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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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Original research

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, 1965, *P. iheringi* Oudemans, 1902, *P. paravargasi* Herrin & Tipton, 1975, *P. torrealbai* Machado-Allison, 1965 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 Mormoopidae; *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 (Dusbábek 1968; Herrin and Tipton 1975; Morales-Malacara 2001; Von Heyden 1826.). Except for *Paraspinturnix*, all of these genera are recorded from Brazil

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(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 of the same family) (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 (Hoffmann 1944).

Because some *Periglischrus* species are similar enough to make morphological identification difficult (Morales-Malacara 2001) or show 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, morphometrics and statistical analyses, and molecular tools with traditional taxonomy is a 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.

Material 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.

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: A054674).

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*	forest/karstic	221059	SPIN065-22	AEV1278	OP964399	742
	♂ DN	*D. rotundus*	forest/karstic	221060	SPIN066-22	AEV1278	OP964400	816
	♀ DN	*D. rotundus*	forest/karstic	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	-	-	-	-

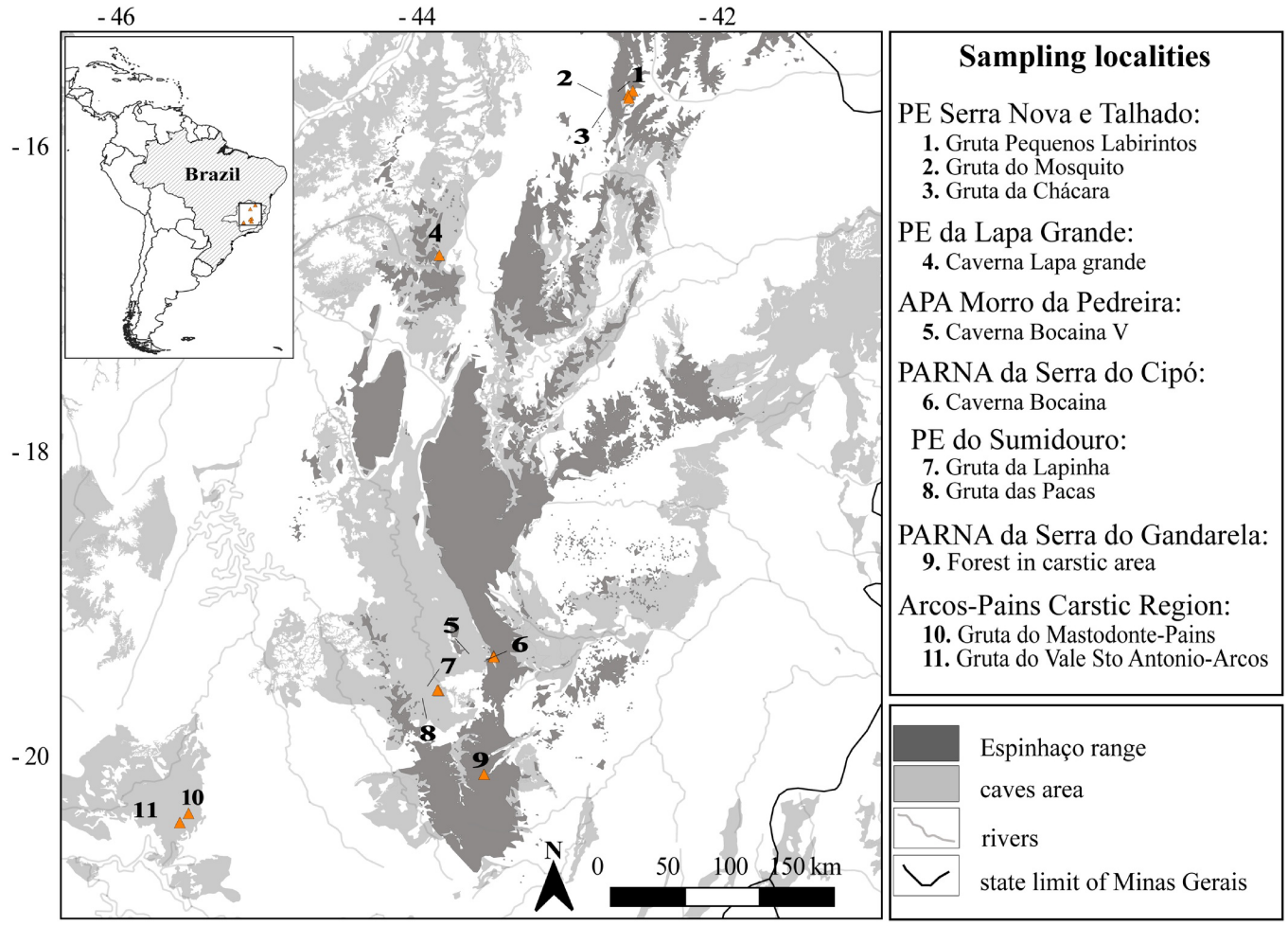

Figure 1 Geographical distribution of sampling localities of *Periglischrus* spp.

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), Machado-Allison and Antequera (1971), Morales-Malacara *et al.* (2018), Rudnick (1960), Almeida *et al.* (2018). The nomenclature for idiosomal chaetotaxy follows Evans (1968) and Domrow (1972), leg chaetotaxy follows Evans (1963). All measurements are in micrometers.

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.

Finally, 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.

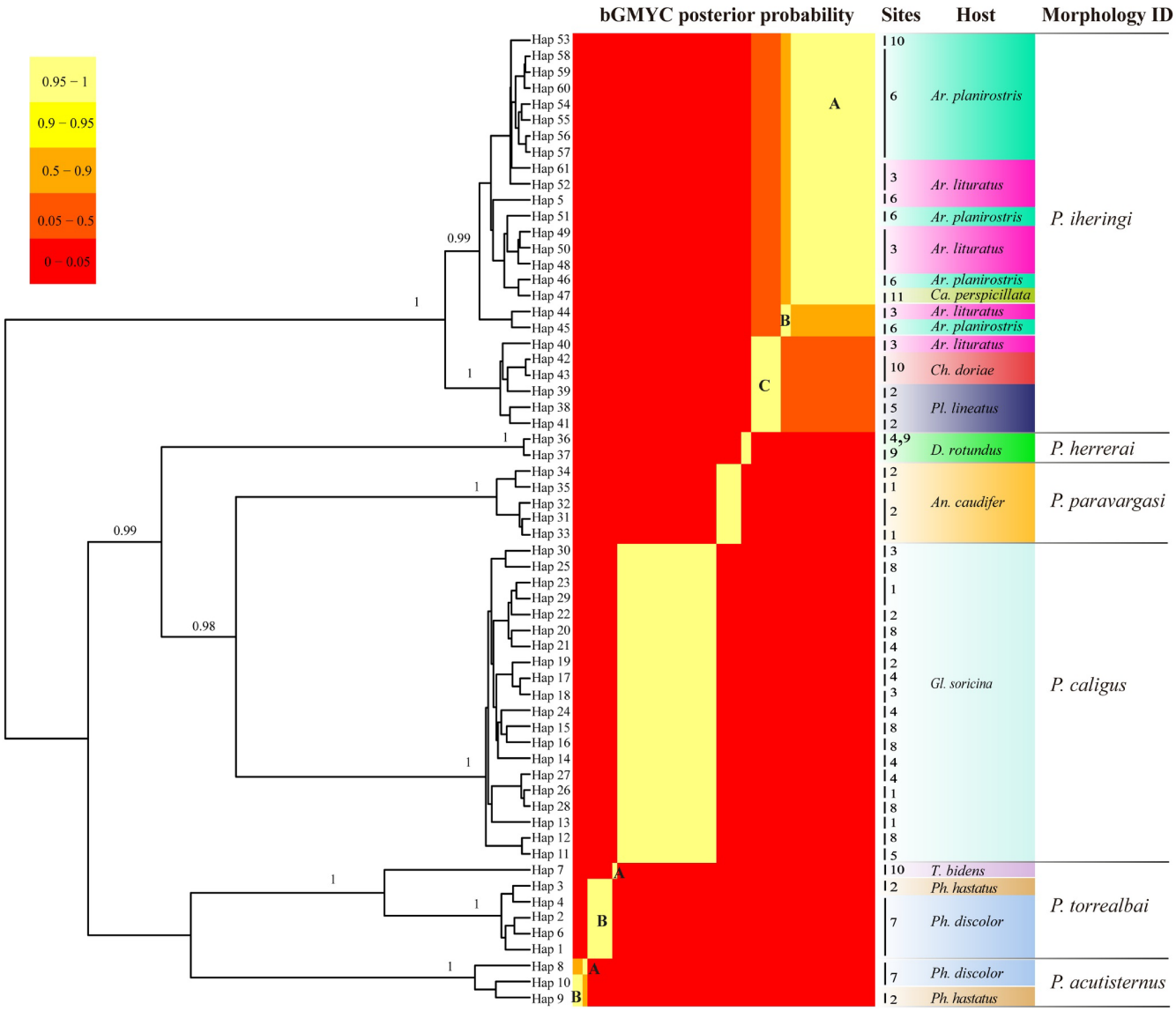

Figure 2 Mitochondrial COI haplotype ultrametric tree inferred in BEAST using strict clock and species delimitation probabilities obtained in bGMYC.

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 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	0.013			
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*	0.164	0.1676	0.1648	0.1678	0.15	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 were 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 (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

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° S, -43.959° E, 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° S, -42.7335° E, 16 Dec. 2021, collected by B. Gomes-Almeida *et al.* (COX1 sequence [voucher code]: OP964373 [UFMG AC 220136]).

Barcode sequences — OP964373 and OP964375 (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):

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		

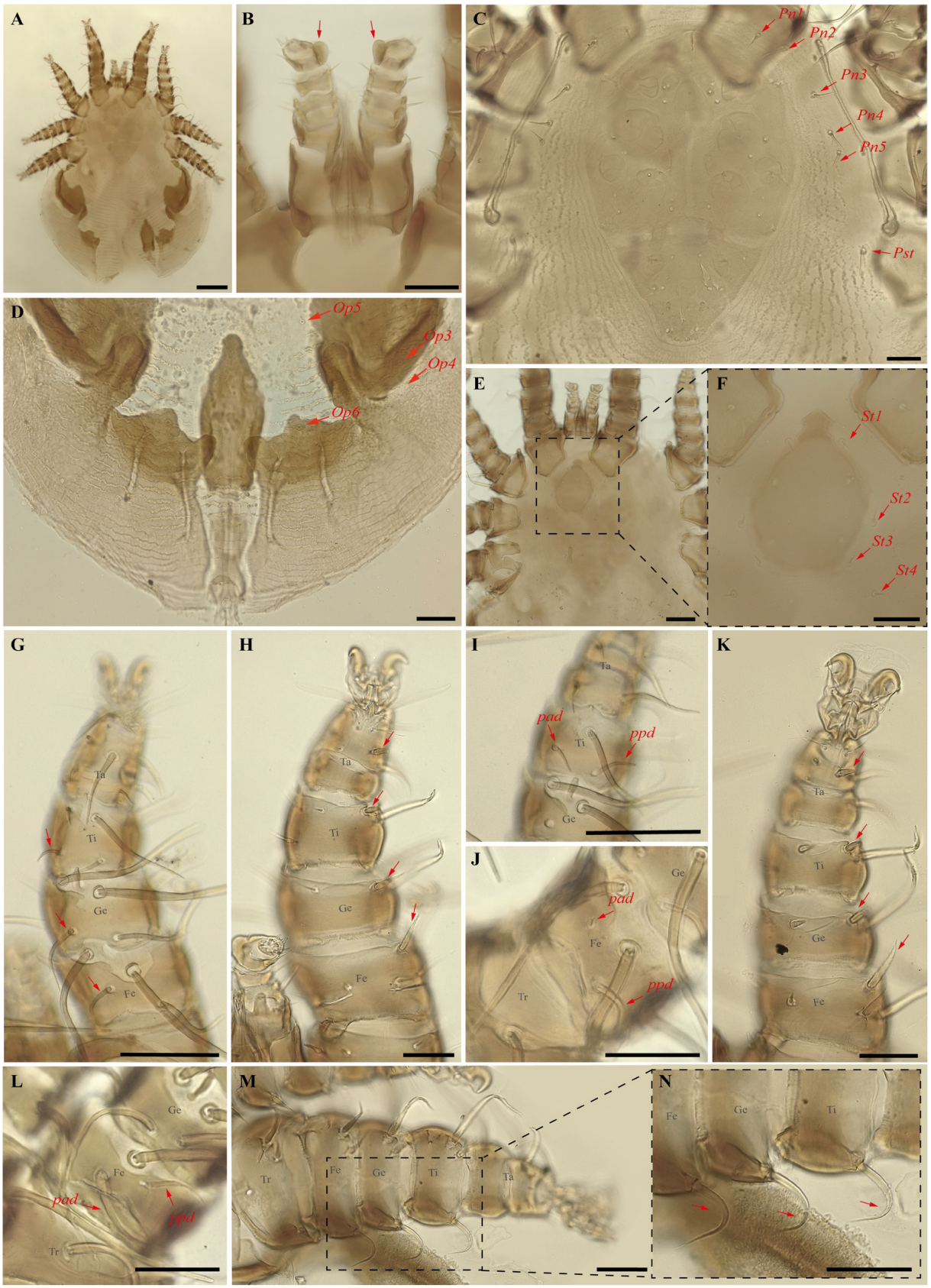

Figure 3 *Periglischrus acustisternus*, female. A – General view; B – Mediodistal lobe of palpal tibia indicated in red arrow; C – Dorsal plate with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; D – Dorsal opisthosoma with hysteronotal setae (*Op3–Op6*) indicated in red arrow; E and F – Sternal plate with sternal setae (*St1–St4*); G – Proximal anterodorsal (*ad*) seta on femur–tibia 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*) and posterodorsal (*pd*) setae on tibia II; J – Proximal *ad* and *pd* setae on femur II; K – Distal *pv* setae on femur II (finely serrated) and genu–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 = 50 µm, E, G, I = 100 µm.

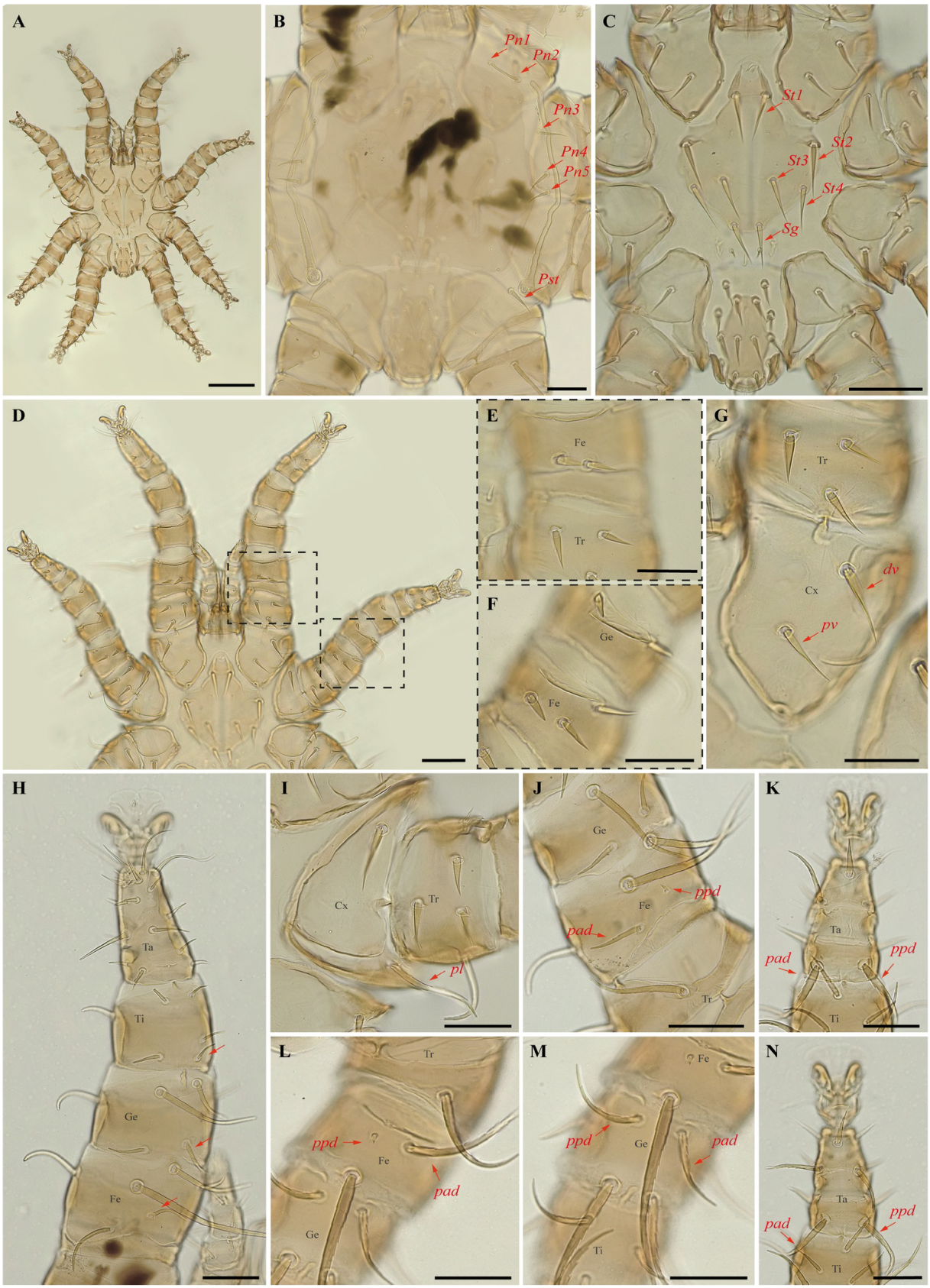

Figure 4 *Periglischrus acustisternus*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with proximal (*pv*) and distal (*dv*) setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

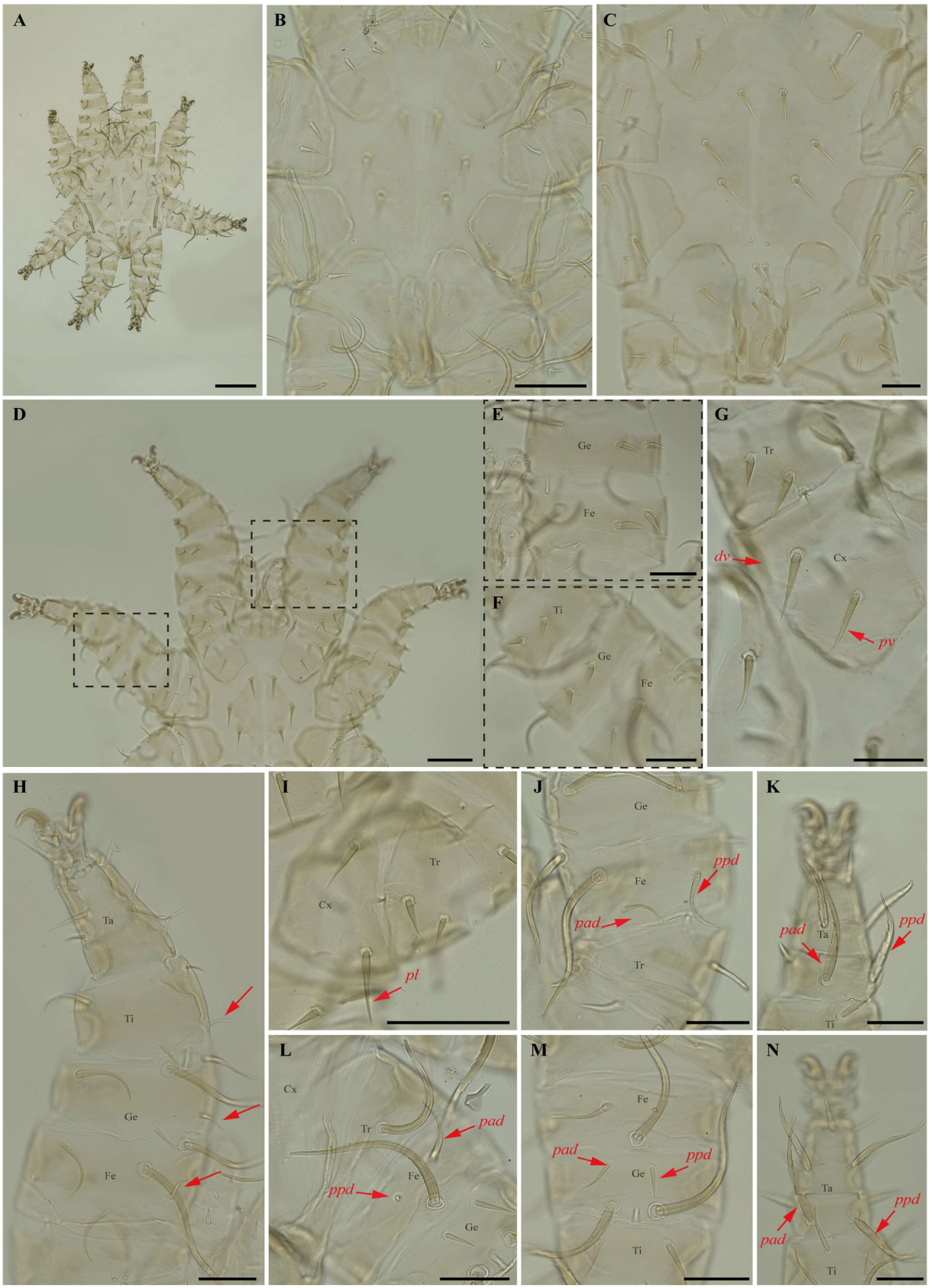

Figure 5 *Periglischrus acustisternus*, protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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*. The record on *Ph. discolor* from Minas Gerais state is new to science. Individuals obtained in this study match the original description and re-descriptions (Machado-Allison 1964; Herrin and Tipton 1975; Furman 1966). This species often co-occurs with smaller *P. torrealbai* Machado-Allison, 1965a, males and nymphs of which may be misidentified as *P. acutisternus* due to the similar size, presence of some distinctly enlarged ventral setae of legs I and II, 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. *P. acutisternus*, however, has some ventral setae on legs I and II 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. 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%).

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° S, -43.9667° E, 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

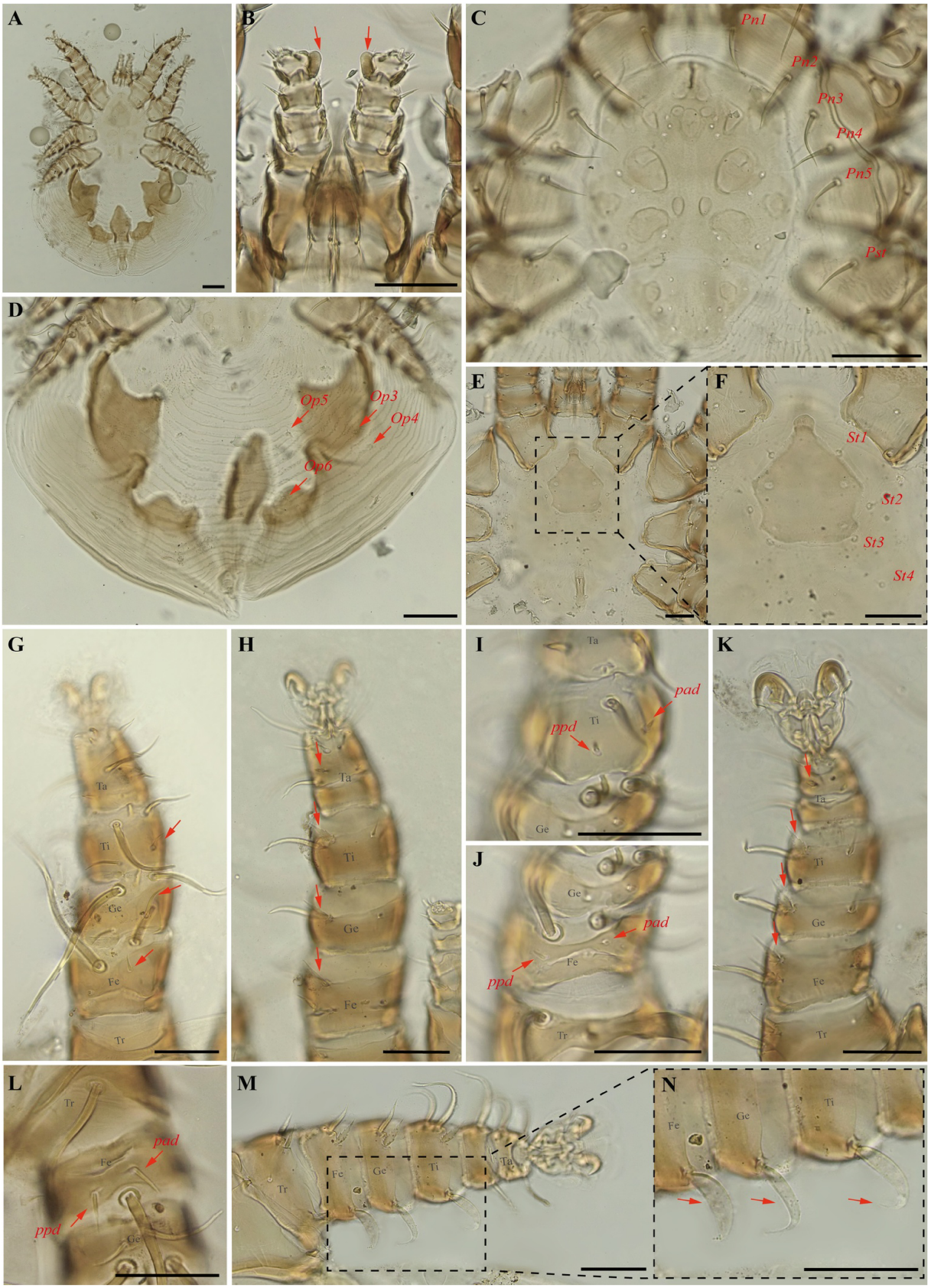

Figure 6 *Periglischrus caligus*, female. A – General view; B – Mediodistal lobe of palpal tibia indicated in red arrow; C – dorsal plate with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; D – Dorsal opisthosoma with hysteronotal setae (*Op3–Op6*) indicated in red arrow; E and F – Sternal plate with sternal setae (*St1–St4*); G – Proximal anterodorsal (*ad*) seta on femur–tibia I; H – Distal posteroventral (*pv*) seta on femur–tibia I (enlarged and serrate) and tarsus I (small and spinelike), indicated in red arrow; I – Proximal antero (*ad*) and posterodorsal (*pd*) setae on tibia II; J – Proximal *ad* and *pd* setae on femur II; K – Distal *pv* setae on femur–tibia II (enlarged and serrate) and tarsus II (small and spinelike), 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.

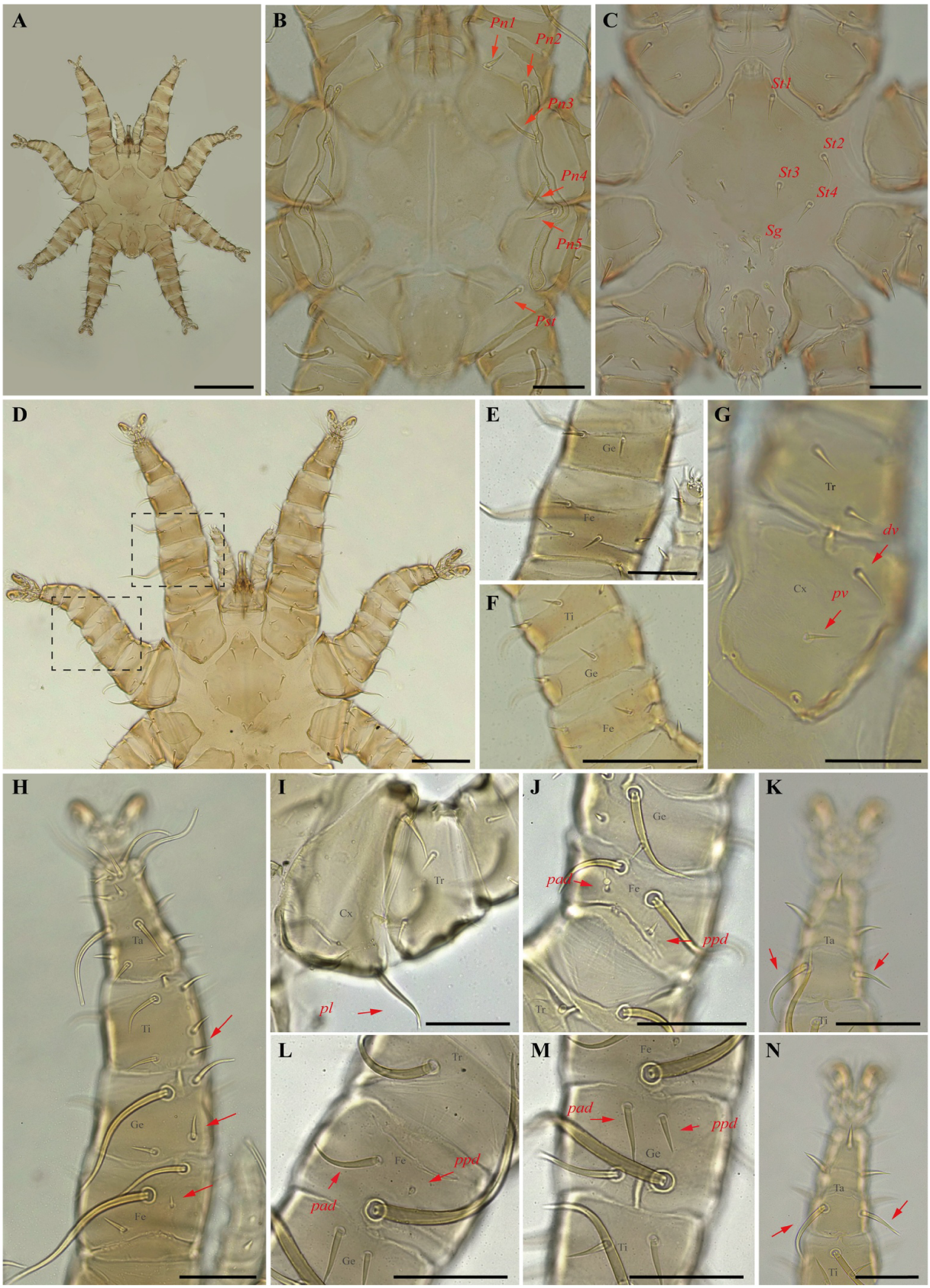

Figure 7 *Periglischrus caligus*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

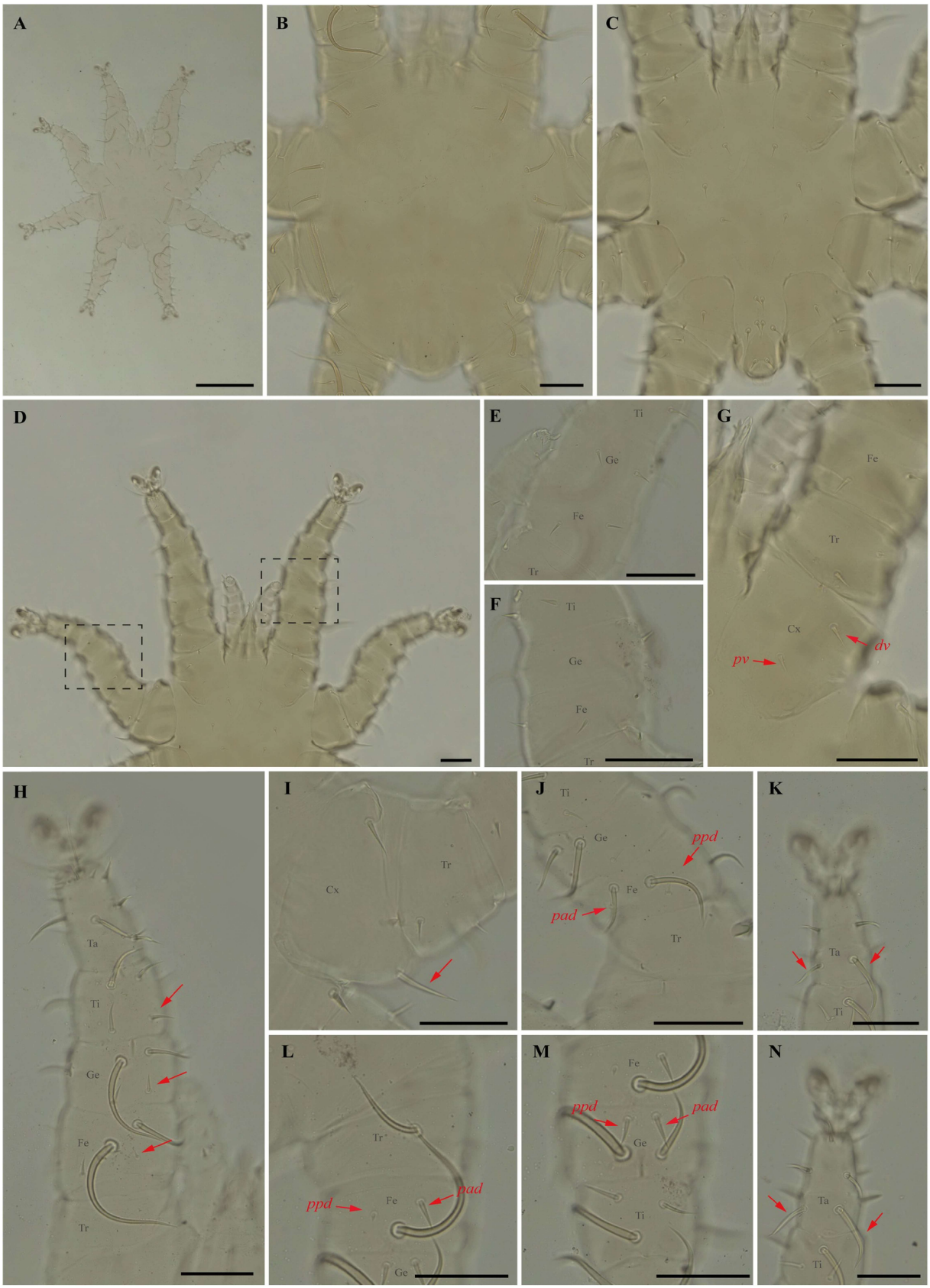

Figure 8 *Periglischrus caligus,* protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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° S, -43.9549° E, 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° S, -42.7295° E, 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° S, -42.7335° E, 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° S, -42.7043° E, 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° S, -43.6032° E, 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 (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%.

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: 300–316.

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° E, 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° S, -43.9549° E, 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

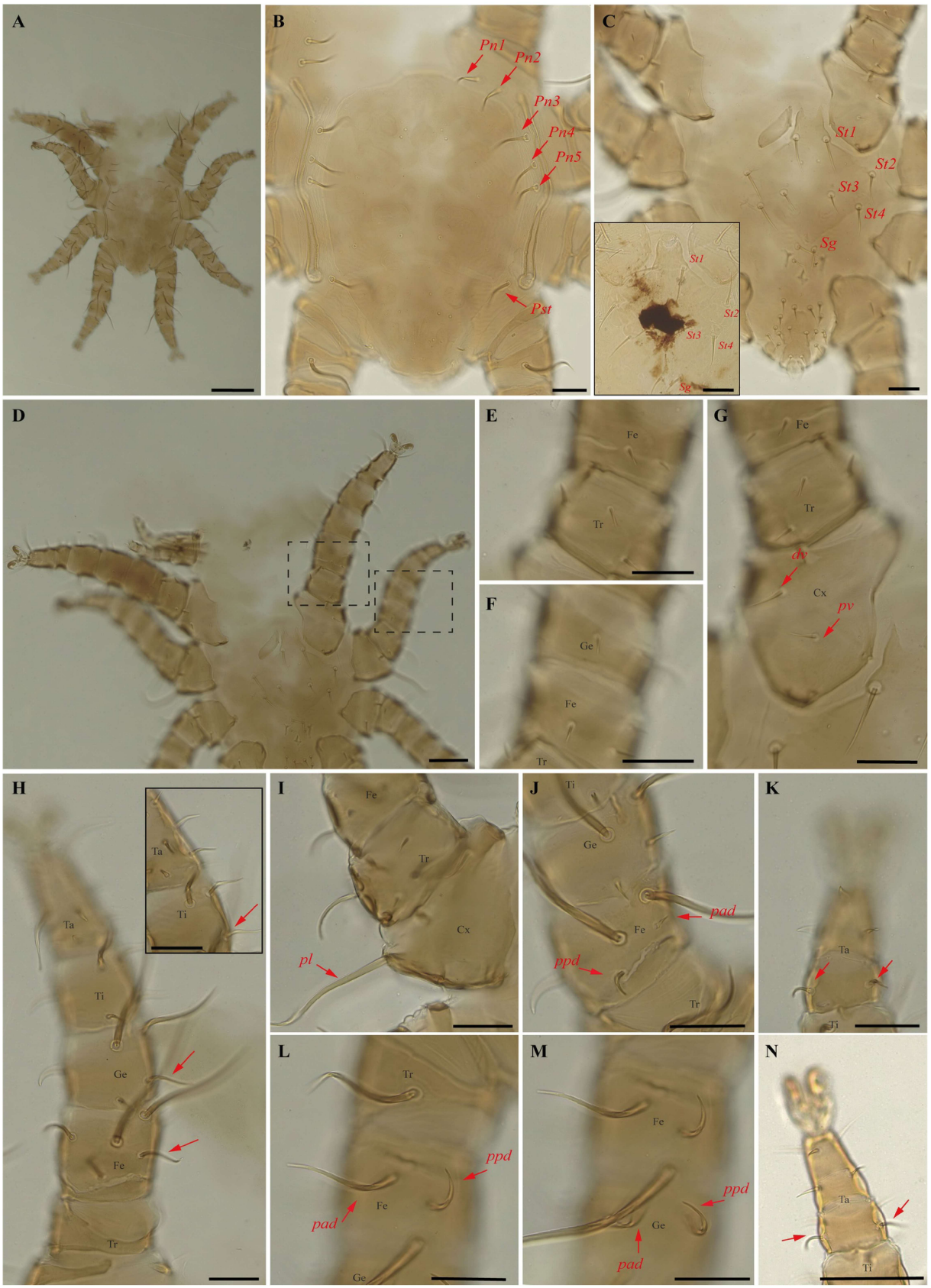

Figure 9 *Periglischrus herrerai*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

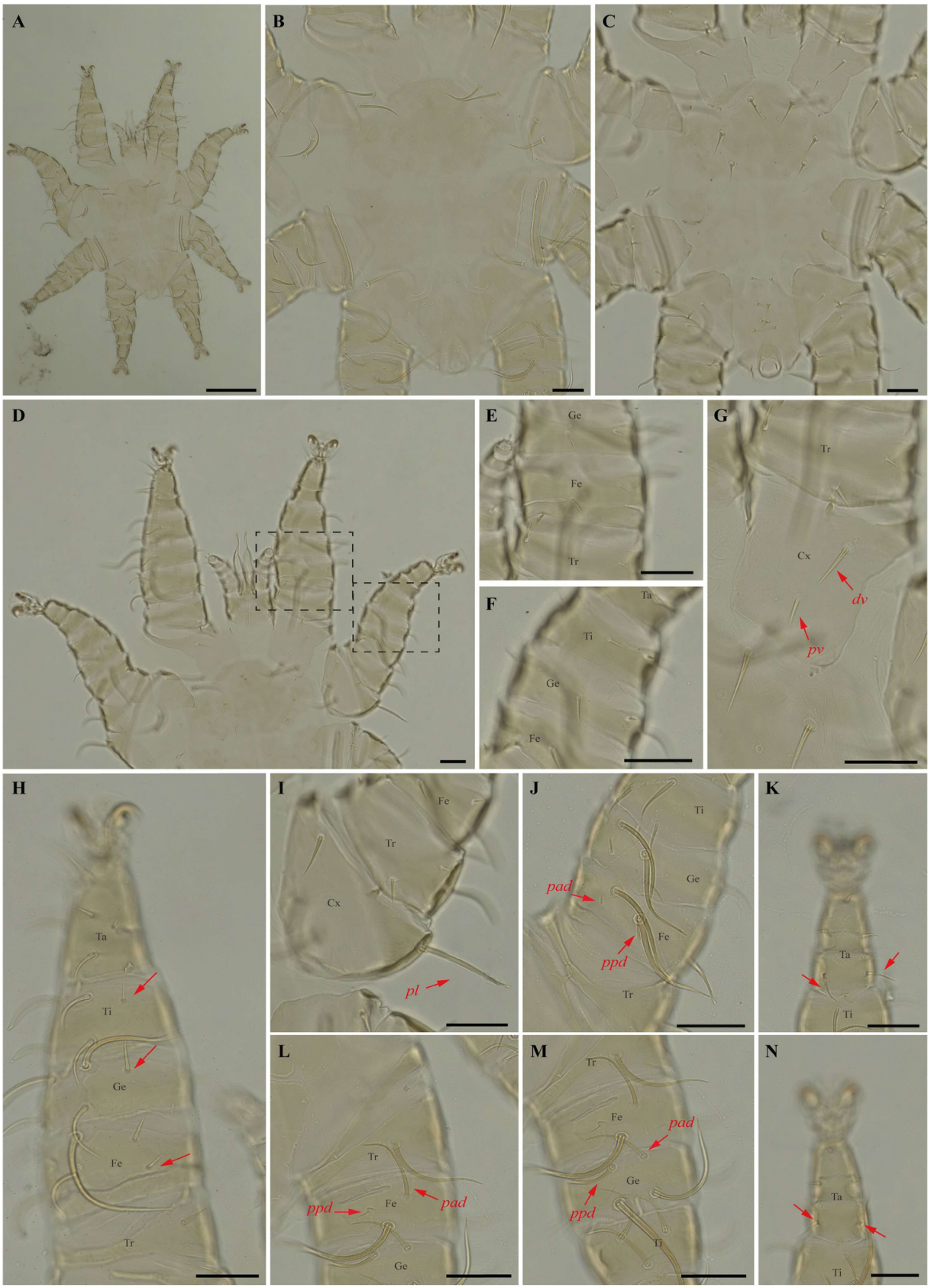

Figure 10 *Periglischrus herrerai*, protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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%.

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° E, 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° S, -43.6032° E, 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° E, 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° S, -42.7295° E, 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° S, -42.7335° E, 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° S, -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° S, -43.6032° E, 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

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 (*Op3–Op6*) indicated in red arrow; E and F – Sternal plate with sternal setae (*St1–St4*); G – Proximal anterodorsal (*ad*) seta on femur–tibia I; H – Distal posteroventral (*pv*) seta on femur–tarsus I (slender), 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–tarsus II (slender), 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.

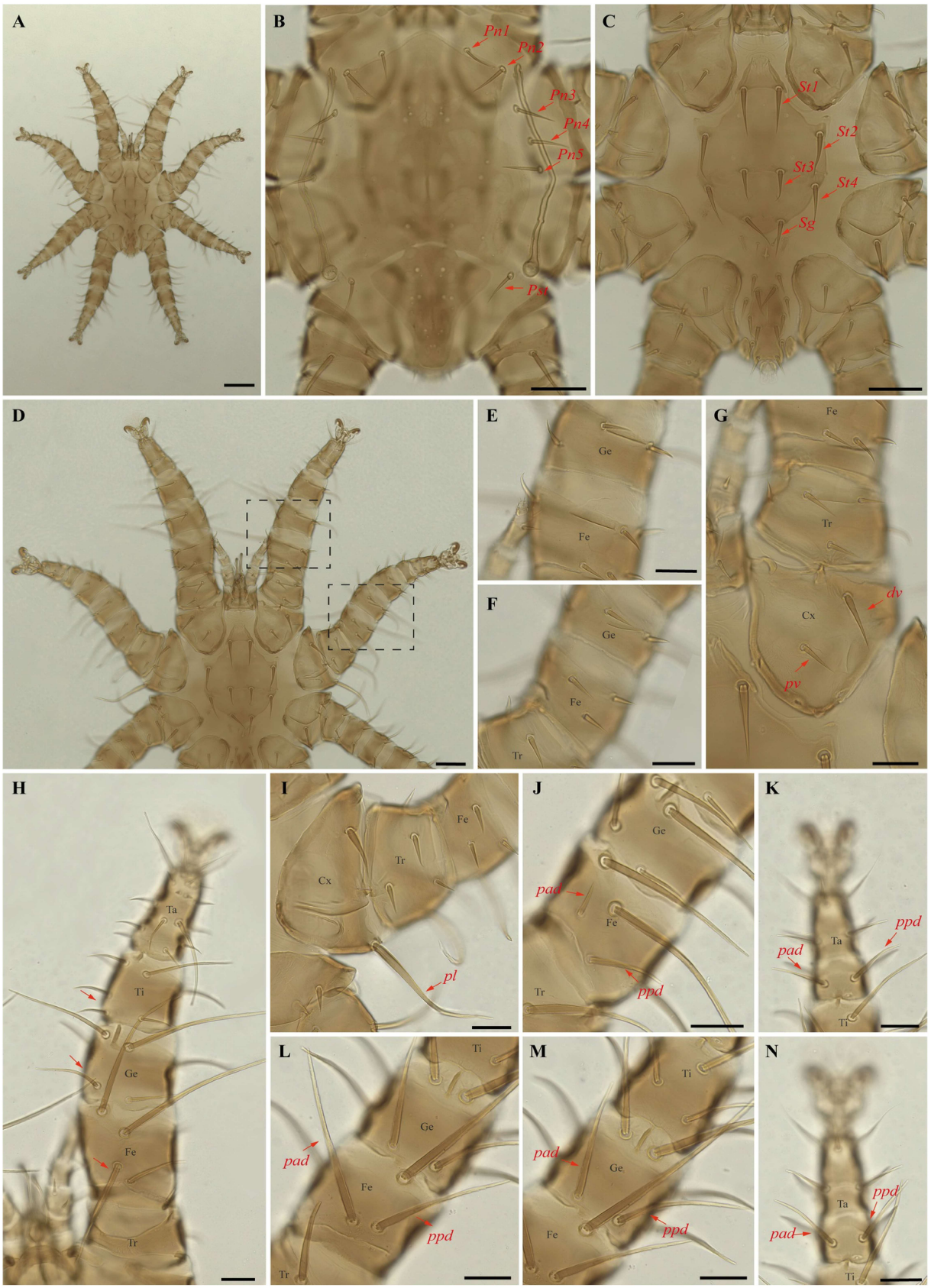

Figure 12 *Periglischrus iheringi*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

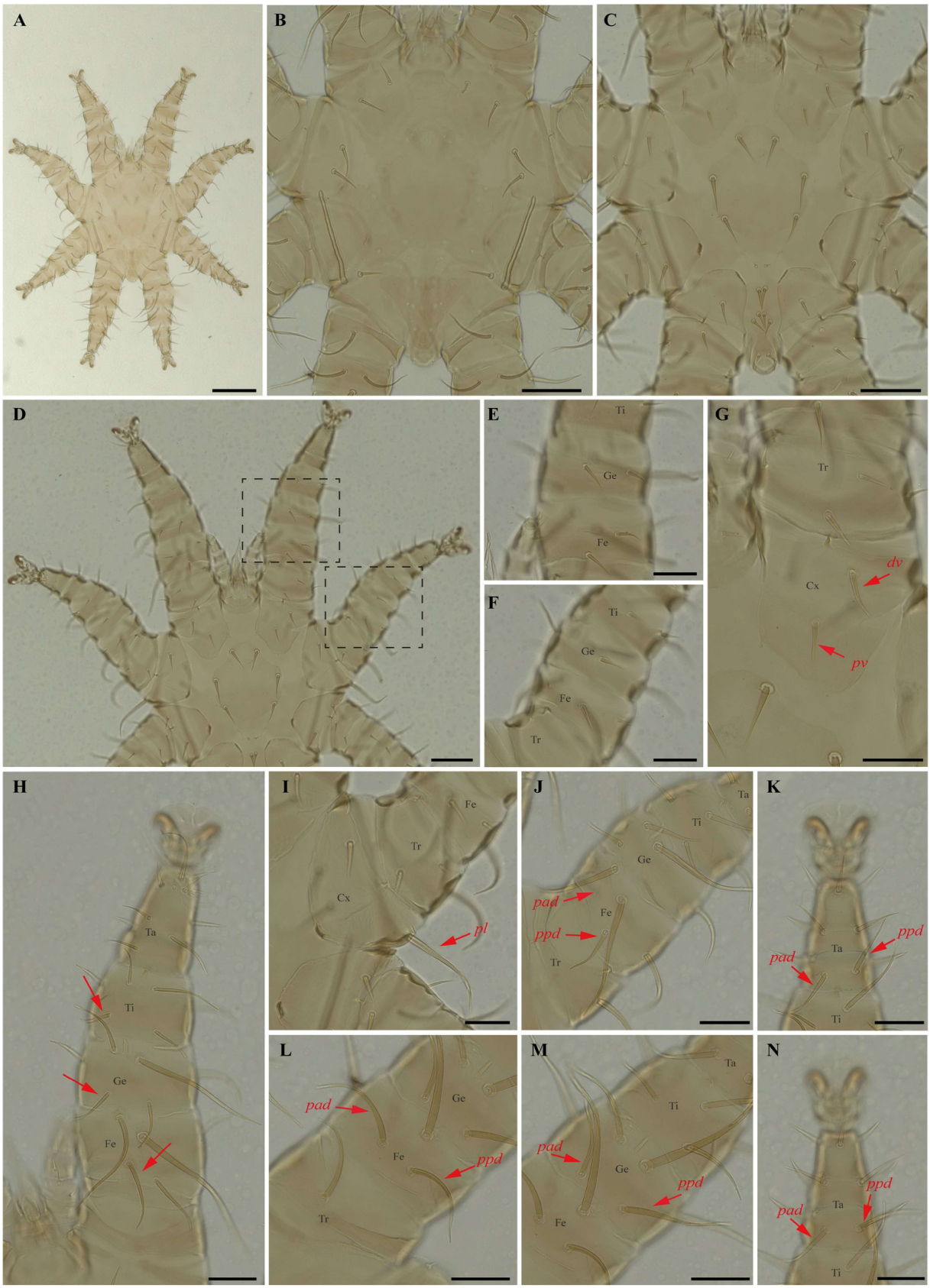

Figure 13 *Periglischrus iheringi*, protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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 and Loomis 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 the presence of a small central pair of foveae in *P. iheringi* 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 in *P. ojastii*), *Pnl* are very small (vs. *Pnl*-*Pn5*). 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 three 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 morphologically 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*.

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° S, -42.7335° E, 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° S, -42.7043° E, 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).

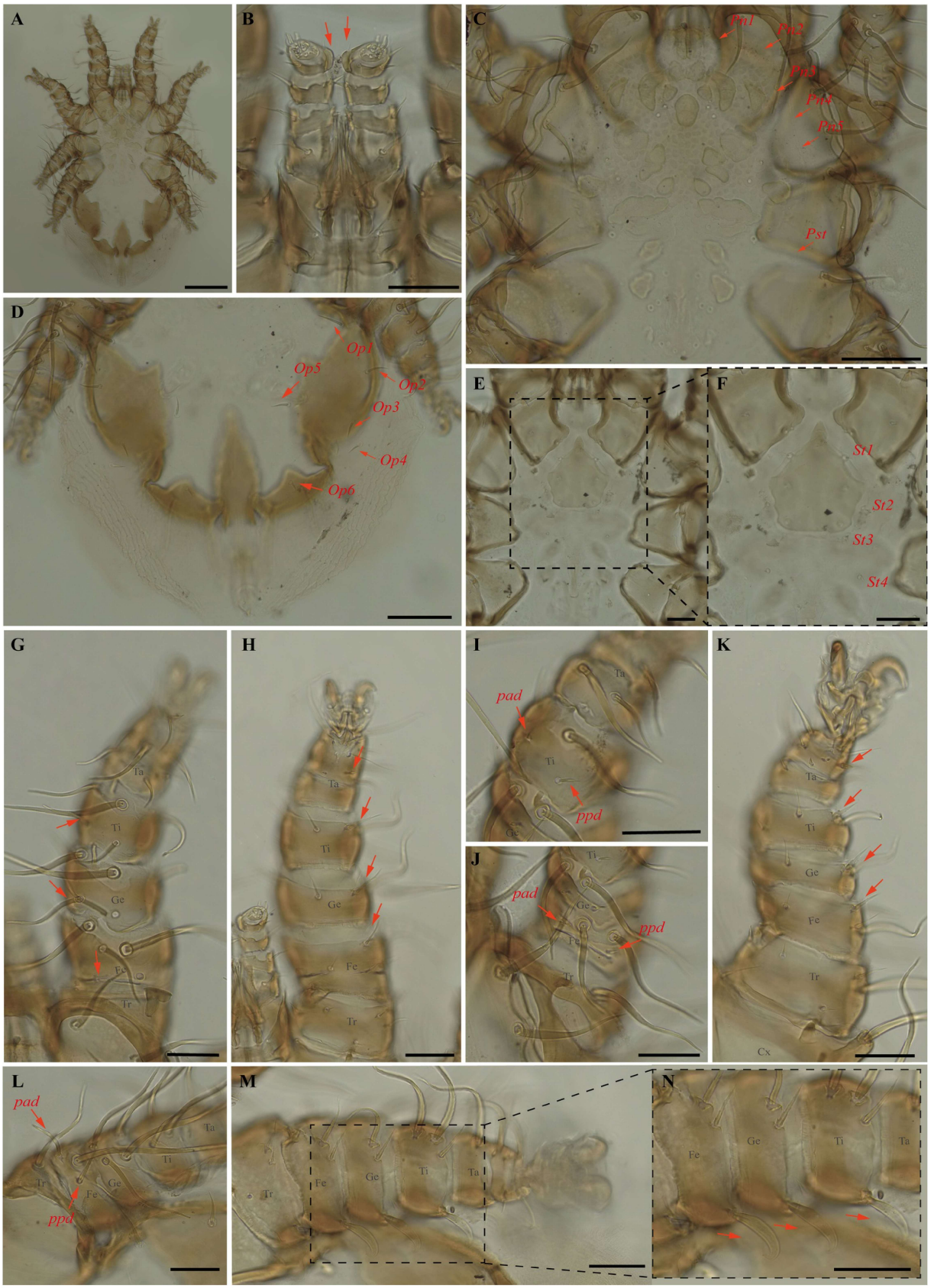

Figure 14 *Periglischrus paravargasi*, female. A – General view; B – Mediodistal lobe of palpal tibia indicated in red arrow; C – Dorsal plate with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; D – Dorsal opisthosoma with hysteronotal setae (*Op1–Op6*) indicated in red arrow; E and F – Sternal plate with sternal setae (*St1–St4*); G – Proximal anterodorsal (*ad*) seta on femur–tibia I; H – Distal posteroventral (*pv*) seta on femur–tibia I (serrated) and tarsus I (small and spinelike), indicated in red arrow; I – Proximal antero (*ad*) and posterodorsal (*pd*) setae on tibia II; J – Proximal *ad* and *pd* setae on femur II; K – Distal *pv* setae on femur–tibia II (serrated) and tarsus II (small and spinelike), 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.

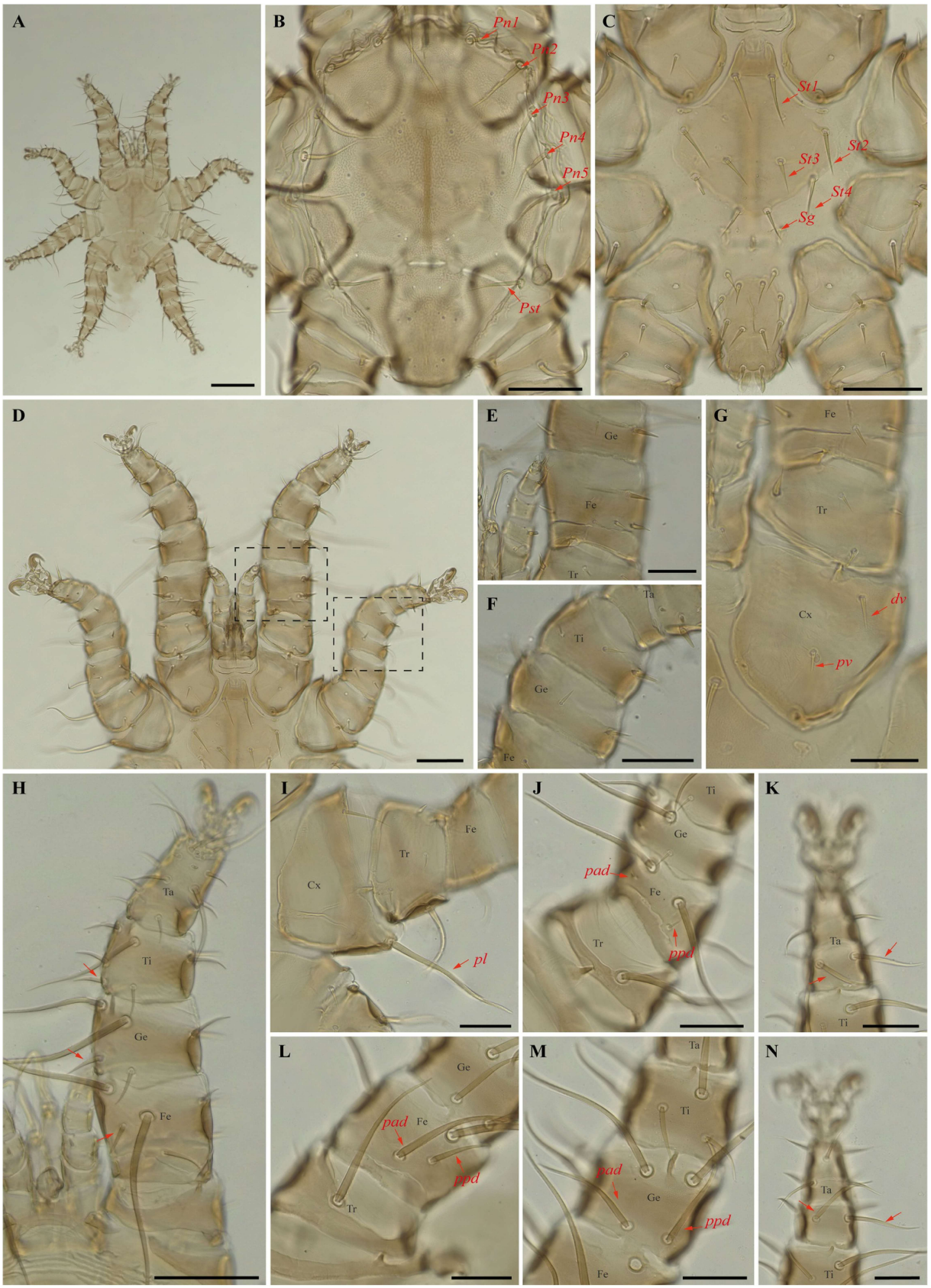

Figure 15 *Periglischrus paravargasi*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

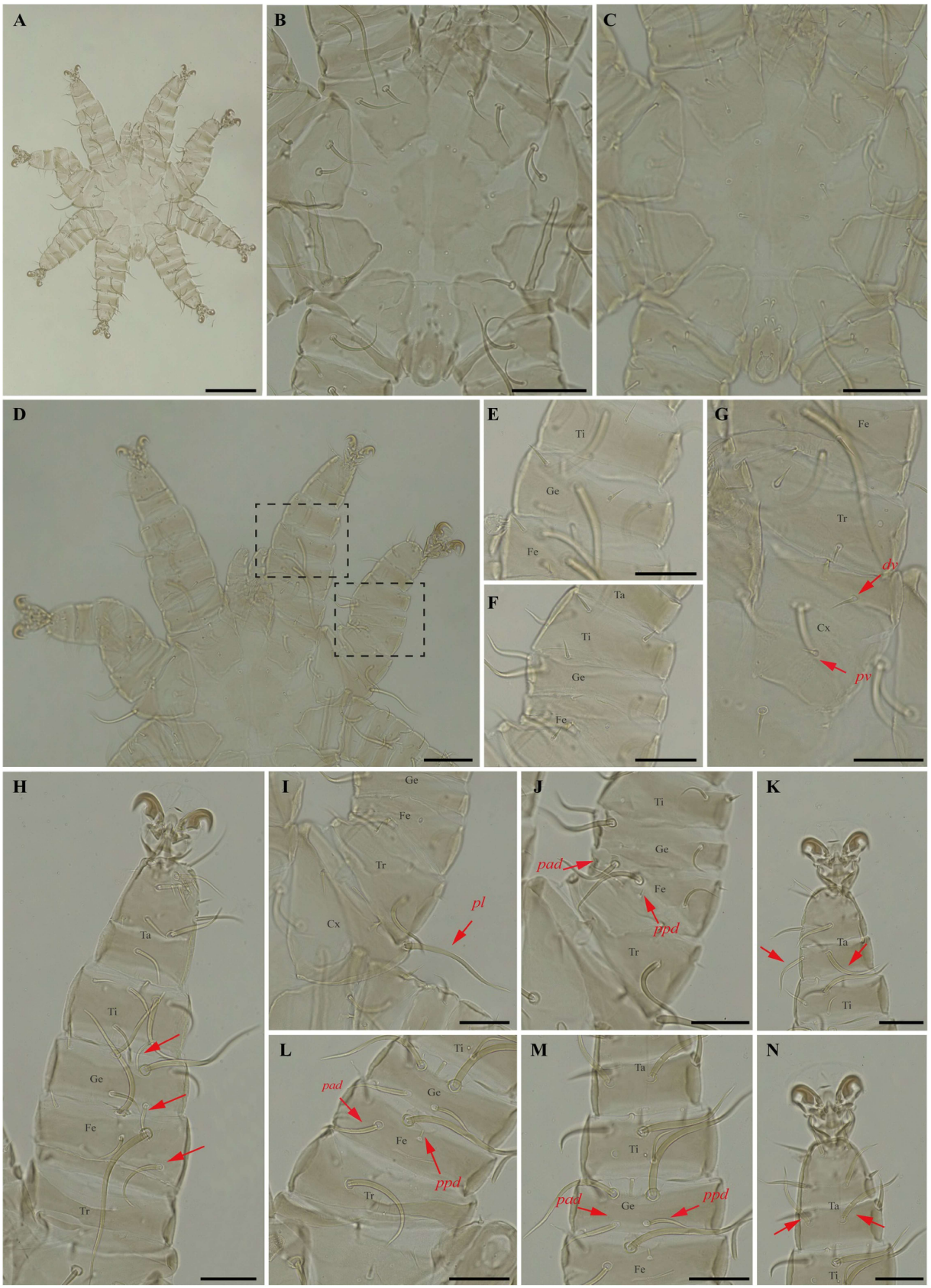

Figure 16 *Periglischrus paravargasi*, protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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%.

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° S, -43.959° E, 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° S, -45.6322° E, 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° S, -42.7335° E, 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° S, -43.959° E, 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 and Graciolli 2013; Silva *et al.* 2017); *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*

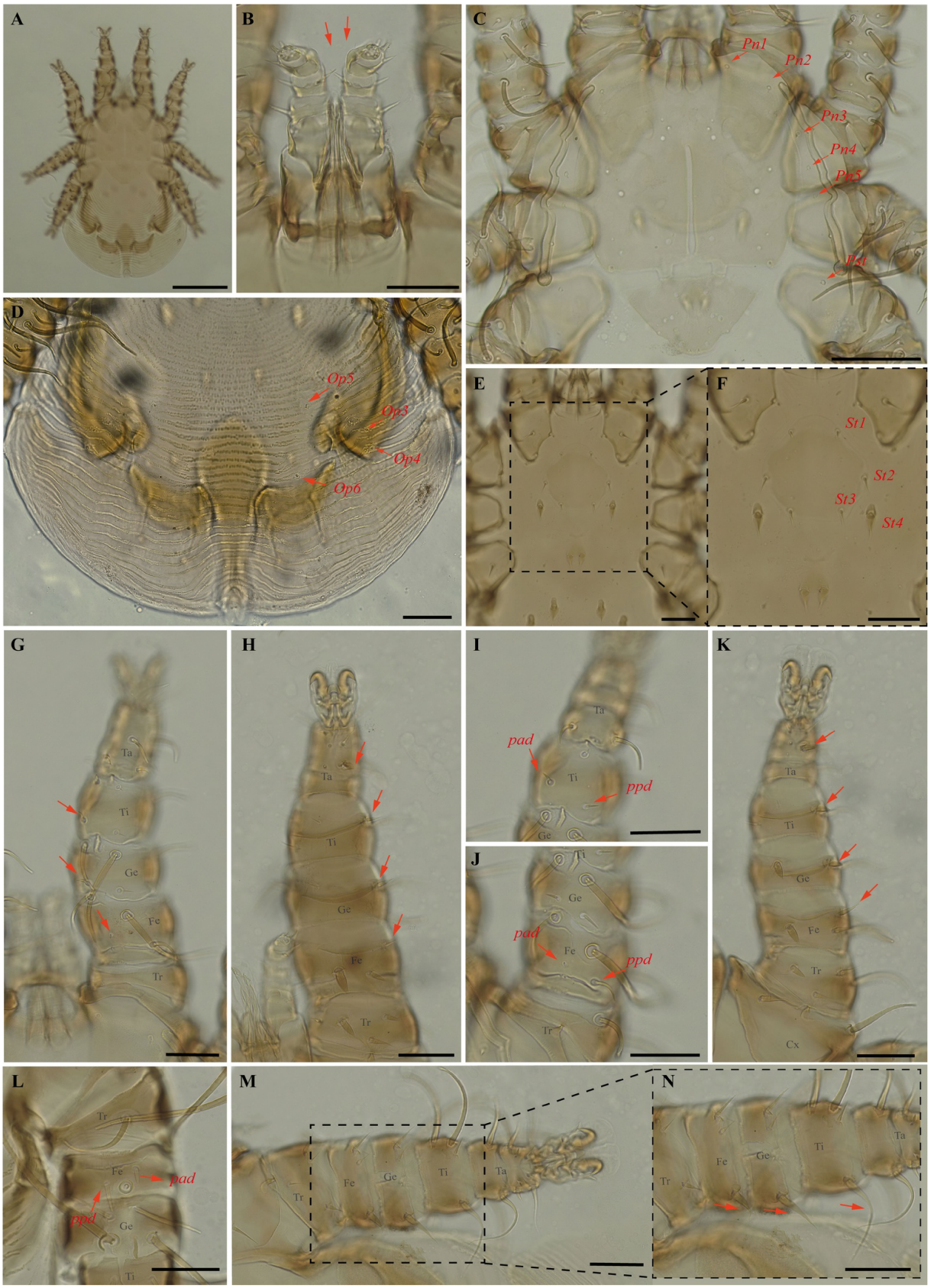

Figure 17 *Periglischrus torrealbai*, female. A – General view; B – Mediodistal lobe of palpal tibia indicated in red arrow; C – Dorsal plate with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; D – Dorsal opisthosoma with hysteronotal setae (*Op3–Op6*) indicated in red arrow; E and F – Sternal plate with sternal setae (*St1–St4*); G – Proximal anterodorsal (*ad*) seta on femur–tibia I; H – Distal posteroventral (*pv*) seta on femur–tibia I (slender) and tarsus I (short, enlarged and peglike), indicated in red arrow; I – Proximal antero (*ad*) and posterodorsal (*pd*) setae on tibia II; J – Proximal *ad* and *pd* setae on femur II; K – Distal *pv* setae on femur II (slender) and genu–tarsus II (short, enlarged 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, F and H = 100 µm, B, D, G–N = 50 µm.

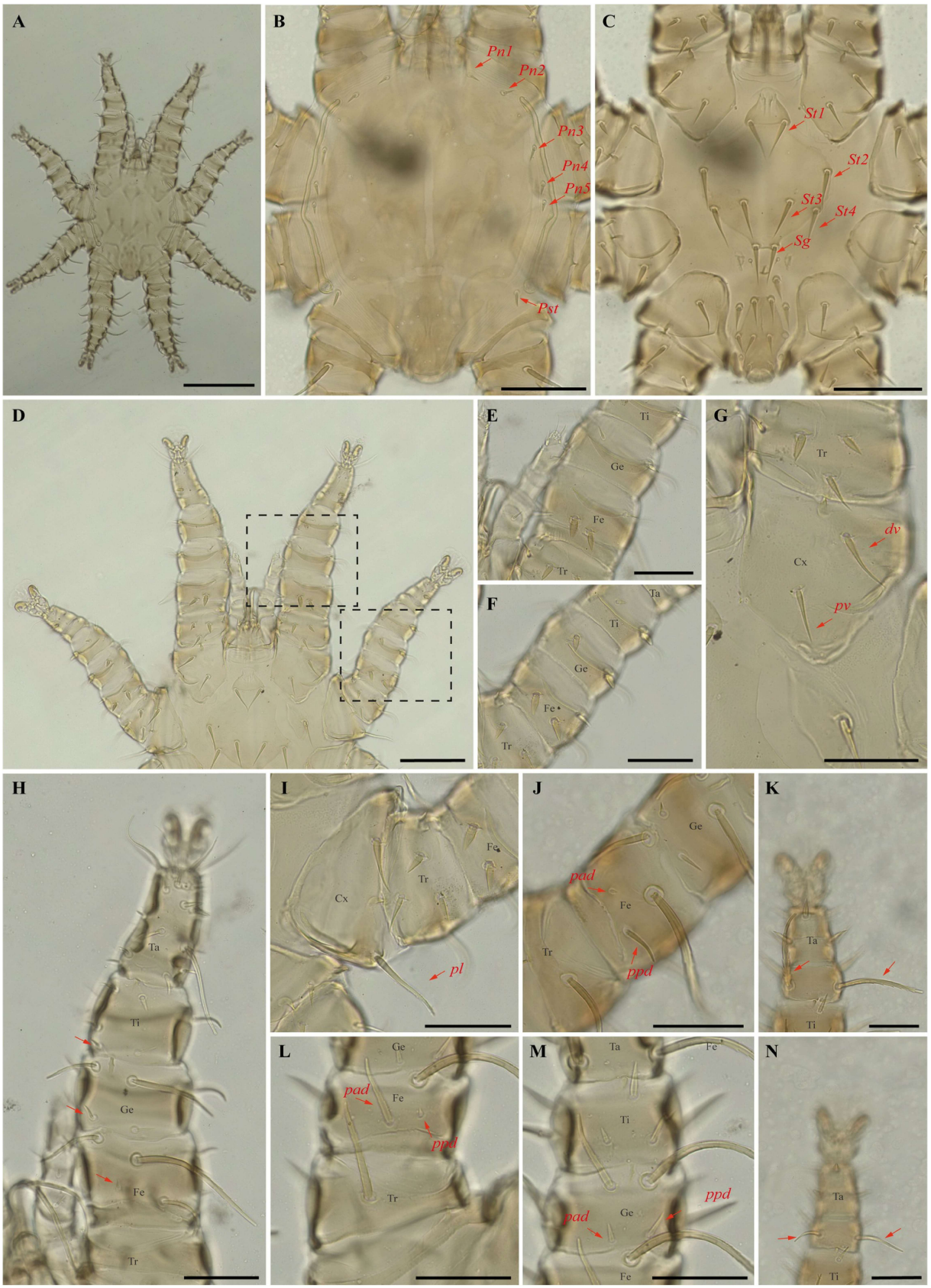

Figure 18 *Periglischrus torrealbai*, male. A – General view; B – Dorsal view with proteronotal setae (*Pn1–Pn5*) and poststigmal seta (*Pst*) indicated; C – Ventral view with sternogenital setae (*St1–St4*) and genital seta (*Sg*) indicated; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated in red arrow; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

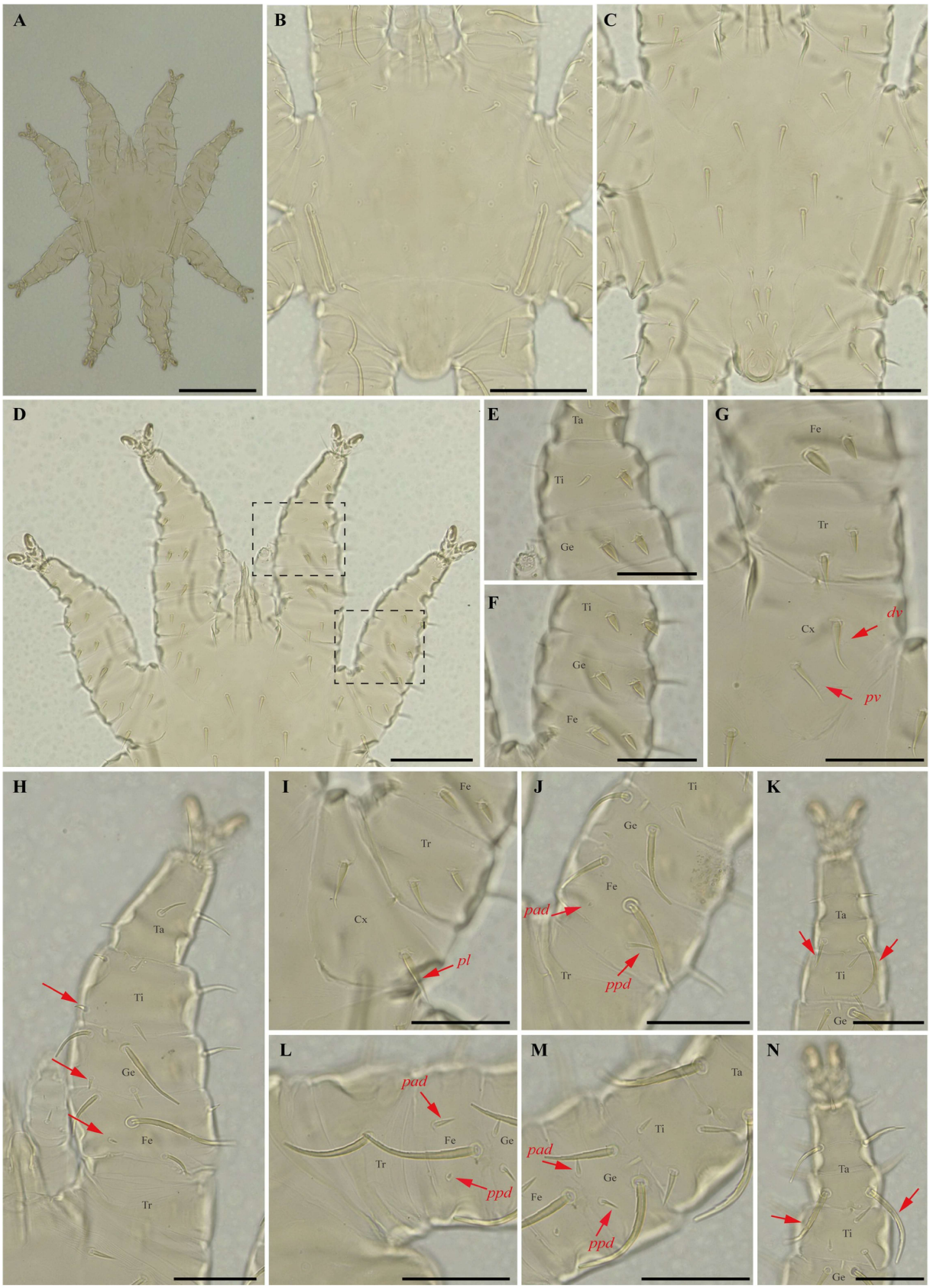

Figure 19 *Periglischrus torrealbai*, protonymph. A – General view; B – Dorsal view; C – Ventral view; D – Ventral setae on legs I and II with details; E – Details ventral setae on leg I; F – Details ventral setae on leg II; G – Coxa I with *pv* and *dv* setae, indicated; H – Femur–tibia I with proximal *ad* setae, indicated; I – Coxa II with posterolateral seta (*pl*) indicated; 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.

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 I–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 results of Almeida *et al.* (2018), who showed 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 suggests 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 on 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 shifts 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 (Morales-Malacara and 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 (Morales-Malacara 2001). 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* and *P. vargasi*) due to 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* from *caligus* species group and included the other species (*P. paracaligus*, *P. paravargasi* and *P. vargasi*) from *vargasi* species-group, along with *P. empheresotrichus* Morales-Malacara, Castaño-Meneses & Klompen, 2020, and *P. calcariflexus* Morales-Malacara & López-Ortega, 2023.

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 setae 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 with *P. ramirezi* Machado-Allison & Antequera, 1971, based on the shape of the 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) categorized *P. ramirezi* from *delfinadoae* species-group, along with *P. delfinadoae* Dusbábek, 1967, still belonging to *acutisternus*-clade. This placement occurred despite limited morphological similarities and its association with a different bat clade. *P. delfinadoae* is associated to stenodermatini bats and *P. ramirezi* to macrotine bats.

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