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DESCRIPTION OF A NEW SPECIES OF BAT-ASSOCIATED ARGASID TICK (ACARI: ARGASIDAE) FROM BRAZIL

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ABSTRACT: A new species of argasid tick (Acari: Argasidae) is described from immature and adult specimens collected from several localities in Brazil. A complete morphological account is provided for all postembryonic life stages, i.e., larva, nymph, female, and male. *Ornithodoros cavernicolous* n. sp. is the 113th in the genus. Morphologically, the new species shares common features, e.g., presence of well-developed cheeks and legs with micromammillate cuticle, with other bat-associated argasid ticks included in the subgenus *Alectorobius*. In particular, the new species is morphologically related to *Ornithodoros azteci* Matheson, with which it forms a species group. Phylogenetic analysis based on the 16S rRNA gene sequences supports the placement of the new species within a large clade that includes other New World bat-associated argasids. However, the new species seems to represent an independent lineage within the genus *Ornithodoros*.

Argasid ticks embrace a diverse group of species, whose genus-level classification has been disputed (Estrada-Peña et al., 2010). In the most recent list of valid tick species, Guglielmone et al. (2010) adopted the classical genus-level classification of the Argasidae proposed by Hoogstraal (1985), who considered the following genera as valid: *Antricola* Cooley & Kohls, *Argas* Latreille, *Nothoaspis* Keirans & Clifford, *Ornithodoros* Koch, and *Otobius* Banks. In this systematic framework, *Ornithodoros* is paraphyletic (Nava et al., 2009) and includes most argasid representatives (Guglielmone et al., 2010). In brief, the main reasons for the controversies surrounding the classification of the Argasidae is the lack of reliable morphological characters for species determination and the considerable, yet underestimated, species diversity within this family. Again, genetic data and information about the biology of most argasid species are also meager and certainly represent a hurdle to be surpassed toward resolving the systematics of the Argasidae.

Insight derived from recent investigations suggests that the Brazilian argasid fauna is probably much more diverse than currently known. For example, 6 new argasid species, namely, *Antricola delacruzi* Estrada-Peña, Barros-Battesti & Venzal; *Antricola guglielmonei* Estrada-Peña, Barros-Battesti & Venzal; *Antricola inexpectata* Estrada-Peña, Barros-Battesti & Venzal; *Ornithodoros fonsecai* (Labruna & Venzal); *Ornithodoros rondoniensis* (Labruna, Terassini, Camargo, Brandão, Ribeiro & Estrada-Peña); and *Nothoaspis amazoniensis* Nava, Venzal & Labruna, have been described from Brazil during the past 6 yr (Estrada-Peña et al., 2004; Labruna et al., 2008; Labruna and Venzal, 2009; Nava et al., 2010). Certainly, this indicates that our current knowledge on the Brazilian argasid fauna has much more to be developed.

Incidentally, the Brazilian tick fauna is currently known to include 63 species, of which only 19 belong to the family Argasidae (Dantas-Torres et al., 2009; Labruna and Venzal, 2009; Nava et al., 2010). The genus *Ornithodoros* is the most representative of the family in Brazil, with the following species: *Ornithodoros brasiliensis* Aragão; *Ornithodoros capensis* Neumann; *O. fonsecai*; *Ornithodoros hasei* Schulze; *Ornithodoros jul* Schulze; *Ornithodoros marinkellei* Kohls, Clifford & Jones; *Ornithodoros mimon* Kohls, Clifford & Jones; *Ornithodoros nattereri* Warburton; *O. rondoniensis*; *Ornithodoros rostratus* Aragão; *Ornithodoros rudis* Karsh; *Ornithodoros setosus* Kohls, Clifford & Jones; *Ornithodoros stageri* Cooley & Kohls; and *Ornithodoros talaje* Guérin-Méneville. The present work adds new data to the Brazilian argasid fauna, with the description of a new tick species belonging to *Ornithodoros*, and this genus now includes 113 species. A complete morphological account is provided for all postembryonic life stages, i.e., larva, nymph, female, and male. Phylogenetic analysis based on the 16S rRNA gene sequences supports the placement of the new species within a large clade that includes other New World bat-associated argasids. However, the new species seems to represent an independent lineage within the genus *Ornithodoros*.

MATERIALS AND METHODS

Tick collection

Between January 1999 and July 2010, 49 ticks (9 larvae, 21 nymphs, 11 females, and 8 males) belonging to a new species were collected crawling freely on the ground, mainly on bat guano; in cracks and crevices on the wall and ceiling of caves; and in mines in different Brazilian states (Bahia, Ceará, Minas Gerais, Pará, and Rio Grande do Norte). Ticks were collected directly from the substrata and immediately placed in labeled vials containing 70% ethanol. In addition, larvae (3 engorged and 1 partially engorged) collected in 10 October 2010 from bats captured in the municipality Orizona, Goiás, were used for the species description. Ticks collected in the present study have been deposited in the following collections: Coleção Nacional de Carrapatos, University of São Paulo, Brazil (CNC); Coleção de Invertebrados Subterrâneos de Lavras, Zoology Sector, Department of Biology, Federal University of Lavras, Minas Gerais, Brazil (ISLA); Acari Collection of the Butantan Institute, Brazil (IBSP); and the U.S. National Tick Collection, Statesboro, Georgia (USNTC).

The tick collection of the Gorgas Memorial Institute provided for the present study 4 nymphs, 5 females, and 4 males of *Ornithodoros azteci* Matheson that were collected by H. Van Horn (collection date 20 March

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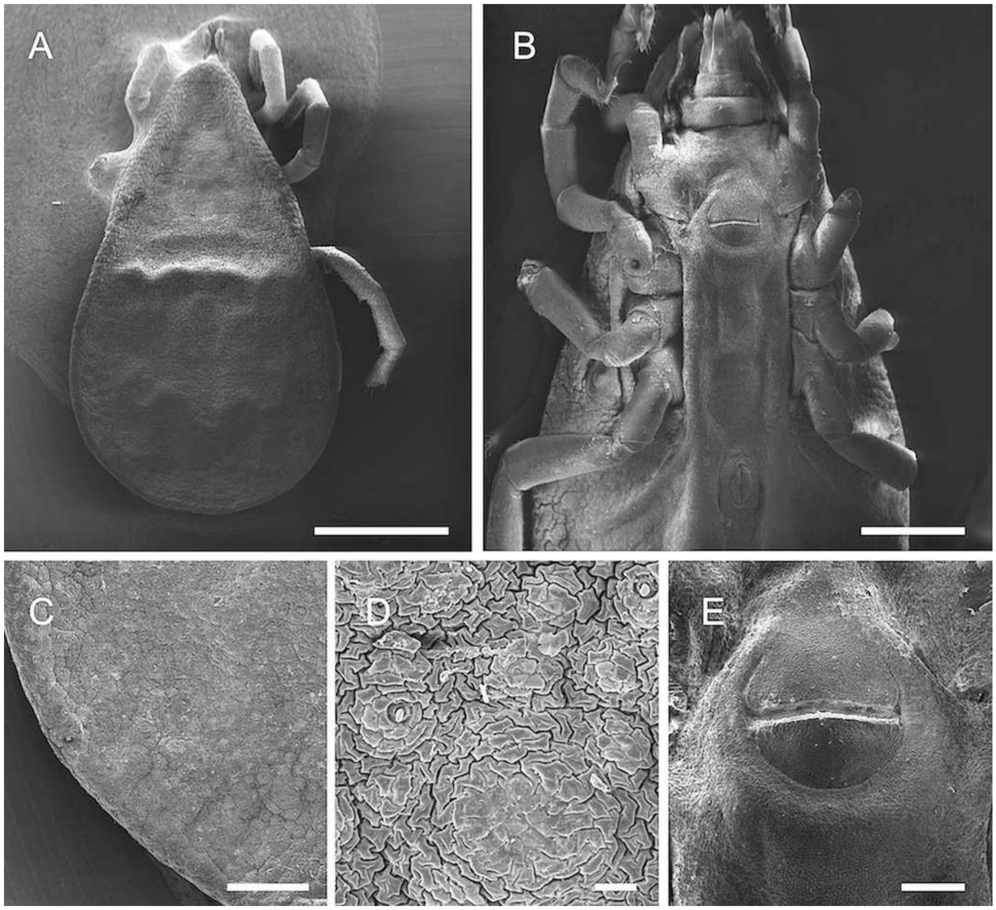

FIGURE 1. *Ornithodoros cavernicolous* n. sp., female. (**A**) Dorsal view. (**B**) Ventral view. (**C**) Dorsal posterolateral integument. (**D**) Dorsal posterolateral mammillae. (**E**) Genital opening. Bars = 100 µm.

1984) in Colón Province, not far (circa 37 km) from the type locality (Panama Canal Zone) of the aforementioned species. Moreover, 1 paratype female of *O. azteci* (IBSP-1063) collected from *Carollia perspicillata* (Linnaeus), by L. H. Dunn (12 November 1930) in Summit, Panama Canal Zone, also was available for morphological comparisons.

Morphological study

Ticks were identified using morphological keys and original species descriptions of *Ornithodoros* spp. (Matheson, 1935, 1941; Cooley and Kohls, 1944; Kohls et al., 1965, 1969; Jones and Clifford, 1972). Measurements for adults (males and females) and nymphs (large specimens only) were made using a stereomicroscope and are provided in millimeters, being expressed as mean followed by standard deviation and range within parentheses. Larvae were mounted in Hoyer's medium to make semi-permanent slides and examined and photographed by light microscopy for morphological and morphometric analyses using an Eclipse E200 optical microscope (Nikon, Tokyo, Japan). Measurements for larvae are in micrometers. For the description, 70 morphological features in total were observed, measured, or both using 9 larvae. Representative specimens of females, males, and nymphs were prepared for scanning electron microscopy as described previously (Corwin et al., 1979).

Molecular study

DNA was extracted from individual tick specimens using the guanidine isothiocyanate-phenol technique, as described previously (Sangioni et al., 2005). Extracted DNA samples were subjected to conventional polymerase chain reaction (PCR) targeting a fragment of approximately 460 base pairs of the mitochondrial 16S rDNA (Mangold et al., 1998). PCR products of the expected sizes were purified and then directly sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems/Perkin Elmer, Foster City, California) with the same primers used in the PCR. The nucleotide sequences generated were deposited in GenBank under accessions JF714963 and JF714964. These sequences were manually aligned using GeneDoc software (http://www.nrbsc.org/downloads/) with sequences previously determined for other argasid species available in GenBank, and also with sequences of *Ixodes holocyclus* Neumann and *Ixodes uriae* White (Ixodidae Murray) that were used as outgroup (accessions of all sequences are shown in the resulting phylogenetic tree). The phylogenetic tree was inferred by the maximum parsimony method using PAUP version 4.0b10 (Swofford, 2002) with 500 replicates of random addition taxa and tree bisection and reconnection branch swapping; all positions were given equal weight.

DESCRIPTION

Ornithodoros cavernicolous Dantas-Torres, Venzal & Labruna n. sp.

(Figs. 1–6)

Female (Figs. 1, 2; measurements based on allotype and 3 paratypes): Body elongate, in outline pyriform, broadly rounded posteriorly and narrowing gradually from behind fourth pair of legs; body 5.16 ± 0.27 (4.85–5.50) in length (from pointed anterior end to posterior body margin) and 3.09 ± 0.20 (2.88–3.32) in maximum width; color light yellow in unfed, preserved specimens; lateral suture absent. Dorsal surface distinctly mammillated with minute setae between mammillae, which are larger and more distinct in the marginal areas; discs and eyes absent. Ventral surface distinctly mammillated, as dorsal surface, with minute setae between mammillae, more evident between legs; median and postanal grooves well

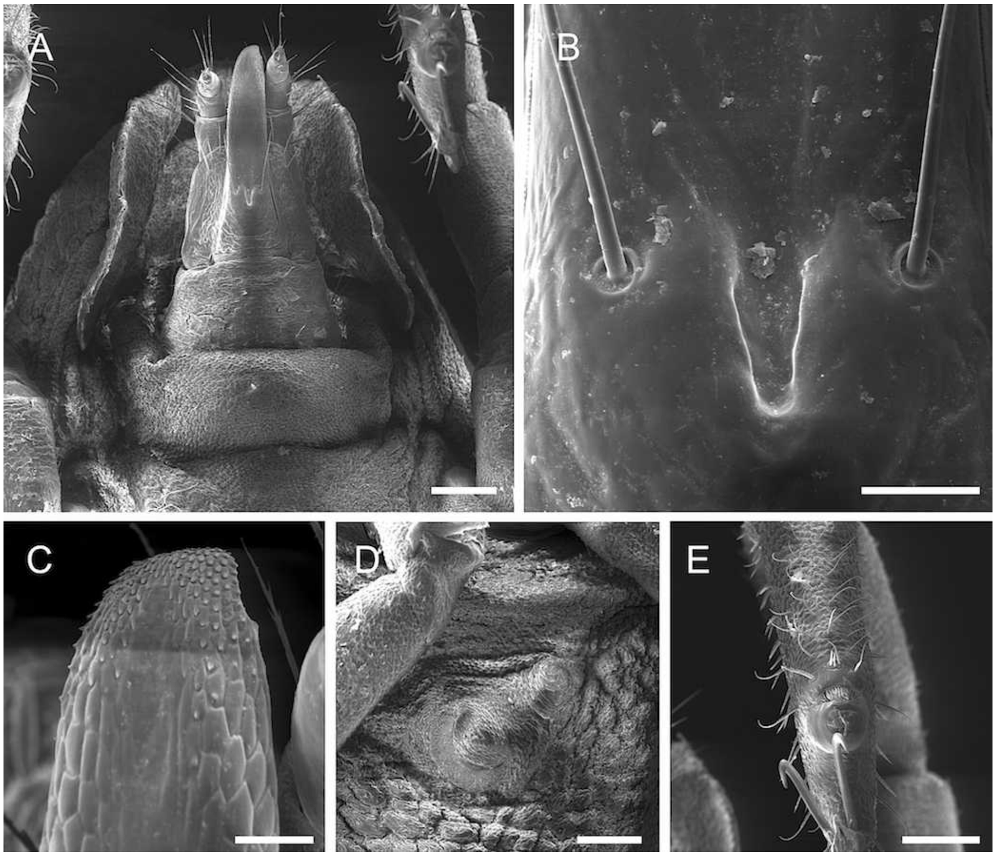

FIGURE 2. *Ornithodoros cavernicolous* n. sp., female. (**A**) Capitulum. (**B**) Hypostome base. (**C**) Hypostome apice. (**D**) Spiracular plate. (**E**) Haller's organ. Bars = 100 μm.

developed; genital opening located anteriorly, between coxae I and II, with anterior and posterior labia subequal in size; spiracular plate small, 0.16 ± 0.03 (0.12–0.18) in maximum diameter, and semi-lunar in shape; anus elliptical, each valve provided with short setae. Basis capituli as wide as long, well chitinized; capitulum 0.60 ± 0.17 (0.44–0.84) long, extendable (when extended, basis capituli visible from above), situated in well-marked camerostome with movable cheeks, provided with short, peg-like setae on free margins; chelicerae elongate, sharply pointed shafts terminating in pointed digits; hypostome long, thin, with very small denticles not in clearly definite files on apical portion; palpi as long as hypostome; article 1 twice as long as article 2 and longer than article 3; article 4 short, pointed, provided with several apical setae; long setae present on all palpal articles. Coxae I and II distinctly separated, first somewhat larger than second; coxae II to IV subequal in size and contiguous; coxal and supracoxal folds prominent; all legs provided with setae, varying in size and number; legs subequal in size, with leg I shortest and leg IV longest; tarsus I 0.63 ± 0.02 (0.60–0.64) long; tarsus IV 0.87 ± 0.12 (0.70–1.00) long; hump (''gibbosity'') distinct on tarsus I, vestigial on tarsi II–IV; pulvilli absent; claws stout.

Male (Figs. 3, 4; measurements based on holotype and 2 paratypes): Body essentially as described for female, except for being slightly smaller; length: 4.83 ± 0.53 (4.25–5.50); width: 2.92 ± 0.32 (2.52–3.20). Dorsum as in female. Venter as in female, except that genital opening crescent-shaped at level of coxa I; spiracular plate small, 0.14 ± 0.01 (0.12–0.16) in maximal diameter. Capitulum as in female; length: 0.61 ± 0.13 (0.44–0.76). Legs as in female; tarsus I 0.59 ± 0.05 (0.54–0.66) long; tarsus IV: 0.73 ± 0.09 (0.60–0.78) long.

Nymph (Fig. 5; measurements based on 8 paratypes): Body as in female, except for being smaller; length: 2.78 ± 0.35 (2.25–3.35); width 1.44 ± 0.23 (1.04–1.68). Dorsum as in female. Venter as in female, except for absence of genital opening; spiracular plate small, 0.11 ± 0.02 (0.08–0.14) in maximal diameter. Capitulum as in female, except palpal articles; article 1 twice as long as article 3; article 2 shorter than article 1; article 4 short, pointed, with several apical setae; length: 0.52 ± 0.11 (0.40–0.72). Legs as in female; tarsus I 0.40 ± 0.02 (0.38–0.42) long; tarsus IV 0.52 ± 0.05 (0.44–0.60) long.

Larva (Fig. 6; measurements based on 9 paratypes): Body narrowly elongate, expansion at level of first pair of legs and narrowed again at level of third pair of legs; length including capitulum 1,660; length without capitulum 1,366; width 927. Dorsal plate triangular in shape, broadest posteriorly; length 165 ± 11 (146–175); width 163 ± 11 (151–180); dorsal surface provided with 15 pairs of setae, 7 anterolateral, 3 central, and 5 posterolateral setae; anterolateral setae (Al): Al_1 length 124 ± 6 (114–131), Al_2 length 116 ± 5 (112–124), Al_3 length 114 ± 8 (105–125), Al_4 length 107 ± 6 (97–112), Al_5 length 112 ± 11 (97–129), Al_6 length 110 ± 11 (97–124), Al_7 length 117 ± 7 (110–124); central setae (C): C_1 length 106 ± 8 (97–112), C_2 length 96 ± 16 (83–114), C_3 length 89 ± 11 (80–102); posterolateral setae (Pl): Pl_1 length 92 ± 4 (88–97), Pl_2 length 106 ± 8 (97–112), Pl_3 length 98 ± 3 (97–102), Pl_4 length 94 ± 3 (90–97), Pl_5 length 90 ± 10 (85–102). Ventral surface with 7 pairs of setae plus pair on anal valves, 1 posteromedian seta present; 3 pairs of sternal setae (St): St_1 length 100 ± 19 (80–117), St_2 length 84 ± 3 (83–88), St_3 length 93 ± 5 (90–97); 1 pair of postcoxal setae (Pc) length 92 ± 6 (85–97); 3 pairs of circumanal setae (Ca): Ca_1 length 71 ± 10 (63–83), Ca_2 length 100 ± 11 (85–110), Ca_3 length 115 ± 11 (102–122); posteromedian setae (PM) length 86 ± 9 (73–93). Basis capituli measuring 204 ± 11 (195–224) from posterior margin to PH_1; length from posterior margin of basis capituli to insertion of hypostome 261 ± 13 (249–285); length from posterior margin to apex of hypostome 420 ± 13 (392–431); width 318 ± 17 (294–343); 2 pairs of post-hypostomal setae; Ph_1 length 13 ± 1 (12–14), Ph_2 length not determined (broken setae); distance between Ph_1 setae 38 ± 2 (37–44), and between Ph_2 setae 118 ± 5 (110–124); palpi total length 304 ± 6 (294–313), segmental length/width from I to IV: (I) 76 ± 3 (73–80)/47 ± 1 (46–49), (II) 84 ± 3 (78–90)/49, (III) 93 ± 3 (85–97)/46 ± 2 (44–49), (IV) 44 ± 1 (44–46)/24; setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, and (IV) 9. Capsule of Haller's organ with reticulations. Hypostome length from Ph_1 to apex 214 ± 7 (207–227), and length from insertion of hypostome

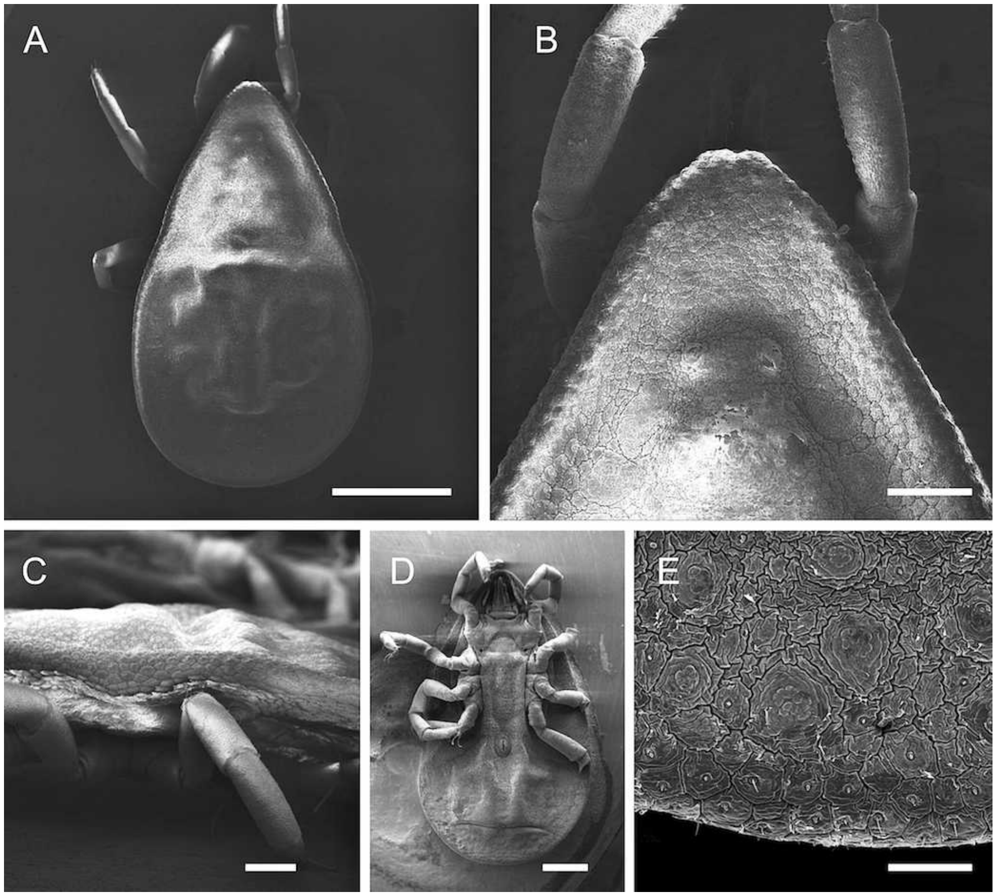

FIGURE 3. *Ornithodoros cavernicolous* n. sp., male. (**A**) Dorsal view. (**B**) Detail on dorsal anterior integument. (**C**) Detail on lateral view. (**D**) Ventral view. (**E**) Detail on dorsal posterior mammillae. Bars = 100 μm.

in basis capituli to apex 161 ± 8 (151–175); width in medial basis portion of hypostome 49 ± 3 (44–53) and in basis portion of hypostome 78 ± 6 (73–90); dental formula 4/4 in the anterior third and 2/2 posteriorly to base; file 1 with 12 to 14 (typically 13) denticles, file 2 with 10 to 13 denticles, file 3 with 4 to 6 denticles, and file 4 with 3 to 5 denticles; corona in apex with 2 or 3 tiny denticles; apex blunt; basis of hypostome enlarged with 2 or 3 denticles towards laterally; some basal denticles crowed and deformed. Three pairs of legs, subequal in size, provided with several setae of variable size and form; tarsus I 364 ± 15 (343–392) long, 94 ± 2 (90–97) wide; setal formula: 1 pair apical (A), 1 distomedian (DM), 5 paracapsular (PC), 1 posteromedian (PM), 3 basal pairs (B), 1 pair apicoventral (AV), 1 pair midventral (MV), 1 pair basiventral (BV), 1 pair anteroventral (AL), 1 pair midlateral (ML), and 1 pair posterolateral (PL).

Taxonomic summary

Holotype: Male, collected in a cave (Gruta do Ubajara) (03°49′53.8″S, 40°53′54.9″W), municipality of Ubajara, state of Ceará, Brazil, 30 December 2006, by M. Souza-Silva. Deposited in the Coleção Nacional de Carrapatos (CNC-1825).

Allotype: Female, same data as holotype. Deposited in the Coleção Nacional de Carrapatos (CNC-1825).

Paratypes (an asterisk indicates that the specimen was measured): 1 nymph*, Toca do Morrinho (cave) (10°12′32″S, 40°55′05″W), Campo Formoso, Bahia, I-1999, R. L. Ferreira (USNTC); 1 nymph* and 2 engorged larvae* (1 larvae measured), Lapa do Caboclo (cave) (15°05′18.74″S, 44°16′02.61″W), Itacarambi, Minas Gerais, 22-VII-2003, R. L. Ferreira (CNC-1828, 1841; USNTC); 2 males and 1 nymph, Lapa do Mosquito (cave) (18°37′34″S, 44°24′45″W), Curvelo, Minas Gerais, IX-2004, L. F. O. Bernardi (ISLA-710); 1 male and 2 nymphs* (1 nymph destroyed for DNA extraction), Gruta do Ubajara (cave) (03°49′53.8″S, 40°53′54.9″W), Ubajara, Ceará, 30-XII-2006, M. Souza-Silva (CNC-1826); 1 larva, Gruta do Morcego Branco (cave) (3°49′58.4″S, 40°54′03.20″W), Ubajara, Ceará, 3-I-2007, M. Souza-Silva (CNC-1897); 2 females* (1 female destroyed for DNA extraction), Furna do Araticum (cave) (03°48′12.6″S, 41°00′03.5″W), Ubajara, Ceará, I-2007, R. L. Ferreira (USNTC); 1 male* and 1 nymph (this nymph was destroyed for DNA extraction), Casa de Pedra (cave) (06°04′16.7″S, 37°53′02.6″W), Martins, Rio Grande do Norte, I-2007, R. L. Ferreira (IBSP-10640); 1 female*, 5 nymphs* (4 nymphs measured), and 1 engorged larva, Gruta do Roncador (cave) (05°35′50.4″S, 37°49′39.5″W), Apodi, Rio Grande do Norte, VII-2007, R. L. Ferreira (CNC-1827, 1842; IBSP-10641); 1 male* and 1 engorged larva*, Gruta da Carrapateira (cave) (05°33′36.8″S, 37°39′49.2″W), Felipe Guerra, Rio Grande do Norte, VII-2007, R. L. Ferreira (CNC-1843; USNTC); 1 nymph, Gruta do Ioio (cave) (12°23′34″S, 41°33′13″W), Palmeiras, Bahia, XII-2008, L. F. O. Bernardi (ISLA-702); 1 nymph, Lapa do Convento (cave) (10°02′56″S, 40°43′37″W), Campo Formoso, Bahia, I-2008, R. L. Ferreira (ISLA-706); 1 nymph, Gruta da Abelha Italiana (cave) (05°33′38.846.5″S, 37°39′39.5″W), Felipe Guerra, Rio Grande do Norte, I-2008, R. L. Ferreira (ISLA-703); 3 nymphs and 2 larvae*, Toca da Barriguda (cave) (10°08′26″S, 40°51′08″W), Campo Formoso, Bahia, VII-2008, R. L. Ferreira (CNC-1844); 1 male and 1 nymph, Toca da Barriguda (cave) (10°08′26″S, 40°51′08″W), Campo Formoso, Bahia, I-2009, R. L. Ferreira (ISLA-701); 1 nymph, Toca do Pitú (cave) (10°07′43.6″S, 40°50′16.7″W), Campo Formoso, Bahia, VII-2009, R. L. Ferreira (ISLA-704); 1 nymph, Toca do Ossos (cave) (10°55′52″S, 41°03′24″W), Ourolândia, Bahia, VII-2009, R. L. Ferreira (ISLA-707); 1 female, Túnel da Fazenda do Sol V (mine) (16°20′35.4″S, 41°27′03.7″W), Medina, Minas Gerais, 15-VII-2009, L. F. O. Bernardi (ISLA-700); 1 male, Gruta dos Três Salões (cave) (20°15′46.5″S, 45°38′08.4″W), Arcos, Minas Gerais, 2009, R. A. Zampaulo (ISLA-709); 1 female, Gruta do Trinta (cave) (05°12′44.3″S, 37°15′51.1″W), Mossoró, Rio Grande do Norte, 11-VI-2010, D. Bento

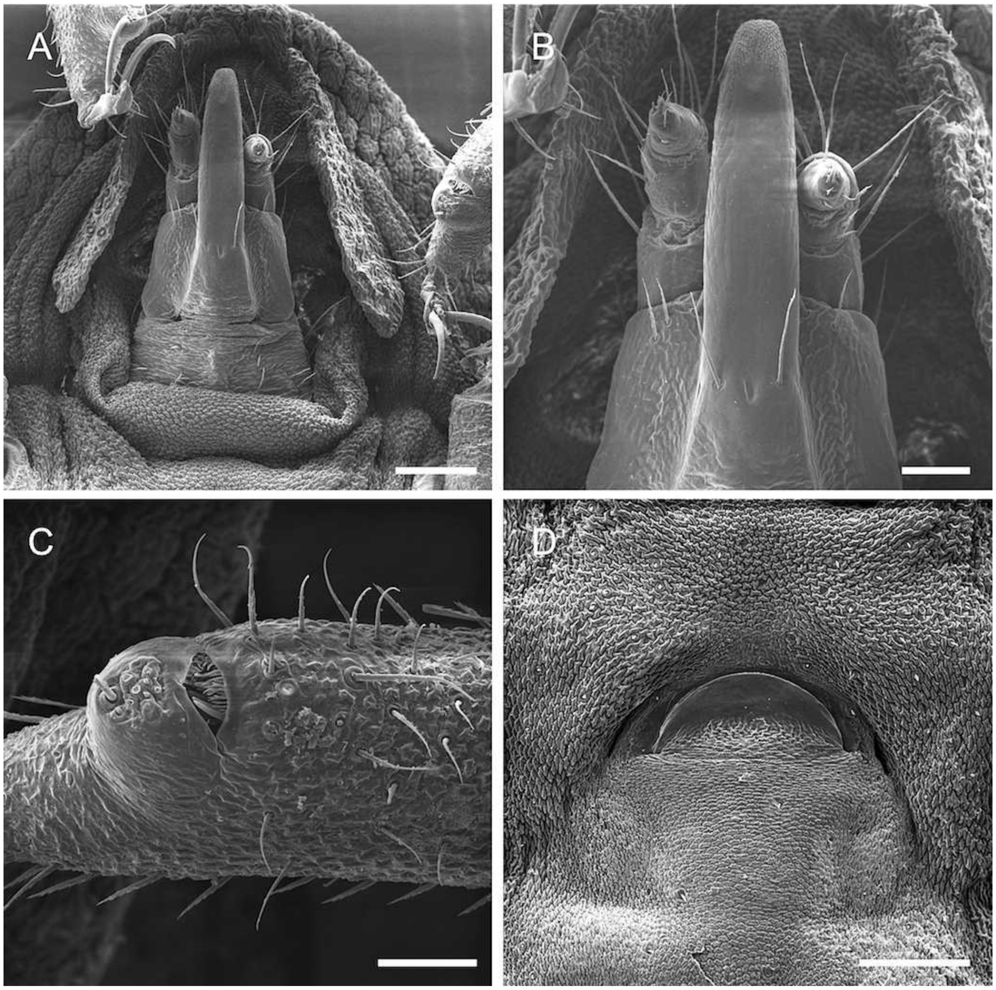

FIGURE 4. *Ornithodoros cavernicolous* n. sp., male. (**A**) Capitulum. (**B**) Hypostome base. (**C**) Haller's organ. (**D**) Genital opening. Bars = 100 μm.

(ISLA-1437); 1 nymph and 4 females, Gruta do Calixto (cave) (13°17′3520″S, 41°03′4790″W), Iramaia, Bahia, 01-I-2010, L. F. O. Bernardi (ISLA-706); 2 larvae*, Furna do Fim do Morro (cave) (10°38′25.8″S, 37°52′02.5″W), Paripiranga, Bahia, I-2010, L. F. O. Bernardi (CNC-1898); 1 larva* from *C. perspicillata*, 2 larvae* from *Desmodus rotundus* (E. Geoffrey), 1 larva (internal contents taken off for DNA extraction) from *Anoura caudifer* E. Geoffroy, Orizona (17°02′02″S, 48°17′52″W), Goiás, 10-X-2010, A. M. Souza and A. D. Cabral (CNC-1830, 1839, 1840; IBSP-10642; USNTC); 1 female, Caverna SL-092 (cave) (05°57′32.48251″S, 49°38′06.24857″W), Parauapebas, Pará, 22-VII-2010, R. A. Zampaulo et al. (CNC-1940).

Hosts and distribution: Larvae of *O. cavernicolous* were collected from 3 species of bats (*A. caudifer*, *C. perspicillata*, and *D. rotundus*). Hosts for nymphs and adults are unknown, but the finding of at least 2 engorged females suggests that they are active feeders. The species is widespread in Brazil, occurring in at least 6 states (Bahia, Ceará, Goiás, Minas Gerais, Pará, and Rio Grande do Norte) located in 4 geographical regions (north, northeast, southeast, and central west). The distribution of *O. cavernicolous* n. sp. is likely to follow the distribution of its bat hosts, which are widespread throughout the country (Reis et al., 2007).

Etymology: The specific epithet derives from the Latin *caverna* (=cavern) and *colō* (=to inhabit), in allusion to the habitat where this species was found.

Remarks

Adults and nymphs of *O. cavernicolous* are easily separated from their congeners by the following combination of characters: body outline pyriform; disks absent; hypostome long, thin, with only very small denticles on the apical portion; capitulum extendable, being basis capituli visible from above when extended; presence 2 setae at the beginning of the posterior third of hypostome; and presence of vestigial humps on tarsi II–IV. Larvae of *O. cavernicolous* are distinct in having 15 pairs of dorsal setae; triangular and small dorsal plate; 3 pairs of basal setae on tarsus I; and hypostomal dentition with 4/4 in the anterior portion, being file 1 with 12 to 14 denticles, file 2 with 10 to 13 denticles, file 3 with 4 to 6 denticles, and file 4 with 3 to 5 denticles.

16S rRNA gene sequences and phylogenetic position

PCR products were amplified from 4 specimens (1 female from Furna do Araticum, Ubajara, Ceará; 1 nymph from Gruta do Ubajara, Ubajara, Ceará; 1 nymph from Casa de Pedra, Martins, Rio Grande do Norte; and 1 larva from Orizona, Goiás) of *O. cavernicolous* and generated 2 genotypes of 428 nucleotides of the 16S rRNA gene, with the female sequence differing by a single nucleotide (G to A) from the nymphal and larval sequences. By BLAST analysis, these partial sequences of the 16S rRNA gene of *O. cavernicolous* indicated a relationship with *O. rondoniensis* (EU090907), *O. capensis* (AB076080), *N. amazoniensis* (HM047069), and *O. mimon* (GU198362), but with relatively low (83–84%) sequence identities. Phylogenetic analysis based on the 16S rRNA gene sequences supports the placement of *O. cavernicolous* within a large clade that includes all New World bat-associated argasids for which sequences are available. However, the new species seems to represent an independent lineage within *Ornithodoros* that will probably include its closest congener *O. azteci*. At least 5 specimens of *O. azteci* from Panama were subjected to DNA extraction and PCR testing, but no amplification was achieved, possibly because these specimens have been conserved in

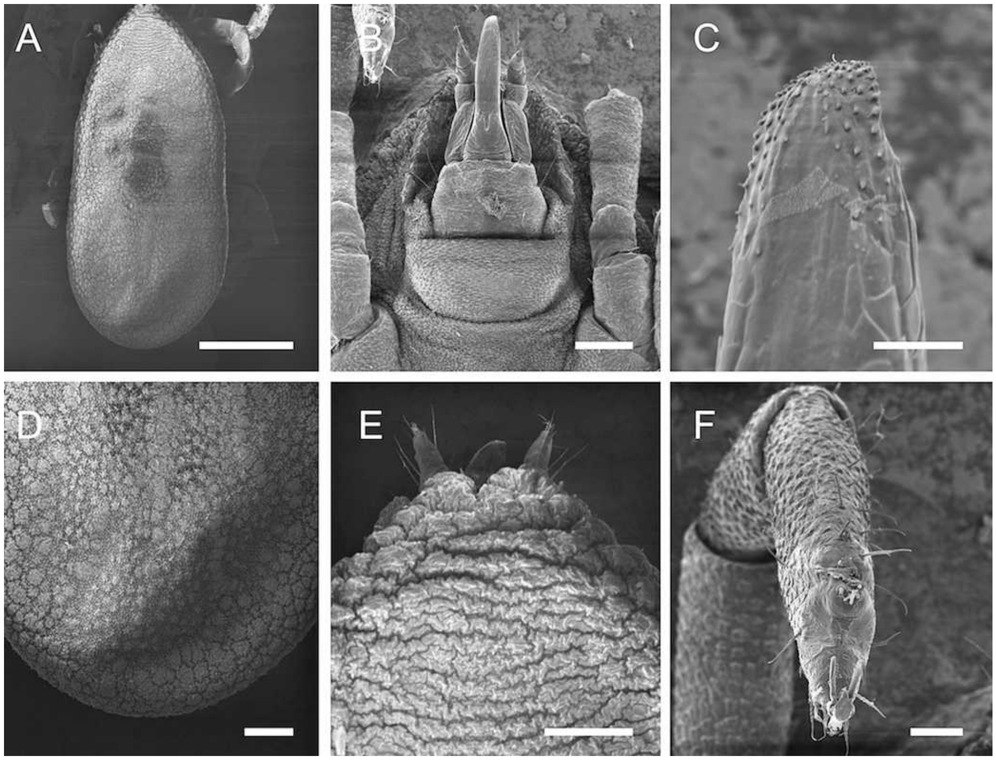

FIGURE 5. *Ornithodoros cavernicolous* n. sp., nymph. (**A**) Dorsal view (bar = 500 μm). (**B**) Capitulum (bar = 100 μm). (**C**) Hypostome apice (bar = 20 μm). (**D**) Detail on dorsal posterior integument (bar = 100 μm). (**E**) Detail on dorsal anterior integument (bar = 100 μm). (**F**) Haller's organ (bar = 50 μm).

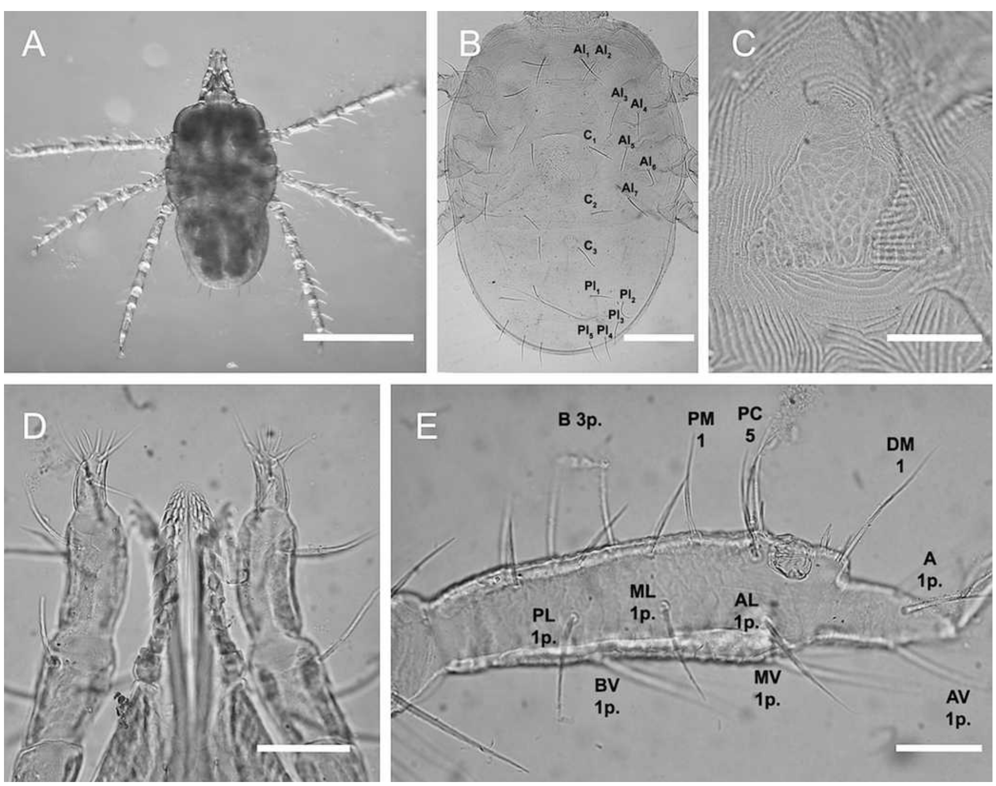

FIGURE 6. *Ornithodoros cavernicolous* larva. (**A**) General view of a slightly engorged larva (bar = 900 μm). (**B**) Dorsal view of dorsal setae: Al, anterolateral setae; C, central setae; Pl, posterolateral setae (bar = 300 μm). (**C**) Dorsal plate (bar = 80 μm). (**D**) Hypostome (bar = 70 μm). (**E**) Tarsus I setae: A, apical: 1 pair; DM, distomedian: 1 seta; PC, paracapsular: 5 setae; PM, posteromedian: 1 seta; B, basal: 3 pairs; AV, apicoventral: 1 pair; MV, midventral: 1 pair l; BV, basiventral: 1 pair; AL, anteroventral: 1 pair; ML, midlateral: 1 pair; PL, posterolateral: 1 pair (bar = 50 μm).

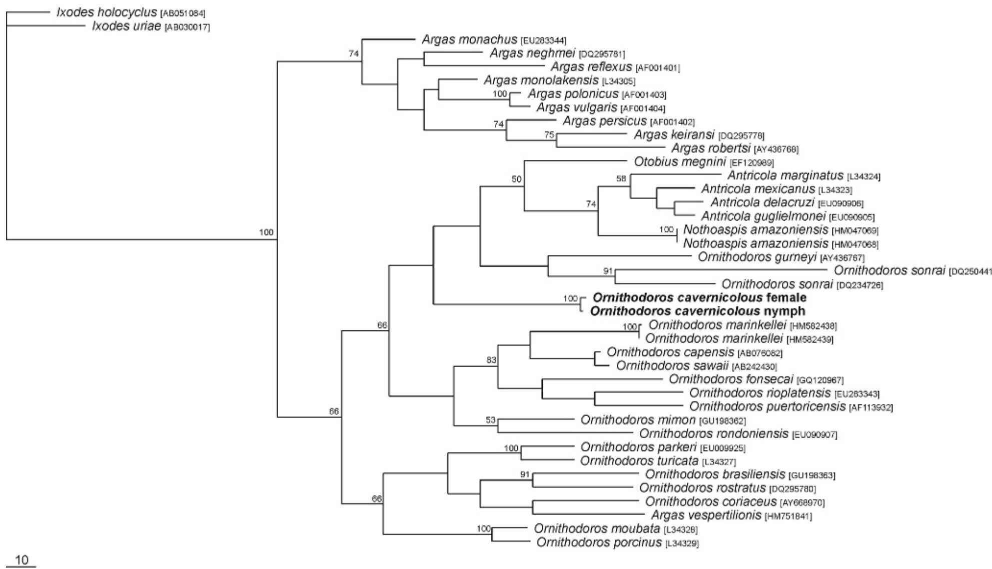

FIGURE 7. Phylogenetic tree based on 34 Argasidae ticks and 2 species of *Ixodes* (outgroup). The alignment of 384 characters was used for maximum parsimony inferences. Bootstrap confidence levels (from 500 replications) ≥50% are shown above the branches. Numbers in parentheses are GenBank accessions.

70% ethanol for a long time (since 1984), resulting in DNA degradation. The phylogenetic tree (Fig. 7) generated here possessed several interesting features. In particular, *Ornithodoros sonrai* Sautet & Witkowski is not monotypic, and *Argas vespertilionis* (Latreille) is included within a group of species belonging to the subgenera *Pavlovskyella* Pospelova-Shtrom and *Ornithodoros* Koch. Remarkably, the latter feature contradicts the idea that *A. vespertilionis* is the type species for *Carios* Latreille species as proposed in the classification of Argasidae of Klompen and Oliver (1993). In the same way, the tree demonstrates that *Argas* is paraphyletic, with the inclusion of *A. vespertilionis*. Certainly, the level of resolution of the tree branch encompassing most *Ornithodoros* species is poor, sometimes being supported by low bootstrap values (<50%). Undoubtedly, new sequences and new gene targets need to be generated to resolve the phylogeny of Argasidae, particularly to better define the generic and subgeneric position of most species included in *Ornithodoros*.

Species relationships

Ornithodoros cavernicolous clearly forms a species group with *O. azteci* (the type species of the group), designated hereafter as the ''*Ornithodoros* (*Alectorobius*) *azteci* group.'' Both species are classified placed into the subgenus *Alectorobius* Pocock that includes argasid species whose adults have cheeks and legs with micromammillate cuticles. Moreover, both species have a body in pyriform outline and hypostome that is long and thin, with minute denticles on the apical portion (Matheson, 1935).

The definition of *Alectorobius* provided by Clifford et al. (1964) is obsolete. Originally, this subgenus included larvae with pointed hypostome, but it now includes 3 species whose larvae present hypostome bluntly pointed anteriorly (*O. azteci*, *O. capensis*, and *O. cavernicolous*). Moreover, the subgenus now contains several species with capsule of Haller's organ with reticulations (*Ornithodoros yumatensis* Cooley & Kohls, *Ornithodoros brodyi* Matheson, *Ornithodoros dyeri* Cooley & Kohls, *O. azteci*, *Ornithodoros rossi* Kohls, Sonenshine & Clifford, and *O. cavernicolous* n. sp.). According to Kohls et al. (1965), larvae of species belonging to *Alectorobius* have a dorsum with 14–25 pairs of setae; dorsal plate present, elongated or pyriform; hypostome usually pointed anteriorly, with denticles throughout its length, dentition 3/3 to 5/5 in anterior portion; and short PH_1 setae. In *O. cavernicolous* larvae, the dorsal plate is typically triangular. In their re-description of the *O. azteci* larva, Kohls et al. (1965) mentioned a moderately large dorsal plate that is triangular to pyriform in shape. Thus, besides the fact that these authors could have been dealing with more than 1 species under the name *O. azteci*, their description for larvae belonging to the subgenus *Alectorobius* also should be amended. Indeed, larvae belonging to this subgenus might present an elongate dorsal plate that is pyriform or triangular in shape; a blunt hypostome; and denticles that may be absent from a portion at the base of hypostome, as in *Ornithodoros rioplatensis* Venzal, Estrada-Peña & Mangold.

The original description of *O. azteci* larva by Matheson (1935) is of little use. For this reason, larval comparisons in the present study were carried out with the re-description performed by Kohls et al. (1965) that is based mainly on specimens collected from bats in Trinidad. In this regard, *O. cavernicolous* larvae have 15 pairs of dorsal setae, whereas *O. azteci* larvae from Trinidad possess from 17 to 21 pairs of dorsal setae. Another important difference is in the dorsal plate that is triangular to pyriform in Trinidadian larvae, averaging 205 μm in length and 185 μm in width. In contrast, *O. cavernicolous* larvae presented a small (165 μm in length and 163 μm in width, on average) triangular dorsal plate. The larva of *O. azteci*, Kohls et al. (1965) have 2 pairs of basal setae (B) on tarsus I, and Klompen (1992) mentioned the presence of additional dorsal setae in *O. azteci*. In *O. cavernicolous* larvae, 3 pairs of basal setae on tarsus I were observed.

Adults of *O. cavernicolous* seem to be slightly wider than those of *O. azteci*. Noteworthy, *O. azteci* adults from Panama have humped tarsi, that is, they present a distinct ''gibbosity'' (hump) on tarsi I to IV. Indeed, in *O. cavernicolous* adults, these protuberances are distinct on tarsus I, but vestigial on tarsi II–IV (Fig. 8). Likewise, *O. azteci* adults from Panama present distinctly elevated mammillae in the preanal region that are not observed in *O. cavernicolous* (Fig. 9). According to Clifford et al. (1964), by definition, adults belonging to the subgenus *Alectorobius* have an integument with distinct mammillae and discs. However, the integument of adult *O. azteci* and *O. cavernicolous* lacks evident discs.

DISCUSSION

In the present study, a new species of *Ornithodoros* is described based on tick specimens collected from different Brazilian regions. The new species, namely, *O. cavernicolous*, forms a species group with *O. azteci* that is designated as the ''*Ornithodoros* (*Alectorobius*) *azteci* group.'' The new species extends the known geographical distribution of this species group toward the south, but it seems to be widespread in South America, Central America, Mexico, and the Caribbean region (Matheson, 1935, 1941; Cooley and Kohls, 1944; Kohls et al., 1965; Guglielmone et al., 2003). Probably, the wide geographical range of members of the *O.* (*A.*) *azteci* group is also a result of the widespread distribution of their

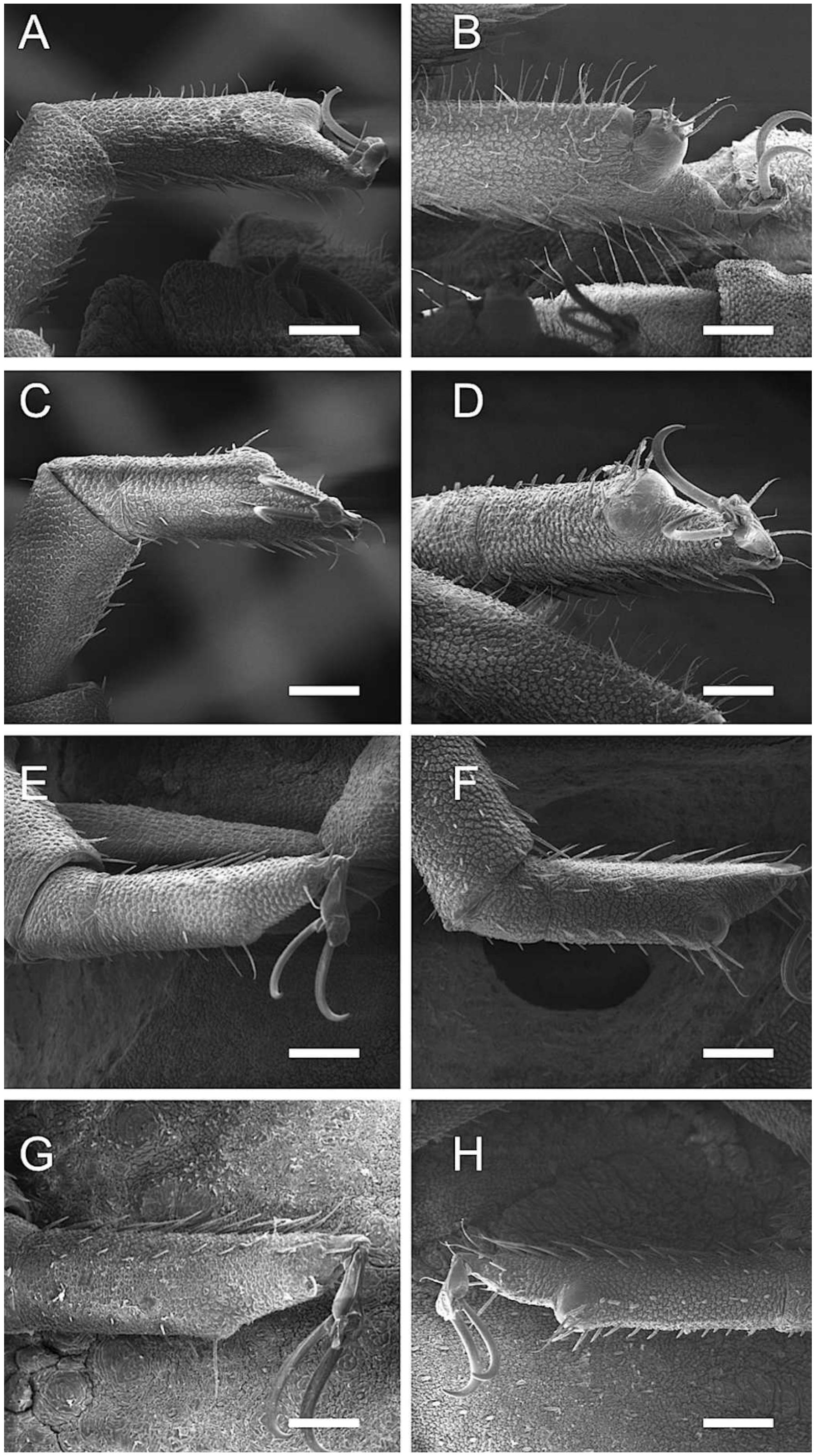

FIGURE 8. *Ornithodoros azteci* species group tarsi I–IV. (**A**) Tarsus I, adult *O. cavernicolous* n. sp. (**B**) Tarsus I, adult *O. azteci*. (**C**) Tarsus II, adult *O. cavernicolous* n. sp. (**D**) Tarsus II, adult *O. azteci*. (**E**) Tarsus III, adult *O. cavernicolous* n. sp. (**F**) Tarsus III, adult *O. azteci*. (**G**) Tarsus IV, adult *O. cavernicolous* n. sp. (**H**) Tarsus IV, adult *O. azteci*. Bars = 100 μm.

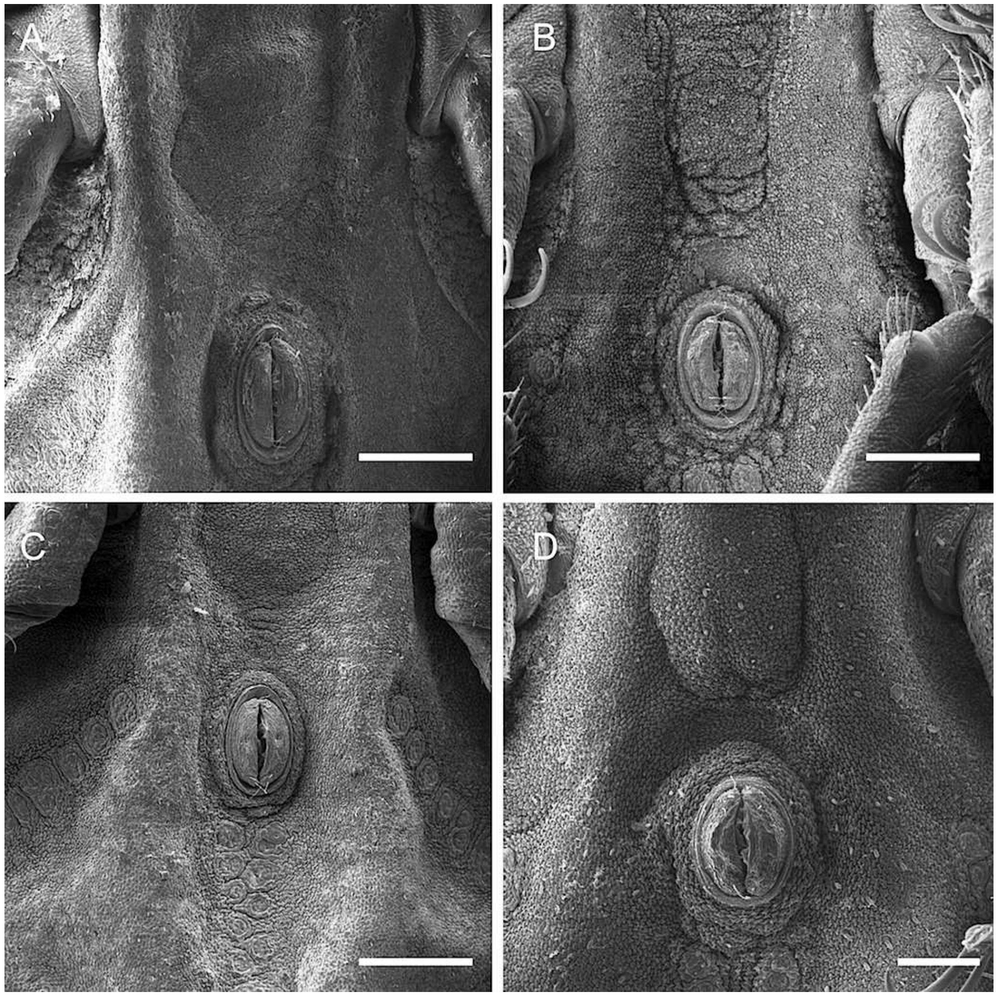

FIGURE 9. *Ornithodoros azteci* species group anus and supra-anal integument. (**A**) Female *O. cavernicolous* n. sp. (**B**) Female *O. azteci*. (**C**) Male *O. cavernicolous* n. sp. (**D**) Male *O. azteci*. Bars = 100 μm.

hosts, i.e., frugivorous, hematophagous, and insectivorous bats (Kohls et al., 1965).

Larvae of *O. cavernicolous* are parasitic on bats. The hosts of *O. cavernicolous* adults are unknown, but the finding of at least 2 engorged females suggests they are active feeders. The mouthparts of adults of argasid ticks vary enormously in terms of morphology, and this has been related to their feeding habits. For example, it has been speculated that adults of *Antricola* spp. probably do not feed because of their poorly developed mouthparts (Oliver, 1989). *Antricola* spp.-like hypostomes also have been observed in a recently described *Ornithodoros* species (Labruna et al., 2008). The hypostome of nymphs and adults of *O.* (*A.*) *azteci* group ticks, i.e., *O. azteci* and *O. cavernicolous*, is unique and suggests that they are very specialized blood feeders. The presence of 1 pair of setae at the beginning of the posterior third of hypostome also indicates that nymphs and adults do not insert the whole hypostome into the host's skin. Again, the existence of very reduced denticles restricted to the apical portion of hypostome suggests that they are superficial feeders. Unfed adults and nymphs of *O. cavernicolous* were found crawling freely on the ground, mainly on bat guano, and in cracks and crevices on the wall and ceiling of several Brazilian caves. Although the possibility that adults and nymphs of *O. cavernicolous* can eventually feed on ground animals cannot be ruled out, it is very likely that they use bats as primary hosts.

Since the works of Kohls et al. (1965, 1969) and Jones and Clifford (1972), the identification of argasid species has largely been based on the morphology of larvae, because nymphs and adults are often inadequate for taxonomy due to the lack of external characters suitable for species identification (Venzal et al., 2008; Estrada-Peña et al., 2010). However, a few Neotropical bat-associated argasid species have been identified based on adult external morphology, e.g., *O. rondoniensis* (Labruna et al., 2008), *O. marinkellei* (Labruna et al., 2011), and *O. azteci* (Cooley and Kohls, 1944). Adults of *O. cavernicolous* also present a unique combination of characters that allow its easy separation from all other species of *Ornithodoros*. The same is true for nymphs of *O. cavernicolous* that are easily distinguished from its congeners. Incidentally, in the present study, only large nymphs were measured, and the number of nymphal instars presented for the new species is unknown.

The present work suggests that ticks previously identified as *O. azteci* might actually represent a distinct species. In fact, the number of dorsal setae (ranging from 17 to 21 pairs) and the shape of dorsal plate (triangular to pyriform) reported for *O. azteci* larvae by Kohls et al. (1965) might indicate that they were dealing with more than 1 species. In this context, further field studies in countries such as Panama, Trinidad, Venezuela, and Brazil are needed to better define the so-called *O.* (*A.*) *azteci* group.

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