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NEW REPORTS OF *ANTRICOLA GUGLIELMONEI* AND *ANTRICOLA DELACRUZI* IN BRAZIL, AND A DESCRIPTION OF A NEW ARGASID SPECIES (ACARI)

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ABSTRACT: Adults of 3 tick species (Acari: Argasidae) identified as *Antricola guglielmonei*, *Antricola delacruzi*, and *Carios rondoniensis* n. sp. were collected on bat guano in a cave in the state of Rondônia, western Amazon, Brazil. Adults of *C. rondoniensis* possess a unique combination of characters that distinguish them from all described adults in the Argasidae, i.e., a large spiracular plate densely filled with small goblets, a well-developed flap covering the female genital opening, and palpi containing several tufts of long setae on articles 2 and 3. Unlike *Ornithodoros* or other *Carios* species, adults of *C. rondoniensis* have a scooplike hypostome devoid of denticles, as in *Antricola* spp. Conversely, the presence of a pair of long posthypostomal setae, and a slitlike transverse fissure at the capsule opening of the Haller's organ, are characters of *C. rondonensis* that are also found in species of *Carios* and *Ornithodoros*, but not in *Antricola* species. Molecular analyses inferred from a portion of the 16S rRNA mitochondrial gene indicate that *C. rondoniensis* is phylogenetically closest to species of *Carios*, followed by species of *Antricola*, and then *Ornithodoros*. Because the highest bootstrap value linking *C. rondoniensis* to *Carios* spp. was 62%, further phylogenetic studies are needed to better evaluate the taxonomic status of the former species.

Antricola was erected by Cooley and Kohls (1942) to include *Antricola coprophilus* (McIntosh), a species originally described in *Ornithodoros*. Currently, there are 16 known *Antricola* species, all restricted to the western hemisphere, particularly the southeastern United States, Mexico, Central America, the Caribbean region, Colombia, Venezuela, and Brazil (Guglielmone et al., 2003; Estrada-Peña et al., 2004). Typically, *Antricola* species live in warm, humid caves inhabited by several species of bats (De la Cruz, 1973). Females are autogenous and only the larval stage is parasitic (De la Cruz, 1973); however, a recent study indicates that early nymphal stages of *A. delacruzi* Estrada-Peña, Barros-Battesti and Venzal feed on vertebrate hosts (Estrada-Peña et al., in press).

Extensive morphological, biological, and a few molecular phylogenetic studies by Klompen (1992), Klompen and Oliver (1993), and Klompen et al. (1996) concluded that all bat-associated argasid genera, (including *Antricola*) are part of a single, monophyletic lineage of ticks, which were transferred to *Carios* Latreille, raised from *Argas* (*Carios*). Because this proposal would require invalidation of *Antricola*, it has not been accepted by researchers from the Neotropical region, who consider it premature and have argued that additional evidence is needed from studies on morphology, life histories, host associations, and molecular taxonomy (Estrada-Peña et al., 2004; Guglielmone et al., 2005). However, Horak et al. (2002) accepted the new classification, relegating all *Antricola* species to *Carios*, although they too state that additional molecular taxonomic studies are needed to clarify this issue. For instance, molecular taxonomy has seldom been applied to Argasidae (Guglielmone et al., 2005).

In the present study, we provide new collection data for 2 *Antricola* species from Brazil. In addition, we describe a new species of *Carios* that shares morphological characters with *Carios* species formerly classified as *Antricola* species, as well as other *Carios* species formerly classified as *Ornithodoros*. The new species is biologically and ecologically similar to *Antricola*, but our molecular analysis, inferred from a fragment of the 16S rDNA gene, indicates that it is more closely related to bat-associated *Ornithodoros* species (now raised to *Carios*) than to *Antricola* species. Because our molecular analysis separates the proposed *Carios* group from *Ornithodoros*, we prefer to describe it as a new species of *Carios*. However, we have retained *Antricola* as a valid genus, which is supported by our phylogenetic analysis.

MATERIALS AND METHODS

Tick collection and morphological study

Ticks were collected on 16 August 2004 from bat guano in a cave in the state of Rondônia, western Amazon, Brazil. The cave is located within a primary Amazon forest area in Porto Velho Municipality (08°40′S, 63°51′W). All specimens were immediately fixed in 70% ethanol.

Ticks were collected and identified according to Estrada-Peña et al. (2004). A group of ticks that could not be identified was selected for describing the new species. Ten unfed specimens of each sex of the new species were measured with the use of the KS400 Zeiss program for analysis of images and morphometry, fitted to a Stemi SV6 Zeiss stereomicroscope. In the description, all measurements, first the range and followed by the mean ± the standard deviation in parentheses, are given in millimeters. Representative specimens were prepared for scanning electron microscopy (SEM) by the method of Corwin et al. (1979).

Molecular study

DNA was individually extracted from representative specimens (1 male and 1 female per species) of the tick species collected in the present study and processed by polymerase chain reaction (PCR) with the use of primers targeting a ≈460–base pair (bp) fragment of the 16S rRNA mitochondrial gene, as previously described (Mangold et al., 1998). In addition, 1 paratype of *A. guglielmonei* Estrada-Peña, Barros-Battesti and Venzal, collected in the state of Sergipe, northeastern Brazil (10°50′S, 37°27′W), by Estrada-Peña et al. (2004) was processed by PCR. PCR products of the expected size were cloned with the use of the TOPO TA Cloning kit (Invitrogen, Carlsbad, California), according to the manufacturer's instructions. Plasmids containing DNA inserts of the expected sizes were sequenced at least 4 times with the use of an ABI automated sequencer (Applied Biosystens/Perkin Elmer, model ABI Prism 310 Genetic, Foster City, California) with the same primers used for PCR.

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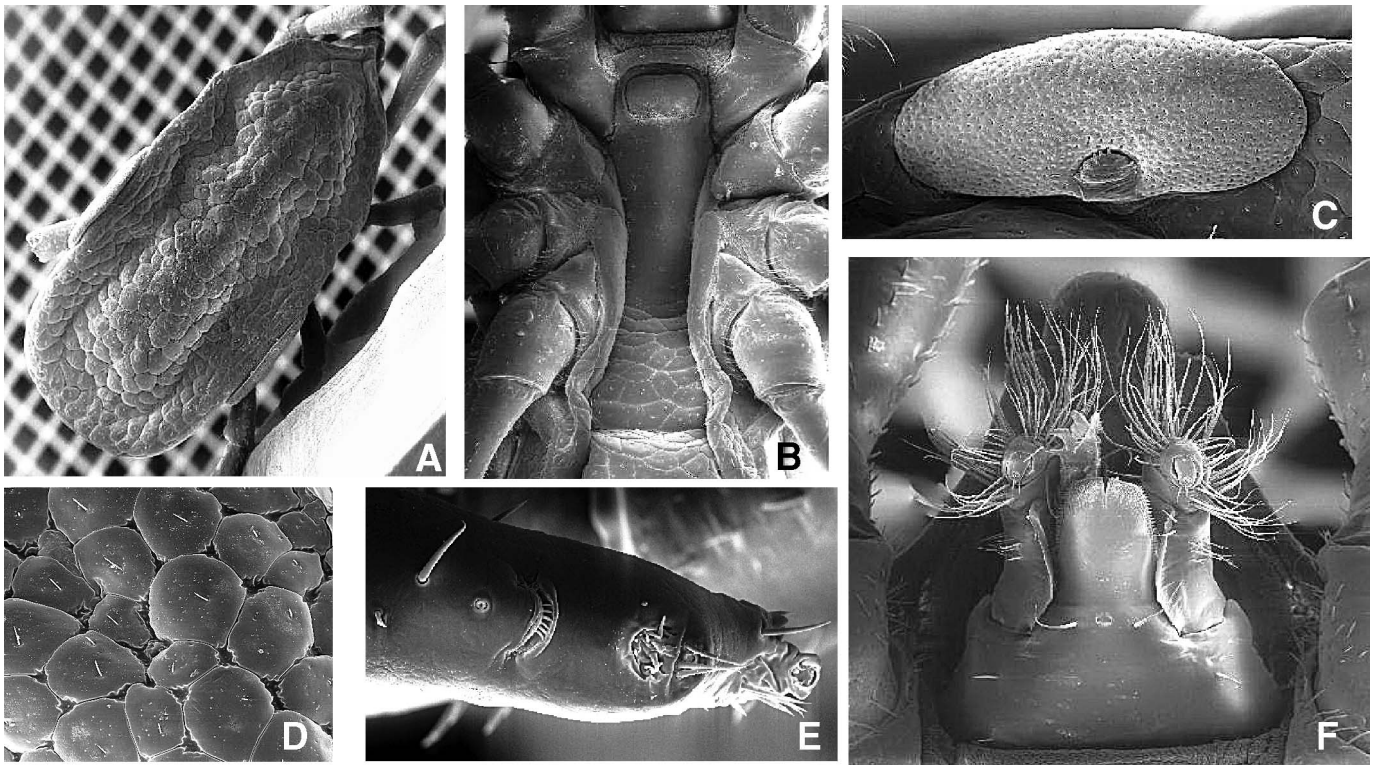

FIGURE 1. Scanning electron microscopy of *Carios rondoniensis* male. (**A**) Dorsal view (18×). (**B**) Coxae and genital opening (30×). (**C**) Spiracular plate (60×). (**D**) Dorsal mammillae (100×). (**E**) Haller's organ and anterior pit (200×). (**F**) Ventral capitulum (70×).

DNA sequences were aligned using the Clustal/W method (Thompson et al., 1994) and edited with the use of Bioedit 7.0.5.3 software (Hall, 1999) to obtain the consensus sequence for each tick specimen. Nucleotides that were obviously misaligned were manually shifted. The corresponding 16S rRNA partial sequences of other argasid species (Fig. 3) and the ixodid species *Ixodes uriae* White (used as outgroup because the Ixodidae is a sister group of Argasidae) available in GenBank were aligned with the sequences obtained in this study with the use of Clustal/W for phylogenetic analysis. Three different methods were applied to build the phylogenetic trees, i.e., maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP). All trees were calculated with the program PAUP 4.0b1 (Swofford, 1999). ML and NJ phylogenies were built with the GTR + G with nonvariables site, considering a gamma shape parameter of 0.5 as indicated by FindModel at http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html. The stability of the trees was assessed by bootstrapping over 1,000 replicates.

RESULTS

A total of 151 tick specimens was collected in the cave, comprising 3 different species, i.e., *A. guglielmonei* (5 males, 20 females), *A. delacruzi* (8 males, 31 females), and a third group of 24 males and 63 females, which were considered a new *Carios* species, as describe below.

DESCRIPTION

Carios rondoniensis n. sp.

(Figs. 1 and 2)

Male (dorsal): Outline oval, pointed anteriorly, broadest at level of spiracular plate (Fig. 1A). Length from pointed anterior end to posterior body margin 4.88–6.75 (5.89 ± 0.56), breadth 2.50–3.38 (2.93 ± 0.25). Submarginal grooves present and distinct, fused anteriorly. Entire idiosoma with smooth, tilelike mammillae of variable shape (rounded, pentagonal, rectangular); mammillae devoid of setae anteriorly, with 1–3 small separated setae posteriorly (Fig. 1D); distinct elongate mammillae dorsal to spiracular plate. Discs in depressed areas along submarginal grooves; 2 pairs of discs in anteromedian portion.

Male (lateral): Mammillae as dorsally; however, anterior mammillae tend to be fused and devoid of setae; a distinct lateral groove present from anterior of body to spiracular plate. Spiracular plates very large, located at level of coxa IV, elliptical (length 0.95–1.77 [1.22 ± 0.26], width 0.47–0.74 [0.60 ± 0.10]); dorsal margin visible from above, protruding above level of idiosoma dorsum, with numerous small goblets; macula located in median–ventral margin (Fig. 1C).

Male (ventral): Mammillae as dorsally, except on central area, from genital opening to level of coxa IV, which is smooth because of fused mammillae (Fig. 1B); fresh or well-preserved specimens with distinct reddish stain in central part of smooth area, at level of coxae III. Genital opening rectangular with rounded angles, located at level of coxa I. Distinct median and transverse postanal grooves; coxal folds extending from coxa II to near posterior end, where they diverge; transverse preanal groove straight. Anal plate elliptical. Anterior end of idosoma with bulbous structure containing fingerlike projection; hood poorly developed but with 2 distinct hornlike projections anteriorly. Camerostome well developed as a depression receiving capitulum; surface micromammillated; cheeks indistinct.

Male (capitulum): Basis capituli tumescent, slightly wider than longer, rounded laterally. Surface smooth and shining, with a few scattered small setae in lateral fields. Hypostome small, amber-colored, scooplike, rounded apically, without distinct denticles; chelicerae well developed. With 2 pairs of posthypostomal setae reaching about three-fourths length of hypostome. Palps rounded laterally, with ventromedial integumental ridgelike extension on article 1. Several tufts of long setae present on articles 2 and 3; tuft of shorter setae laterally on article 1 (Fig. 1F). Some difficulty in clearly observing magnitude of the palpal tufts by optical microscopy because setae tend to be entangled in alcohol-preserved specimens.

Male (legs): Long and smooth, sparsely setose, except for tarsi II–IV, which are densely setose. All coxae contiguous, decreasing in size

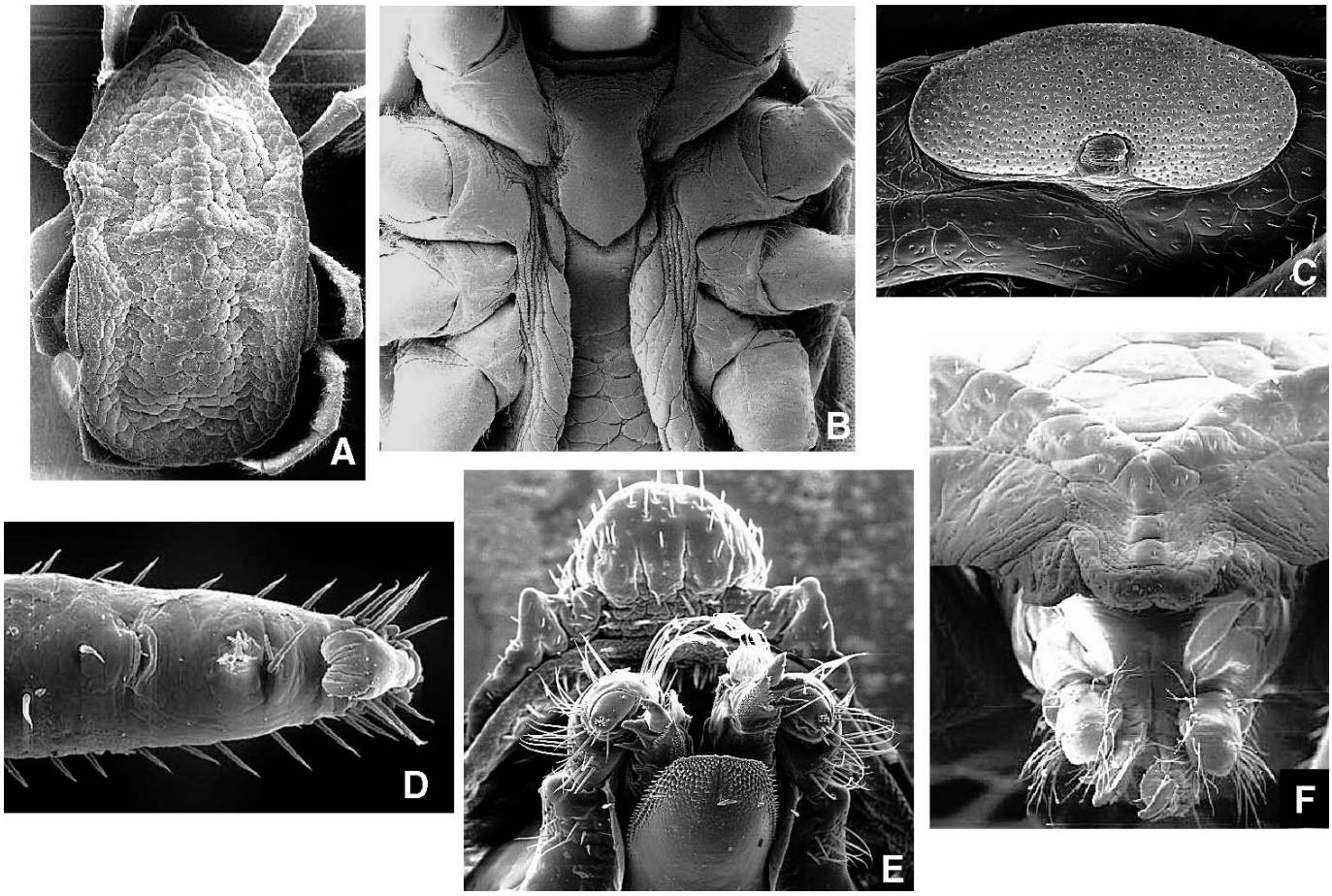

FIGURE 2. Scanning electron microscopy of *Carios rondoniensis* female. (**A**) Dorsal view (16×). (**B**) Coxae and genital opening (35×). (**C**) Spiracular plate (70×). (**D**) Haller's organ and anterior pit (200×). (**E**) Ventral capitulum and anterior of idiosoma (130×). (**F**) Dorsal capitulum and anterior of idiosoma (76×).

from I to IV, without spurs. Small triangular pointed medial spur on trochanters I–IV. Claws absent. All tarsi lacking subapical dorsal humps. Anterior tip of Haller's organ with distinct anterior and posterior sections; capsule opening transverse, slitlike (Fig. 1E).

Female (dorsal): Body outline oval, pointed anteriorly, broadest at level of spiracular plates (Fig. 2A). Length from pointed anterior end to posterior body margin 5.50–6.00 (5.78 ± 0.18), width 2.63–3.25 (2.94 ± 0.19). Other features as in male.

Female (lateral): Spiracular plates very large (Fig. 2C), elliptical (length 0.98–1.37 [1.24 ± 0.12], width 0.47–0.63 [0.56 ± 0.06]), at level of coxa IV. Other features as in male.

Female (ventral): Mammillae as dorsally, except in central area from genital opening to level of coxae IV, which is smooth due to fused mammillae. Genital opening covered by flap consisting of calyx-shaped integumental extension (Fig. 2B); a pair of distinct small folds between coxae II and integumental extension. In 2 specimens, this flap was intentionally removed and the genital opening was found to be U shaped, situated at level of coxae II. Median and transverse postanal grooves clearly visible; coxal folds extending from coxa II almost to posterior margin, where they diverge; transversal preanal groove straight. Anal plate elliptical. Anterior of idiosoma and camerostome as in male (Fig. 2E, F).

Female (capitulum): As in male.

Female (legs): As in male (Fig. 2D).

Taxonomic summary

Hosts: Adult ticks collected on bat guano.

Material examined: Holotype male (CNC 1039) and allotype female (CNC 1039), collected in a cave at Porto Velho (08°40′S, 63°51′W), state of Rondônia, Brazil, 16 August 2004, by M. B. Labruna and F. A. Terassini, deposited in the ''Coleção Nacional de Carrapatos,'' Faculty of Veterinary Medicine of the University of São Paulo, São Paulo, SP, Brazil; 15 paratype males and 53 paratype females deposited in the Faculty of Veterinary Medicine of the University of São Paulo with same collection data, except CNC-1040; 2 paratype males and 3 paratype females deposited in the Tick Collection of the Instituto Butantan, São Paulo, Brazil (IBSP 9698); 1 paratype male and 1 paratype female deposited in the Tick Collection of the Department of Parasitology, Veterinary Faculty (Zaragoza, Spain); 3 paratype males and 3 paratype females deposited in the Department of Veterinary Parasitology, Veterinary Faculty (Montevideo, Uruguay); 2 paratype males and 2 paratype females deposited in the U.S. National Tick Collection, Georgia Southern University (Statesboro, Georgia).

Location: Brazilian cave, associated with bat guano.

Etymology: The species is named for the state of Rondônia, Brazil, where the type specimens were collected.

Molecular analysis

PCR products were amplified from adults of every species studied. Generated sequences had 415–427 nucleotides, corresponding to a portion of the 3′ half of the 16S rRNA gene of tick sequences available in GenBank. All 3 *A. guglielmonei* specimens (2 from Rondônia and 1 from Sergipe) had exactly the same nucleotide sequence and were represented by a single sequence in the similarity tree (Fig. 3). The same procedure was adopted for 2 *A. delacruzi*, and for 2 *C. rondoniensis* specimens, which generated the same sequence for each species. Because 16S rRNA gene partial sequences of some Argasidae species in GenBank available are shorter than 415 nucleotides, we used corre-

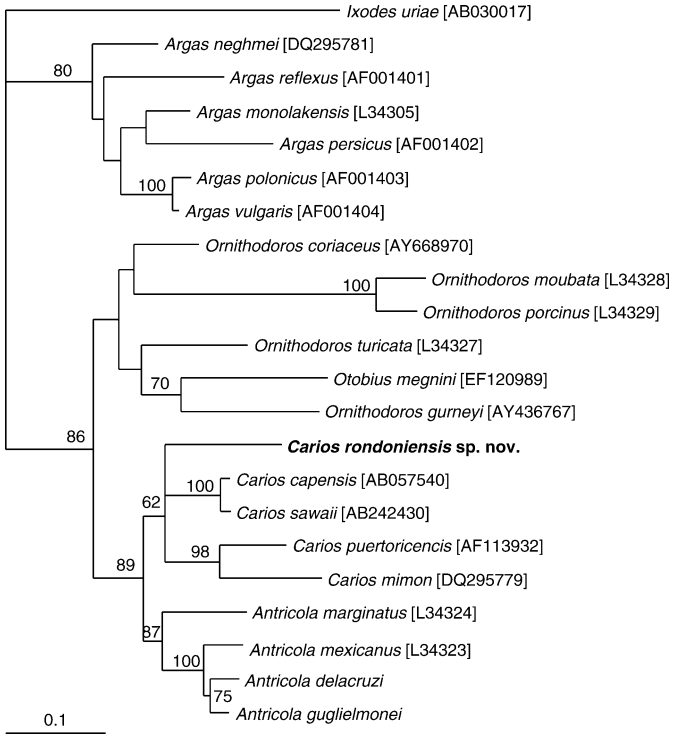

FIGURE 3. Similarity tree constructed to compare the 16S rRNA partial sequence (391 bp) of *Carios rondoniensis* with corresponding sequences of other argasid species. The corresponding 16S rRNA sequence of *Ixodes uriae* was used as outgroup. The tree was constructed with the use of the neighbor-joining method with GTR + G (scale bar). Bootstrap confidence levels (from 1,000 replications) higher than 60% are shown above the branch tested. Numbers in brackets are GenBank accession numbers.

sponding fragments of 371–392 nucleotides to construct the similarity tree, which showed 4 distinct clades, each represented by *Argas*, *Ornithodoros*, *Carios*, and *Antricola* species. These 4 clades were supported by moderate to high bootstrap values (62–89%). The inclusion of the sequence of *C. rondoniensis* in the *Carios* clade was supported by a 62% bootstrap value in the NJ tree (Fig. 3). Both the ML and the MP trees showed the same topology as observed for NJ, but bootstrap values of *C. rondoniensis* within *Carios* spp. were 55 and <50%, respectively (data not shown). Nucleotide sequences of the 16S rDNA region generated in the present study have been deposited in GenBank as follows: *A. guglielmonei* (EU090905), *A. delacruzi* (EU090906), *C. rondoniensis* (EU090907).

DISCUSSION

Previous reports of cave-dwelling ticks in Cuba referred to different *Carios* (reported as *Ornithodoros*) and *Antricola* species occurring in the same cave (Cerny, 1967; De la Cruz, 1976, 1978). Similarly, in the state of Rondônia, we found 2 *Antricola* species and a new *Carios* species coinhabiting a cave. In an earlier study, Estrada-Pena et al. (2004) found the same 2 *Antricola* species in a cave in the state of Sergipe, located ≈2,900 km east of Rondônia. Molecular analysis of specimens from these 2 disparate Brazilian populations of *A. guglielmonei* yielded identical 16S rRNA partial sequences, indicating that these 2 populations have not been isolated for an extended period of time. Additional *A. guglielmonei* and *A. delacruzi* populations may exist in caves situated between these 2 widely distant sites. Our results contrast with collections from Cuba, where several of the 11 described *Antricola* species were found, each in a different cave (De la Cruz, 1978; De la Cruz and Estrada-Peña, 1995).

Adults of *C. rondoniensis* possess a combination of unique characters that distinguish them from all known adults ticks in the Argasidae, i.e., large spiracular plate densely filled with small goblets that resembles the spiracular plates in some ixodids, a well-developed flap covering the female genital opening, and palpi with several tufts of long setae on articles 2 and 3. Unlike *Ornithodoros* or other *Carios* species, adults of *C. rondoniensis* have a scooplike hypostome devoid of denticles, as in *Antricola* spp. Conversely, the presence of a pair of long posthypostomal setae, and a slitlike transverse fissure at the capsule opening of the Haller's organ, are characters of *C. rondonensis* that are also found in species of *Carios* and *Ornithodoros*, but not in *Antricola* species. The capsule of Haller's organ in *Antricola* species comprises a solid circular roof with a small opening at the center (De la Cruz and Dusbábek, 1989; Estrada-Peña et al., 2004).

Despite the peculiarities of *C. rondoniensis*, our molecular analysis indicates that it is phylogenetically closest to species of *Carios*, clustering with *Antricola* spp., and then with *Ornithodoros*. However, in all methods used to build our phylogenetic trees, the highest bootstrap value was *C. rondoniensis* within *Carios* spp., at 62%. Further phylogenetic studies based on molecular and morphological features, and incorporating greater numbers of *Carios*, *Antricola*, *Ornithodoros*, and *Nothoaspis* species, would help to elucidate the taxonomic status of *C. rondoniensis* and related genera more precisely.

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