UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Departamento de Zoologia
Programa de Pós-Graduação em Zoologia

Brenda Karolina Gomes Almeida

TAXONOMIA INTEGRATIVA DE ÁCAROS ECTOPARASITAS DE MORCEGOS EM CAVERNAS BRASILEIRAS (ACARI: MACRONYSSIDAE, SPINTURNICIDAE E LEEUWENHOEKIIDAE)

Belo Horizonte
2023

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Orientador: Prof. Dr. Almir Rogério Pepato

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Taxonomia integrativa de ácaros ectoparasitas de morcegos em cavernas brasileiras (Acari: Macronyssidae, Spinturnicidae, Leeuwenhoekiidae)

BRENDA KAROLINA GOMES ALMEIDA

Esta tese foi apresentada em sessão pública e submetida a avaliação em 28 de agosto de 2023, sendo aprovada pela Banca Examinadora composta pelos seguintes membros:

Prof. Dr. Adalberto José dos Santos (Membro / UFMG)

Prof. Dr. Almir Rogério Pepato (Orientador / UFMG)

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Prof. Dr. Juan Bibiano Morales-Malacara (Membro / UNAM)

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UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DO CIÊNCIAS BIOLÓGICAS
PÓS-GRADUAÇÃO EM ZOOLOGIA

ATA DE DEFESA DE TESE

BRENDA KAROLINA GOMES ALMEIDA

Ao vigésimo oitavo dia do mês de agosto do ano de dois mil e vinte e três, às treze horas, realizou-se, por webconferência, a defesa de Doutorado da Pós-Graduação em Zoologia, de autoria da Doutoranda **Brenda Karolina Gomes Almeida** intitulada: "Taxonomia integrativa de ácaros ectoparasitas de morcegos em cavernas brasileiras (Acari: Macronyssidae, Spinturnicidae, Leeuwenhoekiidae)". Abrindo a sessão, o Presidente da Banca, Prof. Dr. Almir Rogério Pepato, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra para a discente para apresentação de seu trabalho. Esteve presente a Banca Examinadora composta pelos membros: Adalberto José dos Santos, Fernando Araújo Perini, Juan Bibiano Morales-Malacara, Gustavo Graciolli, e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da discente. Após a argüição, apenas a Banca Examinadora permaneceu na sala para avaliação e deliberação acerca do resultado final, a saber: o trabalho foi APROVADO SEM ALTERAÇÕES

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A minha filha, mãe, pai, irmão e irmã pelos
momentos perdidos em família devido à
distância geográfica que nos separou.
Ademais, a todos os meus familiares e amigos
que se foram, vítimas da pandemia Covid-2019.
Dedico!

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"[...] Se olhe, se valorize
E se permita errar
Se dê de presente a chance
De pelo menos tentar
Se o SE for bem usado,
O impossível sonhado
Pode se realizar."
(Bráulio Bessa – Se)

RESUMO

Os morcegos são cruciais para o fluxo de matéria e energia dentro de cavernas, pois a ausência de produção primária por meio da fotossíntese torna o guano (fezes) produzido por esses animais fundamentais para a manutenção dos organismos em muitas cavidades. Consequentemente, os parasitas a eles associados são de especial relevância, incluindo alguns ácaros ectoparasitas como os das famílias Spinturnicidae e Macronyssidae (Ordem Mesostigmata), e larvas do gênero *Whartonia* (Leeuwenhoekiidae, Trombidiformes). Parte desses ácaros vivem durante todo o ciclo de vida sobre os morcegos (Spinturnicidae), enquanto outros passam parte do seu ciclo de vida no assoalho das cavernas, sendo importantes componentes dessas comunidades (alguns Macronyssidae e *Whartonia*). No Brasil, foram registradas 67 espécies de ácaros associados a morcegos, distribuídas em 10 famílias, o que é uma pequena fração da diversidade esperada se comparada a outras regiões, mesmo com uma diversidade menor de hospedeiros. Esta tese teve como objetivo contribuir para o conhecimento taxonômico dos ácaros ectoparasitas de morcegos em cavernas no Brasil. Foram analisados espécimes de ácaros ectoparasitas de morcegos cavernícolas depositados na coleção Acarológica do Centro de Coleções Taxonômicas da UFMG e provenientes de novas coletas, compreendendo cavidades de Minas Gerais, Pará e Rio de Janeiro. Para tal, foi utilizada uma abordagem integrativa, compreendendo dados moleculares, morfológicos e ecológicos. A morfologia foi descrita usando técnicas microscópicas e fotográficas tradicionais, de acordo com as práticas adotadas na literatura taxonômica dos diferentes grupos abordados. O DNA dos ácaros foi extraído e foram sequenciados marcadores moleculares como os gene 16S, COI e 28S de acordo com a disponibilidade de marcadores em bases públicas de dados como o *GenBank* e o *BOLD System V4*. Os marcadores moleculares foram utilizados para inferência filogenética ou técnicas de delimitação de espécies como o *bGMYC* e o *Stacey*. Os dados de zonação nas cavernas também foram disponibilizados quando possível. Como resultados, foi descrito um novo gênero e nova espécie de Macronyssidae, *Chiasmanyssus cavernicola* Gomes-Almeida & Pepato, 2021, baseado em exemplares encontrados em cavernas de Minas Gerais, Pará e Rio de Janeiro, bem como todos os estágios ativos. Foram descritos os estágios pós-larvas de duas espécies de *Whartonia*, *W. pachywhartoni* and *W. nudosetosa*, antes conhecidas apenas por larvas, utilizando marcadores moleculares para associação dos estágios heteromórficos. Finalmente foi criado um banco de dados de DNA Barcoding para a identificação das espécies de *Periglischrus* (Spinturnicidae) encontradas em cavernas de Minas Gerais, publicado no BOLD System V4, juntamente com sequências COI e a fotodocumentação dos exemplares testemunho. Os dados moleculares obtidos permitiram concluir que *W. pachywhartoni* tem maior diferenciação genética e parece ter a sua estruturação genética influenciada pela distribuição dos ambientes subterrâneos onde seus estágios pós-larvas habitam. Já *P. iheringi* tem menor diferenciação genética, aparentemente mais intimamente ligada aos seus hospedeiros morcegos.

Palavras-chave: *Chiasmanyssus cavernicola*; *Whartonia; Periglischrus;* novas espécies; associação de estágios pós-larvas; banco de dados de DNA Barcoding.

ABSTRACT

Bats are crucial for the flow of matter and energy within caves, as the absence of primary production through photosynthesis makes the guano produced by these animals essential for the maintenance of organisms in many cavities. Consequently, parasites associated with them are of particular relevance, including ectoparasitic mites such as Spinturnicidae and Macronyssidae (Order Mesostigmata), and larvae of the genus *Whartonia* (Leeuwenhoekiidae, Trombidiformes). Some of these mites live their entire life cycle on bats (Spinturnicidae), while others spend part of their life cycle on cave soil being important components of these communities (some Macronyssidae and *Whartonia*). In Brazil, 67 species of mites associated with bats have been recorded, distributed among 10 families, which is a small fraction of the expected diversity compared to other regions, even with a lower diversity of hosts. The objective of this thesis is to contribute to the taxonomic knowledge of ectoparasitic mites of bats in Brazilian caves. Specimens of ectoparasitic mites from cave-dwelling bats deposited in the Acarological Collection of the Taxonomic Collections Center of UFMG and from new collections, including cavities in Minas Gerais, Pará and Rio de Janeiro, were analyzed. An integrative approach was used, including molecular, morphology, and ecology data. Morphology is described using traditional microscopic and photographic techniques, following practices employed in taxonomic literature for the different groups studied. The mite DNA has been extracted and sequenced using molecular markers, such as 16S, COI, and 28S genes, according to the marker availability in public databases, including GenBank and BOLD System V4. Molecular markers were used for phylogenetic inference or species delimitation techniques, bGMYC and Stacey. Data on cave zonation was provided whenever possible. As result, a new genus and species of Macronyssidae, *Chiasmanyssus cavernicola* Gomes-Almeida & Pepato, 2021 was described based on specimens from caves in Minas Gerais, Pará and Rio de Janeiro, including all active stages. The post-larval stages of two *Whartonia* species, *W. pachywhartoni* and *W. nudosetosa*, previously known only as larvae, are described using molecular markers for heteromorphic stages association. Finally, a DNA Barcoding database is created for identification of *Periglischrus* species (Spinturnicidae) found from caves in Minas Gerais, which is published in BOLD System V4, along with COI sequences and photodocumentation of voucher specimens. The molecular data obtained allowed concluding that *W. pachywhartoni* has greater genetic differentiation and appears to have its genetic structure influenced by the distribution of underground environments where its post-larval stages inhabit. On the other hand, *P. iheringi* shows lower genetic differentiation, seemingly more closely linked to its bat hosts.

Keywords: *Chiasmanyssus cavernicola*; *Whartonia*; *Periglischrus*; new species; association of post-larval stages; DNA Barcoding database.

SUMÁRIO

1 INTRODUÇÃO GERAL

1.1 As cavernas

As cavernas, tocas, lapas ou grutas são espaços naturais subterrâneos em rochas que se formam ao longo do tempo devido a processos dependentes da litologia do terreno. No caso de rochas calcárias, por exemplo, a ação química e da água superficial com ácido carbônico que penetra no subsolo dissolve a rocha (Culver & Pipan 2019). O desenvolvimento das cavernas pode levar a outras formações como lapiás, dolinas, sumidouros e surgências que estão interconectadas no subsolo pelas diaclases e outros espaços menores da matriz rochosa (Auler & Zogbi 2005).

Isso forma um sistema único e complexo que se estende muito além do entorno da caverna (Auler & Piló 2019), ainda que as cavernas em si compartilhem algumas características peculiares em relação ao meio epígeo (superfície) como ausência permanente ou parcial de luz, escassez alimentar, alta umidade e temperatura praticamente constante (Culver 1982).

Algumas cavernas são tão profundas que um gradiente de luz, de nutrientes e condições climáticas podem ser encontrados criando zonas ambientais distintas em relação a influência da superfície: zona de entrada, zona de penumbra, zona de transição ou afótica e zona profunda (Mammola 2019). Quanto mais profundo, a luz e os nutrientes vão diminuindo e a estabilidade climática aumenta, sendo as zonas profundas menos afetadas pela sazonalidade e ciclos diários do que a zona de entrada e penumbra.

Tais condições ambientais dificultam o estabelecimento de uma alta diversidade e abundância de espécies, mas, por outro lado, as especializações subterrâneas para viver nesses ambientes aumentam em resposta à pressão seletiva que é imposta neste meio. Paralelamente, a própria litologia influencia a ocupação por diferentes organismos, pois o tipo de rocha é responsável por diferenças estruturais como a dimensão (tamanho), nível de compactação, disponibilidade de espaços internos e conectividade da caverna com ambientes externos (Culver & Pipan 2019).

A seleção natural para as condições do ambiente subterrâneo leva a especializações morfológicas conhecidas como troglomorfismos (Romero 2009), além de adaptações fisiológicas e comportamentais. Como veremos, no entanto, o desconhecimento da biologia, distribuição e relações filogenéticas de muitas espécies cavernícolas, dificulta a compreensão da sua relação com o ambiente cavernícola.

No Brasil, a maior parte das cavidades subterrâneas são encontradas em áreas de rochas carbonáticas, como calcários, dolomitos e mármores mas, devido a fatores geológicos, geomorfológicos e climáticos, elas também ocorrem em arenito, quartzito, formações ferríferas, cangas, em granito, gnaisse, rochas metamórficas variadas, como micaxistos e filitos, além de coberturas de solos (Piló & Auler 2019).

Até o momento, 21.838 cavidades subterrâneas brasileiras já foram registradas no Cadastro Nacional de Informações Espeleológicas (Canie) (Cecav 2021). Embora esse número seja alto, ele representa apenas 7% das 310.000 cavernas estimadas no país (Piló & Auler 2013). Os estados de Minas Gerais e Pará têm maior ocorrência de cavernas, 9.960 (45,61%) e 2.835 (12,98%), respectivamente (Cecav 2021).

1.2 Os morcegos

Os morcegos, além de desempenhar importantes serviços ecológicos na superfície, como polinizadores, dispersores de sementes e predadores de vertebrados e invertebrados, são essenciais para a manutenção dos ecossistemas cavernícolas, onde são os principais responsáveis pela entrada de matéria orgânica por meio do guano (fezes), que sustenta a vida de comunidades complexas de invertebrados (Gnaspini & Trajano 2000; Ferreira 2019) e outros organismos que habitam estes ambientes.

Embora também sejam capazes de se abrigar em estruturas naturais no ambiente epígeo como árvores ocas, folhas, pedrais etc. e artificiais, como construções humanas, as cavernas são abrigos ideais para os morcegos, pois são ambientes termicamente estáveis e úmidos que os protegem do clima e dos predadores, sendo assim usadas para interações sociais, procriação, cuidado da prole e como hibernáculo (Kunz 1982), permitindo a manutenção de colônias com poucos ou milhares de indivíduos.

Atualmente, os morcegos podem ser classificados como: *essencialmente cavernícolas* – espécies que se abrigam majoritariamente em caverna; *cavernícola oportunista* – espécies que usam cavernas de forma oportuna, mas rotineiramente utilizam outro tipo de abrigo; e *não cavernícolas* – espécies não registradas em cavernas ou que tenham preferência por outros abrigos (incluindo espécies amostradas em apenas uma caverna) (Guimarães & Ferreira 2014).

No Brasil, foram registradas 182 espécies de morcegos, distribuídas em 68 gêneros e nove famílias (Garbino et al. 2020; Garbino et al. 2022). Dessas, cerca de 44% das espécies foram registradas usando cavernas como abrigo principal ou alternativo (Pereira et al. 2022). Sabe-se que essa seleção de cavernas pelos morcegos ocorre principalmente as condições ambientais estáveis no interior das cavernas, mas também pela disponibilidade de recursos na paisagem do entorno ou requerimento espécie-específico ou interespecífico (Kunz 1982; Barros et al. 2020; Vargas-Mena et al. 2020).

Além dos morcegos serem essenciais para os seus ecossistemas, eles abrigam uma enorme variedade de ectoparasitas, distribuídos nas ordens Ixodida, Trombidiformes, Sarcoptiformes, Mesostigmata, Dermaptera, Diptera, Hemiptera e Siphonaptera (Whitaker et al. 2009), que podem ser encontrados fixos ou movendo-se nas asas, pelos ou orelhas, alimentando-se de sangue ou do tecido epidérmico. Os clados de ectoparasitas podem ser exclusivos de morcegos ou não e variam desde espécies-específicos, associados a um conjunto limitado de espécies de morcegos relacionadas, até espécies bastante generalistas (Dick 2007; Dick et al. 2016; Rudnick 1960; Radovsky 2010; Whitaker et al. 2009).

1.3 Subclasse Acari e os ácaros ectoparasitas de morcegos

Dentre os ectoparasitas de morcegos, os ácaros (que inclui os carrapatos) pertencem à subclasse Acari, um grupo megadiverso de aracnídeos que inclui mais de 58.000 espécies descritas (Zhang 2011; Liu et al. 2013). Os ácaros são divididos em duas linhagens principais, Acariformes e Parasitiformes. Dunlop & Alberti (2008) revisaram os caracteres morfológicos que unem os ácaros e separam as duas linhagens, demonstrando a fragilidade das evidências morfológicas que tradicionalmente as reúnem em Acari. As filogenias moleculares apresentam resultados conflitantes com estudos recuperando Acari como monofilético (Lozano-Fernandez et al. 2019) ou difilético (Dabert et al. 2010; Pepato et al. 2010; Pepato e Klimov 2015; Arribas et al. 2020; Ontano et al. 2021; Ballesteros et al. 2022).

Os ácaros apresentam enorme diversidade morfológica, de hábitats e modos de vida, incluindo linhagens que remontam ao paleozóico (ver Pepato et al. 2022 para os Acariformes). Esses pequenos animais são encontrados em todos os ambientes aquáticos e terrestres do planeta, seja em vida livre (como predadores, fitófagos, consumidores de depósitos orgânicos e associados a diversos animais como comensais, ou para dispersão) e endo ou ectoparasitas de vertebrados e invertebrados (Krantz & Walter 2009; Walter & Proctor 2013).

Entre os ácaros ectoparasitas de vertebrados, aqueles que parasitam os morcegos incluem representantes de quatro ordens da subclasse Acari: Ixodida e Mesostigmata (Parasitiformes), Sarcoptiformes e Trombidiformes (Acariformes) (Krantz & Walter 2009). Estas ordens incluem parasitas com diversos ciclos de vida, alguns que passam todas as fases da vida no hospedeiro, outros que alternam períodos no hospedeiro com períodos no abrigo ou no assoalho da caverna, lá vivendo sem se alimentar ou como predadores.

No Brasil, são conhecidas 52 espécies, classificadas em 10 famílias, de ácaros associados aos morcegos (Silva et al. 2017; Gomes-Almeida & Pepato 2021). No entanto, acredita-se que a quantidade real de espécies seja muito maior, considerando o potencial de diversidade de espécies existentes dentro da subclasse Acari (Dantas-Torres et al. 2009). Também é importante destacar que, no contexto dos organismos ectoparasitas de morcegos, a maioria dos estudos publicados no país estão relacionados a dípteros, ao passo que os ácaros fazem parte de um dos grupos que têm sido bastante negligenciados (Graciolli & Bernard 2002; Graciolli 2004; Graciolli & Bianconi 2007; Graciolli & Carvalho 2012; Rui & Graciolli 2005; Teixeira & Ferreira 2010; Graciolli 2023; Graciolli & Hrycyna 2023).

Nesta tese, dedicaremos especial atenção a dois grupos de ácaros: as duas famílias de Mesostigmata, Spinturnicidae Oudemans, 1902 e Macronyssidae Oudemans, 1936, e o gênero de Trombidiformes *Whartonia* Ewing 1944, dada a elevada frequência em que são encontrados em morcegos cavernícolas e a distinta relação parasita-hospedeiro que cada táxon mantém com os morcegos.

1.3.1 Família Spinturnicidae

Spinturnicidae inclui ácaros parasitas que se alimentam exclusivamente do sangue de morcegos e apresentam adaptações morfológicas e biológicas para viver aderidos às membranas das asas e da cauda desses hospedeiros, especialmente no plagiopatágio, uma região da asa altamente vascularizada (Dusbabek 2002; Almeida, Serra-Freire & Peracchi 2015).

Esses ácaros têm distribuição cosmopolita e passam todo o ciclo de vida (ovo, larva, protoninfa, deutoninfa e adultos) no hospedeiro, sendo ovovivíparos e com estágios ativos de protoninfa (primeiro estágio vágil), deutoninfa (macho ou fêmea, não sexualmente ativos) e adultos (macho e fêmea, sexualmente ativos) (Rudnick 1960).

São ácaros que permanecem por longos períodos no mesmo morcego devido à sua capacidade limitada de dispersão entre hospedeiros, limitada ao contato direto em agregações nos abrigos, da mãe à prole (transmissão vertical), ou durante a cópula (Gettinger & Gribel 1989; Clayton & Tompkins 1994; Christe et al. 2000; Ter Hofstede & Fenton 2005). Associações com tal grau de especificidade leva a expectativa de grande congruência entre as topologias da árvore do hospedeiro e parasita (Mayr 1957), o que inclui eventos de co-especiação, co-dispersão, e raras transferências interespecíficas (Bruyndonckx et al. 2009).

1.3.2 Família Macronyssidae

Já Macronyssidae inclui ácaros parasitas que se alimentam do sangue de diversos grupos de vertebrados, incluindo morcegos, marsupiais, roedores, aves, répteis e humanos (Radovsky 1966; Radovsky 2010). Esses ácaros têm distribuição cosmopolita e alternam períodos no hospedeiro e no abrigo (ou ninho) do hospedeiro durante o seu ciclo de vida.

O ciclo de vida dos macronissídeos tem cinco estágios: ovo, larva, protoninfa, deutoninfa e adultos. A maioria é ovípara (Saunders 1975), podendo depositar seus ovos e efetuar as mudas no abrigo do hospedeiro (Fonseca 1948), e poucos são larvíparos (Radovsky 1967). Radovsky (2010) relatou que apenas a protoninfa e adultos são hematófagos, a fase de larva é curta e a de deutoninfa é mais longa, mas inativa. A deutoninfa torna-se um adulto sem se alimentar e é encontrada no substrato do abrigo de seu hospedeiro (Radovsky 1966). Ao contrário, os adultos alimentam-se ativamente, principalmente a fêmea que precisa de grande quantidade de sangue, enquanto os machos permanecem a maior parte do tempo nos abrigos de seus hospedeiros, sendo raros os machos encontrados sobre o hospedeiro (Dowling 2006).

Embora estes ácaros sejam parasitas semi-permanentes, em contraste aos spinturnicídeos, eles também exibem um alto nível de especificidade ao táxon hospedeiro. Dos 35 gêneros válidos de Macronyssidae (Radovsky 2010; Bassini-Silva et al. 2021; Gomes-Almeida & Pepato 2021), 18 são encontrados exclusivamente em morcegos, com 12 ocorrendo na região neotropical e seis apenas no Velho Mundo. Dois outros gêneros, *Steatonyssus* Kolenati, 1858 (cosmopolita) e *Trichonyssus* Domrow 1959 (Velho Mundo) estão associados a morcegos e outros mamíferos.

1.3.3 Gênero *Whartonia* (Leeuwenhoekiidae Womersley 1945)

O gênero *Whartonia*, pertencente à família Leeuwenhoekiidae, assim como outros ácaros Parasitengona, possui um ciclo de vida complexo, dividido em sete estágios: ovo, pré-larva, larva, protoninfa, deutoninfa, tritoninfa e adultos (macho ou fêmea). Dentre esses estágios, as protoninfas e tritoninfas são inativas. Dos estágios ativos, o gênero inclui larvas parasitas que se alimentam da pele de morcegos (raramente de outros mamíferos) por digestão extracorpórea, e pós-larvais (deutoninfas e adultos) predadores de vida livre, frequentes no assoalho de cavernas, onde se alimentam de pequenos invertebrados (Takahashi et al. 2006).

A larva parasita, após o contato com o hospedeiro, fixa-se à pele inserindo suas quelíceras e desenvolve um tubo conhecido por estilostoma que inicia o processo de digestão extracorpórea através de secreções salivares, o que causa sérias lesões em seus hospedeiros (Goff et al. 1982). A associação com um hospedeiro ocupa um curto período do ciclo de vida das espécies de *Whatonia*, o restante do tempo despendido como um habitante do solo de vida livre.

Ao contrário de Spinturnicidae e Macronyssidae, as larvas parasitas não são específicas em relação às espécies hospedeiras. Em Trombiculidae, grupo mais próximo para o qual há um conhecimento mais detalhado da biologia, a seleção do hospedeiro por uma larva é mais bem explicada pela co-ocorrência do que pela coevolução (congruência entre as árvores de hospedeiros e parasitas, Shatrov & Kudryashova 2006). Neste caso, os pós-larvas demonstram preferência por um micro-habitat e suas larvas parasitam oportunisticamente todas ou a maioria das espécies de vertebrados que ocupam este habitat (Kudryashova 1998). No caso dos gêneros que exploram morcegos, como *Whartonia*, podemos estar diante de uma exceção parcial a essa regra, já que eles dificilmente são encontrados em outras ordens de mamíferos (Shatrov & Kudryashova 2006; Shatrov & Kudryashova 2008; Zajkowska & Mąkol 2022).

1.4 Justificativa e relevância

Cavernas são ecossistemas únicos e frágeis que podem ser facilmente afetados por mudanças em seu entorno. Algumas das ameaças mais comuns incluem a perda do habitat circundante devido ao desmatamento, urbanização, agricultura e atividades industriais, supressão parcial ou total pela mineração, poluição por agroquímicos, turismo e mudança climática (Piló & Auler 2019).

Embora morcegos usem comumente as cavernas como abrigos e sejam uma das principais fontes de entrada de energia e matéria neste ambiente, apenas 2% das cavernas brasileiras possuem informações sobre a sua quiropterofauna (Guimarães & Ferreira 2014). A informação disponível sobre a fauna de ácaros ectoparasitas associada a cavernas é ainda mais exígua, restrita à descrição de oito espécies ectoparasitas de morcegos (Bassini-Silva et al. 2022; Zampaulo & Henrique-Simões 2022).

As colônias de morcegos em cavernas compreendem até milhares de indivíduos em uma mesma caverna ou em sistema de cavernas (Leal & Bernard 2021; Ito et al. 2022) A carga parasitária de ácaros em morcegos que utilizam cavernas como abrigo é maior do que em ambientes não confinados (Postawa & Szubert-Kruszyńska 2014). Tanto a presença de ectoparasitas pode afetar a saúde e o comportamento dos morcegos (Lourenço & Palmeirin 2007), que podem ser vetores de patógenos (Amaral et al. 2018), quanto a composição taxonômica da comunidade de morcegos e seu modo de vida interfere na composição da comunidade dos parasitas (Gordon & Owen 1999; Christe et al. 2007; Bruyndonckx et al. 2010).

Os grupos de ácaros ectoparasitas de morcegos abordados nesta tese têm sido estudados principalmente quanto a sua morfologia externa, em trabalhos descritivos. Como outros grupos de ácaros, a ausência de especialistas desencoraja o uso mais disseminado do grupo em trabalhos abordando as comunidades subterrâneas (Bernardi et al. 2009; Senna et al. 2013; Silva et al. 2017). Isso justifica a produção de chaves de identificação e bases de dados moleculares que, ainda que não substituam a necessidade de formar especialistas, permite o engajamento de um maior número de pesquisadores no estudo dessas comunidades.

Em Spinturnicidae, a uniformidade fenotípica dificulta a acurácia da identificação, com prováveis espécies crípticas e considerável variação intraespecífica (Morales-Malacara 2001; Deunff et al. 2004). Por outro lado, na família Macronyssidae, a ausência de uma análise formal dos caracteres morfológicos diagnósticos e das relações filogenéticas entre as espécies pode resultar em alocações taxonômicas imprecisas, devido à dificuldade na identificação precisa das espécies (Micherdzinski 1980; Radovsky 2010). Todas as espécies do gênero *Whartonia* eram conhecidas apenas pelas suas larvas, que não são associáveis utilizando apenas a morfologia aos estágios pós-larvas, nunca descritos antes deste trabalho (Nielsen et al. 2021). Dadas as diferenças drásticas em sua morfologia (Zhang 1998) a associação de larvas e pós-larvas só é possível através da criação experimental desses ácaros ou métodos de delimitação de espécies com base em dados moleculares (ex. Stålstedt et al. 2016; Costa et al. 2019; Alarcón-Elbal et al. 2020).

Nesse três casos, a taxonomia integrativa, ao combinar diferentes tipos de dados, especialmente morfológicos e moleculares, para a identificação de espécies facilita a abordagem das dificuldades específicas de cada um deles (Dayrat 2005; Will et al. 2005). Como em tantos outros componentes da biodiversidade Neotropical, a lacuna Linneana, isto é, a falta de descrição e identificação de espécies (Hortal et al. 2015), é um dos principais obstáculos para uma apreciação correta da dinâmica desses grupos.

A taxonomia integrativa permite um maior rigor na formulação de hipóteses para a delimitação de espécies, entendidas como segmentos de linhagens evoluindo independentemente (De Queiroz 2007). A ideia não é substituir a taxonomia tradicional, como argumentam alguns defensores mais entusiasmados do uso de “Barcoding” de DNA (e.g. Hebert et al. 2003; Tautz et al. 2003), mas usar a complementaridade entre os diferentes conjuntos de evidências para a formulação de hipóteses taxonômicas que estão sujeitas a verificação em face a novas evidências (Schlick-Steiner et al. 2010; Yeates et al. 2011).

Essa integração de dados pode ser feita de duas maneiras: por acumulações, em que vários tipos de dados (como morfológicos, genéticos e ecológicos) são analisados separadamente, mas nem todas as evidências precisam convergir entre si; e por estruturas de congruência, em que as diferentes fontes de dados são analisadas em conjunto, a fim de identificar padrões de concordância entre elas, ou seja, todas as evidências devem sugerir a mesma hipótese taxonômica (Padial et al. 2010).

2 OBJETIVOS

Esta tese tem por objetivo contribuir com conhecimento taxonômico dos ácaros ectoparasitas de morcegos cavernícolas no Brasil, especialmente das famílias Macronyssidae, Spinturnicidae e Leeuwenhoekiidae, com uma abordagem integrativa, combinando especialmente dados morfológicos e moleculares:

i. Elaborar descrições de novas espécies baseadas no maior número possível de estágios do seu ciclo de vida, combinando dados morfológicos, moleculares e reportando dados ecológicos;

ii. No caso particular do gênero *Whartonia,* utilizar os marcadores Citocromo c Oxidase I (COI, mitocondrial) e o gene para o RNA da Subunidade maior do ribossomo (28S, nuclear), para associar as larvas e aos pós-larvas das espécies *Whartonia pachywhartoni* e *W. nudosetosa*, descrevendo pela primeira vez os estágios pós-larvas de representantes desse gênero.

iii.Elaborar ferramentas de identificação: a) chaves dicotômicas ou interativas para a identificação a menor nível taxonômico possível; b) Criar um banco de dados Barcode com foto documentação dos indivíduos testemunho.

3 ESTRUTURA DA TESE

A tese é apresentada em quatro capítulos, que correspondem a dois artigos publicados (Capítulo I, publicado na revista *Acarologia* e Capítulo III, publicado na *Systematic & Applied Acarology*). O segundo capítulo foi submetido como nota científica à revista *Acarologia,* e o quarto capítulo está pronto para ser submetido nessa mesma revista. A última seção consiste nas considerações finais sobre os dados obtidos nessa tese, incluindo resultados preliminares de pesquisas futuras usando esses dados. Os artigos publicados são referidos nos capítulos e apresentados como anexos da tese. O artigo pronto para submissão e as considerações finais são apresentados integralmente como capítulo IV e na seção final da tese, respectivamente.

Capítulo I. Dedicado à descrição de um novo gênero da família Macronyssidae, incluindo a descrição detalhada dos adultos e protoninfa. Além disso, inclui análises filogenéticas do gene 16S, dados ecológicos de zonação em cavernas e um mapa de distribuição a partir dos indivíduos depositados na coleção Acarológica do Centro de Coleções Taxonômicas da UFMG. São também reportadas ocorrências a partir de coletas feitas no marco do doutorado.

Capítulo II. Neste capítulo, são reportados pela primeira vez os hospedeiros de *Chiasmanyssus cavernicola* e novas localidades cársticas a partir das coletas realizadas posteriormente à publicação do capítulo I.

Capítulo III. O Capítulo descreve pela primeira vez os estágios pós-larvais de duas espécies do gênero *Whartonia,* já conhecidas para o Brasil a partir de suas larvas. A associação foi feita utilizando os marcadores COI e 28S.

Capítulo IV. Este capítulo dedica-se à família Spinturnicidae, apresentando uma base de dados de sequências de DNA *barcode*, um guia visual para identificação, novas ocorrências e associações com hospedeiros de seis espécies de ácaros do gênero *Periglischrus* no estado de Minas Gerais.

Considerações finais. Como conclusão, os dados moleculares permitiram concluir que *W. pachywhartoni* tem maior diferenciação genética e parece ter a sua estruturação genética influenciada pela distribuição dos ambientes subterrâneos onde seus estágios pós-larvas habitam. Já *P. iheringi* tem menor diferenciação genética, aparentemente mais intimamente ligada aos seus hospedeiros morcegos.

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CAPÍTULO I – A new genus and new species of macronyssid mite (Mesostigmata: Gamasina: Macronyssidae) from brazilian caves including molecular data and key for genera occurring in Brazil

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A new genus and new species of macronyssid mite (Mesostigmata: Gamasina: Macronyssidae) from Brazilian caves including molecular data and key for genera occurring in Brazil

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Original research

ABSTRACT

A new genus and a new species of macronyssid mites is described based on material collected from caves in Minas Gerais state, Southeastern Brazil, and additional occurrences from caves in Rio de Janeiro (Southeast) and Pará (Eastern Amazon region). The new genus may be distinguished from other Macronyssidae genera by the combination of sternal shield with a distinct X-shaped area; sternal shield bearing three pairs of setae and two pairs of pores; sternal gland absent; anterior spur on coxa II lacking; adults with dorsal shield entire and j and J series complete; adults and protonymphs with setae J5 minute; males with strong-pores on dorsal shield and peritrema short extending from posterior margin of coxae II to the anterior margin of coxae IV. A key for genera occurring in Brazil is provided, as well sequences from the mitochondrial large subunit of ribosome (16S).

Keywords Acari; Dermanyssoidea; Ectoparasitic mites; Integrative taxonomy; key
Zoobank http://zoobank.org/9C3C87C0-5371-4A8E-80B1-2F4E89632BDC

Introduction

The family Macronyssidae Oudemans, 1936 includes mites that can be associated with a variety of vertebrates, such as birds, reptiles and mammals, including humans (Radovsky 2010; Orlova *et al.* 2017; Bassini-Silva *et al.* 2019). The life cycle of these mites comprises five stages of development: egg, larva, protonymph, deutonymph and adults (male or female) (Radovsky 1966; 1967). Of these, only protonymph and adults are hematophagous, other stages are inactive and can be found on the substrate or roost of hosts (Radovsky 2010).

Radovsky (2010) reviewed the Macronyssidae family, recognizing seven new genera for the family, from new species discovered by him or by splitting other genera, mainly *Ornithonyssus* Sambon, 1928, which included at least a third of the Macronyssid genera (Zumpt and Till 1953; Fonseca 1954; 1960; Radovsky 1966; 1969; Micherdzinski 1980; Shepherd and Narro 1983).

Currently, Macronyssidae comprises 34 valid genera, with occurrences worldwide. Of these, at least 23 genera occur in the Neotropical region associated with different host taxa: *Synasponyssus* Radovsky and Fuman 1969, *Parichoronyssus* Radovsky 1966, *Radfordiella* Fonseca 1948, *Chiroecetes* Herrin and Radovsky 1974, *Mitonyssoides* Yunker, Lukoschus and Giesen 1990, *Macronyssoides* Radovsky 1966, *Nycteronyssus* Saunders and Yunker 1973,

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Mitonyssus Yunker and Radovsky 1980, *Cryptonyssus* Radovsky 1966, *Chiroptonyssus* Augustson 1945, and *Macronyssus* Kolenati 1858 are associated exclusively with bats; *Steatonyssus* Kolenati 1858 with bats and other mammals; *Acanthonyssus* Yunker and Radovsky 1966, *Argitis* Yunker and Saunders 1973, *Lepidodorsum* Saunders and Yunker 1975, *Lepronyssoides* Fonseca 1941 and *Lagidonyssus* Radovsky 2010 with rodents, despite *Lepronyssoides* can be found associated with other mammals; *Pellonyssus* Clark and Yunker 1956 with birds; *Ornithonyssus* Sambon 1928 with birds and mammals, including humans; *Draconyssus* Yunker and Radovsky 1966, *Ophionyssus* Megnin 1884, *Endophionyssuus* Radovsky 2010 on lizards and snakes; and, finally, *Thigmonyssus* Radovsky 2010 found associated with anteaters.

Thirteen macronyssid genera are recorded from Brazil thus far: *Chiroptonyssus*, *Lepronyssoides, Macronyssoides*, *Macronyssus*, *Mitonyssoides, Mitonyssus*, *Ophionyssus, Ornithonyssus, Parichoronyssus*, *Pellonyssus, Radfordiella, Steatonyssus* and *Thigmonyssus* (Bassini-Silva *et al.* 2020). They comprise twenty-seven species of mites occurring in localities in the Southeast (Minas Gerais, Rio de Janeiro and São Paulo), Middle-west (Mato Grosso, Mato Grosso do Sul and Goiás), Northeast (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí and Rio Grande do Norte) and South (Paraná and Rio Grande do Sul) (Fonseca 1948; Fonseca 1954; Radovsky 1967; Whitaker Jr and Mumford 1977; Azevedo *et al.* 2002; Almeida *et al.* 2011; Nieri-Bastos *et al.* 2011; Moras *et al.* 2013; Silva *et al.* 2017; Bassini-Silva *et al.* 2019; 2020; Oliveira *et al.* 2020).

Herewith, we aim at describing a new genus and new species based on adults and protonymphs found in caves located in the states of Minas Gerais, Rio de Janeiro (Southeastern) and Pará (Eastern Amazon region).

Material and methods

Specimens were collected during surveys for the subterranean fauna, in iron ore and limestone caves in Minas Gerais and Rio de Janeiro states, Southeastern Brazil and from caves in Pará state, North Brazil (Figure 1A). Mites were found freely dwelling on the cave floor, from entrance, penumbra and aphotic zone, during the dry and rainy seasons (Table 1; Figure 1B).

Specimens were mounted as permanent microscope slides using Hoyer's medium. Measurements and illustrations were made using the Leica DM 750 optical microscope with an ICC50 W digital camera attached. All measurements are given in micrometers (µm). Illustrations were prepared using the live image overlay technology adapted from Sidorchuk and Vorontsov (2014). Map was produced with QGIS 3.18.1 program and figures were prepared with Krita v.4.4.2 program. Terminology of dorsal and ventral chaetotaxy and the nomenclature of the dorsum based on Radovsky (2010). The terminology of sternal pores was reported according to Evans and Till (1979).

Holotype, paratypes and additional individuals (Supplementary material 1) are deposited at the Acarological Collection, Centro de Coleções Taxonômicas, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte City. Collection acronym: UFMG AC.

Molecular procedures

DNA extraction — Specimens were preserved in 95–100 % ethanol and stored at −20 °C. Genomic DNA was extracted from single specimens using a QIAamp DNA Micro kit (Qiagen) following the manufacturer's protocol, except by using two steps of the final elution, leading to a final volume of 50 µl.

16S rRNA gene amplification — A fragment of 16S rRNA was chosen due its availability for other Macronyssidae, since was employed in previous studies on the Macronyssid genus *Ornithonyssus*. The amplification of mitochondrial large ribosomal subunit (16S) fragment of ~ 410 bp was conducted using the primers proposed by Mangold *et al.* (1998): 16S + 1 (5′-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3′); 16S–1 (5′ -CCG GTC TGA ACT CAG ATC AAG T-3′). Amplifications were performed in 20 µl of final volume, with Platinum

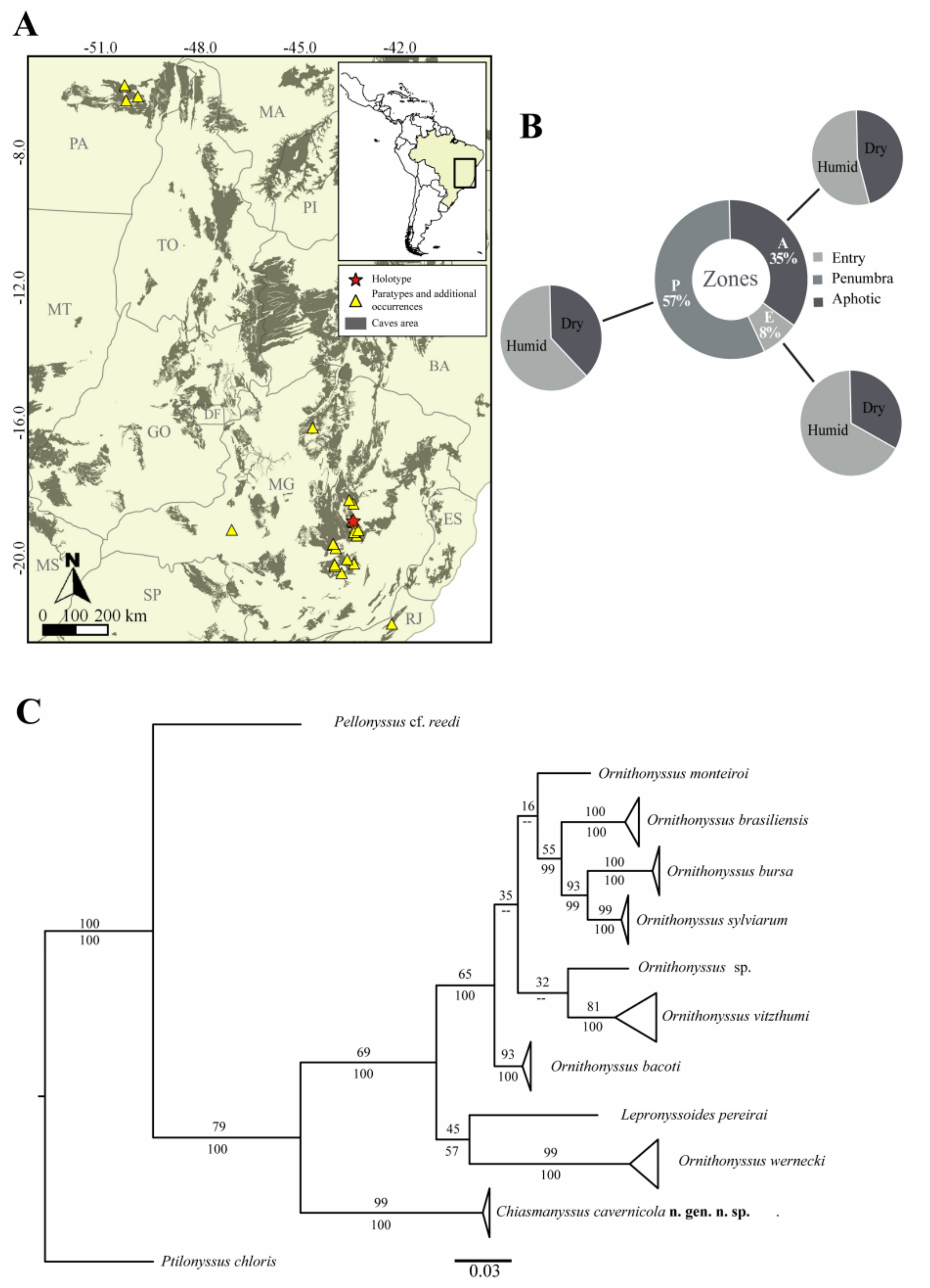

Figure 1 A – Sample distribution map with distribution across Brazilian territory and details of the distribution in the state of Pará and Minas Gerais, respectively; B – Graphic detail of the cave zone and period of collection of 39 specimens, of a total of 188 samples examined; C – Maximum Likelihood Tree inferred from sequences of mitochondrial 16S from Macronyssidae deposited in GenBank, in addition to sequences obtained from individuals of *Chiasmanyssus cavernicola* **n. sp.** Values above branches are Bootstrap support and bellow Posterior Probabilities (both in %). Clades absent in the Bayesian analyses are indicated by "–".

Table 1 Detailed sampling data of specimens with zoning information in the cave, season or collection period. Abbreviations: Protonymph (PN) female (F), male (M), Minas Gerais state (MG).

AC-UFMG	Instar/Sex	Cave	Zone	Season	Date	Latitude/Longitude	Municipality	State
171097	F	CAI-03	Entrance	-	27-29/II/01-09/III/2012	18°56'10.6"S 43°24'45.5"W	Conceição do Mato Dentro	MG
174528	F	CCB-0052	Penumbra	Humid	22-24/I/2018	20°09'58.9"S 43°22'59.7"W	Mariana	MG
174529	F	CCB-0052	Penumbra	Humid	22-24/I/2018	20°09'58.9"S 43°22'59.7"W	Mariana	MG
174550	F	CCB-0052	Penumbra	Dry	11-13/IX/2018	20°09'58.9"S 43°22'59.7"W	Mariana	MG
174537	F	CCB-0063	Penumbra	Dry	11-13/IX/2017	20°09'58.1"S 43°23'16.8"W	Mariana	MG
174554	F	CCB-0063	Penumbra	Dry	11-13/IX/2018	20°09'58.1"S 43°23'16.8"W	Mariana	MG
180365	F	CSS-0001	Penumbra	Humid	30/IV a 05/V/2018	18°55'02.1"S 43°25'43.7"W	Conceição do Mato Dentro	MG
180368	M	CSS-0001	Penumbra	Humid	30/IV a 05/V/2018	18°55'02.1"S 43°25'43.7"W	Conceição do Mato Dentro	MG
180371	F	CSS-0001	Penumbra	Humid	30/IV a 05/V/2018	18°55'02.1"S 43°25'43.7"W	Conceição do Mato Dentro	MG
180376	F	CSS-0001	Penumbra	Humid	30/IV a 05/V/2018	18°55'02.1"S 43°25'43.7"W	Conceição do Mato Dentro	MG
180348	F	CSS-0005	Penumbra	Humid	30/IV a 05/V/2018	18°55'03.8"S 43°25'42.7"W	Conceição do Mato Dentro	MG
180364	F	CSS-0005	Penumbra	Humid	30/IV a 05/V/2018	18°55'03.8"S 43°25'42.7"W	Conceição do Mato Dentro	MG
180339	F	CSS-0006	Aphotic	Humid	30/IV a 05/V/2018	18°55'04.3"S 43°25'43.6"W	Conceição do Mato Dentro	MG
180340	M	CSS-0007	Penumbra	Humid	30/IV a 05/V/2018	18°55'08.0"S 43°25'13.6"W	Conceição do Mato Dentro	MG
180341	M	CSS-0007	Penumbra	Humid	30/IV a 05/V/2018	18°55'08.0"S 43°25'13.6"W	Conceição do Mato Dentro	MG
180361	F	CSS-0007	Penumbra	Humid	30/IV a 05/V/2018	18°55'08.0"S 43°25'13.6"W	Conceição do Mato Dentro	MG
180367	F	CSS-0084	Penumbra	Humid	30/IV a 05/V/2018	18°55'00.3"S 43°25'48.0"W	Conceição do Mato Dentro	MG
170372	F	CSS-0111	Entrance	-	31/V-12/VI/2016	18°23'48.7"S 43°24'49.3"W	Conceição do Mato Dentro	MG
171374	F	HOLC-0090/97	Aphotic	Humid	21/XI-02/XII/2016	19°36'10.5"S 44°00'43.1"W	Pedro Leopoldo	MG
171375	F	HOLC-0090/97	Aphotic	Humid	21/XI-02/XII/2016	19°36'10.5"S 44°00'43.1"W	Pedro Leopoldo	MG
180121	F	MCFC-0009	Aphotic	Dry	26/VI-06/VII/2018	19°19'52.2"S 43°18'41.8"W	Itambé do Mato Dentro	MG
180093	PN	MCFC-0012	Aphotic	Dry	26/VI-06/VII/2018	19°20'35.0"S 43°18'28.8"W	Itambé do Mato Dentro	MG
180094	F	MCFC-0012	Aphotic	Dry	26/VI-06/VII/2018	19°20'35.0"S 43°18'28.8"W	Itambé do Mato Dentro	MG
180106	F	MCFC-0013	Entrance	Dry	26/VI-06/VII/2018	19°20'38.8"S 43°18'16.8"W	Itambé do Mato Dentro	MG
180419	F	MGB-0061	Penumbra	Dry	I/VIII/2018	20°26'37.0"S 43°45'55.1"W	Ouro Preto	MG
180473	F	MGB-0063	Penumbra	Dry	03-06/IX/2018	20°26'37.2"S 43°45'55.3"W	Ouro Preto	MG
180483	F	MGB-0063	Penumbra	Dry	03-06/IX/2018	20°26'37.2"S 43°45'55.3"W	Ouro Preto	MG
180489	F	MGB-0063	Aphotic	Dry	03-06/IX/2018	20°26'37.2"S 43°45'55.3"W	Ouro Preto	MG
180067	F	MS-0004	Aphotic	Humid	26-28/III/2018	20°11'01.7"S 43°58'19.8"W	Nova Lima	MG
180073	F	MS-0004	Aphotic	Dry	28-29/IX/2017	20°11'01.7"S 43°58'19.8"W	Nova Lima	MG
180074	PN	MS-0004	Aphotic	Dry	28-29/IX/2017	20°11'01.7"S 43°58'19.8"W	Nova Lima	MG
180076	F	MS-0004	Penumbra	Dry	28-29/IX/2017	20°11'01.7"S 43°58'19.8"W	Nova Lima	MG
180077	F	MS-0004	Penumbra	Dry	28-29/IX/2017	20°11'01.7"S 43°58'19.8"W	Nova Lima	MG
180065	F	MS-0025	Entrance	Humid	26-28/III/2018	20°14'52.7"S 43°57'59.6"W	Itabirito	MG
180068	F	MS-0025	Entrance	Humid	26-28/III/2018	20°14'52.7"S 43°57'59.6"W	Itabirito	MG
180052	F	MS-0030	Penumbra	Humid	26-28/III/2018	20°12'07.8"S 43°58'03.7"W	Nova Lima	MG
180053	F	MS-0030	Aphotic	Humid	26-28/III/2018	20°12'07.8"S 43°58'03.7"W	Nova Lima	MG
180049	PN	MS-0031	Aphotic	Humid	26-28/III/2018	20°12'08.7"S 43°58'03.4"W	Nova Lima	MG
180050	F	MS-0031	Aphotic	Humid	26-28/III/2018	20°12'08.7"S 43°58'03.4"W	Nova Lima	MG

Taq DNA Polymerase (Invitrogen) in a Mastercycler nexus (Eppendorf) thermocycler. Master mix for initial PCR contained 2.0 µl of PCR buffer (1X), 1.4 µl $MgCl_2$ (50 mM), 1.4 µL of dNTP (10 mM each), 0.8 µl of each oligonucleotide primer. Amplifications used 1–3 µl of genomic DNA and included an initial denaturing step of 2 min at 94 °C, followed by 35 cycles, comprising: denaturing at 94 °C for 30s, annealing for 35s, and extension at 72 °C for 45 s. The annealing temperature for the first seven cycles increased in 0.3 °C every cycle, ranging from 47 °C to 48.8 °C, remaining 28 cycles used an annealing temperature of 50 °C. Final extension of 5 min. at 72 °C.

Sequencing and editing chromatogram — PCR products found positive in 1% agarose gel electrophoresis were purified using the Ampure® (Agencourt) kit and sequenced using a 3730 DNA Analyzer. Sequencing primers as for PCRs. Chromatograms were resolved in ChromasPro 1.41 (Technelysium Pty Ltd).

Sampling, alignment and 16S secondary structure — Newly obtained sequences and those downloaded from GenBank are reported in Table 2, along voucher material collection numbers. This fragment was chosen because it led to the largest sample of terminals identified to species and with vouchering data. It is beyond the objectives of this article perform a throughout phylogenetic analyses of Macronyssidae, something that would require more markers and terminals. The aim of including this fragment is just to verify if it supports the inclusion of the new genus in the relatively well sampled *Ornithonyssus*. Preliminary automatic alignment was performed using MUSCLE (Edgar 2004) and then manually aligned with aid of sequence editor BioEdit 7.2.1 (Hall 1999) considering the 16S secondary structure as reported for other arthropods (Smith and Bond 2003; Gillespie *et al.* 2006). A full annotated alignment is provided in the supplementary material. Therein, matching parentheses and dots were used to indicate stem and loop regions, respectively; structural helix numbering is given after Gillespie *et al.* (2006). Length variable regions inferred to be ambiguously aligned were classified into regions of expansion and contraction (REC), non-pairing regions of ambiguous alignment (RAA) and excluded from downstream phylogenetic analyses. Access to the Brazilian genetic heritage was registered in SisGen under register number A68A31E.

Molecular Phylogenetic Analysis — Model for sequence evolution was chosen using the AICc criterion implemented in jModeltest (Darriba *et al.* 2012). Maximum Likelihood inference was run in PhyML (Guindon *et al.* 2010) online at http://www.atgc-montpellier.fr/phyml/ (accessed January 18nd 2021) and Bayesian analyses in MrBayes 3.2.7 (Ronquist *et al.* 2012).

Table 2 GenBank accession numbers and vouchering for 16S gene. The sequence of the Rhinonyssid *Ptilonyssus chloris* (Rhinonyssidae) was used as outgroup in some analyses. * In Radovsky (2010) the current taxonomic position of this species is placed in the genus *Lepronyssoides*.

Terminal	Voucher	GenBank
Rhinonyssidae		
P. chloris (Fain 1962)		NC_045208
Macronyssidae		
Pellonyssus cf. *reedi*	LEcEN: SIA:Ornis70	MK131079
	IBSP10372	HM059863
	IBSP10371	HM059862
	IBSP10370	HM059861
	IBSP10369	HM059860
	IBSP10368	HM059859
	IBSP10367	HM059858
	IBSP10366	HM059857
Ornithonyssus brasiliensis (Fonseca 1939)	IBSP10365	HM059856
	IBSP10364	HM059855
	IBSP10363	HM059854
	IBSP10362	HM059853
	IBSP10361	HM059852
	IBSP10360	HM059851
	IBSP10359	HM059850
	IBSP10358	HM059849
	IBSP10357	HM059848
Ornithonyssus vitzthumi (Fonseca 1941)	IBSP8546	HM059847
	IBSP10356	HM059846
	IBSP10355	HM059845
	IBSP10354	HM059844
	IBSP10353	HM059843
	IBSP10352	HM059842
	IBSP10351	HM059841
Ornithonyssus wernecki	IBSP10348	HM059837
	IBSP10347	HM059836
	IBSP10346	HM059835
Lepronyssoides pereirai *	IBSP8770	HM059834
Ornithonyssus bacoti	IBSP10350	HM059840
	IBSP10349	HM059839
Ornithonyssus monteiroi	IBSP10388	HM059838
Ornithonyssus bursa	LEcEN SIA Ornis31	MK131067
	LEcEN SIA Ornis14	MK131059
	LEcEN SIA Ornis51	MK131071
	LEcEN SIA Ornis32	MK131068
	LEcEN SIA Ornis109	MK131086
	LEcEN SIA Ornis102	MK131085
	LEcEN SIA Ornis101	MK131084
	LEcEN SIA Ornis99	MK131083
	LEcEN SIA Ornis98	MK131082
	LEcEN SIA Ornis97	MK131081
	LEcEN SIA Ornis72	MK131080
	LEcEN SIA Ornis69	MK131078
	LEcEN SIA Ornis67	MK131077
	LEcEN SIA Ornis65	MK131076
	LEcEN SIA Ornis55	MK131074
Ornithonyssus sylviarum	LEcEN SIA Ornis54	MK131073
	LEcEN SIA Ornis52	MK131072
	LEcEN SIA Ornis48	MK131070
	LEcEN SIA Ornis39	MK131069
	LEcEN SIA Ornis27	MK131066
	LEcEN SIA Ornis26	MK131065
	LEcEN SIA Ornis24	MK131064
	LEcEN SIA Ornis23	MK131063
	LEcEN SIA Ornis22	MK131062
	LEcEN SIA Ornis21	MK131061
	LEcEN SIA Ornis15	MK131060
	LEcEN SIA Ornis4	MK131057
	LEcEN SIA Ornis2	MK131056
Ornithonyssus sp.	LEcEN SIA Ornis91	MK131087
Chiasmanyssus cavernicola **n. gen. n. sp.**	UFMG AC 200347	MW522521
	UFMG AC 200348	MW522522
	UFMG AC 200349	MW522523
	UFMG AC 200350	MW522524
	UFMG AC 200380	MW522525
	UFMG AC 200381	MW522526
	UFMG AC 200382	MW522527
	UFMG AC 200383	MW522528
	UFMG AC 200384	MW522529
	UFMG AC 200385	MW522530
	UFMG AC 200386	MW522531
	UFMG AC 200387	MW522532

Inter and intraspecific distances were calculated in MEGA7 (Kumar *et al.* 2016).

Result

DNA sequencing and analyses

The final alignment counted with 288 positions after having their 3′ and 5′ extremities trimmed, and regions of ambiguous alignment excluded. Among them, 186 positions were conserved across all macronyssid species and 78 parsimony informative. Whereas intraspecific mean p-distances among sequences varied from 0.0005 in *O. sylviarum* (Canestrini and Fanzago 1877) (n= 24) to 0.0094 in *O. wernecki* (Fonseca 1935a) (n= 3), among *Ornithonyssus* and *Lepronyssoides* species, interspecific p-distances varied from 0.0462 (*O. bacoti* (Hirst 1913) x *O monteroi* (Fonseca 1941) and 0.1435 (*O. bursa* (Berlese 1888) x *O. wernecki* and *L. pereirai* (Fonseca 1935a). Pairwise mean interspecific p-distances are summarized in Table 3. The results suggest the 16S fragment sequenced here is a useful barcoding marker for molecular identification.

The model HKY+G was chosen using jModeltest and used in Maximum likelihood and Bayesian analyses. They agree to each other and similar to Nieri-Bastos *et al.* (2011) recovers *L. pererai* and *O. wernecki* in a basal position relative to remaining *Ornithonyssus* species (Figure 1C). *Chiasmanyssus cavernicola* **n. gen. n. sp.** branches basal in the topology obtained, being recovered in an isolated position relative to *Ornithonyssus* and *Lepronyssoides* species from which 16S sequences are available.

Taxonomy

Family Macronyssidae Oudemans, 1936

Diagnosis according to Radovsky (2010).

Chiasmanyssus n. gen.

Zoobank: 1C2CF818-9683-4DB2-BE34-DAD69DC435F5

(Figures 3–10)

Type species: *Chiasmanyssus cavernicola*, new species.

Diagnosis — Female large, its sternal shield square, distinctly sclerotized, with a smoother area on central sternal shield X-shaped; sternal shield bearing three pairs of setae and two pairs of pores; without sternal glands; anterior median protusion in epigynal shield extends under the sternal shield; anterior spur on coxa II lacking. Adults with dorsal shield entire, reticulated

Table 3 Pairwise mean interspecific p-distances values between sequences of 16S from species of macronyssid mites deposited in GenBank and newly sequenced from the new genus. Values with cells shadowed in gray are intra-specific mean distances in case of more than one sequence available for that species.

	1	2	3	4	5	6	7	8	9	10	11
1. *Pellonyssus*	--										
2. *O. brasiliensis*	0. 1828	0. 0013									
3. *O. vitzhumi*	0. 1816	0. 0602	0. 0078								
4. *O. wernecki*	0. 1799	0. 1274	0. 1094	0. 0094							
5. *L. pereirai*	0. 1825	0. 1239	0. 1156	0. 1115	--						
6. *O. bacoti*	0. 1707	0. 0609	0. 0561	0. 1162	0. 0943	0. 0036					
7. *O. monteroi*	0. 1944	0. 0605	0. 0732	0. 1256	0. 1021	0. 0462	--				
8. *O. bursa*	0. 1815	0. 0667	0. 0839	0. 1435	0. 1435	0. 0738	0. 0731	0. 0018			
9. *O. sylvarium*	0. 1825	0. 0545	0. 0726	0. 1254	0. 1297	0. 0628	0. 0632	0. 0476	0. 0005		
10. *Ornithonyssus* sp.	0. 1905	0. 0700	0. 0550	0. 1176	0. 1163	0. 0686	0. 0504	0. 0921	0. 0778	--	
11. *C. cavernicola* **n. gen. n. sp.**	0. 1620	0. 1479	0. 1432	0. 1567	0. 1543	0. 1381	0. 1437	0. 1569	0. 1554	0. 1388	0. 0017

and with j and J series complete. Adults and protonymphs with setae J5 minute; all setae on idiosoma, legs and gnathosoma slender and smooth, except palp with disto-ventral seta on genu spatulate. Females and protonymphs with chelae simple and slender, without spines or other processes. Male with four pairs of conspicuous pores near setae j3, z4, J3 and one laterally between J4 and J5 setae; peritrema short, extending from posterior margin of coxae II to the anterior margin of coxae IV and peritrematic shield with nodule fused to the middle of the shield.

Etymology — The generic name comes from Greek word "*chiasma*" referring to the distinct X-shaped area on sternal shield and "*nyssus*" , to prick, referring to the parasitic life.

Chiasmanyssus cavernicola n. gen. n. sp.

Zoobank: A34F4774-19C6-41E7-AFCB-023910A345A0

Diagnosis — *Female:* Elongated dorsal shield, holotrichous, bearing 27 pairs of setae, all setae long and subequal, except j2, z2, s1–s2 small (and J5 minute). Unarmed dorsum with approximately 84 pairs of long setae and ventral opisthosoma with approximately 48 pairs of setae; peritrema long, extending from level of anterior margin of coxae II to the middle of coxae IV; palpal trochanter with a small blade-like ventral process. *Male:* anterior margin of the dorsal shield not discernible from the soft cuticle; with neotrichous condition on opisthonotal region; a pair of additional minute setae, laterally to J4 and J5, on neotrichous area of opisthonotal region. Four pairs of caudal setae on unarmed dorsum and four pairs of setae on unarmed venter. *Protonymph:* Subrectangular podonotal shield with lateral borders biconcave at level of z2 and z4, posterior region almost square; with j2 very close to j1. Trapezoidal pygidial shield with J4 and Z4 small than others (except J5 minute). Unarmed dorsum with 12 pairs of long setae. Unarmed venter with four pairs of median setae between ventral shields and a pair of caudal setae, without setae flanking the anus.

Description. Female — Measurements summarized in table 4.

(Figures 2–4)

Idiosoma — (Figures 2A–B). Oval, moderately sclerotized and striated integument; all setae smooth. *Dorsum* (Figure 2A). Dorsal shield entire, elongated, longer than wide, and narrow, even at its widest, distinctly tapered in the middle of opisthonotal region; reticulated sculpture on all its area; holotrichous condition with 27 pairs of setae, 16 on podonotum and 11 on opisthonotum; j and J series complete with j1, j2 and j3 on dorsal shield; all setae long and subequal except j2, z2, s1–s2 small and J5 as microsetae (Figure 3A); one pair of lyrifissures near on margin lateral to setae j1 and four pair of pores on margin lateral to setae j3 and J4 and near s4 and z6. Unarmed dorsum with approximately 84 pairs of long setae. *Venter* (Figure 2B). Tritosternal base not expanded; Tritosternum bipartite with long and fimbriate laciniae (Figure 3B). Presternal area is weakly sculptured. Sternal shield nearly square, anterior margin with a small medial prominence; strongly sclerotized and punctate, except for a smoother X-shaped portion on central sternal shield (Figure 3C); anterior and posterior corners absent; three pairs of sternal setae relatively long, st1 and st2 on the sclerotized part and st3 inserted on X-shaped region on shield; two pairs of sternal pores. Metasternal setae (st4) on unarmed integument with third pair of pores accompanying metasternal setae. Epigynal shield expanded anteriorly, median projection extends under the sternal shield, no anterior scale-like markings; posterior margin pointed (Figure 3C); one pair of genital setae, approximately at the middle of epigynial plate. Anal plate pyriform; long and narrow cribrum; one pair of adanal setae (pan) and one postanal seta (pon) (Figure 3D). Endopodal plates small and triangular between level of coxae II and III. Opisthosoma with approximately 48 pairs of setae ventrally, slender, except caudal setae, being thicker, similar to the setae on unarmed dorsum. Peritreme long, extending from anterior margin of coxae II to the middle of coxae IV (Figure 2B). Peritrematic shield free from dorsal shield but interrupted by two "nodules" on the anterior dorsum between coxae I and II and in the middle ventral between coxae II and III (Figure 2A–B). Peritrematic shield posterior to the stigma fused with the parapodal shield around the posterior margin of coxa IV (Figure 2B).

Table 4 Metric data of females, males and protonymphs specimens from new genus.

	FEMALE				MALE			PROTONYMPH		
Characters	**Holotype**	**n**	**range**	**mean**	**n**	**range**	**mean**	**n**	**range**	**mean**
idiosoma L	792	10	726-847	782	2	520-693	606	2	400-424	412
idiosoma W.	432	10	377-507	451	2	294-384	339	2	264-288	276
dorsal shield L.	624	10	569-658	625	2	534-605	570	-	-	-
anterior dorsal plate W.	-	-	-	-	2	216-233	225	-	-	-
middle dorsal plate W.	-	-	-	-	2	313-319	316	-	-	-
posterior dorsal plate W.	-	-	-	-	2	79-93	86	-	-	-
dorsal shield W. at j3	199	10	191-218	202	-	-	-	-	-	-
dorsal shield W. at j6	202	10	173-225	202	-	-	-	-	-	-
dorsal shield W. at J5	41	10	33-42	38	-	-	-	-	-	-
podonotal shield L.	-	-	-	-	-	-	-	2	204-211	207
podonotal shield W. at z2	-	-	-	-	-	-	-	2	132-139	136
podonotal shield W. at j5	-	-	-	-	-	-	-	2	176-184	180
podonotal shield W. at j6	-	-	-	-	-	-	-	2	132-134	133
anterior pygidial shield L.	-	-	-	-	-	-	-	2	71-73	72
pygidial shield W. at Z3	-	-	-	-	-	-	-	2	107-112	109
pygidial shield W. at S5	-	-	-	-	-	-	-	2	86-89	88
pygidial shield W. at Z5	-	-	-	-	-	-	-	2	49-60	54
j1	51	10	40-61	52	2	27-33	30	2	33-38	35
j2	22	10	22-32	27	2	18-19	19	2	24-29	27
j3	54	9	43-59	52	2	30-37	34	2	40-42	41
j4	65	9	64-76	68	1	57	-	2	42-57	49
j5	61	7	60-78	66	2	31-47	39	2	51-64	58
j6	85	9	69-94	83	1	60	-	2	80-80	80
z2	27	10	24-32	28	2	23-24	24	2	20-23	21
z4	54	8	45-59	52	1	39	-	2	60-63	62
z5	83	9	64-86	78	1	56	-	2	64-64	64
z6	75	10	68-86	74	1	55	-	-	-	-
s1	28	10	23-34	28	-	-	-	-	-	-
s2	32	10	24-37	31	2	45-49	47	-	-	-
s3	60	10	58-76	67	2	52-94	73	-	-	-
s4	63	10	63-83	73	1	55	-	2	69-74	72
s5	70	8	58-72	66	2	52-52	52	-	-	-
s6	70	9	52-74	68	2	45-60	53	2	91-92	91
J1	81	9	65-82	76	1	62	-	2	81-89	85
J2	73	8	65-79	74	1	56	-	2	87-97	92
J3	63	8	50-70	60	1	57	-	2	96-96	96
J4	55	6	42-61	53	1	43	-	2	18-20	19
J5	-	-	-	-	1	10	-	-	-	-
Z1	71	10	59-76	68	-	-	-	-	-	-
Z2	61	7	57-71	65	-	-	-	-	-	-
Z3	56	7	46-57	54	-	-	-	2	92-96	94
Z4	47	6	33-48	41	-	-	-	2	10-10	10
Z5	73	9	62-84	72	1	60	-	2	102-106	104
S5	56	8	54-64	59	-	-	-	2	88-92	90
peritrema to stigma	238	10	213-254	234	2	104-106	105	2	62-62	62
sternal plate L. at st.3	124	10	112-124	118	-	-	-	2	160-161	161
sternal plate W.at st.1	133	10	129-146	137	-	-	-	2	65-70	67
sternal plate W. at st.2	121	10	117-129	122	-	-	-	2	96-97	96
sternal plate W.at st.3	126	10	122-132	127	-	-	-	2	69-70	70
holoventral shield L.	-	-	-	-	1	468	-	-	-	-
holoventral shield W. at st.2	-	-	-	-	2	87-93	90	-	-	-
holoventral shield W. at gen.	-	-	-	-	2	44-45	44	-	-	-
holoventral shield W. at pan.	-	-	-	-	1	52	-	-	-	-

Table 4 Continued

	FEMALE				MALE			PROTONYMPH		
Characters	**Holotype**	**n**	**range**	**mean**	**n**	**range**	**mean**	**n**	**range**	**mean**
st.1	52	8	50-63	55	2	31-36	34	2	42-44	43
st.2	52	7	46-56	52	2	27-32	30	2	44-45	44
st.3	59	7	50-83	64	2	35-41	38	2	50-50	50
st.4	60	7	50-64	57	2	27-41	34	2	33-33	33
epigynial plate W.	66	10	65-70	67	-	-	-	-	-	-
epigynial plate L.	292	10	289-321	306	-	-	-	-	-	-
genital setae L.	42	7	35-44	40	-	-	-	-	-	-
anal shield L.	137	10	109-137	125	-	-	-	2	68-71	69
anal shield W.	59	10	57-80	68	-	-	-	2	43-44	43
pan. setae	45	7	38-50	45	1	32	-	2	50-60	55
pon. setae	51	9	39-59	51	2	33-37	35	2	54-56	55
tarsus I L.	312	10	259-324	308	2	212-220	216	2	203-213	208
tibia I	150	10	130-154	144	2	101-104	103	2	84-88	86
Genu I	127	10	110-139	125	2	89-95	92	2	71-80	75
femur I	183	10	170-202	185	2	104-134	119	2	105-107	106
trocanter I	73	10	61-74	69	2	41-45	43	2	48-49	49
coxa I	95	10	87-97	93	2	64-74	69	2	62-70	66
leg I L.	940	10	855-958	914	2	615-667	641	2	572-607	590
tarsu II	223	10	186-239	218	2	128-133	130	2	143-148	145
tibia II	111	10	93-112	104	2	72-77	75	2	68-69	68
genu II	110	10	81-113	97	2	60-61	61	2	66-69	68
femur II	150	10	124-154	143	2	93-101	97	2	85-93	89
trocanter II	69	10	46-69	60	2	48-50	49	2	42-44	43
coxa II	61	10	45-74	61	2	39-53	46	2	30-33	31
leg II L.	724	10	615-738	683	2	442-473	458	2	440-449	445
tarsu III	219	10	182-233	217	2	111-137	124	2	138-139	139
tibia III	86	10	84-107	96	2	68-70	69	2	62-64	63
genu III	78	10	78-92	84	2	61-71	66	2	58-60	59
femur III	116	10	110-137	123	2	74-93	84	2	82-84	83
trocanter III	64	10	58-74	65	2	48-52	50	2	45-50	47
coxa III	68	10	58-77	67	2	44-50	47	2	34-41	38
leg III L.	630	10	598-685	652	2	416-463	439	2	425-433	429
tarsu IV	307	10	260-315	300	2	176-188	182	2	200-201	200
tibia IV	140	10	132-148	140	2	93-99	96	2	87-90	89
genu IV	119	10	113-137	124	2	76-92	84	2	81-82	81
femur IV	186	10	168-208	188	2	121-125	123	2	106-123	115
trocanter IV	80	10	80-107	89	2	63-77	70	2	70-73	71
coxa IV	66	10	58-81	67	2	52-53	53	2	38-46	42
leg IV L.	899	10	858-968	908	2	582-634	608	2	586-611	598
tritosternum L.	214	9	197-225	211	1	113	-	2	104-132	118
hyp.1	42	10	40-49	44	2	29-29	29	2	25-30	27
hyp.2	35	10	30-44	37	2	22-23	23	2	19-25	22
hyp.3	43	9	34-50	43	2	31-32	32	2	31-36	33
gnathosoma setae (pc)	44	10	44-56	49	2	28-29	28	2	26-34	30
chelicera L. with chela	223	10	213-231	224	2	127-129	128	2	152-159	156
spermatheca	-	-	-	-	2	37-40	38	-	-	-
palps	258	10	235-266	252	2	200-225	212	2	227-244	236

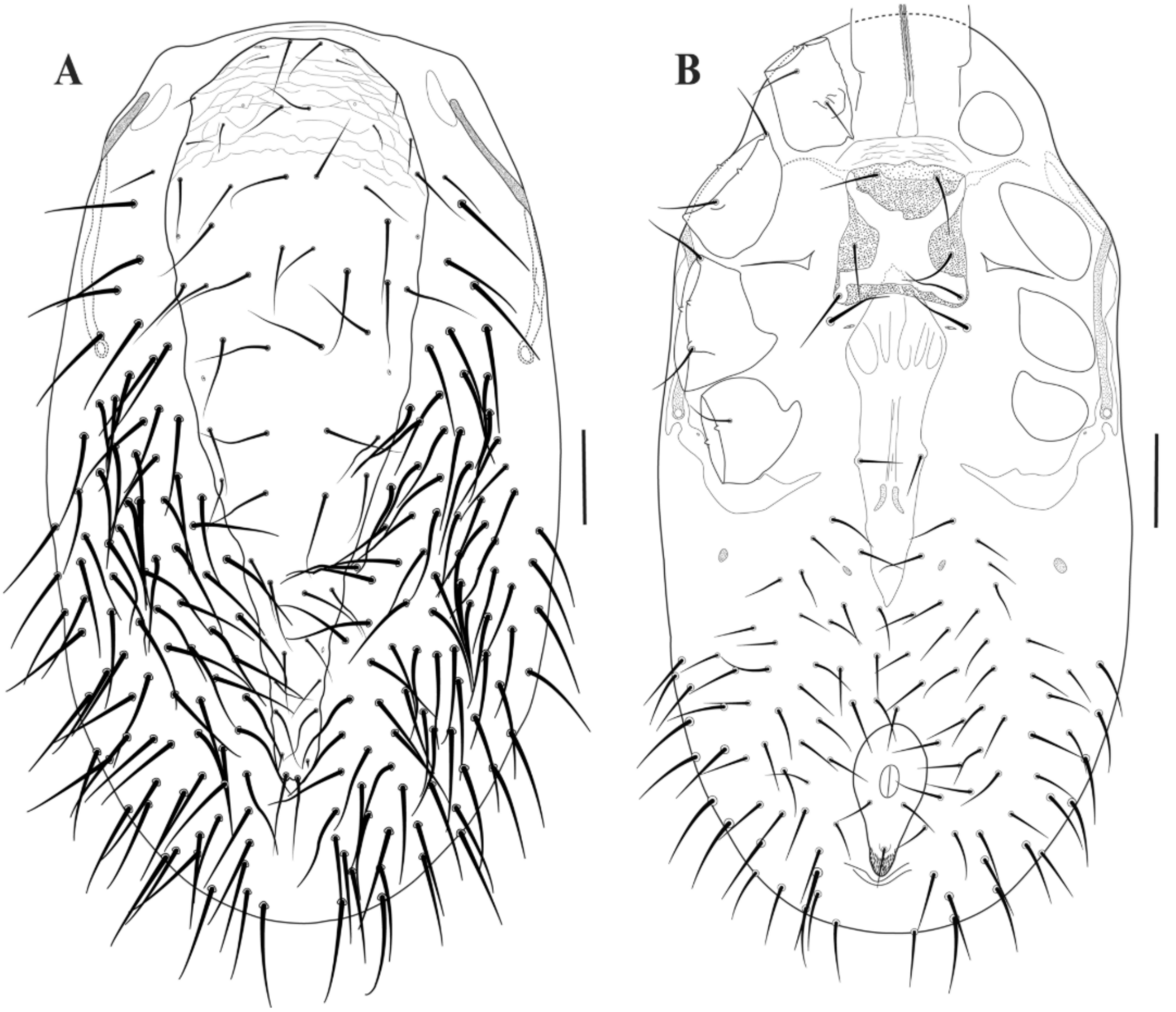

Figure 2 *Chiasmanyssus cavernicola* **n. sp.** female. A – Idiosomatic dorsum; B – Idiosomatic ventral. Scale Bars: A & B 20 µm.

Legs — Legs long and slender; legs I and IV longer than others; leg setae smooth. Coxae II and III may have posterior seta on a low protuberance; coxae II lacks anterodorsal spines; coxae lack ventral spurs or spur-like spines and ridges. Chaetotaxy of legs: I= 0 0/1 0/1 0 (2), 1 0/2 1/1 1 (6), 2 3/1 2/3 2 (13), 2 3/2 3/1 2 (13), 2 3/2 3/1 2 (13), 4 8/4 2/2 6/3 4 (33) + 6 solenidia + 1 macroseta; II= 0 0/1 0/1 0 (2), 1 0/2 0/1 1 (5), 2 3/1 2/2 1 (11), 2 3/1 2/1 2 (11), 2 2/1 2/1 2 (10), 3 3/2 1/1 3/2 3 (18); III= 0 0/1 0/1 0 (2), 1 1/2 0/1 0 (5), 1 2/1 1/0 1 (6), 2 2/1 2/1 2 (10), 2 1/1 2/1 2 (9), 3 3/2 1/1 3/2 3 (18) and IV= 0 0/1 0/0 0 (1), 1 1/2 0/1 0 (5), 0 2/2 1/0 1 (6), 2 2/1 3/1 1 (10), 2 1/1 3/1 2 (10), 3 3/2 1/1 3/2 3 (18). Sensory field of tarsus I has distal setae (d1–d2) on pedicels; setae s4 (solenidion) fused at base; two setae (solenidion) additional in tarsus ("s?"), a seta near ds and d2, and a very posterior seta; one macroseta, long and slender with distinctly wide socket on dorsal (seta "m", probable).

Gnathosoma — (Figure 4A–F). Deutosternal groove with nine denticles arranged 1–1–

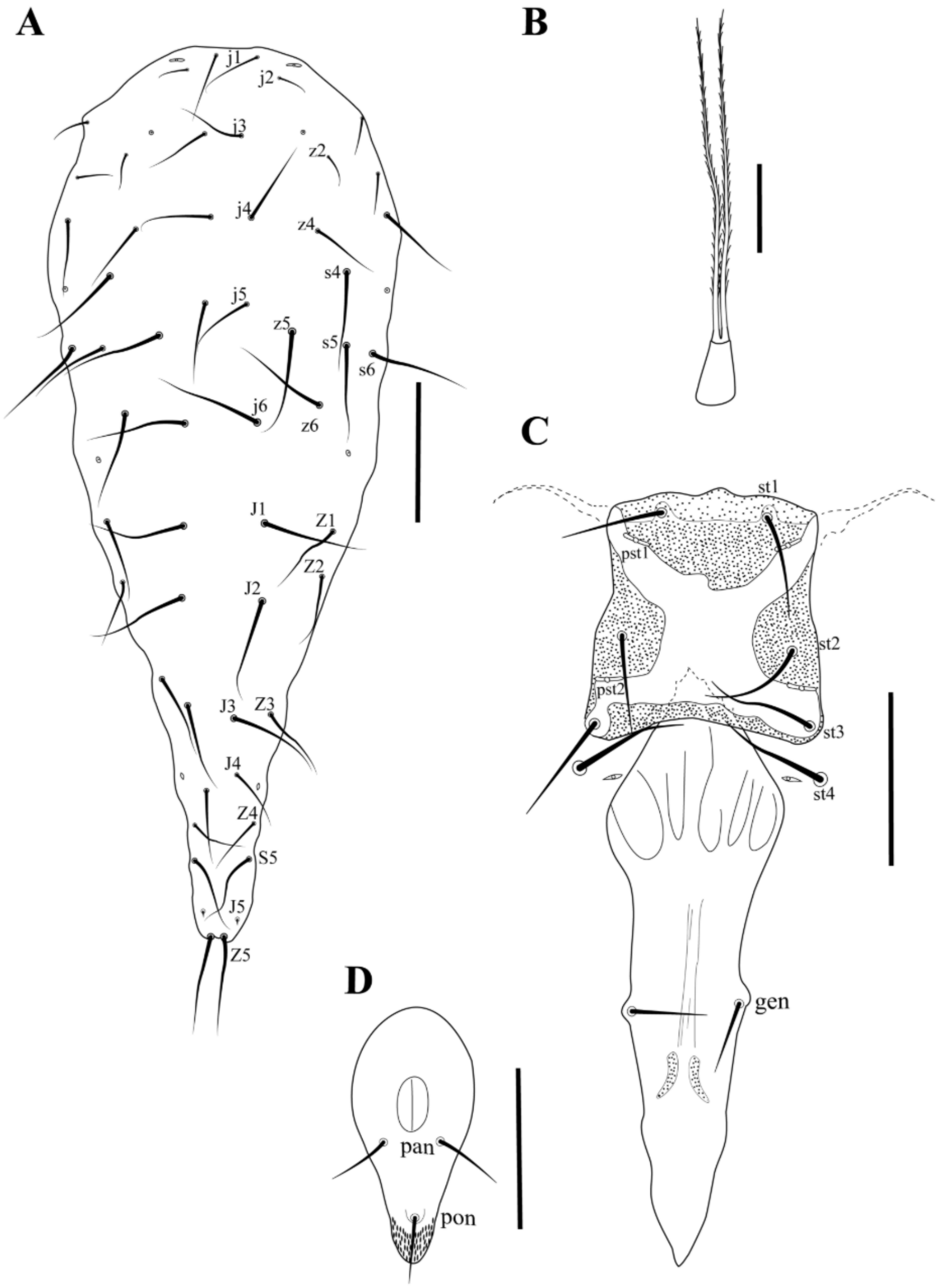

Figure 3 *Chiasmanyssus cavernicola* **n. sp.** female. A – Dorsal shield; B – Tritosternum; C – Sternal shield, note the distinct X-shaped sclerotization and epigynal shield; D – Anal shield. Scale bars: A 20 µm; B, C & D 50 µm.

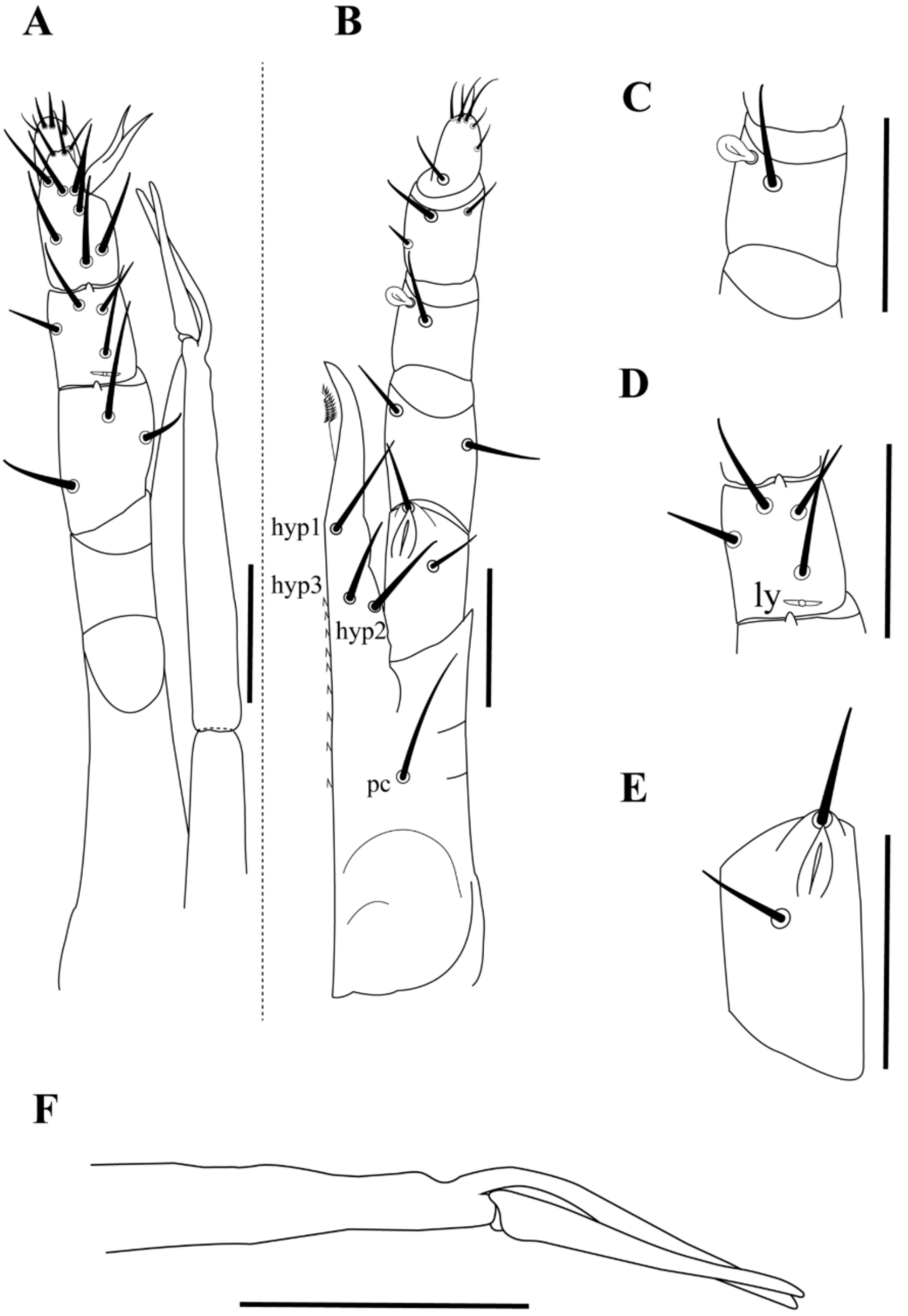

Figure 4 *Chiasmanyssus cavernicola* **n. sp.** female. A – Gnathosomatic dorsal; B – Gnathosomatic ventral; C – Venter palpal genu with distinct circular (spatulate) and membranous seta; D – Dorsal palpal genu with lyrifissure; E – Palpal trochanter with ventral process; F – Chelicera. Scale bars: 50 µm.

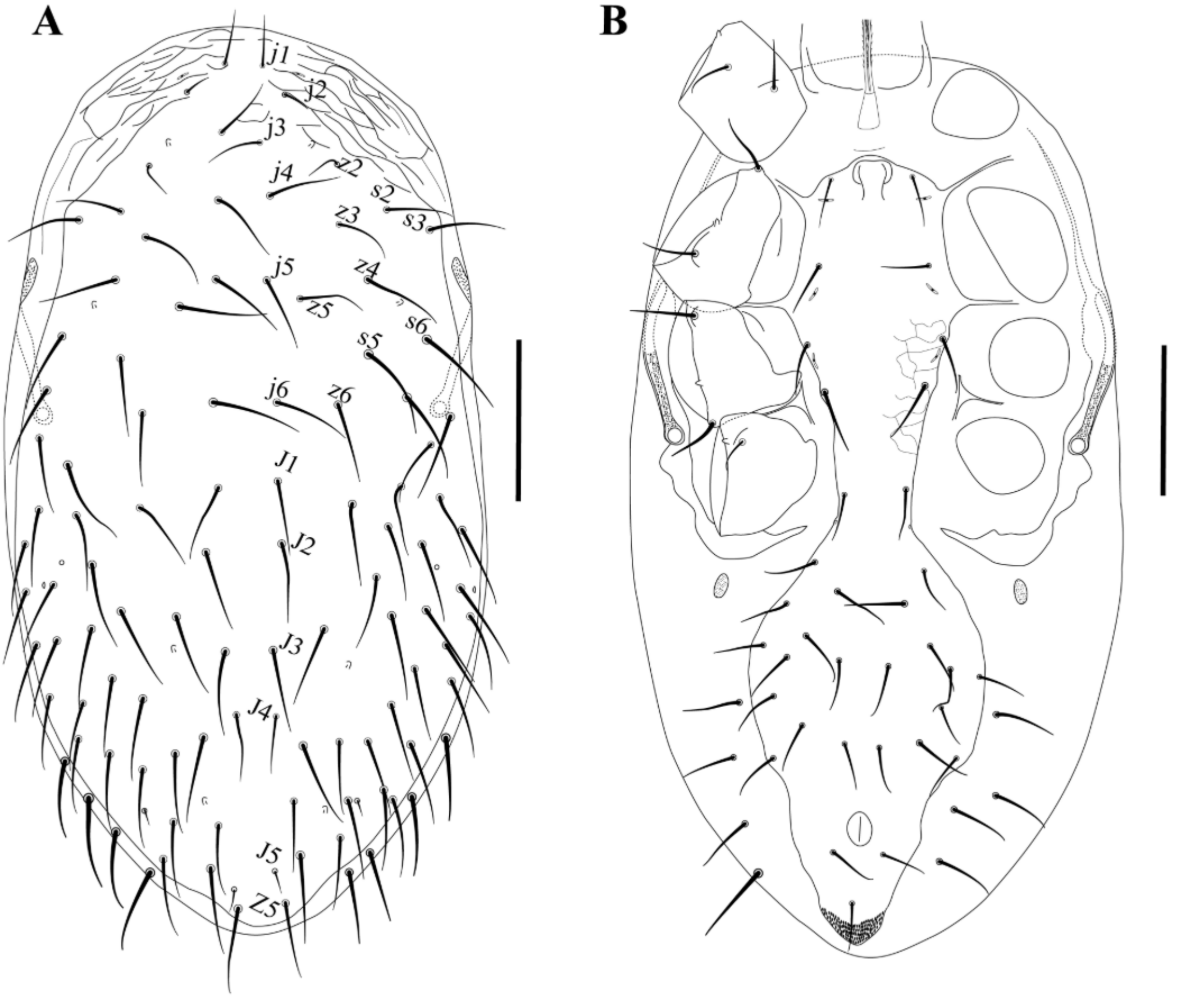

Figure 5 *Chiasmanyssus cavernicola* **n. sp.** male. A – Idiosomatic dorsum; B – Idiosomatic ventral. Scale bars: 100 µm.

1–1–1–1–1–1–1. Gnathosoma with three pairs of hypostomal setae (hyp1, hyp2 and hyp3) slender and subequal and one pair of gnathosomal setae (pc). Trochanter–tarsus chaetotaxy formula 0/2 (2); 1 1/0 1/0 1/0 1 (5); 2 1/0 1/0 1/0 1 (6); 2 2/1 2/0 3/1 3 (14) and tarsus with 16 setae + apotele forked. Disto-ventral with circular (spatulate) and membranous seta (Figure 4C) and one pair of dorsal lyrifissure on posterior margin of palpal genu (Figure 4D). Palpal trochanter has a small blade-like ventral process, not extending beyond the segment (Figure 4E). Chelicera with chelae simple and slender, without spines or other process (Figure 4F).

Description. Male — Measurements summarized in table 4

(Figures 5–7)

Idiosoma — (Figure 5A–B). As in female but smaller. *Dorsum* (Figure 5A). Dorsal shield entire, occupying most of the dorsal idiosoma; anterior margin of the dorsal shield not discernible from the soft cuticle; posterior narrow to setae J5; reticulate sculpture; dorsal shield with j and J series complete, j1, j2 and j3 on dorsal shield and J5 minute, bears approximately 56 pairs of setae; neotrichous area on opistonothal region; a pair of additional minute setae,

Figure 6 *Chiasmanyssus cavernicola* **n. sp.** male. A–C: Legs II, III and IV with chaetotaxy of setae dorsal and ventral (black). Scale bars: A 20 µm; B & C 50 µm.

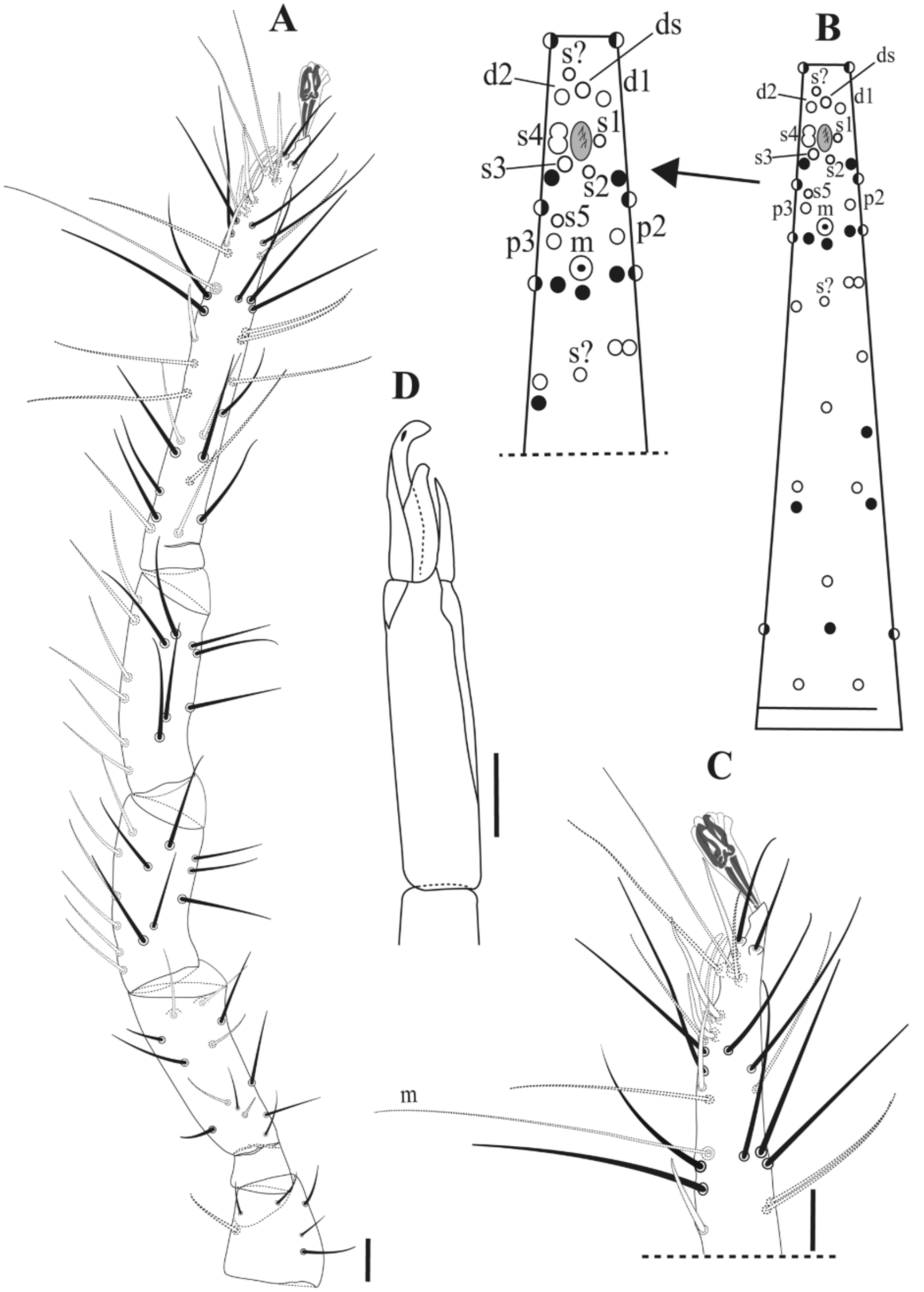

Figure 7 *Chiasmanyssus cavernicola* **n. sp.** male. A – Leg I; B – Chaetotaxy of setae in tarsus I; C – Tarsus I with setae in sensillary field; D – Chelicera. Scale bars: A, B & C 50 µm; D 20 µm.

laterally to J4 and J5, on neotrichous area of opistonothal region; one pair of lyrifissures near on margin lateral to setae j1 and six pairs of pores visible on dorsal shield, four pairs of conspicuous pores near setae j3, z4, J3, one laterally to J4 and J5 setae, and two supplementary pairs positioned close together, in the dorsal shield margin, laterally the setae J2; four pairs of caudal setae on unarmed dorsum. *Venter* (Figure 5B). Tritosternum as in female. Venter has a holoventral shield; reticulated sculptured; bears two pairs of lyrifissures and three pairs of setae on sternal region; third pair of pores near the metasternal and genital setae on shield; ventral region broadened posteriorly, with 10–11 setae on each side. Anal region with three post-anal setae, cribrum wide and anal ring present. Endopodal plates small and triangular, between coxae III and IV. Unarmed ventre with four pairs of setae. Peritrema much shorter than in female, extending from posterior margin of coxa II to anterior margin of coxa IV. Peritrematic shield anteriorly fused to dorsal shield, with nodule fused to the middle of the shield. Peritrematic shield posterior to stigma as in females.

Legs (Figure 6A–C and Figure 7A–C). Legs as in female. Chaetotaxy of legs as in female, including tarsus I sensory field (Figure 7B–C).

Gnathosoma — As in female. Palpal trochanter lacks a ventral process and chelicera with spermatodactyl short (Figure 7D).

Description. Protonymph — Measurements summarized in table 4.

(Figures 8–9).

Idiosoma — (Figure 8A–B). Smaller than adults. *Dorsum* (Figure 8A)*:* Subrectangular podonotal shield with lateral borders biconcave at level of z2 and z4, posterior region almost square; some line sculpture on dorsal shield surface; 11 pairs of setae, with j2 very close to j1; two pairs of pores near setae j3 and s5 on podonotal shield. Dorsum with two pairs of medial setae between the shields (J1–J2) and a lateral pair (J3). Mesonotal shield (or median diastema or platelets) comprising three pairs of platelets. Pygidial shield trapezoidal, as long as wide, with pairs of setae: J4–J5, Z3–Z5 and S5; J4 and Z4 much smaller than other setae (except J5, minute) on the pygidial shield; three pairs of pores near setae Z3, Z4 and J5 on pygidial shield. Unarmed dorsum has three pairs of free "nodules", two pairs large near to lateral edges and one pair very small on anterior region. Unarmed dorsum with 12 pairs of long setae, dorsal and ventral caudal setae may be present 1 or 2 minute barbs (clearly observed just under immersion). *Venter* (Figure 8B). Tritosternum as in adults. Sternal shield resembles a pentagon; three pairs of slender and long sternal setae; two pairs of lyrifissures posterior to st1 and st2. Unarmed venter with five pairs of setae, four pairs of median setae between ventral shields and a pair of caudal setae (without seta flanking the anus); setae pair between coxae IV are slender than other ventral setae. Anal shield subtriangular with postanal setae long and subequal; Peritreme short, extending from middle of coxae III to middle of coxae IV.

Legs — (Figure 9A–D). Chaetotaxy of the legs: I= 0 0/1 0/1 0 (2), 1 0/2 0/0 1 (4), 2 3/1 1/1 2 (10), 1 2/1 2/1 1 (8), 1 2/1 2/1 1 (8), 4 6/3 1/1 6/3 3 (27) + 6 solenidia+ 1 macroseta; II= 0 0/1 0/1 0 (2), 1 0/1 0/1 1 (4), 1 2/1 2/1 1 (8), 1 2/0 2/0 1 (6), 1 1/1 2/1 1 (7), 3 3/2 1/0 3/2 3 (17); III= 0 0/1 0/1 0 (2), 1 0/1 0/1 1 (4), 1 2/1 1/0 0 (5), 1 2/0 2/0 1 (6), 1 1/1 2/1 1 (7), 3 3/2 1/0 3/2 3 (17) and IV= 0 0/1 0/0 0 (1), 1 1/2 0/0 0 (4), 0 2/1 1/0 0 (4), 1 2/0 2/0 0 (5), 1 1/1 2/1 1 (7), 3 3/2 1/0 3/2 3 (17). Tarsus I sensory field has setae as in adults.

Gnathosoma — The gnathosoma, including chelicerae, similar to females, except the trochanter–tarsus palp chaetotaxy formula: 0/1 (1); 1 0/0 1/0 1/0 1 (4); 1 1/0 1/0 1/0 1 (5); 1 4/1 4/1 1 (12) and tarsus with 15 setae + forked apotele. Palpal trochanter lacks a ventral process.

Deutonymph and Larva — Unknown.

Host — Unknown

Etymology — The specific name "*cavernicola*" refers to its habitat.

Material studied — *Holotype:* Female [UFMG AC 171121], on 12-26/Aug./2013 at 18°56′10.6″S 43°24′45.5″W, in cave **CSS-09**, Conceição do Mato Dentro municipality. *Paratypes:* Female [UFMG AC 180094], on 26/Jun- 06/Jul./2018, at 19°20′35.0″S 43°18′28.8″W, in cave **MCFC-0012**, Itambé do Mato Dentro municipality; Female [UFMG AC AC171375], on 21/Nov. and 02/Dec./2016, in cave **HOLC-0090/97**, at 19°36′10.5″S

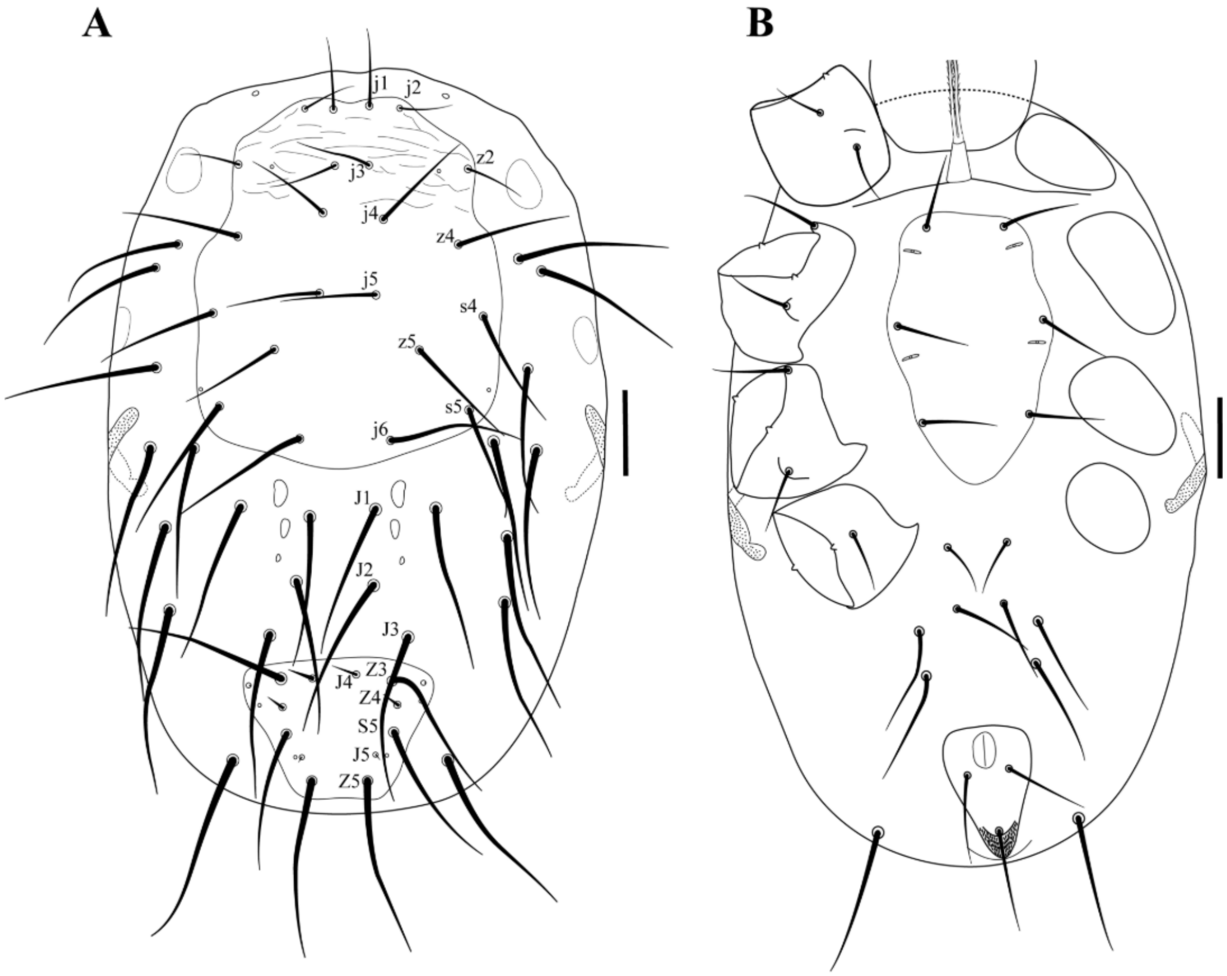

Figure 8 *Chiasmanyssus cavernicola* **n. sp.** protonymph. A – Idiosomatic dorsum; B – Idiosomatic venter. Scale bars: 50 µm.

44°00′43.1″W, Pedro Leopoldo municipality; Female [UFMG AC 180044], on 26-28/Mar./2018, at 20°12′07.8″S 43°58′03.7″W, in cave **MS-0030**, Nova Lima municipality; Female [UFMG AC 170277], on 16-26/Jan./2017 at 19°09′58.7″S 43°15′59.3″W, in cave **SPT-0180**, Conceição do Mato Dentro municipality; Three female [UFMG AC 160229, 160327, 160214] and one male [UFMG AC 160322], on 10-27/Apr./2015, at 16°08′56.0″S 44°37′39.0″W, in cave **Lapa Sem Fim**, Luizlândia municipality; Female [UFMG AC 180121], on 26/Jun. and 06/Jul./2018, at 19°19′52.2″S 43°18′41.8″W, in cave **MCFC-0009**, Itambé do Mato Dentro municipality; Female [UFMG AC 200027], male [UFMG AC 180341] and protonymph [UFMG AC 200032], on 30/Apr. and 05/May/2018, at 18°55′08.0″S 43°25′13.6″W, in cave **CSS-0007**, Conceição do Mato Dentro municipality; Protonymph [UFMG AC 180049], on 26-28/Mar./2018, 20°12′08.7″S 43°58′03.4″W, in cave **MS-0031**, Nova Lima municipality. All localities in Minas Gerais state, Brazil. Coordinates given in WGS-84 All mites were collected freely dwelling on cave floor by the staff of the company Carste Ciência e Meio Ambiente. The additional material analyzed can be seen in the supplementary material 1.

Figure 9 *Chiasmanyssus cavernicola* **n. sp.** protonymph. A–D: Legs I, II, III and IV with chaetotaxy of setae dorsal and ventral (black). Scale bars: A & B 50 µm; C & D 20 µm.

Discussion — The new genus differs from *Synasponyssus*, *Bewsiella* Domrow 1958, *Glauconyssus* Uchikawa 1991, *Ichoronyssus* Kolenati 1858 and *Parichoronyssus* by bearing less than three pairs of pores on the sternal shield. These genera have the third pair of sternal pores on the shield or as in *Synasponyssus* on the complex sternal-median shield, included in the so-called group "A" by Radovsky (2010).

Chiasmanyssus cavernicola **n. gen. n. sp.** resembles all other genera of Macronyssidae by sharing the peritrematic shield posterior to the stigma fused to the parapodal shield around the posterior margin of coxa IV in adults, a character regarded as apomorphic in the family, but absent in several genera (*Bewsiella*, *Glauconyssus*, *Ichoronyssus*, *Synasponyssus* and *Parichoronyssus*) probably close to the laelapine stem (Radovsky 2010), and by the presence of an extra seta in the tibia III, genu III and IV.

Those setae are most likely lost in some derived genera: i) all extra seta absent from *Chiroecetes*; ii) only extra setae on genu III in *Megistonyssus* Radovsky 1966; iii) tibia III and genu IV both with one extra seta in *Mitonyssoides* and *Acanthonyssus*; iv) only genu IV with one extra seta in *Macronyssus* and *Radfordiella* (one species *R. oudemansi* Fonseca 1948); v) *Pellonyssus*, *Atrichonyssus* Radovsky 2010 and *Ophionyssus* have two extra setae in genu IV. Radovsky (2010) suggests that absence or presence of one or more extra setae on any or all tibia III, genu III and IV is useful for setting apart closely related genera within the family.

Chiasmanyssus **n. gen.** differs from all other genus described by the morphology of the sternal shield in females with its distinct "X" shaped area. Some genera have the sternal shield with some distinct sclerotization but not comparable to this: *Chelanyssus* Zumpt and Till 1953 and *Steatonyssus* have a sclerotized band in posterior margin (Till and Evans 1964; Till and Evans 1966; Radovsky 1966); *Chiroptonyssus* has a heavily sclerotized posterior band extending anteriad on the sides of the shield and *Lagidonyssus* has the shield divided into two parts, which anterior part shortest and a posterior part (from first pair of pores) more strongly sclerotized and with setae st2 and st3 (Fonseca 1935b; Radovsky 1967; 2010).

The presence of the seta J5 minute is similar to *Macronyssus* and *Megistonyssus*, among the most derived genera. However, it is distinguished from these genera by the absence of anterior spur of coxa II. In *Macronyssus*, the protonymphs have only 10 setae in shield podonotal, with j1 setae are anterior to the podonotal shield and in *Megistonyssus* the pygidial shield has only four setae pairs and a setae pair flanking the anal shield in the unarmed venter (Radovsky 1966; 1967; 2010).

Chiasmanyssus **n. gen.** is closely related to genus *Cryptonyssus* Radovsky 1966. It can be set apart, however, by only five pairs of setae on venter, four between the shields and one caudal (without setae flanking the anus); shape of pygidial and podonotal shield and presence of only 12 setae pairs on the dorsal integument. Furthermore, females lack marginal setae on dorsal shield, while males lack stout or spinose anterior setae on coxae II and III (Radovsky 1966; 1967).

Despite the scanty molecular data that could be retrieved from GenBank, the 16S fragment sequenced apparently is a good bar-coding marker, since it has a considerable gap between intra- and inter-specific distances. This fragment also supported *Chiasmanyssus* **n. gen.** as an independent lineage, highly supported in a clade recovered as sister-group of *Ornithonyssus* and *Lepronyssoides*.

Finally, it remains as a mystery the host of the new species. As the large number of individuals dwelling on caves floor, vertebrates that use them as nesting sites or shelter are candidates. However, authors have been surveying bats for several months, the most abundant vertebrates found in those caves and, despite finding most of mesostigmata lineages usually associated to bats in neotropics, could not find *Chiasmanyssus* **n. gen.** on them thus far.

Keys for genus of Macronyssidae from Brazil

Females

1. Peritrematic shield posterior to the stigma not fused with the parapodal shield around the posterior margin of coxa IV; three pairs of pores on sternal shield; setae z3 on dorsum; setae J5 absent; dorsal shield with lateral and posterior portions darkened and submerged beneath striated cuticula; cinctures absent in chelae . . . . *Parichoronyssus*
— Peritrematic shield posterior to the stigma fused to the parapodal shield around the posterior margin of coxa IV; less than three pores pairs on sternal shield; setae z3 absent; setae J5 present or absent; dorsal shield without portions submerged; cinctures may be present in chelae . . . (2)

2. Genu III with 9 setae; cinctures may be present on chelae . . . . (3)
— Genu III with 10 setae; cinctures absent on chelae . . . . (5)

3. Ventral process of palpal trochanter absent; cinctures absent on chelae; coxa II without anterior spur; tibia III with nine setae . . . . *Mitonyssoides*
— Ventral process of palpal trochanter present; cinctures present on chelae; coxa II with anterior spur; tibia III with less than nine setae . . . . (4)

4. Setae J5 absent; sternal glands absent on sternal shield; accessory setae absent on epigynal shield; ventral shields lack sculpturing . . . . *Radfordiella*
— Setae J5 present and minute; sternal glands present, lateral to the first pair of pores and the glands are variable in structure on shield; accessory setae present or absent on epigynal shield; ventral shields with some distinct sculpturing . . . . *Macronyssus*

5. Epigynal shield flanked by the genital setae inserted on the unarmed integument; chelicera basally swollen . . . . *Ophionyssus*
— Epigynal shield with genital setae inserted on shield; chelicera basally not as above . . . . . (6)

6. Some or most dorsal and caudal setae of idiosoma barbed . . . . (7)
— Barbed setae absent on idiosoma (or at most 1–2 pairs of caudal setae notched or feathered) . . . . (10)

7. Ventral process of palpal trochanter absent; sternal shield with less than 3 pairs of setae . . . . *Thigmonyssus*
— Ventral process of palpal trochanter present; sternal shield with 3 pairs of setae . . . . (8)

8. Setae J3 present; J5 usually present (absent in *C. cubensis* Dusbábek 1969)]; some caudal setae are peg-like and with tip multiply barbed; setae on the legs smooth; sternal shield with a heavily sclerotized posterior band extending anteriad on the sides of shield; claw I much smaller than the others claws; leg II stout than the others legs . . . . *Chiroptonyssus*
— Setae J3 and J5 absent; caudal setae are not peg-like and with single barbed tip; setae on the legs smooth or barbed; sternal shield lacking a sclerotized band; subequal claws and legs in width . . . . (9)

9. Setae j3 present; all setae on legs smooth; sternal shield with smooth oval structures posterior to st1 and posteromedian to the first pair of pores; always three pairs of setae on sternal shield; coxa III with a posterior triangular, round-tipped spur . . . . *Lepronyssoides*
— Setae j3 absent; usually legs with barbed setae, but a single barb; sternal shield lacking ovals structure; usually three pairs of setae on sternal shield, rarely st3 are off the shield (some *O. sylviarum* and *O. hypertrichus* Radovsky 2007); coxa III with a setiform posterior seta . . . . *Ornithonyssus*

10. Dorsal shield divided . . . . (11)
— Dorsal shield entire . . . . (12)

11. Setae j3 and J5 absent; 1–2 pores pairs on sternal shield; sternal shield lacking sclerotized band; genu IV with two extra setae (11 setae) . . . . *Pellonyssus*
— Setae j3 usually present (absent in e.g. *S. evansi* Delfinado 1960, *S. benoiti* Till and Evans 1964), often much reduced; J5 usually present, rarely absent; two pores pairs on sternal shield; sternal shield with a sclerotized band in posterior margin; genu IV with one extra setae (10 setae) . . . . *Steatonyssus*

12. Setae J5 absent; dorsal shield always non-neotrichous; some coxae with ridges; claws subequal . . . . *Macronyssoides*
— Setae J5 present; dorsal shield may be neotrichous; coxae without ridges; claws may be unequal . . . . (13)

13. Dorsal shield neotrichous; setae J5 small, subequal to Z5; most 1–2 pairs of caudal setae notched or feathered; sternal shield lacking distinct sclerotization; without an anterior protusion on epigynal shield; claw I reduced relative to other claws . . . . *Mitonyssus*
— Dorsal shield non-neotrichous; setae J5 minute; none of caudal setae notched or feathered; sternal shield with distinct X-shaped area; anterior edge of epigynal shield with a protusion; claws subequal . . . . *Chiasmanyssus* **n. gen.**

Males

1. Peritrematic shield posterior to the stigma not fused, without the parapodal shield around the posterior margin of coxa IV; tarsus I with 2–4 strongly pedicellate distal setae; setae J5 absent; tibia III, genu III and IV without extra setae . . . . *Parichoronyssus*
— Peritrematic shield posterior to the stigma fused, with the parapodal shield around the posterior margin of coxa IV; tarsus I with 0–1 pedicellate distal setae setae J5 present or absent; some tibia III, genu III and IV with extra setae . . . . (2)

2. Genu III with 9 setae . . . . (3)
— Genu III with 10 setae . . . . (5)

3. Setae st1 arise in the presternal area; neotrichous on dorsal shield . . . . *Mitonyssoides*
— Setae st1 arise in the esternal shield; non-neotrichous on dorsal shield . . . . (4)

4. Setae pair J5 present and minute; ventral process of palpal trochanter with a strong longitudinal ridge; dorsal and/or caudal margin may bear some setae long and flagelliform; fixed chela with a tiny pilus dentilis; leg I slender than or equal to the other legs; all claws small; coxa II with anterior spur entire (except *M. contrictus* with bifid tip) . . . . *Macronyssus*
— Setae pair J5 absent; without ventral process of palpal trochanter or at most with a line showing its position; dorsal and/or caudal margin never flagelliform; fixed chela without a tiny pilus dentilis; leg I and II stout but leg II may be slightly to much stronger than the other legs; all claws are strong; coxa II with anterior spur bifid or 2 subequal spur . . . . *Radfordiella*

5. Some setae barbed on idiosoma at least caudally with 1–3 barbed setae . . . . (6)
— All setae smooth (or 1–2 pairs of caudal setae notched or feathered) on idiosoma . . . . (9)

6. Setae j1 and j2 off dorsal shield . . . . *Thigmonyssus*
— Setae j1 and j2 on dorsal shield . . . . (7)

7. Palpal femur without enlarged lateral setae and one pedicel; J5 usually present (absent *C. cubensis*, small); venter usually has sternogenital and ventrianal shields or rarely holoventral shield . . . . *Chiroptonyssus*
— Palpal femur with spinose seta and on a lateral pedicel; J5 absent; venter always has a holoventral shield . . . . (8)

8. Setae j3 absent; setae in the opisthonotal region, not including neotrichae behind J2; 1 or 2 enlarged lateral setae on palp femur, of which the anterior one on a prominent pedicel; all legs with some dorsal setae barbed . . . . *Ornithonyssus*
— Setae j3 present; setae in the opisthonotal region, including about 3–4 neotrichae behind J2; palpal femur has only 1 enlarged lateral seta on pedicel; legs setae lack barbs . . . . *Lepronyssoides*

9. Venter with sternogenital and anal shields, the ventral region unarmed; genital setae inserted on the unarmed integument flanking the sternogenital shield; Chelicera is basally swollen as in the female . . . . *Ophionyssus*
— Venter with a holoventral shield or sternogenital and ventrianal shields; genital setae on the shield; Chelicera basally not as above . . . . (10)

10. 1–2 pairs of caudal setae notched or feathered; claws I greatly reduced relative to other claws . . . . *Mitonyssus*
— None setae as above; claws I not reduced relative to other claws . . . . (11)

11. Genu IV with 2 extra setae (11 setae) . . . . *Pellonyssus*
— Genu IV with 1 extra seta (10 setae) . . . . (12)

12. Setae J5 absent; some setae on dorsal shield with expanded tip . . . . *Macronyssoides*
— Setae J5 present or absent; none setae on dorsal shield with expanded tip . . . . (13)

13. Setae J5 minute; j3 subequal to other setae on shield; anterior spur in coxa II absent; coxa III has an anterior seta setiform . . . . *Chiasmanyssus* **n. gen.**
— Setae J5 when present small, not minute; setae j3 very reduced or absent; anterior spur in coxa II present with tip divided into several denticles; coxa III has an anterior seta ranging from normal setiform to regular spinose to spinose with a blade-like proximal expansion . . . . *Steatonyssus*

Protonymphs

1. Setae j1 anterior to podonotal shield or may be on margin of podonotal shield . . . . (2)
— Setae j1 on podonotal shield . . . . (4)

2. Setae j2 off podonotal shield . . . . *Thigmonyssus*
— Setae j2 on podonotal shield . . . . (3)

3. Podonotal shield with 10 setae pairs; setae j3 present; J5 present and minute; pygidial shield with 6–7 setae pairs; 2 median setae between dorsal shield; normally 4 setae pairs between ventral shields but may be more than 10 setae pairs when neotrichous . . . . *Macronyssus*
— Podonotal shield with 8–9 setal pairs; setae j3 absent; J5 absent; pygidial shield with 1–3 setae pairs; 3 median setae between dorsal shield; 2–3 setae pairs between ventral shields, never neotrichous . . . . *Pellonyssus*

4. Usually no more than two median setal pairs between dorsal shields, a posterior pair (J3) may be present in integument, in which case it is shifted laterally . . . . (5)

— About 3–8 or more median setal pairs between dorsal shields, setae J3 pair when present on integument, not laterally shifted . . . . (9)

5. Chelicera with a swollen base; on tarsus I, seta s2 small much less than half the length of seta s1 . . . . *Ophionyssus*
— Chelicera with an approximately uniform base in width throughout its length; seta s2 on tarsus I not as above . . . . (6)

6. Setae J5 present and minute; pygidial shield with six setae pairs . . . . *Chiasmanyssus* **n. gen.**
— Setae J5 absent; pygidial shield no more than four setae pairs . . . . (7)

7. Pygidial shield with two setae pairs; sensory field of tarsus 1 with 2–4 pedicillate distal setae; genital setae pair absent; two pairs flanking the anal shield; none extra seta on tibia III, genu III and IV . . . . *Parichoronyssus*
— Pygidial shield with 3–4 setae pairs; sensory field of tarsus 1 with 0–1 pedicillate distal setae; genital setae pair present; 0–1 pairs flanking the anal shield; all tibia III, genu III and IV with one extra seta or at least genu IV with an extra seta . . . . (8)

8. Setae j3 present, may be reduced but always represented by the trichopores; pygidial shield with a pair of enlarged anterolateral pores; pygidial shield normal or much reduced; none extra seta in tibia III and genu III but genu IV may be with one extra seta; claw I not larger than other legs; peritreme lacking a post-stigmal part . . . . *Radfordiella*
— Setae j3 present and subequal other shield setae; pygidial shield lacking enlarged pores; pygidial shield always normal; one extra seta on tibia III, genu III and IV; claw I larger than those of the other legs; peritreme with a post-stigmal part . . . . *Macronyssoides*

9. Three median setal pairs between dorsal shields (non-neotrichous) . . . . (10)
— Four or more median setal pairs between dorsal shields (neotrichous) . . . . (12)

10. All setae smooth on idiosoma . . . . *Steatonyssus*
— Barbed setae on idiosoma, usually single barb . . . . (11)

11. Podonotal shield with 10 pairs of setae; j3 absent; Z4 not reduced; all legs with some barbed setae . . . . *Ornithonyssus*
— Podonotal shield with 11 pairs of setae; j3 present; Z4 reduced; all legs with smooth setae . . . . *Lepronyssoides*

12. Venter with only 3 pairs of setae between the ventral shield . . . . *Mitonyssoides*
— Venter with more than 3 pairs of setae between the ventral shields . . . . (13)

13. Venter neotrichous with about 5 or 6 pairs of setae between shields (except *C. venezolanus*); some caudal setae on the pygidial shield peg-like and/or with short barbs; leg II much stouter than other legs; claws II much larger than other claws and claws I as claws III and IV . . . . *Chiroptonyssus*
— Venter neotrichous with about 8–11 pairs of setae between shields; some caudal setae on the pygidial shield; leg II with normal thickness as the other legs; claws II subequal other claws but claws I much reduced or absent . . . . *Mitonyssus*

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CAPÍTULO II – Host discovery and new locality records of cave-dwelling mite *Chiasmanyssus cavernicola* Gomes-Almeida & Pepato, 2021 (Acari: Macronyssidae) from Minas Gerais state, Brazil

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Abstract

The family Macronyssidae comprises 35 genera that parasitize a wide range of vertebrates. Among them, *Chiasmanyssus* is a recently described monotypic genus in Brazil, known exclusive from specimens collected on cave soil in Minas Gerais, Pará, and Rio de Janeiro states. However, the host for this mite species remains unknown. Here, we report for the first time the occurrence of *C. cavernicola* associated with bat hosts, including *Anoura caudifer, Carollia perspicillata*, *Chrotopterus auritus*, *Desmodus rotundus*, *Diphyla ecaudata, Micronycteris megalotis* and *Mimon bennetti*. Additionally, we present new records within and surrounding caves in the karstic region of Pains and four state conservation units: Parque Serra do Rola Moça, Parque do Sumidouro, Parque da Lapa Grande and Parque Serra Nova e Talhado, expanding the geographical range for this mite from north to south in Minas Gerais state, Brazil.

Keywords

Host bats; distribution; ectoparasites mites; Dermanyssoidea; Mesostigmata; new records; caves

Introduction

The family Macronyssidae Oudemans, 1936 comprises 35 genera known worldwide (Radovsky 2010; Bassini-Silva *et al.* 2021; Gomes-Almeida and Pepato 2021). This family includes mainly ectoparasitic mites that are blood-feeding (hematophagous) and infest various vertebrates, such as bats, marsupials, rodents, birds, reptiles and humans (Radovsky 1966; Radovsky 2010). Some species of this family have medical and economic importance (Teixeira *et al.* 2020; Bassini-Silva *et al.* 2022). Macronyssid mites have a semi-permanent ectoparasitic life cycle, alternating between periods on host and on substrate, or roost or nest of host. Out of the five life cycle stages, egg, larva, protonymph, deutonymph, and adults (male and female), only the protonymph and adults actively feed on the host (Fonseca 1948; Dowling 2006; Saunders and Yunker 1975; Radovsky 1966; 1967; 2010).

The genus *Chiasmanyssus* is monotypic and recently described from Brazil, with its type species *Chiasmanyssus cavernicola*. According to Gomes-Almeida and Pepato (2021), this genus may be distinguished from other Macronyssidae genera by: sternal shield with a distinct X-shaped area, bearing three pairs of setae and two pairs of pores; sternal gland absent; anterior spur on coxa II lacking; adults with dorsal shield entire and j and J series complete; adults and protonymphs with setae J5 minute; males with strong-pores on dorsal shield and peritrema short extending from posterior margin of coxae II to the anterior margin of coxae IV. Despite knowing that the species is parasitic, many individuals collected had been engorged, the host was unknown until now, since the specimens examined and used for describing the species were all collected on cave soil in Minas Gerais, Pará, and Rio de Janeiro states (Gomes-Almeida and Pepato 2021).

Thus, here, we report for the first time the occurrence of cave-dwelling mites *C. cavernicola* associated with bat hosts, seven Phyllostomidae bat species. Additionally, we report new records within and surrounding caves in protected and non-protected areas from Minas Gerais, which expands the geographical range of this mite from north to south of the state.

Material and methods

Mites were collected from 25 localities on caves, epigean habitats and artificial galleries in Minas Gerais State, Brazil (Table 1). Most mites were preserved in 70% alcohol at room temperature and kept in 95–100% ethanol at -20°C upon acceptance at the *Centro de Coleções Taxonomicas da Universidade Federal de Minas Gerais* (CCT-UFMG) acarological collection and in the Collection of Subterranean Invertebrates from Lavras (ISLA-UFLA), Federal University of Lavras, Biology Department, Zoology Sector, Lavras, Minas Gerais, Brazil. Individuals newly collected by authors were preserved directly in 95–100% alcohol, refrigerated in the field, and then stored at -20 °C. The latter was collected under ICMBio, SISBIO 71120–4 license, along with the "Instituto Estadual de Florestas (IEF 009/2020)" of Minas Gerais state, besides following the ethics committee for animal use in research "Comissão de Ética no Uso de Animais (CEUA)" of UFMG, with protocol number 50/2020).

Mites were actively sampled on cave soil and on the body of seven phyllostomid bats, representing seven species distributed in seven genera, which were captured with mist nets. Mites parasitizing hosts were delicately removed using fine pincers and alcohol-soaked brushes, and preserved in absolute ethanol (95–100%) for identification. Hosts were identified in the field following keys proposed by Gardner (2008), supplemented by Lemos *et al.* (2020) and Velazco and Patterson (2019) for *Carollia* species, and Hurtado-Miranda and Pacheco-Torres (2014) for *Mimon* species. Subsequently, hosts were released at the exact location of capture and, in cases of uncertain identification, up to two voucher specimens being euthanized for detailed laboratory identification and later deposited at the CCT-UFMG Mastozoological collection.

Mites were mounted on permanent microscope slides using Hoyer's medium (Walter and Krantz 2009) for morphology examination. Identification and microphotographs were performed with a Leica DM 750 optical microscope equipped with an ICC50W digital camera. Besides, mites identification followed the keys proposed by Radovsky (2010) and Gomes-Almeida and Pepato (2021). The terminology for dorsal and ventral chaetotaxy, as well as the nomenclature of the dorsum, followed Radovsky (2010). Sternal pores terminology is based on Evans and Till (1979). The preparation of the map was performed using R Software (v4.1.2; R Core Team 2021). All figures were prepared using Krita program (v4.4.2; Krita 2020).

Results and Discussion

A total of 172 specimens of macronyssid mites, including protonymphs and adults, have been collected parasitizing bats from Phyllostomidae family and on cave soil from 25 hypogean or associated localities in Minas Gerais, Brazil. These mites have been identified morphologically as *Chiasmanyssus cavernicola* (Figures 1-2).

Our findings revealed seven Phyllostomidae species as hosts of *C. cavernicola*. It is noteworthy that even mites collected off-host have been found in caves and others subterranean habitat inhabited by bats, vertebrates that use caves as shelter efficiently and permanently (Kunz 1982).

Of the nine families of the order Chiroptera that occur in Brazil, Phyllostomidae is the most diverse and abundant, with 94 species distributed in 43 genera (Nogueira *et al.* 2014; Garbino *et al.* 2020; Garbino *et al.* 2022). They are widely distributed bats occupying a variety of trophic guilds and habitats in different biomes of Brazil, and provide important ecosystem services (Kunz *et al.* 2010). Their wide geographic distribution suggests that geographic distribution of *C. cavernicola* mite may also be extensive due to its association with them (Figure 3).

Anoura caudifer, Diphylla ecaudata, *Chrotopterus auritus*, *Desmodus rotundus*, *Carollia perspicillata, Micronycteris megalotis* and *Mimon bennettii*, all of which are commonly found in Brazilian caves (Guimarães and Ferreira 2014) (Figure 4), are among the phyllostomid bat species infested by *C. cavernicola*. This association suggests a possible close relationship between this semi-permanent parasitic mite and cave-dwelling habitat.

Furthermore, high affinity with Phyllostomidae is observed for *C. cavernicola*, but is not specific to a single bat species, indicating a wide host range, similar to other genera of Macronyssidae (Radovsky 2010).

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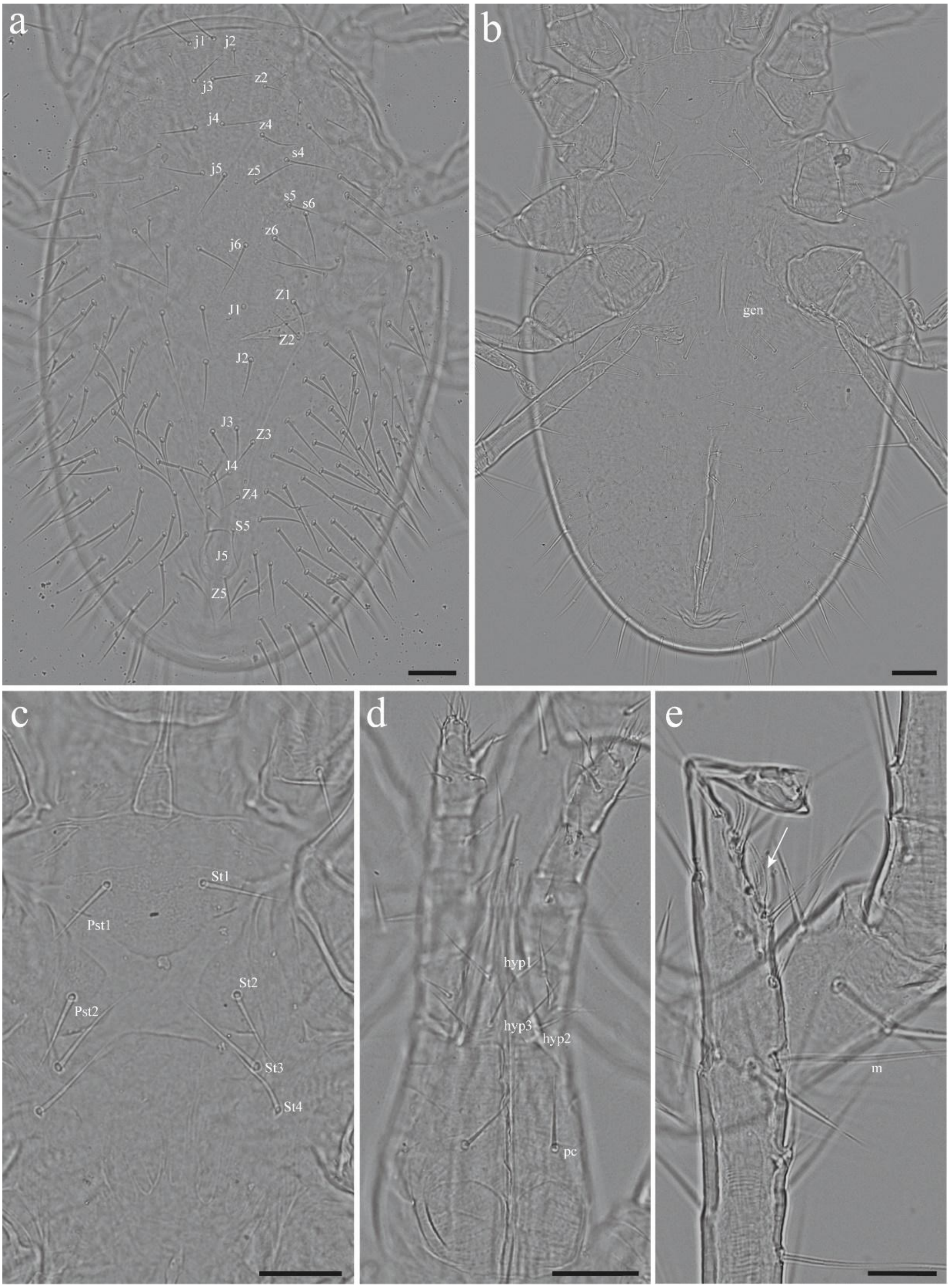

Figure 1 *Chiasmanyssus cavernicola* female. a – Idiosoma, dorsum, b – Idiosoma, ventral, c –Sternal shield, note the distinct X-shaped sclerotization, d – Gnathosoma, ventral, e – Tarsus I with setae in the sensillary field. Scale bars: a & b 100 µm, c & d 50 µm, e 25 µm.

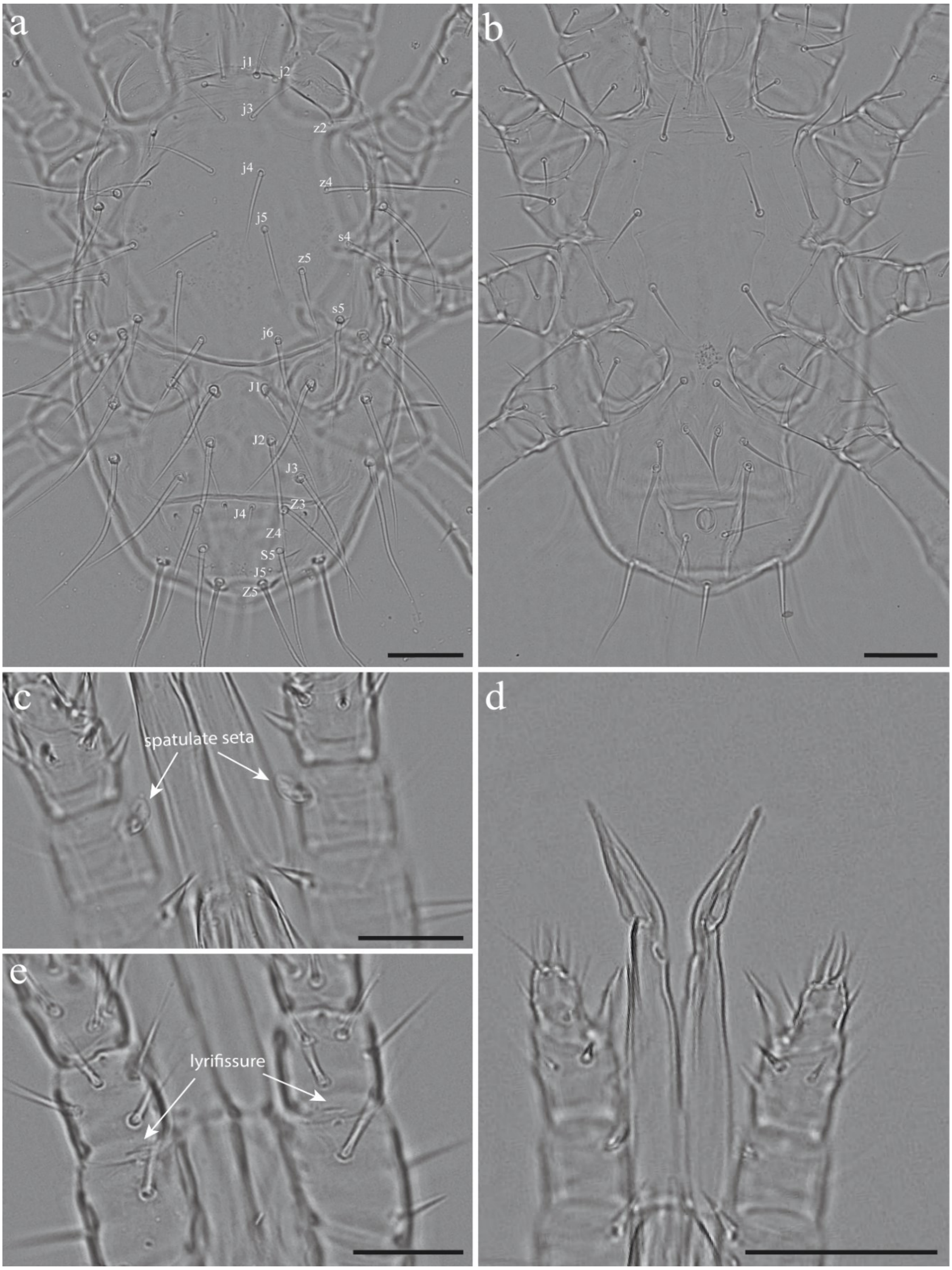

Figure 2 *Chiasmanyssus cavernicola* protonymph. a – Idiosoma, dorsum, b – Idiosoma, ventral, c – Venter palpal genu with distinct circular (spatulate) and membranous seta, d – Chelicera, e – Dorsal palpal genu with lyrifissure. Scale bars: a, b & d 50 µm, c & e 25 µm.

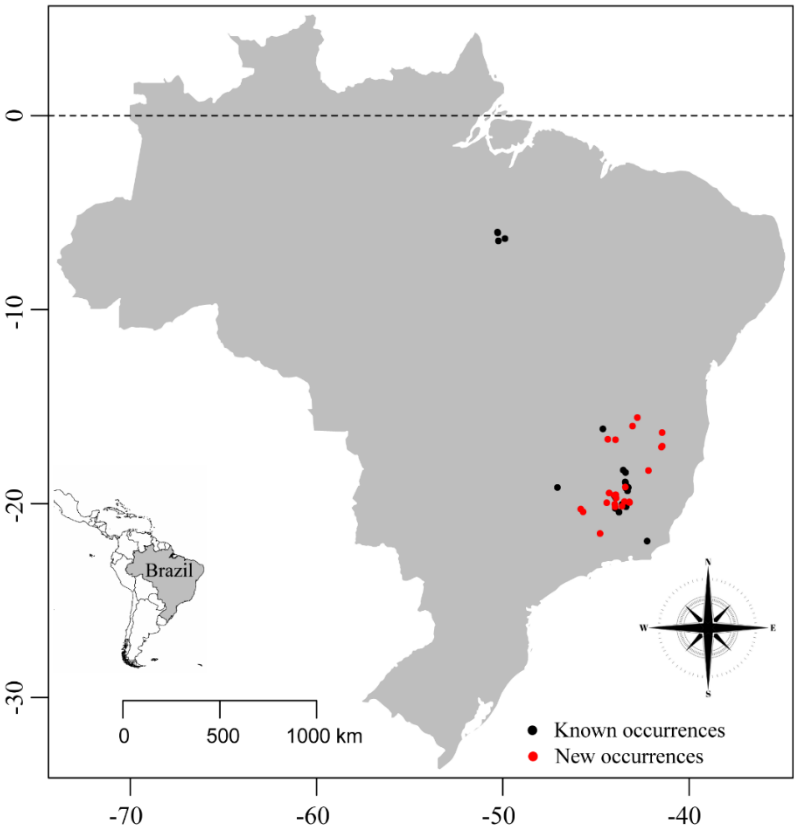

Figure 3 Map showing the distribution of *Chiasmanyssus cavernicola* from Brazil. Black points are literature reports and red points are new occurrences at subterranean habitat or karst areas.

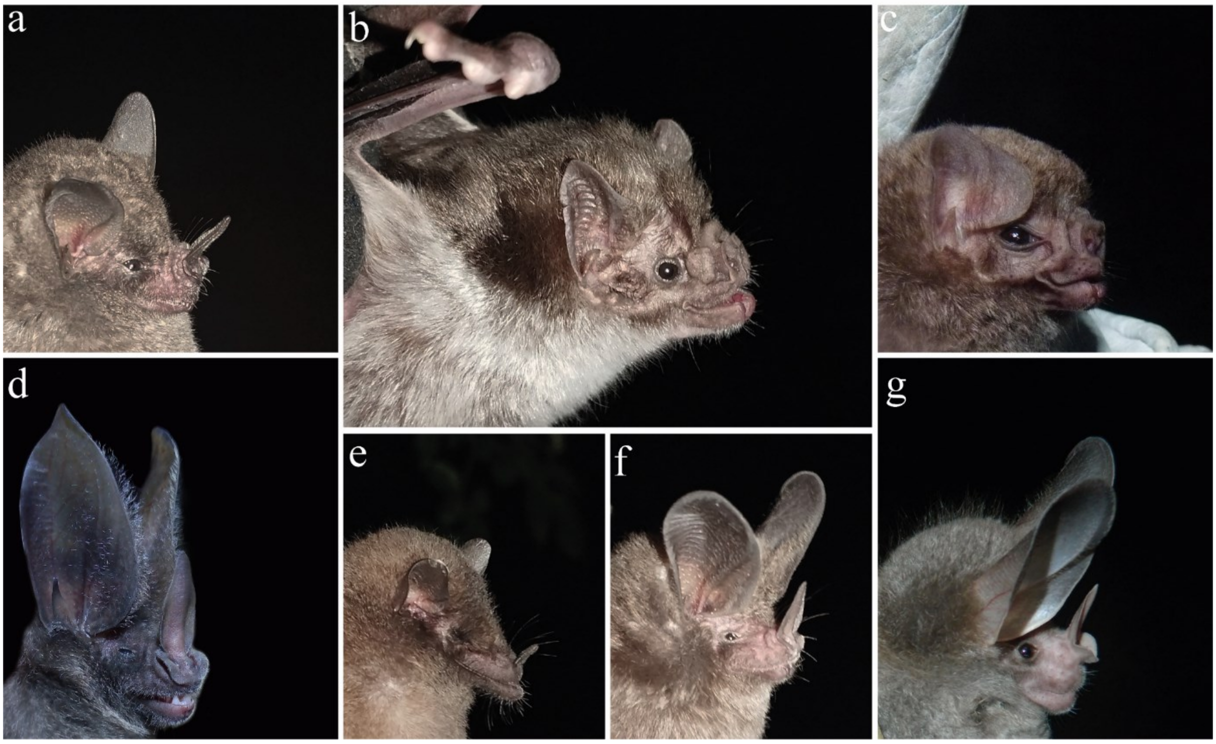

Figure 4. Hosts bat of *Chiasmanyssus cavernicola*. a – *Carollia perspicillata*, b – *Desmodus rotundus*, c – *Diphylla ecaudata*, d – *Mimon bennettii*, e – *Anoura caudifer,* f – *Micronycteris megalotis,* g – *Chrotopterus auritus*. Photos: Fred Victor Oliveira.

Table 1. Detailed sampling data of specimens with collection number, stage, sex, host, cave and locality.

Deposited	Instar/sex	Bat host or cave soil	Habitat	Locality, Data, Colector, Coordinates
UFMG AC 220032	PN	*Desmodus rotundus* (É. Geoffroy, 1810)	Cave, Lapa do Sumidouro	Parque Estadual do Sumidouro, Lagoa Santa, 10.viii.2021, Gomes-Almeida B, Ribeiro DB, Letícia Oliveira, Lat -19.5418 Long -43.9411
UFMG AC 220033	F	*De. rotundus*	Cave, Lapa do Sumidouro	Parque Estadual do Sumidouro, Lagoa Santa, 10.viii.2021, Gomes-Almeida B, Ribeiro DB; Oliveira LL, Lat -19.5418, Long -43.9411
UFMG AC 220035	PN	*De. rotundus*	Cave, Lapa do Sumidouro	Parque Estadual do Sumidouro, Lagoa Santa, 10.viii.2021, Gomes-Almeida B, Ribeiro DB, Oliveira LL, Lat -19.5418, Long -43.9411
UFMG AC 220081	PN	*Carollia perspicillata* (Linnaeus, 1758)	Cave RM 33	Parque Estadual Serra do Rola Moça, Nova Lima, 24.viii.2021, Gomes-Almeida B, Oliveira LL, Bárbara Faleiro, Lat -20.0349, Long -43.9946
UFMG AC 220082	PN	*Ca. perspicillata*	Cave RM 33	Parque Estadual Serra do Rola Moça, Nova Lima, 24.viii.2021, Gomes-Almeida B, Oliveira LL, Bárbara Faleiro, Lat -20.0349, Long -43.9946
UFMG AC 220106	PN	*Diphylla ecaudata* Spix, 1823	Lapa Grande cave	Parque Estadual Lapa Grande, Montes Claros, 13.xii.2021, Gomes-Almeida B, Ribeiro DB, Oliveira LL, Lat -16.7067, Long -43.9549
UFMG AC 220170	F	*Mimon bennetti* (Gray, 1838)	Artificial gallery	Parque Estadual Serra Nova e Talhado, Distrito Serra Nova, Rio Pardo de Minas, 17.xii.2021, Gomes-Almeida B, Ribeiro DB, Oliveira LL, Lat -15.5642, Long -42.7781
UFMG AC 220499	PN	soil	Cave, Campi-024	Pedro Leopoldo, 24.ix.2014, Equipe Spelayon, Lat -19.5697, Long -44.0111
UFMG AC 220500	F	soil	Cave 003	Dumbá, Vespasiano, 08.viii.2014, Equipe Spelayon, Lat -19.7145, Long -43.9153
UFMG AC 220502	F	soil	Cave Simmons	São Gonçalo do Rio Abaixo, X.2016, Bernardi LFO, -19.8986, Long -43.4727
UFMG AC 220504	PN	soil	Cave 0021/22	Morro do pilar, 12-24.ix.2021, Renata Andrade *et al.*, Lat -19.1488, Long -43.4291
UFMG AC 220506	M	soil	Cave, Lapa sem fim	Luislândia, 18.iv.2014, Carste *et al.*, Lat -16.1488, Long -44.6275

UFMG AC 220507	PN	soil	Cave, Lapa sem fim	Luislândia, 18.iv.2014, Carste *et al.*, Lat -16.1488, Long -44.6275
UFMG AC 220511	F	soil	Cave, unnamed	Coração de Jesus, iv.2014, unknown collectors and geographical coordinates
UFMG AC 220514	PN	soil	Cave, unnamed	Riacho dos Machados, xi.2015, unknown collectors and geographical coordinates
UFMG AC 220515	PN	soil	Cave, unnamed	Riacho dos Machados, xi.2015, unknown collectors and geographical coordinates
UFMG AC 221074	PN	*De. rotundus*	Cave, Lapa das Pacas	Parque Estadual do Sumidouro, Lagoa Santa, 15.XII.2020, Gomes-Almeida B, Geremias S, Torres R, Lat -19.5606 Long -43.9667
UFMG AC 221085	PN	*Ca. perspicillata*	Cave, Gruta RM 33	Parque Estadual Serra do Rola Moça, Nova Lima, 24.VIII.2021, Gomes-Almeida B, Oliveira LL, Bárbara Faleiro, Lat -20.0349 Long -43.9946
UFMG AC 221131	PN	*Chrotopterus auritus* (Peters, 1856)	Epigean habitat	Karst area, Pains, 27.I.2021, Tahara A, Genelhú S, Lat -20.4248, Long -45.6928
ISLA	F	soil	Cave, AGL 007	Rio Piracicaba, 04.vii.2023, Ativo Ambiental Consulting, Lat -19.914491 Long -43.210046
ISLA	F, PN	soil	Cave, AGL 006	Rio Piracicaba, 05.iii.2021, Ativo Ambiental Consulting, Lat -19.949161 Long -43.201096
ISLA	F, PN	*De. rotundus*	Artificial gallery	Mateus Leme, 15.vii.2009, Bernardi LFO, Lat -19.959395 Long -44.422326
ISLA	F, PN	*Di. ecaudata*	Artificial gallery	Mateus Leme, 15.vii.2009, Bernardi LFO, Lat -19.959395 Long -44.422326
ISLA	F, PN	*Micronycteris megalotis* (Gray 1842)	Artificial gallery	Mateus Leme, 15.vii.2009, Bernardi LFO, Lat -19.959395 Long -44.422326
ISLA	F, PN	soil	Artificial gallery	Mateus Leme, 15.vii.2009, Bernardi LFO, Lat -19.959395 Long -44.422326
ISLA	F, PN	*De. rotundus*	Artificial gallery	Medina, 2009, Bernardi LFO, vii.2009, Lat -16.340325 Long -41.451245
ISLA	F, PN	*Mic. megalotis*	Artificial gallery	Medina, 2009, Bernardi LFO, vii.2009, Lat -16.340325 Long -41.451245

ISLA	F, PN	soil	Artificial gallery	Medina, 2009, Bernardi LFO, vii.2009, Lat -16.340325 Long -41.451245
ISLA	F, PN	soil	Artificial gallery	São José da Safira, vii.2009, Bernardi LFO, Lat -18.296562 Long -42.183090
ISLA	F, PN	*Anoura caudifer* (É. Geoffroy, 1818)	Artificial gallery	Padre Paraíso, vii.2008, Bernardi LFO, Lat -17.040842 Long -41.444535
ISLA	F, PN	soil	Artificial gallery	Padre Paraíso, vii.2008, Bernardi LFO, Lat -17.040842 Long -41.444535
ISLA	F	soil	Cave, Cap1-0003	Serra do Gandarela, Nova Lima, 21.iii.2022, Lat -20.134061 Long -43.603512
ISLA	F	soil	Cave Veneza	Nova Lima, 2022, Ativo Ambiental Consulting, Lat -20.170081 Long -43.978197
ISLA	F, PN	soil	CGP-008	Sete Lagoas, xi.2021, Candiani DF, Guarda DD, Lat -19.456947 Long -44.293854
ISLA	F	soil	JAT 96/97	Doresópolis, xii.2022, Reis A, Lat -20.285208 Long -45.817338
ISLA	F, PN	soil	Cave Mandembe	Luminárias, 2007, Bernardi LFO, Lat -21.543917 Log -44.784006°
ISLA	F	*Mic. megalotis*	Cave Fazenda Boa Vista	Padre Paraíso, vii.2008, Bernardi LFO, Lat -17.089338 Long-41.489056
ISLA	PN	*De. rotundus*	Cave Fazenda Boa Vista	Padre Paraíso, vii.2008, Bernardi LFO, Lat -17.089338 Long-41.489056
ISLA	F, PN	soil	Cave Fazenda Boa Vista	Padre Paraíso, vii.2008, Bernardi LFO, Lat -17.089338 Long-41.489056

CAPÍTULO III – First multi-instar descriptions of cave-dwelling *Whartonia* Ewing, 1944 (Parasitengona, Leeuwenhoekiidae) from Brazil through integrative taxonomy

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First multi-instar descriptions of cave-dwelling *Whartonia* Ewing, 1944 (Parasitengona, Leeuwenhoekiidae) from Brazil through integrative taxonomy

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Abstract

For the first time deutonymphs and adults (females and males) of species belonging to *Whartonia*, a genus with post-larval stages commonly found in caves and larvae as ectoparasites of bats, are described. Two species from new localities in states of Pará (Eastern Amazon) and Minas Gerais (southeastern Brazil) are reported. Post-larval cave dwelling mites could be unambiguously associated with *W.* (*W*). *pachywhartoni* and *W.* (*W.*) *nudosetosa* larvae using mitochondrial COI and nuclear ribosomal 28S sequence data, despite apparent oversplitting by species delimitation methods. They are distinguished from closely related *Albeckia senase*, a species with described deutonymphs, by having eyes and by the shape of idiosomal setae. They are very similar to each other, being distinguished mainly by the distribution of special setae on legs, and shape of idiosomal setae. Detailed redescriptions of the two larvae are presented based on individuals found on bats belonging to nine species, with six new host species association: *Anoura caudifer*, *Diphylla ecaudata*, *Mimon bennettii*, *Platyrrhinus lineatus*, *Sturnira lilium* (Phyllostomidae) and *Peropteryx trinitatis* (Emballonuridae).

Keywords: Chiggers, post-larval, species delimitation, bat ectoparasites, soil cave

Introduction

Whartonia Ewing, 1944, currently classified in the family Leeuwenhoekiidae Womersley, 1944, comprises 40 described species, with occurrences over tropical and subtropical regions around the world (Nielsen *et al.* 2021; Bassini-Silva *et al.* 2022a). The genus is classified into four subgenera according to Nielsen *et al.* (2021): *Whartonia* s.s. Ewing, 1944, *Asolentria* Vercammen-Grandjean, 1968, *Fascutonia* Vercammen-Grandjean, Langston & Audy, 1973, and *Brennanella* Radford, 1954. The first three subgenera are defined by Vercammen-Grandjean *et al.* (1973) by leg chaetotaxy of larvae: *Whartonia* has 6, 5, 4 femoral branched setae on legs I, II, and III, with a solenidion (ω) on the posterior tarsus III; *Asolentria* also has 6, 5, 4 femoral branched setae, but without ω on tarsus III; *Fascutonia* has 6, 6, 5, without ω on the tarsus III. The subgenus *Brennannella* was previously described by Radford (1954) as a separated genus based on *W.* (*B.*) *longispina* (Radford, 1954) and later included by Vercammen-Grandjean *et al.* (1965) in the subgenus *Whartonia*. Both articles, however, did not include a complete account of *W.* (*B.*) *longispina* chaetotaxy, precluding a complete comparison of this subgenus with the others without a restudy of the type material of *W.* (*B.*) *longispina*.

Similar to other Parasitengona, *Whartonia* deutonymphs and adults (males and females) differ from larvae (Zhang 1998), the former being free-living predators on soil, while the latter being mostly bat associated ectoparasites, rarely associated with other mammals (Takahashi *et al.* 2006). It precludes the description of post-larval individuals, requiring experimental rearing of mites or species delimitation methods based on molecular data.

No species belonging to *Whartonia* had their post-larval stages described. A closely related species, previously included in the genus, *Albeckia senase* (Greenberg, 1952), had the deutonymph described and may be useful for comparisons (Crossley 1960). Larvae belonging to the genus *Albeckia* Vercammen-Grandjean & Watkins, 1966 differ from *Whartonia* by its reduced palptarsal chaetotaxy (only four setae 4Bω), absence of dorsal teeth on cheliceral blades and microsetae on genua I–II, and stubby or clubbed microsetae on tibiae I (Vercammen-Grandjean & Watkins 1966; Reed & Brennan 1975), otherwise being similar. Indeed, *Albeckia senase* was referred to as belonging to *Whartonia* in recent articles (e.g., Takahashi *et al.* 2006).

Three larval species are recorded from Brazil thus far: *Whartonia* (*Whartonia*) *nudosetosa* (Wharton, 1938), *W.* (*W.*) *pachywhartoni* Vercammen-Grandjean, 1966 and *W.* (*W.*) *parauapebensis* Bassini-Silva *et al.*, 2022. *Whartonia* (*W.*) *nudosetosa* is a widespread species, occurring from Mexico and Central America to Minas Gerais state; *Whartonia* (*W.*) *pachywhartoni* occurs only in Brazil, originally described from a specimen collected on bat *Micronycteris megalotis* (Gray, 1842) in the XIX century and later reported on bat *Carollia perspicillata* (Linnaeus, 1758) (Silveira *et al.* 2015); and *W.* (*W.*) *parauapebensis* was recently described, reported on soil from caves in the state of Pará (Bassini-Silva *et al.* 2022a).

Here we present the first description including all active instars of *Whartonia* (*W.*) *pachywhartoni* and *W.* (*W.*) *nudosetosa*: larvae, deutonymphs, and adults (males and females). This could be achieved by employing an integrative approach using multigene species delimitation techniques (Costa *et al.* 2019). Furthermore, six new bat hosts could be reported and the zonation inside caves were recorded for most post-larval individuals of both species.

Material and methods

Specimen sampling and preservation

A total of 52 *Whartonia,* individuals comprising various developmental stages were collected in 27 localities in Minas Gerais and Pará states, Brazil (Figure 1A and Table 1). Most mites were preserved in 70% alcohol at room temperature and kept in 95–100% ethanol at -20 °C upon acceptance at the *Centro de Coleções Taxonomicas da Universidade Federal de Minas Gerais* (CCT-UFMG) acarological collection. Individuals newly collected by authors were preserved directly in 95–100% alcohol, refrigerated in the field, and then stored at -20 °C. The latter was collected under ICMBio, SISBIO 71120–4 license, authorized by the Instituto Estadual de Florestas (IEF 009/2020) of Minas Gerais state, and in accordance with the precepts of the ethics committee for animal use in research "Comissão de Ética no Uso de Animais (CEUA)" of Universidade Federal de Minas Gerais (UFMG), protocol number 50/2020. Access to the genetic heritage from Brazilian mites was registered in SisGen (register number: A0C4F58).

Larval mites were collected from bats by careful examination of these animals, using fine pincers and brushes soaked in alcohol for their removal. Most bats were freed after being identified using keys to neotropical species (Vizotto & Taddei 1973; Jones & Hood 1993; Gardner 2007), and a small fragment of patagium skin was collected for future DNA barcoding identification. Post-larval stages of mites were obtained by active search on cave floor and epigean environment (Wynne *et al.* 2019). Caves were divided into zones according to the incidence of epigeal light through the researcher's visual accuracy, being (1) entrance zone, close to an opening, where light incidence is direct; (2) twilight zone, where light incidence is indirect; (3) aphotic zone, where there is no external light, total absence of light. Winkler's extractors were used additionally to search for Parasitengona in the epigean environment around and further away from the cave, for which random samples of litter were collected, and subsequently taken for extraction for 7 days.

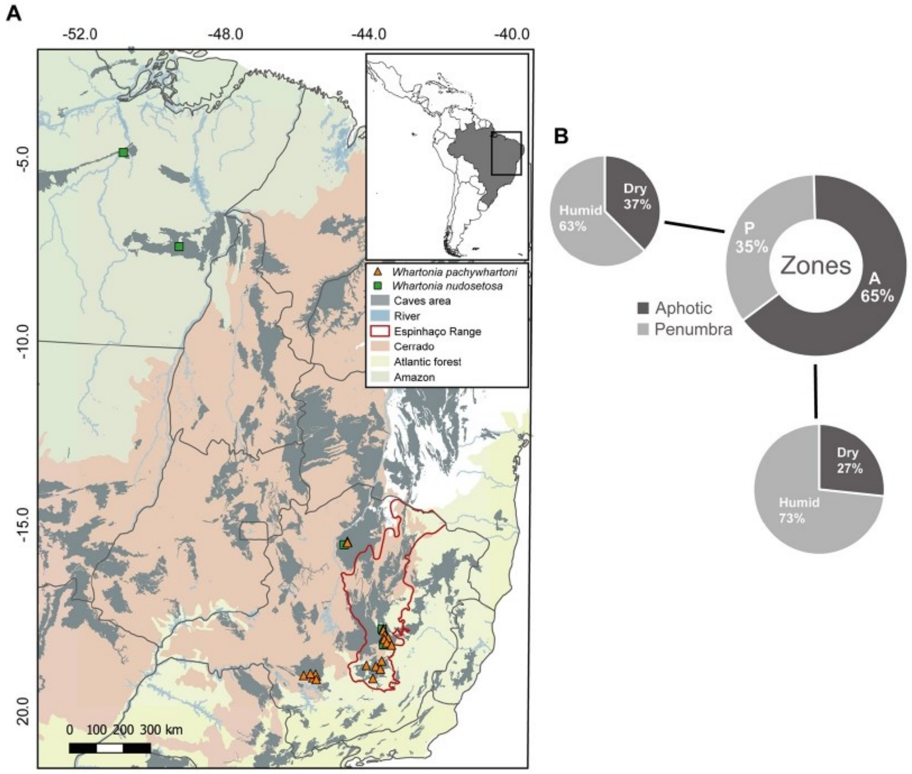

FIGURE 1. A: Geographical distribution of sampling localities of *Whartonia* sp.; B: Graphic detail of distribution across cave zonation and season of 23 out 31 post-larval for which the data is available.

Morphological procedures

Mounting, identification and terminology. All mites had their genomic DNA extracted and the exoskeleton recovered from extraction was mounted as permanent microscope slides using Hoyer's medium (Walter & Krantz 2009) for morphological examination. Identification of larval specimens was made by comparing them with original descriptions (Wharton 1938; Vercammen-Grandjean 1966), species redescriptions based on types (Brennan & Dalmat 1960; Reed & Brennan 1975) and comparison to specimens employed as vouchers of recent occurrences (Silveira *et al.* 2015).

For terminology and abbreviations, see Wharton *et al.* (1951), Crossley (1960), Goff *et al.* (1982), Gabryś (1999) and Wohltmann *et al.* (2007). Besides, Grandjean (1939) with adaptations by Kethley (1990) for the dorsal opisthosomal setae and prodorsal setae.

TABLE 1. Detailed sampling data of specimens and GenBank accession numbers. Abbreviations: larva (L), deutonymph (DN), female (F), male (M), Minas Gerais (MG), Pará (PA), dry (D) and humid (H).

Species of *Whartonia*	Locality						GenBank Accession	
		Coordinates	ID UFMG -AC	Instar/Sex	Bat host or cave zone	Season	COI	28S
W. (*W.*) *pachywhartoni*	PE Serra do Rola Moça, Belo Horizonte, MG, Brazil, June 2nd, 2013, col. A.R. Pepato *et. al.*	-20.0346, -43.9944	130432	L	*C. perspicillata*	D	ON145636	
	Gruta do Brega, Pains, MG, Brazil, August 3rd, 2020, col. A. Tahara & S. Genelhú.	-20.4174, -45.7717	210162	L	*C. perspicillata*	D	ON145656	ON146212
	Caverna Simmons, São Gonçalo do Rio Abaixo, October 2016, col. unknown.	-19.8986, -43.4727	210081	♀	unknown	H	ON145647	ON146213
	São Roque de Minas, MG, Brazil, November 2nd- 3rd, 2017, col. F.V. Oliveira.	-20.3499, -46.0741	174468	L	*C. perspicillata*	H	ON145649	
	São Roque de Minas, MG, Brazil, November, 2017, col. F.V. Oliveira.	-20.3499, -46.0741	174467	L	*A. caudifer*	H	ON145634	ON146219
	Cave SPT-0316, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016–January 26th, 2017, col. Carste Co.	-19.2218, -43.3906	170291	♂	twilight	H	ON145650	ON146214
	Cave SPT-0316, Conceição do Mato Dentro, MG, July 26th — October 13th, 2016, col. Carste Co.	-19.2213, -43.365	170018	♀	aphotic	D	ON145654	
	Cave SPT-0316, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th., 2017, col. Carste Co.	-19.2218, -43.3906	170289	♂	aphotic	H	ON145651	
	Cave SPT-0316, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.2218, -43.3906	170255	♂	aphotic	H	ON145653	
	Cave SPT-0316, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.2218, -43.3906	170288	♀	aphotic	H	ON145628	
	Cave SPT-0490, Conceição do Mato Dentro, MG, July 26th — October 13th, 2016, col. Carste Co.	-19.2183, -43.3863	161056	♀	twilight	D	ON145648	
	Cave SPT-0160, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.1651, -43.2792	170258	♀	aphotic	H	ON145652	ON146215
	Cave SPT-0081, Conceição do Mato Dentro, MG, Brazil, July 26th – October 13th, 2016, col. Carste Co.	-19.1663, -43.2711	170002	DN	aphotic	D	ON145637	
	Cave SPT-0131, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.0907, -43.3653	170297	♂	twilight	H	ON145633	
	Gruta do Nadinho, Pains, MG, Brazil, August 11th, 2020, col. A. Tahara & S. Genelhú.	-20.3678, -45.6572	210157	L	*D. rotundus*	D	ON145657	ON146215
	Cave SPT-0585, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.1749, -43.2650	170230	♀	aphotic	H	ON145670	
	Cave SPT-0585, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.1749, -43.2650	170325	♂	twilight	H	ON145640	
	Cave SPT-0585, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.1749, -43.2650	170228	♂	aphotic	H	ON145642	
	Cave SPT-0585, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.1749, -43.2650	170326	DN	twilight	H	ON145638	
	Cave SPT-0131, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.0854, -47.1673	170281	♀	aphotic	H	ON145645	
	Cave SPT-0495, Conceição do Mato Dentro, MG, Brazil, December 16th, 2016 – January 26th, 2017, col. Carste Co.	-19.2188, -43.3861	170329	♀	twilight	H	ON145639	
	Cave SPT-0151, Conceição do Mato Dentro, MG, Brazil, November 29th – December 16th, 2016, col. Carste Co.	-19.0929, -43.3648	170270	♀	aphotic	H	ON145641	
	Cave MOES-0002, Santa Maria de Itabira, MG, Brazil, August 06th – 10th, 2018, col. Carste Co.	-19.4046, -43.1442	180266	DN	twilight	D	ON145643	
	Cave MCFIN-0005, Itambé do Mato Dentro, MG, Brazil, June 26th – July 06th, 2018, col. Carste Co.	-19.3317, -43.3075	180252	♂	aphotic	D	ON145644	
	Cave CSS-0113, Conceição do Mato Dentro, MG, Brazil, November 22nd – December 15th, 2016	-18.9382, -43.4123	170452	♀	unknown	H	ON145632	
	Cave CSS 0015, Conceição do Mato Dentro, MG, Brazil, April 26th – 29th, 2016, col. Carste Co.	-18.9105, -43.4286	160176	♀	aphotic	H	OM401566	OM641865 *
	Cave CSS-0015, Conceição do Mato Dentro, MG, Brazil, April 26th – 29th, 2016, col. Carste Co.	-62.3391	160177	♀	aphotic	H	ON145625	
	Cave MGB-0063, Ouro Preto, MG, Brazil, September 03rd – 6st, 2018, col. Carste Co.	-20.4436, -43.7653	180490	♂	aphotic	D	ON145626	
	Gruta da Paca, Formiga, MG, Brazil, May 9th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210180	L	*D. ecaudata*	D	ON145629	ON146216
	Gruta da Paca, Formiga, MG, Brazil, April 1st, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210194	L	*D. ecaudata*	H	ON145660	ON146217
	Gruta da Paca, Formiga, MG, Brazil, April 1st, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210248	L	*D. ecaudata*	H	ON145666	ON146218
	Gruta da Paca, Formiga, MG, Brazil, May 9th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210232	L	*C. perspicillata*	D	ON145661	ON146221
	Gruta da Paca, Formiga, MG, Brazil, May 9th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210241	L	*M. bennettii*	D	ON145662	ON146222
	Gruta da Paca, Formiga, MG, Brazil, May 9th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.4743, -45.6595	210190	L	*A. caudifer*	D	ON145658	ON146224
	Gruta da Coruja, Doresópolis, MG, Brazil, May 5th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.2933, -45.8535	210246	L	*D. rotundus*	D	ON145665	ON146223
	Gruta da Coruja, Doresópolis, MG, Brazil, May 5th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.2933, -45.8535	210244	L	*D. rotundus*	D	ON145664	
	Gruta da Coruja, Doresópolis, MG, Brazil, August 08th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.2937, -45.8539	210161	L	*D. rotundus*	D	ON145630	ON146220

.....continued on the next page

TABLE 1. (Contimed)

Species of *Whartonia*	Locality						GenBank Accession	
		Coordinates	ID UFMG -AC	Instar/Sex	Bat host or cave zone	Season	COI	28S
W. (*W.*) *nudosetosa*	Gruta da Coruja, Doresópolis, MG, Brazil, May 5th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.2933, -45.8535	210243	L	*D. rotundus*	D	ON145663	
	Lapa sem Fim, Luislândia, MG, Brazil, April 10th – 27th. 2015, col. Carste Co.	-16.1488, -44.6275	160212	♀	unknown	H	ON145627	ON146225
	Cave RM-38, Nova Lima, MG, Brazil, January 1st, 2021, col. B. Gomes-Almeida et al.	-20.0135, -43.9786	210251	♀	aphotic	H	ON145631	
	Gruta Olhos d'água, Pains, MG, Brazil, May 13th, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.3115, -45.6846	210173	L	*S. lilium*	D	ON145635	ON146226
	Gruta Olhos d'água, Pains, MG, Brazil, March 31st, 2020, col. Arthur Tahara & Sebastião Genelhú.	-20.3115, -45.6846	210195	L	*C. perspicillata*	H	ON145659	ON146227
	Gruta Olhos d'água, Pains, MG, Brazil, May 13th, 2020, col. A. Tahara & S. Genelhú.	-20.3115, -45.6846	210174	L	*Pl. lineatus*	D	ON145646	ON146213
	Cave Planaltina, Brasil Novo, PA, Brazil, April 04th, 2017, col. B. Gomes-Almeida et. al	-03.3227, -52.2746	210082	L	*Pe. trinitatis*	H	ON145667	ON146208
	Lapa das Pacas, Lagoa Santa, MG, Brazil, December 15th, 2020, col. B. Gomes-Almeida et. al	-19.5606, -43.9667	210083	L	*G. soricina*	H	ON145668	ON146211
	Lapa das Pacas, Lagoa Santa, MG, Brazil, December 15th, 2020, col. B. Gomes-Almeida et. al	-19.5606, -43.9667	210084	L	*G. soricina*	H	ON145669	
	Cave GEM-1614, Canaã dos Carajás, PA, Brazil, November 19th -24th, 2014, col. Carste Co.	-06.4561, -50.2419	160070	DN	unknown	H	ON145620	
	Cave GEM-1614, Canaã dos Carajás, PA, Brazil, November 06th. - 19th, 2015, col. Carste Co.	-06.4561, -50.2419	160193	♀	unknown	H	ON145621	ON146207
	Cave GEM-1614, Canaã dos Carajás, PA, Brazil, May 2nd. - 13th. 2016, col. Carste Co.	-06.4561, -50.2419	172125	♀	unknown	D	ON145655	
	Lapa sem Fim, Luislândia, MG, Brazil, April 10th-27th, 2015, col. Carste Co.	-16.1488, -44.6275	160219	♀	unknown	H	ON145622	ON146209
	Lapa sem Fim, Luislândia, MG, Brazil, April 10th-27th, 2015, col. Carste Co.	-16.1488, -44.6275	160216	♀	unknown	H	ON145623	ON146210
	Cave SPT-0351, Conceição do Mato Dentro, MG, Brazil, January 16-26th, 2017, col. Carste Co.	-19.2219, -43.3952	170263	♂	twilight	D	ON145624	

Measurements, illustrations and collection. Measurements and illustrations were made using a Leica DM 750 optical microscope with an ICC50 W digital camera attached. All measurements are given in micrometers (*μm*). Illustrations were prepared using the live image overlay technology adapted from Sidorchuk & Vorontsov (2014). Maps were produced with the QGIS 3.18.1 program (http://www.qgis.org) using the Google satellite image provider. All figures were prepared with Krita v.4.4.2 program (https://krita.org/en/).

Voucher mite specimens are deposited at the Acarological Collection, Centro de Coleções Taxonômicas, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte city, Brazil. Collection acronym: UFMG AC.

Molecular procedures

DNA extraction, PCR and sequencing. Genomic DNA was extracted from single specimens using QIAamp® DNA Micro kit (Qiagen) for larvae and the Wizard® Genomic DNA Purification Kit (Promega) for post larval stages following the manufacturer's protocol, except by using two steps of the final elution in the former, leading to a final volume of 50 µl and by removing and preserving the specimen's exoskeleton after the protein digestion step.

Two target DNA fragments were PCR-amplifIed and sequenced, the mitochondrial cytochrome c oxidase subunit I (COI) and nuclear D3-D4 region in 28S rDNA. All DNA amplification was performed in 20 µl of final volume, with Platinum Taq DNA Polymerase (Invitrogen) in a Mastercycler nexus (Eppendorf) thermocycler. The master mix for initial PCR contained 2.0 µl of PCR buffer (1X), 1.4 µl MgCl 2 (50 mM), 1.4 µL of dNTP (10 mM each) and 0.8 µl of each oligonucleotide primer (10uM), to which 1–3 µl of genomic DNA was added or alternatively 0.5 µl of parent PCR products. For single step reactions of 28S rDNA, 1 µl of DMSO was added as PCR enhancer. In nested PCRs, the child primers were appended with M13 Forward (5'-TGTAAAACGACGGCCAGT-3') and M13 Reverse (5'-CAGGAAACAGCTATGACC-3') oligonucleotides, which were employed for sequencing (Supplementary table 1).

Due to the poor preservation of mites deposited in collection (since many were kept at room temperature in 70% alcohol) different approaches were employed to obtain sequences. They are summarized in Supplementary table 1 and were based on previously published protocols (Klimov *et al.* 2018; Pepato *et al.* 2019; Costa *et al.* 2019; Whiting *et al.* 1997). All PCR products found positive in 1% agarose gel electrophoresis were purified using the Ampure® (Agencourt) kit and sequenced using a 3730 DNA Analyzer and BigDye™ Terminator v3.1 (Applied Biosystems) according to the manufacturer's protocol.

Forward and reverse chromatograms were checked, edited and assembled into contigs using software ChromasPro 1.41 (Technelysium Pty Ltd). All sequences generated for this study were compared with available mites' sequences using the BLAST feature (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.* 1997) and deposited in GenBank database (Table 1).

Sequence alignment. After preliminary automatic alignment with the aid of the MUSCLE software (Edgar 2004) sequences had their extremities trimmed and checked for the maintenance of the reading frame in the case of COI mtDNA in the program MEGA 7.0 (Kumar *et al.* 2016). In the case of 28S rDNA, sequences were manually aligned with the aid of sequence editor BioEdit 7.2.1 (Hall 1999) considering the secondary structure obtained from previous studies (Pepato & Klimov 2015).

Gene tree inference. Best-fitting models of nucleotide substitutions were found in ModelFinder (Kalyaanamoorthy *et al.* 2017) using Corrected Akaike Information Criterion (AICc), as implemented in IQ-Tree (Nguyen *et al.* 2015). This algorithm tests the best fit models for each partition and the best partitioning scheme. The partitions taken into account were codon positions (1st, 2nd and 3rd) in COI and a single partition for 28S. A maximum likelihood tree was obtained for each gene in IQ-Tree for exploratory purposes. They are presented in supplementary material. For ML analysis a single terminal was employed as an outgroup, *Acomatacarus arizonensis* (BMOC 08-0602-002, GenBank: KY922406 (COI) and KY922029 (28S)).

Species delimitation. We employed the bGMYC model (Bayesian General Mixed Yule Coalescent, Reid & Carstens 2012) to estimate the delimitation putative species, associating instars with different haplotypes as a single species. The method uses the distinct expectations on branching patterns between interspecific divergence and intraspecific diversification to distinguish between species and population processes, assigning a threshold to the waiting times between successive branching events on an ultrametric tree (Pons *et al.* 2006; Fujisawa & Barraclough 2013). In its Bayesian implementation, it takes into account the uncertainty in gene tree estimation by taking a population of trees sampled during the Markov chain Monte Carlo (MCMC) instead of a point estimation of gene tree as in the original method (Reid & Carstens 2012).

For these analyses, we removed the repeated haplotypes and obtained ultrametric gene trees using mentioned models of substitution, a strict clock (Bouckaert *et al.* 2014), and the Yule process for modeling lineage divergence in BEAST. Analyses were run for 10000000 generations sampled every 1000. Trees were summarized using the software TreeAnnotator v1.8.4 (Drummond *et al.* 2012), to obtain the maximum clade credibility (MCC) tree, with a burn-in of 10%. Phylogenetic trees were visualized and edited in the program FigTree v.1.4.2 (Rambaut 2014). The final figures were edited using Adobe Illustrator CC.

We used a subsample of 100 BEAST stationary trees; for each of these subsampled trees, we calculated species membership probabilities in the R package bGMYC (available at https://sites.google.com/site/noahmreid/software).

Information from COI and 28S sequences were combined for species delimitation using the STACEY algorithm, with substitution and clock models chosen as above and Yule model for tree topology, and constant population size for the coalescent model. In the taxon sets panel in BEAUTi 2, each individual was set to a different species. The ploidy was set at two for nuclear D3-D4 region in 28S rDNA sequences and 0.5 for mitochondrial. Relative to a usual *BEAST analysis, the STACEY model has two extra parameters. One controls the degree of approximation, named *collapseheight*, and is usually a small value, set in our analyses to ε=0.0001. The second controls the prior distribution of the numbers of species, named *collapseweight*, or ω. A flat prior distribution was employed for this: (1,1), that results in the same a priori probability from one to the total number of terminals as species (Jones 2016).

Results

Molecular analyses

Data set characteristics and Gene Tree inference. Our dataset resulted in 52 mitochondrial COI sequences, 426 bp long, after trimming all columns with missing data, among which 115 were variable and 101 parsimony informative, distributed in 19 haplotypes (Table 2). The best-fit model according to AICc was TIM2+F+I for the first and second codon position and HKY+F+G4 for the third. This partitioned model was employed in all downstream analyses, except by TIM2 (that implies rates AC=AT, CG=GT) that was replaced by HKY in Bayesian analyses. Our study could include 24 28S sequences, which were 608 bps long, without gaps among ingroup samples but two positions shorter than *Acomatacarus* sequences were employed as outgroup. The ingroup sequences presented three haplotypes: Hap. 1 including UFMGAC 16093; Hap. 2 UFMGAC 210082, UFMGAC 160219, UFMGAC 160216, and UFMGAC 210083; and Hap. 3 all other sequences. The alignment presented 14 variable positions, all parsimony informative. The best fit model according to AICc was HKY+F+I. Maximum likelihood trees for COI and 28S sequences are shown in Supplementary figure 1.

Association among larvae and post-larval stages and species delimitation. Despite unequal sampling, as well as the limited success in obtaining 28S sequences, the results from bGMYC and STACEY matched and pointed to five well-defined clades recovered as species. They do not correspond to species delimitation using morphology, splitting *W.* (*W.*) *pachywhartoni* in two and *W.* (*W.*) *nudosetosa* in three clades (Figure 2A and B), despite both species being recovered as monophyletic.

The partition of *W.* (*W.*) *pachywhartoni* in two clades was supported by 78% of MCMC samples in STACEY, a moderate support at best. It is noteworthy that both clades comprise larvae, females and males, making the association among stages and sexes unambiguous. In *W.* (*W.*) *nudosetosa* two lineages are from the Eastern Amazon region (Clades A and B) while the third includes only mites from southeast Brazil. The latter is the only clade which simultaneously includes larvae, males and females.

In the following descriptions, the divergence among clades is ignored since no morphological support to their distinction in separate species could be detected (see discussion). However, the association among different heteromorphic stages could be inferred beyond reasonable doubts.

Ecological notes

Among 23 free living adults and deutonymphs with complete account on sampling, 15 were obtained from the aphotic zone, eight from the twilight, and none for the cave entrance (Figure 1B). During field trips, samples from epigean litter were taken and post-larval *Whartonia* could not be found, despite several other Parasitengona did. Concerning larval association to bat hosts (the only mammal order examined for mites in this study) they were found on Phyllostomidae (*Anoura caudifer* (É. Geoffroy, 1818), *Carollia perspicillata*, *Diphylla ecaudata* Spix, 1823, *Desmodus rotundus* (É. Geoffroy, 1810), *Glossophaga soricina* (Pallas, 1766), *Mimon bennettii* (Gray, 1838), *Platyrrhinus lineatus* (É. Geoffroy, 1810), *Sturnira lilium* (É. Geoffroy, 1810)) and one species on Emballonuridae (*Peropteryx trinitatis* Miller, 1899). *W.* (*W.*) *pachywhartoni* was collected on *A. caudifer*, *D. ecaudata*, *M. bennettii*, *Pl. lineatus* and *S. lilium* are new bat host-associations, the same for *W. (W.) nudosetosa* associated with *Pe. trinitatis* (Table 1).

Description of larval and post-larval stages

Taxonomy

Family Leeuwenhoekiidae (Womersley, 1944)

Diagnosis

LARVAE: Leg segmentation 6-6-6 (7-6-6 in some *Comatacarus* species). True stigma opening on each side between gnathosoma and coxae I, from the atrium of which tracheal tubes transverse the body; prodorsal sclerite furnished with two *vi* [=AM] setae, two *ve* [=AL] setae and two *se* [=PL] setae (*se* off the prodorsal sclerite in *Apolonia*), as well as a pair of nude flagelliform trichobothria (*si*); prodorsal sclerite with or without an anteromedial tongue-like processes (naso); all tarsi with similar claws and median claw-like empodium (Womersley 1945).

POST-LARVAL: Body not constricted (except the genus *Hannemania*); smooth tectum with a pair of *vi* (=AM) setae; a pair of long, nude and flagelliform trichobothria (*si*); eyes present or absent, 1/1 or 2/2; anus circular, not obviously divided into two shoelike portions. Setae on palpgenu less numerous than those on palpfemur; sternal area sclerotized but no true sternum formed by a crossbar connecting coxae II; precoxal plates never present; two accessory claws on palpal tibia (Crossley, 1960)

Genus *Whartonia* Ewing, 1944

Type species: *Hannemania nudosetosa* Wharton, 1938.

Generic diagnosis for larvae according to Stekolnikov (2018)

Palptarsus with 7 branched setae (7B); odontus divided by 2–8 prongs; adoral setae (*cs*) branched or nude; prodorsal sclerite wide, sometimes striplike, without nasus, with 2 *vi* [=AM], 2 *ve* [=AL]; and 2 *se* [=PL]> setae; trichobothria (*si*) flagelliform; cheliceral blade long, with large, recurved ventral row of teeth and dorsal teeth or hooks; eyes 2/2; tracheae and stigmae absent or present. Posterior sternal setae (*3a*) between coxae III; coxa I with two setae; leg segmentation 6-6-6, not divided into basifemur and telofemur; onychotriches absent; dorsal eupathidium (ζ') and companion seta (z) or sometimes setae branched closely associated with ζ' (condition scored as seta (z) absent as in our case) on tarsus I, genu solenidia formula 2σ, σ, σ; solenidia (φ) on tibia III, mastiseta or solenidion (ω) on tarsus III sometimes present, mastiseta on tibia III rarely present; mastiseta on genu and femur III absent. Parasites of bats.

Generic diagnosis for post-larval stages (deutonymph and adults)

Deutonymph and adult idiosoma oval, not constricted (Figures 5A, 7A, 12A, and 14A); idiosomal setae modified, referred as canoe-like leaf, with outer surface covered by setules and margins curved dorsally, with slightly serrated margins. They are situated on short and stout peduncles, set on cylindrical papillae on striated cuticle (Figures 5B–F and 12B–F). Legs, sternum, genital area, palpgenu and palptibia with expanded, leaf-like setae (Figures 6E, G and I; 7E and G; 8B–E; 13D and G; 14E, G–H, K and 15B–E). Tectum rounded, edges smooth, bearing a pair of *vi*. Posterior trichobothria (*si*) area sagittate with a posterior process; pair of *vi* leaf-like, pair of posterior trichobothria (*si*) nude, flagelliform; eyes present, 1/1 (Figures 5G–J and 12G–J); solenidia (θ) present on basifemur (Figures 8F–G and 15F–G); tarsus I pyriform with claws smaller than in other legs (Supplementary figures 2A–3A). Deutonymphs with two spines (paradonts) in tandem and none spinelike seta accessory (basidont) on palptibia; fewer eupathidia (ζ) distally on palptarsus; incomplete genitalia with two pairs of genital acetabula. Adults have three spines (paradonts) in tandem and one accessory spine on palptibia; more eupathidia (ζ) distally on palptarsus; complete genitalia with three pairs of genital acetabula. Males with fan-like membranous setae on centrovalves and internal genital armature with nude setae (Figures 6G–I and 7E–K, 13G and 14E–L).

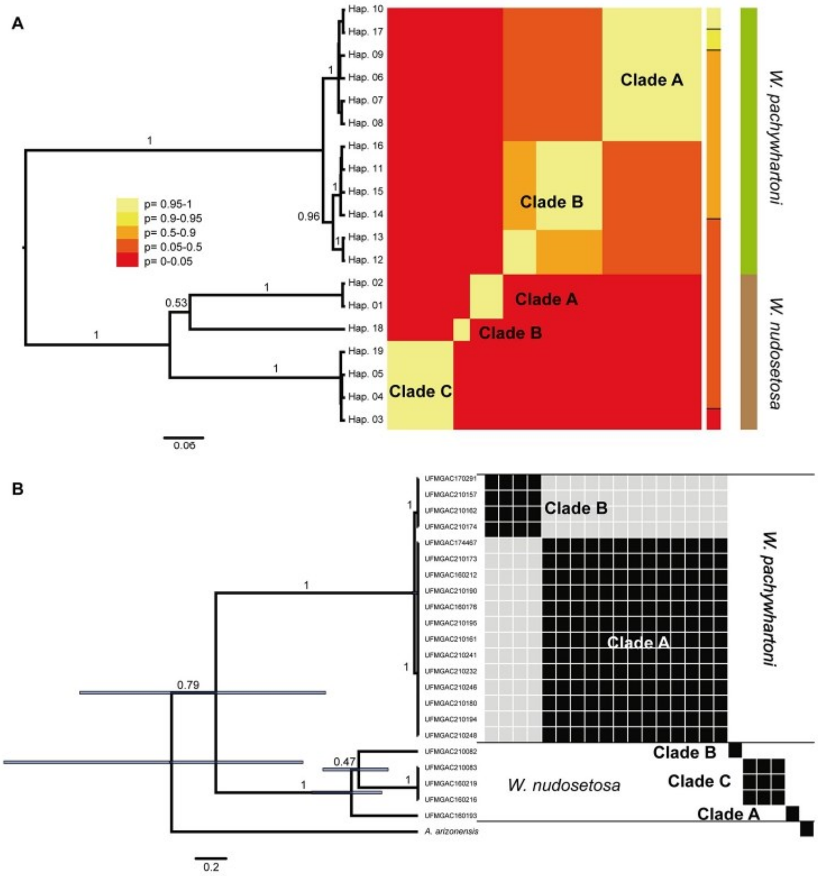

FIGURE 2. A: Mitochondrial COI haplotype ultrametric tree inferred in BEAST using strict clock and species delimitation probabilities obtained in bGMYC; B: Species tree inferred in STACEY from sequences of mitochondrial COI and nuclear ribosomal 28S and similarity matrix summarizing posterior probabilities of grouping samples into species. Values on branches are Posterior Probabilities. Scales are proportional to branch lengths assuming root=1.

***Whartonia* (*W.*) *pachywhartoni* Vercammen-Grandjean, 1966**

Whartonia pachywhartoni Vercammen-Grandjean, 1966: 282; Takahashi *et al.*, 2006: 132. Silveira *et al.*, 2015: 1; Bassini-Silva *et al.*, 2022b:153.

(Figures 3–9, Tables 4 and 5)

Studied specimens summarized in Table 1.

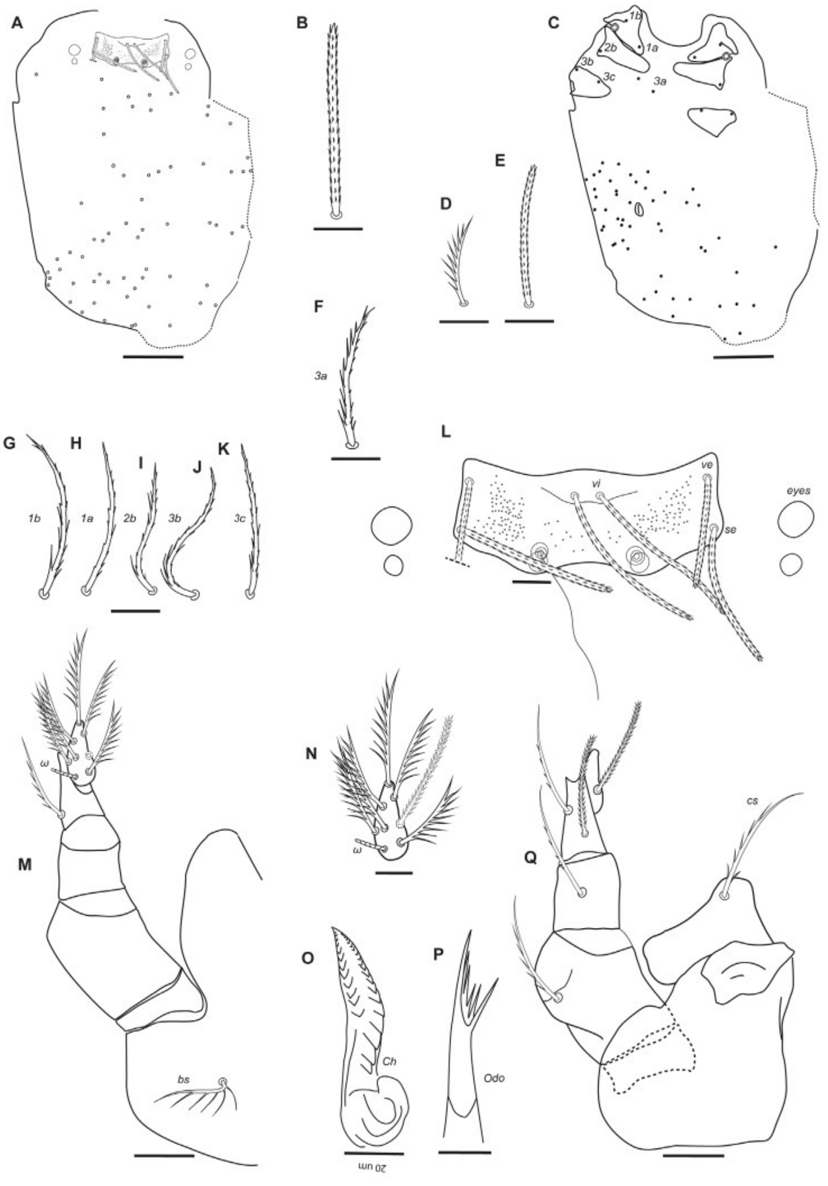

FIGURE 3. *Whartonia (W.) pachywhartoni*, larva. A: idiosoma, dorsal view, open circles indicate dorsal setae; B: C4, seta 4 of row C [=H, humeral seta] in dorsal opisthosomal seta; C: idiosoma, ventral view, solid circles indicate ventral setae; D: Ventral opisthosomal setae [=preanal setae or true ventral setae]; E: Ventral opisthosomal setae [=postanal setae]; F: *3a* seta; G: 1b seta; H: *1a* seta; I: *2b* seta; J: 3b seta; K: *3c* seta; L: Prodorsal sclerite and eyes; M: gnathosoma, venter; N: palptarsus; O: Chelicera; P: odontus; Q: gnathosoma, dorsum. *Symbols: 1a*, seta on proximal coxa I; *1b*, seta on distal coxa I; *2b*, coxa II seta; *3a*, sternal setae; *3b*, seta on distal coxa III; *3c*, seta on proximal coxa III; *ve*[=AL], external vertical setae; *vi*[=AM]; internal vertical setae; *se* [=PL], external scapular setae; *Ch*, chelicera; *Odo*, odontus; *bs*, subcapitular setae; *ω*, solenidion on palptarsus and *cs*, adoral setae. Scale Bars: A and C=100 µm; B, D–M, O and Q=20 µm; N and P=10 µm.

Diagnosis

LARVAE (n=18): Palpal setal formula (fPp) B/B/BBB/7Bω; odontus six-pronged; adoral setae (*cs*) branched; subcapitular setae (*bs*) sparsely branched; prodorsal sclerite rectangular with small punctations, more than four times wider than long; trichobothria (*si*) nude and flagelliform; prodorsal sclerite setae stiff and covered by many barbulae, with *vi* [=AM] >*se* [=PL]> *ve* [=AL]; femur branched setae formula 6B, 5B, 4B; genu solenidia formula 2σ, σ, σ with solenidia on genu I at same level, inserted proximally; tibia solenidia formula 2φ, 2φ, φ; tarsus solenidion formula ω, ω, ω; cheliceral blade with minute saw-like edges on dorsal and ventral surfaces; 115–120 total number of idiosomal setae, coxal setae excluded; leg index 1158–1515 µm, with long tarsus (I=157–185 µm, II=135–158 µm, III= 160–189 µm) and tarsus I with 50–56 branched setae.

POST LARVAL (deutonymph, n=3; female, n=14; male, n=8): Deutonymph and adults with small canoe-like leaf idiosomal setae (height= 6–9, width=17–21 as indicated in 5C); posterior process long and discernible on crista metopica; one pair of eyes, without ocular plate; lanceolate leaf-like setae on palp, sternum, genital epivalves and legs. Deutonymphs with 32 hypostomal barbed setae (n=2); palpgenu bearing 11–19 expanded and leaf like setae and 17–21 barbed slender setae; palptibia with 16–18 barbed setae; palptarsus with 16–19 pectinate setae and two subterminal eupathidia (ζ); six or seven barbed setae on centrovalves; anal valves bearing five or six long barbed setae; solenidia (θ) on basifemur formulae 0, 2, 4, 0–1. Adults with 54 hypostomal barbed setae (n=1); palpgenu bearing 47–56 expanded and leaf like setae and 24–42 barbed slender setae (n=5); palptibia bearing 32–43 barbed slender setae (n=5); palptarsus bearing 38–42 pectinate setae (n=7) and five to six subterminal eupathidia (ζ) (n=8). Adults with numerous setae on genital plates (female: 59–62 slender barbed setae with smooth apex on centrovalves and 50–55 expanded leaf-like setae on epivalves (n=1); male: 54–59 on centrovalves and 55–61 on epivalves (n=1), similar barbed setae); males with 16 pairs of fan-like membranous setae on centrovalves (n=1) and four pairs smooth seta on internal genital armature (n=3) (absent in females). Adults with more than 10 long stiff barbed setae on anal valves (female:11–14 setae (n=6); male: 13–16 setae (n=3)). Solenidia (θ) on basifemur formulae 0, 5–10, 3–12, 3–7 (female, n=14) and 0, 5–8, 4–12, 2–6 (male, n=8).

Description. Studied specimens summarized in Table 1.

LARVAE (n=18; Figures 3 and 4). Measurements summarized in Table 3.

Idiosoma (Figure 3A–E). Ovoid, live larvae whitish, 115–120 opisthosomal setae. *Dorsum* (Figure 3A–B). Total of 61–63 dorsal opisthosomal setae (n=2), with dense barbulae along its axis, arranged in irregular rows. *Venter* (Figure 3C–E). Total of 52–59 ventral setae, including a pair of sternal setae branched (*3a*); opisthosomal ventral setae divided in two shapes (Figure 3D–E), 36–42 setae alike sternal setae but shorter (Figure 3F), and 16–17 similar to opisthosomal dorsal setae. All coxal setae branched (Figure 3G–K). Prodorsal sclerite (Figure 3L). Rectangular, broader than long with small punctations conspicuous; nude and flagelliform trichobothria (*si*) bases inserted posterior to *se* in examined specimen; prodorsal sclerite with *vi* [=AM] >*se* [=PL]> *ve* [=AL]; prodorsal sclerite setae stiff and barbed. Two pairs of eyes, without ocular plates. Gnathosoma (Figure 3M–Q). Palpal setal formula (fPp)=B/B/BBB/7Bω; subcapitular setae (*bs*) branched; cheliceral blade with minute saw-like edges on dorsal and ventral surfaces (Figure 3O); odontus six-pronged and adoral setae (*cs*) branched (Figure 3P and Q). Legs (Figure 4A–C). All legs six-segmented, ending in pair of claws and claw-like empodium, without onychotriches. Tarsus I with a dorsal eupathidium (ζ*'*) without a companion seta (z). No mastisetae on legs I–III. Coxae I–III bisetose. *Leg chaetotaxy:* Leg I: Cx—2B; Tr—1B; Fe—6B; Ge—4B, 2 σ at same level, inserted proximally, k; Ti—8B, 2 φ, k; Ta—50–56B, ω, ε, ζ’, ζ”; Leg II: Cx—1B; Tr—1B; Fe—5B; Ge—4B, σ, k; Ti—6B, 2 φ; Ta—41–46B, ω, ε, ζ.; Leg III: Cx—2B; Tr—1B; Fe—4B; Ge—4B, σ; Ti—6B, φ; Ta—40–43B, ω.

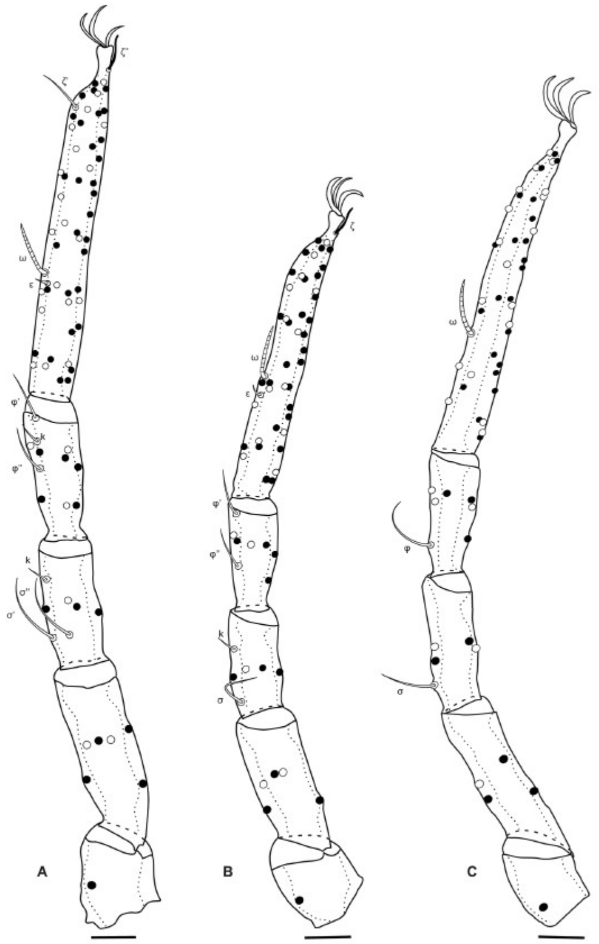

FIGURE 4. *Whartonia (W.) pachywhartoni*, larva. A: leg I; B: leg II; C: leg III. Solid circles indicate ventral leg setae; open circles indicate dorsal leg setae. *Symbols:* ζ', ζ, dorsal eupathidia on Ta I and II; ζ'', subterminal eupathidium on Ta I; ω, solenidion on Ta I, II and III; σ', σ'', solenidion on Ge I; σ, solenidion on Ge II and III; κ, microsetae on Ge I and II and Ti I; φ', φ'', solenidia on Ti I and II; φ, solenidia on Ti III; ε, famulus on Ta I and II. Scale Bars: A–C=20 µm.

DEUTONYMPHS (n=3; Figures 5–6; Figure 9H–L;). Measurements summarized in Table 4. Idiosoma (Figure 5A–F). Color in life orangeish. Idiosoma oval, not constricted, anterior region narrows from leg II to palps. Idiosoma densely covered by canoe-like leaf setae. Their outer surfaces are covered by setules and margins are bent upward dorsally, with slightly serrated margins. They are pedunculate and set on cylindrical papillae in striated cuticle. The wall seems reinforced by bifurcating ribbons. The following measurements were taken from five such setae from anterolateral idiosoma of three individuals (indicated in Figure 5C): height, 6–9; width, 17–21 (Figure 5B–F). Crista metopica (Figure 5G–J). Well sclerotized, with narrow stem, anterior setae (*vi*) on a rounded tectum, trichobothria (*si*) area sagittate, with posterior process easily discernible; a pair of anterior setae (*vi*) expanded, leaf-like (Figure 5H); a pair of posterior trichobothria (*si*) nude and flagelliform.

One pair of eyes, without ocular plate, on each side of prodorsum; anterior to sensillary area, leveled to middle length crista metopica. Gnathosoma (Figure 6A–F). Hypostome blunt, triangular, height= 248–278, basal width=144–170 (n=3), bearing 32 branched setae, gnathosoma base with 52–53 weakly expanded setae (n=2). Chelicerae base stout, cheliceral blades curved, with minute teeth (Figure 6A). Palps robust, moderate in size. Palptrochanter short, without setae. Palpfemur and genu bearing two kinds of setae, 27–36 and 11–12 expanded and leaf like, 11–19 and 17–21 barbed slender setae, respectively; palptibia bearing 16–18 slender barbed setae, one spinelike seta (basidont) ventrally and two dorso-lateral spines (paradonts) in tandem; palptibia with odontus entire, slender, shorter than palptarsus; palptarsus cylindrical, elongate bearing 16–19 pectinate setae, of these two ventro-lateral setae robust; one proximal solenidion (ω) and two subterminal eupathidia (ζ) (Figure 6B–F). Genital valves (Figure 6G–I). Placed posterior to coxae IV; epivalves bearing 18–22 expanded leaf like setae; 6–7 slender barbed setae with smooth apex on centrovalves; incomplete genitalia with two pairs of genital acetabula. Anal valves (Figure 6J–K). Bearing 5–6 stiff barbed setae, apart from genital valves by 92–107 (n=2). Legs. All legs shorter than idiosoma, leg I and IV much longer than legs II-III (Figure S2A–C). Two kinds of normal setae on all legs: coxae to tibiae with expanded, lanceolate leaf-like, similar to those in palp, sternum and genital epivalves; tarsi I to IV with unexpanded barbed setae (sometimes also on distal tibiae) (similar to male, Figure 8B–E). *Specialized setae on all legs* (as normal setae are imbricated and precluded accurate counting for some segments only the minimum number of such setae indicated) (n=3): leg I: bFe—0 θ, tFe—3 θ, ca 17–19 ζ; Ge—5 σ, > 20 ζ; Ti—9–16 φ, > 20 ζ; Ta > 90 ω (4–5 robust), > 50 ζ small and curved tip; leg II: bFe—2 θ; tFe—ca 0–1 θ, 6–7 ζ; Ge—0 σ, 12–18 ζ; Ti—3–5 φ, 8–11 ζ; Ta—10–16 ω (with one robust), k and ζ distal, close to the claw; leg III: bFe—4 θ; tFe—0–1 θ, ca 3–6 ζ; Ge—0–1 σ, 6–11 ζ; Ti—ca 3–7 φ, 7–10 ζ; Ta—8–17 ω (with one robust) and ζ dorsal, close to claw fossae; leg IV: bFe—0–1 θ; tFe—0–2 θ, 4–6 ζ; Ge—σ, 19–20 ζ; Ti—ca 3–5 φ, ca 12–31 ζ; Ta—8–13 ω and ζ dorsal, close to claw fossae. Solenidia (θ) absent from basifemur IV, except by a single specimen (similar to male, Figure 8F and G); eupathidia (ζ) numerous on dorsum of telofemur to tibia I-IV (similar to female, Figure 9A–C); solenidia (σ) absent on genu II; tarsus I pyriform with claw reduced relative to other legs (similar to male, Figure 9D–G). Solenidia (*ω*) and eupathidia (ζ) especially numerous on tarsus I with various robust solenidia (Figure 9H and I); microseta (k) could be found on tarsus II only (Figure 9J); some robust solenidia (*ω*) on tarsus II and III; dorsal eupathidia (ζ) close to claw fossae on tarsus III to IV (Figure 9K); and distal eupathidia (ζ) only close to claws on tarsus II (Figure 9L).

FEMALES (n=14; Figure 7E–G and M; Figure 8A, E–F; Figure 9A–C, D–F). Measurements summarized in Table 4.

Idiosoma, crista metopica, gnathosoma, genital valves, anal valves and legs. As in deutonymphs except by hypostome with 54 barbed setae, and gnathosoma base with 206 weakly expanded barbed setae (n=1) (Figure 8A). Palpfemur and Palpgenu bearing two kinds of setae, 65–87 and 28–38 expanded and leaf like (male: 59–61 and 24–30 setae, n=2), 47–56 and 28–42 barbed slender setae, respectively (male: 47–52 and 28–38 setae, n=2) (n=4); palptibia bearing 38–43 barbed setae unexpanded (n=4) (male: 32 setae, n=1), one spinelike seta (basidont) ventrally, three dorso-lateral spines (paradonts) in tandem and one accessory dorso-lateral spine; palptarsus bearing 38–42 pectinate setae (n=4) (male: 39–40 setae, n=3), one solenidion (ω) and five to six subterminal eupathidia (ζ) (n=7). Genital valves with 50–55 expanded leaf-like setae on genital epivalve and 59–62 slender barbed setae with smooth apex on centrovalves (n=1) and complete genitalia with three pairs of genital acetabula (Figure 7E–G). Anal valves (n=6) bearing 11–14 stiff barbed setae, apart from genital valves by 105–283 (n=11) (Figure 7M). Solenidia (θ) on basifemur formulae 0, 5–10, 3–12, 3–7 and solenidia (σ) on genu II (n=14).

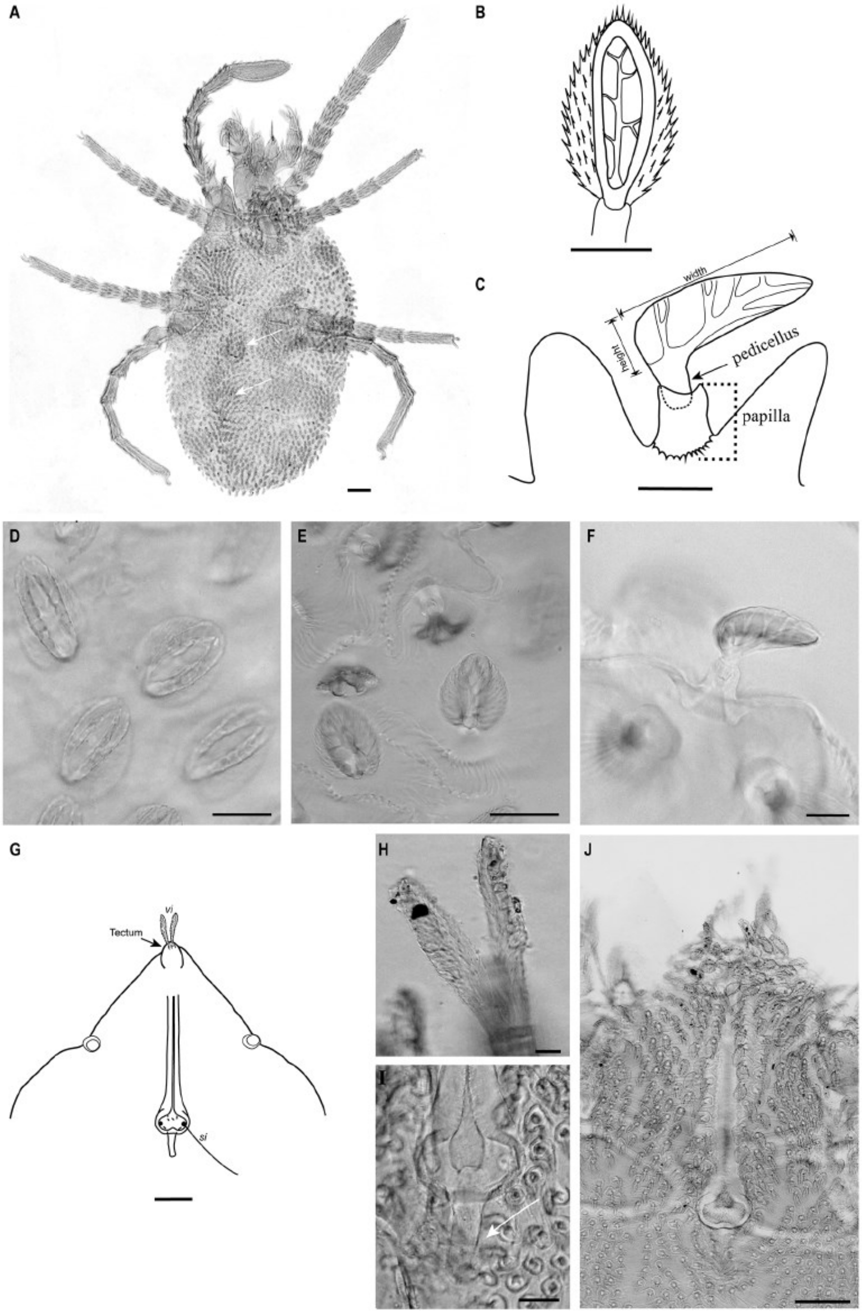

FIGURE 5. *Whartonia* (*W.*) *pachywhartoni*. Deutonymph, A: General view, B–C: Opisthosomal seta with details; Male, D–F: Opisthosomal seta; Deutonymph, G: Crista metopica and eyes; Female, H: *vi* setae; Deutonymph, I: posterior process on crista metopica and J: Crista metopica and dorsal setae. Scale Bars: A, G and J=100 µm; B, C, F and H=10 µm; D and F=20 µm; I=25 µm.

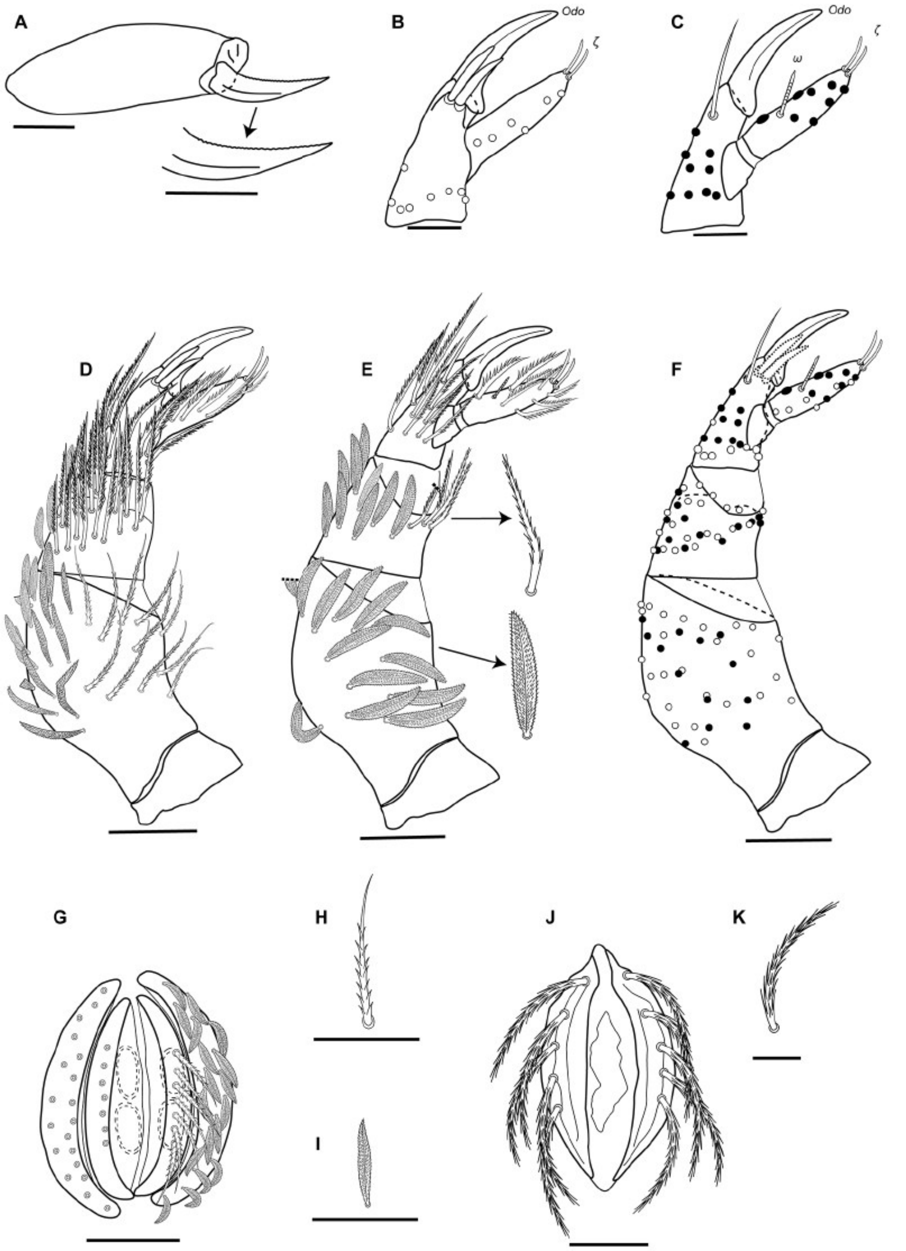

FIGURE 6. *Whartonia* (*W.*) *pachywhartoni*, deutonymph. A: Chelicera with blade detail; B: Dorsal tibia-tarsus on palp; C: Ventral tibia-tarsus on palp; D: Dorsal palp view; E: Ventral palp view; F: Chaetotaxy of setae in palp; G: Genital pore, H: Genital centrovalve seta, I: Genital epivalve seta; J: Anal pore; K: Anal seta. Scale Bars: A, D–F and G=50 µm; B–C, H and I=25 µm; J=20 µm; K=10 µm.

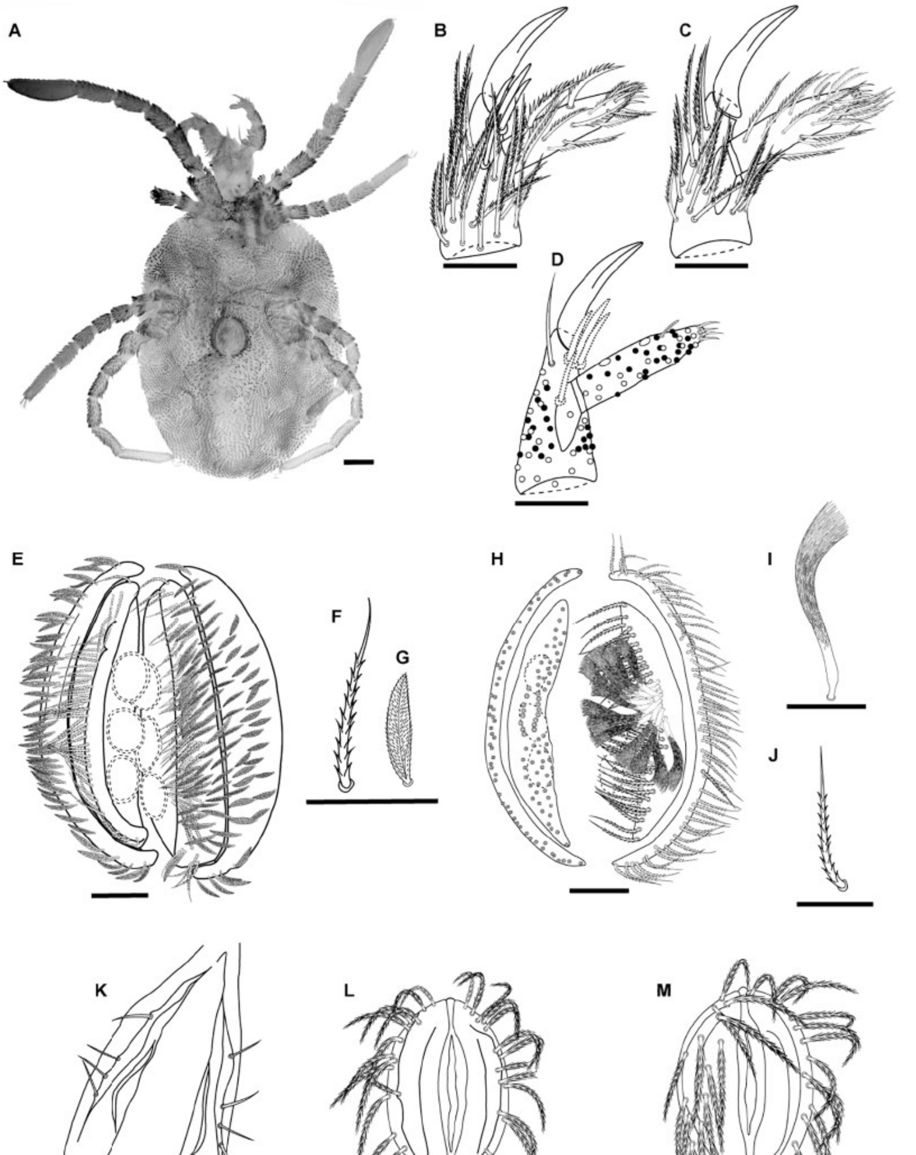

FIGURE 7. *Whartonia (W.) pachywhartoni*. Male, A: General view, B: Dorsal tibia-tarsus on palp, C: Ventral tibia-tarsus on palp, D: Chaetotaxy of tibia-tarsus in palp; Female, E: Genital pore, F: Genital centrovalve seta, G: Genital epivalve seta; Male, H: Genital pore, I: Genital centrovalve seta, J: Genital epivalve seta, K: Internal genital armature, L: Anal pore; Female, M: Anal pore. Scale Bars: A=200 µm; B–E and H=50 µm; F–G and I–J=25 µm; K–M=20 µm.

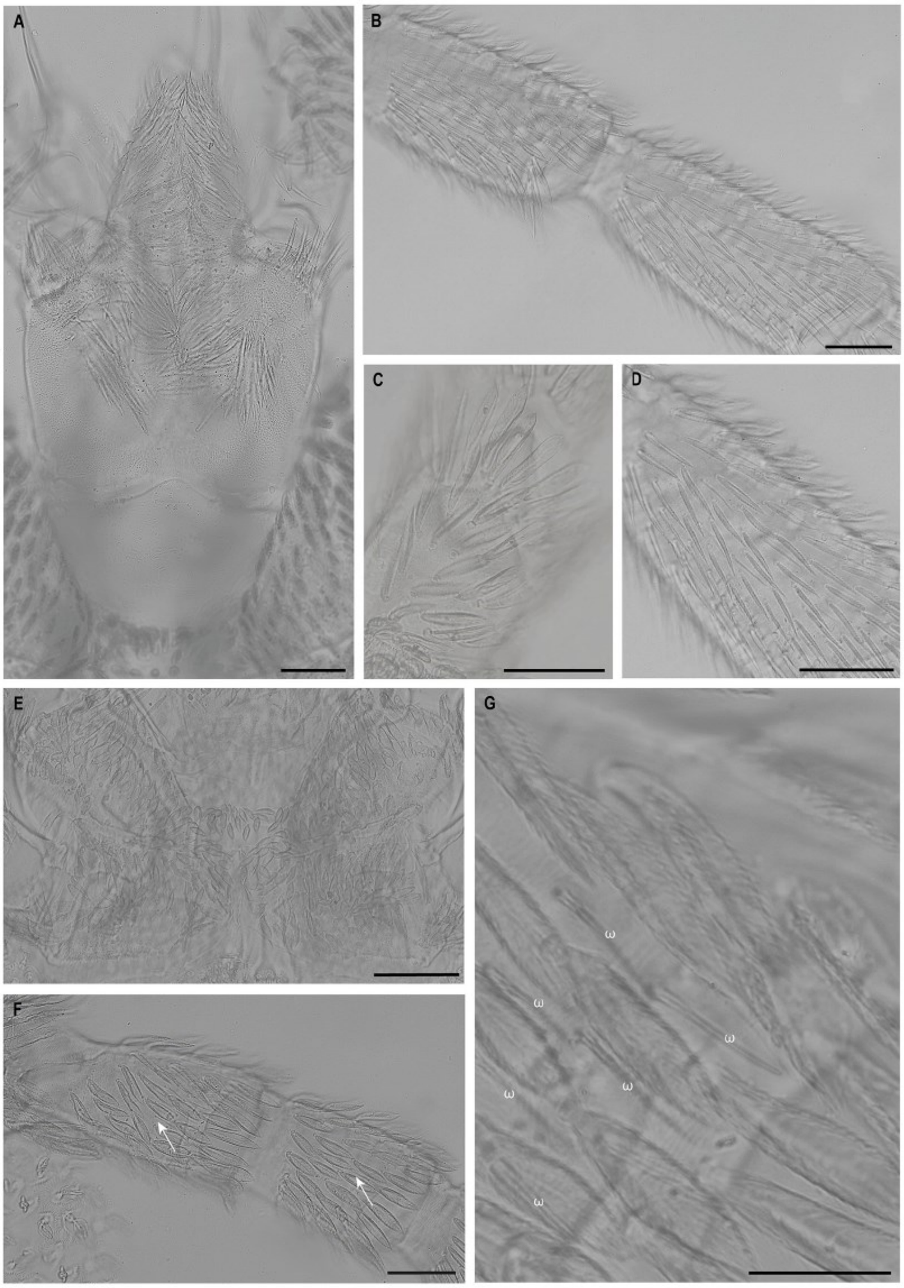

FIGURE 8. *Whartonia (W.) pachywhartoni*. Female, A: Hypostomal setae; Male, B: Genu-tibia I; Female, C: Setae on trochanter I; Male, D: Setae on tibia I; Male, E: Sternum and coxae; Male, F–G: Solenidia (θ) on basifemur to telofemur. Scale Bars: A–D and F=50 µm; E=100 µm; G=20 µm.

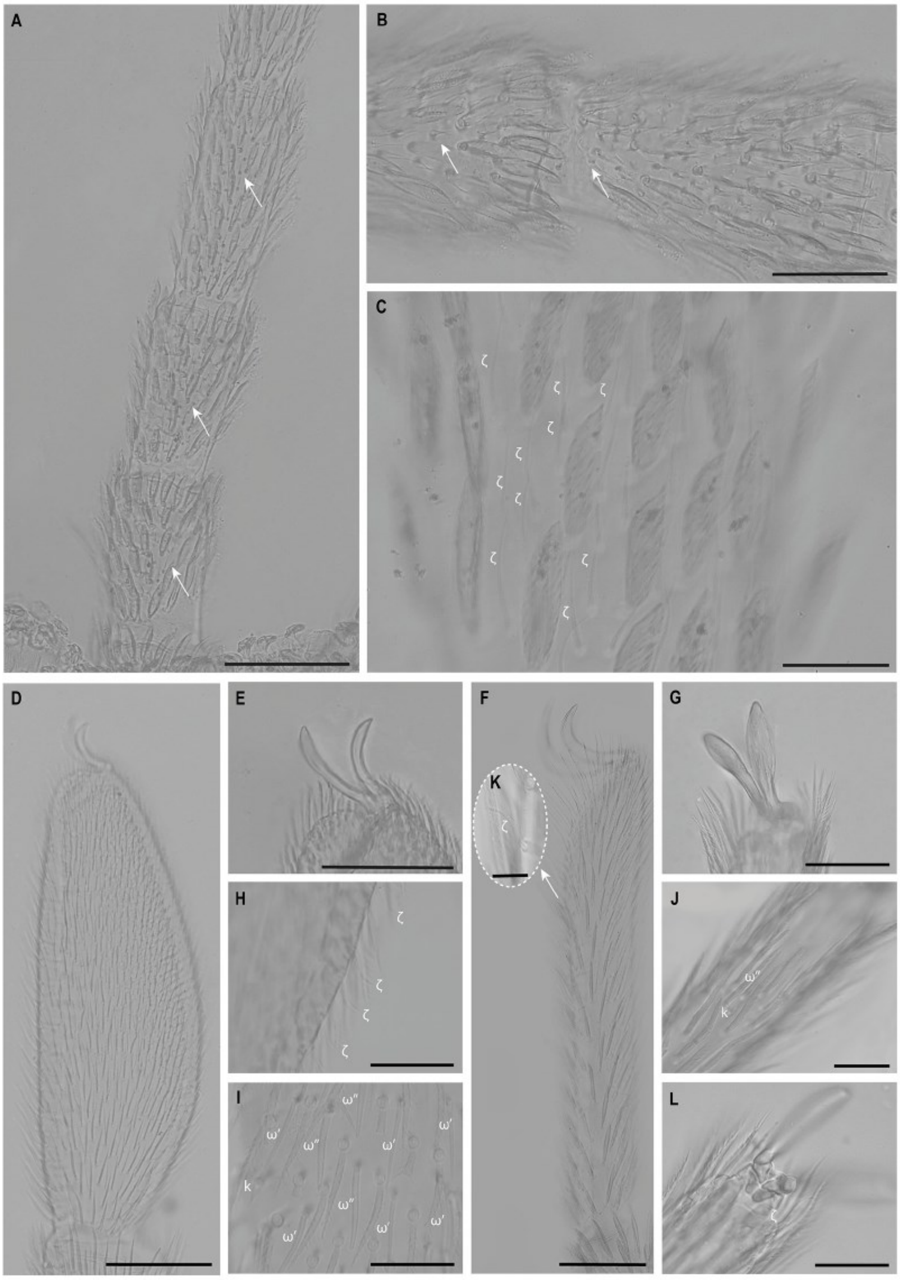

FIGURE 9. *Whartonia (W.) pachywhartoni*. Female, A: Eupathidia (ζ) numerous on dorsum of legs, B–C: Details of eupathidia (ζ); Male, D: Tarsus I, E: Claw I; F: tarsus III, G: Claw III; Deutonymph, H: Eupathidia (ζ) on tarsus I, I: Solenidia (ω) on tarsus I, J: Microseta (k) and solenidia (ω) on tarsus II, K: Dorsal eupathidia (ζ) close to claw fossae, L: Distal eupathidia (ζ) close to the claw on tarsus II. Scale Bars: A and D=100 µm; B, E–G=50 µm; C, H–J and L=20 µm; K=10 µm.

MALES (n=8; Figures 7A–D and H–L, 8B–D and 9D–G). Measurements summarized in Table 4.

Idiosoma, crista metopica, genital and anal valves, gnathosoma and legs similar to females, differing by genital area with fan-like membranous setae, internal genital armature present, and in the number of setae on genital and anal valves. Epivalves and centrovalves with similar barbed setae, 55–61 on epivalves and 54–59 on centrovalves (n=1). Additionally, centrovalves inner edges bear 16 pairs of fan-like membranous setae located approximately at middle length of each sclerite (n=1) (Figure 7H–J); four pairs of smooth and slender setae on the internal genital armature (n=3) (Figure 7K). Anal valves (n=3) bearing 13–16 long stiff barbed setae, apart from genital valves by 109–329 (n=8) (Figure 7L). Solenidia (θ) on basifemur formulae 0, 5–8, 4–12, 2–6 (n=8).

Remarks

See remarks on *W. nudosetosa*.

***Whartonia* (*Whartonia*) *nudosetosa* (Wharton, 1938)**

Hannemania nudosetosa: Wharton, 1938: 142; Hoffman, 1944: 56; Vercammen-Grandjean, 1968: 126. *Whartonia nudosetosa*: Ewing, 1944: 102; Hoffman, 1949: 189; Wharton & Fuller, 1952: 104; Brennan & Jones, 1959: 11; Chen & Hsu, 1959: 555; Brennan & Dalmat, 1960: 185; Hoffman, 1960:4; Vercammen-Grandjean, 1968:126; Reed & Brennan, 1975: 34–35; Webb & Loomis, 1977: 66–68, 96; Hoffman, 1990: 224; Takahashi *et al.*, 2006: 131. Silveira *et al.*, 2015: 3.

Diagnosis. LARVAE (n=3): Palpal setal formula (fPp)= B/B/B(N or B) (N or B)/7Bω; odontus five-pronged; adoral setae (*cs*) nude or sparsely branched; subcapitular setae (*bs*) sparsely branched; prodorsal sclerite rectangular with small punctations, more than four times wider than long; trichobothria (*si*) flagelliform; prodorsal sclerite setae stiff and almost smooth, with minute barbulae; with *vi* [=AM] >*se* [=PL]> *ve* [=AL]; branched setae on femora 6,5,4; solenidia (*σ*) on genua 2,1,1 with solenidia on genu I set proximal and distal; solenidia (φ) on tibiae 2,2,1; solenidia (*ω*) on tarsi 1,1,1; cheliceral blade with minute saw-like edges on dorsal and ventral surfaces; total number of idiosomal setae 98–101, coxal setae excluded; leg index 1134–1189 µm, with shorts tarsus (I=134–140 µm, II=108–114 µm, III=132–135 µm) and tarsus I with 36–39 branched setae.

POST LARVAL (deutonymph, n=1; female, n=4 and male, n=1): Deutonymph and adults with large canoe-like leaf idiosomal setae (height=11–14 and width=24–26) (Figure 12 B–F); posterior process short and barely discernible on crista metopica; one pair of eyes on each side of prodorsum, without ocular plate; elliptical leaf-like setae on palp, sternum, genital epivalves and legs. Deutonymphs with 18 hypostomal barbed setae; palpgenu bearing 19 expanded leaf-like and 17 barbed slender setae; palptibia with 13 barbed setae; palptarsus with 14 pectinate setae and three subterminal eupathidia (ζ); five barbed setae on centrovalves; anal valves bearing four long barbed setae on each valve; solenidia (θ) on basifemur I to IV following the formula 3, 2, 2, 2. Adults with 42 hypostome barbed setae (n=1); palpgenu bearing 18–32 expanded and leaf like setae and 23–28 barbed slender setae (n=4); palptibia with 20–24 barbed setae unexpanded (n=4); palptarsus bearing 25–31 pectinate setae and five subterminal eupathidia (ζ) (n=4). Adults with fewer setae on genital valves (female: 26–30 barbed setae on centrovalves and 43 leaf-like setae on epivalves (n=1); male: 38–45 barbed setae on centrovalves and 54 leaf-like setae on epivalves); male with 12 pairs fan-like membranous setae on centrovalves located approximately at middle length of each sclerite and four pairs of smooth setae on internal genital armature. Adults less than 10 long barbed setae on each anal valve (females: 9–10 setae (n=2); male: 9 setae). solenidia (θ) on basifemur I to IV following the formula 3–6, 5–10, 6–9, 6–8 (females, n=4); 1, 5,?,? (male, basifemur III–IV not clearly visible from specimen).

TABLE 2. Cytochrome Oxidase I haplotypes related to individuals, their ontogenetic stages and distribution according to the coding and clades indicated in Figure 2A. Coordinates given in decimal WGS84 latitude and longitude.

Haplotype	**Individuals (stages)**	**Range**	**Clade (Range)**	**Morphospecies**
Hap. 10	210161 (L); 210232 (L); 210241 (L); 210246 (L).	-20.293, -20.474, -45.660, -45.854	**Clade A:**	***W.* (*W.*) *pachywhartoni***
Hap. 17	210244 (L).	-20.293, -45.854	-16.149—20.474, -43.265—46.074	-16.149–-20.474, -43.144—47.167
Hap. 09	210180 (L); 210194 (L); 210248 (L).	-20.474, -45.660		
Hap. 06	160177 (♀); 160212 (♀); 210251 (♀); 170452 (♀); 174467 (L); 210173 (L); 210195 (L); 210243 (L).	-16.149, -20.350, -43.412, -46.074		
Hap. 07	180490 (♂).	-20.444, -43.765		
Hap. 08	160176 (♀); 170288 (♀); 170297 (♂); 210190 (L); 170230 (♀).	-18.911, -20.474, -43.265, -43.429		
Hap. 16	210162 (L)	-20.417, -45.772	**Clade B:**	
Hap. 11	130432 (L); 210174 (L); 174468 (L); 170291 (♂); 170289 (♂); 210157 (L);	-19.222, -20.368 -43.391, -46.074	-19.086—20.417, -43.144—47.167	
Hap. 15	161056 (♀); 170258 (♀); 170255 (♂); 170018 (♀).	-19.165– -19.222 -43.279– -43.391		
Hap. 14	210081 (♀).	-19.899, -43.473		
Hap. 13	170281 (♀);	-19.086, -47.167		
Hap. 12	170002 (DN); 170326 (DN); 170329 (♀); 170325 (♂); 170270 (♀); 170228 (♂); 180266 (DN); 180252 (♂).	-19.093– -19.405 -43.144– -43.386		
Hap. 02	160193 (♀); 172125 (♀).	-06.456, -50.242	**Clade A:**	***W.* (*W.*) *nudosetosa***
Hap. 01	160070 (DN).	-06.456, -50.242	-06.4561, -50.2419	-03.323–-19.561, -43.3952—52.275
Hap. 18	210082 (L).	-03.323, -52.275	**Clade B**	
Hap. 19	210083 (L); 210084 (L).	-19.561, -43.967	**Clade C:**	
Hap. 05	170263 (♂);	-19.2219, -43.3952	-16.149—19.561, -43.3952—44.628	
Hap. 04	160216 (♀);	-16.149, -44.628		
Hap. 03	160219 (♀).	-16.149, -44.628		

Description. Studied specimens summarized in table 1.

LARVAE (n=3; Figures 10 and 11). Measurements summarized in Table 3.

Idiosoma (Figure 10A–E**)**. Ovoid, color in life is unknown. 100–101 opisthosomal setae (n=2). *Dorsum* (Figure 10A–B). Total of 38 dorsal opisthosomal setae (n=2), with very short barbulae regularly spaced along its axis (Figure 10B), arranged in regular rows: C (c1–5), D (d1–4), E (e1–4), F (f1–4), H (h1–2) [=setae formula: 2H-8-8-8-8-4]. *Venter* (Figure 10C–E). Total of 62–63 ventral setae, including a pair of sternal setae branched (*3a*); opisthosomal ventral setae divided in two shapes (Figure 10D–E), 40–44 setae alike sternal setae but shorter (Figure 10D and F), and 18–23 similar to opisthosomal dorsal setae. All coxal setae branched (Figure 10G–K). Prodorsal sclerite (Figure 10L). Rectangular, broader than long with small and sparse punctations conspicuous; trichobothria (*si*) bases inserted posterior to *se* seta but unknown shape in examined specimen; prodorsal sclerite with *vi* [=AM] >*se* [=PL]> *ve* [=AL]; prodorsal sclerite setae stiff and almost smooth, with minute barbulae. Two pairs of eyes, without ocular plates. Gnathosoma (Figure 10M–Q). Palpal setal formula (fPp) = B/B/BBN/7Bω; subcapitular setae (*bs*) branched; cheliceral blade with minute saw-like edges on dorsal and ventral surfaces (Figure 10O); odontus five-pronged and adoral setae (*cs*) nude or sparsely branched (Figure 10P and Q). Legs (Figure 11A–C). All legs six-segmented, ending in pair of claws and claw-like empodium, without onychotriches. Tarsus I with a dorsal eupathidium (ζ') without a companion seta (z). No mastisetae on legs I-III. Coxa I-III bisetose. *Leg chaetotaxy:* Leg I: Cx—2B; Tr—1B; Fe—6B; Ge—4B, 2σ with solenidia positioned proximal and latero-distal, k; Ti—8B, 2 φ, k; Ta—36–39B, ω, ε, ζ', ζ''; Leg II: Cx—1B; Tr—1B; Fe—5B; Ge—4B, σ, k; Ti—6B, 2 φ; Ta—28–30B, ω, ε, ζ.; Leg III: Cx—2B; Tr—1B; Fe—4B; Ge—4B, σ; Ti —6B, φ; Ta—28–29B, ω.

DEUTONYMPH (n=1; Figures 12–13 and Figure 16H–L). Measurements summarized in Table 4.

TABLE 3. Metric data of larvae from *Whartonia (W.) pachywhartoni* and *W. (W.) nudosetosa*.

Characters	Larvae						Characters	Larvae					
	W. (W.) pachywhartoni (n=18)			*W. (W.) nudosetosa* (n=3)				*W. (W.) pachywhartoni* (n=18)			*W. (W.) nudosetosa* (n=3)		
	n	mean	range	n	mean	range		n	mean	range	n	mean	range
AW	18	122	111–128	3	106	103–110	Ge II	18	52	44–60	3	42	38–45
PW	18	133	116–141	3	115	113–118	Ti II	18	52	45–60	3	41	38–44
Sba	18	12	9–14	3	11	10–11	Ta II	18	143	135–158	3	111	108–114
Sbp	18	50	44–55	3	41	40–43	Leg II (with coxa)	18	437	339–483	3	359	348–372
ASB	17	38	26–43	3	30	30–30	Cx III	18	72	66–80	3	61	58–63
PSB	17	9	5–12	3	8	8–9	Tr III	18	47	38–56	3	44	43–45
SD	17	48	38–52	3	38	38–38	Fe III	18	79	63–87	3	64	64–64
AP	17	26	19–32	3	19	19–20	Ge III	18	62	52–69	3	50	46–52
vi	16	91	85–96	3	71	62–83	Ti III	17	62	49–69	3	50	49–51
ve	17	53	46–59	3	45	43–48	Ta III	16	175	160–189	3	134	132–135
se	17	85	78–90	3	59	56–62	Leg III (with coxa)	18	474	262–529	3	402	392–409
si	2	127	122–131	NA	NA	NA	IP	18	1390	1158–1546	3	1163	1134–1189
chelicera	10	72	71–76	2	70	69–71	σ'' I posterior	15	34	20–45	3	35	31–42
palptrochanter	18	15	12–18	3	13	12–14	σ' I anterior	14	35	28–47	3	32	21–47
palpfemur	18	47	40–58	3	51	45–54	κ I	17	12	10–15	2	11	10–13
palpgenu	18	22	19–26	3	21	20–21	φ'' I proximal	15	21	18–27	2	20	19–20
palptibia	18	27	16–35	3	25	24–26	φ' I distal	17	25	21–30	2	28	28–28
palptarsus	18	21	16–25	3	18	16–19	κ I	15	13	10–19	2	11	10–12
Odo	18	29	23–35	3	26	25–27	ω I	15	23	18–26	2	28	28–29
IL	8	512	247–582	2	344	314–374	ε I	15	5	4–8	2	6	6–6
IW	9	300	226–404	2	207	183–230	z I	13	23	16–30	2	21	18–24
3a	14	46	34–57	2	44	41–47	ζ' I proximal	13	22	19–25	2	29	28–29
Seta H	14	73	61–90	2	64	61–66	ζ'' I distal	16	16	13–22	2	16	16–17
Cx I	18	75	72–82	3	63	59–65	σ II	13	42	28–57	1	49	49–49
Tr I	18	39	29–47	3	34	31–36	κ II	14	12	10–14	2	11	9–13
Fe I	18	82	74–92	3	68	67–70	φ'' II proximal	17	19	16–21	2	17	17–18
Ge I	18	59	52–69	3	48	47–48	φ' II distal	17	23	16–28	2	22	21–23
Ti I	18	60	54–68	3	52	49–57	ω II	17	24	21–27	2	23	22–24
Ta I	18	165	157–189	3	138	134–140	ε II	15	5	3–7	2	4	4–5
Leg I (with coxa)	18	480	457–539	3	402	395–408	ζ II	17	15	13–19	2	19	17–21
Cx II	17	88	83–102	3	70	66–73	σ III	15	47	32–65	1	51	51–51
Tr II	18	39	28–47	3	37	36–38	φ III	13	38	23–53	2	43	25–61
Fe II	18	68	58–89	3	58	56–60	ω III	16	24	17–28	2	24	23–24

Idiosoma (Figure 12A–F). Color in life is unknown. Idiosoma oval, not constricted, with anterior region narrowing from leg II to palps. Idiosoma densely covered by canoe-like leaf setae, having the surfaces covered by setules and margins bent upward dorsally, slightly serrated margins, and the wall seems reinforced by bifurcating ribbons; set on cylindrical papillae in a striated cuticle. The following measurements were taken from five such setae from anterolateral idiosoma of three individuals (indicated in Figure 12C): height, 11–14; width, 24–26 (Figure 12B–F). Crista metopica (Figure 12G–J). Region well sclerotized, has a narrow stem, tectum with smooth borders, bearing setae *vi*, which is long and leaf-like (Figure 12H). Posterior trichobothria (*si*) area sagittate, with a faint posterior process; trichobothria (*si*) nude and flagelliform. One pair of eyes, without ocular plate, on each side of prodorsum; anterior to sensillar area, leveled to in middle length crista metopica. Gnathosoma (Figure 13A–F). Hypostome blunt, triangular, height=240, basal width=156, bearing 18 barbed setae, gnathosoma base with 55 weakly expanded barbed setae. Chelicerae base stout, cheliceral blade curved, with minute teeth (Figure 13A). Palptrochanter without setae. Palpfemur and palpgenu bearing two kinds of setae, 19 and 13 expanded and leaf like, 19 and 17 barbed slender setae, respectively; palptibia bearing 13 barbed setae unexpanded, one spinelike seta (basidont) ventrally and two dorso-lateral spines (paradonts) in tandem; palptibia with odontus entire, slender, shorter than palptarsus; palptarsus cylindrical, elongate bearing 16–19 (asymmetrical in individual studied) pectinate setae, of these two ventro-lateral setae robust; one proximal solenidion (ω) and two subterminal eupathidia (ζ) (Figure 13B–F). Genital valves (Figure 13G). Placed posterior to coxae IV; epivalves bear 10–14 expanded leaf-like setae; five barbed setae with smooth apex on centrovalves; incomplete genitalia with two pairs of genital acetabula. Anal valves (Figure 13H). Apart from genital valves by 63, bearing 4 barbed setae, approaching the leaf-like type, Legs. All legs shorter than idiosoma, legs I and IV much longer than legs II-III (Figure S3A–C). Two kinds of normal setae on all legs: coxae to tibiae with expanded, elliptical leaf-like, similar to those in palp, sternum and genital epivalves; tarsi I to IV with slender barbed setae (sometimes also on distal tibiae, similar to female, Figure 15B–E). *Specialized setae on all legs* (as normal setae are imbricated and precluded accurate counting for some segments only the minimum number of such setae indicated): leg I: bFe - 3 θ; tFe - 7 θ, ca 17 ζ; Ge - ca 9 σ, 20 ζ; Ti - 15 φ, 30 ζ; Ta - >90 ω (with 6 strongly robust) and > 50 ζ small and curved tip; leg II: bFe - 2 θ; tFe - θ, 4 ζ; Ge - 3 σ, 7 ζ; Ti - 9 φ, 7 ζ; Ta - >50 ω (with one robust), k and ζ distal, close to the claw; leg III: bFe - 2 θ; tFe - 2 θ, 5 ζ; Ge - 4 σ, 5 ζ; Ti - 10 φ, 10 ζ; Ta - > 50 ω (with one robust) and ζ dorsal, close to claw fossae; bFe - 2 θ; tFe - 2 θ; Ge - 4 σ, 15 ζ; Ti - 15 φ; 18 ζ; Ta - > 50 ω and ζ dorsal, close to claw fossae. Solenidia (θ) on basifemur I to IV (similar to female, Figure 15F and G); eupathidia (ζ) numerous in dorsum on telofemur to tibia I-IV (similar to female, Figure 16A–C); solenidia (σ) present on genu II; tarsus I pyriform with claw reduced relative to other legs (similar to female, Figure 16D–G), solenidia (ω) and eupathidia (ζ) especially numerous on tarsus I with various robust solenidia (Figure 16H and I); microseta (k) on tarsus II (Figure 16J); some robust solenidia (ω) on tarsus II and III (Figure 16J–K), dorsal eupathidia (ζ) close to claw fossae on tarsus III to IV (Figure 16K); and distal eupathidia (ζ) close to the claw on tarsus II (Figure 16L).

FEMALES (n=4; Figure 14A and E–G and O; Figures 15A–G and 16A–G). Measurements summarized in Table 4.

Idiosoma, crista metopica, genital and anal valves, gnathosoma and legs. As in deutonymphs except by hypostome with 42 barbed setae, and base gnathosoma with 108 weakly expanded setae (n=1) (Figure 15A). Palpfemur and genu bearing two kinds of setae, 40–44 and 18–23 expanded and leaf like (male: 35 and 32 setae), 18–35 and 23–28 barbed slender setae, respectively (male: 34 and 28 setae) (n=3); palptibia with about 20–24 slender barbed setae (male: 21 setae), one spinelike seta (basidont) ventrally, three dorso-lateral spines (paradonts) in tandem and one accessory dorso-lateral spine; palptarsus bearing 25–26 pectinate setae (male: 31 setae), one solenidion (ω) and five subterminal eupathidia (ζ) (n=3). Genital valves bearing 43 leaf-like setae on genital epivalve and 26–30 slender barbed setae on genital centrovalve (n=1); complete genitalia with three pairs of genital acetabula (Figure 14E–G). Anal valves (n=2) bearing 9–10 long barbed setae on each valve, apart from genital valves by 123–150 (Figure 14O). Solenidia (θ) on basifemur I to IV following the formula 3–6, 5–10, 6–9, 6–8 (n=4).

MALES (n=1; Figures 14B–D and H–M). Measurements summarized in Table 4.

Idiosoma, crista metopica, genital and anal valves, gnathosoma and legs. Similar to females, except by genital area, with fan-like membranous setae on genital centrovalve and internal genital armature, and the number of setae on genital and anal valves. One epivalve with 54 barbed setae, the other non-suitable to study; two setae morphology on centrovalves, 38–45 barbed setae, and 12 fan-like membranous setae around middle length of each valve (Figure 14H–K). Four pairs of smooth and slender setae on the internal genital armature (Figure 14L). Anal valves bearing nine pairs long barbed setae on each valve, apart from genital valves by 149 (Figure 14M). Solenidia (θ) on basifemura I and II 1 and 5, respectively (basifemur III–IV not clearly visible from male specimen).

Studied UFMG-AC specimens summarized in table 1.

TABLE 4. Metric data of deutonymphs, males and females of *Whartonia (W.) pachywhartoni* and *W. (W.) nudosetosa*.

Characters	Deutonymphs				Females						Males				
	W. (W.) pachywhartoni (n=3)			*W. (W.) nudosetosa* (n=1)	*W. (W.) pachywhartoni* (n=7)			*W. (W.) nudosetosa* (n=4)			*W. (W.) pachywhartoni* (n=6)			*W. (W.) nudosetosa* (n=1)
	n	mean	range	160070	n	mean	range	n	mean	range	n	mean	range	170263
IL	3	1497	1302–1678	1121	7	2349	2070–2565	4	1675	1097–2112	5	2186	1836–2587	NA
IW	3	868	766–1041	1121	7	1392	1222–1600	4	1049	968–1127	6	1368	1056–1617	1081
Sba	3	13	8–17	29	6	16	12–21	3	13	9–16	6	14	7–21	NA
Sbp	3	35	32–39	41	7	49	44–57	3	50	49–50	6	50	44–56	65
Vvi	3	52	51–53	59	5	83	58–95	3	71	66–76	6	61	42–73	NA
si	0	NA	NA	NA	2	179	172–185	1	225	225	2	187	141–234	NA
ISD	3	274	255–291	NA	7	490	420–535	3	413	372–436	6	466	430–503	NA
Crista metopica`s common setae	2	49	43–55	54	5	74	65–81	2	66	65–68	4	71	26–78	NA
ACW (=CMW)	0	NA	0–0	NA	3	9	7–13	1	17	17	3	16	15–19	NA
PCW	3	9	7–11	6	6	12	8–14	3	8	6–10	6	12	10–15	16
ASAW	0	NA	NA	NA	4	85	66–107	2	80	70–89	5	76	61–95	NA
PSAW	3	72	68–74	69	7	93	85–107	3	94	86–101	6	91	83–99	111
CML	3	343	324–357	392	7	599	531–677	2	517	472–562	6	582	519–682	NA
O	3	21	20–22	23	6	30	22–34	3	31	30–31	6	26	21–32	NA
O–O	3	304	241–348	337	5	451	392–553	2	448	413–483	6	486	406–572	NA
OAS	3	121	42–162	111	4	228	146–366	2	179	105–254	6	218	148–293	NA
ECO	3	21	20–22	23	6	30	22–34	3	31	30–31	6	26	21–32	NA
Eye Ring	3	35	27–41	31	6	41	33–52	3	42	40–44	6	37	31–42	NA
EC–EC	3	304	241–348	337	4	466	394–553	2	448	413–483	6	486	406–572	NA
EC–Vi	3	176	115–283	NA	4	259	131–378	2	239	186–292	6	241	186–301	NA
GL	3	238	236–241	250	5	462	433–485	4	351	318–368	6	355	287–410	350
GW	2	168	143–193	156	5	257	223–290	4	227	206–244	6	223	185–266	230
Pa Tr (L)	3	39	36–41	46	6	68	58–83	4	47	43–52	6	63	58–67	54
Pa Tr (W)	3	69	66–70	60	6	107	90–123	4	76	62–90	6	103	91–111	91
PaFe (L)	3	160	156–164	133	6	230	145–275	4	203	184–213	6	255	246–265	195
PaFe (W)	3	94	89–98	79	6	165	135–262	4	101	91–112	6	130	125–139	85
PaGe (L)	3	72	71–73	62	6	130	120–148	4	92	86–101	6	126	120–136	80
PaTi (L)	3	79	76–81	67	6	130	122–149	4	94	89–105	6	130	126–136	103
PaTa (L)	3	70	67–74	69	6	115	106–128	4	105	97–113	6	111	102–118	98
PaTa (W)	3	19	16–21	20	6	29	26–33	4	27	26–28	6	28	27–29	30
Odo (L)	3	62	59–67	67	6	91	80–105	4	89	84–100	6	95	91–103	111
DS	3	28	25–31	27	4	24	22–25	4	29	25–33	6	28	24–38	44
VS	3	23	20–26	21	4	27	23–33	4	22	20–24	5	27	23–34	NA
Cx I	3	162	155–166	172	7	256	238–285	3	227	207–239	6	258	243–269	222
Tr I	3	118	114–122	74	7	183	169–195	4	134	119–148	6	178	170–188	175
BFe I	3	154	146–170	159	7	292	255–327	4	229	197–278	6	268	227–291	176
TFe I	3	135	115–152	118	7	211	200–223	4	193	179–204	6	199	181–221	192

......continued on the next page

TABLE 4. *(Continued)*

Characters	Deutonymphs				Females						Males			
	W. (W.) pachywhartoni (n=3)			*W. (W.) nudosetosa* (n=1)	*W. (W.) pachywhartoni* (n=7)			*W. (W.) nudosetosa* (n=4)			*W. (W.) pachywhartoni* (n=6)			*W. (W.) nudosetosa* (n=1)
	n	mean	range	160070	n	mean	range	n	mean	range	n	mean	range	170263
Ge I	3	138	121–148	123	7	233	206–255	4	196	186–212	6	217	195–229	201
Ti I	3	164	154–169	153	7	281	249–307	4	234	227–245	6	271	257–283	248
Ta I (L) (without claw)	3	269	265–276	260	7	431	398–457	4	372	356–383	6	413	405–425	354
Ta I (W)	3	99	92–106	111	7	144	126–158	4	143	131–154	6	147	138–162	161
Leg I (without Cx)	3	1013	917–1100	887	7	1671	1510–1861	4	1358	1292–1447	6	1546	1512–1592	1346
Cx II	3	164	150–176	135	7	252	221–295	3	211	176–230	6	242	210–276	208
Tr II	3	103	100–107	77	7	163	146–174	4	150	142–161	6	168	160–175	187
Bfe II	3	112	97–120	81	7	176	142–205	4	149	125–171	6	195	184–208	168
Tfe II	3	96	84–105	94	7	158	150–169	4	135	116–147	5	157	140–170	114
Ge II	3	101	99–105	80	7	174	150–193	4	154	140–163	6	169	164–179	140
Ti II	3	116	114–118	97	7	230	199–307	4	176	166–186	6	206	194–224	204
Ta II (L) (without claw)	3	211	210–212	208	7	345	246–399	4	321	296–347	6	333	311–353	278
Leg II (without Cx)	3	738	707–758	636	7	1246	1187–1325	4	1085	1036–1158	6	1203	1071–1253	1090
Cx III	3	138	112–159	129	7	207	192–221	4	179	146–222	6	203	184–230	164
Tr III	3	93	73–114	108	6	166	158–177	3	151	146–157	6	177	160–198	116
Bfe III	3	116	107–133	101	6	209	165–237	3	147	120–163	6	219	191–256	142
Tfe III	3	96	86–110	98	6	172	151–211	3	140	136–148	6	161	155–172	129
Ge III	3	106	97–111	98	6	187	167–233	4	145	131–154	6	174	167–186	157
Ti III	3	120	116–124	104	6	229	216–247	4	181	171–192	6	220	209–227	172
Ta III (L) (without claw)	3	213	205–217	195	6	336	302–388	4	315	294–337	6	325	291–340	302
Leg III (without Cx)	3	745	698–782	704	6	1299	1220–1474	4	970	645–1126	6	1276	1212–1336	1018
Cx IV	3	163	137–182	130	7	272	219–310	4	219	203–239	6	253	202–286	206
Tr IV	3	139	119–149	148	7	253	230–270	3	191	161–215	6	244	213–260	270
Bfe IV	3	123	109–142	127	6	242	228–262	4	187	163–212	6	236	221–248	186
Tfe IV	3	105	83–117	130	6	205	186–226	4	178	159–206	6	204	192–212	175
Ge IV	3	131	118–140	121	7	239	214–262	4	192	173–200	6	230	220–247	220
Ti IV	3	180	152–204	156	7	290	236–325	4	263	249–279	6	304	295–318	278
Ta IV (L) (without claw)	3	253	249–260	243	7	421	294–514	4	407	383–447	6	419	403–441	397
Leg IV (without Cx)	3	931	842–978	925	7	1586	1388–1730	4	1370	1201–1529	6	1638	1594–1696	1526
IP	3	3426	3163–3613	3151	7	5617	4872–6015	4	4783	4408–5259	6	5662	5551–5804	4980
AC1	3	28	27–29	29	6	43	38–47	4	39	35–42	6	39	35–43	NA
AC2	3	29	25–33	24	6	39	36–45	4	35	33–39	6	36	33–38	NA
AC3	0	NA	NA	NA	6	37	33–43	4	36	31–41	6	31	27–36	NA
Gen L	3	135	128–149	132	7	288	256–310	4	245	232–267	6	271	215–292	249
Gen W	3	95	86–105	85	7	234	183–297	4	168	137–202	6	223	187–267	263
An L	2	65	62–68	59	7	89	81–97	4	78	72–83	6	91	80–102	79
An W	2	34	32–35	42	7	52	45–61	4	54	49–61	6	52	48–57	52
PprL	3	47	44–50	21	7	69	59–75	2	70	69–71	6	68	57–72	NA
PprW	3	18	13–21	17	6	24	18–30	2	24	24–25	6	22	17–27	NA

Remarks

The larvae of *W. (W.) pachywhartoni*, *W. (W.) nudosetosa* and *W. (W.) parauapebensis* share the following characteristics: coxal chaetotaxy formula 2, 1, 2; femur chaetotaxy formula 6, 5, 4; genu solenidia formula 2σ, σ, σ; tibia solenidia formula 2φ, 2φ, φ; tarsus solenidia formula ω, ω, ω; absence of companion seta (z) on Tarsus I; coxa III bisetose; and presence of ω on Ta III and absent plate ocular. *W. (W.) pachywhartoni* and *W. (W.) nudosetosa* also share subterminal eupathidium on palptarsus and ocular plate are absent (Silveira *et al.* 2015; Bassini-Silva *et al.* 2022a). Two additional shared characters may be the branched adoral (*cs*) setae and palpal chaetotatxy B/B/BBB in the latter two species, nevertheless, Silveira *et al.* (2015) observed varying conditions of these in most of Brazilian material for *W.* (*W.*) *nudosetosa*: adoral (*cs*) setae, nude in most specimens but sparsely branched setae in some their specimens; and palpal setal formula (fPp), B/N/BNN (one specimen with sparse pectination (only two barbulae) on the lateral setae of the tibia, UFMG-AC1200331) and B/B/B/BNN (most specimens), instead of B/B/BBB of the holotype. Here, we consider these variations to be intraspecific but suggest testing it with new material and molecular data from the type locality and across species geographical range.

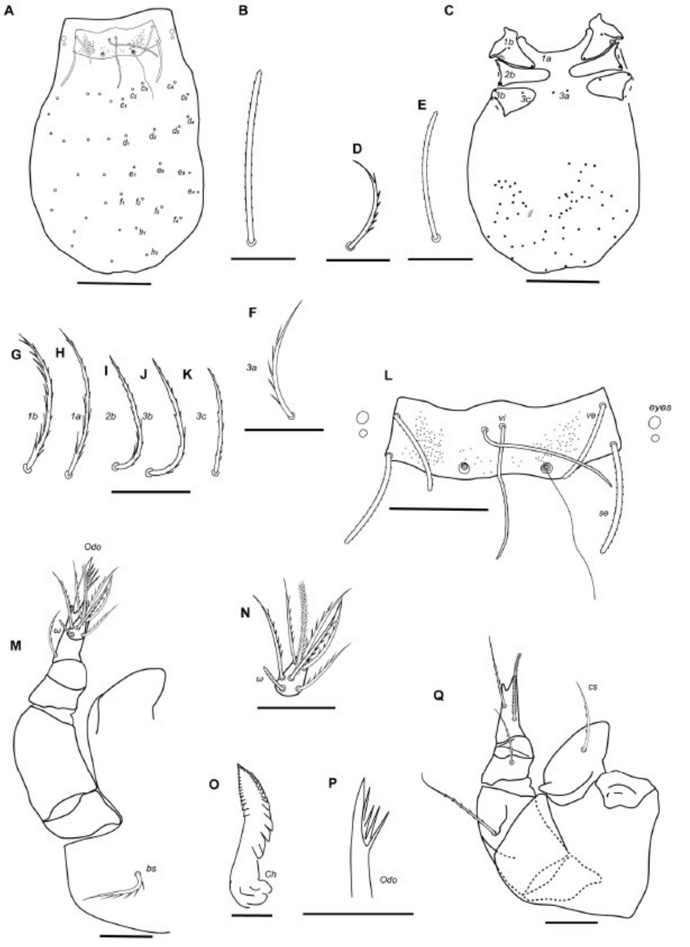

FIGURE 10. *Whartonia (W.) nudosetosa*, larvae. A: Idiosoma, dorsal view, open circles indicate dorsal setae; B: *C4*, seta 4 of row C [=H, humeral seta] in dorsal opisthosomal seta; C: Idiosoma, ventral view, solid circles indicate ventral setae; D: Ventral opisthosomal setae [=preanal setae or true ventral setae]; E: Ventral opisthosomal setae [=postanal setae]; F: *3a* seta; G: *1b* seta; H: *1a* seta; I: *2b* seta; J: *3b* seta; K: *3c* seta; L: Prodorsal sclerite and eyes; M: Gnathosoma, venter; N: Palptarsus; O: Chelicera; P: Odontus; Q: Gnathosoma, dorsum. *Symbols: 1a*, seta on proximal coxa I; *1b*, seta on distal coxa I; *2b*, coxa II seta; *3a*, sternal setae; *3b*, seta on distal coxa III; *3c*, seta on proximal coxa III; *ve*[=AL], external vertical setae; *vi*[=AM]; internal vertical setae; *se*[=PL], external scapular setae; *Ch,* Chelicera; *Odo*, odontus; *bs*, subcapitular setae; *ω*, solenidion on palptarsus and *cs*, adoral setae. Scale Bars: A and C=100 µm; B, D–E and M–Q=20 µm; F–K=25 µm; L=50 µm.

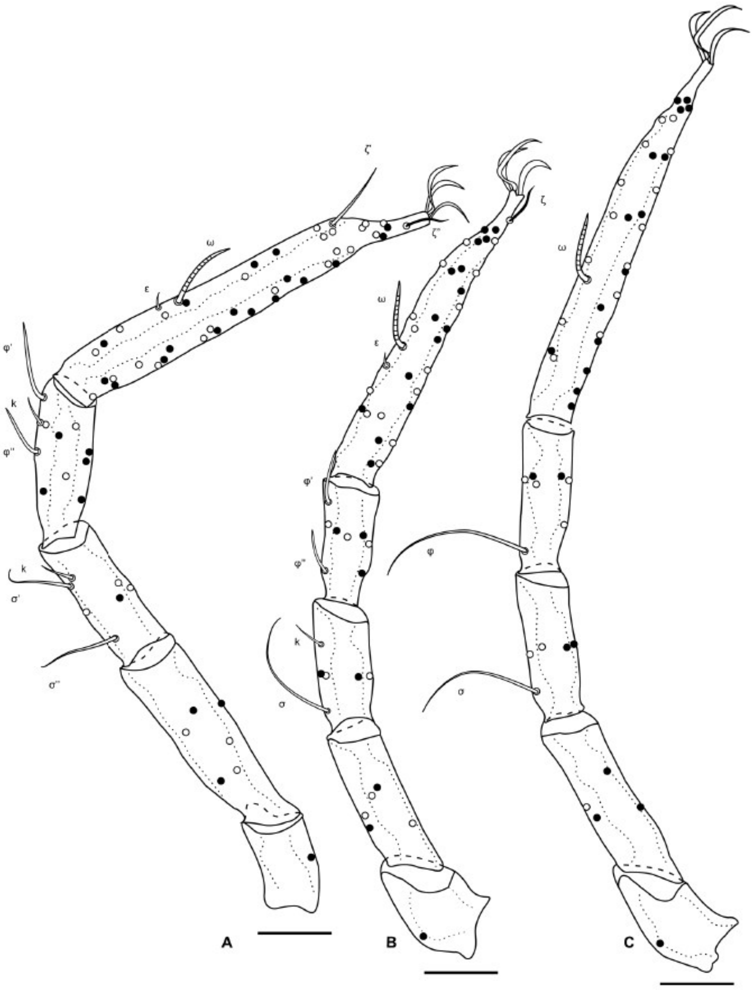

FIGURE 11. *Whartonia (W.) nudosetosa,* larvae. A: leg I; B: leg II; C: leg III. Solid circles indicate ventral leg setae; open circles indicate dorsal leg setae. *Symbols:* ζ', ζ, dorsal eupathidia on Ta I and II; ζ'', subterminal eupathidium on Ta I; ω, solenidion on Ta I, II and III; σ', σ'', solenidion on Ge I; σ, solenidion on Ge II and III; κ, microsetae on Ge I and II and Ti I; φ', φ'', solenidia on Ti I and II; φ, solenidia on Ti III; ε, famulus on Ta I and II. Scale Bars: A–C=25 μm.

Whartonia. (W.) pachywhartoni differs from the other two species by having solenidia on genu I at same level, placed proximally; prodorsal sclerite setae stiff with very dense barbs; tarsus I with 50–56 branched setae; and many opisthosomal setae (total number of idiosomal setae: 115–148). On other hand, the other two species share solenidia on genu I apart from each other; prodorsal sclerite setae stiff and almost smooth, with minute barbulae; tarsus I with 36–39 branched setae; and fewer opisthosomal setae (total number of idiosomal setae: 90–101). However, *W.* (*W.*) *parauapebensis* has an eupathidion on palptarsus; odontus with two larger supplementary prongs; and two pairs of eyes set on ocular plates.

Deutonymphs and adults of *W. (W.) pachywhartoni* and *W. (W.) nudosetosa* share the idiosoma oval, not constricted; crista metopica with narrow stem, tectum with smooth borders, *vi* leaf-like, posterior trichobothria (*si*) nude and flagelliform, its insertion area sagittate with a posterior process; one pair of eyes, without ocular plates. They also share idiosoma densely covered by canoe-like leaf setae (Figures 5B–F, 12B–F). This setae morphology finds some parallels among Microtrombidiidae, and the terminology employed by Gabryś (1999) may be tentatively employed for its description (see specially Gabryś 1999's Fig. 10). Setae are born on almost cylindrical papillae, with short peduncle and canoe-like folded leaf covered by tiny setulae and reinforced by ribbons. The setae stem delimits a space or chamber, with a wide opening.

Deutonymphs and adults of *W. (W.) pachywhartoni* differ from *W. (W.) nudosetosa* by the size and shape of these idiosomal setae (setae body excluding peduncle height=6–9; width=17–21, vs height=11–14, width, 24–26 in deutonymphs); lanceolate leaf-like setae on palp, sternum, genital epivalves and legs (Figures 6E, G, 8F–G) vs elliptical leaf-like on these structures (Figures 13 D, F–G); and absence of solenidion (θ) on basifemur I vs presence.

Meristic characters that must be confirmed with further sampling are: *W. (W.) pachywhartoni* deutonymphs differs from *W. (W.) nudosetosa* by bearing two subterminal eupathidia (ζ) on palptarsus (instead of three ζ); six or seven barbed setae on centrovalves (instead of five); anal valves bearing five or six long slender barbed setae (instead of four); and solenidia (θ) on basifemur following the formula 0, 2, 4, 0-1 (instead of 3, 2, 2, 2); males with 16 pairs of fan-like membranous setae (instead of 12) on centrovalves located approximately at middle length of each sclerite.

Discussion

Molecular data in integrative studies of Parasitengona

The use of molecular data in integrative taxonomy often employs the same tools to achieve two objectives: species delimitation that aims at correct assignment of individuals to species and association among heteromorphic stages, otherwise possible by rearing experiments only (e.g., Stålstedt *et al.* 2016; Costa *et al.* 2019). The strengths and limitations of the set of tools (including DNA markers and analytical approaches), as well as the sampling design are different for the two purposes.

For example, it is clear that methods based on multispecies coalescent model may delimit the genetic structure instead of species (Sukumaran & Knowles 2017). It seems to be the case in this study, especially for individuals identified as *W.* (*W.*) *nudosetosa.* The clades recovered as distinct species are geographically apart from each other. Furthermore, the markers employed here certainly are far from ideal. The mitochondrial genome has a population effective size of a quarter, making usually sharper the geographic structuring and the 28S fragment obtained proved to be almost with no intraspecific variation. Future studies must be designed to sample across Brazil to bridge the geographic gap and new informative nuclear markers must be obtained.

On the other hand, the sampling of individuals and markers included in this study allowed associating larvae and post-larval stages beyond doubt. It was possible because even if the strictest criterion was employed, we could obtain individuals belonging to all stages in the same strongly supported clades.

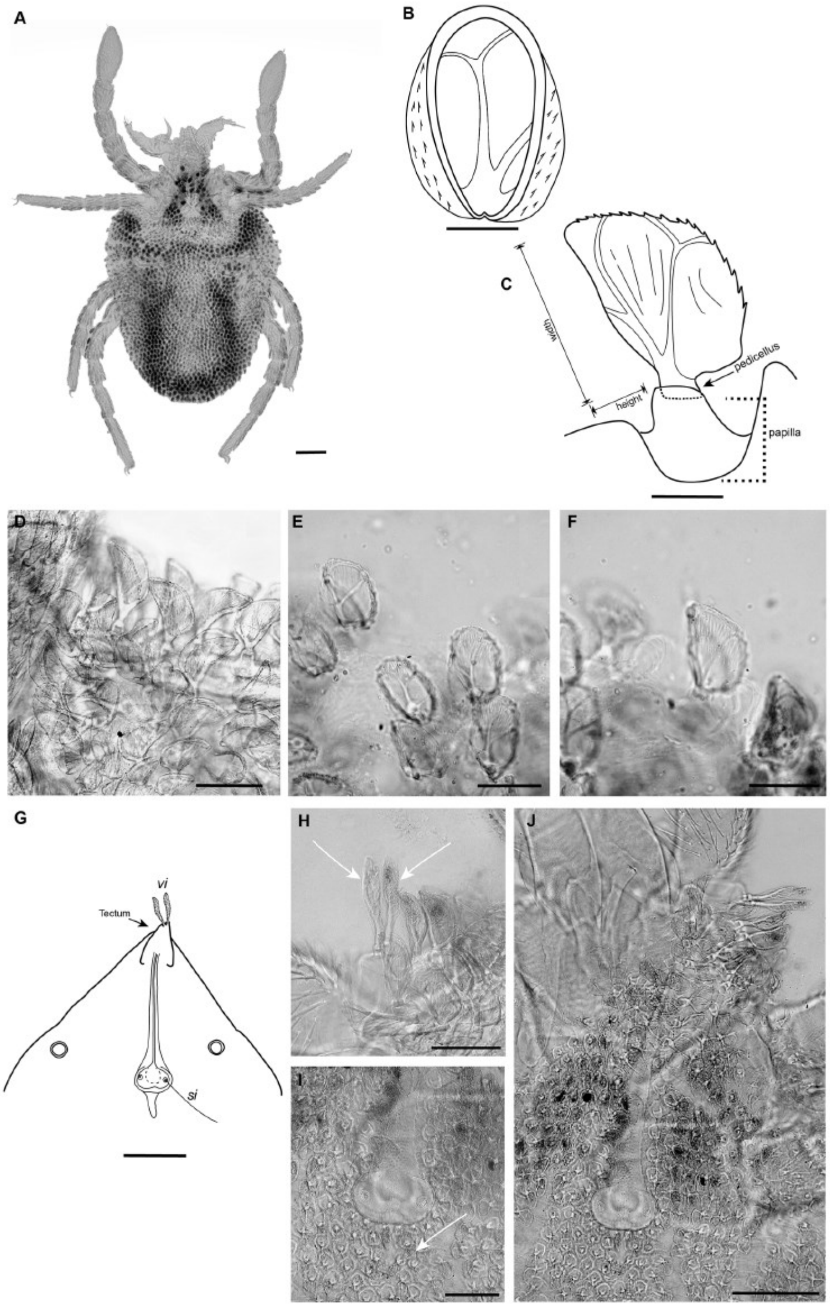

FIGURE 12. *Whartonia (W.) nudosetosa*. Deutonymph, A: General view, B–C: Opisthosomal seta with details; Male, D–F: Opisthosomal seta; Deutonymph, G: Crista metopica and eyes; Female, H: *vi* setae; Deutonymph, I: Posterior process on crista metopica and J: Crista metopica and dorsal setae. Scale Bars: A, G and J=100 μm; B–C, 10 μm; D, H–I=50 μm; E–F=20 μm.

FIGURE 13. *Whartonia (W.) nudosetosa*, deutonymph. A: Chelicera with blade detail; B: Dorsal palp view; C: Chaetotaxy of dorsal setae in tibia-tarsus palpal; D: Ventral palp view; E: Chaetotaxy of ventral setae tibia-tarsus in tibia-tarsus palpal; F: Chaetotaxy of setae in palp; G: Genital pore; H: Genital centrovalve seta; I: Genital epivalve seta; J: Anal pore; K: Anal seta. Scale Bars: A and G=50 µm; B–F and H=20 µm.

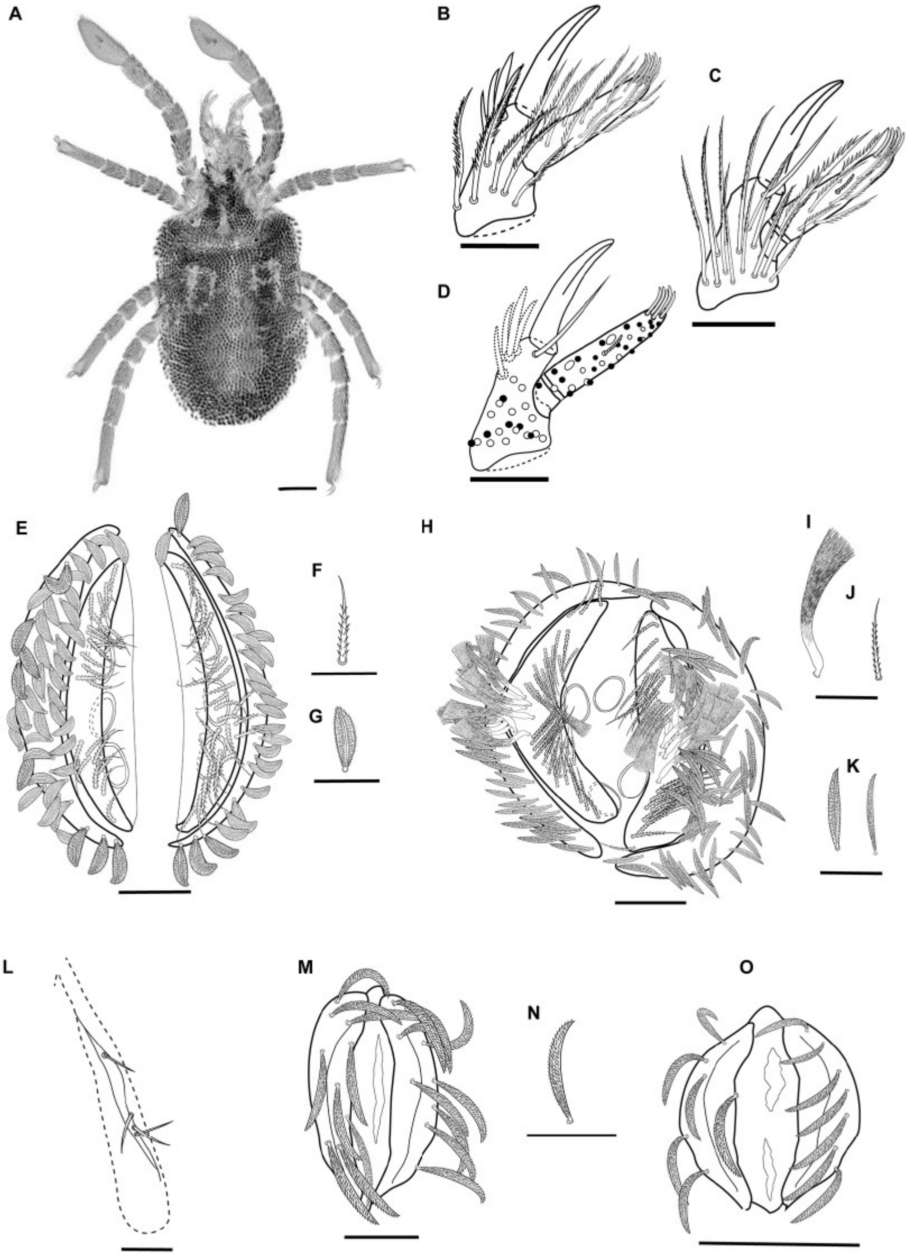

FIGURE 14. *Whartonia (W.) nudosetosa*. Male, A: General view, B: Dorsal tibia-tarsus on palp, C: Ventral tibia-tarsus on palp, D: Chaetotaxy of tibia-tarsus in palp; Female, E: Genital pore, F: Genital centrovalve seta, G: Genital epivalve seta; Male, H: Genital pore, I–J: Genital centrovalve setae, K: Genital epivalve seta, L: Internal genital armature, M: Anal pore, N: Anal seta; Female, O: Anal pore. Scale Bars: A=200 µm; B–E, H and O=50 µm; F–G, I–K and M–N=25 µm; L=20 µm.

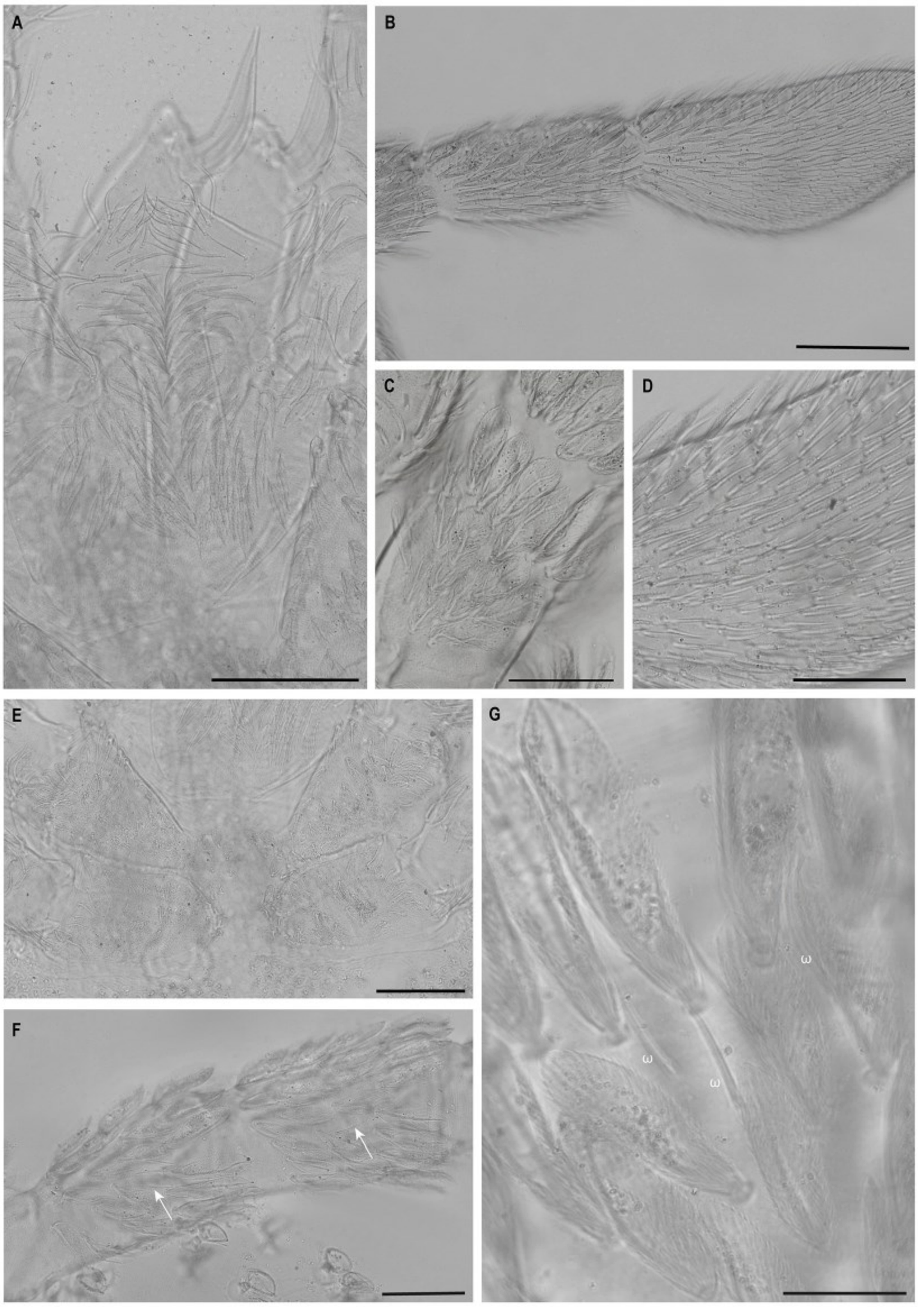

FIGURE 15. *Whartonia (W.) nudosetosa*, female. A: Hypostomal setae; B: Tibia-tarsus I; C: Setae on trochanter I; D: Setae on tarsus I; E: Sternum and coxae; F–G: Solenidia (θ) on basifemur to telofemur. Scale Bars: A–B, E= 100 µm; C–D, F=50 µm; G=20 µm.

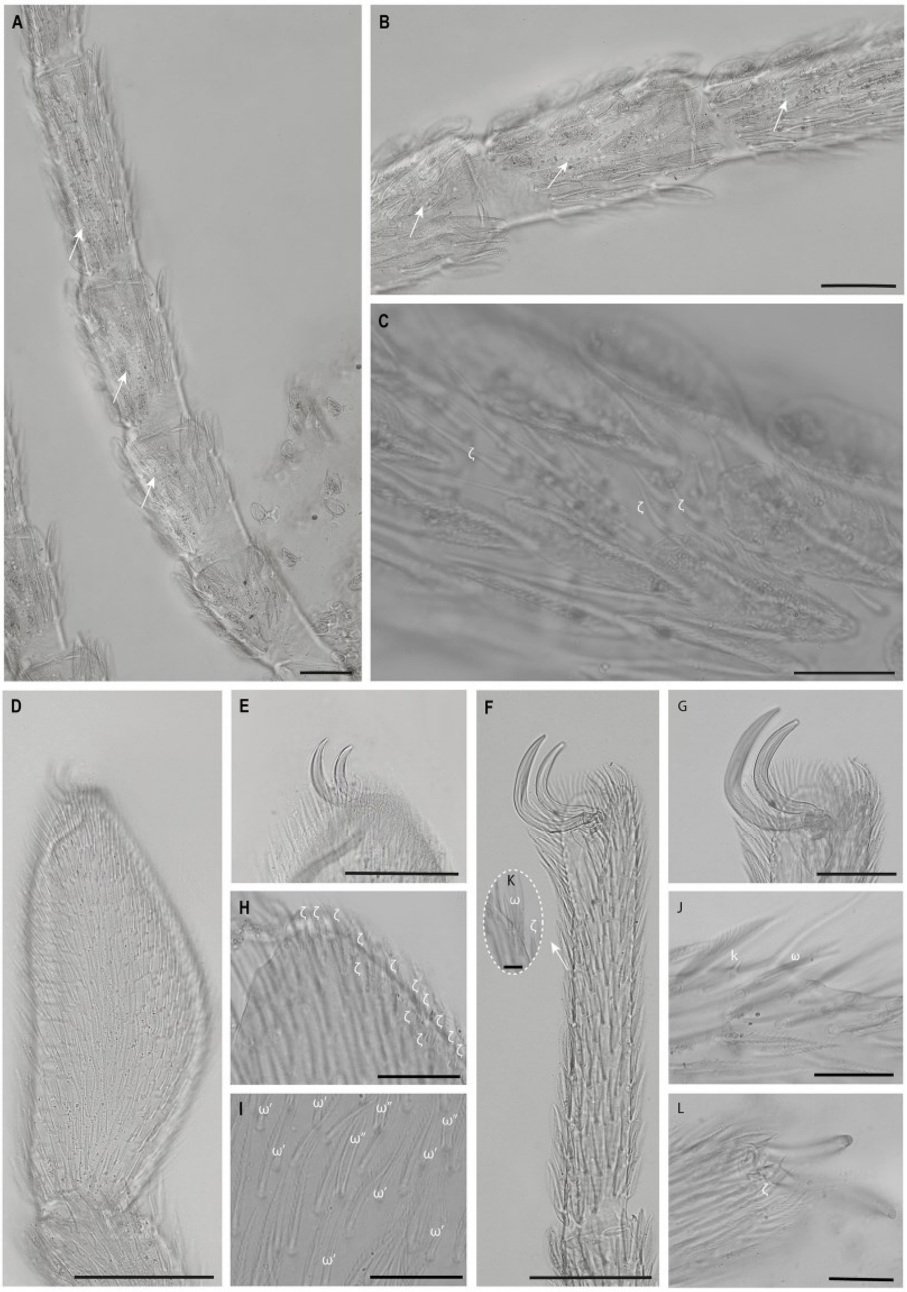

FIGURE 16. *Whartonia (W.) nudosetosa*. Female, A: Eupathidia (ζ) numerous on dorsum of legs, B–C: Details of eupathidia (ζ), D: Tarsus I, E: Claw I, F: Tarsus IV, G: Claw IV; Deutonymph, H: Eupathidia (ζ) on tarsus I, I: Solenidia (ω) on tarsus I, J: Microseta (k) and solenidia (ω) on tarsus II, K: Dorsal eupathidia (ζ) close to claw fossae, L: Distal eupathidia (ζ) on tarsus II. Scale Bars: A–B, E and G=50 µm; C, H–J and L=20 µm; D and F=100 µm; K=10 µm.

We preferred being conservative and assigning the individuals obtained to the two species previously described from larvae. If the clades recovered here as different species reflect a cryptic diversity at species level or an artifact related to geographic or intraspecific variation remains to be better explored.

Postlarval morphology

A total of 279 species from 34 genera belonging to the Leeuwenhoekiidae family are known worldwide (Nielsen *et al.* 2021). Of these, only eight species (~2.8%) belonging to six genera have postlarval individuals, in this case deutonymphs, described, all by Crossley (1960). The taxonomy of leeuwenhoekiid mites is based almost entirely on their larvae, as most Parasitengona (Shatrov & Kudryashova 2008; Jacinavicius *et al.* 2018).

No species belonging to *Whartonia* had their post-larval stages described, but a closely related species, previously included in the genus, *Albeckia senase*, had the deutonymph described and may be useful for comparisons (Crossley 1960). *W. (W.) pachywhartoni* and *W. (W.) nudosetosa* differs from *A. senase* by: eye presence (vs. absent); 16–18 and 13 barbed setae on palptibia, respectively (vs. six setae); 16–19 and 14 branched setae on palptarsus and two and three eupathidia (ζ) distally, respectively (vs. seven branched setae and five eupathidia (ζ) distally); *vi* not forked (vs. forked); hypostome with 32 and 18 distal barbed setae and gnathosoma base with 52–53 and 55 weakly expanded setae, respectively (vs. 6–8 weakly distal barbed setae and 10 expanded setae); shape of idiosomal setae that instead of canoe-like leaf setae are arborescent, branched setae (see Crossley 1960, figure 234).

Deutonymphs and adults provide additional characters for chigger taxonomy and, whenever possible, should be considered in parallel with larval characters in species identification (Wharton 1951; Crossley 1960; Singer 1971). Here, we note that the distribution of special setae (eupathidia and solenidia) on legs of poslarval *Whartonia* species that occurs on basifemur, something unusual among poslarval Trombiculidae in which this character was described thus far, with special setae appearing from telofemur onwards (Audy *et al.* 1965; Moniuszko *et al.* 2017).

We could also register ontogenetic changes that were expected because they also occur in other Trombiculidae and Leeuwenhoekiidae. *Deutonymphs*—two spines (paradonts) in tandem and none spine accessory on palptibia; fewer eupathidia (ζ) distally on palptarsus; incomplete genitalia with two pairs of genital acetabula. *Adults*—three spines (paradonts) in tandem and one spine accessory on palptibia; more eupathidia (ζ) distally on palptarsus; complete genitalia, three pairs of genital acetabula and, besides, males have fan-shaped genital setae on centrovalves of each sclerite (André 1929; Wharton & Hardcastle 1946; Wharton 1951; Southcott 1996; Makol *et al.* 2010; Moniuszko *et al.* 2017).

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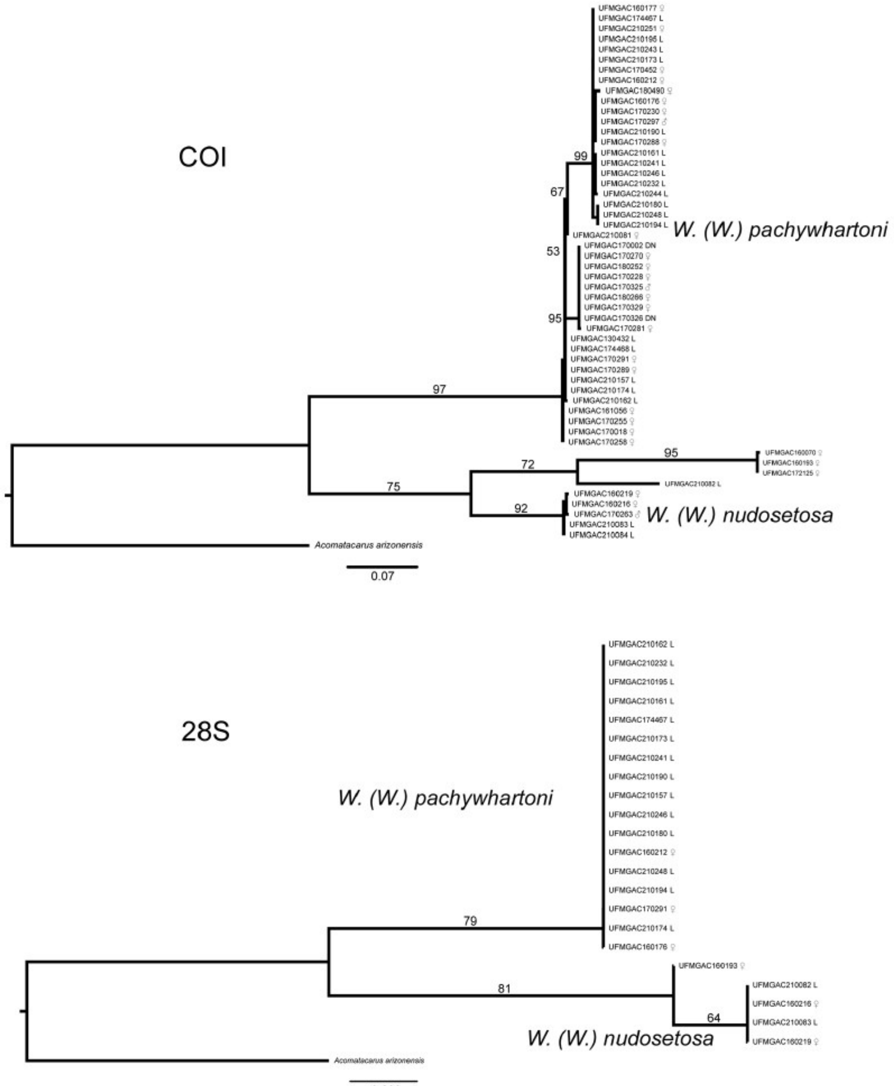
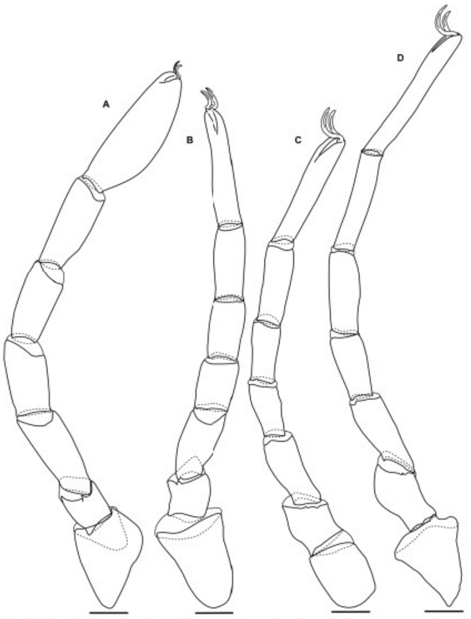
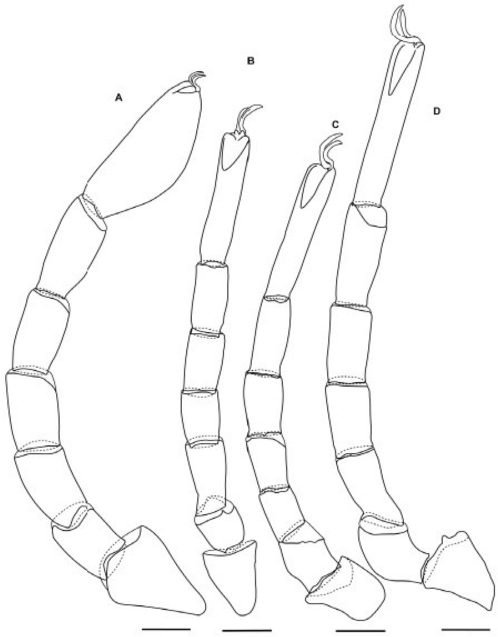
Appendix



SUPPLEMENTARY FIGURE 1. Maximum likelihood trees inferred from COI and 28S sequences. Values on branches are Ultrafast bootstrap proportions (1000 replicates). Scales are in substitution per site.



SUPPLEMENTARY FIGURE 2. *Whartonia (W.) pachywhartoni*, deutonymph. A: leg I; B: leg II; C: leg III; D: leg IV. Scale Bars: A, B and C=50 µm.



SUPPLEMENTARY FIGURE 3. *Whartonia (W.) nudosetosa*, deutonymph. A: leg I; B: leg II; C: leg III; D: leg IV. Scale Bars: A, B and C=100 µm.

CAPÍTULO IV – DNA barcoding, visual-guide resource, new localities and host associations of genus *Periglischrus* Oudemans, 1902 (Acari: Mesostigmata, Spinturnicidae) from Minas Gerais, Brazil

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DNA barcoding, visual-guide resource, new localities and host associations of genus *Periglischrus* Oudemans, 1902 (Acari: Mesostigmata, Spinturnicidae) from Minas Gerais, Brazil

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Abstract

The family Spinturnicidae (Oudemans, 1902) comprises hematophagous mites found exclusively on bats. In this article, we present DNA barcodes (mitochondrial Cytochrome c Oxidase I) for 71 specimens morphologically assigned to six spinturnicid species from 34 bat specimens (11 species) obtained from 10 caves and one forest fragment in karst areas from Minas Gerais state, southeast Brazil. A visual guide with diagnostic characters for *Periglischrus acutisternus* Machado-Allison, 1964, *P. caligus* Kolenati, 1857, *P. herrerai* Machado-Allison, 1965a, *P. iheringi* Oudemans, 1902, *P. paravargasi* Herrin & Tipton, 1975, *P. torrealbai* Machado-Allison, 1965a is provided. Along DNA barcode data, photo-documentation of vouchering material of each individual sequenced have been deposited at BOLD (Barcoding of Life Data System v4) database. *Periglischrus herrerai*, *P. caligus* and *P. paravargasi* are reported for the first time in Minas Gerais state, extending their distribution ranges.

Keywords

Bat-associated mites; BOLD; COI; ectoparasites; integrative taxonomy

Introduction

The cosmopolitan family Spinturnicidae (Oudemans, 1902) includes hematophagous mites found exclusively as ectoparasites on bats. Their life cycle comprises five stages, all occurring on the host: egg, larva, protonymph, deutonymph, and adult. Of these, the egg and larval stages occur inside the pregnant female (Rudnick 1960), nymphs and adults mostly inhabit the plagiopatagium of bats (Almeida *et al.* 2015).

Currently, Spinturnicidae comprises 12 genera and 110 species (Beron 2020). Among genera, five occur across Americas, associated more or less specifically with four bat families: *Cameronieta* Machado-Allison, 1965b with Mormopidae; *Periglischrus* Kolenati, 1857 with Phyllostomidae; *Spinturnix* Von Heyden, 1826, a cosmopolitan genus, with Vespertilionidae bats, *Paraspinturnix* Rudnick, 1960, a monotypic genus, associated with the anal orifice of bats belonging to the genus *Myotis* (Vespertilionidae); and *Mesoperiglischrus* (Dusbábek, 1968) associated with Natalidae (Herrin and Tipton 1975; Morales-Malacara 2001). Except by *Paraspinturnix*, all these genera are recorded from Brazil (Kolenati 1857; Oudemans 1902, 1903; Rudnick 1960; Confalonieri 1976; Gettinger and Gribel 1989; Azevedo *et al.* 2002; Almeida *et al.* 2007, 2011, 2015, 2016a, 2016b, 2018; Dantas-Torres *et al.* 2009; Silva *et al.* 2009, 2017; Silva and Graciolli 2013; Moras *et al.* 2013; Lourenço *et al.* 2016; Bezerra and Bocchiglieri 2018; Lourenço *et al.* 2020; Vidal *et al.* 2021).

Periglischrus includes 26 species recorded from Neotropics (Morales-Malacara and López-Ortega 2023). Some closely related species are similar enough to make accurate identification difficult. Some authors proposed species groups (e.g., *acutisternus* species group proposed by Morales-Malacara 2001) according to morphological similarity and affinity to their Phyllostomidae bat hosts. Host specificity ranges from monoxenous (a single host species), stenoxenous (a single host genus) to oligoxenous (occur in two or more host genera) (Herrin and Tipton 1975; Morales-Malacara 2001).

Thus far, fourteen species have been recorded in Brazil (Almeida *et al.* 2016b; Silva *et al.* 2017; Lourenço *et al.* 2020): *Periglichrus acutisternus* Machado-Allison, 1964, *P. caligus* Kolenati, 1857, *P. herrerai* Machado-Allison, 1965a, *P. hopkinsi* Machado-Allison, 1965a, *P. iheringi* Oudemans, 1902, *P. micronycteridis* Furman, 1966, *P. ojastii* Machado-Allison, 1964, *P. paracutisternus* Machado-Allison & Antequera, 1971, *P. paravargasi* Herrin & Tipton, 1975, *P. parvus* Machado-Allison, 1964, *P. ramirezi* Machado-Allison & Antequera, 1971, *P. tonatii* Herrin & Tipton, 1975, *P. torrealbai* Machado-Allison, 1965a, and *P. vargasi* Hoffmann, 1944.

Because some *Periglischrus* species are similar enough to make difficult morphological identification (Morales-Malacara 2001) or extensive intraspecific phenotypic variation related to host species (Almeida *et al.* 2018; Morales-Malacara *et al.* 2018, 2020; Zamora-Mejías *et al.* 2022), the inclusion of molecular data and careful specimen documentation is useful to make identification and occurrence ranges more reliable and reproducible.

The convergence of different methodologies, such as biological or breeding studies, morphometry and statistical analyses, and molecular tools with traditional taxonomy is the desirable scenario (Łaydanowicz and Makol 2010; Dabert *et al.* 2011), conducted as part of an integrative taxonomic approach (Knowles and Carstens 2007; Schlick-Steiner *et al.* 2010).

Herewith, we generated and made available DNA barcoding sequences and high-resolution photographs in the BOLD (Barcoding of Life Data System v4) database (Ratnasingham and Hebert 2007) of six species of *Periglichrus* collected on 11 bat species obtained from Minas Gerais state and provided a visual guide to the diagnostic characters useful to their identification.

Materials and methods

Specimen sampling, preservation and licenses — We examined a total of 81 specimens, consisting of males, females and nymphs from Minas Gerais state, Brazil. Of these, seventy-one individuals of *Periglischrus* had the cytochrome c oxidase subunit I (COI) gene successfully sequenced. Collection Numbers, BOLD IDs, BOLD Bin, instar/sex, host details, locality, coordinates and GenBank accession numbers are provided in Table 1. Localities are summarized in the map at Figure 1.

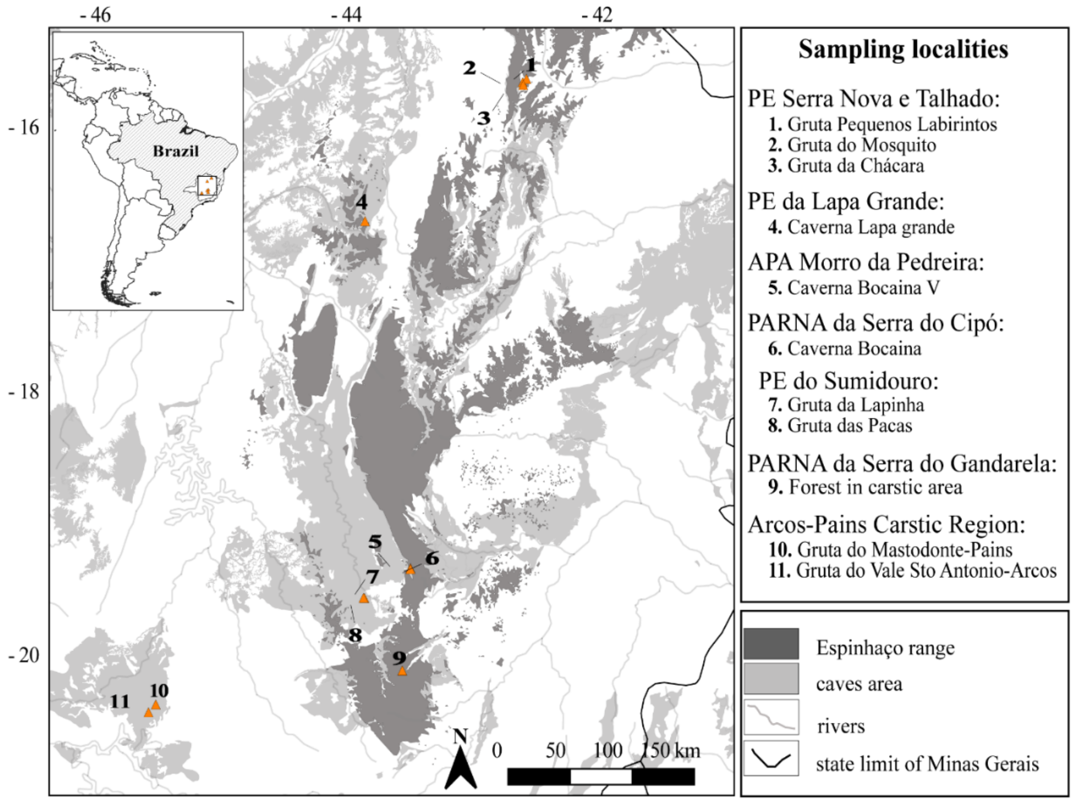

Figure 1. Geographical distribution of sampling localities of *Periglischrus* spp.

Mites were removed from their live bat hosts in the field by careful examination of these animals, using fine pincers and alcohol-soaked brushes, and immediately preserved in 96% ethanol, refrigerated in the field, and stored at -20 °C upon arrival.

Bats were collected under ICMBio license SISBIO 71120–4, authorized by the Instituto Estadual de Florestas do Estado de Minas Gerais (IEF 009/2020), in accordance with the precepts of the ethics committee for animal use in research "Comissão de Ética no Uso de Animais (CEUA)" of Universidade Federal de Minas Gerais (UFMG), protocol number 50/2020. Access to the genetic heritage from Brazilian mites was registered in SisGen (register number: [provided upon acceptance]).

DNA extraction, mounting, mites ID, photo-vouchers and collection — Genomic DNA was extracted from single specimens using Chelex based solution Instagene® (BIORAD) incubated for 30 min at 54°C, followed by 8 min at 100°C. The solution was spun and approximately 170 µl of supernatant was obtained for PCR reactions. Exoskeletons recovered from extraction were mounted on permanent microscope slides using Hoyer's medium (Walter and Krantz 2009) for morphology examination and kept as voucher material. Identification and photo-documentation of vouchers were performed using a Leica DM 750 optical microscope with an ICC50 W digital camera attached. Besides, mites identification followed the keys proposed by Herrin and Tipton (1975) and Morales-Malacara (2001), supplemented by original description and re-descriptions, such as Furman (1966), Machado-Allison (1964, 1965a,1965b), Morales-Malacara *et al.* (2018), Rudnick (1960), Almeida *et al.* (2018).

The voucher materials are deposited at the Acarological Collection, Centro de Coleções Taxonômicas, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte City. Collection acronym: UFMG AC. The map was prepared using QGIS 3.22.1 (https://www.qgis.org/ko/site/).

PCR, sequencing and chromatogram checking — The amplification of mitochondrial cytochrome c oxidase subunit I (COI) fragment of ~ 1200 bp was conducted using the primers and protocols described by Klimov *et al.* (2018). Amplifications were performed in 20 µl of final volume, with Platinum Taq DNA Polymerase (Invitrogen) in a Mastercycler nexus (Eppendorf) thermocycler. The master mix for initial PCR contained 2.0 µl of PCR buffer (1X), 1.4 µl MgCl 2 (50 mM), 1.4 µL of dNTP (10 mM each) and 0.8 µl of each oligonucleotide primer (10uM), to which 7–10 µl of genomic

DNA was added or alternatively 0.5 µl of parent PCR products. All PCR products found positive in 1% agarose gel electrophoresis were purified using the Ampure® (Agencourt) kit and sequenced using a 3730 DNA Analyzer and BigDyeTM Terminator v3.1 (Applied Biosystems) according to the manufacturer's protocol, employing M13 forward and reverse primers.

Forward and reverse chromatograms were checked, edited and assembled into contigs using software ChromasPro 1.41 (Technelysium Pty Ltd). All sequences generated for this study were compared with available mites sequences using the BLAST feature (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.* 1997) and deposited in GenBank database (Table 1).

Alignment and phylogenetic inference — After preliminary automatic alignment with the aid of the MUSCLE software (Edgar 2004) sequences had their extremities trimmed and checked for the maintenance of the reading frame in the program MEGA 7.0 (Kumar *et al.* 2016). All sequences were uploaded into BOLD (Project-SPIN: DNA barcode library for Spinturnicidae mites from Brazil) database that assigned Barcode Index Number (BIN) (Ratnasingham and Hebert 2013). Pairwise mean interspecific and intraspecific p-distances values were calculated in MEGA 7.0. Haplotypes were identified employing DnaSP V. 6. and the alignment filtered from redundant sequences. The best-fitting models of nucleotide substitutions were found in ModelFinder (Kalyaanamoorthy *et al.* 2017) using Bayesian information criterion (BIC) as implemented in IQTree (Nguyen *et al.* 2015). This algorithm tests best fit models for each partition and the best partitioning scheme. The partitions taken into account were codon positions (1st, 2nd and 3rd).

Maximum likelihood (ML) tree was obtained in IQTree, with support being estimated using UltraFast Bootstrap (UFBoot) and SH-like approximate likelihood ratio test (SH-aLRT) was calculated in IQTree with 1,000 replicates. The bayesian inference (BI) was run on Beast v. 2.4.6 (Bouckaert *et al.* 2014). The analyses were run for $3x10^8$ generations, sampled every $3x10^4$ generations, under the Yule tree model. Convergence check using Tracer v.1.6.0 (Rambaut *et al.* 2014). The resulting trees are summarized using the software TreeAnnotator v1.8.4 (Drummond *et al.* 2012), with a 10% burnin, on the maximum clade credibility tree displaying the median heights for the tree nodes. Leading to an ultrametric bayesian tree.

At last, two methods of putative species delimitation were performed: the Bayesian implementation of the Generalized Mixed Yule Coalescent algorithm (bGMYC) model (Reid and Carstens 2012) was used to delimit putative species belonging to different haplotypes following Costa *et al.* (2019) and Gomes-Almeida *et al.* (2023); and *Assemble Species by Automatic Partitioning* (ASAP) analysis (Puillandre *et al.* 2021), based on genetic distance calculated between DNA sequences and ranked by their ASAP-scores, was performed without the outgroup, with default settings and Kimura K80 substitution model (ts/tv=2.0) through the web-server accessible at via the webserver (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), using fasta files for locus COI as input file.

Results

Molecular analyses

We obtained 71 COI sequences of *Periglischrus* mite specimens morphologically assigned to six species, collected from 11 species of bats from Minas Gerais state. Sequences ranged from 657 to 953bp due to poor chromatogram quality due to repeated bases or mixed trace signals (multiple peaks) on their extremities. Among the 71 sequences, trimmed to 654 bp, 61 different haplotypes were identified. Intraspecific mean p-distances among sequences varied from 0.0006 (0.06%) in *P. herrerai* (n= 5) to 0.0322 (3.22%) in *P. torrealbai* (n= 6), among *Periglischrus* species, interspecific p-distances varied from 0.1255 (12.55%, *P. herrerai* x *P. caligus*) and 0.1833 (18.33%, *P. caligus* x *P. acutisternus*). Pairwise mean intraspecific and interspecific p-distances are summarized in Table 2.

Table 1. Details on barcoded specimens of *Periglischrus* mites from Minas Gerais state.

Species *Periglischrus*	Instar/ Sex	Host	Habitat	Voucher UFMG-AC	Bold ID	Bin	Genbank	bp
P. acutisternus	♀	*Phyllostomus discolor*	cave	220038	SPIN002-22	AEV1280	OP964374	953
	♀	*Ph. discolor*	cave	220055	SPIN006-22	AEV1281	OP964375	953
	PN	*Ph. hastatus*	cave	220136	SPIN027-22	AEV1280	OP964373	953

	♂	*Ph. hastatus*	cave	220819	-	-	-	-
	♀	*Ph. hastatus*	cave	220820	-	-	-	-
	♂	*Ph. discolor*	cave	221025	-	-	-	-
	♂	*Ph. discolor*	cave	221026	-	-	-	-
P. caligus	PN	*Glossophaga soricina*	cave	220057	SPIN007-22		OP964398	657
	♀	*Gl. soricina*	cave	220058	SPIN008-22		OP964397	657
	PN	*Gl. soricina*	cave	220059	SPIN009-22		OP964396	657
	PN	*Gl. soricina*	cave	220060	SPIN010-22		OP964395	657
	♂	*Gl. soricina*	cave	220066	SPIN011-22		OP964376	657
	♂	*Gl. soricina*	cave	220104	SPIN020-22		OP964394	657
	♂	*Gl. soricina*	cave	220107	SPIN021-22		OP964393	657
	♂	*Gl. soricina*	cave	220108	SPIN022-22		OP964392	657
	♂	*Gl. soricina*	cave	220109	SPIN023-22		OP964391	657
	PN	*Gl. soricina*	cave	220124	SPIN026-22		OP964390	657
	♀	*Gl. soricina*	cave	220147	SPIN029-22		OP964389	657
	♂	*Gl. soricina*	cave	220148	SPIN030-22	AEV1279	OP964388	873
	PN	*Gl. soricina*	cave	220183	SPIN035-22		OP964387	657
	PN	*Gl. soricina*	cave	220184	SPIN036-22		OP964386	657
	♂	*Gl. soricina*	cave	220185	SPIN037-22		OP964385	657
	♀	*Gl. soricina*	cave	220186	SPIN038-22		OP964384	657
	♀	*Gl. soricina*	cave	220214	SPIN039-22		OP964383	657
	PN	*Gl. soricina*	cave	220216	SPIN040-22		OP964382	657
	♂	*Gl. soricina*	cave	220808	SPIN046-22	AEV1279	OP964381	873
	♂	*Gl. soricina*	cave	221016	SPIN055-22		OP964380	657
	♀	*Gl. soricina*	cave	221031	SPIN062-22		OP964379	657
	PN	*Gl. soricina*	cave	221032	SPIN063-22		OP964378	657
	♂	*Gl. soricina*	cave	221035	SPIN064-22		OP964377	657
	♀	*Gl. soricina*	cave	220218	-	-	-	-
	♀	*Gl. soricina*	cave	220149	-	-	-	-
	♀	*Gl. soricina*	cave	220181	-	-	-	-
P. herrerai	PN	*Desmodus rotundus*	cave	220113	SPIN024-22		OP964403	742
	PN	*D. rotundus*	cave	220114	SPIN025-22		OP964402	730
	♂	*D. rotundus*	mata/carste	221059	SPIN065-22	AEV1278	OP964399	742
	♂ DN	*D. rotundus*	mata/carste	221060	SPIN066-22	AEV1278	OP964400	816
	♀ DN	*D. rotundus*	mata/carste	221061	SPIN067-22		OP964401	730
P. iheringi	♀ DN	*Ar. lituratus*	cave	220043	SPIN004-22	AAF9243	OP964421	953
	PN	*Pl. lineatus*	cave	220085	SPIN012-22	AAF9243	OP964412	953
	♂	*Artibeus planirostris*	cave	220090	SPIN013-22	AAF9243	OP964410	953
	PN	*Ar. planirostris*	cave	220091	SPIN014-22	AAF9243	OP964409	812
	♀	*Ar. planirostris*	cave	220092	SPIN015-22	AAF9243	OP964408	953
	♂	*Ar. planirostris*	cave	220093	SPIN016-22	AAF9243	OP964407	953
	PN	*Ar. planirostris*	cave	220094	SPIN017-22	AAF9243	OP964406	953
	♂	*Ar. planirostris*	cave	220095	SPIN018-22	AAF9243	OP964405	953
	♀	*Pl. lineatus*	cave	220157	SPIN031-22	AAF9243	OP964411	953
	♂	*Ar. lituratus*	cave	220221	SPIN041-22	AAF9243	OP964404	953
	♂	*Ar. lituratus*	cave	220224	SPIN042-22	AAF9243	OP964419	953
	♂	*Ar. lituratus*	cave	220226	SPIN043-22	AAF9243	OP964418	953
	♂ DN	*Ar. lituratus*	cave	220227	SPIN044-22	AAF9243	OP964417	953
	♂ DN	*Ar. lituratus*	cave	220230	SPIN045-22	AAF9243	OP964416	953
	PN	*Pl. lineatus*	cave	220828	SPIN048-22	AAF9243	OP964415	953
	♀	*Ar. lituratus*	cave	221011	SPIN050-22	AAF9243	OP964431	953
	♀	*Ar. lituratus*	cave	221012	SPIN051-22	AAF9243	OP964430	909
	♂	*Ar. lituratus*	cave	221013	SPIN052-22	AAF9243	OP964429	953
	PN	*Ar. lituratus*	cave	221014	SPIN053-22	AAF9243	OP964428	953
	PN	*Ar. lituratus*	cave	221015	SPIN054-22	AAF9243	OP964427	953
	PN	*Ar. planirostris*	cave	221017	SPIN056-22	AAF9243	OP964426	953
	♂ DN	*Ar. planirostris*	cave	221018	SPIN057-22	AAF9243	OP964425	953

	♀ DN	*Ar. planirostris*	cave	221020	SPIN058-22	AAF9243	OP964424	953
	♂	*Ar. planirostris*	cave	221021	SPIN059-22	AAF9243	OP964423	953
	♀ DN	*Ar. planirostris*	cave	221022	SPIN060-22	AAF9243	OP964422	953
	♂	*Ar. planirostris*	cave	221113	SPIN068-22	AAF9243	OP964420	953
	♂	*Ch. doriae*	cave	221114	SPIN069-22	AAF9243	OP964432	953
	♀	*Ch. doriae*	cave	221115	SPIN070-22		OP964414	723
	♂	*Ca. perspicillata*	cave	221127	SPIN073-22	AAF9243	OP964413	953
P. paravargasi	♀	*An. caudifer*	cave	220162	SPIN032-22	AEW4949	OP964434	953
	PN	*An. caudifer*	cave	220166	SPIN033-22	AEW4949	OP964438	953
	♀ DN	*An. caudifer*	cave	220178	SPIN034-22	AEW4949	OP964433	953
	♀	*An. caudifer*	cave	220813	SPIN047-22	AEW4949	OP964436	953
	♀ DN	*An. caudifer*	cave	221009	SPIN049-22	AEW4949	OP964437	953
P. torrealbai	♂	*Ph. discolor*	cave	220041	SPIN003-22	AEW4950	OP964442	953
	PN	*Ph. discolor*	cave	220054	SPIN005-22	AEW4950	OP964444	953
	♀	*Ph. discolor*	cave	220098	SPIN019-22	AEW4950	OP964441	818
	♂	*Ph. hastatus*	cave	220139	SPIN028-22	AEW4950	OP964440	953
	♂	*Ph. discolor*	cave	221030	SPIN061-22	AEW4950	OP964439	953
	♂	*T. bidens*	cave	221129	SPIN072-22	AEW4948	OP964443	953
	♂	*Ph. hastatus*	cave	220137	-	-	-	-
	♂	*Ph. hastatus*	cave	220138	-	-	-	-
	♂	*Ph. hastatus*	cave	220823	-	-	-	-

Table 2. Pairwise mean interspecific p-distances values between sequences of COI from species of *Periglischrus* mites. Values with cells shadowed in gray are intra-specific mean distances.

	1	**2**	**3**	**4**	**5**	**6**
1. *P. torrealbai*	0.0322					
2. *P. acutisternus*	0.1394	0.0245				
3. *P. caligus*	0.1492	0.1833	0.0130			
4. *P. paravargasi*	0.1651	0.1713	0.1419	0.0113		
5. *P. herrerai*	0.1569	0.1363	0.1255	0.1416	0.0006	
6. *P. iheringi*	0.1640	0.1676	0.1648	0.1678	0.1500	0.0222

ModelFinder chose as best partition/model according to Bayesian information criterion (BIC) the first, second and third codon positions merged in a single partition under the model TIM+F+I+G4. Bayesian Inference and Maximum Likelihood phylogenetic inference resulted in six groups of *Periglischrus* species with clades that are highly supported, corroborating the previous morphological identification of the mites (Table 3; Figure 2; Supplementary figure 1-2). Eight BINs identified by BOLD, five created from our data and three that matched existing BINs (Supplementary Figure 3). For bGMYC analyses, identified 10 species, with posterior probability being $p < 0.95$ (Figure 2), while the best partition provided by ASAP identified six hypothetical species that match six morphological species delimitations, observed a barcode gap of about 8-14% at the threshold distance of 10.57 % (K80; 2.0) which has the best ASAP-score (1.50) within the available molecular data. In ASAP analysis, all haplotypes have been identified into a six group, corresponding to morphological identification (Supplementary figure 3).

Below, we provide an annotated list reporting the six species of *Periglischrus* mites found, along with information on their distribution, host, COX1 barcode sequence data, and high-resolution photographs of diagnostic characters. All localities in Minas Gerais state, Brazil. Coordinates are given in WGS-84 format.

Taxonomy

Family Spinturnicidae Oudemans, 1902
Genus *Periglischrus* Kolenati, 1857

1. Periglischrus acutisternus Machado-Allison (Figures 3–5)

Periglischrus acutisternus Machado-Allison, 1964: 200–202 (original designation).
Periglischrus tiptoni Furman, 1966: 144–147.

Specimens examined — 2♀ (UFMG AC 220038, 220055) on bats *Phyllostomus discolor* (Wagner, 1843) (2 ex.) and 2♂ (UFMG AC 221025-26) on *P. discolor* (1 ex.): Lagoa Santa, **Lapinha cave**, PE Sumidouro, -19.5616, -43.959, 11 Aug. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964374 [UFMG AC 220038], OP964375 [UFMG AC 220055]). 1♀ (UFMG AC 220820), 1♂ (UFMG AC 220819) and 1 protonymph (UFMG AC 220136) on *Phyllostomus hastatus* (Pallas, 1767) (1 ex.): Rio Pardo de Minas, **Mosquito cave** (unregistered), PE Serra Nova e Talhado, -15.6545, -42.7335, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964373 [UFMG AC 220136]).

Barcode sequences: OP964373 and 75 (Table 1).

Distribution — Brazil, Colombia, Costa Rica, Mexico, Panama, Peru, Trinidad and Venezuela (Gettinger 2018; Beron 2020).

Hosts and records from Brazil: *Mimon bennettii* (Gray, 1838): Rio de Janeiro (Almeida *et al.* 2011); *Phyllostomus discolor* Wagner, 1843: Pernambuco (Dantas-Torres *et al.* 2009), Distrito Federal (Gettinger and Gribel 1989), Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (present study); *Phyllostomus hastatus* (Pallas, 1767): Maranhão, Paraná and São Paulo (Confalonieri 1976), Minas Gerais (Confalonieri 1976 and present study), Rio de Janeiro (Lourenço *et al.* 2016); Mato Grosso do Sul (Silva *et al.* 2017).

Differential diagnosis — *Female*: large, idiosoma length less than 2.000 µm (n=3, 1.167–1.352, 1.279) (Figure 3A); dorsal opisthonotal area with four pairs of minute setae (Figure 3D); sternal plate has a flask-shaped with a narrow, subtriangular, median sclerotized projection at the anterior end of the plate (Figure 3E–F); distal posteroventral (*pv*) setae on tibia–tarsus I, genu–tarsus II and distal anteroventral (*av*) seta on genu-tibia II and tibia III are short, blunt and peg-like; and posteroventral (*pv*) setae of femur and genu I-II robust with finely serrated on entire surface (Figure 3H and K). *Male*: Smaller than female (n=3, 492–558, 518); has distinctly longer sternogenital (*St1–St4*) setae, extending almost to level of second pair of setae and second–fourth pairs of sternal setae extending beyond bases of adjacent posterior setae (Figure 4C); seven pairs of setae on intercoxal IV and one pair of minute setae posterior to sternogenital plate; ventral setae on legs I-II mostly normal, setiform, and slender, however, some may be enlarged, spinelike (Figure 4D–F); large dorsal setae of tarsi III–IV coarsely barbed or serrated (Figure 4K and N); proximal anterodorsal (*ad*) seta of femur–tibia I and genu IV medium to large in size (Machado-Allison 1964; Furman 1966; Herrin and Tipton 1975; Morales-Malacara 2001).

Nymphs: similar to males with regard to above features, except by ontogenetic differences: Protonymph is smaller and less sclerotized than deutonymphs and adults; peritreme is short, over coxa III; four pairs of proteronotal setae, usually lack *Pn5* seta; sternal shield plate not completely developed and lacks st4 and genital seta; anal-intercoxal plate not completely developed but smaller than in deutonymphs and male; intercoxal IV area with five pairs of setae, including adanal pair. Deutonymph female and male are smaller and less sclerotized than adults; peritreme with a long and narrow extension anteriorly; pair of proteronotal setae as adults; sternal shield plate not completely developed but *st4* and genital setae are present and off shield; anal-intercoxal plate not completely developed but smaller than in male (Deunff *et al.* 2011).

Remarks — This species is stenoxenous on bats of the genus *Phyllostomus* (Herrin and Tipton 1975), found here on bats *Ph. discolor* and *Ph. hastatus*, being the record on *Ph. discolor* from Minas Gerais state new to science. Individuals obtained in this study matches original description and re-descriptions (Machado-Allison 1964; Herrin and Tipton 1975; Furman 1966), something regarded here as intraspecific variation. This species often co-occurs with smaller *P. torrealbai* Machado-Allison, 1965a, which males and nymphs may be misidentified as *P. acutisternus* due to the similar size, presence of some ventral setae of legs I and II distinctly enlarged, large dorsal setae of tarsi III–IV coarsely barbed or serrated; males with long sternogenital setae and intercoxal IV area bearing seven pairs of setae. *acutisternus*, however, has some ventral setae on legs I and II enlarged, spinelike (instead of blunt and fusiform) and proximal anterodorsal (*ad*) seta of femur–tibia I and genu IV medium to large in size (instead of always small) (Herrin and Tipton 1975).

In our bGMYC species delimitation analyses (Figure 2) *P. acutisternus* is represented by three haplotypes (8, 9 and 10) out of three sequences, with haplotypes 9 and 10 were recovered as a single species with large posterior probability (pp. >95%), whereas haplotype 8 was associated with a lower posterior probability (pp. 76%).

Table 3. Details of haplotypes of *Periglischrus* mites sampled from Minas Gerais state.

Species ID	**Haplotypes**	**N**	**Sequences ID**	
P. torrealbai	Hap 1	1	OP964442	
	Hap 2	1	OP964439	
	Hap 3	1	OP964440	
	Hap 4	1	OP964441	
	Hap 6	1	OP964444	
	Hap 7	1	OP964443	
P. acutisternus	Hap 8	1	OP964374	
	Hap 9	1	OP964373	
	Hap 10	1	OP964375	
P. caligus	Hap 11	1	OP964394	
	Hap 12	1	OP964396	
	Hap 13	1	OP964386	
	Hap 14	1	OP964393	
	Hap 15	1	OP964379	
	Hap 16	1	OP964377	
	Hap 17	1	OP964391	
	Hap 18	1	OP964380	
	Hap 19	1	OP964389	
	Hap 20	1	OP964376	
	Hap 21	1	OP964378	
	Hap 22	1	OP964388	
	Hap 23	1	OP964387	
	Hap 24	1	OP964392	
	Hap 25	2	OP964397	OP964395
	Hap 26	2	OP964384	OP964382
	Hap 27	2	OP964390	OP964381
	Hap 28	1	OP964398	
	Hap 29	1	OP964385	
	Hap 30	1	OP964383	
P. paravargasi	Hap 31	1	OP964434	
	Hap 32	1	OP964438	
	Hap 33	1	OP964437	

	Hap 34	1	OP964436			
	Hap 35	1	OP964433			
P. herrerai	Hap 36	4	OP964403	OP964399	OP964400	OP964402
	Hap 37	1	OP964401			
P. iheringi	Hap 5	1	OP964421			
	Hap 38	1	OP964412			
	Hap 39	1	OP964411			
	Hap 40	3	OP964431	OP964429	OP964417	
	Hap 41	1	OP964415			
	Hap 42	1	OP964432			
	Hap 43	1	OP964414			
	Hap 44	1	OP964416			
	Hap 45	1	OP964424			
	Hap 46	2	OP964405	OP964410		
	Hap 47	1	OP964413			
	Hap 48	2	OP964404			
	Hap 49	1	OP964430			
	Hap 50	1	OP964427			
	Hap 51	1	OP964422			
	Hap 52	1	OP964419			
	Hap 53	1	OP964420			
	Hap 54	1	OP964409			
	Hap 55	1	OP964407			
	Hap 56	1	OP964425			
	Hap 57	1	OP964423			
	Hap 58	1	OP964408			
	Hap 59	1	OP964426			
	Hap 60	1	OP964406			
	Hap 61	2	OP964418	OP964428		

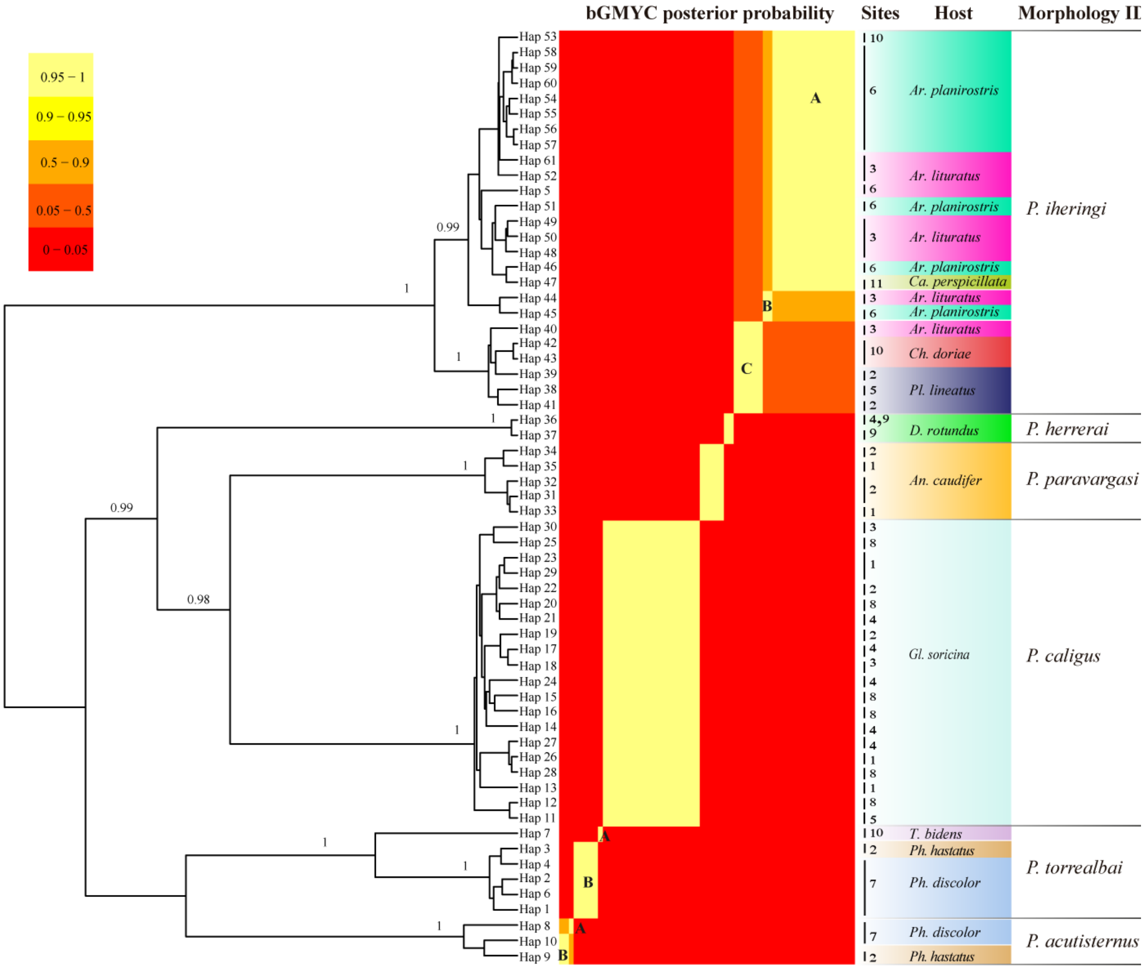

Figure 2. Mitochondrial COI haplotype ultrametric tree inferred in BEAST using strict clock and species delimitation probabilities obtained in bGMYC.

2. *Periglischrus caligus* Kolenati (Figures 6–8)

Periglischrus caligus Kolenati, 1857:60 (original designation).
Periglischrus setosus Machado-Allison 1964:199–200.

Specimens examined — 2♀ (UFMG AC 220058, 221031), 2♂ (UFMG AC 220066, 221035) and 3 protonymphs (UFMG AC 220057, 220059–60) on bat *Glossophaga soricina* (4 ex.): Lagoa Santa, **Pacas cave**, PE Sumidouro, -19.5606, -43.9667, 12 Aug. 2021, collected by B. Almeida *et al.* (COX1 sequence [voucher code]: OP964376 [UFMG AC 220066], OP964377 [UFMG AC 221035], OP964379[UFMG AC 221031], OP964395[UFMG AC 220060], OP964396[UFMG AC 220059], OP964397[UFMG AC 220058], OP964398[UFMG AC 220057]); 4♂ (UFMG AC 220107–09, 220808) and 2 protonymphs (UFMG AC 220124, 221032) on *Glossophaga soricina* (3 ex.): Montes Claros, **Lapa Grande cave**, PE da Lapa Grande, -16.7067, -43.9549, 13-14 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964378[UFMG AC 221032], OP964381[UFMG AC 220808], OP964390[UFMG AC 220124], OP964391[UFMG AC 220109], OP964392[UFMG AC 220108], OP964393[UFMG AC 220107]); 2♀(UFMG AC 220214, 220218), 1♂ (UFMG AC 221016) and 1 protonymph (UFMG AC 220216) on *Glossophaga soricina* (2 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Chácara cave** (unregistered), PE Serra Nova e Talhado, -15.6758, -42.7295, 20 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964380[UFMG AC 221016], OP964382[UFMG AC 220216], OP964383[UFMG AC 220214]); 2♀(UFMG AC 220147, 220149) and 1♂ (UFMG AC 220148) on *Glossophaga soricina* (1 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Mosquito cave** (unregistered), PE Serra Nova e Talhado, -15.6545, -42.7335, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964388 [UFMG AC 220148] and OP964389[UFMG AC 220147]); 2♀(UFMG AC 220186, 220181), 1♂ (UFMG AC 220185) and 2 protonymphs (UFMG AC 220183-84) on *Glossophaga soricina* (2 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Pequenos Labirintos cave** (unregistered), PE Serra Nova e Talhado, -15.6293, -42.7043, 18 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964384–87); 1♂(UFMG AC 220104) on *Glossophaga soricina* (1 ex.): Brazil, Minas Gerais, Santana do Riacho, **Bocaina V cave**, APA Morro da Pedreira, -19.3346, -43.6032, 17 Sep. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964394 [UFMG AC 220104]).

Barcode sequences: OP964376–78, OP964381, OP964383–92, OP964394, OP964397 (Table 1).

Distribution — Bolivia, Brazil, Mexico, Panama, Peru, Suriname, Venezuela (Beron 2020).

Hosts and records from Brazil — *Artibeus lituratus* (Olfers, 1818): Rio de Janeiro (Lourenço *et al.* 2020); *Artibeus planirostris:* Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017); *Glossophaga soricina* (Pallas, 1766): Ceará, Mato Grosso (Almeida *et al.* 2016b), Rio de Janeiro, São Paulo (Confalonieri 1976), Distrito Federal (Gettinger and Gribel 1989), Brazil (Rudnick 1960), Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Rio Grande do Sul (Silva *et al.* 2009) and Minas Gerais (new report in present study); *Platyrrhinus lineatus*: Mato Grosso do Sul (Silva and Graciolli 2013); unknown bat: Mato Grosso (Confalonieri 1976).

Differential diagnosis — *Female*: four pairs of small to minute setae on dorsal opistonothal area (Figure 6D); sternal plates subpentagonal, narrow anterior border with anterior projection narrowly rounded (Figure 6E–F); posterolateral (*pl*) setae on femur-tibia IV greatly inflated, with slender recurved end (Figure 6M–N). *Male*: sternogenital seta *St1* small, extending posteriorly about two-thirds the distance to first pair of pores; intercoxal IV area bears eight pairs of setae with first pair as microsetae posterior to sternogenital plate (Figure 7C); coxa II with seta *pv* much shorter than the width of coxa II (Figure 7I) (Herrin and Tipton 1975; Morales-Malacara 2001; Morales-Malacara and López-Ortega 2001). *Protonymph*: similar to males, except by ontogenetic differences (Deunff *et al.* 2011) (Figures 8A–N).

Remarks — *P. caligus* is reported for the first time to Minas Gerais fauna. This is a stenoxenous species on glossophagine bats of the genus *Glossophaga*. Morphological characters of examined specimens agree with original description and re-descriptions (Machado-Allison 1964; Herrin and Tipton 1975; Furman 1966). *Periglischrus caligus* protonymph may be misidentified as *P. herrerai* that differs by having first pair of setae on intercoxal area IV small (instead minute in *P. caligus*, Figures 8C and 10C); tarsus III with proximal posterodorsal (*pd*) seta medium to small (vs large) and proximal anterodorsal (*ad*) seta similar in size with *pd* (vs small, Figure 8K and 10K); Proximal anterodorsal (*ad*) on femur IV medium (vs small, Figure 8L and 10L) and proximal antero and posterodorsal setae on genu IV medium (vs small, Figure 8M and 10M).

In our bGMYC species delimitation analyses (Fig. 2) *Periglischrus caligus* is represented by 20 haplotypes (11 to 30) out of 23 sequences and all haplotypes were recovered as a single putative species with pp. >95%.

3. Periglischrus herrerai Machado-Allison (Figures 9–10)

Periglischrus herrerai Machado-Allison, 1965a:282–284 (original designation).
Periglischrus desmodi Furman, 1966: 139–141.
Periglischrus herrerai, Herrin & Tipton, 1975:55; *Periglischrus herrerai*, Morales-Malacara *et al.*, 2018:1–17.

Specimens examined — 1♂ (UFMG AC 221059), 1 deutonymph ♀ (UFMG AC 221061) and 1 deutonymph ♂ (UFMG AC 221060) on *Desmodus rotundus* (1 ex.): Brazil, Minas Gerais, Rio Acima, **carste/mata**, PN Serra do Gandarela, -20.1105, -43.6661, 27 Mar. 2020, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964399 [UFMG AC 221059], OP964400[UFMG AC 221060] and OP964401[UFMG AC 221061]); 2 protonymphs (UFMG AC 220113–14) on *Desmodus rotundus* (1 ex.): Brazil, Minas Gerais, Montes Claros, **Lapa Grande cave**, PE da Lapa Grande, -16.7067, -43.9549, 13-14 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964402 [UFMG AC 220114] and OP964403 [UFMG AC 220113]).

Barcode sequences: OP964399–403 (Table 1).

Distribution — Brazil, Colombia, Costa Rica, Mexico, Panama, Paraguay, Peru, Trinidad, Venezuela (Gettinger 2018; Beron 2020).

Hosts and records from Brazil — *Artibeus planirostris*: Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017); *Desmodus rotundus* (E. Geoffroy, 1810): Distrito Federal (Gettinger and Gribel 1989), Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (new report in present study), Rio de Janeiro (Confalonieri 1976; Almeida *et al.* 2011), São Paulo (Confalonieri 1976); *Myotis nigricans*: Mato Grosso do Sul (Silva and Graciolli 2013); *Sturnira lilium*: Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017).

Differential diagnosis — *Female*: distance between first and second pairs of dorsal proteronotal setae less than or equal to distance between second and third pairs; six opistonothal setae short to very short (first pair just posterior to coxa IV largest). Sternal plate subpentagonal in shape, with narrow anterior border, and an acute small and triangular tip (homomorphic), or with sternal plate with subpentagonal shape gradually distorted to a spade-shaped outline with a broad arrow head pointed tip (maximum heteromorphic form); proximal anterodorsal seta of tibia II large (Morales-Malacara *et al.* 2018). *Male*: narrow longitudinal and cross-shaped unsclerotized crack in the center of dorsal plate (Morales-Malacara 2001; Morales-Malacara *et al.* 2018) (Figure 9B); intercoxa IV area with nine pairs of setae, plus one pair of adanal setae: first pair small to medium sized and situated posterior to sternogenital plate (Herrin and Tipton 1975) with a unique reticulated sclerotized pattern over most of plate (Figure 9C) (Morales-Malacara 2001; Morales-Malacara *et al.* 2018).

Nymphs (Figure 10A–N): similar to males with regard to above features, except by ontogenetic differences (Deunff et al. 2011). Deutonymph female has 13 pairs setae on intercoxal IV area, including adanal pair, and seven pairs of hysteronotal setae (one pair poststigmal setae and six pairs opisthosomal setae) and plus one unpaired seta on caudal dorsum. Deutonymph male has 10 pairs setae on intercoxal IV area, including adanal pair, and five pairs hysteronotal setae (one pair poststigmal setae and four pair opisthosomal setae) and plus one unpaired seta on caudal dorsum (similar to adult male). Protonymph has only two pairs hysteronotal setae (one pair poststigmal setae and one pair opisthosomal setae) and plus one unpaired seta on caudal dorsum.

Remarks — *P. herrerai* is reported for the first time from Minas Gerais. This species is monoxenous associated with the vampire bat, *D. rotundus*. Morphological characters of examined specimens match those of the original description and re-descriptions (Machado-Allison 1965a; Furman 1966; Herrin and Tipton 1975; Morales-Malacara *et al.*, 2018), except by four pairs of dorsal opisthosomal setae on male deutonymph, instead of three pairs.

In our bGMYC species delimitation analyses (Figure 2), both *Periglischrus herrerai* haplotypes (haplotypes 36 and 37) out of five sequences recovered as a single putative species with pp. >95%.

4. *Periglischrus iheringi* Oudemans (Figures 11–13)

Periglischrus iheringi Oudemans, 1902: 38.
Periglischrus jheringi (sic) Oudemans, 1903:135.
Periglischrus meridensis Hirst, 1927: 335.
Spinturnix ewingia Wharton, 1938: 139.
Spinturnix artibiensis Radford, 1951: 97.

Specimens examined — Male (UFMG AC 221127) on *Carollia perspicillata* (1 ex.): Brazil, Minas Gerais, Arcos, **Vale Sto Antônio cave,** -20.3673, -45.5756, 26 jan. 2021, collected by A. Tahara *et al.* (COX1 sequence [voucher code]: OP964413[UFMG AC 221127]); deutonymph female (UFMG AC 220098) on *Artibeus lituratus* (PNSC0092): Brazil, Minas Gerais, Santana do Riacho, **Bocaina cave,** PN da Serra do Cipó, -19.3419, -43.6032, 19 Sep. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964421[UFMG AC 220098]); female (UFMG AC 221115), male (UFMG AC 221114) on *Chiroderma doriae* (1 ex.) and male (UFMG AC 221113) on *Artibeus planirostris* (1 ex.): Brazil, Minas Gerais, Pains, **Mastodonte cave**, -20.4270, -45.6322, 03 Mar. 2020, collected by A. Tahara *et al.* (COX1 sequence [voucher code]: OP964414[UFMG AC 221115], OP964420[UFMG AC 221113] and OP964432[UFMG AC 221114]); 2♀(UFMG AC 221011-12), 4♂ (UFMG AC 220221, 220224, 220226, 221013), 2 deutonymph♂ (UFMG AC 220227, 220230) and 2 protonymphs (UFMG AC 221014–15) on *Artibeus lituratus* (3 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Chácara cave** (unregistered), PE Serra Nova e Talhado, -15.6758, -42.7295, 20 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964404[UFMG AC 220221], OP964416[UFMG AC 220230], OP964417[UFMG AC 220227], OP964418[UFMG AC 220226], OP964419[UFMG AC 220224], OP964427[UFMG AC 221015], OP964428[UFMG AC 221014], OP964429[UFMG AC 221013], OP964430[UFMG AC 221012], OP964431[UFMG AC 221011]); 1 ♀(UFMG AC 220157) and 1 protonymph (UFMG AC 220828) on *Platyrrhinus lineatus* (1 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Mosquito cave** (unregistered), PE Serra Nova e Talhado, -15.6545, -42.7335, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964411[UFMG AC 220157], OP964415[UFMG AC 220157]); 1♀ (UFMG AC 220092), 4♂ (UFMG AC 220090, 220093, 220095, 221021), 2 deutonymph ♀ (UFMG AC 221020, 221022), deutonymph ♂(UFMG AC 221018), 3 protonymphs (UFMG AC 220091, 220094, 221017) on *Artibeus planirostris* (4 ex.): Brazil, Minas Gerais, Santana do Riacho, **Bocaina cave**, PN da Serra do Cipó, -19.3419, -43.6032, 19 Sep. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964405, [UFMG AC 220095], OP964406[UFMG AC 220094], OP964407[UFMG AC 220093], OP964408[UFMG AC 220092], OP964409[UFMG AC 220091], OP964410[UFMG AC 220090], OP964422[UFMG AC 221022], OP964423[UFMG AC 221021], OP964424[UFMG AC 221020], OP964425[UFMG AC 221018], OP964426[UFMG AC 221017]); 1 protonymph (UFMG AC 220085) on *Platyrrhinus lineatus* (1 ex.): Brazil, Minas Gerais, Santana do Riacho, **Bocaina V cave**, APA Morro da Pedreira, -19.3346, -43.6032, 17 Sep. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964412[UFMG AC 220085]).

Barcode sequences: OP964404–08, OP964412–19, OP964421, OP964423–29 (Table 1).

Distribution — Bolivia, Brazil (detailed below), Colombia, Costa Rica, Cuba, Guatemala, Honduras, Mexico, Panama, Paraguay, Peru, Puerto Rico, Surinam, Trinidad, Venezuela and Virgin Islands (Gettinger 2018; Beron 2020).

Hosts and records from Brazil — *Anoura caudifer* (É. Geoffroy, 1818)*:* Rio Grande do Sul (Silva *et al.* 2009); *Anoura* sp.: Rio Grande do Sul (Silva *et al.* 2009)*; Artibeus fimbriatus:* Rio de Janeiro (Lourenço *et al.* 2016, 2020); Rio Grande do Sul (Silva *et al.* 2009); *Artibeus lituratus* (Olfers, 1818): Ceará, Mato Grosso (Almeida *et al.* 2016b), Distrito Federal (Gettinger and Gribel 1989), Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (Azevedo *et al.* 2002; present study), Paraná and São Paulo (Confalonieri 1976), Pernambuco (Dantas-Torres *et al.* 2009), Rio de Janeiro (Confalonieri 1976; Almeida *et al.* 2010, 2011; Almeida *et al.* 2015; Lourenço *et al.* 2016), Rio Grande do Sul (Silva *et al.* 2009), Santa Catarina (Rudnick 1960), Sergipe (Bezerra and Bocchiglieri 2018), Brazil (Webb *et al.* 1977); *Artibeus obscurus*: Rio de Janeiro (Almeida *et al.* 2011; Lourenço *et al.* 2016); *Artibeus planirostris*: Ceará (Almeida *et al.* 2016b; Confalonieri 1976); Distrito Federal (Gettinger and Gribel 1989); Mato Grosso (Almeida *et al.* 2016b); Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017); Minas Gerais (present study), Pernambuco (Dantas-Torres *et al.* 2009); Rio de Janeiro (Almeida *et al.* 2011; Confalonieri 1976; Lourenço *et al.* 2016) and Sergipe (Bezerra and Bocchiglieri 2018); *Carollia perspicillata:* Ceará (Almeida *et al.* 2016b; Confalonieri 1976), Minas Gerais (present study), Rio de Janeiro (Almeida *et al*. 2011; Lourenço *et al.* 2020); *Chiroderma doriae*: Rio de janeiro (Lourenço *et al.* 2016), Mato Grosso do Sul (Silva *et al.* 2017), Minas Gerais (present study); *Chiroderma vizottoi*: Ceará (Almeida *et al.* 2016b); *Chrotopterus auritus*: São Paulo (Confalonieri 1976); *Dermanura cinerea* (Gervais, 1856): Distrito Federal (Gettinger and Gribel 1989), Sergipe (Bezerra and Bocchiglieri 2018); *Desmodus rotundus* (E. Geoffroy, 1810): Rio de janeiro (Lourenço *et al.* 2016), São Paulo (Confalonieri 1976); *Eptesicus brasiliensis* (Desmarest, 1819): Pará (Confalonieri 1976); *Glossophaga soricina* (Pallas, 1766): Rio de Janeiro (Lourenço *et al.* 2020), Rio Grande do Sul (Silva *et al.* 2009); *Lophostoma brasiliense*: Sergipe (Bezerra and Bocchiglieri 2018); *Lophostoma silviculum*: Mato Grosso do Sul (Silva *et al.* 2017); *Micronycteris microtis* (Miller, 1898): Rio de Janeiro (Lourenço *et al.* 2020); *Myotis nigricans*: Rio de Janeiro (Almeida *et al.* 2010); *Noctilio albiventris*: Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al*. 2017); *Peropteryx macrotis*: Ceará (Confalonieri 1976); *Phyllostomus discolor*: Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Sergipe (Bezerra and Bocchiglieri, 2018); *Platyrrhinus incarum* (Thomas, 1912): Mato Grosso (Almeida *et al.* 2016b); *Platyrrhinus lineatus* (E. Geoffroy, 1810): Ceará (Almeida *et al.* 2016b), Distrito Federal (Gettinger and Gribel 1989), Mato Grosso (Almeida *et al.* 2016b, Mato Grosso do Sul Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (present study), Pernambuco (Dantas-Torres *et al.* 2009), Rio de Janeiro (Confalonieri 1976; Lourenço *et al.* 2016), São Paulo (Oudemans 1902); *Platyrrhinus recifinus* (Thomas, 1901): Rio de Janeiro (Lourenço *et al.* 2016, 2020);

Platyrrhinus sp.: Rio de Janeiro (Confalonieri 1976); *Pygoderma bilabiatum*: Rio de Janeiro (Lourenço *et al.* 2016); *Sturnira lilium* (E. Geoffroy, 1810): Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (Azevedo *et al.* 2002), Pernambuco (Dantas-Torres *et al.* 2009), Rio de Janeiro (Almeida *et al.* 2011; Confalonieri 1976; Lourenço *et al.* 2016); *Sturnira tildae*: Espírito Santo (Confalonieri 1976); *Tonatia silvicola* (d'Orbigny, 1836): Mato Grosso do Sul (Silva and Graciolli 2013); *Vampyressa pusilla* (Wagner, 1843): Rio de janeiro (Lourenço *et al.* 2016, 2020), *Vampyrodes caraccioli* (Thomas, 1889): Pará (Confalonieri 1976).

Differential diagnosis — *Females*: first pair of dorsal proteronotal setae minute and on the anterolateral margin of the dorsal plate, while the other proteronotal setae large and off the plate (Figure 11C); sternal plate pear shaped (Figure 11E–F); proximal anterodorsal seta of femur II minute and proximal posterodorsal seta of femur II medium sized (Figure 11J); posteroventral (*pv*) seta of femur-tibia IV straight and bladelike (Figure 11M–N). *Male*: intercoxa IV area with eight pairs of setae, first pair of setae posterior to sternal plate short to minute; sternogenital setae long, first pair extending to or beyond level of second pair of setae (Figure 12C); proximal antero- and posterodorsal setae of femur IV long, subequal in length (Figure 12L) (Herrin and Tipton 1975). *Nymphs*: similar to males with regard to above features, except by ontogenetic differences (Deunff et al. 2011) (Figure 13A–N). Deutonymph female has 13 pairs setae on intercoxal IV area, including adanal pair, and five pairs of hysteronotal setae (one pair poststigmal setae and four pairs opisthosomal setae) and plus one unpaired seta on caudal dorsum. Deutonymph male has eight pairs setae on intercoxal IV area, including adanal pair, two pairs hysteronotal setae (one pair poststigmal setae and one pair opisthosomal setae) and plus one unpaired seta on caudal dorsum (similar to adult male). Protonymph has pairs of hysteronotal setae and caudal dorsum similar to deutonymph male.

Remarks — This is the first record of *P. iheringi* on bats *Ar. planirostris, Ca. perspicillata, Ch. doriae* and *Pl. lineatus* from Minas Gerais. The species is oligoxenous, associated with numerous phyllostomid bats, especially with Sternodermatini bats (Herrin and Tipton 1975), and closely related to *P. ojastii* Machado-Allison, 1964. they share pronounced shoulders on the anterolateral outline of the dorsal plate and similarly shaped sternal and sternogenital plates, and posteroventral setae of femur-tibia IV are straight and bladelike in females (Herrin and Tipton 1975; Morales-Malacara 2001). They may be distinguished by *P. iheringi* shows a small central pair of foveae are associated with a longitudinal medial keel, the end of which is joined with the anterocentral unpaired fovea, such that it looks like an arrow (vs absent), Pnl are very small (vs Pnl-Pn5), on *Artibeus* (vs *P. ojastii* on *Stunira*). In both sexes distance between the first and second pairs is distinctly greater than that between the second and third (vs *P. ojastii* the distance between the first and second pairs of podosomal setae is distinctly less than the distance between the second and third pairs). Morphological characters of examined specimens match original description and re-descriptions (Rudnick 1960; Herrin and Tipton 1975; Furman 1966).

In our bGMYC species delimitation analyses (Figure 2), 25 haplotypes (5, 38 to 61) out of 29 sequences from individuals morphologically assigned to *P. iheringi* were recovered split into two well supported species (pp >95%). The first putative species hereinafter referred to as *P. iheringi* A includes haplotypes 5, 46 to 61 and the second, as *P. iheringi* B, includes haplotypes 44 to 45 and third, as *P. iheringi* C includes haplotypes 38 to 45.

On other hand, the posterior probability of including all mites identified morphologycally as *P. iheringi* in a single species is less than 50%, suggesting *P. iheringi* as a species complex. Interesting, when we turn to hosts: *P. iheringi* A was found on *Artibeus planirostris Artibeus lituratus* and *Carollia perspicillata; P. iheringi* B was found on *Artibeus planirostris* and *Art. lituratus*; and *P. iheringi* C was found on *Art. lituratus, Platyrrhinus lineatus* and *Chiroderma doriae*. Hence, all clades of *P. iheringi* were found on *Artibeus lituratus*.

5. *Periglischrus paravargasi* Herrin & Tipton (Figures 14–16)

Periglischrus paravargasi Herrin & Tipton, 1975: 46.

Specimens examined — 1♂ (UFMG AC 221122) on *Anoura caudifer* (1 ex.): Brazil, Minas Gerais, Formiga, **carste/mata** of Gruta Paca, 26 Jan. 2021, collected by A. Tahara *et al.* (COX1 sequence [voucher code]: OP964435[UFMG AC 221122]); 2♀(UFMG AC 220162, 220813) and 1 protonymph (UFMG AC 220166) on *A. caudifer* (1 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Mosquito cave** (unregistered), PE Serra Nova e Talhado, -15.6545, -42.7335, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964434[UFMG AC 220162], OP964436[UFMG AC 220813], OP964438[UFMG AC 220166]); 2 deutonymphs ♀ (UFMG AC 220178, 221009) on *A. caudifer* (1 ex.): Brazil, Minas Gerais, Rio Pardo de Minas, **Pequenos Labirintos cave** (unregistered), PE Serra Nova e Talhado, -15.6293, -42.7043, 18 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964433[UFMG AC 220178], OP964437[UFMG AC 221009]).

Barcode sequences: OP964433–38 (Table 1).

Distribution — Brazil, French Guiana, Peru and Venezuela (Gettinger 2018; Beron 2020).

Hosts and records from Brazil — *Anoura caudifer*: Distrito Federal (Gettinger and Gribel 1989), Rio de janeiro (Lourenço et al. 2016) and Minas Gerais (this study).

Differential diagnosis — *Female*: dorsal plate distinctive ornamentation with numerous small darker circular areas, some larger irregularly shaped lighter areas, and very small circular pores or setal bases. (Figure 14C); dorsal opisthosoma with six pairs of setae, first pair behind level of coxa IV rather large; next three pairs medium sized; last two pairs (posteriomost) small to minute (Figure 14D); sternal plates subpentagonal, distinctly longer than wide, with longer anterior end border that narrows abruptly, forming narrow anterior projection (Figure 14E–F); posterolateral setae of femur to tibia IV greatly inflated, with slender recurved end (Figure 14M–N). *Male*: sternogenital setae are long, the setae *St1* extending posterior to or slightly beyond the level of the first pair of pores; intercoxal IV area with seven pairs of setae plus one pairs of subterminal adanal setae; first pair posterior to sternogenital plate minute, and coxa II with setae *pv* long (length at least equal to the width of coxa II) (Herrin and Tipton 1975; Morales-Malacara 2001). *Nymphs*: similar to males with regard to above features, except by ontogenetic differences (Deunff et al. 2011) (Figure 16A–N). Deutonymph female has 13 pairs setae on intercoxal IV area, including adanal pair, and seven pairs of hysteronotal setae (one pair poststigmal setae and six pairs opisthosomal setae) and plus one unpaired seta on caudal dorsum. Protonymph has two pairs hysteronotal setae (one pair poststigmal setae and one pair opisthosomal setae) and plus one unpaired seta on caudal dorsum (similar to adult male).

Remarks — *P. paravargasi* is reported for the first time from Minas Gerais state. This species seems to be monoxenous, exclusive to *Anoura caudifer.* Examined specimens match those of the original description and re-descriptions (Herrin and Tipton 1975; Morales-Malacara 2001). In our bGMYC species delimitation analyses (Figure 2), *Periglischrus paravargasi* led to five haplotypes (31-35) out five sequences and was recovered as a single species with pp. >95%.

6. *Periglischrus torrealbai* Machado-Allison (Figures 17–19)

Periglischrus torrealbai Machado-Allison, 1965a:276–279.
Periglischrus inflatiseta Furman, 1966: 134–135.

Specimens examined — 2♂ (UFMG AC 220041, 221030) and 1 protonymph (UFMG AC 220054) on *Phyllostomus discolor* (3 ex.): Brazil, Minas Gerais, Lagoa Santa, **Lapinha cave**, PE Sumidouro, -19.5616, -43.959, 11 Aug. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964439[UFMG AC 221030], OP964442[UFMG AC 220041], OP964444[UFMG AC 220054]); 1♂ (UFMG AC 221129) on *Tonatia bidens* (1 ex.): Brazil, Minas Gerais, Pains, **Mastodonte cave**, -20.4270, -45.6322, 27 Jan. 2021, collected by A. Tahara *et al.* (COX1 sequence[voucher code]: OP964443[UFMG AC 221129]); 4♂ (UFMG AC 220137, 220138, 220823, 220139) on *Phyllostomus hastatus* (PESNT112): Brazil, Minas Gerais, Rio Pardo de Minas, **Mosquito cave** (unregistered), PE Serra Nova e Talhado, -15.6545, -42.7335, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence[voucher code]: OP964440[UFMG AC 220139]); ♀(UFMG AC 220043) on *Phyllostomus discolor* (1 ex.): Brazil, Minas Gerais, Lagoa Santa, **Lapinha cave,** PE Sumidouro, -19.5616, -43.959, 11 Aug. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964441[UFMG AC 220043]).

Barcode sequences: OP964441–44 (Table 1).

Distribution — Brazil, Colombia, Costa Rica, Panama, Peru and Venezuela (Gettinger 2018; Beron 2020).

Hosts and records from Brazil — *Artibeus planirostri*s (Spix, 1823): Ceará (Almeida *et al.* 2016b); *Lophostoma silviculu*m: Mato Grosso do Sul (Silva *et al.* 2017; Silva and Graciolli 2013); *Phyllostomus discolor* (Wagner, 1843): Ceará (Almeida *et al.* 2016b; Almeida *et al.* 2018); Distrito

Federal (Gettinger and Gribel 1989); Mato Grosso (Almeida *et al.* 2018); Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017), Minas Gerais (present study); *Phyllostomus hastatus* (Pallas, 1767): Minas Gerais and São Paulo (Confalonieri 1976); Mato Grosso (Almeida *et al.* 2018); Mato Grosso do Sul (Silva and Graciolli 2013; Silva *et al.* 2017); Rio de Janeiro (Almeida e*t al.* 2011; Almeida *et al.* 2018; Lourenço *et al.* 2016; Lourenço *et al.* 2020); *Tonatia bidens* (Spix, 1823): Ceará (Almeida *et al.* 2016b; Almeida *et al.* 2018), Minas Gerais (present study).

Differential diagnosis — *Female*: small, idiosoma length up to 779 µm (Figure 17A); dorsal opisthosoma with 4-5 pairs of minute setae (Figure 17D); sternal plate broadly pear shaped; five pairs of ventral body setae (metasternal setae, genital setae, and three pairs of ventral opisthosomal setae posterior to the sternal plate) expanded basally but with acute tips (Figure 17E–F); certain ventral setae of legs I and II short and spinelike to peglike in both sexes. Ventral setae on trochanters I–II, femora l–II, genua II, and one posteroventral seta of each tarsi III short, enlarged and peglike. Setae *pv* on femur-genu IV inflated and bladelike rather than setiform and recurved. *Male*: sternogenital setae long, with first pair extending well beyond level of first pair of pores and the second through fourth sternogenital setae longer, extending beyond bases of adjacent posterior setae; some setae of ventral intercoxa IV area enlarged and expanded basally; many ventral setae of legs I and II distinctly enlarged, blunt and fusiform; large dorsal setae of tarsi III–IV coarsely barbed or serrated; proximal anterodorsal (*ad*) seta of femur–tibia I and genu IV always small. *Nymphs:* similar to males with regard to above features, except by ontogenetic differences (Deunff *et al.* 2011) (Figure 19A–N).

Remarks — This is the first record of *P. torrealbai* on bats *Ph. discolor* and *T. bidens* to Minas Gerais state. This is a stenoxenous species, associated with the phyllostomine bats *Phyllostomus* and *Tonatia*, and the smallest species associated with genus *Phyllostomus* Lacépède, 1799 (the other are *P. acutisternus* and *P. grandisoma* Herrin & Tipton, 1975) (Herrin and Tipton 1975; Almeida *et al.* 2018). Morphological characters match original description and re-descriptions (Machado-Allison 1965a; Furman 1966; Herrin and Tipton 1975; Almeida *et al.* 2018).

In our bGMYC species delimitation (Figure 2), *Periglischrus torrealbai* is represented by six haplotypes (1-4 and 6-7) out of six sequences and recovered as two putative species with pp. >95%: *P. torrealbai* A (7) from *Tonatia bidens* and *P. torrealbai* B (1 to 4 and 6) from *Phyllostomus hastatus* and *Ph. discolor*. This result is in part consistent with Almeida *et al.* (2018) shows that *P. torrealbai* morphology varies on a morphometric basis among the host bat species *P. discolor*, *P. hastatus* and *T. bidens* with three morphologically distinct species with host specificity, and suggest that *P. torrealbai* includes at least two distinct species of *Periglischrus*, one with *Phyllostomus* and *Tonatia* genus.

Discussion

The sequenced COI fragment provided a confident range of >10% for interspecific divergence indicates that there is sufficient genetic differentiation among species to reliably distinguish them using this molecular marker. The maximum likelihood (ML) analysis showed consistency with morphological identification of *Periglischrus* species based on descriptions, redescriptions and taxonomic keys (Machado-Allison 1964, 1965a; Furman 1966; Herrin and Tipton 1975; Morales-Malcara 2001). The nodes were supported by high bootstrap values (100%) further strengthening the confidence in the accuracy of species identification based on DNA barcoding.

Species clustering and delimitation by BINs, ASAP and bGMYC yielded distinct but partially consistent results. For ASAP analysis identified a barcode gap and supported the six species*,* matching the morphological identification, while BINs supported eight species, as it subdivided *P. acutisternus* and *P. torrealbai*. In contrast, bGMYC identified at least 10 putative species, as it subdivided *P. acutisternus* and *P. torrealbai* into two species each (concordant with BINs), and further subdivided *P. iheringi* into three species.

The observed subdivisions within *P. acutisternus*, *P. torrealbai*, and particularly *P. iheringi* suggest that BINs and bGMYC oversplit these species, something previously observed (Gomes-Almeida *et al.* 2023). The co-occurrence of putative species in the same locations and even in the same host allows us to discard geographic barriers as a cause for the genetic structure observed today. Instead, the observed genetic structure may be explained by past vicariance events caused by host shift and a recent re-encounter in the case of *P. iheringi* A, B and C sampled in the same host. Nevertheless, to test these hypotheses more specimens and genes, especially nuclear genes, must be analyzed.

On a positive note, genetic barcoding has proved to be a valuable and effective tool to unravel genetic diversity and aid in the identification of *Periglischrus* mite species. Moreover, this approach has enabled reliable identification of immature stages. Notably, our study reports *Periglischrus caligus*, *P. herrerai* and *P. paravargasi* for the first time from Minas Gerais, extending their distribution ranges.

Morphological character-based taxonomy

Herrin and Tipton (1975) assigned species to groups based mainly on sternal plate outline, size and location of the proteronotal setae, and size of proximal dorsal setae on femur I-IV. However, Morales-Malacara (2001) later added new morphological characters of the idiosoma to the type-based taxonomy of *Periglischrus*, and renamed and re-defined these species groups and subgroups. Herewith, the species recorded are classified in six species groups according to Morales-Malacara 2001.

Periglischrus acutisternus is a member of *acutisternus* species group, alongside with *P. tonatii* Herrin & Tipton, 1975, *P. paracutisternus* Machado-Allison & Antequera, 1971, *P. dusbabeki* Machado-Allison & Antequera, 1971, and *P. steresotrichus* Morales-Malacara & Juste, 2002. They share female sternal plates with a distinct anterior median projection, subtriangular or elongated in shape, distinct constriction anterior to first sternal setae, and the anterolateral corners of sternogenital plate are weakly defined and long sternogenital setae (Morales-Malacara 2001). Herrin and Tipton (1975) called it subgroup B from Group I, and included *P. grandisoma* Herrin & Tipton, 1975 too. To them, the subgroup is based on the presence of a medium-sized to prominent mediodistal lobe on the palpal tibia in addition to above mentioned similarity in female sternal plate shape.

Periglischrus torrealbai belongs to the *torrealbai* species-group, that also comprises *P. paratorrealbai* Herrin & Tipton, 1975 and *P. eurysternus* Morales-Malacara & Juste, 2002. According to Herrin and Tipton (1975) and Morales-Malacara (2001), they share broad pear-shaped sternal plate with a somewhat narrow or broadly rounded anterior border, some enlarged ventral hysterosomal setae, proteronotal and poststigmal setae very small in females and males, and sternogenital plate with rounded lateral borders.

Periglischrus caligus is closely related to *P. leptosternus* Morales-Malacara & López-Ortega, 2001 from *caligus* species group. They share the dark foveae pattern on dorsal plate and a sternal plate with a subpentagonal outline and a pointed anterior border, and males with a small sternogenital plate with weakly sclerotised anterolateral borders and small sternogenital setae (Morales-Malacara 2001). Herrin and Tipton (1975) previously included *P. caligus* in subgroup A from group II (*P. paracaligus* Herrin and Tipton, 1975*, P. paravargasi* Herrin & Tipton, 1975, and *P. vargasi* Hoffmann, 1944) due their morphological similarity (all dorsal proteronotal setae large, long, stout, with first and second pairs apart from each other by a distance larger than second and third pairs; proximal anterodorsal (*ad*) seta of femur–tibia I, and tibia II small to minute), and host associations (genera *Glossophoga* (*P. caligus*), *Leptonycteris* (*P. paracaligus*) and *Anoura* (*P. paravargasi and P. vargasi*). Meanwhile, Morales-Malacara 2001 indicated *P. caligus* as a separate group, and included the other species, along with *P. empheresotrichus* Morales-Malacara, Castaño-Meneses & Klompen, 2020, and *P. calcariflexus* Morales-Malacara & López-Ortega, 2022 in the *vargasi* species-group.

Periglischrus paravarvagi is a member of *vargasi* species-group, along *P. vargasi*, *P. paracaligus*, *P. calcariflexus*, and *P. empheresotrichus*. The group share a sigilla or foveal arrangement, unique scale-like interfoveal ornamentation pattern on dorsal plate, long proteronotal setae and a subpentagonal sternal plate with anterior subtriangular border on female, and males sternogenital plate usually with unsclerotized anterolateral corners, with medium or moderately long sized sternal setae (Morales-Malacara 2001). Additionally, this species-group is associated with three genera of Glossophagini bats: *Anoura*, *Monophyllus*, and *Leptonycteris* (Morales-Malacara *et al.* 2020; Morales-Malacara and López-Ortega 2023).

Periglischrus herrerai is a member of *hopkinsi* species group, similar to *P. hopkinsi* Machado-Allison, 1965a in having sternal plates with a subpentagonal outline and a subtriangular anterior border. A small cross-shaped crack on the dorsal plate occurs in males of *P. herrerai*, and both sexes of *P. hopkinsi* (Morales-Malacara 2001), and males of *P. herrerai* have a unique reticulated sternogenital shield. Furthermore, sharing the distance between Pn1–Pn2 ≤ Pn2–Pn3 and proximal anterodorsal (ad) seta of tibia II large (Herrin and Tipton 1975). *P. herrerai* is associated with bats of the genus *Desmodus*, while *P. hopkinsi* is associated with the Glossophagini bat *Lionycteris spurelli* and *Lonchophylla robusta* (Herrin and Tipton 1975).

Periglischrus iheringi belongs to *iheringi* species-group, along *P. ojastii* Machado-Allison, 1964, sharing the pronounced shoulders on anterolateral dorsal plate and sternal and sternogenital plates similarly shaped (Herrin and Tipton 1975; Morales-Malacara 2001). Nevertheless, *P. iheringi* females have Pn1 minute to other setas and on dorsal plate (vs subequal in length to the other setae and off dorsal plate), and both sexes with distances between Pn1-Pn2 > Pn2-Pn3 (vs Pn1-Pn2 < Pn2-Pn3) (Herrin and Tipton 1975). Herrin and Tipton (1975) also grouped these two species (their group III), along *P. ramirezi* Machado-Allison & Antequera, 1971, based on shape of sternal plate (especially *P. ojastii* and *P. iheringi*) and males with 7-8 pairs of setae on intercoxal area IV and sternogenital setae longer, St1 seta extending posteriorly to or beyond level St2 setae bases or just beyond first pair of pores. On the other hand, Morales-Malacara 2001 classified *P. ramirezi* in *delfinadoae* species-group, still belonging to *acutisternus*-clade, with *P. delfinadoae* Dusbábek, 1967, despite having few morphological similarities and association with different bat clade, stenodermatini and macrotine bats, respectively.

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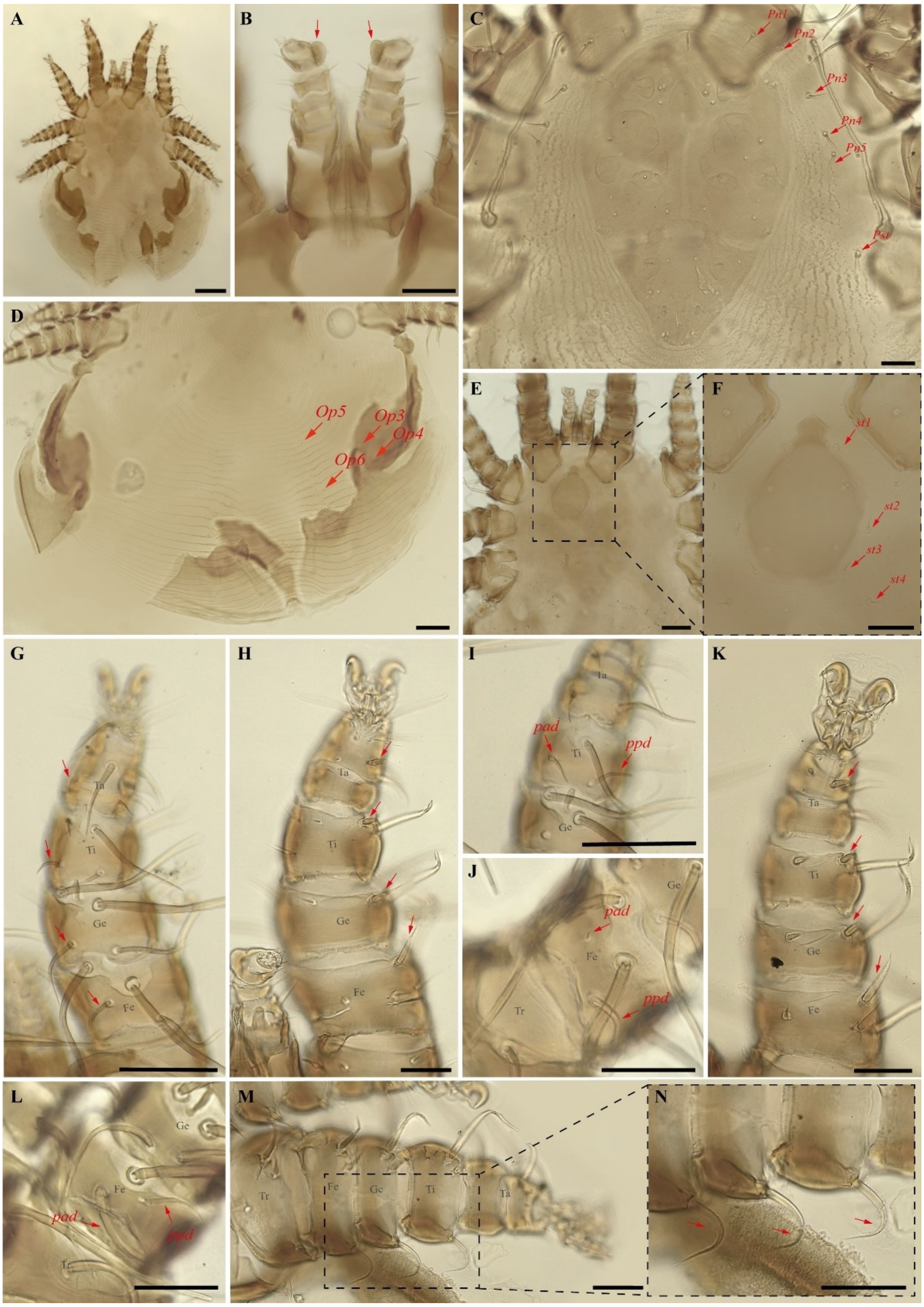


Figure 3. *Periglischrus acustisternus*, female. A — General view; B — Mediodistal lobe of palpal tibia indicated in red arrow; C — dorsal plate with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; D — dorsal opisthosoma with hysteronotal setae (*Op1*-*Op4*) indicated in red arrow; E and F — Sternal plate with details; G — Proximal anterodorsal (*ad*) seta on femur-genu I; H — Distal posteroventral (*pv*) seta on femur-genu I (finely serrated) and tibia-tarsus I (blunt and peglike), indicated in red arrow; I — Proximal antero (*ad*) - posterodorsal (*pd*) setae on tibia II; J — Proximal *ad* and *pd* setae on femur II; K — Distal *pv* setae on femur-genu II and tibia-tarsus II (blunt and peglike), indicated in red arrow; L — Proximal *ad* and *pd* setae on femur IV; M and N — Posterolateral (*pl*) setae on femur-tibia IV with details, indicated in red arrow. Scale bars: A= 200 µm, B–C, F, H, J, K–N= 50 µm, D, E, G, I= 100 µm.

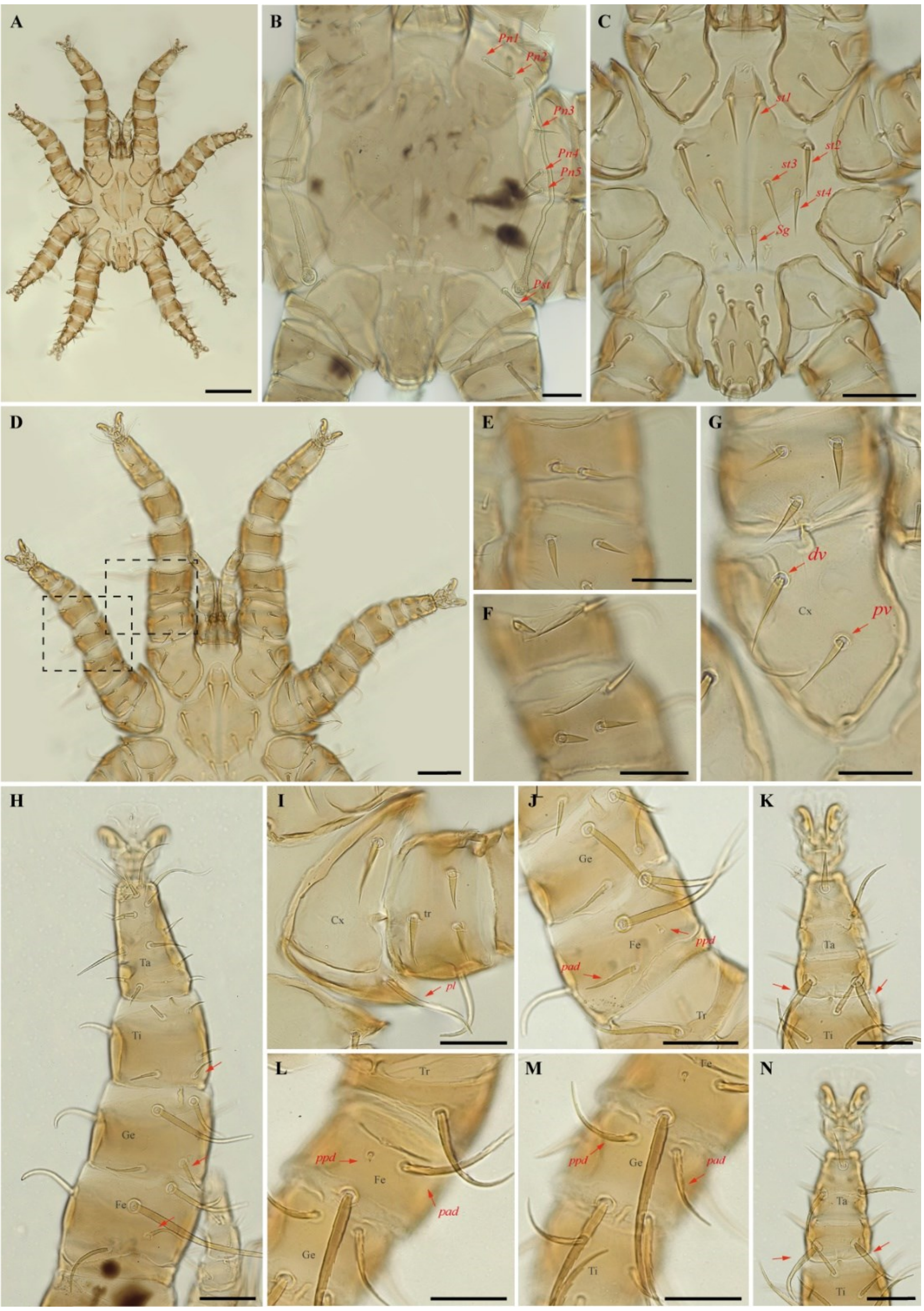


Figure 4. *Periglischrus acustisternus*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A=200 µm, B, E, F, G, H, I, J, K–N= 50 µm, C, D = 100 µm.

Figure 5. *Periglischrus acustisternus*, protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, C–N= 50 µm, B= 100 µm.

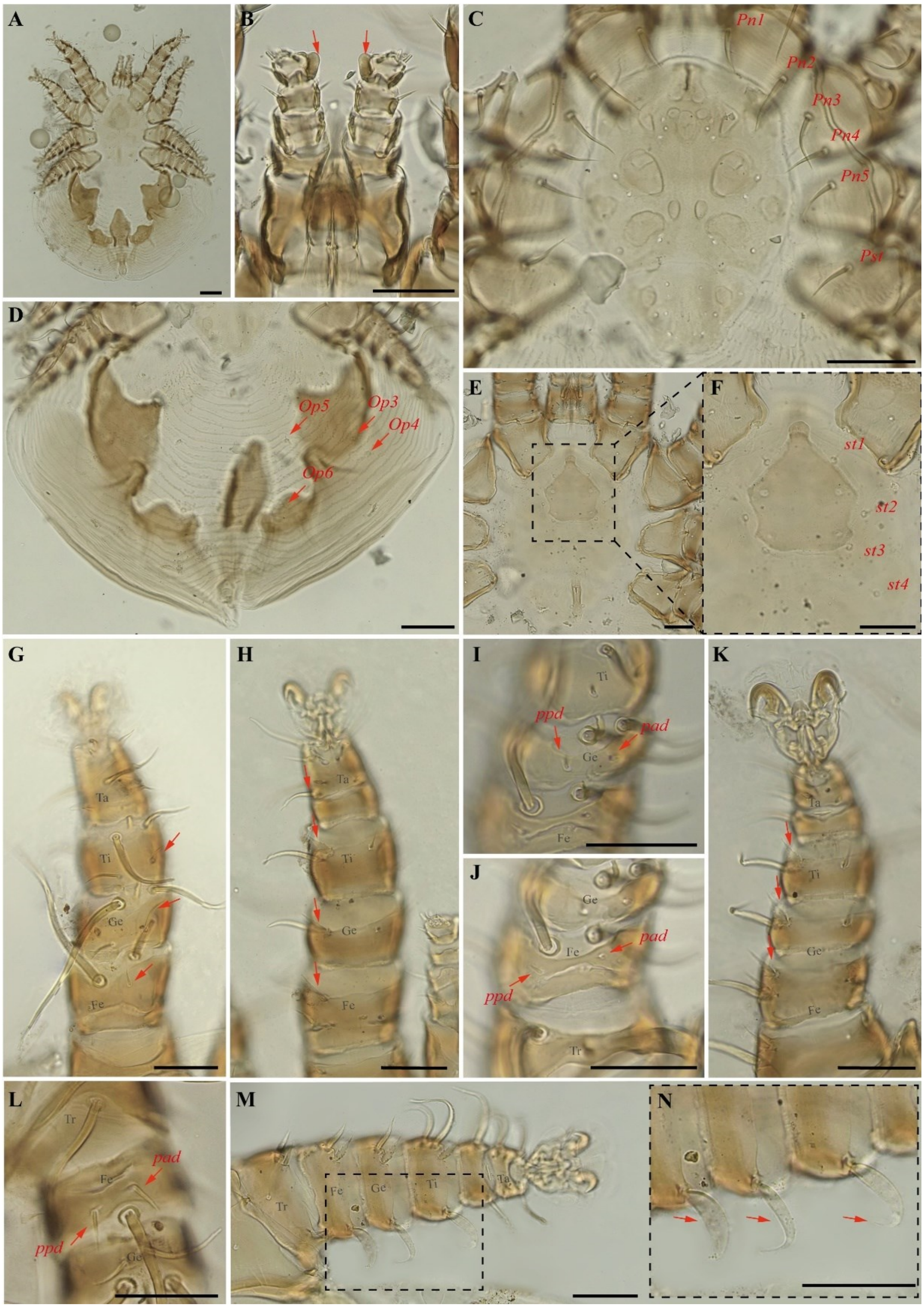


Figure 6. *Periglischrus caligus*, female. A — General view; B — Mediodistal lobe of palpal tibia indicated in red arrow; C — dorsal plate with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; D — dorsal opisthosoma with hysteronotal setae (*Op1*-*Op4*) indicated in red arrow; E and F — Sternal plate with details; G — Proximal anterodorsal (*ad*) seta on femur-genu I; H — Distal posteroventral (*pv*) seta on femur-genu I (finely serrated) and tibia-tarsus I (blunt and peglike), indicated in red arrow; I — Proximal antero (*ad*) - posterodorsal (*pd*) setae on tibia II; J — Proximal ad and pd setae on femur II; K — Distal *pv* setae on femur-genu II and tibia-tarsus II (blunt and peglike), indicated in red arrow; L — Proximal *ad* and *pd* setae on femur IV; M and N — Posterolateral (*pl*) setae on femur-tibia IV with details, indicated in red arrow. Scale bars: A=200 µm, B, F–N= 50 µm, C–E= 100 µm.

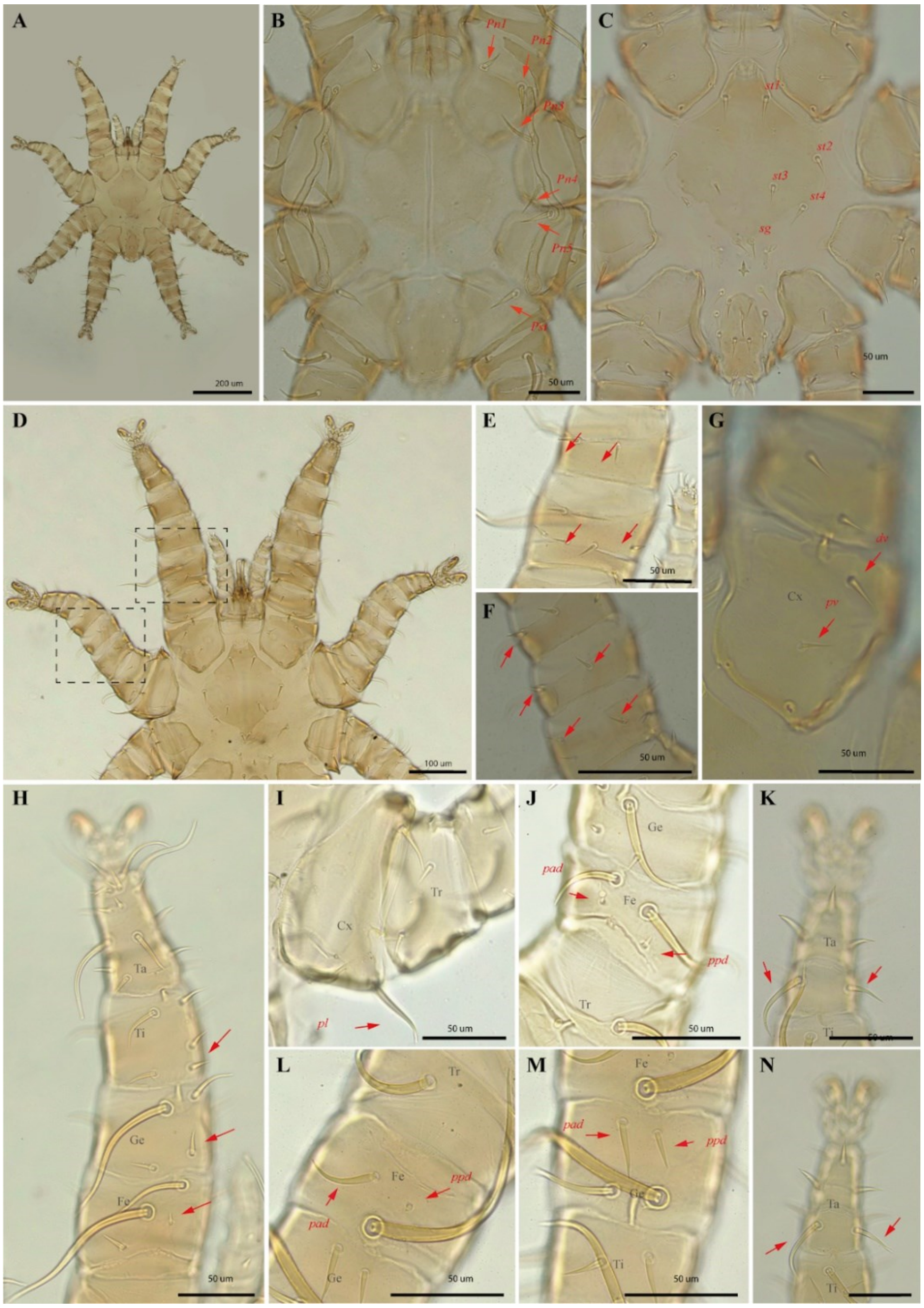


Figure 7. *Periglischrus caligus*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A=200 µm, B, C, E–N= 50 µm, D= 100 µm

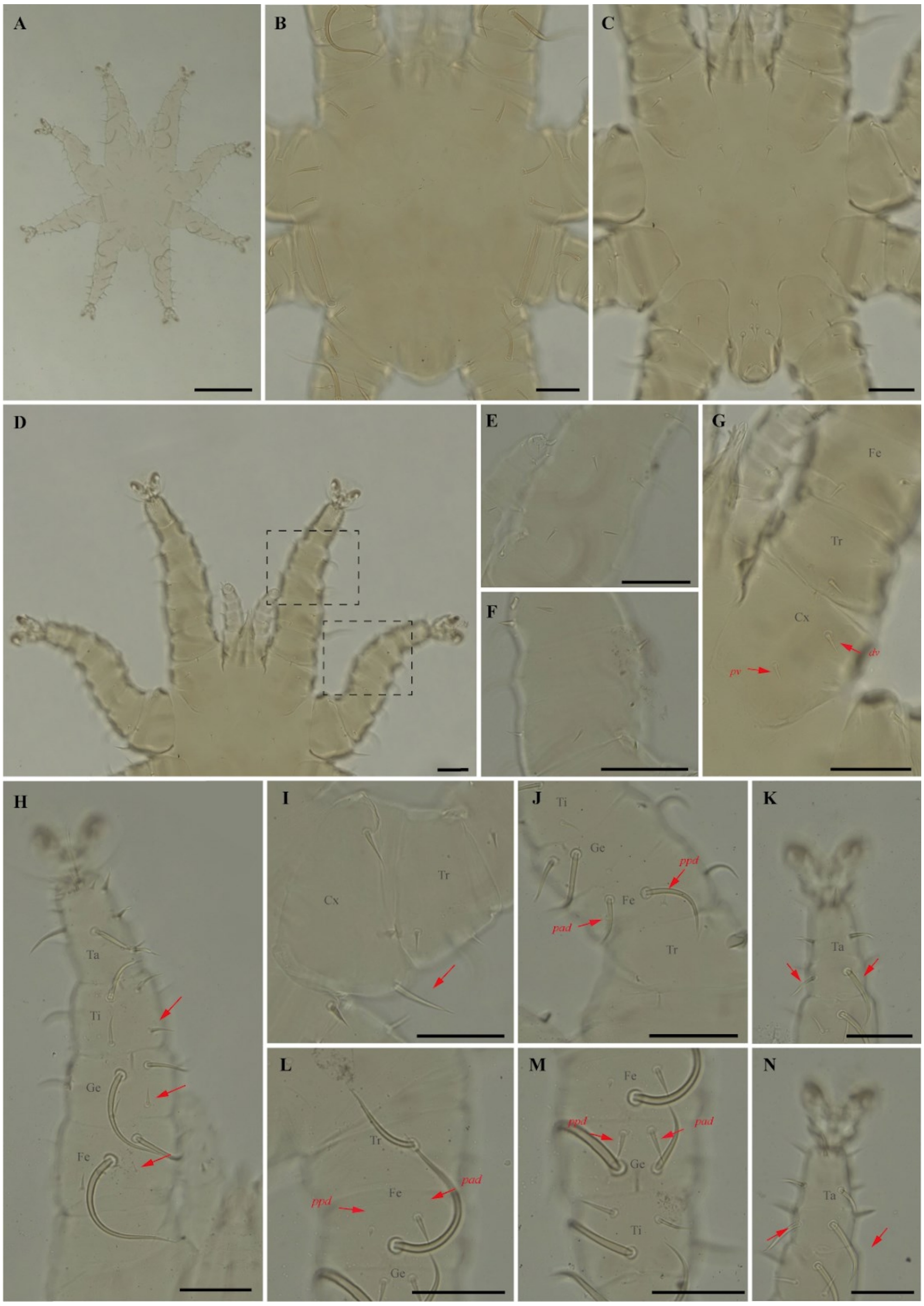


Figure 8. *Periglischrus caligus,* protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A=200 µm, B–N= 50 µm.

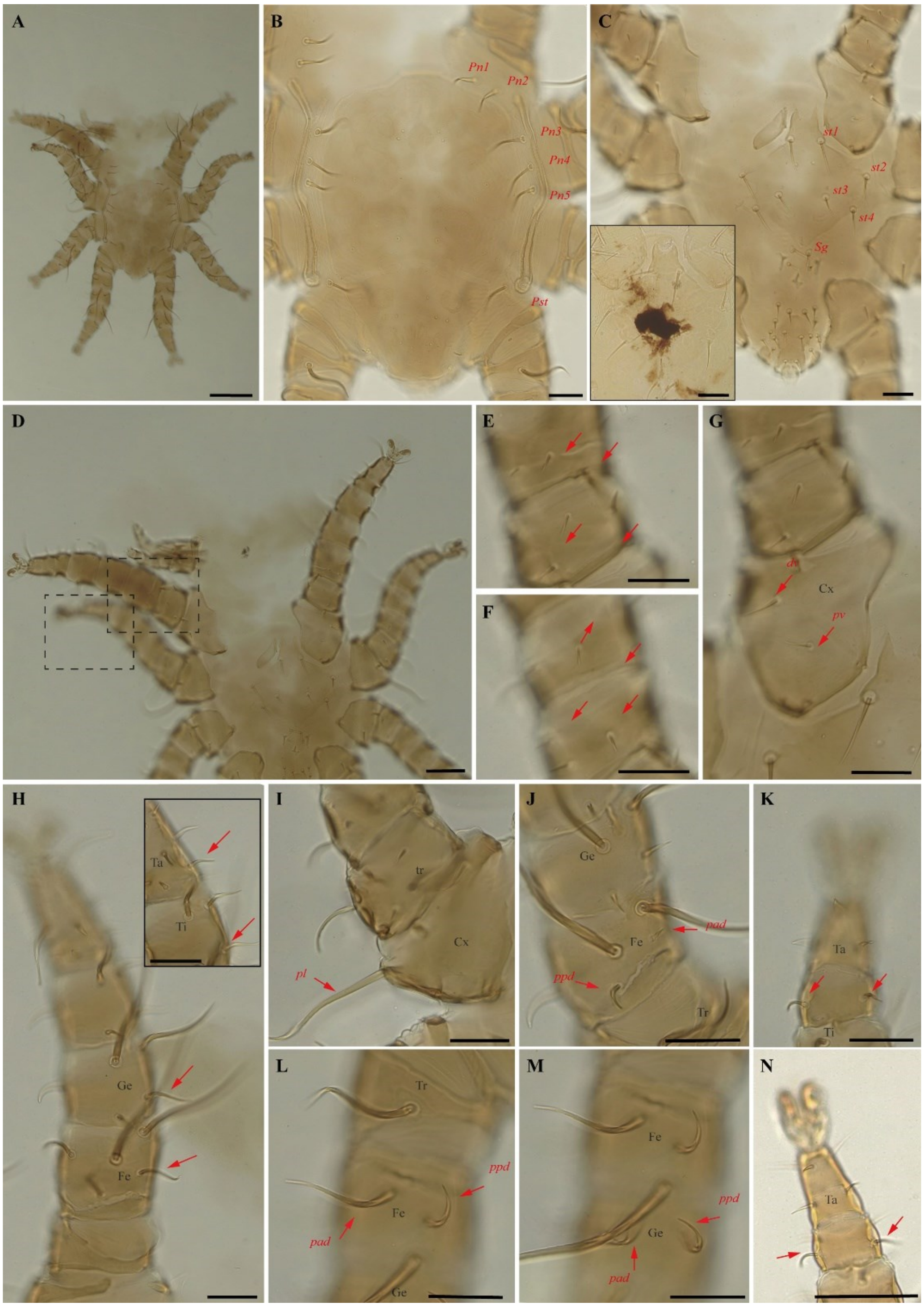


Figure 9. *Periglischrus herrerai*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A=200 µm, B–C, E–M= 50 µm, D and N= 100 µm.

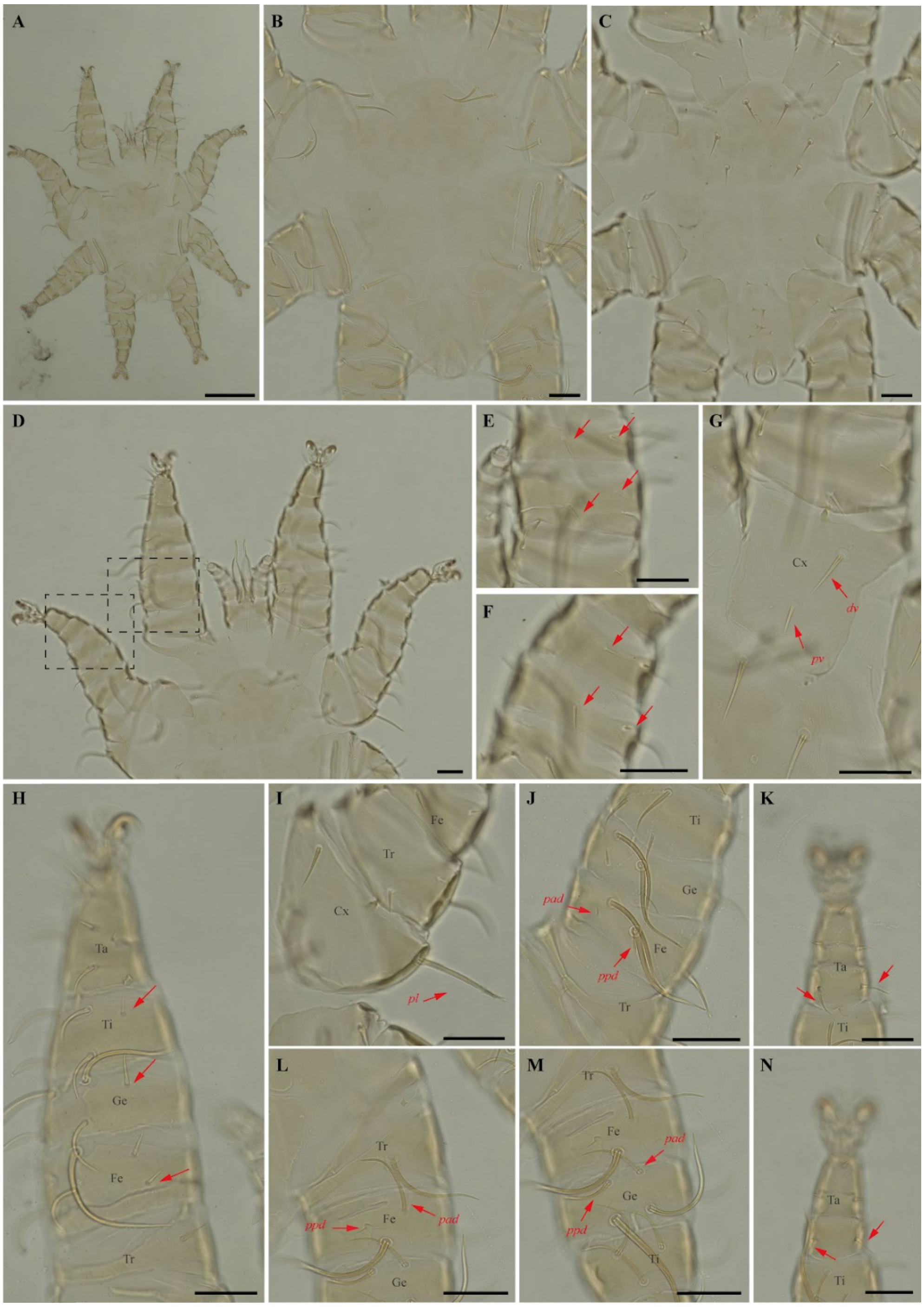


Figure 10. *Periglischrus herrerai*, protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A–N= 50 µm.

Figure 11. *Periglischrus iheringi*, female. A — General view; B — Mediodistal lobe of palpal tibia indicated in red arrow; C — dorsal plate with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; D — dorsal opisthosoma with hysteronotal setae (*Op1*-*Op4*) indicated in red arrow; E and F — Sternal plate with details; G — Proximal anterodorsal (*ad*) seta on femur-genu I; H — Distal posteroventral (*pv*) seta on femur-genu I (finely serrated) and tibia-tarsus I (blunt and peglike), indicated in red arrow; I — Proximal antero (*ad*) - posterodorsal (*pd*) setae on tibia II; J — Proximal *ad* and *pd* setae on femur II; K — Distal *pv* setae on femur-genu II and tibia-tarsus II (blunt and peglike), indicated in red arrow; L — Proximal *ad* and *pd* setae on femur IV; M and N — Posterolateral (*pl*) setae on femur-tibia IV with details, indicated in red arrow. Scale bars: A, C, D= 100 µm, B, E–N= 50 µm.

Figure 12. *Periglischrus iheringi*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D= 100 µm, E–N= 50 µm.

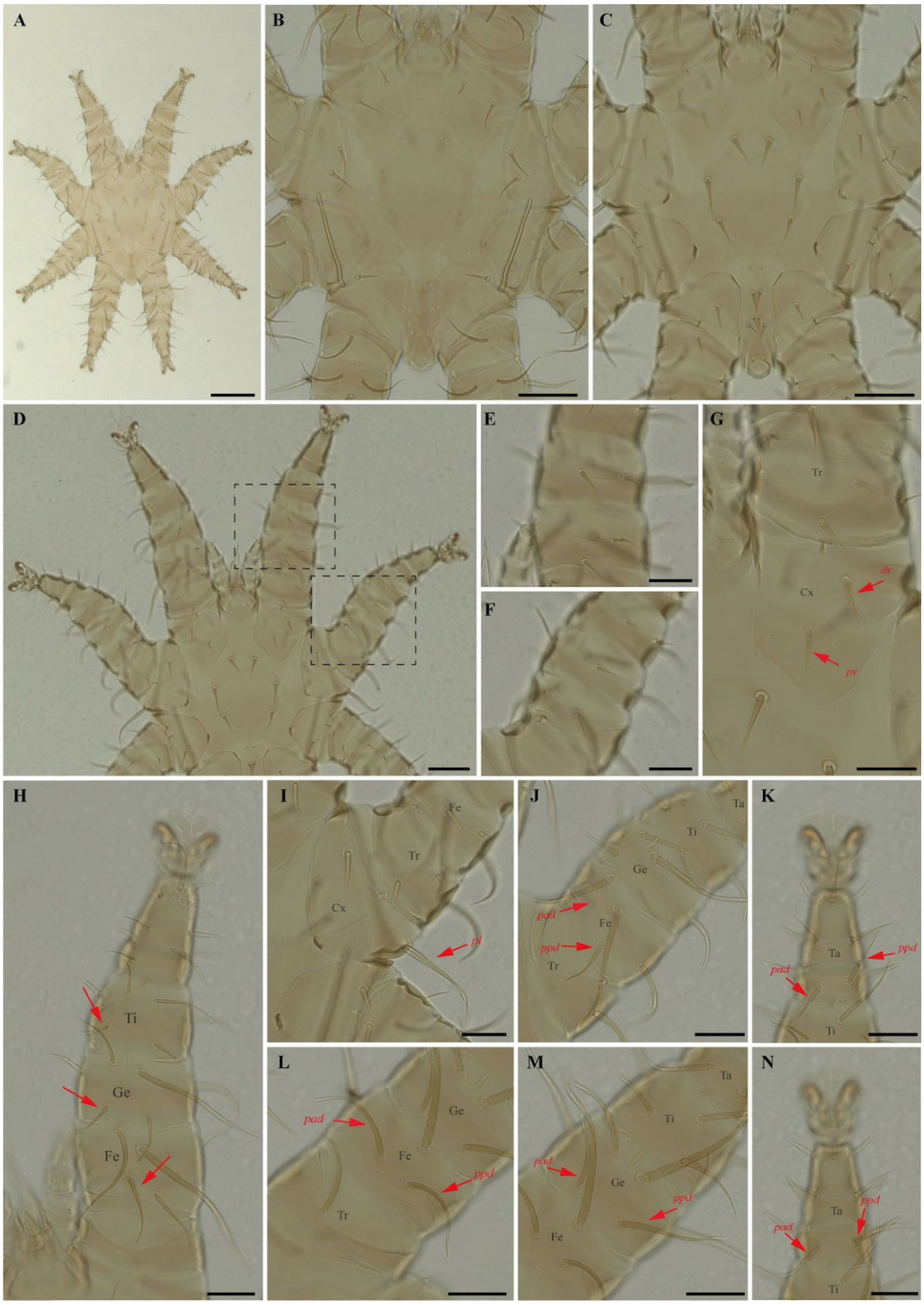


Figure 13. *Periglischrus iheringi*, protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D= 100 µm, E–N= 50 µm.

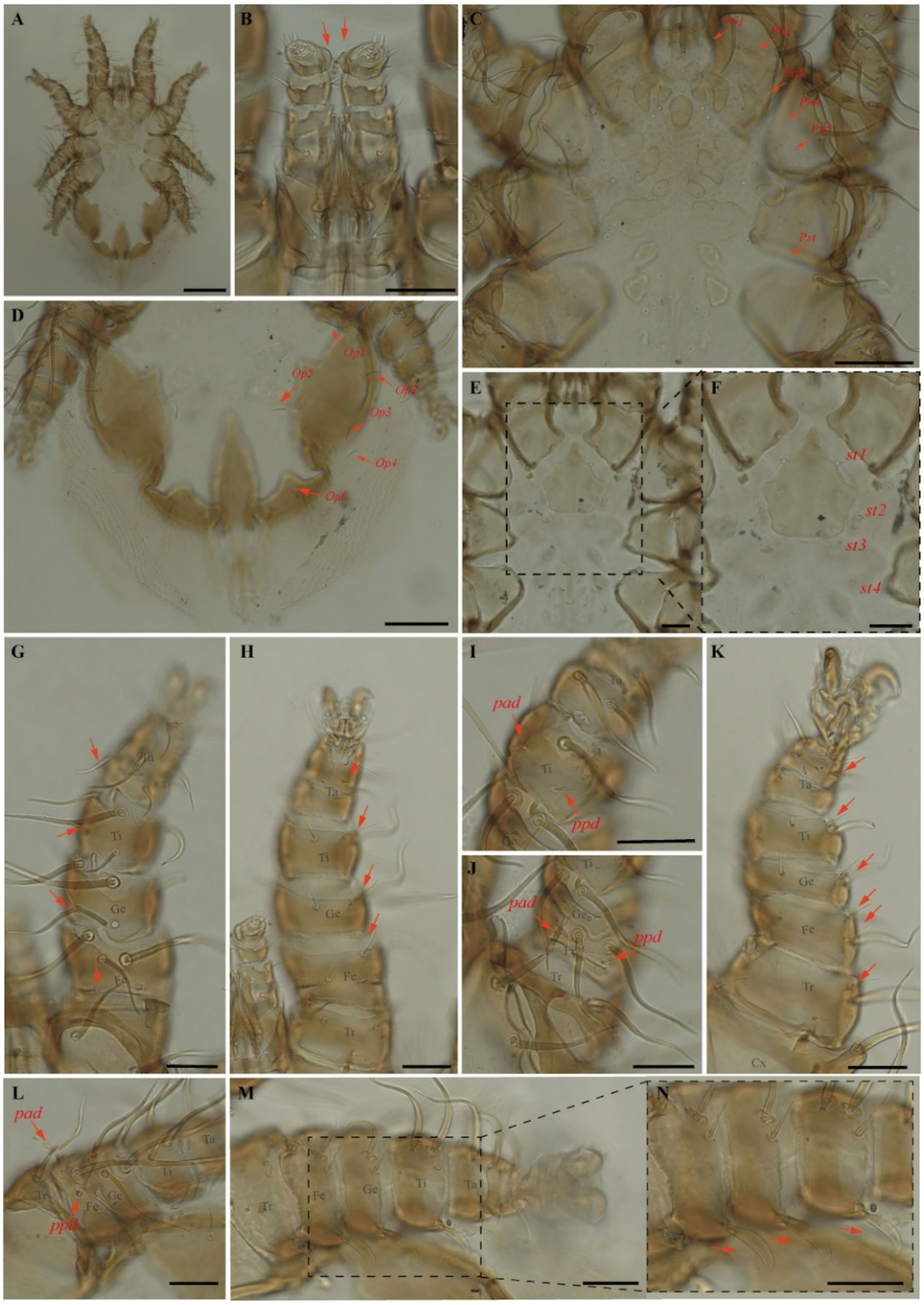


Figure 14. *Periglischrus paravargasi*, female. A — General view; B — Mediodistal lobe of palpal tibia indicated in red arrow; C — dorsal plate with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; D — dorsal opisthosoma with hysteronotal setae (*Op1*-*Op4*) indicated in red arrow; E and F — Sternal plate with details; G — Proximal anterodorsal (*ad*) seta on femur-genu I; H — Distal posteroventral (*pv*) seta on femur-genu I (finely serrated) and tibia-tarsus I (blunt and peglike), indicated in red arrow; I — Proximal antero (*ad*) - posterodorsal (*pd*) setae on tibia II; J — Proximal *ad* and *pd* setae on femur II; K — Distal *pv* setae on femur-genu II and tibia-tarsus II (blunt and peglike), indicated in red arrow; L — Proximal *ad* and *pd* setae on femur IV; M and N — Posterolateral (*pl*) setae on femur-tibia IV with details, indicated in red arrow. Scale bars: A= 200 µm, C–D= 100 µm, B, E–N= 50 µm.

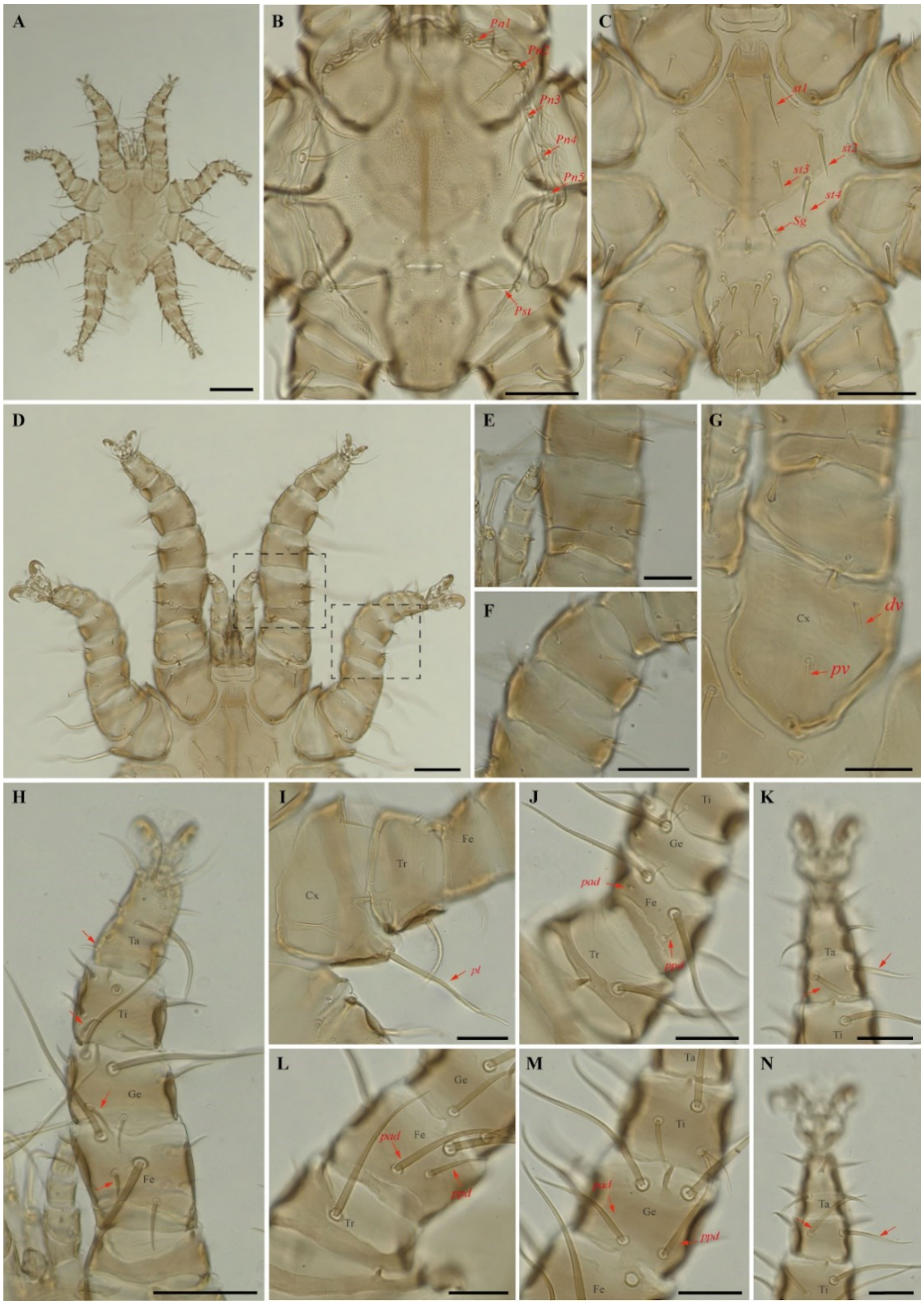


Figure 15. *Periglischrus paravargasi*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D and H= 100 µm, E–G, I–N= 50 µm.

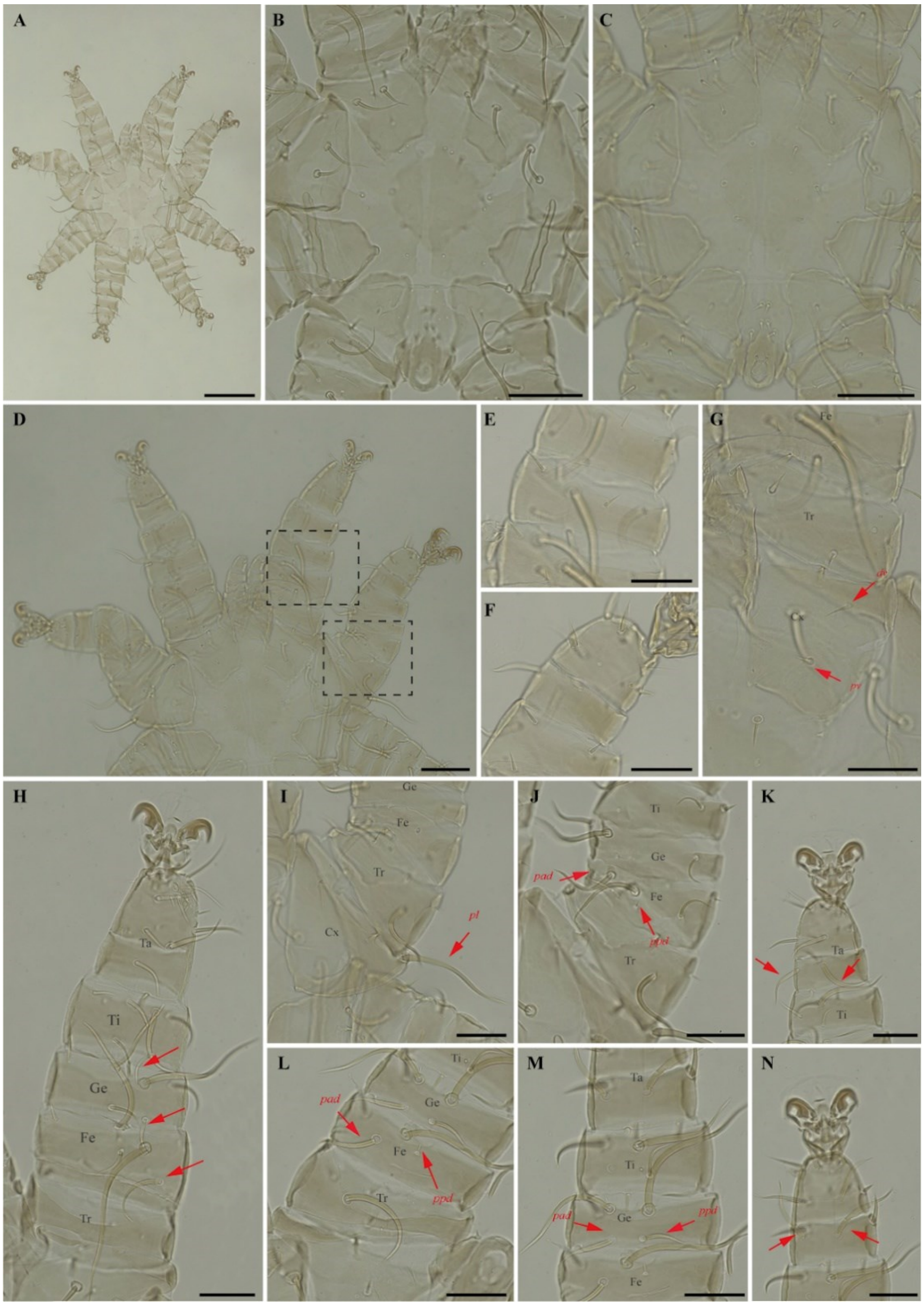


Figure 16. *Periglischrus paravargasi*, protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D= 100 µm, C, E–N= 50 µm.

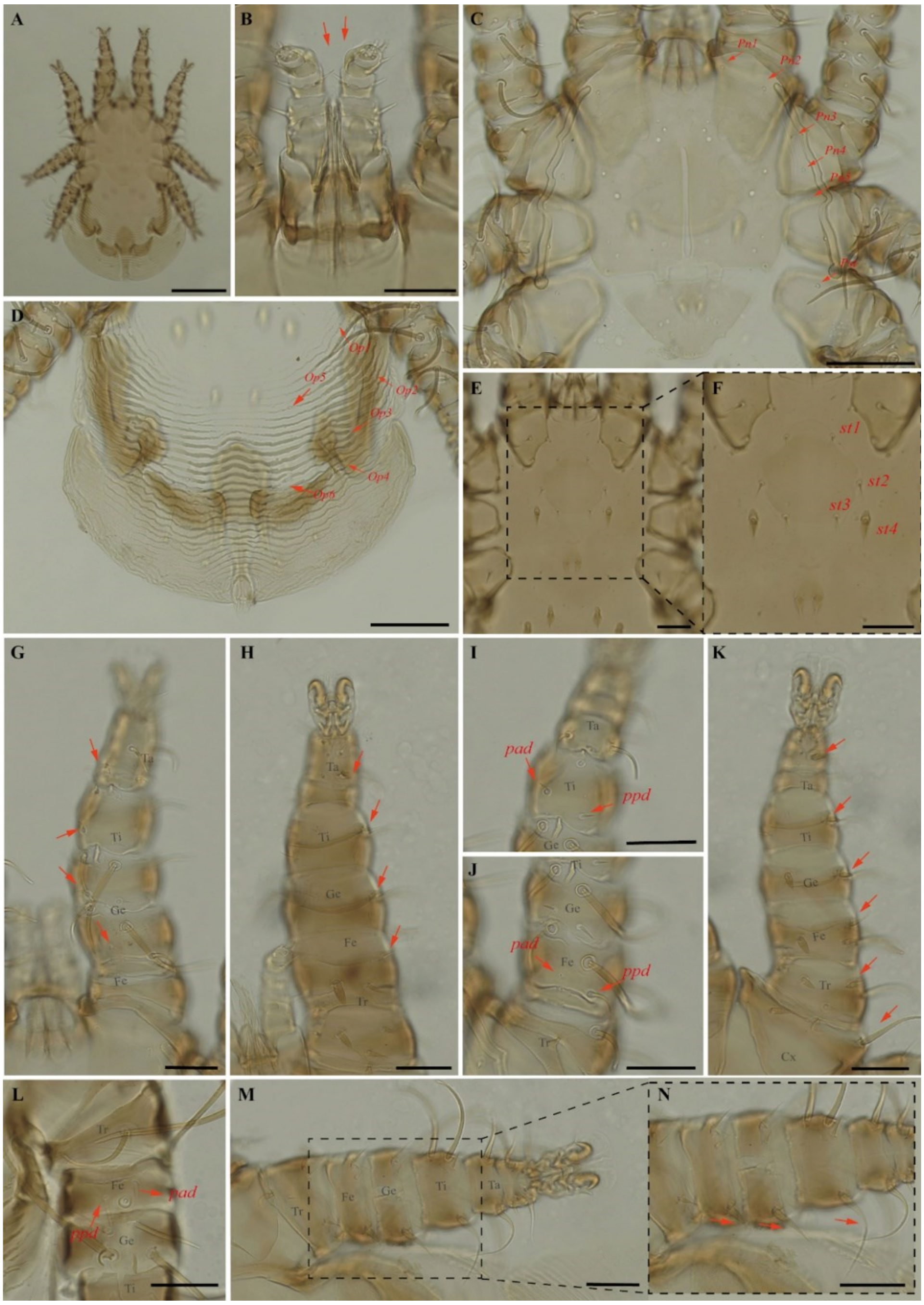


Figure 17. *Periglischrus torrealbai*, female. A — General view; B — Mediodistal lobe of palpal tibia indicated in red arrow; C — dorsal plate with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; D — dorsal opisthosoma with hysteronotal setae (*Op1*-*Op4*) indicated in red arrow; E and F — Sternal plate with details; G — Proximal anterodorsal (*ad*) seta on femur-genu I; H — Distal posteroventral (*pv*) seta on femur-genu I (finely serrated) and tibia-tarsus I (blunt and peglike), indicated in red arrow; I — Proximal antero (*ad*) - posterodorsal (*pd*) setae on tibia II; J — Proximal *ad* and *pd* setae on femur II; K — Distal *pv* setae on femur-genu II and tibia-tarsus II (blunt and peglike), indicated in red arrow; L — Proximal *ad* and *pd* setae on femur IV; M and N — Posterolateral (*pl*) setae on femur-tibia IV with details, indicated in red arrow. Scale bars: A= 200 µm, C, D–F and H= 100 µm, B, G–N= 50 µm.

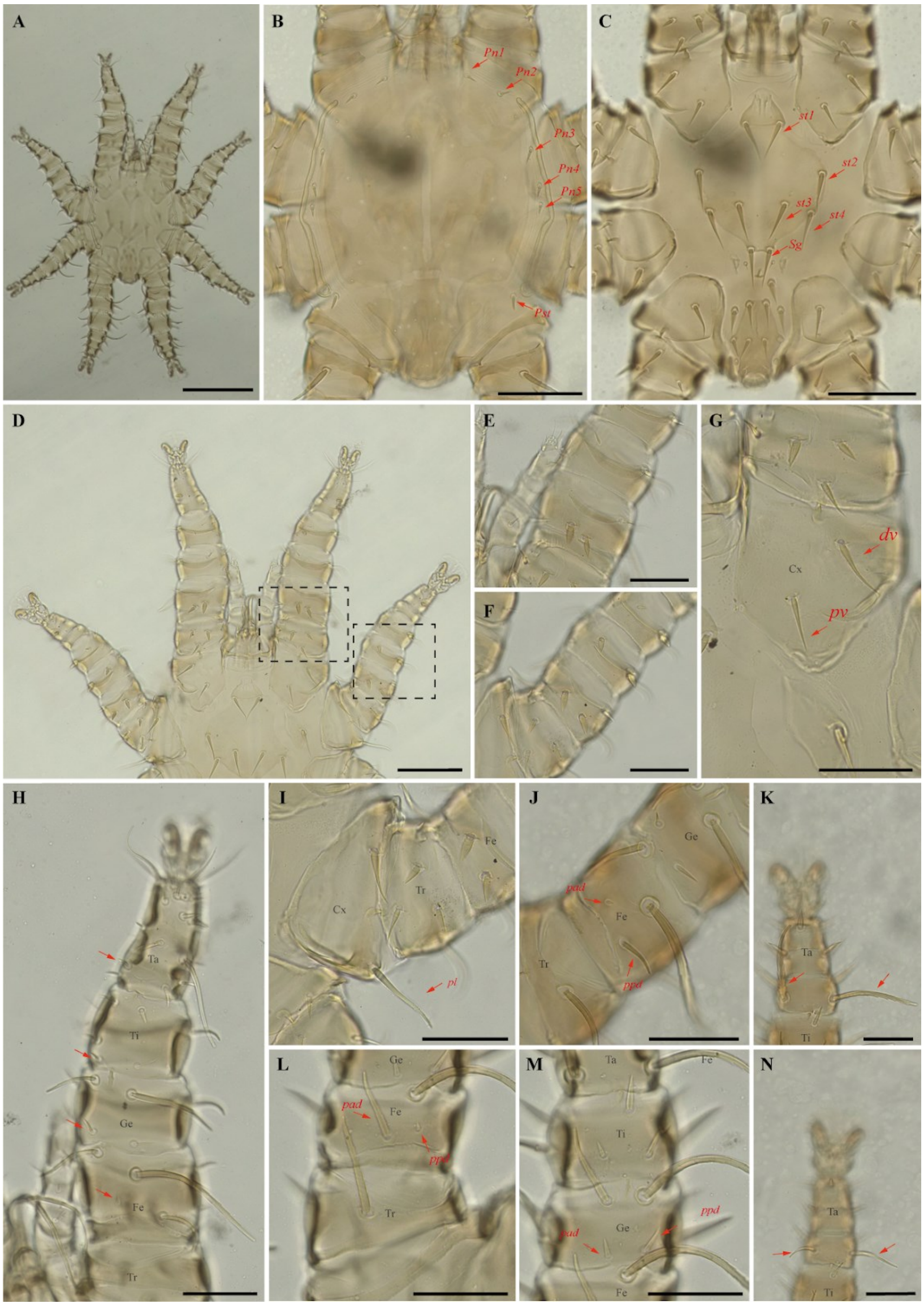


Figure 18. *Periglischrus torrealbai*, male. A — General view; B — Dorsal view with proteronotal setae (*Pn1*-*Pn5*) and poststigmal seta (*Pst*) indicated; C — Ventral view with sternogenital setae (*St1*-*St4*) and genital seta (*Sg*) indicated; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated in red arrow; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D=100 µm, E–N= 50 µm.

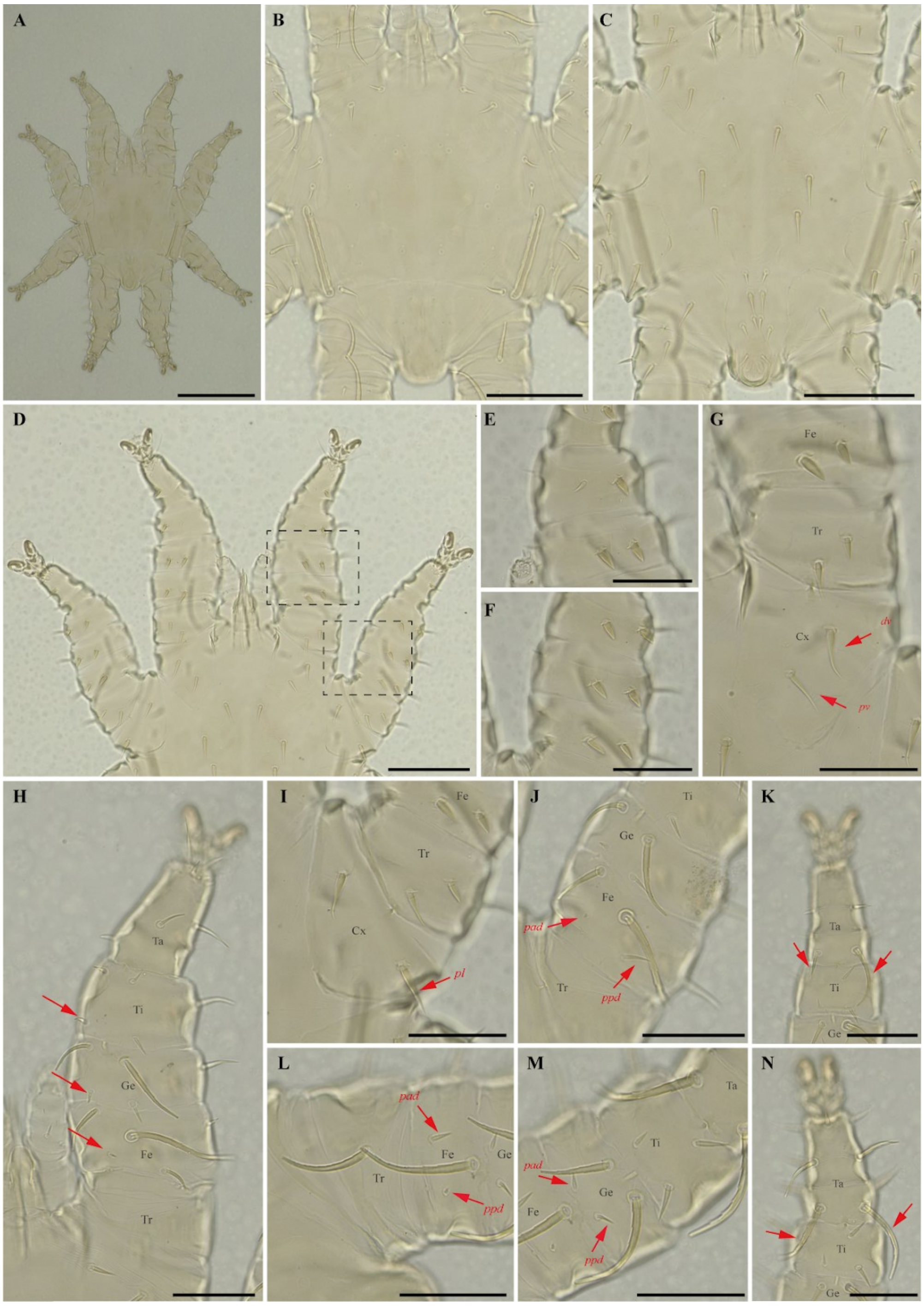
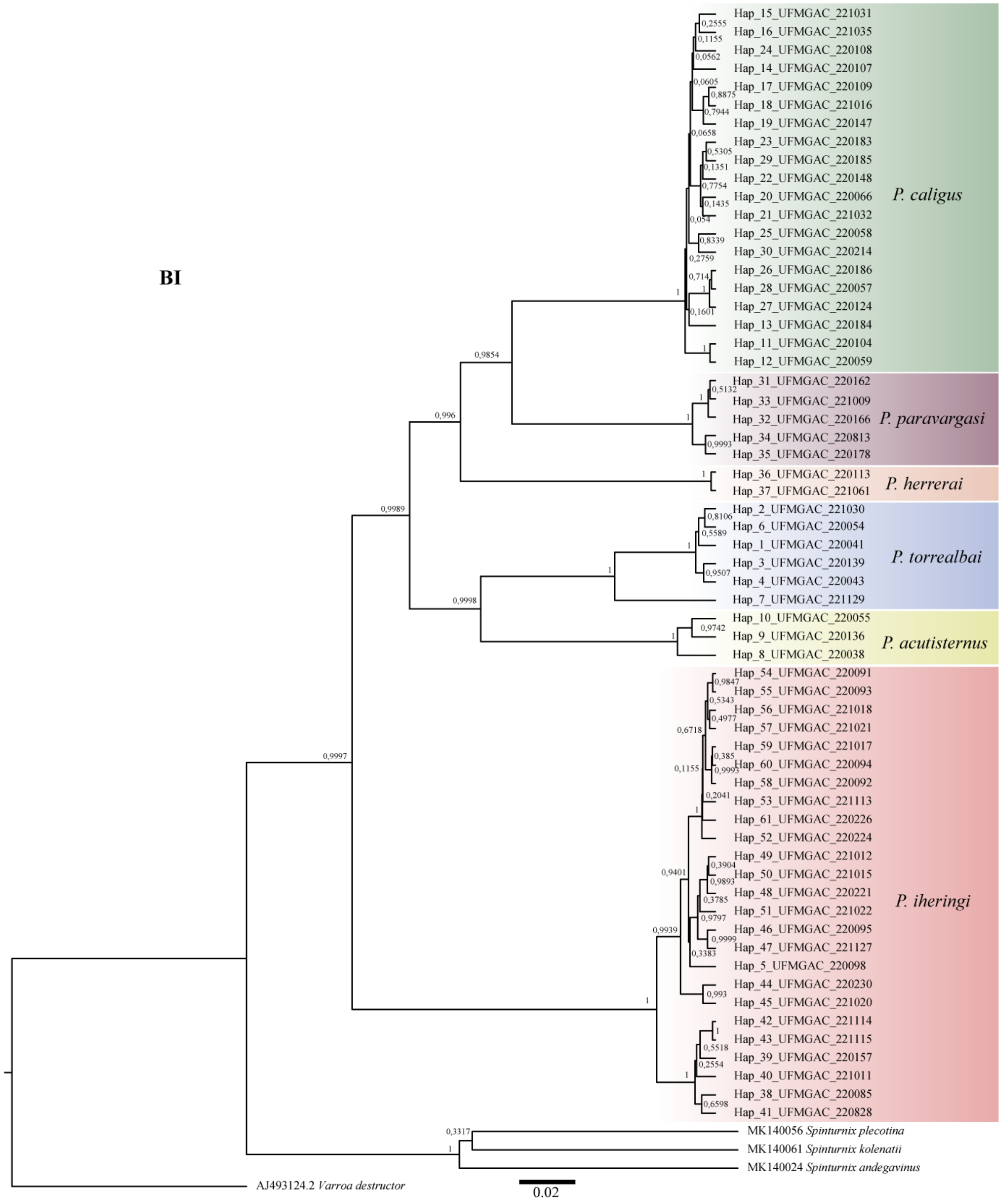
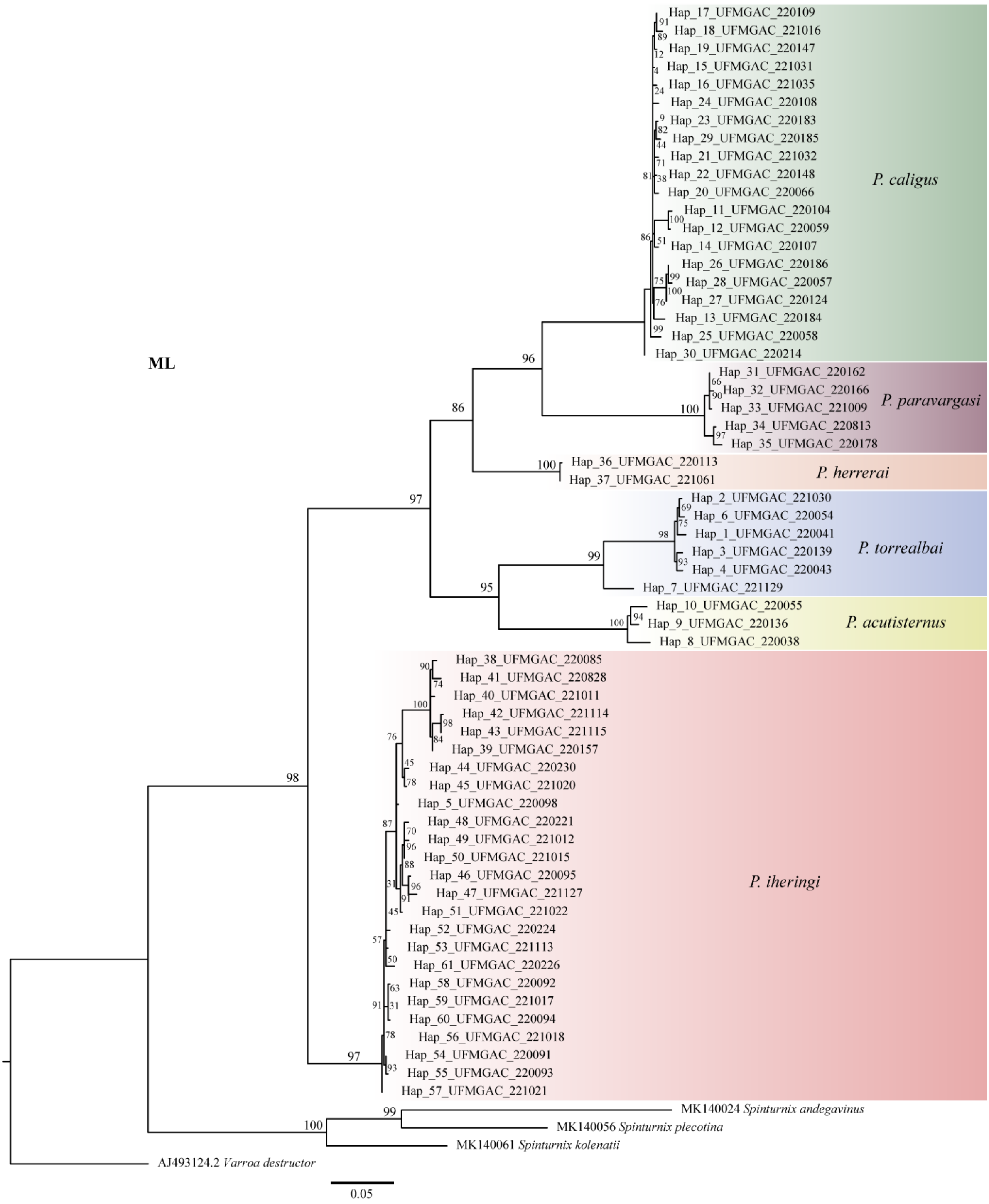


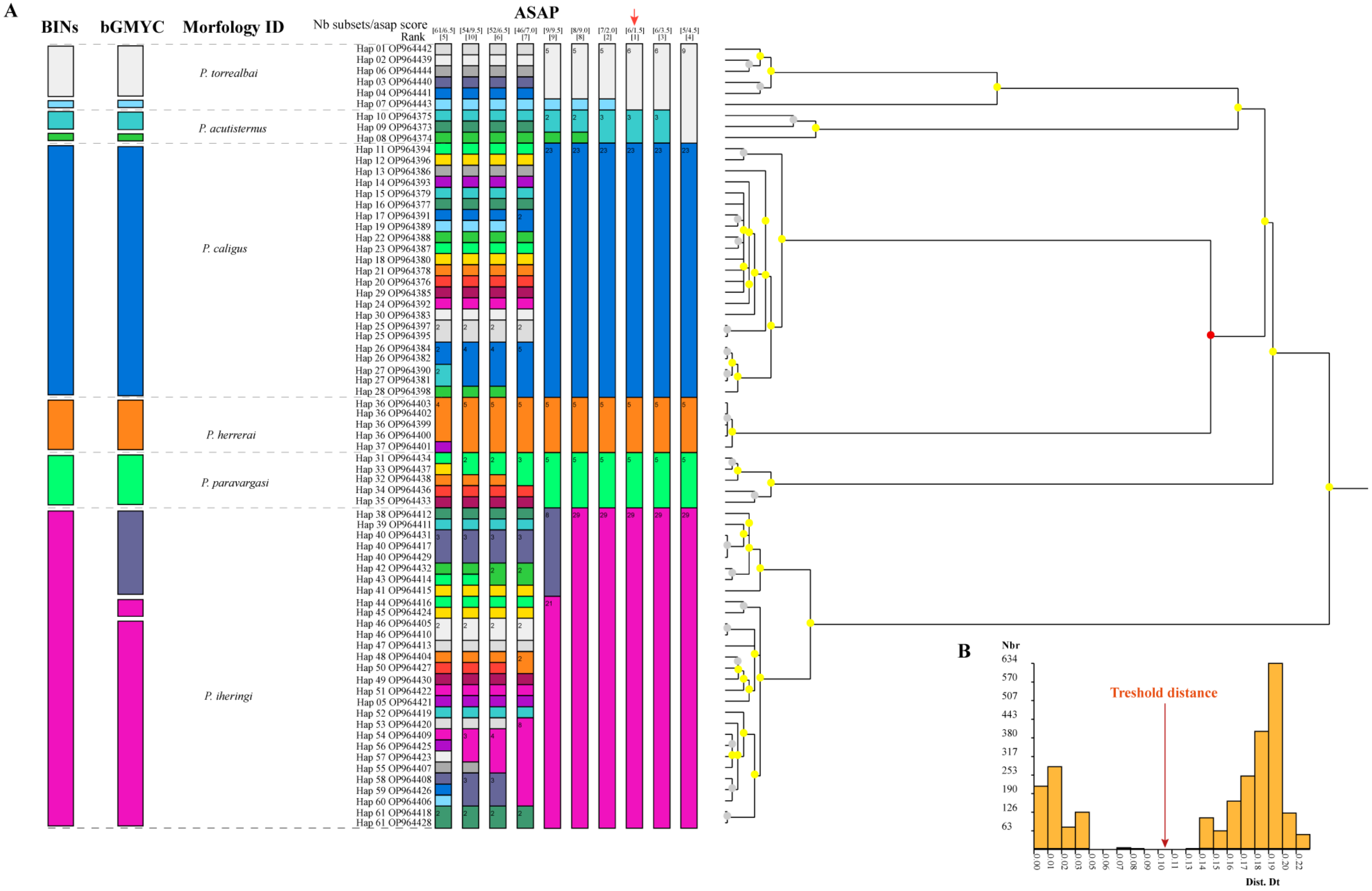
Figure 19. *Periglischrus torrealbai*, protonymph. A — General view; B — Dorsal view; C — Ventral view; D — Ventral setae on legs I and II with details; E — Details ventral setae on leg I; F — Details ventral setae on leg II; G — Coxa I with setae, indicated; H — Femur-tarsus I with proximal *ad* setae, indicated; I — coxa II; J — Femur II; proximal *ad* and *pd* setae, indicated; K — proximal *ad* and *pd* on tarsus III, indicated; L — Femur IV; proximal *ad* and *pd* setae, indicated; M — Genu IV; proximal *ad* and *pd* setae, indicated; N — proximal *ad* and *pd* on tarsus IV, indicated. Scale bars: A= 200 µm, B–D= 100 µm, E–N= 50 µm.



Supplementary Figure 1. Bayesian maximum clade credibility (MCC) tree inferred for the mitochondrial COI sequences of *Periglischrus* sp. obtained from BEAST.



Supplementary Figure 2. Maximum Likelihood (ML) tree inferred for the mitochondrial COI sequences of *Periglischru*s sp. obtained from IQ-TREE.



Supplementary Figure 3. **A —** COI gene species delimitation of *Periglischrus* by BINs, bGMYC and ASAP. The colors represent partitions**.** Every field contains the number of individuals. Above the colorful bars, the coefficient asap-score (the lower value) and number of species (the upper value) recognized for whole dataset are presented. The cladogram supports the species delimitation presented on the bar charts. **B —** Distribution of pairwise differences by ASAP.

CONSIDERAÇÕES FINAIS – Hipóteses a respeito da filogeografia dos ácaros cavernícolas associados a morcegos

Resumo

Os dados obtidos no marco desta tese, além de avançar com o conhecimento taxonômico a respeito dos grupos de ácaros abordados, também permitem formular hipóteses preliminares sobre a estrutura populacional de duas espécies de ácaros comuns em cavernas. Neste capítulo, esses dados são empregados para investigar a estrutura populacional de *Whartonia pachywhartoni* (Leeuwenhoekiidae) e *Periglichrus iheringi* (Spinturnicidae), que possuem ciclos de vida distintos. Em *W. pachywhartoni*, cujo ciclo de vida inclui larvas parasitas de morcegos e estágios pós-larvais predadores de vida livre, parece ter a sua estruturação genética mais claramente influenciada pela distribuição dos ambientes subterrâneos em que os últimos habitam. Por outro lado, *P. iheringi* apresenta menor diferenciação genética e parece ter a sua genética de populações atrelada de maneira mais estreita aos seus hospedeiros morcegos.

Introdução

Principais Resultados

Dentre os principais resultados desta tese estão a descrição de um novo gênero e nova espécie de um ácaro da família Macronyssidae, *Chiasmanyssus cavernicola*, reportando todos os seus estágios ativos, sequências de um fragmento do gene 16S, informações de zonação em cavernas e associação com hospedeiros; descrição dos estágios pós-larvais de *Whartonia* através da associação molecular das larvas, ninfas e adultos; desenvolvimento ferramentas de identificação, tais como chaves taxonômicas para os gêneros de Macronyssidae do Brasil e um banco de dados de código de barras do DNA mitocondrial do gene COI para seis espécies de *Periglischrus* (Spinturnicidae) que foram disponibilizadas publicamente no BOLD com fotodocumentação do material testemunho, incluindo espécies com alta e baixa especificidade parasitária em morcegos cavernícolas de Minas Gerais.

Contribuições da Pesquisa e Hipóteses abordadas

A abordagem integrativa adotada, além de permitir a resolução de questões taxonômicas dos grupos de ácaros aqui estudados, levou a obtenção de inédito conjunto de dados moleculares para os grupos abordados. Com base nos dados moleculares obtidos nesta tese, é possível apontar *Whartonia pachywhartoni* e *Periglichrus iheringi* como espécies candidatas para futuros estudos que visem entender a estruturação genética dos ectoparasitas de morcegos, sejam eles permanentes e não-permanentes. Passamos a seguir a abordar duas hipóteses com base nos dados moleculares obtidos na tese: a) Será que parasitas com estágios no assoalho de cavernas apresentam distribuições distintas dos restritos (ou quase) ao hospedeiro? b) A estruturação genética desses ácaros ectoparasitas é devido a sua associação com as diferentes espécies de morcegos, biomas ou cavernas (geografia)?

Material e métodos

Referência aos dados obtidos nos capítulos anteriores

As análises de delimitação de espécies utilizando o marcador mitocondrial COI (utilizando a implementação bayesiana do modelo geral misto Yule-coalescente, bGMYC) apresentados nos Capítulos III (Figura 2, anexo II) e IV (Figura 2) sugeriram a presença de pelo menos duas espécies crípticas em *W. pachywhartoni* e três em *P. iheringi*, respectivamente, com todos os clados altamente suportados tendo uma probabilidade posterior de 0.95 a 1. Com base nisso, conduzimos uma investigação preliminar da estrutura genética de duas espécies de ácaros comuns como ectoparasitas de morcegos: *Whartonia pachywhartoni*, parasita apenas durante o estágio larval e predador de vida livre nos estágios pós-larvais, e *Periglischrus iheringi*, um parasita permanente. Para isso, utilizamos sequências do gene COI, que foram geradas nos capítulos III e IV desta tese. Os alinhamentos foram feitos com as sequências traduzidas para aminoácidos, no programa Mega 7, não apresentaram inserções/deleções nem códons de parada, evidências de pseudogenes ou erros de sequenciamento. As tabelas 1 (Capítulos III (anexo II) e IV a sumarizam as sequências utilizadas para as duas espécies.

Alinhamentos, redes de haplótipos, análise especial de variância molecular (SAMOVA) e Análise de Variância Molecular (AMOVA)

Utilizamos o software DnaSP v6.0 para estimar o número de haplótipos (Rozas *et al.* 2017). Redes de haplótipos foram inferidas através do método *Median Joining Network* (Bandelt *et al.* 1999) implementadas no PopART (Leigh & Bryant 2015).

A presença de estruturação e o eventual agrupamento das populações foi inferido comparando diferentes particionamentos através do critério de informação bayesiano (BIC) (Meirmans 2012, Pepato *et al.* 2019). Foram realizadas análise espacial de variância molecular (SAMOVA) (Dupanloup, Schneider & Excoffier 2002); AMOVA hierárquica (Excoffier, Smouse & Quattro 1992), considerando os grupos populacionais encontrados na SAMOVA; AMOVA Total (não-hierárquica); e FST par-a-par (Wright 1951);

Na SAMOVA 2, foram realizadas 1.000 simulações com valores de K (números de grupos) variando de 2 a 3 para avaliar a estrutura genética e a diferenciação entre os grupos de populações. Para cada valor de K, além do BIC, o índice FCT foi examinado para encontrar o mais alto e estatisticamente significativo. Adicionalmente, avaliamos a estrutura populacional dos grupos de populações obtidos pela SAMOVA e, em cada valor de K (2 a 3), avaliamos a significância dos resultados da Análise de Variância Molecular (AMOVA) que estima a variação genética entre os grupos de populações (FCT), entre as populações dentro de grupos (FST) e dentro das populações (FSC).

O modelo com as populações divididas em grupos foi comparado com uma AMOVA total (não-hierárquica), equivalente a ausência de estruturação, avaliada através do valor de BIC. Essas análises foram realizadas usando *pairwise differences* com 10.000 permutações e significância estatística de $p<0.05$, utilizando o programa Arlequin v3.5.2 (Excoffier & Lischer 2015).

Resultados preliminares

a) Whartonia pachywhartoni:

O alinhamento do gene COI contou com sequências de 43 indivíduos, totalizando 426 pares de bases. Entre as 23 populações amostradas de *W. pachywhartoni*, foram identificados doze haplótipos (H1-12) (Tabela 1). A rede de haplótipos permitiu detectar três haplogrupos, I e II separados por dez passos mutacionais, II e III separados por seis passos mutacionais (Figura 1A). Os haplogrupos indicam uma ligeira estruturação geográfica: O haplogrupo III, composto pelos haplótipos H7 e H8, é restrito à região do cipó no espinhaço, enquanto, o haplogrupo I, composto pelos haplótipos H1-H5 e H12, e o haplogrupo II, formado pelos haplótipos H6, H9, H10 e H11, apresentam uma ampla distribuição, tanto fora quanto ao longo do espinhaço (Figura 1B). Esses três haplogrupos também foram recuperados como clados altamente suportados em nossas análises bayesianas anteriores.

A análise SAMOVA foi restrita a testar a subdivisão das amostras em três grupos, já que muitas das populações foram pouco amostradas, o que dificulta a aplicação do método. Os valores obtidos (K=2, FCT 0.56930 (56.93%), BIC=95.61467; K=3, FCT 0.66276 (66.27%), BIC=81.91056), comparada com a AMOVA não-hierárquica (K=1) (FST=0.39459 (39%), BIC=112.72302), indicam diferenciação genética significativa ($p<0.001$) entre as populações. Já, as comparações par a par de diferenciação genética (FST par a par) entre as populações resultaram em poucas comparações significativas ($p<0.05$): Gruta coruja *versus* SPT316, Gruta Paca *versus* SPT316 e Gruta Paca *versus* SPT585. O que sugere que apenas essas populações são geneticamente distintas entre si. Esses resultados estão relacionados à escassa amostragem obtida até agora.

A melhor configuração de grupos de acordo com K=3, apresenta a seguinte composição: Grupo 1: Cipó, compreendendo amostras dos municipios de Itambé do mato dentro (população C3), Santa Maria de Itabira (população C4) e Conceição do Mato dentro (populações C5, C6, C7, C11, C12); Grupo 2: Norte, Cipó, Quadrilatero a Pains, compreendendo amostras de Conceição do Mato dentro (C1, C2), Ouro Preto (QF22), Nova Lima (QF21), Doresópolis (AP13), Formiga (AP14), Pains (AP17) e Luislândia (N23); Grupo 3: Cipó, Quadrilatero à Pains, compreendendo amostras de Conceição do Mato dentro (C8, C9, C10), São Gonçalo do Rio Abaixo (QF20), Belo Horizonte (QF19), Pains (AP15) e São Roque de Minas (AP18).

b) *Periglichrus iheringi*

O alinhamento contou com sequências de 29 indivíduos, com comprimento de 720 pares de bases. Foi observada alta diversidade haplotípica, com 25 haplótipos (H1-25), identificados nas sete populações analisadas. A rede de haplótipos indica pouca estruturação genética entre as populações, mesmo em diferentes hospedeiros e nenhuma em relação à geografia.

Todas as configurações de K (de 2 a 3) testadas na análise SAMOVA para a diferenciação genética entre os grupos de populações foram estatisticamente significativas (K=2, FCT=0.37930 (37.93%), BIC=30.43977; K=3, FCT=0.32301 (32.30%), BIC=29.49469; $p<0.05$). No entanto, o FSC foi alto (>50%) em todos os casos (K=2, FST=55.31%; K=3, FST=64.20%), indicando que a maior parte da diversidade genética (variação) ocorre dentro das populações, e apenas uma pequena proporção da variação ocorre entre grupos e entre populações dentro de grupos.

A AMOVA total (K=1) teve o valor de FST igual a 0.23037 (23.04%), indicando pouca ou nenhuma diferenciação genética entre as populações ($p<0.01$). Já, as comparações par a par de diferenciação genética (FST par a par) entre as populações encontraram poucas diferenças significativas estatisticamente ($p<0.05$) de FST: Gruta Bocaina *versus* Gruta Mosquito, Gruta Bocaina *versus* Gruta Chacara e Gruta Bocaina *versus* Gruta Mastodonte. Ou seja, apenas essas populações são geneticamente distintas entre si.

O Critério de Informação Bayesiano (BIC) reflete essa situação, com variação pequena entre as diferentes partições (BIC K1=32.15665; BIC K2=30.43977; BIC K3=29.49469) de forma que o modelo de partição escolhido é o sem estruturação, com menos parâmetros. Assim, o melhor valor de K é 1.

Discussão das Hipóteses

Hipótese 1: Será que parasitas com estágios no assoalho de cavernas apresentam distribuições distintas dos restritos (ou quase) ao hospedeiro?

Com base nos resultados obtidos para as duas espécies de ácaros, é possível sugerir que parasitas com estágios no assoalho de cavernas, como *Whartonia pachywhartoni*, podem apresentar maior diferenciação genética e geográfica. A existência de uma subdivisão específica no cipó indica que esses ácaros podem estar mais suscetíveis à formação de grupos de populações geneticamente distintas entre regiões, o que pode ser atribuído à sua associação com habitats específicos que pode restringir o fluxo gênico entre as populações. Por outro lado, parasitas como *Periglichrus iheringi*, que possuem um ciclo de vida permanente no hospedeiro, podem apresentar menor diferenciação genética e geográfica sugerindo maior fluxo gênico entre suas populações devido a alta capacidade de dispersão e mobilidade dos hospedeiros voadores a que estão associados.

Hipótese 2: A estruturação genética desses ácaros ectoparasitas é devido a sua associação com as diferentes espécies de morcegos, biomas ou cavernas (geografia)?

As duas espécies apresentaram padrões distintos de estruturação genética. Para *Whartonia pachywhartoni*, os resultados indicam que a estruturação genética parece ser influenciada pelas características geográficas do ambiente em que é encontrado, com a subdivisão específica no cipó indicando algum nível de restrição ao fluxo genético a partir dessa região para as outras amostradas. Por outro lado, para *Periglichrus iheringi*, a baixa diferenciação genética e ausência de estruturação geográfica sugere que outros fatores, possivelmente relacionados à associação com diferentes espécies de morcegos, exercem maior influência na estruturação genética desses ácaros.

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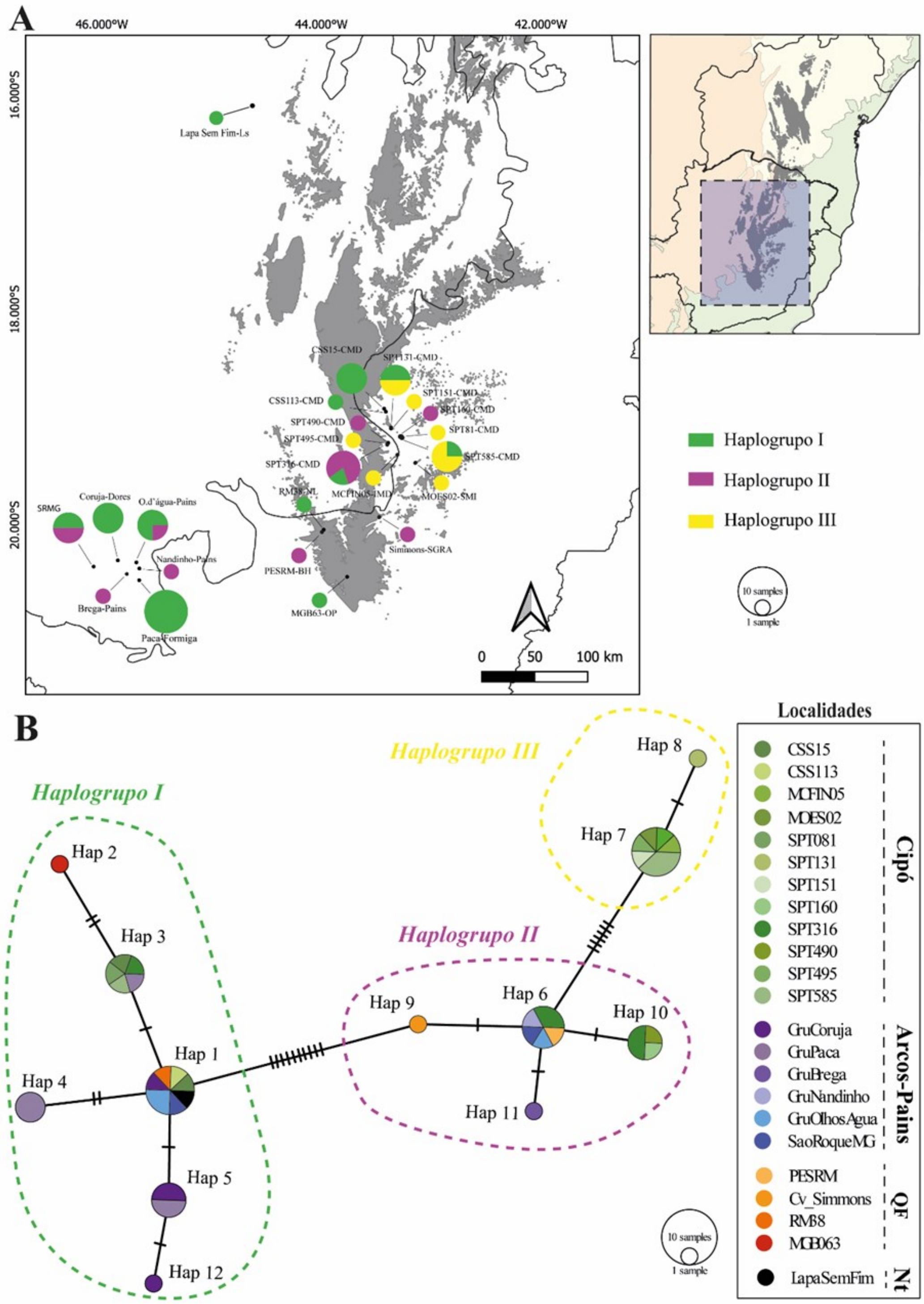

Figura 1. *Whartonia pachywhartoni*. A: mapa de distribuição dos haplótipos; B: Rede de haplótipos construída através do método median-joining usando o programa PopArt (Os traços verticais pretos indicam a distância entre os haplótipos em relação ao número de mutações pontuais).

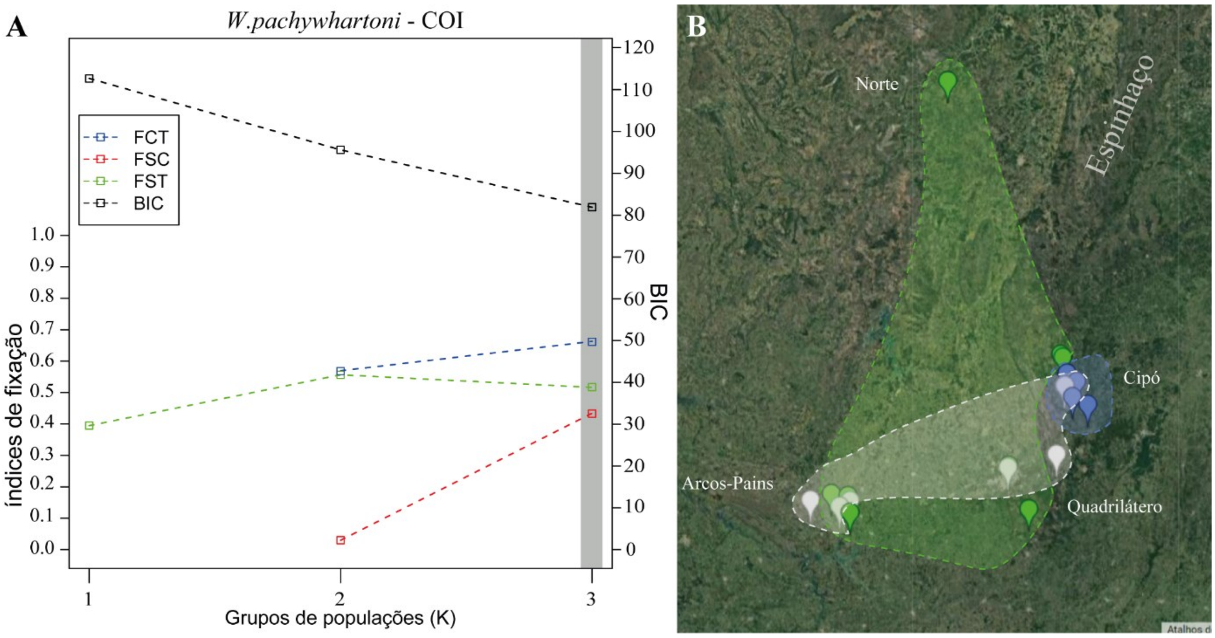

Figura 2. Análise AMOVA (K=1) e SAMOVA (K=2-3) realizadas com 23 populações de *Whartonia pachywhartoni*, usando o gene COI. A: Gráfico mostrando os índices de fixação e BIC para cada valor de K. A Barra cinza representa o melhor K de acordo com o critério BIC (menor valor de BIC). B: Distribuição dos grupos de populações de acordo com K=3 em relação às áreas geográficas pré-definidas. As cores representam as populações em cada grupo: Azul=Grupo 1; Verde=grupo 2, Branco=Grupo 3.

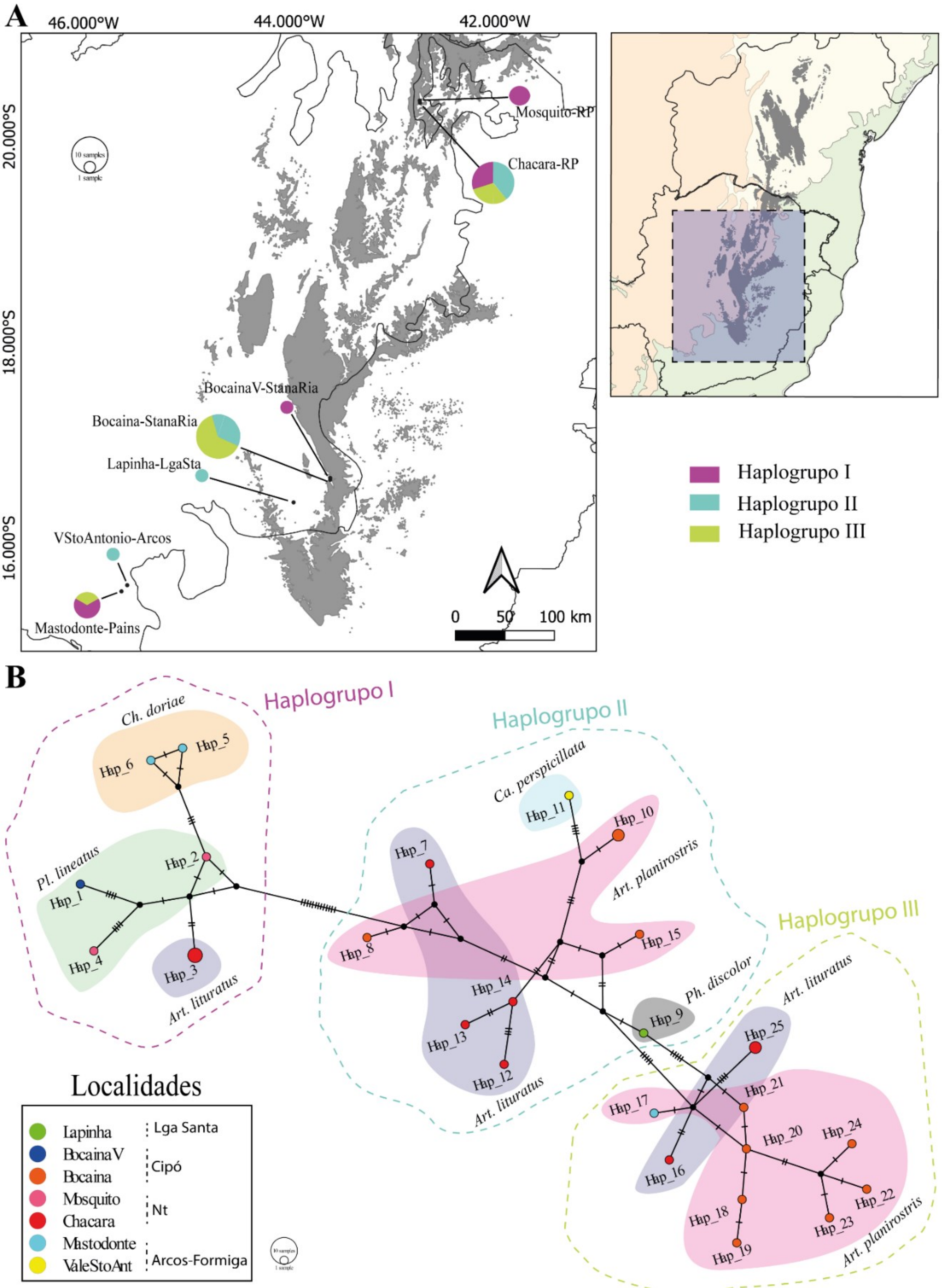

Figura 3: *Periglichrus iheringi*. A: mapa de distribuição dos haplótipos; B-C: Rede de haplótipos construída através do método *median-joining* (Os traços verticais pretos indicam a distância entre os haplótipos em relação ao número de mutações pontuais).

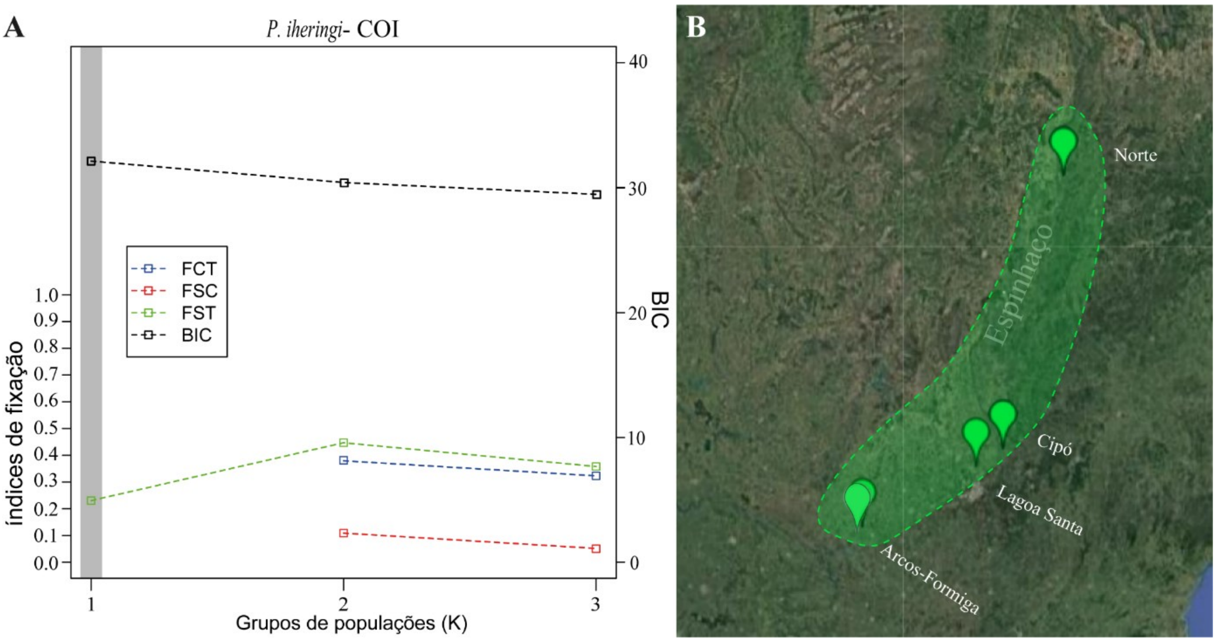

Figura 4. Análise AMOVA (K=1) e SAMOVA (k=2-3) realizadas com sete populações de *P. iheringi*, usando o gene COI. A: Gráfico mostrando os índices de fixação e BIC para cada valor de K. A Barra cinza representa o melhor K de acordo com o critério BIC (quando a diferença do BIC é pequena, escolhemos o modelo com menos parâmetros). B: Distribuição das populações de acordo com K=1 e em relação as áreas pré-definidas.

Tabela 1. Detalhes das 23 populações de *Whartonia pachywhartoni* pré-analisadas. Legenda: Pop= sigla do grupos geográfico + número de população.

Local de coleta	**Pop**	**Grupo GEO**	**Hospedeiro**	**Haplotipos(N)**
CSS15, Conceição do mato dentro (CMD)	C1	Cipó (C)	Solo	Hap1(1),Hap3(1)
CSS13, CMD	C2		Solo	Hap1(1)
MCFIN05, Itambé do mato dentro	C3		Solo	Hap7(1)
MOES02_Santa Maria de Itabira	C4		Solo	Hap7(1)
SPT081, CMD	C5		Solo	Hap7(1)
SPT131, CMD	C6		Solo	Hap3(1),Hap8(1)
SPT151, CMD	C7		Solo	Hap7(1)
SPT160, CMD	C8		Solo	Hap10(1)
SPT316, CMD	C9		Solo	Hap3(1),Hap6(2),Hap10(2)
SPT490, CMD	C10		Solo	Hap10(1)
SPT495, CMD	C11		Solo	Hap7(1)
SPT585, CMD	C12		Solo	Hap3(1),Hap7(3)
Gruta Coruja,	AP13	Arcos-Pains	*De. rotundus*	Hap1(1),Hap5(2),Hap12(1)

Doresópolis		(AP)		
Gruta Paca, Formiga	AP14		*Dh. Ecaudata, Ca.perspicillata, Mi. Bennetti, An. caudifer*	Hap3(1),Hap4(3),Hap5(2)
Gruta do Brega, Pains	AP15		*Ca.perspicillata*	Hap11(1)
Gruta do Nandinho, Pains	AP16		*De. rotundus*	Hap6(1)
Gruta Olhos d'água, Pains	AP17		*St. Lilium, Ca.perspicillata, Pl. lineatus*	Hap1(2),Hap6(1)
São Roque de Minas	AP18		*Ca.perspicillata, An. caudifer*	Hap1(1),Hap6(1)
Parque Estadual Serra do Rola Moça, Belo Horizonte	QF19	Quadrilátero Ferrífero (QF)	*Ca.perspicillata*	Hap6(1)
Caverna Simmons, São Gonçalo do Rio Abaixo	QF20		Solo	Hap9(1)
RM38, Nova Lima	QF21		Solo	Hap1(1)
MGB063, Ouro Preto	QF22		Solo	Hap2(1)
Lapa Sem Fim, Luislândia	N23	Norte (N)	Solo	Hap1(1)

Tabela 2. Detalhes das sete populações de *Periglischrus iheringi* pré-analisadas. Legenda: Pop= sigla do grupos geográfico + número de população.

Local de coleta	**Pop**	**Grupo GEO**	**Hospedeiro**	**Haplotipos(N)**
Gruta do Mosquito, Rio Pardo	N1	Norte	*Pl.lineatus*	Hap2 (1),Hap4 (1)
Gruta da Chácara, Rio Pardo	N2		*Art.lituratus*	Hap3(3),Hap7(1),Hap12(1),Hap13(1),Hap14(1),Hap16(1),Hap25(2)
Gruta Mastodonte, Pains	AF3	Arcos-Formiga	*Ch.doriae*	Hap5(1),Hap6(1)
			Art.planirostris	Hap17(1)
Vale Sto Antonio, Arcos	AF4		*Ca.perspicillata*	Hap11(1)
Bocaina V, Santana do Riacho	C5	Cipó	*Pl.lineatus*	Hap1(1)
Bocaina, Santana do Riacho	C6		*Art.planirostris*	Hap8(1),Hap10(2),Hap15(1),Hap18(1),Hap19(1),Hap20(1),Hap21(1),Hap22(1),Hap23(1),Hap24(1)
Lapinha, Lagoa Santa	L7	Lagoa santa	*Ph. discolor*	Hap9(1)