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DESCRIPTION OF A NEW ARGASID TICK (ACARI: IXODIDA) FROM BAT CAVES IN BRAZILIAN AMAZON

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ABSTRACT: *Nothoaspis amazoniensis* n. sp. (Acari: Ixodida: Argasidae) is described from adult and immature ticks (nymph II, nymph I, larva) collected from bat caves in the Brazilian Amazon. Also, 16S rDNA sequences are provided. The diagnostic characters for adults are the presence of false shield or nothoaspis, an anteriorly projecting hood covering the capitulum, a medial extension of palpal article I (flaps), genital plate extending from coxa I to IV, absence of 2 setae on the internal margin of the flaps, a minute hypostome without denticles, presence of a central pore in the base of hypostome, and a reticulate surface pattern on the posterior half of the nothoaspis in males. The nymph II stage is characterized by a hood that is small in relation to the capitulum, short coxal setae, palpal flaps lacking setae on the internal margin, long hypostome, pointed with dentition 4/4 apically, and the anterior half of the body is covered by a cell-like configuration. Nymph I stage is characterized by a hood, small in relation to the capitulum, dorsum of the body covered by a cell-like configuration, venter integument covered by a cell-like configuration, and hypostome dentition 4/4 with apices that are “V”-shaped. Diagnostic characters of the larvae are the number and size of dorsal setae, and the shape of scutum and hypostome. The new species appears to have a life cycle with a larva that feeds on bats, a non-feeding nymphal stage (nymph I), a feeding nymphal stage (nymph II), and adults that probably represent non-feeding stages.

Argasid (soft) ticks include approximately 190 species around the world, 81 of which are present in the Neotropics, and 66 are exclusive of this region (Guglielmone et al., 2003, 2009; Labruna and Venzal, 2009; Nava et al., 2009). Currently, the taxonomic classification and phylogenetic relationships among the members of Argasidae are the subject of controversy. Different classifications have been proposed based on morphological characters (Filippova, 1961; Clifford et al., 1964; Pospelova-Shtrom, 1969; Camicas and Morel, 1977; Hoogstraal, 1985; Klompen and Oliver, 1993), without reaching consensus among tick researchers. In addition, molecular phylogenetics analyses performed in the last few years have failed to produce a robust and well-fitting scheme of classification, principally due to the poor representation of several groups (Nava et al., 2009).

In the Neotropical region, new species of soft ticks belonging to different genera have been recently described (Estrada-Peña et al., 2003, 2004; Labruna et al., 2008; Venzal et al., 2008; Labruna and Venzal, 2009). The present report suggests that the specific richness of Argasidae is probably underestimated in the Neotropics. One genus that has not been the subject of extensive study is *Nothoaspis*. At the moment, the only known species of this genus is *Nothoaspis reddelli* Keirans and Clifford, described from ticks collected in hot and humid bat caves in Mexico. The diagnostic characters of *Nothoaspis* include: anterior half of the dorsum covered by a smooth leathery integument resembling scutum of hard ticks (“false shield or nothoaspis”), capitulum situated ventrally beneath an anteriorly projecting hood, medial extension of palpal article I forming a ventral sheath for a minute hypostome, and a genital plate extending from coxa I to IV (Keirans and Clifford, 1975). This diagnosis is only based on the

morphology of males, because females are unknown. Also, only 1 nymphal stage was described, and the larva was determined as *N. reddelli* by association, since it did not have uniquely nothoaspine characters to allow its unequivocal assignment to *Nothoaspis* (Keirans et al., 1977).

In the present study, we describe all stages of a new species of *Nothoaspis* from recently collected specimens in the Brazilian Amazon. Also, 16S rDNA sequences of each stage are provided in order to confirm that they belong to the same taxon; biological data are presented with the aim of contributing to the knowledge of the life cycle of this new tick species.

MATERIALS AND METHODS

Study site and collection of ticks

Free-living ticks (adults, nymphs, and larvae) were collected during January 2009, July 2009, and January 2010 in 2 bat caves (1 and 2) located within the Porto Velho Municipality, state of Rondonia, western Brazilian Amazonia. The ticks were determined as species of *Nothoaspis* according to Keirans and Clifford (1975) and Keirans et al. (1977).

Cave 1 (08°40'43"S, 63°51'05"W, altitude 113 m) is located forward to the eastern margin of Madeira River, whereas cave 2 (08°37'59"S, 63°57'29"W, altitude 81 m) is located forward to the western margin of this river. The straight distance between the main entrances of the 2 caves is 12.7 km. Both caves are located inside primary Amazon forests. Cave 1 is typically hot and humid, with deep internal air temperature between 33 and 38 C, and a foul atmosphere (strong ammonia). In the hot part of the cave, various specimens of *Antricola guglielmonei* Estrada-Peña, Barros-Battesti, and Venzal, *Antricola delacruzii* Estrada-Peña, Barros-Battesti, and Venzal, and a few *Carios rondoniensis* Labruna, Terassini, Camargo, Brandão, Ribeiro, and Estrada-Peña, were recently collected. The *Nothoaspis* specimens of the present study were collected not so deeply in the cave, only ~2 m from the cave entrance, inside cracks and crevices on the cave ceiling, where mean temperature was 30.8 ± 0.9 C. In this part of the cave, *C. rondoniensis* ticks were also present. During tick collection in the cave, outside air temperature was approximately 25–27 C.

In cave 2, collections of ticks were performed deep inside the cave, inside cracks and crevices on the ceiling and walls, where mean temperature was 31.9 ± 2.1 C; the surrounding relative humidity (RH) was 97.0 ± 1.0%; and ammonia was present, but not overwhelming. There was a shallow stream inside the cave, 1–2 m wide, flowing through the main branch. During January 2010, we collected 15 bats in this cave. Each bat was carefully examined for ticks, which were collected and taken to the laboratory. To assess aspects of the life cycle related to developmental phases, free-living engorged and flat nymphs, and 1 engorged larva

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collected on a bat in cave 2 were taken alive to the laboratory, where they were held in a cabinet at 29 C, and 95–99% RH in total darkness.

Preparation of specimens for morphological examinations

Ticks were cleaned with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1. Non-engorged larvae were mounted in Hoyer's medium to create semi-permanent slides for light microscopy. Ten non-engorged specimens of each stage were measured using a stereoscope Nikon® C-PS (all measurements are given in mm as the mean followed by the range in parentheses). Scanning electron photomicrographs of adults and immature ticks were taken at the Servicio de Microscopía Electrónica, Museo de La Plata, Universidad Nacional de La Plata, Argentina, using a JEOL/JSM 6360 LV® Digital Scanning Microscope. Larval chaetotaxonomic terminology and measures follow Sonenshine et al. (1962) and Kohls et al. (1965), with the modifications proposed by Venzal et al. (2008). To allow an appropriate comparison, whenever it was possible, the description of all tick stages follows in general lines the format of the descriptions of male, nymph, and larva of *N. reddelli* given by Keirans and Clifford (1975) and Keirans et al. (1977).

Molecular analysis

DNA extraction and polymerase chain reaction (PCR) amplification were carried out on a representative specimen for each of the morphotypes collected in the cave, namely, larva, nymph I, nymph II, male, and female, following the methodology described by Mangold et al. (1998). The amplified PCR products were used to obtain an ~400-bp fragment of the mitochondrial 16S rDNA gene. Sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), and alignment was made with the program Clustal W (Thompson et al., 1994).

Examination of type specimens

Larvae (paratypes) of *Ornithodoros setosus* Kohls, Clifford, and Jones (RML49559), and *Ornithodoros marinkellei* Kohls, Clifford, and Jones (RML47294 and RML47300), and adult males (paratypes) of *N. reddelli* (RML63679), which have been deposited in the U.S. National Tick Collection (USNTC, Georgia Southern University, Statesboro, Georgia), were examined and compared with ticks collected in the present study. In addition, we also examined 1 nymph (RML65732) and the only known larval specimen of *N. reddelli* (RML66043), both deposited in the USNTC, and 1 male of *N. reddelli* deposited in the Colección Nacional de Acaros, UNAM, Mexico.

DESCRIPTION

Nothoaspis amazoniensis n. sp. Nava, Venzal & Labruna (Figs. 1–8)

Diagnosis (male [Figs. 1, 2]): Dorsum: Length from tip of hood to posterior margin 6.7 (6.5–7.0), maximum body width 3.94 (3.6–4.1). Anterior half of body covered dorsally by smooth integument or nothoaspis (Figs. 1A, B), length 3.26 (3.0–3.4), width 2.21 (2.0–2.6); nothoaspis delimited by paired groove beginning at apex of nothoaspis, diverging posteriorly, and curving medially in posterior portion to become indeterminate, with reticulate surface pattern on posterior half of nothoaspis (Figs. 1A, B). Six to 8 muscle insertions indicated by shallow depressions across the nothoaspis, 2 anterior and rest on posterior one-third. Integument in rest of body surface covered by cell-like configuration, with cells larger medially, and smaller and more numerous on marginal fields. Most cells with single short seta (Fig. 1C). Hood bluntly rounded, visible dorsally (Figs. 1A, B) with large papillae and setae ventrally (Fig. 2A).

Venter (Fig. 1D): Integument similar to that of lateral and postero-dorsal surface, with exception of area that surrounds genital aperture extending from coxa I to IV (genital plate), where integument is smooth; cells becoming larger on posterior field. Genital aperture oval situated between coxae I, length 0.23 (0.22–0.25); width 0.44 (0.4–0.48). Anal valves each with 1 pair of setae. Spiracular plate as illustrated (Fig. 1E), 0.29 (0.26–0.34) long, 0.31 (0.26–0.37) wide, irregularly circular, located laterally to coxa IV, with macula eccentric and situated on medial side.

Capitulum: Length from palpal apices to posterior margin 0.80 (0.78–0.84), width 0.68 (0.64–0.75). Basis capituli rectangular in outline, with 1

pair of post-hypostomal setae and at least 10 pairs of sublateral setae, and post-palpal setae absent (Figs. 2A, B). Palpi 0.58 (0.56–0.70) long, with several setae, longer and more numerous dorsally; segment I with medial integumental extension (flap), rectangular in shape covering hypostome (Figs. 2A, C), length of flaps 0.29 (0.26–0.33); 1 central pore at base of hypostome (Figs. 2C, D). Hypostome minute, without denticles (Fig. 2D).

Legs (Fig. 1D): All coxae inserted in anterior half of body, covered by numerous and long setae; coxae II–IV contiguous, I and II separated. Tarsi long, narrow apically, without subapical dorsal humps, but with numerous setae; tarsus I 0.85 (0.75–0.93) long, 0.22 (0.18–0.24) wide; tarsus IV 1.11 (1.05–1.2) long, 0.19 (0.18–0.22) wide.

Diagnosis (female [Figs. 3, 4]): Dorsum: Length from tip of hood to posterior margin 7.24 (6.90–7.70), maximum body width 4.18 (3.91–4.60). Nothoaspis covered by cell-like configuration (cell smaller than in rest of dorsal surface), lacking smooth-like surface as present in males (Figs. 3A, B); nothoaspis delimited by paired groove beginning at apex of nothoaspis, diverging posteriorly, and curving medially in posterior portion to become indeterminate. Integument in rest of body surface also covered by cell-like configuration, with cells larger medially, and smaller and more numerous on marginal fields (Figs. 3A, B). Most of cells with single short seta. Hood bluntly rounded, visible dorsally, with large papillae and setae ventrally (Figs. 3B, D).

Venter (Fig. 3C): Integument similar to that of lateral and dorsal surface, with exception of area surrounding genital aperture extending from coxa I to IV (genital plate), where integument is smooth; cells becoming larger on posterior field. Genital aperture as illustrated (Fig. 3C), situated between coxae I and II, with anterior and posterior labia subequal, length 0.42 (0.35–0.50), width 0.63 (0.55–0.70). Anal valves each with 1 pair of setae. Spiracular plate equal to that of male, 0.30 (0.25–0.33) long, 0.33 (0.32–0.36) wide.

Capitulum: Length from palpal apices to posterior margin 0.85 (0.79–0.90), width 0.61 (0.48–0.66). Basis capituli rectangular in outline, with 1 pair of post-hypostomal setae and at least 10 pairs of sublateral setae, and post-palpal setae absent (Figs. 3D, 4A). Palpi 0.68 (0.60–0.75) long, with several setae, longer and more numerous dorsally; segment I with flap rectangular in shape covering hypostome (Figs. 3D), length of flaps 0.32 (0.28–0.35); 1 central pore in base of hypostome (Fig. 4B). Hypostome minute, without denticles but with small crenulations (Fig. 4B).

Legs (Fig. 3C): All coxae inserted in anterior half of body and covered by numerous and long setae; coxae II–IV contiguous, I and II separated. Tarsi long, narrow apically, without subapical dorsal humps, but with numerous setae; tarsus I 0.86 (0.75–0.93) long, 0.19 (0.18–0.21) wide; tarsus IV 0.96 (0.85–1.0) long, 0.19 (0.18–0.23) wide.

Diagnosis (nymph II [Fig. 5]): Dorsum: Length from tip of hood to posterior margin 5.09 (4.80–5.46), maximum body width 2.27 (1.90–2.40); body approximately twice as long as wide, anterior portion narrower than posterior portion. Nothoaspis covered by cells (the cells irregular in shape and size), lacking smooth-like surface present in males (Fig. 5A); nothoaspis delimited by paired groove beginning at apex of nothoaspis, diverging posteriorly, and curving medially in posterior portion to become indeterminate, with posterior limit inconspicuous (Fig. 5A). Integument in rest of body surface also covered by cell-like configuration, cells characterized to be irregular in shape and size, and most of them at least with 1 seta; setae short, with exception of posterior margin of body, where setae are larger. Hood small in relation to capitulum, covering only small portion of posterior part of capitulum (Fig. 5B), with papillae and setae ventrally.

Venter (Fig. 5C): Integument covered by cell-like configuration, with exception of narrow area located between coxae I and III. Anus posteriorly to level of coxa IV; anal valves each with 1 pair of setae. Spiracular plate similar to that of male, 0.29 (0.26–0.33) long, 0.26 (0.25–0.28) wide.

Capitulum (Fig. 5D): Length from palpal apices to posterior margin 1.24 (1.10–1.30), width 0.92 (0.80–0.96). Basis capituli subrectangular in outline, with 1 pair of post-hypostomal setae and at least 7 pairs of sublateral setae, bordered posteriorly by integumental fold; postpalpal setae absent. Palpi 0.88 (0.82–0.93) long, with several setae, longer and more numerous dorsally; segment I longer than II, with elongated flap covering hypostome, length of flaps 0.70 (0.66–0.75). Hypostome long, pointed, dentition 4/4 in apical one-third, 5/5 to base, denticles sharply pointed, minute at apex, gradually enlarging in size posteriorly (Fig. 5E).

Legs (Fig. 5C): All coxae inserted in anterior one-half of body and covered by short setae; all coxae contiguous, decreasing in size from I to IV. Tarsi long, narrow apically, without subapical dorsal humps, but with

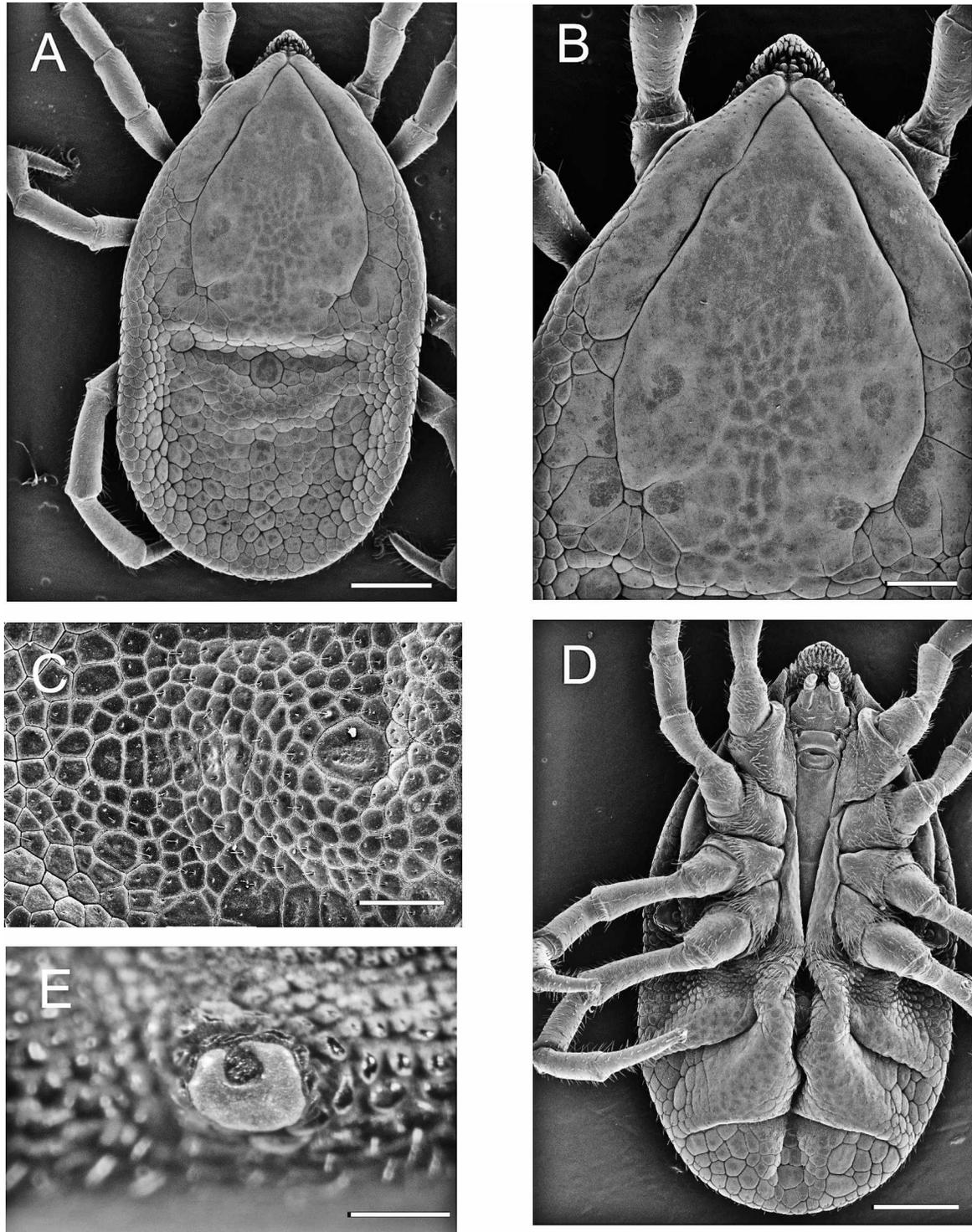


FIGURE 1. Scanning electron microscopy of the male of *Nothoaspis amazoniensis* n. sp. (A) Dorsum (bar = 1 mm). (B) Dorsum: Anterior half of the body (view of nothoaspis) (bar = 0.5 mm). (C) Integument of the of body surface (posterodorsal) (bar = 0.5 mm). (D) Venter (bar = 1 mm). (E) Spiracular plate (bar = 0.3 mm).

numerous setae; tarsus I 0.79 (0.70–0.85) long, 0.16 (0.15–0.18) wide; tarsus IV 0.84 (0.75–1.0) long, 0.16 (0.15–0.17) wide.

Diagnosis (nymph I [Fig. 6]): Dorsum: Length from tip of hood to posterior margin 4.12 (3.90–4.30), maximum body width 2.42 (2.28–2.60); body approximately twice as long as wide, anterior portion narrower than posterior portion. Dorsal surface covered by cell-like configuration

(Fig. 6A); cells with 1 short seta, with exception of posterior margin of body, where setae are larger. Hood small in relation to capitulum (Fig. 6A), covering only small portion of posterior part of capitulum, with papillae and setae ventrally.

Venter (Fig. 6B): Integument covered by cell-like configuration, with exception of areas located below capitulum and between coxae I and IV.

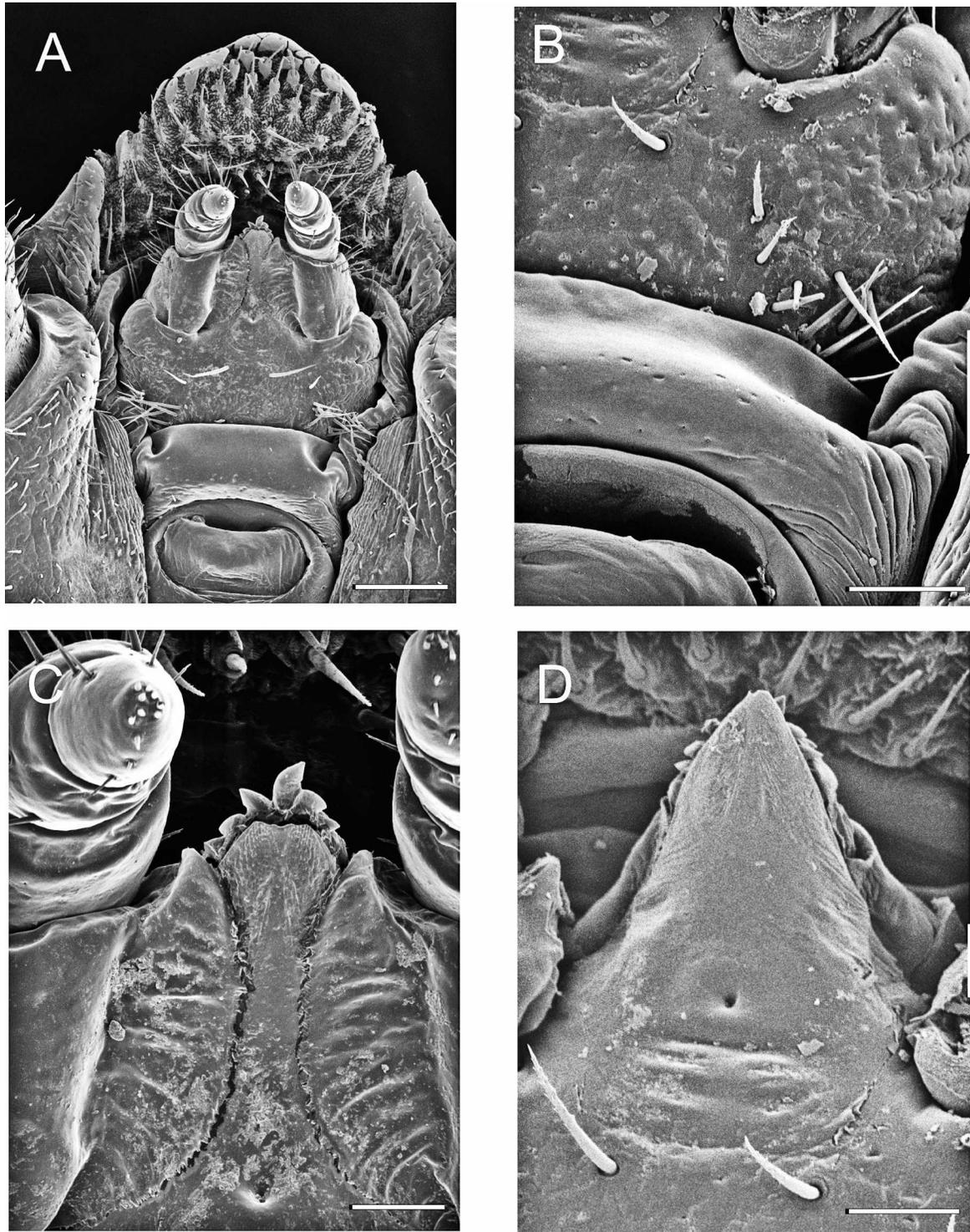


FIGURE 2. Scanning electron microscopy of the male of *Nothoaspis amazoniensis* n. sp. (A) Capitulum (bar = 0.2 mm). (B) Post-hypostomal and sublateral setae of the basis capituli (bar = 0.1 mm). (C) Palpal flaps (bar = 0.05 mm). (D) Hypostome (flaps were removed) (bar = 0.05 mm).

Anus situated posteriorly to level of coxa IV; anal valves each with 1 pair of setae. Spiracular plate as illustrated (Fig. 6C), 0.18 (0.16–0.20) long, 0.15 (0.15–0.15) wide, circular, located lateral to coxa IV.

Capitulum (Fig. 6D): Length from palpal apices to posterior margin 0.60 (0.59–0.61), width 0.60 (0.58–0.62). Basis capituli rectangular in outline, with 1 pair of post-hypostomal setae and at least 4 pairs of sublateral setae, and bordered posteriorly by small integumental fold;

post-palpal setae absent. Palpi 0.44 (0.40–0.50) long, with setae concentrated on dorsal surface of palpal articles; segment I similar in length to segment II, with flap rectangular in shape, partially covering hypostome, length of flaps 0.20 (0.16–0.22). Hypostome with dental formula 4/4 in files of 12–13 denticles, with “V”-shape apices (Fig. 6E).

Legs (Fig. 6B): All coxae inserted in anterior one-half of body, with very small, hardly perceivable, setae; all coxae contiguous, coxae I–III

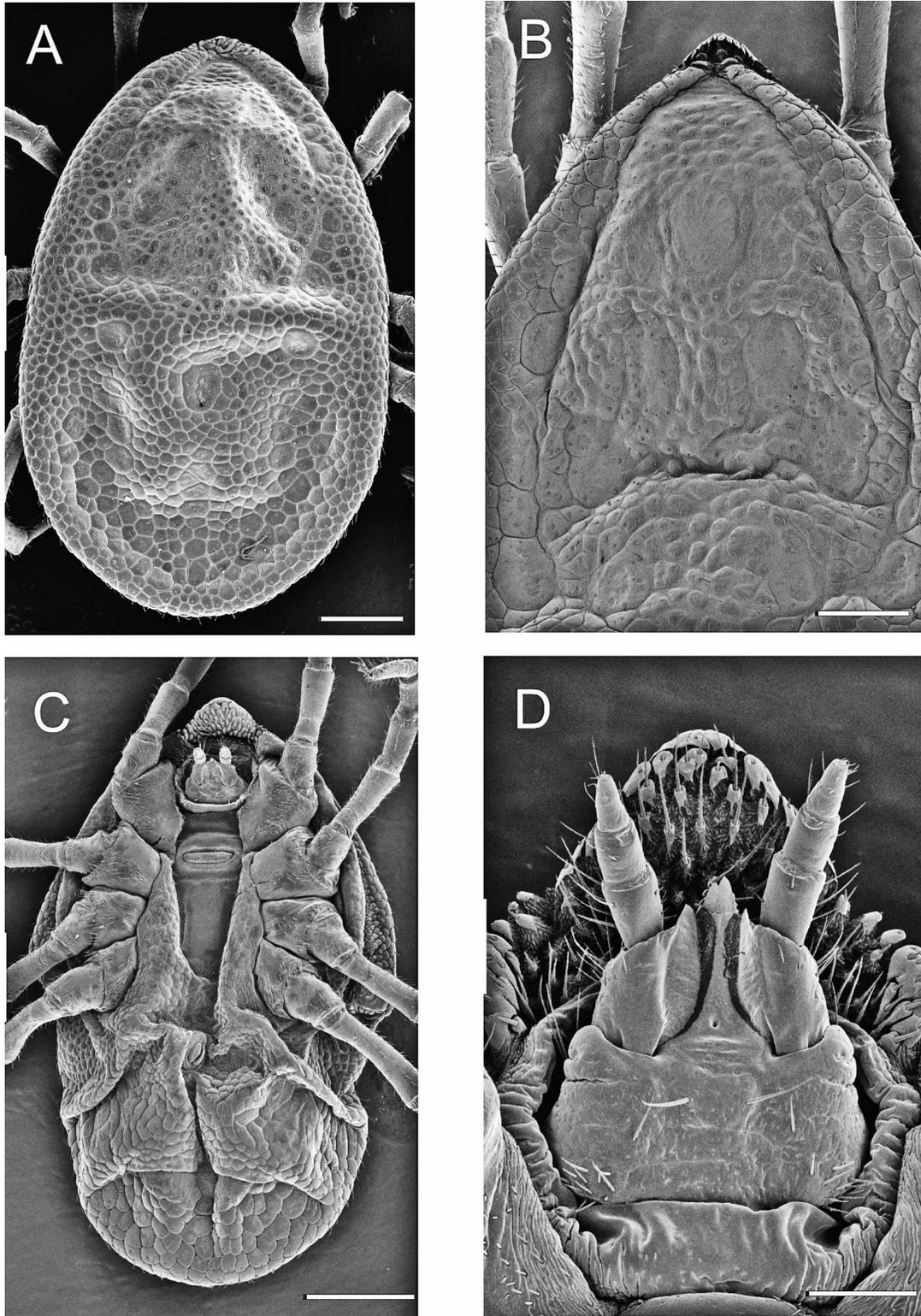


FIGURE 3. Scanning electron microscopy of the female of *Nothospis amazoniensis* n. sp. (A) Dorsum (bar = 1 mm). (B) Dorsum: Anterior half of the body (bar = 0.5 mm). (C) Venter (bar = 1 mm). (D) Capitulum (bar = 0.2 mm).

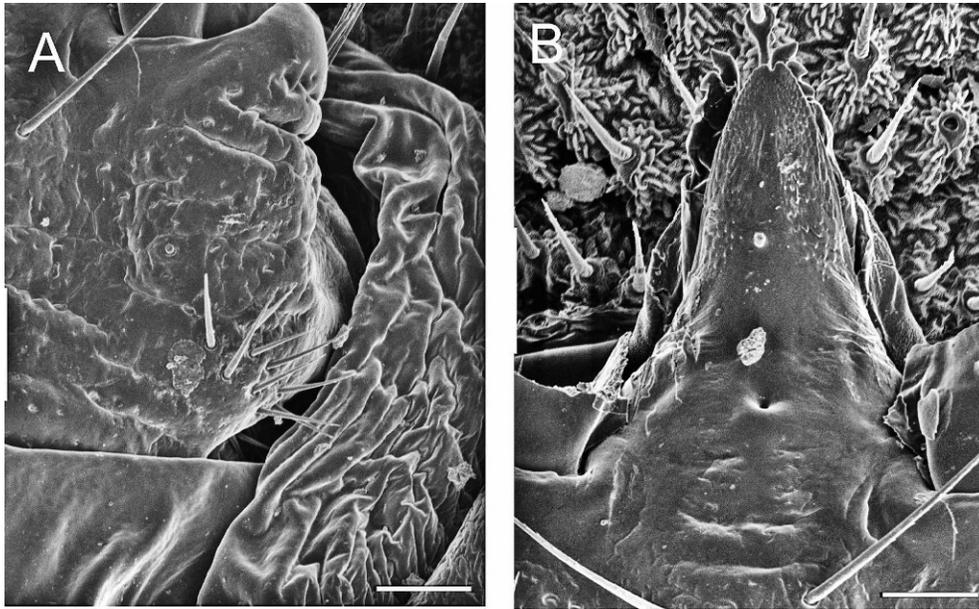


FIGURE 4. Scanning electron microscopy of the female of *Nothoaspis amazoniensis* n. sp. (A) Post-hypostomal and sublateral setae of the basis capituli (bar = 0.1 mm). (B) Hypostome (flaps were removed) (bar = 0.05 mm).

subequal, coxa IV notably smaller than the 3 previous ones. Tarsi long, narrow apically, without subapical dorsal humps, but with numerous setae; tarsus I 0.52 (0.50–0.54) long, 0.16 (0.15–0.20) wide; tarsus IV 0.66 (0.65–0.68) long, 0.16 (0.15–0.17) wide.

Diagnosis (larva [Figs. 7, 8]): Body: Body outline elongate, posterior margin broadly rounded, length (including capitulum) 1.55 (1.52–1.58), width 0.68 (0.57–0.76).

Dorsum (Fig. 7A): Dorsal plate elongate, triangular, surface smooth, posterior margin concave, length 0.75 (0.72–0.76), width 0.33 (0.31–0.36). Dorsum with 12–13 setae pairs (Fig. 8A), 5–6 anterolateral, 3 central, and 4 posterolateral setae; anterolateral setae (Al): Al₁ length 0.05 (0.04–0.06), Al₂ length 0.06 (0.05–0.07), Al₃ length 0.07 (0.06–0.07), posterolateral setae (Pl): Pl (last setae) length 0.09 (0.08–0.10).

Venter (Figs. 7B, 8E): Venter with 10 pairs of setae plus 1 pair on anal valves; posteromedian setae (PMD) absent; 3 pairs of sternal setae (St): St₁ length 0.10 (0.10–0.11), St₂ length 0.09 (0.08–0.09), St₃ length 0.10 (0.09–0.11); 1 pair of post-coxal setae (Pc) length 0.06 (0.05–0.07); 5 pairs of circumanal setae (Ca): Ca₁ length 0.06 (0.05–0.07), Ca₂ length 0.07 (0.06–0.08), Ca₃ length 0.10 (0.08–0.11), Ca₄ length 0.11 (0.10–0.13), Ca₅ length 0.08 (0.07–0.08); 1 pair of ventral posteromarginal setae (PM) length 0.08 (0.07–0.10).

Capitulum (Figs. 7C, D): Basis capituli pentagonal, length from posterior margin of basis capituli to PH₁ 0.29 (0.28–0.31), length from posterior margin of basis capituli to insertion of hypostome 0.37 (0.34–0.39), and length from posterior margin of basis capituli to apex of hypostome 0.78 (0.76–0.79), width 0.38 (0.37–0.40); lateral margins with a small bulge; posterior margin with 2 small auricula-like projections. Two pairs of post-hypostomal setae; Ph₁ length 0.04 (0.03–0.04), Ph₂ length 0.08 (0.07–0.09). Distance between Ph₁ setae 0.05 (0.04–0.05), and between Ph₂ setae 0.015 (0.013–0.016). Palpi total length 0.52 (0.49–0.55), segmental length/width from I–IV: (I) 0.12 (0.12–0.13)/0.06 (0.05–0.06), (II) 0.20 (0.19–0.21)/0.06 (0.06–0.07), (III) 0.14 (0.12–0.14)/0.05 (0.05–0.06), (IV) 0.07 (0.06–0.08)/0.03 (0.03–0.04). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9.

Hypostome (Fig. 7E): Length from Ph₁ to apex 0.48 (0.46–0.49), length from insertion of hypostome in basis capituli to apex 0.42 (0.40–0.43), width in basis portion of hypostome 0.14 (0.13–0.15); dental formula 2/2 throughout length, file 1 with 21–23 (typically 22) denticles and file 2 with 20–22 denticles; without corona of denticles in apex; apex blunt.

Legs: Tarsus I 0.41 (0.39–0.43) long, 0.13 (0.11–0.16) wide. Setal formula: 1 pair apical (A), 1 distomedian (DM), 5 paracapsular (PC), 1 posteromedian (PM), 1 pair basal (B), 1 pair apicoventral (AV), 1 pair anteroventral (AL), 1 pair pre-midventral (PMV), 1 pair midventral (MV).

Capsule of Haller's organ with reticulations and 6 setae in the anterior pit (Fig. 8F). Two small triangular spurs in coxae I, and 1 in coxae II and III (Fig. 7B).

Taxonomic summary

Type locality: Bat cave, Porto Velho Municipality, state of Rondônia, Brazil (08°37'59"S, 63°57'29"W).

Material examined: Holotype ♂ and allotype ♀, 10 paratype ♂♂, 10 paratype ♀♀, 10 paratype nymphs II, 10 paratype nymphs I, and 10 paratype larvae, collected 19 July 2009 and 30 January 2010 by M. B. Labruna, J. M. Venzal, S. Nava, and F. A. Terassini, deposited in the Coleção Nacional de Carrapatos (CNC) of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil (CNC-1560, 1562–1566); paratypes 10 ♂♂, 10 ♀♀, 10 nymphs II, 5 nymphs I, and 2 larvae, deposited in the tick collection of INTA Rafaela, Santa Fe, Argentina (accession number INTA-2126); paratypes 10 ♂♂, 10 ♀♀, 10 nymph II, 10 nymph I, and 2 larvae, deposited in the Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay (accession number DPVURU-593); paratypes 5 ♂, 3 ♀, 3 nymph II, 1 nymph I, and 1 larva deposited in the U.S. National Tick Collection, Georgia Southern University, Statesboro; paratypes 5 ♂, 3 ♀, 3 nymph II, 1 nymph I, and 1 larva deposited in the Instituto Butantan, São Paulo, Brazil. In cave 2, 15 bats (8 *Pteronotus parnellii* [Gray], 5 *Anoura caudifer* Geoffroy, and 2 *Carollia perspicillata* L.) were examined for ticks. Only 3 *P. parnellii* were found infested by ticks (all identified as *N. amazoniensis* larvae), with 2 larvae on 1 bat, and 1 larvae on each of the other 2 bats.

Etymology: The specific epithet "amazoniensis" refers to the ecological region (Amazonia) where the type specimens were collected.

Other material deposited at the CNC: Two engorged nymphs II collected in cave 1 (08°40'43"S, 63°51'05"W), Porto Velho, Rondônia, 30 January 2009, M. B. Labruna, J. M. Venzal, S. Nava, and F. A. Terassini (CNC-1389); 12 ♂♂ free-living; details as previous collection (CNC-1570); 58 ♂♂, 33 ♀♀, 33 nymphs II, 1 nymph I, and 23 larvae collected in cave 2 (08°37'59"S 63°57'29"W), Porto Velho, Rondônia, 19 July 2009 and 30 January 2010, M. B. Labruna, J. M. Venzal, S. Nava, and F. A. Terassini (CNC-1567, 1569, 1571–1573).

Remarks

The diagnostic characters unique to the adults of *N. reddelli* and *N. amazoniensis* include the presence of false shield or nothoaspis, an anteriorly projecting hood covering the capitulum, medial extension of

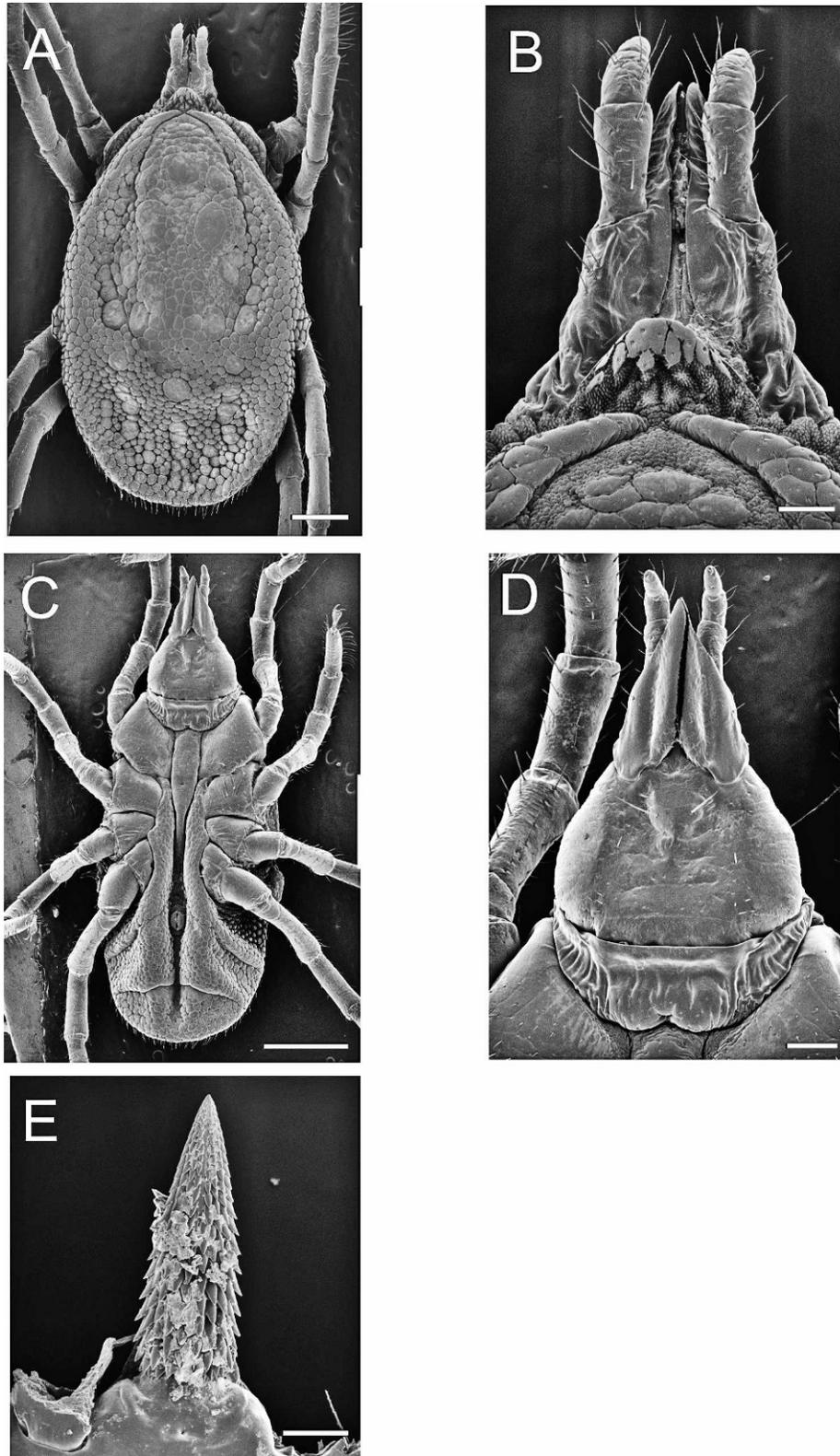


FIGURE 5. Scanning electron microscopy of the nymph II of *Nothoaspis amazoniensis* n. sp. (A) Dorsum (bar = 0.5 mm). (B) Hood and capitulum (dorsal view) (bar = 0.1 mm). (C) Venter (bar = 1 mm). (D) Capitulum (ventral view) (bar = 0.2 mm). (E) Hypostome (flaps were removed) (bar = 0.1 mm).

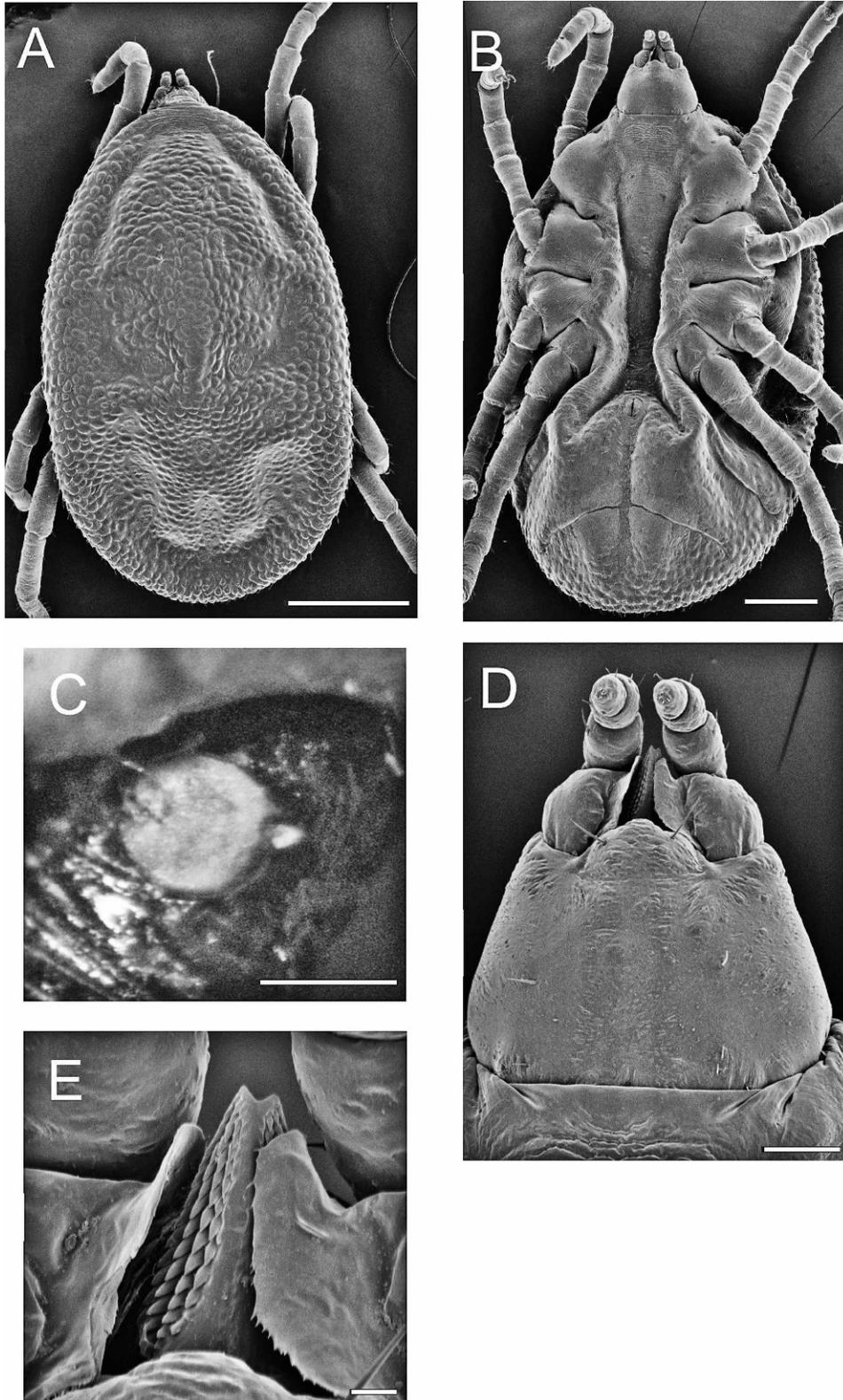


FIGURE 6. Scanning electron microscopy of the nymph I of *Nothoaspis amazoniensis* n. sp. (A) Dorsum (bar = 1 mm). (B) Venter (bar = 0.5 mm). (C) Optical micrograph of the spiracular plate (bar = 0.2 mm). (D) Scanning electron microscopy of capitulum (ventral view) (bar = 0.1 mm). (E) Scanning electron microscopy of the hypostome (bar = 0.02 mm).

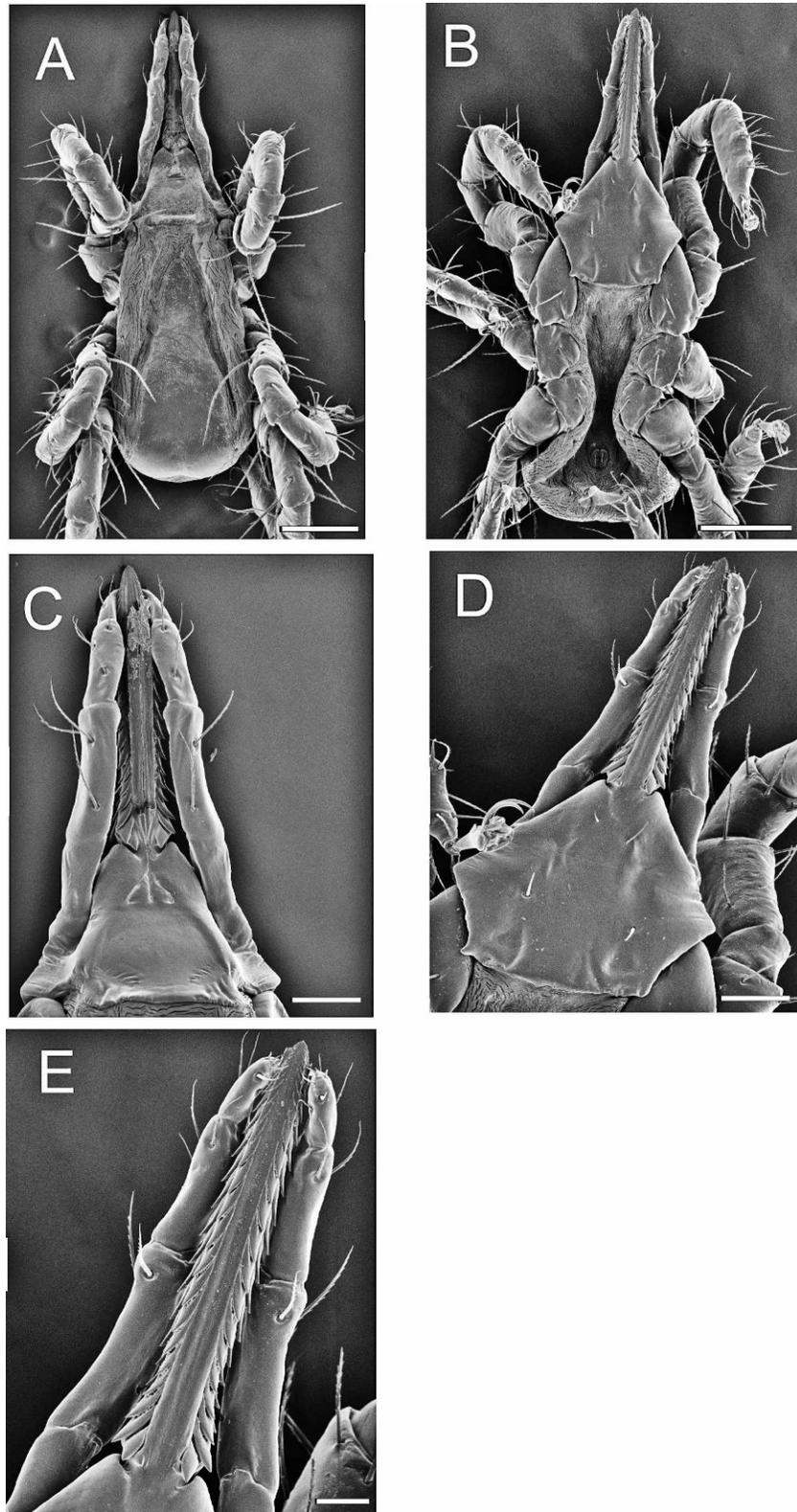


FIGURE 7. Scanning electron microscopy of the larva of *Nothoaspis amazoniensis* n. sp. (A) Dorsum (bar = 0.2 mm). (B) Venter (bar = 0.2 mm). (C) Capitulum (dorsal view) (bar = 0.1 mm). (D) Capitulum (ventral view) (bar = 0.1 mm). (E) Hypostome and palpi (bar = 0.5 mm).

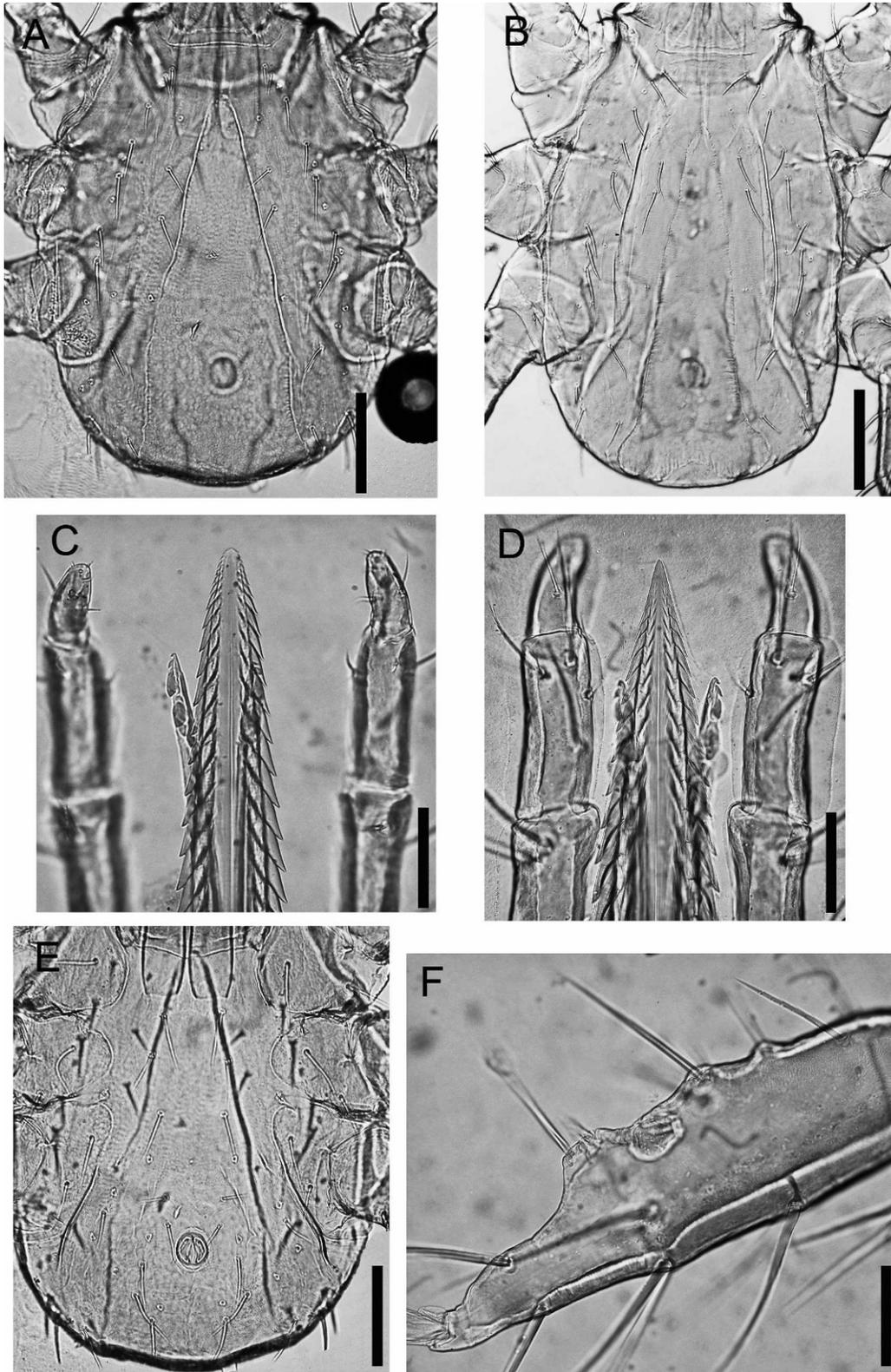


FIGURE 8. Optical micrographs of the larva of *Nothoaspis amazoniensis* n. sp. (A) Dorsal plate and dorsal setae (bar = 0.2 mm); (C) hypostome (bar = 0.1 mm); (E) ventral setae (bar = 0.1 mm); (F) reticulations in capsule Haller's organ (bar = 0.1 mm). Optical micrographs of the larva of *Nothoaspis reddelli*: (B) dorsal plate and dorsal setae (bar = 0.2 mm); (D) hypostome (bar = 0.1 mm).

palpal article I (flaps) forming a ventral sheath for a minute hypostome, and a genital plate extending from coxa I to IV. Males of *N. amazoniensis* can be distinguished from those of *N. reddelli* by the absence of 2 setae on the internal margin of the flaps (present in *N. reddelli*), a reticulate surface pattern on the posterior half of the nothoaspis (absent in *N. reddelli*), a minute hypostome without denticles (dentition 3/3 or 4/4 in *N. reddelli*), and the presence of a central pore in the base of hypostome (this structure is difficult to see using ordinary light microscopy, and they are not observable in the scanning electron microscopy figures of *N. reddelli* given by Keirans and Clifford [1975], nor was it described by these authors). Another noteworthy difference is the number of sublateral setae. Males of *N. amazoniensis* have at least 10 pairs of sublateral setae, but Keirans and Clifford (1975) describe the male of *N. reddelli* with about 4 pairs of sublateral setae. Nevertheless, we examined a paratype male specimen of *N. reddelli*, and we observed at least 8 pairs of sublateral setae. Therefore, this character should be used carefully to compare these 2 species.

The diagnostic characters for the female of *N. amazoniensis* (the female of *N. reddelli* is unknown) are similar to those of males, i.e., the presence of an anteriorly projecting hood covering the capitulum, the medial extension of palpal article I forming a ventral sheath for a minute hypostome, and a genital plate extending from coxa I to IV. However, there is a notable difference between the morphology of males and females (obviously, besides the shape of genital aperture). The males have a nothoaspis with a smooth integument, whereas in females, this area of the body is covered with a cell-like configuration.

The nymph II from Brazil is morphologically similar to the nymph of *N. reddelli* described by Keirans et al. (1977). They can be differentiated because in the nymph II of *N. amazoniensis*, the hood (dorsal view) is small in relation to the capitulum, covering only a minute portion of the posterior part of the capitulum, the setae on coxae are short, although well perceivable, the internal margins of the flaps lack setae, and the nothoaspis is covered by a cell-like configuration, with cells irregular in shape and size. Conversely, in the nymph of *N. reddelli* the hood (dorsal view) covers at least a half of the capitulum, the setae on coxae are numerous and long, the internal margins of flaps have 2 large setae, and the nothoaspis is not covered with a cell-like configuration, but it has a leathery quality.

The nymph I of *N. amazoniensis* is easily determined by a combination of a hood (dorsal view) that is small relative to the capitulum and covers only a very small portion of its posterior part, the dorsum of the body is covered entirely by a cell-like configuration, the integument of the venter is covered by a cell-like configuration, with the exception of the areas located below of the capitulum and between coxae I and IV, the palpal segment I is similar in length to segment II, with flaps rectangular in shape partially covering the hypostome, internal margin of the flaps without setae, and the hypostome dentition 4/4 with “V”-shape apices.

Morphologically, larvae of *N. amazoniensis* are closely related to the larvae of *N. reddelli*, *O. setosus*, and *O. marinkellei*, which are also parasites of bats in the Neotropical region. The principal differences between larvae of *N. amazoniensis* and *N. reddelli* are the shape of the hypostome apex, pointed in *N. reddelli* and blunt in *N. amazoniensis* (Figs. 8C, D), and the number and size of the dorsal setae, 14 pairs in *N. reddelli* and 12–13 in *N. amazoniensis* (Figs. 8A, B). Additionally, the larva of *N. reddelli* is larger than that of *N. amazoniensis*. The morphological differences between larvae of *N. amazoniensis* and *N. reddelli* are summarized in Table I.

Nothoaspis amazoniensis and *O. setosus* larvae share a dorsal plate with similar size and shape, basis capituli with lateral bulges, and the presence of reticulations in capsule of Haller's organ. However, *O. setosus* larvae are easily differentiable from *N. amazoniensis* due to the absence of small auricula-like projections in the capitulum, the hypostome with apical dentition 3/3 (2/2 in *N. amazoniensis*), and 4 pairs of circumanal setae (Ca) (5 pairs in *N. amazoniensis*).

The presence in *O. marinkellei* of a corona of denticles in the hypostome apex and the auricula-like projections in the capitulum larger than in *N. amazoniensis* distinguish the larvae of these 2 species.

The 16S rDNA sequences of male (GenBank accession number: HM047066), female (GenBank accession number: HM047065), nymph II (GenBank accession number: HM047068), nymph I (GenBank accession number: HM047067), and larva (GenBank accession number: HM047069) were identical. Thus, all collected ticks of each stage can be clearly assigned to the same taxon.

Life-cycle biology

The engorged larva of *N. amazoniensis* that was found attached to *P. parnellii* molted to a nymph I in 17 days. Thereafter, this nymph molted without feeding to a nymph II after 16 days. The nymph I was engorged like those collected in the cave. Flat nymphs II were allowed to feed on juvenile mice (hairless) and inside cotton sleeves glued to the shaved back of rabbits. In both mice and rabbits, at least 10 nymphs II fed to repletion, looking like the engorged nymphs II that were collected in the ceiling of the cave. Feeding period varied from 50 to 90 min. Unfortunately, all these engorged nymphs II that fed on laboratory animals died in the cabinet before molting. However, 6 engorged nymphs II collected in cave 2 molted to adults (3 males, 3 females) in the cabinet. The molting period varied from 20 to 35 days. The 6 adults obtained looked to be partially engorged, just like the adults that were collected in the cave.

Considering these data, we propose that *N. amazoniensis* has a life cycle with a larva that feed on bats, a non-feeding nymphal stage (nymph I), a feeding nymphal stage (nymph II), and adults that probably represent non-feeding stages. In this way, the presence of a hypostome, seemingly functionless in nymph I and adults, supports the hypothesis that they are non-feeding stages. All these biological data must be considered as preliminary information, and additional work focused on the life cycle of *N. amazoniensis* should be carried out.

Finally, it is important to note that flat nymphs II were aggressive to humans, with at least 3 episodes of tick infestation on researchers during the fieldwork in the cave.

DISCUSSION

Until now, the only records of *Nothoaspis* spp. have been in bat caves from tropical areas of Mexico (*N. reddelli*) and Brazil (*N. amazoniensis*), characterized by similar environmental conditions (hot and humid environment). Thus, it is almost certain that the distribution of these tick species is determined by the presence of caves with high temperature and humidity, and colonized by bats. There have been 2 findings of larvae of *N. reddelli* (identified as *Carios reddelli*) in the state of Rio de Janeiro, Brazil (Guerra et al., 2001; Botão-Miranda et al., 2002). We judge these findings as doubtful, because the methodology for the taxonomic determination is not explained, figures are not included, and the collections where the ticks were deposited are not indicated.

A geographical range larger than that reported is expected for both *N. amazoniensis* and *N. reddelli*, because the hosts of larvae and nymphs are bats, which are animals with manifest vagility and a high capacity for dispersal. In fact, movement of >1,000 km are known for some bat species (Popa-Lisseanu and Voigt, 2009). *Pteronotus parnellii*, the only apparent host of the larvae of *N. amazoniensis*, has rather broad distribution, from central Mexico to the Amazon and Matto Grosso regions of Brazil (Herd, 1983). Consequently, it is probable that *Nothoaspis* spp. ticks also are present in hot caves of other tropical and subtropical areas of the Neotropics.

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TABLE I. Comparative measurements of larvae of *Nothospis amazoniensis* and *Nothospis reddelli*. Measurements are in millimeters.

Character	<i>N. reddelli</i>	<i>N. amazoniensis</i>
Body length*†	1.69	1.55 (1.52–1.58)
Body width	0.68	0.68 (0.57–0.76)
Dorsal plate: length	0.81	0.74 (0.72–0.76)
Dorsal plate: width	0.36	0.33 (0.31–0.36)
Dorsal setae (pairs): total	14	12–13
Dorsal setae (pairs): dorsolateral	11	9–10
Dorsal setae (pairs): central	3	3
Dorsal anterolateral setae: Al ₁	0.084	0.051 (0.043–0.055)
Dorsal anterolateral setae: Al ₂	0.079	0.056 (0.050–0.065)
Dorsal anterolateral setae: Al ₃	0.081	0.067 (0.065–0.072)
Dorsal posterolateral setae: Pl	0.077	0.087 (0.081–0.098)
Ventral setae (pairs): total	10 +1 anal, PMS absent	10 +1 anal, PMS absent
Sternal setae: St ₁	0.096	0.106 (0.101–0.115)
Sternal setae: St ₂	0.093	0.092 (0.089–0.096)
Sternal setae: St ₃	0.096	0.10 (0.096–0.11)
Circumanal setae: Ca ₁	0.067	0.063 (0.055–0.072)
Circumanal setae: Ca ₂	0.077	0.074 (0.067–0.081)
Circumanal setae: Ca ₃	0.101	0.097 (0.084–0.105)
Circumanal setae: Ca ₄	0.125	0.111 (0.098–0.127)
Circumanal setae: Ca ₅	0.091	0.079 (0.072–0.084)
Post-coxal setae: Pc	0.067	0.059 (0.050–0.072)
Posteromarginal setae: Pm	0.084	0.085 (0.072–0.098)
Length of basis capituli†	0.313	0.298 (0.285–0.313)
Length of basis capituli‡	0.0361	0.0369 (0.034–0.039)
Length of basis capituli§	0.798	0.779 (0.760–0.788)
Width of basis capituli	0.437	0.386 (0.370–0.408)
Post-hypostomal setae Ph ₁	0.045	0.039 (0.031–0.043)
Post-hypostomal setae Ph ₂	0.055	0.084 (0.072–0.096)
Distance of Ph ₁	0.036	0.046 (0.043–0.048)
Distance of Ph ₂	0.134	0.149 (0.132–0.163)
Palpal length	0.551	0.521 (0.494–0.551)
Length article I	0.122	0.126 (0.120–0.134)
Length article II	0.204	0.197 (0.189–0.204)
Length article III	0.153	0.139 (0.127–0.146)
Length article IV	0.079	0.071 (0.065–0.077)
Width article I	0.067	0.059 (0.055–0.060)
Width article II	0.072	0.063 (0.060–0.065)
Width article III	0.060	0.053 (0.050–0.055)
Width article IV	0.041	0.034 (0.031–0.038)
Setae of palpal article I	0	0
Setae of palpal article II	4	4
Setae of palpal article III	5	5
Setae of palpal article IV	9	9
Hypostome: length	0.494	0.482 (0.465–0.494)
Hypostome: length#	0.437	0.419 (0.408–0.427)
Hypostome: width	0.142	0.140 (0.133–0.152)
Apex	Pointed	blunt
Apical dental formula	2/2	2/2
Median dental formula	2/2	2/2
Basal dental formula	2/2	2/2
Denticles in hypostomal row 1	22	21 to 23
Denticles in hypostomal row 2	22	20 to 22
Tarsus I: length	0.446	0.410 (0.389–0.427)
Tarsus I: width	0.142	0.131 (0.114–0.161)
Capsule of Haller's organ	with reticulations	with reticulations

*† Including capitulum.

† Length of basis capituli to Ph₁.

‡ Length of basis capituli to insertion of hypostome.

§ Length of basis capituli to final hypostome.

|| Measured to point to Ph₁.

Measured to point of insertion of hypostome in basis capituli.

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