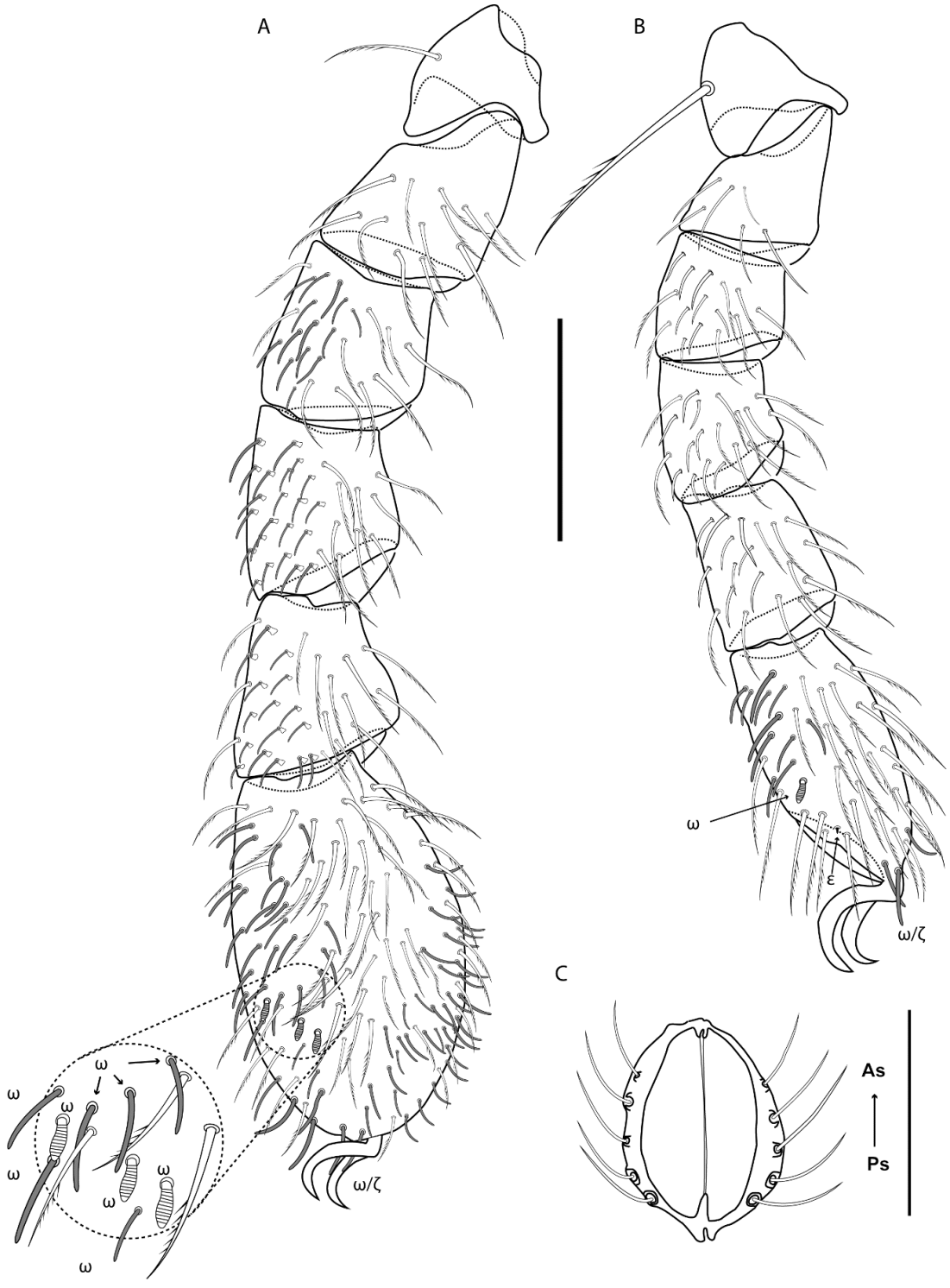

Figure 7. *Centrotrombidium krenak* sp. nov. male. A: Leg I, detail showing normal solenida and club-shaped (short and wide) solenidia. B: Leg II, seta indicating club-shaped solenidion. C: Anal valves. Spatial location is indicated by arrows: posterior side (Ps) and anterior side (As). See figure 3 for setal types. Scale bars: A, B and C= 100 µm.

Centrotrombidium krenak sp. nov. differs from *C. approximatum* by the barbed (vs. smooth) and relatively short *ve*, *ve/si*=1.38 (vs. long, *ve/si*=2.54); from *C. australasiae* by sensilla (*vi*) twice as long as wide and with a globose end (vs. seven times longer, with a clavate end); from *C. delamarei* by the shorter *ve* (47 vs. 65), two spine-like setae on the palp (vs. 3, due to an additional distal and dorsal spine) and length/width=1.72 (vs. 2.06–2.11); from *C. distans* by the palp genu shorter than palp tibia (vs. much longer), barbed *ve* (vs. smooth) and longer ventral setae, especially, on coxae I and II, 48–86 (vs. approximately 25, estimated based on drawings); from *C. hadroseta* by the thin and simple dorsal setae (vs. modified and enlarged) and barbed *ve* (vs. smooth); from *C. schneideri* by the long ventral setae, 48–86 (vs. 25–32) and barbed *ve* (vs. smooth); and from *C. misellum* by sensillae (*vi*) short and with a globose end (vs. long and slender). The original description of *C. motasi* (Feider, 1945) lacks detail necessary for its accurate identifications and is considered as a *nomen dubium* (Wohltmann *et al.*, 2004).

Figure 8. *Centrotrombidium krenak* sp. nov. male. A: Leg I. B: Leg II. See figure 3 for setal types. Scale bar: 100 µm.

Etymology

The new species is named after the Krenak, an indigenous people of Brazil; here used as a noun in apposition.

Discussion

Our two new Johnstonianidae species *Newellia* (mistakenly identified as *Johnstoniana*) and *Centrotrombidium,* together with *Diplothrombium* Berlese, 1910, represented the Family Johnstonianidae in Pepato *et al.* 2022 study. In their timetree *Newellia* and *Centrotrombidium* were recovered as sister groups that diverged near the K-Pg boundary (66 Mya, 95% confidence interval 33–106 Mya). While *Diplothrombium* was recovered as an older lineage that diverged from the previous two in the Cretaceous (117 Mya, 95% confidence interval 64–169 Mya). The whole Johnstonianidae lineage diverged from the remaining Trombidioidea in the Triassic (233 Mya, 95% confidence interval 195–270 Mya).

The molecular data now associated with the new species, allow further testing of species boundaries. We hope that soon, larvae, females, or any heteromorphic specimen may be associated with the aid of this data.

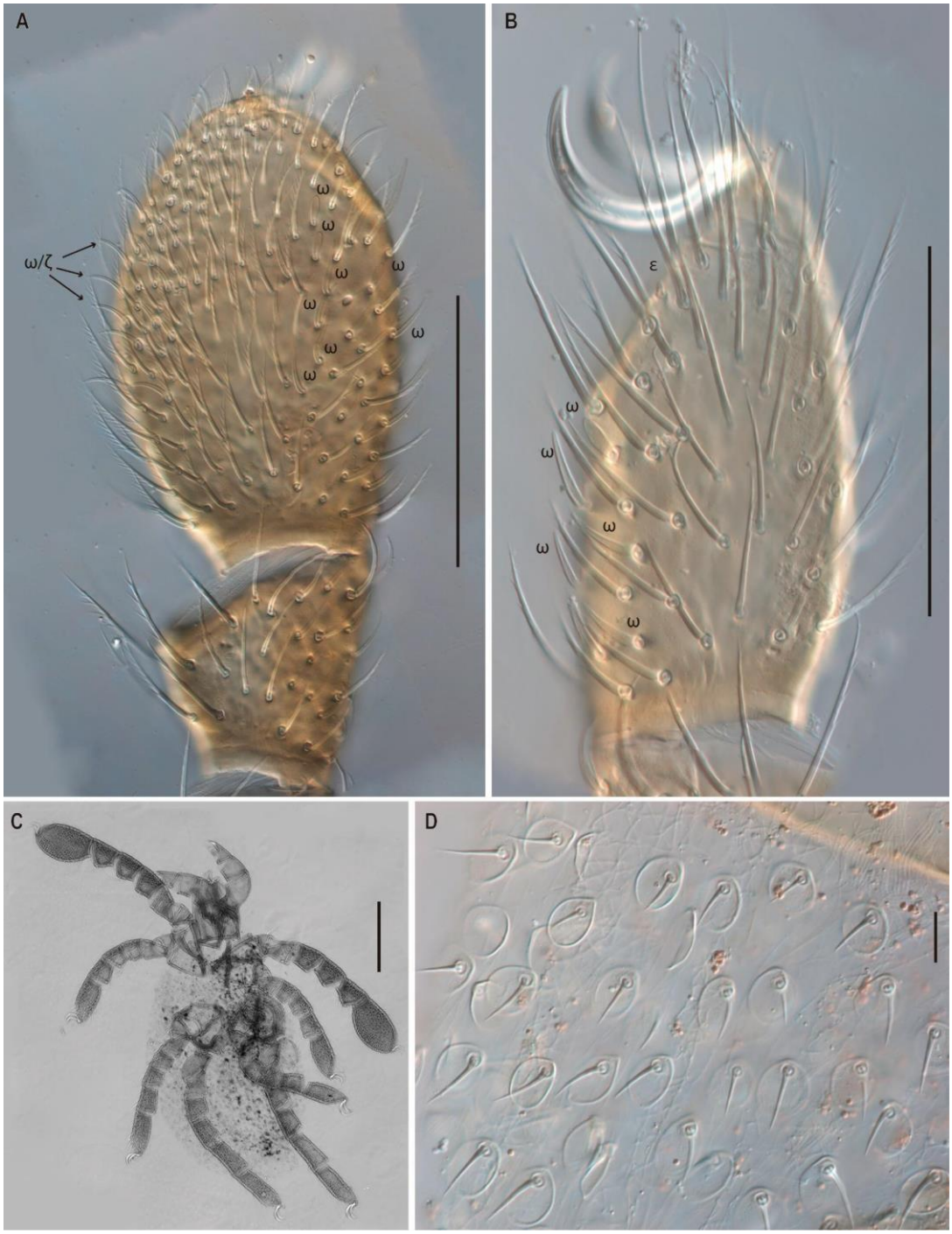

Figure 9. *Centrotrombidium krenak* sp. nov. male. A: Tarsi and tibia I. B: Tarsi II. C: General view, relative lengths of appendages. D: Dorsal setae. Scale bar: A, B= 100 µm, C= 500 µm, D= 10 µm.

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Capítulo 1.5 – Allotanaupodidae

Neste Capítulo é apresentada pela primeira vez a descrição da fase larval de Allotanaupodidae. A posição filogenética dessa família supostamente basal e incomum por ser a única linhagem terrestre sem sensilas é posteriormente inferida na filogenia do capítulo 2.

Capítulo 1.5.1: The unusual morphology of larval Allotanaupodoidea revealed by association of a larva with a deutonymph using DNA barcoding.

SAMUEL GEREMIAS DOS SANTOS COSTA* [1, 2], ZHI-QIANG ZHANG[3,4] & ALMIR ROGÉRIO PEPATO [1,2].

[1] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.*

[2] *Tyumen State University, 10 Semakova Str., 625003 Tyumen, Russia.*

[3]*Manaaki Whenua–Landcare Research, Private Bag 92170, Auckland, New Zealand; zhangz@landcareresearch.co.nz; https://orcid.org/0000-0003-4172-0592*

[4] *School of Biological Sciences, The University of Auckland, Auckland, New Zealand*

** Corresponding author. E-mail:* estoupa.bob@gmail.com

Abstract

Parasitengona mites have a life cycle that includes three active instars consisting in a parasitic larva and radically heteromorphic predatory deutonymph and adult. Allotanaupodoidea is a superfamily of Parasitengona represented only by the family Allotanaupodidae known solely by the post-larval instars. The family occurs in New Zealand and Chile and drastically differs from the remaining Parasitengona due to the lack of trichobothria in the prodorsal shield. In the present study we describe the larva of *Allotanaupodus winksi* Zhang & Fan 2007 from New Zealand associated with the deutonymph employing cytochrome oxidase I. The description reveals a remarkable similarity between larval and post larval specimens of *A. winksi* previously seen only in Calyptostomatoidea (Parasitengona).

Key words: Parasitengona, *Allotanaupodus*, Neotropical, heteromorphic life cycle

Introduction

The Parasitengona is the most diverse group of Prostigmata and are mainly characterized by the complex life cycle that includes *parasitic larvae and free-living* deutonymphs and adults which are predatory, with a few exceptions where the larva is predatory or phytophagous (*e.g.*, *Balaustium* von Heyden, 1826). The life cycle of the Parasitengona includes great morphological changes occurring between larvae and deutonymphs/adults. Consequently, associating larval and post-larval Parasitengona usually requires the cultivation of the specimens in the laboratory or the employment of DNA barcoding techniques (Costa *et al.* 2019). These extra steps resulted in most species being described by many authors based solely on either larvae or post-larval instars (Mąkol & Wohltmann 2012).

A second remarkable condition of most post-larval terrestrial Parasitengona is their hypertrichious body and legs. The numerous setae include chemical receptors (solenidia and eupathidia) and normal setae (non-differentiated setae that may work as mechanoreceptors). Their number is especially high on the tarsus I. These setae are hard to count precisely, and often present intraspecific variation as observed in Leewenhoekiidae, Johnstonianidae, Erythraeidae, Smarididae, among others (*e.g.*, Costa *et al.* 2019, 2021, 2023; Gomes-Almeida *et al.* 2023). This condition makes most authors ignore the number of setae in post larval specimen's legs during taxonomic studies.

On the other hand, the number of normal setae is relatively stable in the larval Parasitengona and their absolute number is often used as diagnostic character. For instance, Saboori *et al.* (2020) observed the same pattern of setae on tibia I, II and III (14, 15, 15 respectively) and a bit more variable in tarsi I, III and III (27–28, 25–26, 25–26 respectively) when reviewing the larvae of the highly diverse erythraeid genus *Leptus* (more than 240 species).

The Calyptostomatoidea Oudemans, 1923 is one exception for the typical Parasitengona heteromorphic life cycle (Mąkol & Wohltmann 2012): its larvae are remarkably similar to the adults and present hypertrichous tarsi I with numerous solenidia (vs. 1–3 in most larvae in Parasitengona).

The superfamily Allotanaupodoidea is represented by a single family, Allotanaupodidae, known solely by its post-larval instars. They are considered an early-derivative group of the Parasitengona and are found in New Zealand and Chile (Zhang & Fan 2007). These mites are characterized by the lack of trichobothria (modified mechanical receptors referred as *vi* and *sci*)

on a big prodorsal sclerite (scutum), the presence of one or two pairs of plates with multiple setae on C to PS rows of dorsal hysterosoma, the presence of only two pairs of genital acetabula in adults, and short, distally inserted palptarsus on the palptibia (Zhang & Fan 2007).

In the present study we describe for the first time the larva of Allotanaupodoidea represented by the larval instar of *Allotanaupodus winksi* Zhang & Fan 2007. The larval specimen was associated with a deutonymph of the same species employing DNA barcoding and is remarkably similar to the deutonymph specimen.

Material and methods

The two specimens of *A. winksi*, a deutonymph (UFMG AC 221502) and a larva (UFMG AC 221478), were collected by Pepato A. R. in the leaf litter near Thames municipality, Waikato Region, New Zealand (37°06'47.6"S 175°31'33.0"E) on 16th December of 2022. The larva (UFMG AC 221478) is deposited in NZAC, whereas the deutonymph is vouchered in UFMG. Collection acronyms follow those in Zhang (2018).

The DNA extraction, microscope slide preparation and the sequencing of the mitochondrial gene cytochrome oxidase subunit I gene (COI) were performed as described by Costa *et. al* (2019) and was used to associate the instars. Illustrations were made with the aid of Zeiss Primo Star compound microscope with a Mshiwi 38MP HDMI Microscope HD camera. Drawings were made with the aid of a simulated camera lucida using the software On Top Replica v3.5.1 and Adobe Illustrator CC 2015. Final artwork was done using Adobe Photoshop CC 2015. All measurements are given in micrometers (µm). Terminology and abbreviations were adapted from Southcott (1961) and Grandjean (1947). In addition, the larval scutelae were numerated 1 to 4 from the anterior to the posterior end of the scutum (Fig. 1A).

DNA analysis results:

The COI sequences were recovered for both specimens were identical and are deposited in GenBank data base, access number to be provided upon acceptance.

Systematics

Family Allotanaupodidae Zhang & Fan, 2007

Diagnosis (Adults and Deutonymph): See Zhang & Fan, 2007.

Diagnosis (Larva): Scutum lacking trichobothria, idiosoma covered by sclerites bearing setae, including one or more pairs of plates with multiple setae (Fig. 1A), Claparède organs present between coxae I and II, ânus present bearing two anal plates with setae (Fig. 1B and 3D), palp genu with two and femur with three normal setae, tarsus of leg I distinctly hypertrichous with more than 10 solenidia.

Genus *Allotanaupodus* Zhang & Fan, 2007

Diagnosis (Adults and Deutonymph): See Zhang & Fan, 2007.

Diagnosis (Larva): Body covered exclusively by smooth setae. Ge I, II and III with 10, 8 and 8 normal setae respectively. Bfe I and Bfe II with 3 and 4 normal setae respectively. Bfe and Tfe III fused. Palpal setal formula: 0-0-3N-2N-3N-4N, 2ζ, 1ω. Palp femur with large punctations. Palp tibia claw with a short basal prong and a distal main prong (Fig. 1D). Scutum with 4 pairs of scutelae, scutelae 1 with similar length of scutelae 2, both shorter than scutelae 3, which is shorter than scutelae 4 (Fig. 1A). Anal valves with 3 pairs of smooth setae. Leg's tarsi divided by tibia equals to 1.5, 1.55 and 1.15 for legs one to three respectively. Leg's tibia divided by genu equals to 1.12, 1.08, 1.15 for legs one to three respectively.

***Allotanaupodus winksi* Zhang & Fan 2007**

(Figs 1–5)

Diagnosis (larva): See genus diagnosis above. Notably the genus diagnosis must be upgraded when more species are found, and morphological diversity can be estimated.

Diagnosis (deutonymph): See Zhang & Fan 2007.

Description

Larva: Idiosoma oval (IL= 537, IW= 291). Antero-dorsal portion covered by a big triangulate scutum with 4 pairs of smooth normal setae and a linear crista metopica, which extends at the posterior ends on two sides forming an upside-down T-shape. Two pairs of eyes hardly observable (this difficulty may be caused by the slide preparation method used). Dorsal setae distributed over 20 dorsal sclerites arranged in four rows (C, D, E, F), with 1 to 5 setae each and summing a total of approximately 37 smooth setae (uncertainty due to damage, Fig. 1A, 3A). Idiosoma ventral side bearing one pair of intercoxala *1a* and *2a* located on weakly

sclerotized and punctate extensions of coxae I and III (Fig. 1B, 3C). Four normal setae located over sclerites located between coxae III and anal pore. Anal valves with 3 pairs of short smooth *ps* setae (Fig. 1B). Ventral setae (H row) located posteriorly to anal valves similar to dorsal ones. Claparède organs easily spotted between coxa I and II and apparently stalked (Fig. 1B and 3D). Notably, this stalked appearance may be an artefact caused during the slide mounting process.

Palps long and robust bearing a single odontus with a main and a proximal prong. Palp tarsus short and located in the distal end of palp tibia. Palp genu with large punctations of irregular shape on the dorsal side (Fig. 1D, 3A–C). Palpal setal formula: 0-0-3N-2N-3N-4N, 2ζ, 1ω. Mouth cone with one pair of smooth hypostomala and galeala (*as* and *cs*). Cheliceral blades short, slightly curved and pointed frontwards (Fig. 1C).

Legs short (375–521), well sclerotized and densely covered by smooth setae (Fig. 2). Legs I and II with seven segments, leg III with six segments due to fused basifemur and telofemur (Fig. 2). Tarsi I, II and III each with one pair of claws and lacking empodium. Tarsi I with more than 67 normal setae and 44 solenidia, some of them placed over conical papilla and with morphology similar to those in deutonymph (Fig. 2A, B and 5). See table I for measurements.

Leg setal formula:

Leg I: Ta I—>67N*, >44ω*, 2ζ; Ti I—14N, 5 φ; Ge I—10N, 2 σ; Tfe—5N; Bfe—3N; Tr—1N; Cx I—1N.

Leg II: Ta II—25N, 2ω, 2ζ, 1Cp; Ti II—10N, 1φ; Ge II—8N 1σ; Tfe—5N; Bfe—4N; Tr—1N; Cx—1N.

Leg III: Ta III—26N; Ti III—10N, 1φ; Ge III—8N; Fe—7N; Tr—2N; C—1N.

* A precise estimation of setae in the palp tarsi is precluded by its high number, optical equipment limitations and lack of additional specimens.

Deutonymph’s comparison.

A morphological comparison of our deutonymph (Fig. 4) with *Allotanaupodus winksi* (Deutonymph), *A. williamsi* (male) and *A. orete* (male) reveals a high similarity to *A. winksi*

(Table II). This similarity is further noticed when comparing metric data of the type specimens with ours (Table III), leading us to conclude that they belong to the same species.

Discussion

Hypertrichious larvae of the Parasitengona, morphologically similar to the adults, have been previously recorded only for the family Calyptostomatidae. Finding a second family of the Parasitengona with this trait raises many questions about early evolution in the Parasitengona.

Soller *et al.* (2001) proposed a morphological phylogeny of the Parasitengona based on the data of Witte (1991, 1995) and Welbourn (1984, 1991). In his phylogeny Calyptostomatoidea was recovered as a sister group of Erythraeidae and Smarididae due to a series of synapomorphies observed in the post-larval active instars: Cheliceral shafts styliform and with piercing function (vs. broad cheliceral shaft provided with hook-like moveable digit, piercing function restricted to moveable digit), protraction of chelicerae direct (vs. protraction mediated through sigmoid pieces), sigmoid pieces transformed to thin-walled tracheal trunks (vs. robust sclerites which mediate chelicera protraction), arcal sclerite present (vs. absent), deep salivary groove (vs. no groove), fat body absent (vs. fat body present), two pairs of genital acetabula (vs. three pairs).

Allotanaupodidae shares with Calyptostomatidae and Erythraeoidea the presence of only two pairs of genital acetabula in the adults (vs. three pairs), in addition to the morphologically similar larval and post-larval instars that is also observed in Calyptostomatoidea. However this condition is also observed in some Trombellidae such as *Neonothrothrombium* and *Durenia* (Robaux 1968) and *Charadracarus* of Johnstonianidae (Newell 1960). These suggest that these taxa may be closely related. Laval Allotanaupodidae also shares with Amphotrombiidae Zhang 1998, Stygothrombiidae Thor 1935, and other water mites the presence of two setae on the palp genu.

Recent molecular phylogenetic studies (Dabert *et al.* 2016 and Pepato *et al.* 2022) were inconclusive about the phylogenetic position of the Calyptostomatidae. These may be explained by the lineage age combined with the lack of closely related taxa. According to Pepato *et al.* (2022) Calyptostomatidae diverged from the remaining Parasitengona in the early Permian, being the second oldest sampled Parasitengona lineage to diverge while Stygothrombiidae is the oldest. The inclusion of the Allotanaupodidae in the molecular phylogenetic analysis of the

Parasitengona may help to solve not only its phylogenetic position, but to increase the resolution of the tree by splitting long branches.

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Figures:

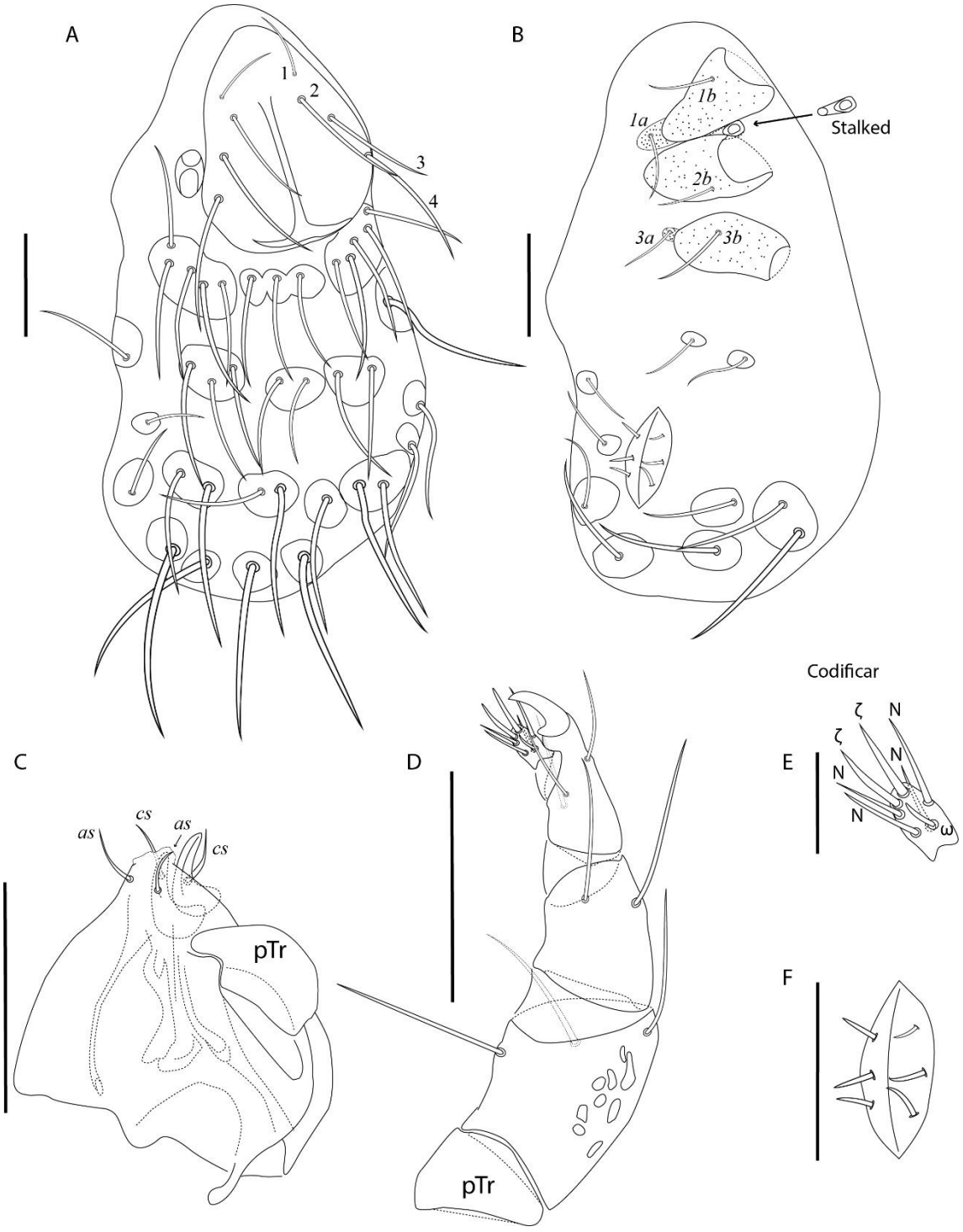

Figure 1. *Allotanaupodus winksi* larva A: Idiosoma dorsal view; B: Idiosoma ventral view; C: Gnathosoma ventro-lateral view; D: Palp; E: Palp tarsus; F: Anal plates. Scale: A–D and F= 100 µm, E= 25 µm.

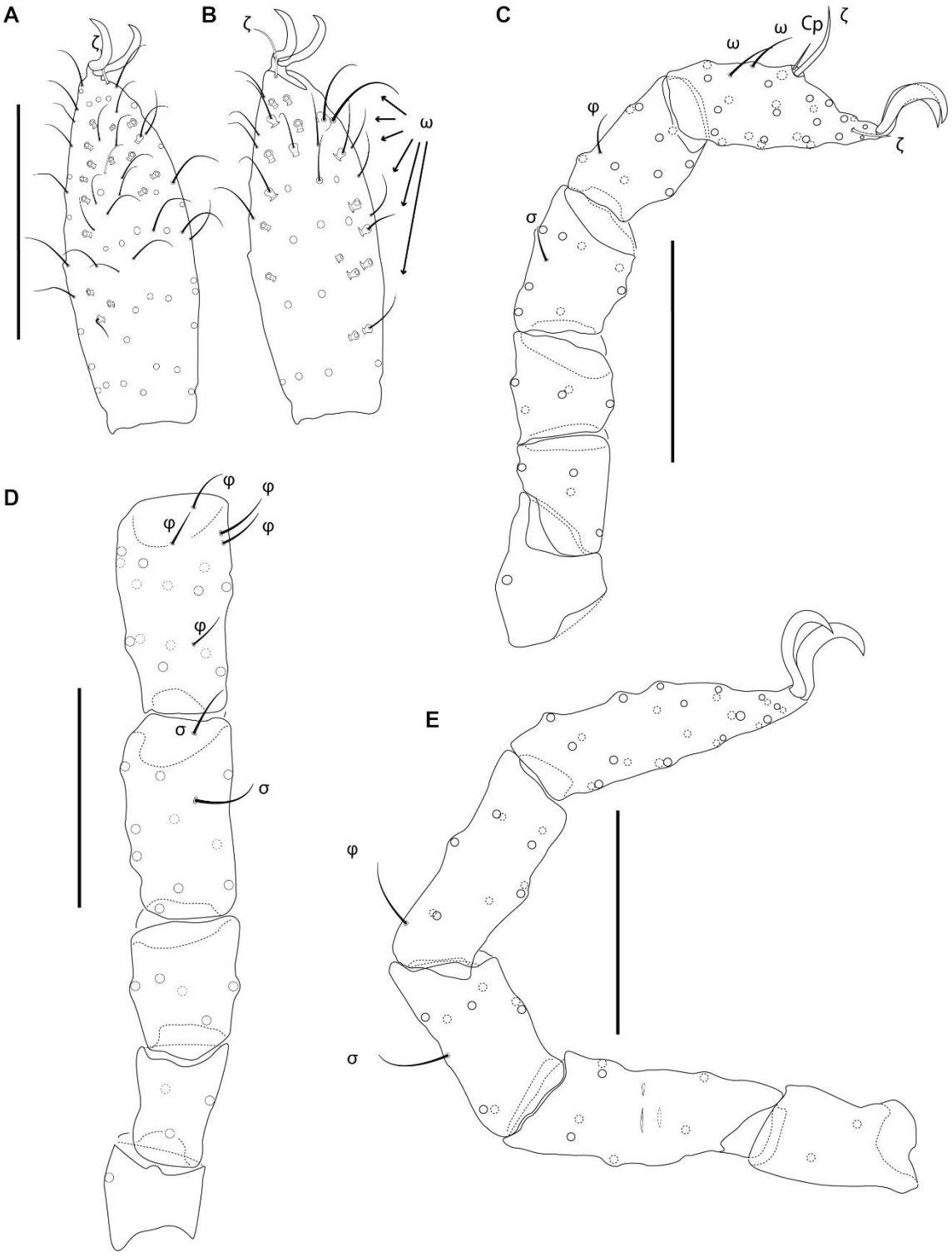

Figure 2. *Allotanaupodus winksi* larva A: Tarsus I anterior side; B: Tarsus I posterior side; C: Leg II; D: Leg I tíbia to trochanter; E: Leg III. Scale: 100 µm.

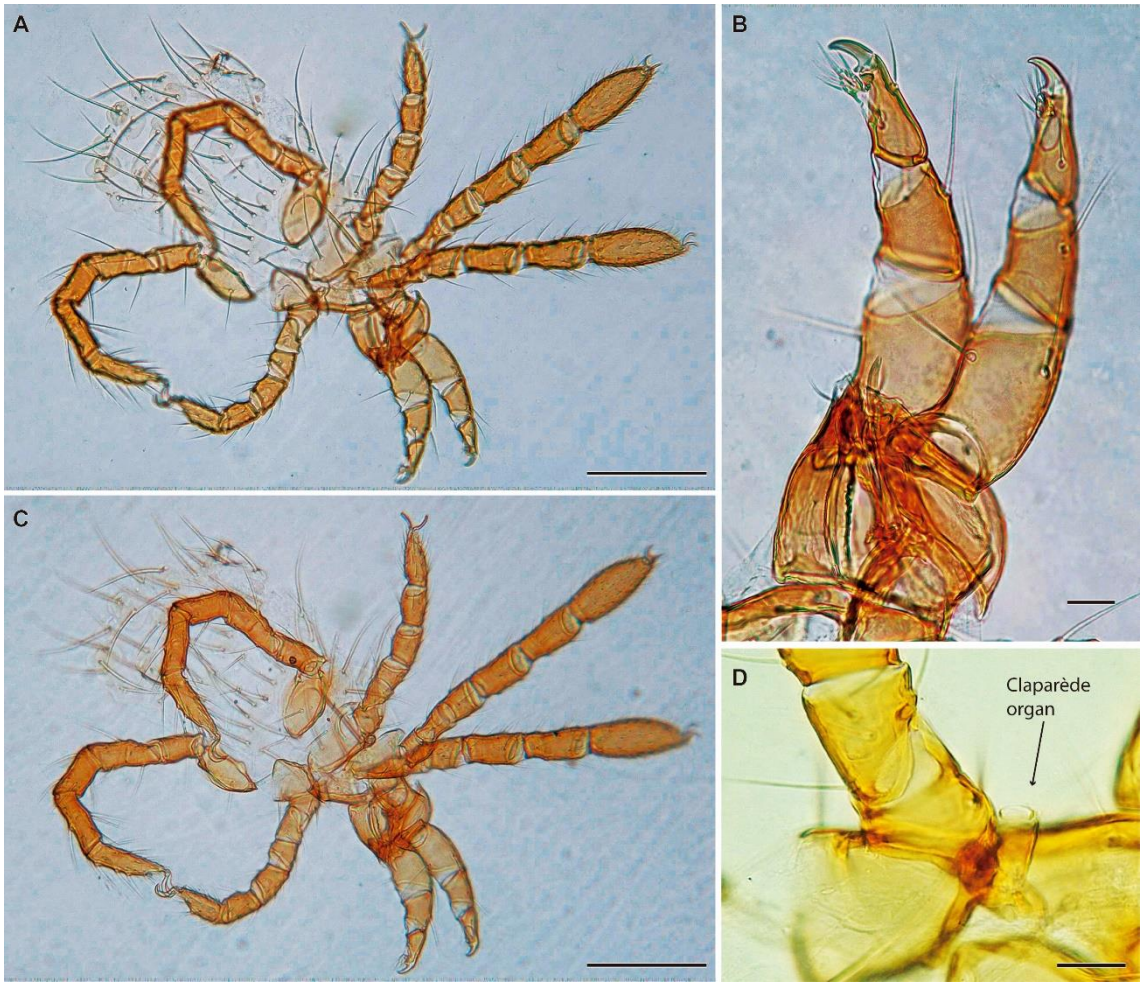

Figure 3. *Allotanaupodus winksi* larva A: General dorsal view; B: Gnathosoma; C: general ventral view; D: Coxa and trochanter I, highlights the stalked Claparède organ that may be an artefact of slide mounting. Scale: 100 µm.

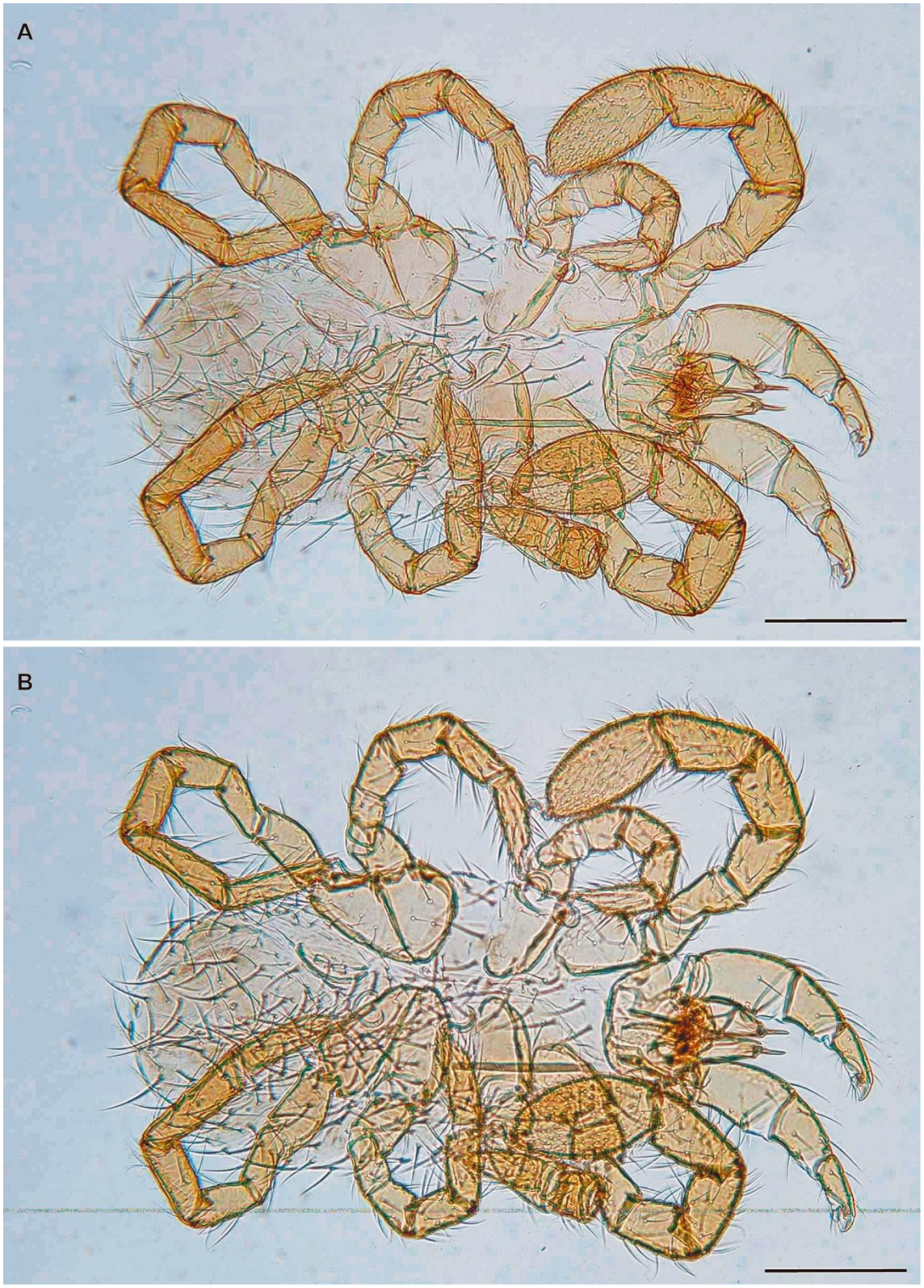

Figure 4. *Allotanaupodus winksi* deutonymph. A: Dorsal general view; B: Ventral general view. Scale: 100 µm.

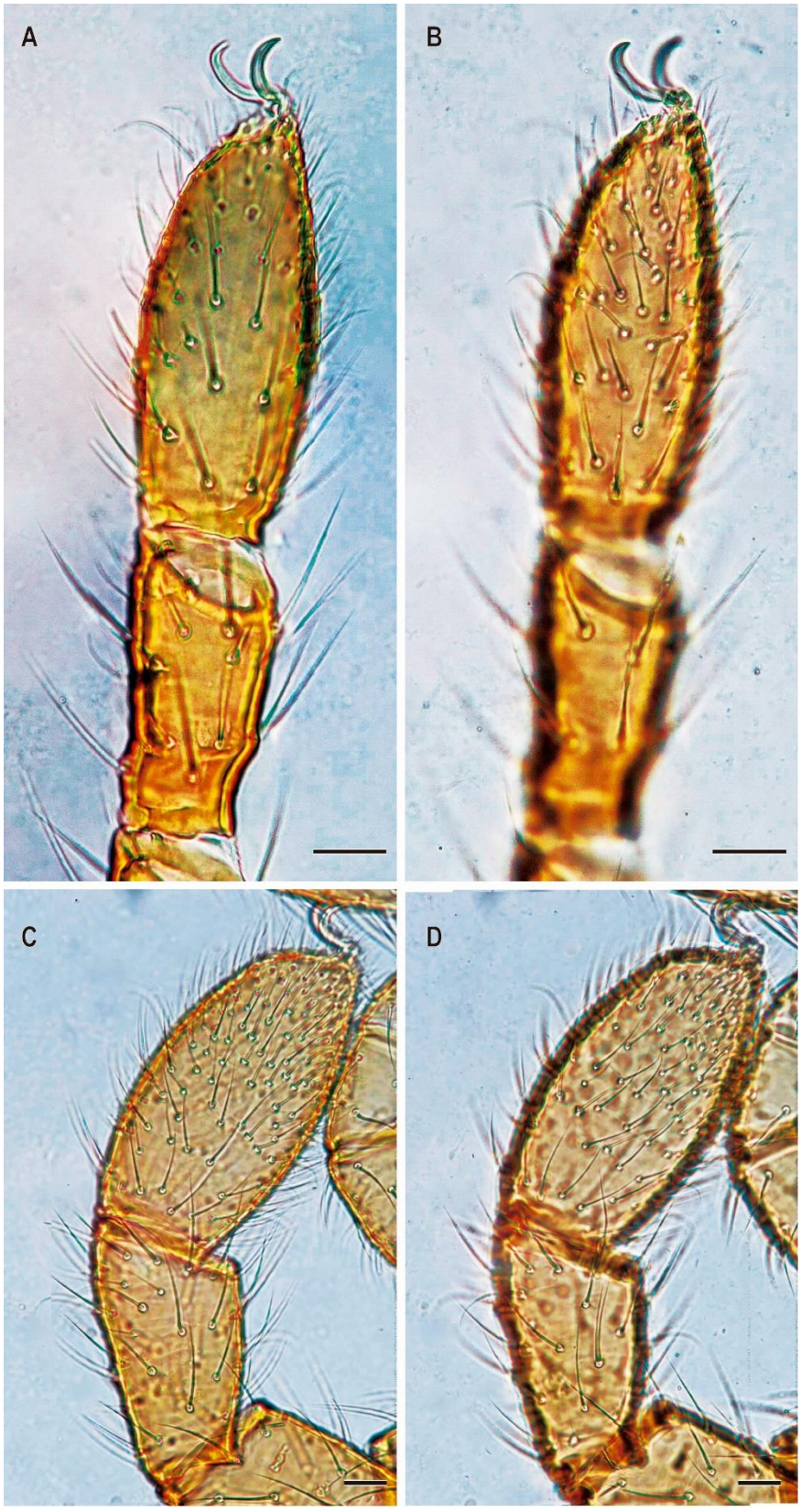

Figure 5. *Allotanaupodus winksi* larva A: Tarsus I anterior view; B: Tarsus I posterior view. Deutonymph C: Tarsus I anterior view; D: Tarsus I posterior view. Scale: 100 µm.

Tables:

Table I – Morphometric data of *Allotanaupodus winksi* (larva).

Character	UFMG AC 221478		Character	UFMG AC 221478
IL	537		Ge I	92
IW	291		tFe I	60
L	212		bFe I	54
W	165		Tr I	57
Distance between sucutelae 1	65		Cx I	90,7
D. between sucutelae 2	64		Leg I	521
D. between sucutelae 3	108,5		Ta II	100,8
D. between sucutelae 4	144,6		Ti II	65
Scutela 1	53		Ge II	60
Scutela 2	107		Tfe II	45,7
Scutela 3	119		Bfe II	51,18
Scutela 4	151		Tr II	53
Dorsal setae	86–136		Cx II	96
Ventral setae	57–112		Leg II	375,68
Anterior eye width	16		Ta III	123
Posterior eye width	16		Ti III	106,4
1a	52,6		Ge III	92,2
1b	75,5		Fe III	111,1
2b	52		Tr III	65,9
3a	46,3		Cx III	117
3b	67		Leg III	498,6
Ta I	155			
Ti I	103			

Table II – Morhpological data of *Allotanaupodus* species (based on Zhang & Fan 2007).

Characters	UFMG AC 221502 (Deutonymph)	*A. winksi* (Deutonymph)	*A. williamsi* (male)	*A. orete* (male)
Length of idiosoma	770	750–950	1250–1450	1500
Shape of prodorsal plate	Sub-pentagonal much longer than wide	Sub-pentagonal much longer than wide	Ovoid slightly longer than wide	Sub-rectangular About as long as wide
Ratio of palptibia length/width	2.9	2.2–2.4	2.2–2.8	3
Ratio of length Cheliceral base/ Cheliceral blade	3.5	2.8–3.2	3.1–3.7	2.8
Ratio of length Anal opening/ genital opening	1.08	1.3	0.6–0.9	0.8
No. of setae on coxa I	11	9–12	22–26	29–32
No. of setae on coxa II	7	6–9	17–22	24–25
No. of setae on trochanter I	6	6–7	12–15	18–19
No. of setae on trochanter IV	6	7	19–22	29

Table III – Morphometric data of *Allotanaupodus winksi* (Deutonymph).

Character	UFMG AC 221502	*A. winksi* type specimens		Character	UFMG AC 221502	*A. winksi* type specimens
IL	770	750–950		Cx II	136	–
IW	430	525–650		Leg II	535,6	520–540
L	308	295		Ta III	156,4	160
W	205	220		Ti III	118,4	125
Dorsal setae	68–109	–		Ge III	96,5	105
Ventral setae	43–85	–		Tfe III	86	105
Eye plate lenght	65	63–65		Bfe III	75,5	82
Ta I	202	235–238		Tr III	92	90
Ti I	158,9	163–175		Cx III	133	–
Ge I	143	140–150		Leg III	624,8	535–635
tFe I	128	118–120		Ta IV	179,3	195
bFe I	91,6	95–105		Ti IV	174,6	180
Tr I	91	–		Ge IV	137	155
Cx I	140	–		Tfe IV	99	107
Leg I	814,5	821–852		Bfe IV	92	130
Ta II	133,6	140		Tr IV	135	–
Ti II	87	90		Cx IV	184,4	–
Ge II	91	86		Leg IV	816,9	660–758
Tfe II	73	82		Genital pore	108	100
Bfe II	80	80		Anal Pore	116,5	125–130
Tr II	71	–				

Capítulo 2 – Enlightening the classification and evolution of terrestrial Parasitengona (Acariformes, Prostigmata) through a comprehensive molecular phylogeny.

Samuel G. S. Costa* [1, 2], Alexandr A. Khaustov[2], Alireza Saboori[4] , Dante B. Ribeiro[1], Javad Noei[5] , Mark S. Harvey[6,7], Matthew D. Shaw[8], Pavel B. Klimov[2,3], Almir R. Pepato [1,2].

[1] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Zoologia, Laboratório de Sistemática e Evolução de Ácaros Acariformes. Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte – MG, Brazil, ZIP: 31270-901.

[2] Tyumen State University, 6 Volodarskogo Str., 625003 Tyumen, Russia.
[3] Purdue University, Lilly Hall of Life Sciences, G-226, 915 W State St, West Lafayette, IN 47907.
[4] Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran.
[5] Department of Plant Protection, Faculty of Agriculture, University of Birjand, Birjand, Iran.
[6] Collections & Research, Western Australian Museum, Welshpool, Western Australia 6106, Australia.
[7] School of Biological Sciences, University of Western Australia, Crawley, Western Australia 6009, Australia.
[8] South Australian Museum, North Tce, Adelaide, 5000, Australia.
** Corresponding author. E-mail: estoupa.bob@gmail.com*

Abstract

Parasitengona is a highly diverse mite lineage with more than 11.000 species found worldwide. The terrestrial Parasitengona can be classified in 18 terrestrial families, from which three are monotypic. Its classification is controversial regarding superfamilies and families' boundaries, and a solution is precluded by the lack of extensive phylogenetic studies to allow a classification based upon the evolutionary history of the major lineages. In this study we present a comprehensive Parasitengona phylogeny focusing on the terrestrial lineages. We included 88 Parasitengona taxa, 14 terrestrial families and 307 outgroups, an addition of 25 taxa and 6 terrestrial Parasitengona families to previous datasets (Allotanaupodidae, Achaemenothrombiidae, Podothrombiidae, Trombellidae, Neotrombidiidae, and Chyzeriidae). The phylogeny was inferred based on five molecular markers with a final filtered alignment of up to 7838 base pairs. We propose a new classification for terrestrial Parasitengona in superfamilies, reducing Trombiculoidea to Trombiculidae and expanding Chyzeroidea to include Trombellidae, Neotrombidiidae, Johnstonianidae, and Chyzeriidae. We also discuss the analyzed water-land transitions and the evolution of the non-heteromorphic Allotanaupodidae larva.

Introduction

Parasitengona are a highly diverse clade with more than 11,000 described species, distributed over all continents except Antarctica (Konikiewicz *et al.* 2016). They share a remarkable life cycle that includes a calyptostatic pre-larva that hatches from the eggs, parasitic larva (with few exceptions), calyptostatic protonymph, active predatorial deutonymph, calyptostatic tritonymphs and predaceous adults (Mąkol & Wohtmann 2012, Krantz & Walter, 2009; Wohltmann, 2000). In most taxa the larval morphology radically differs from the post larval instars except by Allotanaupodidae Zhang & Fan, 2007 (personal observation), something that makes its taxonomy especially difficult, since larvae and postlarval stages must be associated by rearing or molecular analyses (Costa *et al.* 2019).

The present study focuses on terrestrial Parasitengona, whose classification into families and superfamilies is controversial, with authors considering more or less groups. Based on a morphological phylogeny, Welbourn (1991) divided terrestrial Parasitengona into six superfamilies Erythraeoidea, Calyptostomatoidea, Tanaupodoidea, Chyzeroidea, Trombiculoidea and Trombidioidea defined mainly by larval traits. In agreement with this classification, Zhang (1998) and Zhang & Fan (2007) proposed more two terrestrial superfamilies Amphothrombioidea and Allotanaupodidae achieving a total of nine. On the other hand, Söller *et al.* 2001 and later Mąkol & Wohltmann (2012) divided the terrestrial Parasitengona in three superfamilies: Erythraeoidea, Calyptostomatoidea and Trombidioidea.

In the family level the main controversy consists in the classification of mites whose larvae are vertebrate's parasites. While Kudryashova (1998) and Shatrov & Kudryashova (2008) considers Trombiculidae a family with four subfamilies (Leeuwenhoekiinae Womersley, 1944, Apoloniinae Wharton, 1947, Trombiculinae Ewing, 1929 and Gahrliepiinae Womersley, 1952), other classify them into three families (Trombiculidae Ewing 1944, Leeuwenhoekiidae Womersley 1944, and Walchiidae Ewing 1946). There is no consensus or strong evidence in favor of a specific classification for Trombiculidae. Therefore, we initially opt to consider Kudryashova (1998) and Shatrov & Kudryashova (2008) classification. If considering the classification of Kudryashova (1998) and Mąkol & Wohtmann (2012), Parasitengona mites can be classified in 18 terrestrial families. The 18 families, the classification of Söller *et al.* (2001) and Zhang & Fan (2007) are compared in table I.

Table I – Terrestrial Parasitengona classification.

Classification according to		**Family**	**Author**	**Distribution**	**Sampled subfamilies**
Söller *et al.* 2001	Zhang & Fan (2007)				
Calyptostomatoidea	Calyptostomatoidea	Calyptostomatidae	Oudemans, 1923	Global	1/1
Erythraeoidea	Erythraeoidea	Erythraeidae	Robineau-Desvoidy, 1828	Global	5/7
		Smarididae	Vitzthum, 1929	Global	2/2
Trombidioidea	Chyzeroidea	Chyzeriidae	Womersley, 1954	Global, except North America	2/3
	Trombidioidea	Microtrombidiidae*	Thor, 1935	Global	3/3
		Neothrombiidae	Feider, 1959	Global	1/1
		Trombidiidae	Leach, 1815	Global	1/4
		Podothrombiidae	Thor, 1935	Austrália and Northern hemisphere	1/1
	-	Achaemenothrombiidae	Saboori, Wohltmann & Hakimitabar (2010)	Iran	1/1
	Amphothrombioidea	Amphotrombiidae (monotípica)	Zhang, 1998	USA	0/1
	Allotanaupodoidea	Allotanaupodidae	Zhang & Fan, 2007	New Zeland and Argentina	1/2
	Tanaupodoidea	Tanaupodidae	Thor, 1935	Northern hemisphere	0/1
	Yurebilloidea	Yurebillidae (monotípica)	Southcott, 1996	Australia	0/1
	Trombiculoidea	Audyanidae (monotípica)	Southcott, 1987	Malasia	0/1
		Johnstonianidae	Thor, 1935	Global	1/2
		Trombellidae	Thor, 1935	Global	1/5
		Neotrombidiidae	Feider, 1955	Global	1/1
		Trombiculidae (after Kudryashova 1998)	Ewing, 1944	Global	2/5

The aquatic Parasitengona can be split into two lineages: Hydrachnidia and Stygothrombiidae. Their phylogenetic position is still a mystery. Previous phylogenetic studies recovered Hydrachnidia as a basal lineage sister of all remaining Parasitengona based on morphology (Söller *et al.* 2001, Witte 1995). Others using molecular data recovered Hydrachnidia and Stygothrombiidae in a clade that is a sister group of Calyptostomatoidea and Erythraeoidea (eg.: Dabert et al. 2016). Or recovered Stygothrombiidae as a basal lineage sister of all remaining Parasitengona, while Hydrachnidia was recovered as a derived independent lineage closely related to Erythraeoidea or Calyptostomatoidea (Peapato et al. 2022).

Dabert *et al.* (2016), in a study concerned mainly with freshwater Parasitengona, sampled seven terrestrial families: Calyptostomatidae, Erythraeidae, Smarididae Chyzeriidae, Trombiculidae, Johnstonianidae, and Microtrombidiidae (including Eutrombidiinae). They recovered a topology identifying the same three main terrestrial lineages as Söller *et al.* (2001): Calyptostomatoidea (Calyptostomatidae), Erythraeoidea (Erythraeidae, Smarididae), and Trombidioidea (Chyzeriidae, Trombiculidae, Johnstonianidae, and Microtrombidiidae). The Topology recovered, despite moderate support and instability across analyses, was: (Trombidioidea, (Erythraeoidea, (Calyptostomatoidea, (Stygothrombiidae, Hydrachnidia)))). It is interesting to note that freshwater Parasitengona are recovered as a monophyletic group, implying a single transition from land to freshwater.

Pepato *et al.* (2022), in a study exploring the among realms (land, sea, and freshwater) transitions of Acariformes mites included eight families, Trombiculidae (after Kudryashova 1998), Neothrombiidae, Johnstonianidae, Trombidiidae, Microtrombidiidae, Erythraeidae, Smarididae, and Calyptostomatidae. Considering the same classification as Söller *et al.* (2001), the topology, despite low support values, was (Stygothrombiidae, ((Trombidioidea, Calyptostomatoidea), (Erythraeoidea, Hydrachnidia))). Interesting the basal placement of Stygothrombiidae and the sister group placement of Halacaroidea, which has as basal offshoot the freshwater family Pezidae, made an aquatic common ancestor of all Parasitengona highly probable.

The present study includes 88 Parasitengona taxa, 52 terrestrial and 31 aquatic, including 14 out of 15 non-monotypic terrestrial families, an addition of six terrestrial families to previous molecular phylogenies (Dabert *et al.* 2016, Pepato *et al.* 2022). The extra families added to Pepato *et al.* (2022) are Allotanaupodidae, Achaemenothrombiidae, Podothrombiidae, Trombellidae, Neotrombidiidae, and Chyzeriidae.

Material and methods

Taxonomic sampling, sequencing, and alignment

This study expands a previously generated dataset (Pepato *et al.*, 2022) as follows: (1) includes a larger sampling of terrestrial Parasitengona with sequences of Chyzeriidae (Chyzeriinae 3 spp; Pteridopodinae, 1 sp.), Neotrombidiidae (2 spp), Achaemenothrombiidae (1 sp.), Trombellidae (2 spp), Neothrombiidae (1 sp.), Allotanaupodidae (1 sp.), Podothrombiidae (1 sp), Erythraeidae (2 spp.), Smarididae (1 sp.), and Calyptostomatidae (1 sp.) summing up 16 new terminals. (2) included six additional Hydrachnidia; (3) included four additional Halacaridae, one of which freshwater (*Lobohalacarus*). The complete dataset comprises 395 terminals. Similar to Pepato *et al.* (2022), we sequenced five genes, 18S (*nuclear small ribosomal subunit RNA gene*), 28S (*nuclear large ribosomal subunit RNA gene*), COI (*cytochrome c oxidase subunit I*), HSP70 (*Hsc70-5 heat shock protein cognate 5*), and SRP54 (*signal recognition particle protein 54 k*). See GenBank accession numbers and vouchering information in supplementary table I. Amplification and sequencing performed as in Pepato *et al.* (2022). Data about the 25 new taxa included in the present study is presented on table II.

Our dataset includes 61 Parasitengona specimens whose vouchering material is deposited at the Acarological collection of the *Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais* (UFMG AC). The geographic distribution of these samples is illustrated in figure 1. Geographic data from samples from other collections are scarce and were omitted.

Table II – Resumed data of the 25 new taxa included in this study. Blue indicates that the gene was successfully sequenced and included in the analysis. Red indicates the oposit.

Organism	**Country**	**Family**	**Voucher UFMGAC**	**18S**	**28S**	**SRP 54**	**HSP 70**	**COI**
Chyzeria	Chile	Chyzeriidae	170766					
Chyzeria	Australia	Chyzeriidae	200014					
Rhynchohydracarus	Brazil	Rhynchohydracaridae	220556					
Clathrosperchon sp	Brazil	Rhynchohydracaridae	220558					
Hydrodroma sp	Brazil	Hydrodromidae	221139					
Lohmannella	Indian Ocean	Halacaridae	221157					
Omartacarus	Brazil	Omartacaridae	220976					
Clathrosperchon sp2	Brazil	Rhynchohydracaridae	220979					
Neotrombidiidae sp	Brazil	Neotrombidiidae	210278					
Perumaropta sp	Brazil	Chyzeridae	210375					
Chyzeria	New Zeland	Chyzeridae	221528					
Achaemenothrombium khashayarshahi	Iran	Achaemenothrombiidae	221539					
Sperchontidae sp	Russia	Sperchontidae	221425					
Trombella	Brazil	Trombellidae	221519					
Rainbowia imperator	Australia	Erythraeidae	221524					
Fessonia	Iran	Smarididae	221551					
Neothrombiidae sp	Brazil	Neothrombiidae	221566					
Nothrotrombidium	Iran	Trombellidae	221558					
Lobohalacarus	Russia	Halacaridae	221390					
Bathyhalacarus	Indian Ocean	Halacaridae	230099					
Halacaridae sp	Brazil	Halacaridae	230014					
Allotanaupodus winksi	New Zeland	Allotanaupodidae	221478					
Abrolophus sp	Russia	Erythraeidae	221438					
Calyptostoma sp	Russia	Calyptostomatidae	221417					
Podothrombidiidae sp	Russia	Podothrombidiidae	221362					

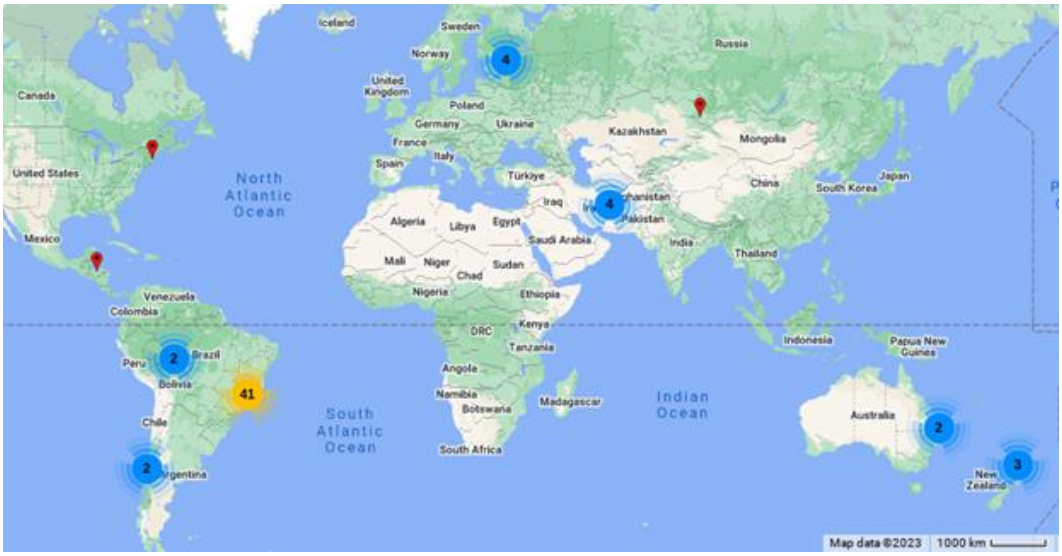

Figure 1. Geographic distribution of Parasitengona mites from the UFMG AC. Source: Google maps and Map Maker (maps.co).

Ribosomal genes were manually aligned using the secondary structure as guide, based on arthropod published structures (Gillespie *et al.*, 2006) and alignments, including our previously published alignments (Pepato & Klimov, 2015). The alignment was performed in the sequence editor BioEdit (Hall, 1999). Nuclear ribosomal RNA sequences were partitioned in loop (single stranded), stem (pairing) regions and regions of ambiguous alignment that were removed from downstream analysis.

Protein coding genes were checked for introns and aligned using the Muscle algorithm (Edgar, 2004) implemented in MEGA (10.2.5) after translated to aminoacids by EMBOSS Transeq tool (Rice *et al.*, 2000). Which genes were partitioned according to codon positions or analyzed as aminoacids. Introns were removed from downstream analysis.

Two different datasets were tested differing in the alignments of ribosomal nuclear genes, (1) one comprising all terminals employed in Pepato *et al.* (2022) plus newly obtained sequences and (2) other restricted to Anystidae + Halacaroidea + Parasitengona clade referred as AHP. Secondary structure alignments are preferred because secondary structure, due functional constraints, evolve at a slower pace than the nucleotide sequence (primary structure) (Kjer, 1995). Secondary structures, however, do evolve, making it useless as criterion for homology for regions where it was not conserved enough. Aligning sequences across an ancient clade such as Acariformes or Euartropoda, as in Pepato *et al.* (2022), necessarily leave out long stretches of sequence in final analyses, some of them may be aligned when just a more recent clade as AHP is considered. Both alignments are provided in the Supplementary material.

Model selection

Saturation tests were performed in DAMBE 6 (Xia, 2017) using the Xia's method (Xia *et al.*, 2003). Best-fitting models of nucleotide substitutions and best partitioning scheme were found in ModelFinder (Kalyaanamoorthy *et al.*, 2017) using the Bayesian Information Criterion (BIC) in IQ-Tree (Nguyen *et al.*, 2015). Four sets of analyses were run: (a) thirteen partitions: 18S Loop, 28S Loop, 18s Stem, 28s Stem, 1st, 2nd, and 3rd codon positions for HSP 70, SRP54 and COI for all 395 terminals, hereafter referred as "AllNucs" analyses; (b) Same initial partitioning but including only the terminals in the Anystidae+Parasitengona-Halacaroidea clade with 182 terminals, and hence the secondary structure alignments specific to them, referred as "AHPNucs"; (c) seven partitions: 18s Stem, and aminoacid sequences of each protein coding gene for all 395 terminals, referred as "AllNucs+AA"; (d) seven partitions, but restricted to the Anystidae+ Halacaroidea + Parasitengona Clade, the "AHPNucs+AA", analyzes.

Phylogenetic analyses, hypothesis testing and phylogenetic signal assessment.

Prior to phylogenetic inference, the Likelihood mapping (Strimmer & von Haeseler, 1997) implemented in IQ-Tree was employed to visualize the phylogenetic content of all datasets.

Additive trees were inferred using Maximum likelihood criterion in IQ-Tree (Nguyen *et al.*, 2015). The UltraFast Bootstrap (UFBoot) and SH-like approximate likelihood ratio test (SH-aLRT) were calculated in IQ-Tree with 1000 replicates. A clade with UFBoot > 95 % and SH-aLRT > 80 % is regarded as well supported (Guindon *et al.*, 2010). Throughout the text and figures, support values will be reported as SH-aLRT/UFBoot.

Genealogical concordance indices (proportion of sequence information that support a clade), gene concordance factor (gCF, percentage of "decisive" gene trees containing that branch) and site concordance factor (sCF, percentage of decisive alignment sites supporting a branch in the reference tree), were estimated in IQ-Tree 2.1.3 (Minh *et al.*, 2020). Sets of trees obtained by the resampling performed during UFBoot were used to graphically show the phylogenetic conflict using consensus networks (Holland & Moulton, 2003) in SplitsTree4 V4.19.1 (Huson & Bryant, 2006). Topologies constrained according to alternative phylogenetic hypotheses were compared by the Approximately Unbiased (AU) test (Shimodaira, 2002) in IQ-Tree.

Reconstruction of ancestral habitats

Ancestral character state estimation was performed in R using the function ace of the package Ape, with the option marginal = TRUE. Models with “Equal rates”, “Symmetrical rate matrix”, and “All rates different” were tested and the best fit model chosen according to the AIC score (Paradis and Schliep, 2019).

Results

Filtering data, model selection, and phylogenetic signal

Nucleotide partitions (rRNA loops and stems, COI, HSP70 and SRP54 codon positions) were tested for saturation. Among the tested partitions, the third position of the protein coding genes presented saturation and were excluded from downstream analyses (Iss > Iss.c: COI: Iss = 1; Iss.cSym = 0.79 ($P < 0.0001$); Iss.cAsym = 0.757 ($P < 0.0001$); HSP70: Iss = 0.865; Iss.cSym = 0.793 ($P = 0.002$); Iss.cAsym = 0.769 ($P < 0.0001$); SRP54: Iss = 0.874; Iss.cSym = 0.793 ($P = 0.0004$); Iss.cAsym = 0.759 ($P < 0.0001$)).

When analysing the whole dataset (AllNucs and AllNucs+AA) the homology between some regions of the ribosomal DNA alignment based on secondary structure couldn’t be established between Parasitengona and distant related taxa like *Apis melifera*. However, some of these regions can be considered and more of the variation observed become parsimony informative, when studying only the internal group AHP. As a result, the creation of AHPNucs and AHPNucs+AA datasets and the realignment of the ribosomal genes using secondary structure allowed us to add a total of 18 positions and recover an extra 267 rRNA positions as parsimony informative. Resulting in 34.4% rRNA positions being parsimony informative in AHPNucs (vs. 28.8% in the AllNucs aligments considering only the AHP taxa). Basic information on each alignment is supplementary table 2.

When considering the 395 terminals alignment, the best fit partitioned model was: (i) merged SSU and LSU loop and stems regions, model GTR+F+I+G4; (ii) merged 1st and 2nd codon positions of HSP70 and SRP54, model SYM+I+G4; (iii) merged 1st and 2nd codon positions of COI, model GTR+F+I+G4. For the Anystidae-Halacaroidea-Parasitengona clade, the best fit partitioning model included the same three partitions: (i) merged SSU and LSU loop and stems regions, model GTR+F+I+G4; (ii) merged 1st and 2nd codon positions of HSP70 and SRP54, model SYM+I+G4; (iii) merged 1st and 2nd codon positions of COI, model TIM2+F+I+G4.

The best fit partitioned model combining rRNA and amino acid considered three partitions: (i) merged SSU and LSU loop and stems regions, model GTR+F+I+G4; (ii) merged HSP70 and SRP54 aminoacid sequences, model LG+I+G4; (iii) COI aminoacid, model mtART+I+G4.

Accessing the phylogenetic signal: likelihood mapping and consensus networks

On figure 2 we present a visual representation of the phylogenetic signal on each dataset employing Likelihood Mapping. The proportion of resolved quartets was slightly lower in AllNucs dataset (91.1 %, Fig. 2A) than in AllNucs+AA dataset (92.2%, Fig. 2B). Similarly, when considering the AHPNucs and AHPNucs+AA, the former showed a lower percentage of resolved quartets (94% vs 94.9).

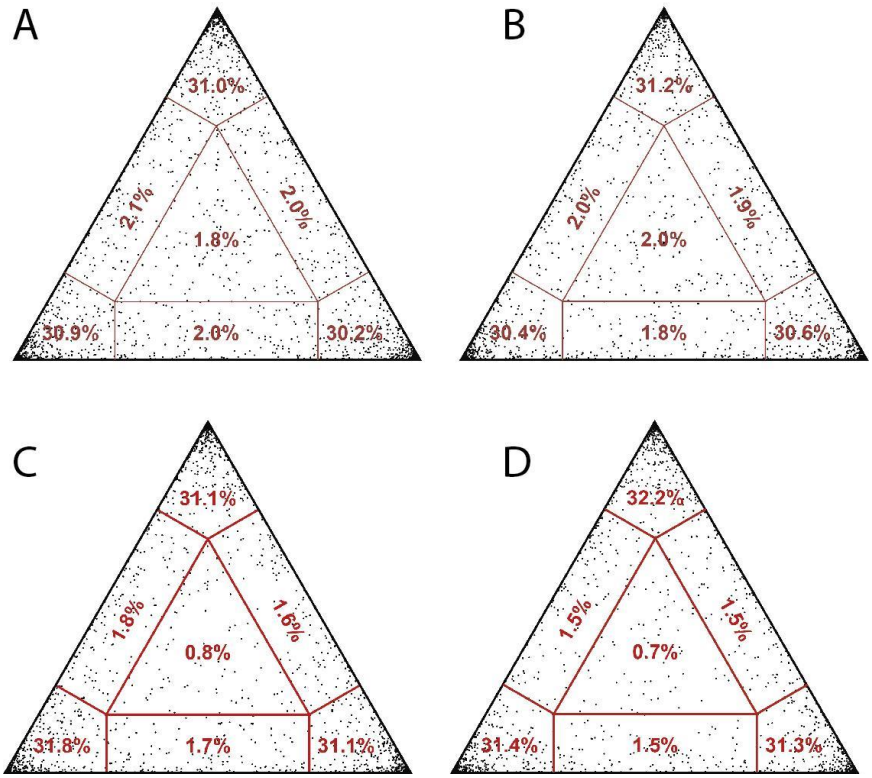

Figure 2. Likelihood mapping for the four datasets. A: AllNucs. B: AllNucs+AA. C: AHPNucs. D: AHPNucs+AA.

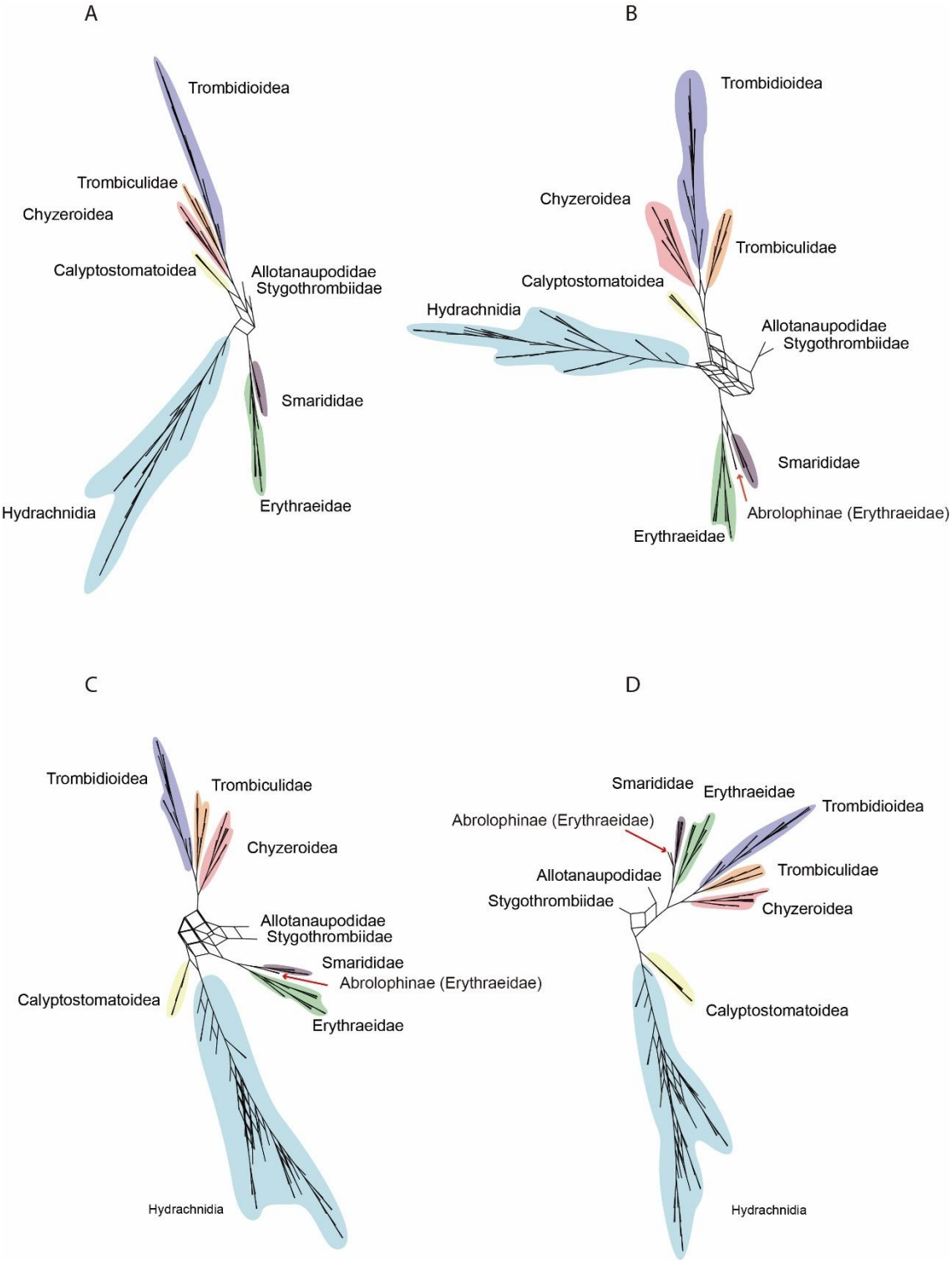

Figure 3. Consensus networks. Each consensus network was calculated from 1000 bootstrap trees (with bipartitions with frequency under 0.1 excluded). Taxon color codes match those on Fig. 4. A: Allnucs dataset. B: Allnucs+AA. C: AHPNucs. D: AHPNucs+AA.

Figure 3 presents the consensus networks of ultrafast bootstrap trees obtained during the maximum likelihood analysis of each dataset. In this figure it is possible to visualize and differentiate well resolved regions of our phylogenetic analysis (Ultrafast support > 90%) from regions where alternative phylogenetic hypotheses were recovered in more than 10% of the bootstrap trees. In these regions, instead of bipartions, a network of alternative hypotheses is illustrated. The most relevant regions of conflict are in the relationships between Stygothrombiidae and Allotanaupodidae. Calyptostomatoidea and Abrolophinae (Erythraeidae) being recovered as a sister group of Smarididae (Fig. 3).

Terrestrial Parasitengona phylogeny

We chose the tree inferred based on the AHPNucs+AA dataset as our best tree. This decision was made due to the higher percentage of resolved quartiles (94.9%, Fig. 2), higher percentage of parsimony informative positions in the ribosomal genes alignment (34.4% vs. 28.8% in AllNucs and AllNucs+AA) and the presence of relatively less conflict in the consensus network (Fig. 3).

A blue and red matrix was added to each tree node where conflict between different datasets were noticed, indicating for which dataset that node is recovered. The complete trees inferred based on AHPNucs+AA (Fig. 4), Allnucs, Allnucs+AA and AHPNucs are presented in the supplementary trees 1, 2, 3, 4, respectively.

Our phylogeny recovered Stygothrombiidae as the most basal Parasitengona, followed by a clade containing Hydrachnidia and Calyptostomatoidea. This clade was recovered as a sister group of the remaining terrestrial Parasitengona for which Allotanaupodoidea was recovered as the most basal lineage. Two large lineages are recovered next (Fig. 4).

The first Erythraeoidea with Erythraeidae and Smarididae as sister groups. Despite a low support, we recovered the two subfamilies of Smaridinae (Smaridinae and Histriosomatinae) as independent lineages in the main tree, but not when considering the AHPNucs dataset where *Fessonia* was recovered as a sister group of *Trichosmaris*. Abrolophinae was recovered as the sister group of Smarididae rendering Smarididae paraphyletic, however this was not observed in the datasets including distant outgroups (AllNucs and AllNucs+AA), which indicates this may be an artefact. We also recovered Erythraeinae as sister group of Leptinae and Callidosomatinae as sister group of Balaustinae (Fig. 4).

The second included three superfamilies. The most basal is a clade containing Johnstonianidae, Chyzeridae, Trombellidae and Neothrombidiidae. We refer to this clade as a superfamily Chyzeroidea expanding Welbourn (1991)'s diagnosis. Within Chyzeroidea, Johnstonianidae was recovered as the most basal lineage. Followed by Chyzeridae, at last, Trombellidae and Neothrombidiidae as sister groups. Chyzeroidea was recovered in all analyses and all internal nodes were observed in all datasets (Fig. 4). Trombiculidae (after Kudryashova 1998) was recovered as an independent lineage with Leewenhoekiinae and Trombiculinae and sister groups. The frog's parasite *Hannemania* was recovered as the most basal Leewenhoekiinae in all datasets. We redefined Trombidioidea as the members of the next clade including Thrombidiidae and Podothrombiidae as sister groups, followed by Achaemenothrombiidae, Neothrombiidae and Microtrombidiidae. Only differing from Welbourn 1991 by the addition of Achaemenothrombiidae. All families were recovered as independent lineages and the phylogenetic relationships between them are relatively well resolved, with all nodes being recovered in all datasets. Except by the relationships within Microthrombidiidae, that were not well resolved precluding us from tagging subfamilies other than Eutrombidiinae. Other phylogenetic relationships as illustrated on figure 4.

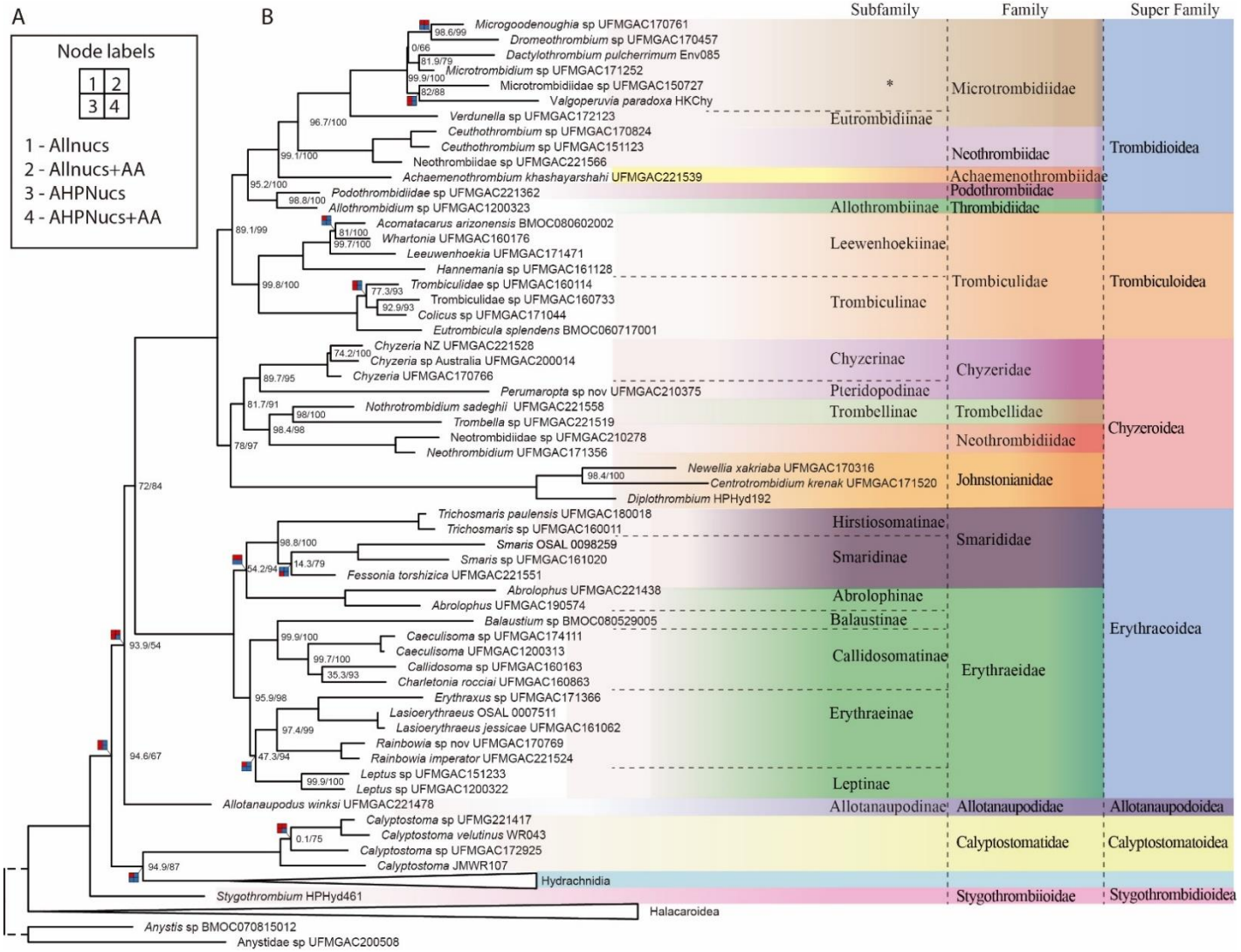

Figure 4. Maximum likelihood tree highlighting terrestrial Parasitengona. Node's matrix indicates whether it is recovered (blue) in other datasets or not (red) and is omitted where there is no conflict between datasets. A: Matrix scheme. B: Topology inferred from the AHPnucs+AA dataset. Branch support numbers are SH-aLRT / ultrafast bootstrap and are omitted when 100% for both. Dashed line indicates pruned branch length to fit the image.

Hypothesis testing using AU statistics

Trombiculoidea was proposed by Welbourn (1991) as a large superfamily including Johnstonianidae, Trombiculidae, Leeuwenhoekiidae, Neotrombidiidae, Trombellidae, and Audyanidae. The group was created due to the presence of solenidium on femur legs I, II and/or III of their larvae. We tested the possibility of recovering this superfamily as a clade using AU statistics and managed to discard this hypothesis when analyzing the Allnucs and AHPNucs datasets (Table III).

Allotanaupodidae was originally described by Zhang & Fan (2007) as a basal lineage, a claim that can be further supported by our observation of larvae that are remarkably similar to the adults, which could reflect the plesiomorphic condition of Acariform mites. We tested the possibility of Allotanaupodidae being the sister group of all remaining Parasitengona and we managed to discard this hypothesis when considering the AHPNucs dataset. A series of other hypotheses tested employing AU statistics were inconclusive and were omitted from this study for the sake of clarity.

Table III – Hypotheses testing using AU statistics.

Hypotheses	All Nucs		All Nucs +AA		AHP Nucs		AHP Nucs+AA	
Allotanaupodoidea + Other Parasitengona	19.10	0.313	**18.72**	**0.036**	**24.97**	**0.027**	**35.15**	**0.010**
Trombiculoidea (Welbourn 1991)	**77.64**	**0.002**	**69.82**	**2.11e-05**	**80.47**	**0.005**	**86.82**	**0.0002**

Reconstruction of ancestral habitats

Reconstruction of ancestral habitats is depicted in Fig. 5, inferred for the AHP Nucs+AA phylogeny. The AIC led to choose Symmetrical Rate model for character evolution for transitions among realms (ER lnL = -47.79, AIC = 97.59; SYM lnL = -43.89, AIC = 93.77; ARD lnL = -42.19, AIC =96.39).

Our results support the hypothesis that the most recent common ancestor of Halacaroidea and Parasitengona was an aquatic mite, probably from fresh water (Fig. 5).

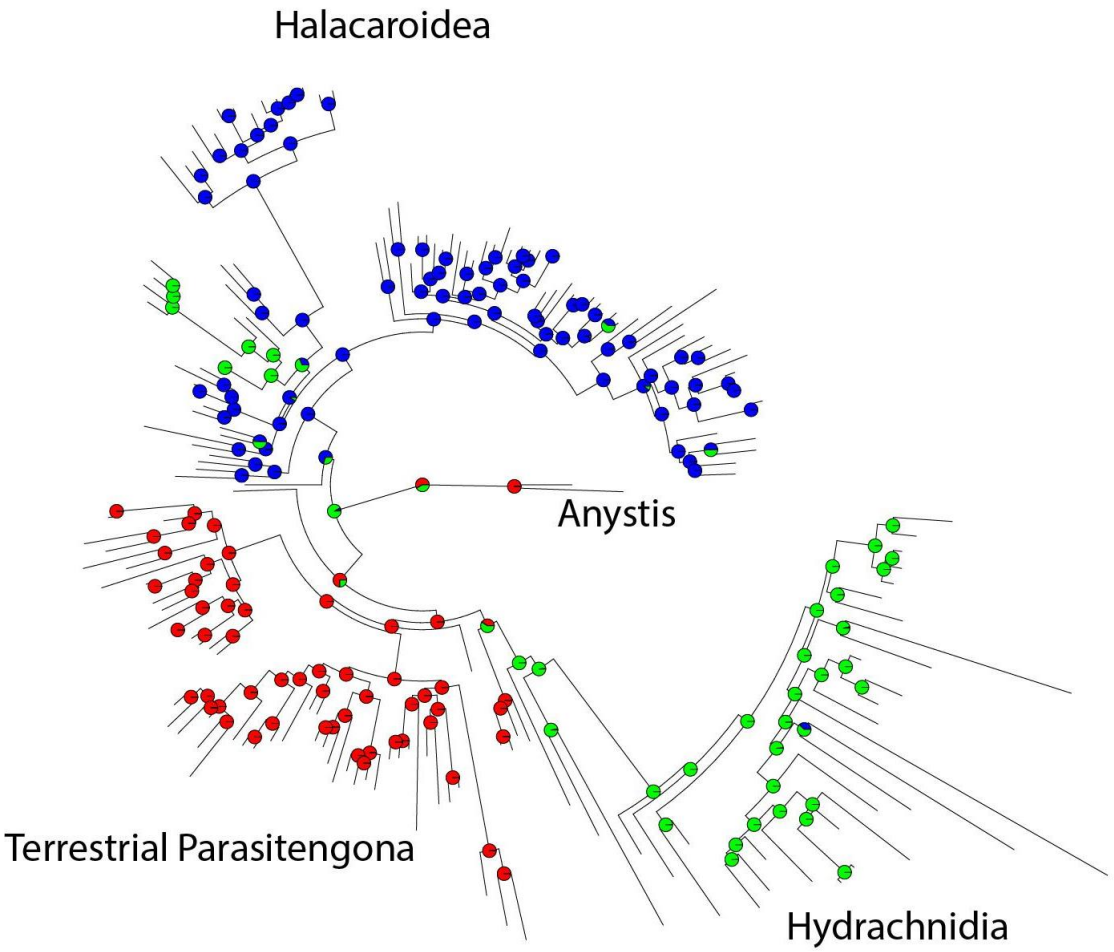

Figure 5. Ancestral state reconstruction inferred over the AHP Nucs+AA using a symmetrical rate model. Note the high likelihood for a freshwater common ancestor between Halacaroidea and Parasitengona.

Systematics

New classification proposal

We suggest reducing Welbourn (1991)'s Trombiculoidea definition to Trombiculidae. Once this is the only independent lineage left from the originally proposed paraphyletic group. Including five subfamilies Leeuwenhoekiinae, Apoloniinae, Trombiculinae and Gahrliepiinae in accordance with Kudryashova (1998) and Shatrov & Kudryashova (2008).

Chyzeroidea was proposed by Welbourn (1991) and included only Chyzeridae. In the present study Chyzeridae was recovered nested in a clade including Trombellidae, Johnstonianidae and Neothrombidiidae. To properly name this clade, we propose an expansion of Chyzeroidea definition to fit all the 4 families.

Chyzeroidea

Below we propose a new diagnosis for Chyzeroidea, expanding Welbourn (1991)'s definition to include Johnstonianidae, Chyzeridae, Trombellidae and Neothrombidiidae.

Diagnosis.

Post larval: Terrestrial or semi-aquatic mites. One or two pairs of sensilla present. Moveable cheliceral digit blade-like, short, not stylet-like and retractable. Pregenital tubercle absent. Palp Femur and genu separated. Dorsal side of palp tibia lacking ctenidium, when present, modified spine-like setae do not form dorsal combs. Pygosomal sclerite absent. Dorsal setae either trifurcate or simple smooth or barbed, but not modified distally expanded. Tarsus of leg I lack a distodorsal tubercle. Palp tibia lacking 2 or 3 distinct spines in tandem on the same side of the tibia.

Larvae: Ânus and claparade organ present. Prodorsal sclerite bearing one or two pairs of trichobothria. Scutellum absent. Palp genu with one pair of setae. Femur of leg I, II and III with solenidia, otherwise anal sclerite present and cheliceral blades with dozens of small teeth.

Based on our results, Welbourn (1991) and Zhang & Fan (2007), we propose a new classification for terrestrial Parasitengona superfamilies (Table IV). Keeping the originally proposed superfamilies that were not included yet in phylogenetic studies until further evidence allows us to review it.

Table IV – Superfamilies of terrestrial Parasitengona.

ALLOTANAUPODOIDEA

Allotanaupodidae f.

AMPHOTHROMBIDIOIDEA

Amphothrombiidae f.

CALYPTOSTOMATOIDEA

Calyptostomatidae f.

ERYTHRAEOIDEA

Erythraeidae f.

Smarididae f.

STYGOTHROMBIDIOIDEA

Stygothrombidiidae f.

CHYZEROIDEA s. f. nov.

Chyzeridae f.

Johnstonianidae f.

Neothrombidiidae f.

Trombellidae f.

TROMBICULOIDEA

Trombiculidae f.

Apoloniinae sf.

Gahrliepiinae sf.

Leeuwenhoekiinae sf.

Trombiculinae sf.

TROMBIDIOIDEA

Achaemenothrombiidae f.

Microtrombidiidae f.

Podothrombiidae f.

Thrombidiidae f.

YUREBILLOIDEA

Yurebillidae f.

Discussion

Similar to Pepato et al (2022) study, our data support the hypothesis of a freshwater ancestor for the Halacaroidea-Parasitegona clade, followed by two independent invasions of the terrestrial helm. The first happening in Calyptostomatoidea ancestors and the second in the common ancestor of Allotanaupodidae and the remaining terrestrial Parasitengona that includes Erythraeoidea and Trombidiina sensu Welbourn (1991). Notably many Trombidiina retain a sami-aquatic lifestyle, either living under humid soil or totally dependent on a hydric environment to survive like Johnstonianidae (Wohltmann, 2000). Illustrating a gradual transition to the terrestrial helm. While Erythraeidae have lineages highly adapted to xeric environments (Wohltmann, 2000).

Calyptostomatoidea was considered a sister group of Erythraeidae due to its retractable gnathosoma and stylet-like chelicerae (Witte 1998, Söller 2001, Welbourn 1991). While older studies suggested that this trait was homoplastic and considered Calyptostoma as closelly related to Johnstonianidae (Robaux 1974, Witte 1984).

Our phylogenetic analysis recovered Calyptostoma as the sister group of Hydrachnidia, while Stygothrombidioidea was recovered as the most basal Parasitengona. Notably, an extensible gnathosoma with retention of the motile cheliceral digit is also observed in Stygothrombidioidea (See Li *et al*. 2021, figure 1 and 2). Suggesting that this condition may be the plesiomorphic state for Parasitengona, retained in Calyptostomatoidea, Stygothrombidioidea and some water mites.

Our analyses reject Allotanaupodidae as the sister-group of all Parasitengona (Table IV). Instead, it was recovered as a basal lineage, sister group of Trombiina + Erythraeoidea (Fig. 4). The non-hetermorphic larva of Allotanaupodidae, unique among Parasitengona, suggests that a heteromorphic larva observed in other taxa may consist in a homoplasy, a trait that evolved more than once within Parasitengona. Which could be a consequence of the larva's parasitic lifestyle. Or that it consists in a reversion of the Parasitengona condition of bearing a heteromorphic larva.

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Supplementary material

Supplementary Tree 1. Maximum likelihood tree inferred from AHPNucs_AA dataset.

Supplementary Tree 2. Maximum likelihood tree inferred from AllNucs dataset.

Supplementary Tree 3. Maximum likelihood tree inferred from AllNucs_AA dataset.

Supplementary Tree 4. Maximum likelihood tree inferred from AllNucs_AA dataset.

Supplementary Table 1. Gen Bank access numbers.

Supplementary Table 2. Summary of alignment features, including length, variable and parsimony informative sites and nucleotide composition after removing regions of ambiguous alignment.

Supplementary File 1. AllNucs 28S rRNA secondary structure alignment.

Supplementary File 2. AllNucs 18S rRNA secondary structure alignment.

Supplementary File 3. AHP 28S rRNA secondary structure alignment.

Supplementary File 4. AHP 18S rRNA secondary structure alignment.

Supplementary File 5. Cytochrome Oxidase subunit I alignment.

Supplementary File 6. Heat Shock Protein 70 alignment.

Supplementary File 7. Signal Recognition Particle 54 alignment.

Supplementary tables and trees are available in the link bellow.

https://drive.google.com/drive/folders/1EHkYj1ge_OLPXo4mqQepRTR7Dhs67mlF?usp=sharing

Supplementary files 1 to 7 will be published together with the paper after peer reviewing.

3 – Conclusão Geral

3.1 – Classificação

Nesta tese foram descritas sete espécies novas pertencentes às famílias Chyzeriidae, Erythraeidae, Johnstonianidae e Smarididae. Sendo seis espécies recentes e uma fóssil do Eoceno. Além disso, foi apresentada a descrição inédita da fase larval de Allotanaupodidae. Dos oito táxons aqui abordados, seis foram incluídos como terminais na análise filogenética apresentada no capítulo 2. Foram também incluídas nessa análise filogenética espécies descritas ou redescritas durante meu mestrado que foram publicadas durante o início do doutorado. Essas espécies são *Charletonia rocciai* Treat & Flechtmann, 1979; *Lasioerythraeus jessicae* Costa, Klompen, Bernardi, Gonçalves, Ribeiro & Pepato 2019; *Leptus maldonadoicus* Haitlinger, 2000 e *Trichosmaris paulensis* Costa, Welbourn, Klimov & Pepato 2021. A classificação de cada uma delas é listada na tabela I.

Tabela I – Classificação dos táxons na tese.

ALLOTANAUPODOIDEA Zhang & Fan 2007

Allotanaupodidae Zhang & Fan 2007

Allotanaupodus Zhang & Fan, 2007

Allotanaupodus winksi Zhang & Fan, 2007 (capítulo 1.4.1)

ERYTHRAEOIDEA Grandjean, 1947

Erythraeidae Robineau-Desvoidy, 1828

Erythraeinae Robineau-Desvoidy, 1828

Erythraeinae gen et. sp. nov. (fóssil, capítulo 1.2.1)

Rainbowia Southcott, 1961

Rainbowia sp. nov. (capítulo 1.2.2).

Smarididae Kramer, 1878

Hirstiosomatinae Southcott, 1946

Trichosmaris Southcott, 1963

Trichosmaris sp. nov. (capítulo 1.1.2).

Smaridinae Vitzthum, 1929

Smaris Latreille, 1796

Smaris hajiqanbari Costa, Gomes-Almeida & Pepato, 2022

CHYZEROIDEA

Johnstonianidae Thor, 1935

Johnstonianinae Thor, 1935

Centrotrombidium krenak Costa, Klimov & Pepato, 2023

Newellia xakriaba Costa, Klimov & Pepato, 2023

Chyzeridae Womersley, 1954

Pteridopodinae Southcott, 1987

Perumaropta Haitlinger, 1999

Perumaropta sp. nov. (capítulo 1.3.1).

3.2 – O monofiletismo de Erythraeidae em relação a Smarididae.

Erythraeidae e Smarididae foram recuperados como grupos irmãos e linhagens independentes em nossa análise considerando os conjuntos de dados completos AllNucs e AllNucs+AA, apesar do suporte moderado. Devido ao baixo suporte, não foi possível descartar a hipótese de que Smarididae seja uma linhagem derivada dentro de Erythraeidae utilizando testes mais rigorosos como o teste estatístico AU. Como demonstrado na figura 3, do capítulo 2 o conflito filogenético em relação ao monofiletismo de Smarididae está relacionado à posição de Abrolophinae Witte, 1995. Uma linhagem basal de Erythraeidae que é encontrada como grupo irmão de Smaridiidae em algumas árvores subótimas e nos alinhamentos que carecem de grupos externos AHPNucs e AHPNucs+AA. O que pode ser causado por atração de ramos longos. Uma maior amostragem de Abrolophinae e Smarididae pode clarificar essa questão. Entretanto, podemos concluir que nada nos leva a crer que Smaridade e Erythraeidae não sejam monofiléticos, uma vez que ambas as linhagens são bem suportadas por caracteres morfológicos (Costa et al. 2021).

3.3 – Allotanaupodidae

A hipótese de que Allotanaupodidae seria a linhagem mais basal entre os Parasitengona e que a semelhança da larva com os adultos refletiria o estado plesiomórfico dos Acariformes pode ser descartada considerando o conjunto de dados AHPNucs utilizando o rigoroso teste estatístico AU. O que sugere que possuir uma larva não heteromórfica consiste em uma reversão do estado observado nos demais Parasitengona. Ou seja, uma homoplasia compartilhada entre os Allotanaupodidae e os Acariformes não Parasitengona.

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