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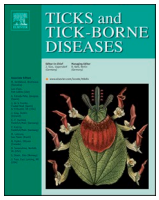

Original article

Description of *Amblyomma monteiroae* n. sp. (Acari: Ixodidae), a parasite of the great horned owl (Strigiformes: Strigidae) in southern Brazil

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ABSTRACT

In 2020, adult hard ticks (males and females) were collected from great horned owls [*Bubo virginianus* (Gmelin, 1788)] in the coastal region in southern Brazil. The engorged females were allowed to oviposit in the laboratory and hatched larvae could be obtained. Analyses of the external morphology of the adult ticks revealed that they represent a new species, which was named *Amblyomma monteiroae* n. sp. Partial sequences of the mitochondrial 16S rRNA gene and the nuclear second internal transcribed spacer (ITS2) were generated from a male and a female. Their 16S rRNA haplotypes were identical to each other and closest (96% identity) to corresponding sequences of *Amblyomma parvitarsum* Neumann, 1901, and 90% identical to *Amblyomma neumanni* Ribaga, 1902. Their ITS2 haplotypes were 95.8 to 96.0 identical to the single ITS-2 partial sequence of *A. parvitarsum* available in GenBank. In the phylogenetic trees inferred by both 16S rRNA and ITS2 partial sequences, *A. monteiroae* n. sp. formed a clade with *A. parvitarsum,* with *A. neumanni* branching sister to this clade. *Amblyomma monteiroae* n. sp. is genetically and morphologically related to *A. parvitarsum.* Both tick species are unique in combining the following morphological characters: scutum extensively ornate; eyes rounded and bulging; coxa I with two moderate pointed spurs, the external longer than the internal; a single triangular short spur on coxae II-III; presence of two spines on the tibia of legs II-IV; hypostomal dentition 3/3, trochanters without spurs. However, the males of the two species can be separated by specific features in palps and festoons, whereas the females differ in specific features of the coxal spurs. The larva of *A. monteiroae* n. sp. can be morphologically distinguished from *A. parvitarsum* only by morphometry, with the former species being slightly smaller. Currently, *A. monteiroae* n. sp. is restricted to southern Brazil, and the only known host is *B. virginianus* (Strigiformes: Strigidae). The present study increases the *Amblyomma* Brazilian fauna to 34 species.

1. Introduction

The genus *Amblyomma* (Acari: Ixodidae) comprises 135 extant species with established populations in different continents, except for Europe and Antarctica (Guglielmone et al., 2015). Nearly half of the *Amblyomma* extant species are found in the Neotropical region, with 54 species being only found in this region (Guglielmone et al., 2021; 2023). Brazil is the country with the largest number of reported *Amblyomma* species (33), of which at least two, *Amblyomma yucumense* (Krawczak, Martins & Labruna, 2015), and *Amblyomma romarioi* (Martins, Luz & Labruna, 2019), were described in the last decade (Krawczak et al., 2015; Martins et al., 2019).

In a recent extensive study about ticks infesting wild raptors (including owls) in Brazil, Teixeira et al. (2020) reported 716 specimens of 15 species of hard ticks (Ixodidae) infesting 33 species of wild raptors in different Brazilian biomes. The genus *Amblyomma* was the most

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diverse with 12 species, all of which have been previously described in the literature. Regarding the typical owls (family Strigidae), Teixeira et al. (2020) reported nine tick species [*Amblyomma auricularium* (Conil, 1878), *Amblyomma cajennense* (Fabricius, 1787) sensu stricto, *Amblyomma calcaratum* Neumann, 1899, *Amblyomma dubitatum* Neumann, 1899, *Amblyomma longirostre* (Koch, 1844), *Amblyomma nodosum* Neumann, 1899, *Amblyomma parkeri* Fonseca & Aragão, 1952, *Amblyomma sculptum* Berlese, 1888, and *Rhipicephalus sanguineus* (Latreille, 1806) sensu lato] from a total of ten host species [*Aegolius harrisii* (Cassin, 1849), *Asio clamator* (Vieillot, 1808), *Asio stygius* (Wagler, 1832), *Athene cunicularia* (Molina, 1782), *Glaucidium brasilianum* (Gmelin, 1788), *Glaucidium minutissimum* (Wied-Neuwied, 1821), *Megascops choliba* (Vieillot, 1817), *Pulsatrix koeniswaldiana* (Bertoni & Bertoni, 1901), *Pulsatrix perspicillata* (Latham, 1790), *Strix hylophila* Temminck, 1825]. The above records on Strigidae owls referred to 110 immature *Amblyomma* spp. (70 larvae, 40 nymphs), and only 19 adult ticks; the later comprised 14 specimens of *A. sculptum* on an undefined owl species, one *A. cajennense* s.s. and one *R. sanguineus* s.l. on two *A clamator*, and one *A. dubitatum* and two *A. sculptum* on two *M. choliba* (Teixeira et al., 2020).

Herein, we present a new species of *Amblyomma* from South America, collected from wild owls in southern Brazil. The new species is morphologically described and genetically characterized based on DNA sequences of mitochondrial (16S rRNA gene) and nuclear (second internal transcribed spacer - ITS-2) markers.

2. Material and methods

2.1. Tick specimens

During July 2020, ticks were collected from two great horned owls [*Bubo virginianus* (Gmelin, 1788)] (Strigiformes: Strigidae) at two distinct municipalities of Rio Grande do Sul state, southern Brazil (Fig. 1). These owls were rescued by the "Centro de Estudos Costeiros, Limnológicos e Marinhos" CECLIMAR, Imbé, RS, under previous authorization by the "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBio permit SISBIO 83208-1). The first owl, rescued on 15 July 2020 at Tramandaí municipality (-30.005771, -50.152245), was found infested by four engorged female ticks, attached to its neck (Fig. S1). These females were manually removed from the owl, and sent alive to our laboratory, where they were weighed and held in an incubator at 23 °C, 95% RH and scotophase for egg laying. The preoviposition period, and incubation period (days from oviposition of the first egg to the hatching of the first larva) of the egg mass of each female tick were evaluated. Part of the hatched larvae, when ≈15 days old, was killed by immersion in hot water and used for the morphological description, along with the females that were preserved in 70% ethanol at the end of oviposition. Attempts to obtain nymphs for morphological description

Fig. 1. Sites within the municipalities of Tramandaí and Balneário Pinhal in the state of Rio Grande do Sul, southern Brazil, where two tick-infested great horned owls (*Bubo virginianus*) were rescued.

were performed by allowing some unfed larvae to feed inside a cotton sleeve glued to the shaved dorsum of a tick-naïve hamster (*Mesocricetus auratus*), as previously described (Soares et al., 2012) and approved by the Ethic Committee on Animal Use of the Faculty of Veterinary Medicine of the University of São Paulo (project number 4425171018); however, the larvae refused to attach on hamster, not being possible to obtain nymphs for the morphological description. The second owl, rescued on 24 July 2020 at Balneário Pinhal municipality (-30.29072, -50.25590), was found infested by four male ticks attached to the neck and head regions (Fig. S2). The ticks were collected and sent to the laboratory, where they were preserved in 70% ethanol.

The region where the two owls were rescued is part of the Pampa biome, located in the northern coastal region of Rio Grande do Sul state. The climate in the region is subtropical humid, with an average annual temperature of 19 °C and uniform rainfall throughout the year (Ferraro and Hasenack, 2009).

2.2. Tick taxonomic identification

Examination of the four female and the four male ticks under a stereomicroscope revealed that each gender represented a single morphotype of the genus *Amblyomma,* which were morphologically similar, albeit distinct, to adults of *Amblyomma parvitarsum* Neumann (1901). This observation raised the possibility that the collected ticks could be a new species of the genus *Amblyomma*. To evaluate this, we performed "side-by-side" morphological comparisons with adult specimens of *A. parvitarsum* and its closely related species, *Amblyomma neumanni* Ribalga, 1902, available at the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) at the Faculty of Veterinary Medicine of the University of São Paulo, São Paulo, SP, Brazil. The following allotments were used for comparisons: CNC-2273: *A. parvitarsum* (four females) ex. *Vicugna vicugna*, Visviri, altitude 4069 m, Arica and Parinacota region, Chile, August 2012; CNC-3476: *A. parvitarsum* (one male, ten females) ex. *Vicugna vicugna*, 'Salinas y Aguada Blanca' National Reserve, altitude ≈4000 m, Arequipa region, Peru, July 2011; CNC-4588: *A. parvitarsum* (two males, two females) ex. vegetation, Parque Nacional San Guillermo, altitude 3600 m, San Juan, Argentina, February 2009; CNC-1650: *A. neumanni* (one male) ex. *Catagonus wagneri*, Rivadavia, Salta Province, Argentina, 21 Sep. 2009; CNC-4589: *A. neumanni* (15 males, four females) ex. vegetation, 'La Luisiana', Dean Funes, Córdoba Province, Argentina, 2005-2006.

In order to confirm the conspecificity of male and female ticks from the two owls, we extirpated four legs from one of the females and from one of the males. Legs from each tick specimen were submitted to DNA extraction using the PureLink Genomic DNA MiniKit (Invitrogen, Carlsbad, CA, USA); a pre-extraction macerated step was performed using "Cell and Tissue Disruptor - L-BEADER 6″. The quantity and quality of the extracted DNA were evaluated using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA of the two ticks were submitted to two PCR protocols, one using primers 16S+1 (5′-CCG GTC TGA ACT CAG ATC AAG T-3′) and 16S-1 (5′-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3′), targeting *a* ≈ 460 bp fragment of the tick mitochondrial 16S rRNA gene, as previously described (Norris et al., 1999), and another using primers RIB-4 (5′-CCA TCG ATG TGA AYT GCA GGA CA-'3) (Zahler et al., 1995) and RIB-3 (5′-GTG AAT TCT ATG CTT AAA TTC AGG GGG T-'3) (McLain et al., 1995), targeting *a* ≈ 1100 bp fragment that contain the complete ITS-2 spacer of *Amblyomma* ticks (≈950–1000 bp), as described (Labruna et al., 2002). PCR products were submitted to electrophoresis through a 1.5% agarose gel and examined by LED transilluminator. Amplicons were purified with the PureLink Kit Invitrogen and sequenced in an automatic sequencer (Sanger) according to the manufacturer's protocol. Generated sequences were aligned to each other and submitted to BLAST analysis (Altschul et al., 1990) to determine the closest identities in GenBank.

2.3. Morphological descriptions

Fed specimens, comprising four females and four males, were measured using the Image-Pro-Plus 5.1 program for analysis of images and morphometry, fitted to an Olympus SZX stereoscope microscope (Olympus Corporation, Tokyo, Japan). As the four females laid eggs, their gnathosoma were retracted; therefore, we used a scalpel blade to remove the gnathosoma of one female, then the hypostome was measured (total length and length of toothed portion) and observed to determine its dental formula. Ten unfed larvae preserved in 70% ethanol were mounted in Hoyer's medium between glass slide and coverslip according to Barros-Battesti et al. (2006), measured using the software LAS V4.12 for image analysis and morphometry, fitted to a LEICA DM3000 LED microscope (Leica Microsystems, Wetzlar, Germany). In all descriptions, the measurements are in mm; the range is given (followed by the mean ± standard deviation in parenthesis). Four specimens (one female, one male, and two larvae) were prepared for scanning electron microscopy (SEM) following techniques described by Corwin et al. (1979). Micrographs were taken with a Zeiss EVO MA 10 scanning electron microscope (Carl Zeiss NTS GmbH, Oberkochen, Germany). Finally, light-microscopy photographs of adult ticks were prepared to show the natural scutal ornamentation pattern using the SteREO Discovery V12 stereomicroscope (Carl Zeiss, Munich, Germany) and Olympus microscope model SZ40 (Olympus, Tokyo, Japan). We used the terminology of Nava et al. (2014) to describe the spots that compose the dark brown background of the scutal ornamentation of males and females.

2.4. Phylogenetic analyses

Partial sequences of the 16S rRNA gene of the new *Amblyomma* species were aligned with 41 corresponding 16S rRNA sequences of 35 different species of the genus *Amblyomma* from the Neotropical region, retrieved from Genbank, using Clustal/W v.1.8.1 (Thompson et al., 1994). A maximum likelihood phylogenetic tree using General Time (GTR + *G*+I) substitution model was generated using Mega 11 software (Tamura et al., 2021) with 1000 bootstrap replicates. The substitution model was select using Mega 11 software (Tamura et al., 2021) according to the lowest Bayesian Information Criterion (BIC) score. The 16S rDNA sequence of *Ixodes uriae* White, 1852 (AB030017) was used as outgroup.

For phylogenetic analysis of the ITS-2 spacer, the sequences of the new species were aligned with 19 other sequences of the genus *Amblyomma*. The sequence of *Amblyomma vikirri* Keirans, Bull & Duffield, 1996 (AF199112) was used as outgroup. For the maximum likelihood method, the sequences were aligned using Clustal/W v.1.8.1 (Thompson et al., 1994) with the help of the BioEdit program. A tree using the General Time + G model, with 1000 bootstrap replicates was generated using Mega 11 software. The substitution model was select according to the lowest BIC score using Mega 11 software. For the Bayesian method, the ITS-2 sequences were aligned under standard configurations using Clustal/W (MEGA 11). We used Gblocks 0.91b (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks) to crop the sequences according to the shortest one and to eliminate poorly aligned and divergent regions. Nucleotide substitution model was acquired with jModelTest 2.1.10 using the Bayesian Information Criterion (BIC). For the reconstruction of the phylogenetic tree through Bayesian Analysis, we used MrBayes 3.2.7 with the MCMC (Markov Chain Monte Carlo) algorithm (Ronquist and Huelsenbec, 2003). We ran two different analyses using the standard configuration with four chains every 100 generations, saving a tree every 1000 generations, with a 25% burn-in, until the standard deviation of split frequencies was below 0.01. Clade support was obtained by examining if the posterior probabilities (PP) at the end of the analysis reached or was very close to 1.0. Resulting tree topologies were visualized in Figtree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/). A similarity matrix for the 16S rRNA and ITS2

partial sequences of the new *Amblyomma* species, *A. parvitarsum* and *A. neumanni* was generated using BioEdit. The resulting matrix provides a visual representation of the similarity among the sequences, with values ranging from 0 (no similarity) to one (complete identity).

3. Results

3.1. Identification of ticks and biological parameters

Analyses of the external morphology of the adult ticks collected from two great horned owls in the present study revealed that they were morphologically distinct from any other known *Amblyomma* species, justifying their description as a new species. Partial 16S rDNA sequences (407 bp) generated from one male (GenBank accession number: OQ546724) and one female (OQ546723) were 100% identical to each other, confirming their conspecificity. The ITS-2 PCR assay generated two 984 bp haplotypes from the male (OQ550041) and female (OQ550040) ticks. The first 20 nucleotides of the 984 bp fragments corresponded to the 5′ end of the 5.8S rRNA gene, whereas nucleotides 21 to 984 (964 bp) corresponded to the nearly complete ITS-2 sequences, partially only at the 3′ end. The two ITS-2 haplotypes differed by only four single nucleotide polymorphisms (at positions 638, 640, 769 and 914 of the ITS-2 sequence) and were 99.6% identical to each other, corroborating their conspecificity.

By BLAST analysis, the 407 bp-16S rDNA sequence was closest (96.0 to 96.4% identities; 100% query cover) to *A. parvitarsum* from Argentina (AY498561, KU285012, KX230479, KX230480), Chile (KX230481, KX230482) and Peru (KY705377). The two ITS-2 haplotypes were closest (95.8 and 96.0 identities; 93% query cover) to the single ITS-2 partial sequence of *A. parvitarsum* available in GenBank (KU285141),

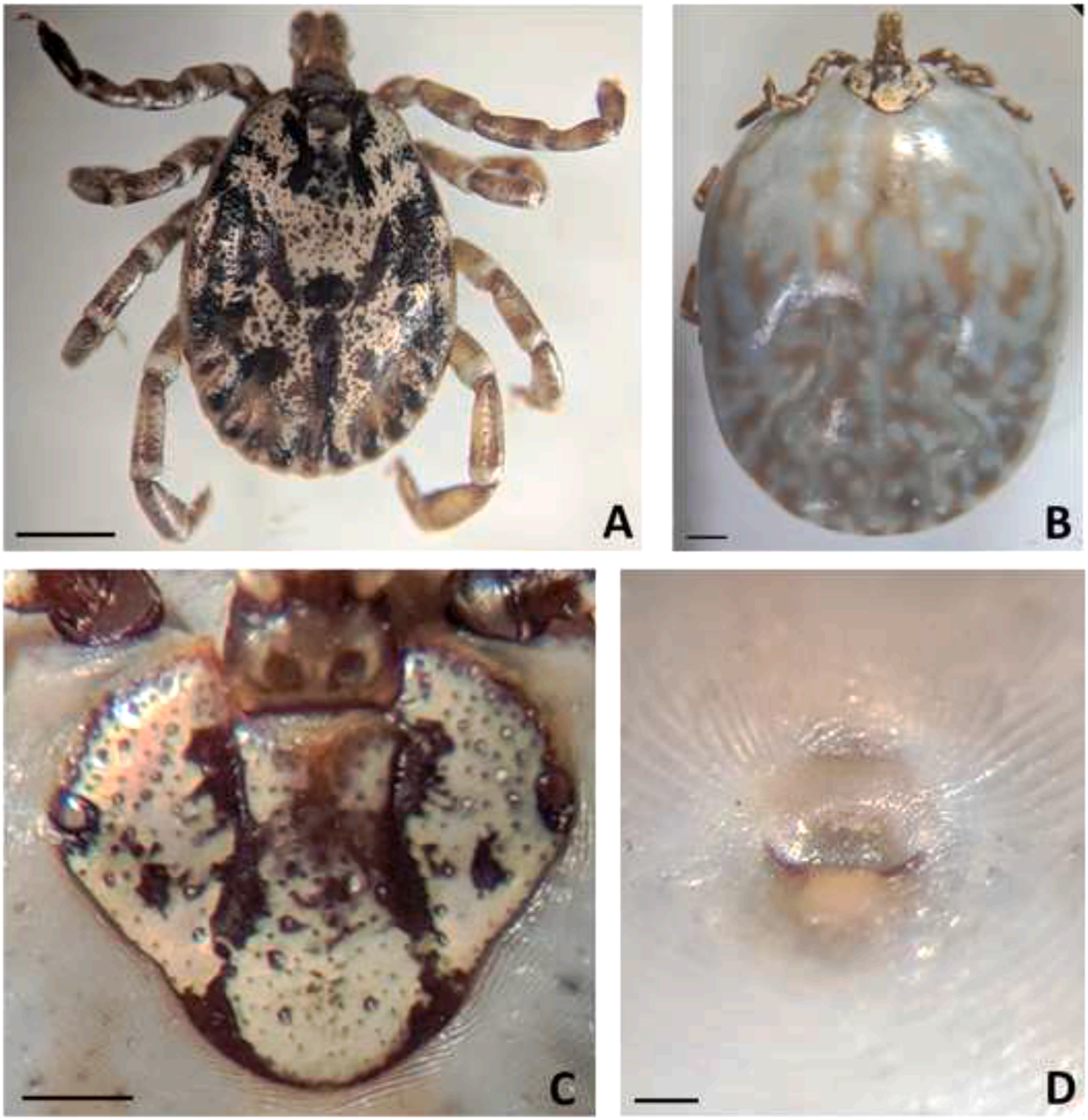

Fig. 2. Light microscopy images of live specimens of *Amblyomma monteiroae* n. sp. (A) Dorsal view of the male (bar: 1 mm). (B) Dorsal view of an engorged female before oviposition (bar: 1 mm). (C) Female scutum (bar: 0.500 mm). (D) Female genital aperture (bar: 0.200 mm).

from Argentina. The% identities between these sequences, including *A. neumanni,* are available in Tables S1 and S2.

The four engorged females had a mean weight of 145 mg (range: 95–191 mg). The mean preoviposition period was 26.0 days (range: 21 – 31 days) for the four females. The mean egg incubation period was 62.5 days (range: 62 – 63 days) for two females, from which less than 100 larvae hatched (<5% hatchability); egg masses of the other two females did not yield any larvae.

3.2. Description

Ixodida Leach, 1815
Ixodidae Koch 1844
Amblyomma Koch, 1844
Amblyomma monteiroae n. sp. Soares, Labruna & Martins (Figs. 2–5)
Male (Figs. 2A and 3). Four fed specimens measured.

Idiosoma. Length from apices of scapulae to posterior body margin 3.85–3.97 (3.91 ± 0.05), maximum breadth 2.85–2.97 (2.92 ± 0.05). Outline oval, broadest at the level of spiracular plates (Figs. 2A, 3A). Genital aperture broadly U-shaped (Fig. 3F). Spiracular plate comma-shaped with elongate macula, numerous minute globlets. Scutum ornate with pale yellow/orange markings on the lateral, median and posterior fields, over a dark brown background; anterior markings give an indication of a pseudoscutum (Fig. 2A). The dark brown background can be divided into a pair of cervical spots; narrow ocular spots (areola); first, second and third lateral spots, the first two fused or interconnected; postero-accessory spots; and limiting spots. There is a postero-median spot, and a central rounded spot between the posterior end of the two limiting spots, just anteriorly to the postero-median spot. Presence of a pair of foveae dorsales, large and deep, well-marked and visible on the central rounded spot, right posterior to the pale markings that delimitate the posterior margin of the pseudoscutum (Fig. 2A). Scutum with

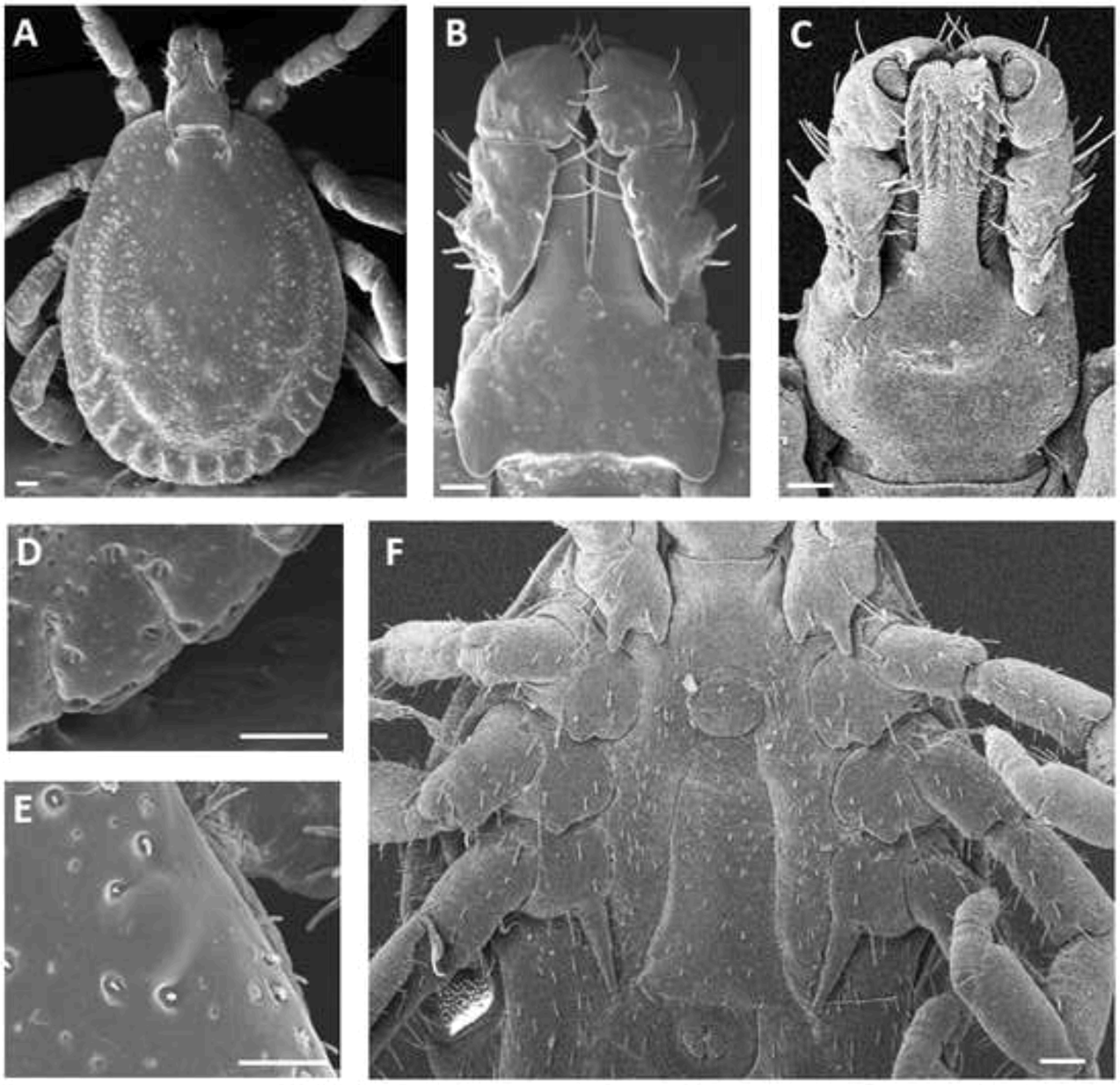

Fig. 3. Scanning electron microscopy of the male of *Amblyomma monteiroae* n. sp. (A) Dorsal view (bar: 0.200 mm). (B) Dorsal gnathosoma (bar: 0.100 mm). (C) Ventral gnathosoma (scale bar: 0.100 mm). (D) Dorsal festoons 3 and 4 with inconspicuous ventral plates (scale bar: 0.200 mm). (E) Right eye (scale bar: 0.100 mm). (F) Ventral idiosoma showing coxae I-IV and genital aperture (scale bar: 0.200 mm).

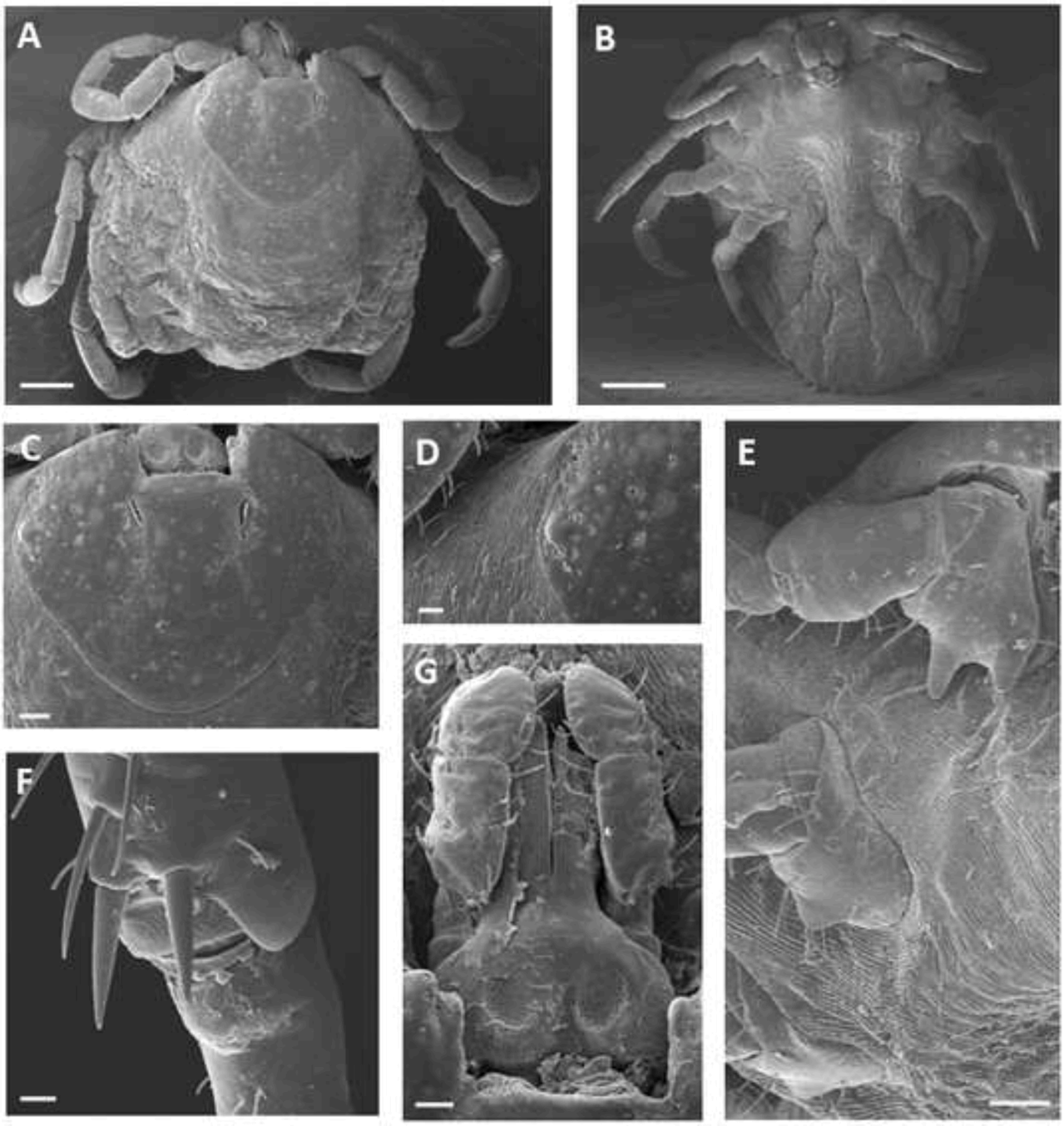

Fig. 4. Scanning electron microscopy (SEM) of the female of *Amblyomma monteiroae* n. sp. (A) Dorsal view (bar: 0.600 mm). (B) Ventral view (bar: 1 mm). (C) Scutum (bar: 0.200 mm). (D) Detail of the left eye (bar: 0.100 mm). (E) Coxae I–III (bar: 0.200 mm). (F) Tibia III showing two spines (bar: 0.040 mm). (G) Dorsal gnathosoma (bar: 0.100 mm). Female specimen was processed by SEM after oviposition in the laboratory, which justifies the irregular contours observed in the idiosoma of the photographed specimen.

numerous large and deep punctations uniformly distributed, except in the central region of the scutum where the punctations are shallow or absent (Fig. 3A). Cervical grooves short, slightly deep, comma shaped. Eyes rounded, bulging, surrounded by dark-colored areola marked by few minute punctations (Figs. 2A, 3E). Marginal groove incomplete, starting at the level of coxa III where it is marked by numerous deep punctations, shallow between the first four festoons and interrupted between festoons five and seven (Figs. 2A, 3A). With 11 festoons, ornate, with some punctations and inconspicuous ventral plates (Fig. 3D).

Gnathosoma. Basis capituli and palpi ornate (Fig. 2A). Palpi long, article I with a pronounced ventral prolongation (Fig. 3C); article II concave laterally with a pronounced retrograde spur-like dorsal extension (Fig. 3B); article III shorter than article II; article IV ventrally to article III. Length of palpal apices to cornua apices 0.99–1.10 (1.05 ± 0.04), breadth 0.63–0.66 (0.64 ± 0.01). Basis capituli subrectangular, posterior margin straight, with the presence of pronounced rounded cornua; few small punctations dorsally (Fig. 3B). Palp length 0.67–0.80 (0.75 ± 0.05); length of palpal article I 0.09–0.12 (0.11 ± 0.01); length of palpal article II 0.34–0.43 (0.39 ± 0.04); length of palpal article III 0.21–0.29 (0.25 ± 0.03), suture between II and III distinct. Hypostome elongate, broadly rounded apically (spatulate) with corona of fine denticles. Total length of hypostome 0.52–0.57 (0.54 ± 0.02); length of

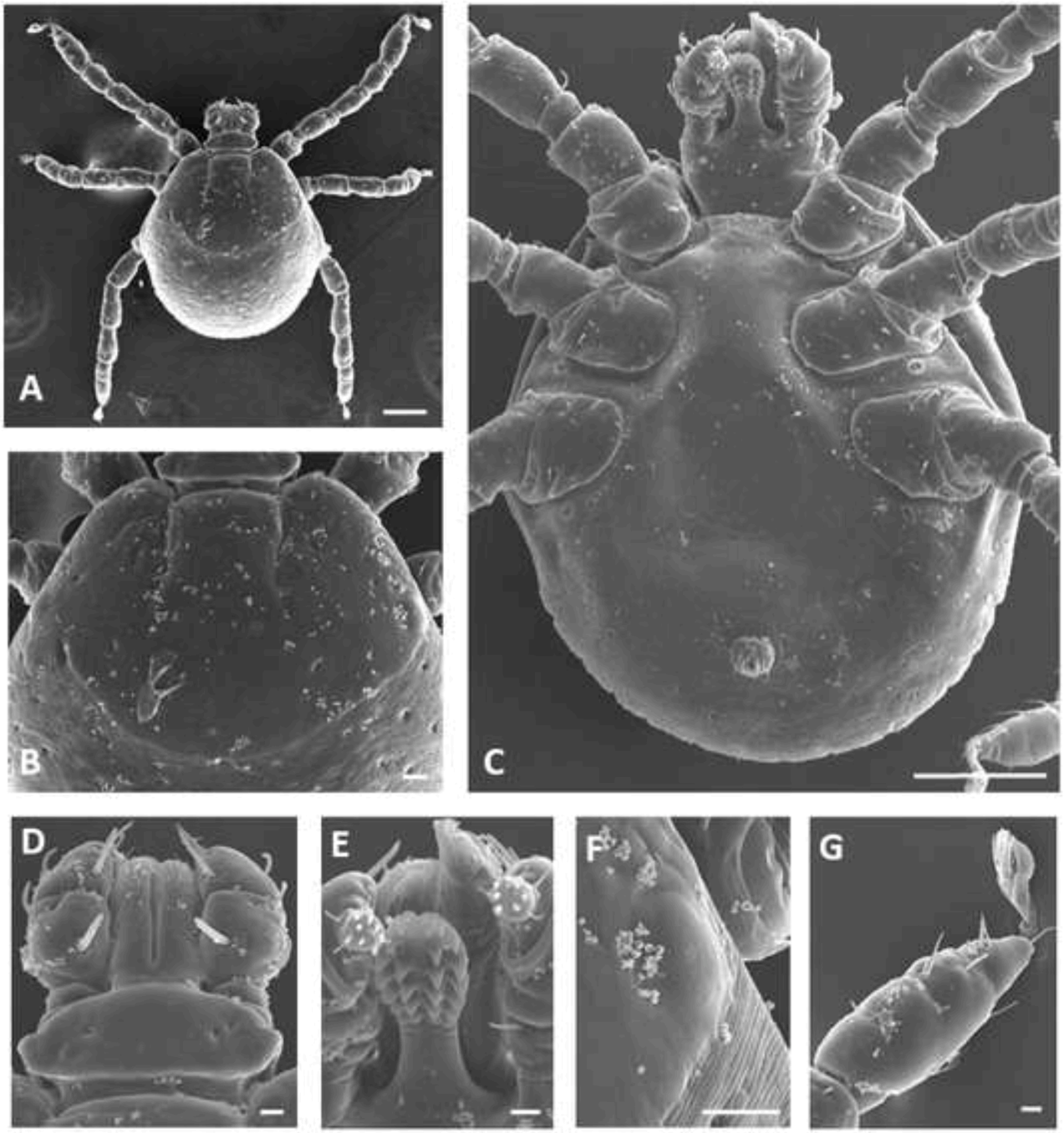

Fig. 5. Scanning electron microscopy of the larva of *Amblyomma monteiroae* n. sp. (A) Dorsal view (bar: 0.100 mm). (B) Scutum (bar: 0.020 mm). (C) Ventral view (bar: 0.100 mm). (D) Dorsal gnathosoma (bar: 0.010 mm). (E) Ventral gnathosoma (bar: 0.010 mm). (F) Detail of the right eye (bar: 0.020 mm). (G) Tarsus I (bar: 0.010 mm).

toothed portion 0.30–0.36 (0.33 ± 0.02). Hypostomal dentition 3/3 with 5–7 teeth per row; teeth of internal row smaller than those of medium and external rows (Fig. 3C).

Legs. Coxa I with two unequal pointed spurs, well separated, the internal smaller, the external less than two times longer than the internal; a single small triangular spur on each of coxae II-III; a single pointed and narrow spur on coxa IV, as long as the length of coxa IV (Fig. 3F). Trochanters without spurs. Presence of two spines on the tibia of legs II-IV (Fig. S3). Tarsus I 0.72–0.85 (0.76 ± 0.05) long, 0.19–0.21 (0.20 ± 0.01) broad. Tarsus IV 0.70–0.75 (0.73 ± 0.02) long; 0.19–0.20 (0.19 ± 0.01) broad.

Female (Figs. 2B–D, 4). Four engorged specimens measured after oviposition.

Idiosoma. Length from apices of scapulae to posterior body margin 6.65–7.76 (7.11 ± 0.53), maximum breadth 4.60–6.22 (5.27 ± 0.68). Outline oval, broadest at the level of spiracular plate (Figs. 2B, 4A,B), with 11 festoons without tubercles. Genital aperture flattened U-shaped (Fig. 2D). Spiracular plate subtriangular with rounded angles, elongate macula surrounded by numerous minute globlets. Scutum length 1.64–1.98 (1.78 ± 0.16), breadth 1.84–2.27 (2.08 ± 0.18). Scutal ornamentation consisting of pale yellow/orange extensively distributed over a dark brown background (Fig. 2C); the dark brown background can be divided into a pair of cervical spots; small accessory spots, and narrow ocular spots (areola). Scutum with numerous punctuations in the

antero-lateral fields, a few large punctations in the central-posterior region of the scutum; cervical grooves deep anteriorly, then diverging at the level of scutal midlength as shallow depressions marked by the scutal dark brown background (Figs. 2C, 4C). Eyes rounded, bulging, surrounded by dark-colored areola marked by few minute punctations, located at lateral scutal angles, between the first and the second thirds of the scutum (Figs. 2C, 4D).

Gnathosoma. Basis capituli and palpi ornate (Fig. 2C), rectangular, without cornua, posterior margin straight (Fig. 4G); length of palpal apices to posterior margin 1.03–1.17 (1.11 ± 0.06), breadth 0.55–0.66 (0.61 ± 0.04). Porose areas large, deeply depressed (Fig. 4G), diameter of one area 0.13–0.17 (0.15 ± 0.02), interporose area 0.10–0.11 (0.11 ± 0.01). Palp length 0.80–0.83 (0.81 ± 0.01); length of palpal article I 0.07–0.10 (0.09 ± 0.01); length of palpal article II 0.33–0.39 (0.37 ± 0.02); length of palpal article III 0.30–0.32 (0.30 ± 0.01), suture between II and III distinct (Fig. 4G). Hypostome elongate, apically rounded (spatulate) with corona of fine denticles. Total length of hypostome 0.56; length of toothed portion 0.31. Hypostomal dentition 3/3 with 6–8 teeth per row; teeth of internal row smaller than those of the other two rows (hypostome measures and dentition based on a single specimen).

Legs. Coxa I with two moderate subequal spurs, well separated, projecting slightly laterally, the external slightly longer than the internal (Fig. 4E); a single small triangular spur on each of coxae II-IV. Trochanters without spurs. Presence of two spines on tibiae II-IV (Fig. 4F). Tarsus I 0.87–0.99 (0.92 ± 0.05) long, 0.18–0.27 (0.22 ± 0.03) broad. Tarsus IV 0.81–0.98 (0.88 ± 0.07) long, 0.16–0.22 (0.18 ± 0.03) broad.

Larva (Fig. 5). Ten unfed specimens measured.

Total length. Length from apex of hypostome to posterior body margin 0.697–0.774 (0.727 ± 0.022).

Idiosoma. Length from apices of scapula to posterior body margin 0.554–0.625 (0.588 ± 0.021), maximum breadth 0.513–0.592 (0.541 ± 0.024), breadth/length ratio 0.886–0.947 (0.918 ± 0.020). Body outline rounded (Fig. 5A), with 11 festoons without tubercles, central festoon 0.064–0.077 (0.072 ± 0.004) breadth, without seta. All festoons separated by prominent grooves, with the central festoon wider than the others ten festoons. Large wax glands: one dorsal pair on lateral margin of alloscutum, above of 1st festoon; five ventral pairs – two pairs on posteroexternal angles of 4th and 5th festoons and one pair located posterior to each coxa. Setae: ten pairs of dorsal body setae – two central pairs and eight marginal pairs; 15 pairs of ventral body setae – three sternal pairs, two pre-anal pairs, four premarginal pairs, five marginal pairs and one pair on anal valves. Anal aperture on central portion of opisthosoma, 0.038–0.048 (0.043 ± 0.003) length, 0.039–0.043 (0.041 ± 0.001) breadth. Scutum (Fig. 5B), 0.307–0.345 (0.327 ± 0.011) length, 0.440–0.500 (0.462 ± 0.019) breadth, breadth/length ratio 1.313–1.452 (1.410 ± 0.048); inornate, surface shagreened, few punctations, outline subtriangular with posterior margin slightly sinuous. Eyes elongate and slightly bulging, located at lateral scutal angles at the level of scutal midlength (Fig. 5F). Setae: three pairs of minute setae, distance between scutal setae (Sc3) 0.055–0.081 (0.068 ± 0.008). Distance between cervical grooves 0.100–0.111 (0.105 ± 0.004), 0.080–0.093 (0.088 ± 0.004) length, slightly convergent, deep and parallel.

Gnathosoma. Length from apex of hypostome to posterior basis margin 0.160–0.181 (0.169 ± 0.006), maximum breadth 0.158–0.172 (0.164 ± 0.004), breadth/length ratio 0.893–1.056 (0.968 ± 0.049). Basis capituli dorsally rectangular in shape, with straight posterior margin, without cornua (Fig. 5D); one pair of dorsal campaniform sensillum (Cs), distance between (Cs) 0.067–0.080 (0.075 ± 0.003), length from (Cs) to posterior basis margin 0.025–0.031 (0.028 ± 0.001); posterior margin convex ventrally, without ventral cornua; one pair of ventral posthypostomal setae (Ph1), distance between (Ph1) 0.027–0.035 (0.030 ± 0.002), length from (Ph1) to posterior ventral margin 0.084–0.103 (0.094 ± 0.006); length from palpal apices to posterior basis margin 0.171–0.187 (0.177 ± 0.004). Palpi robust, 0.110–0.129 (0.119 ± 0.005) length. Article I 0.014–0.023 (0.018 ± 0.002) length, 0.037–0.046 (0.040 ± 0.003) breadth, without ventral prolongation. Article II 0.043–0.053 (0.047 ± 0.003) length, 0.051–0.065 (0.058 ± 0.005) breadth. Article III 0.050–0.058 (0.053 ± 0.002) length, 0.048–0.060 (0.053 ± 0.004) breadth. Article IV apically projected from article III. Articles II and III each more than twice as long as I and IV; sutures between all articles distinct. Setae: without on article I, four dorsally and two ventrally on article II, five dorsally and one ventrally on article III, eight apically and four laterally on article IV. Hypostome compact (Fig. 5E), rounded apically, length from apices to (Ph1) 0.081–0.090 (0.085 ± 0.002), maximum breadth 0.037–0.049 (0.042 ± 0.003), length of toothed portion 0.046–0.057 (0.052 ± 0.003), dentition 2/2 throughout with 6–7 teeth per row, apical corona with four minute denticles.

Legs. Legs relatively long and robust. Coxa I 0.097–0.110 (0.105 ± 0.004) length, 0.092–0.115 (0.105 ± 0.007) breadth, with one minute rounded ridge-like spur (Fig. 5C); antero lateral seta 0.028–0.037 (0.029 ± 0.002) length. Coxa II 0.090–0.130 (0.111 ± 0.013) length, 0.111–0.144 (0.133 ± 0.009) breadth, with one vestigial ridge-like spur. Coxa III 0.107–0.130 (0.113 ± 0.007) length, 0.109–0.129 (0.117 ± 0.007) breadth, with one minute rounded spur. Trochanter without spur. Tarsus I 0.157–0.178 (0.168 ± 0.006) length, 0.059–0.072 (0.066 ± 0.004) breadth. Tarsus I setae: dorsal – two in dI group, five in dII group, two in dIII group, two in dIV group, without in dV group, and two in dVI group; ventral – two in vI group, two in vII group, and two in vIII group; lateral anterior – one in laI group, and three in laII group; lateral posterior – one in lpI group, and three in lpII group. Haller's organ, with transverse capsular aperture slit-like and six setae in anterior pit (Fig. 5G). Tarsus III 0.131–0.155 (0.143 ± 0.007) length, 0.045–0.053 (0.047 ± 0.002) breadth.

3.2.1. Types

HOLOTYPE male was collected ex *B. virginianus* (Strigiformes: Strigidae) from Balneário Pinhal municipality (-30.29072, -50.25590; altitude 3 m), state of Rio Grande do Sul, Brazil, 24 July 2020; collector: D. B. de Amorim; ALLOTYPE Female collected ex *B. virginianus* from Tramandaí municipality (-30.005771, -50.152245; altitude 6 m), state of Rio Grande do Sul, Brazil, 15 July 2020; collector: D. B. de Amorim. Holotype and Allotype were deposited in the United States National Tick Collection (Statesboro, Georgia, USA) under accession numbers USNMENT01786504, CEN/RML130753; and USNMENT1786499, CEN/RML 130754, respectively.

3.2.2. Paratypes

One male, one female, same data as for Holotype and Allotype, respectively, deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (São Paulo, SP, Brazil) under accession numbers CNC-4583 and −4584, respectively; one male, one female, same data as Holotype and Allotype, respectively, deposited in the Acari Collection "Coleção Acarológica do Instituto Butantan" (São Paulo, SP, Brazil) under accession numbers IBSP-18,720 and -18,721, respectively (these two specimens were used for SEM); one male, one female, same data as Holotype and Allotype, respectively, deposited in the "Coleção de Vetores do Laboratório de Protozoologia e Rickettsioses Vetoriais" (ProtozooVet) collection of the Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, under accession numbers ProtozooVet-426 and −427, respectively (the extirpated legs used for DNA extractions were from the latter two paratypes). Paratype larvae consisted of 32 unfed larvae that hatched from eggs laid by paratype females in the laboratory, and were deposited in the above tick collections with the following accession numbers: CNC-4585 (20 larvae; include ten slide-mounted specimens), IBSP-18,722 (5 larvae), and ProtozooVet-428 (7 larvae).

3.2.3. Etymology

The new species is named in honor of Dr. Silvia Gonzalez Monteiro, Professor at the Federal University of Santa Maria (UFSM), in

recognition of her contribution to the study of Brazilian veterinary parasitology.

3.3. Phylogenetic analyses

In the phylogenetic tree inferred by partial sequences of the mitochondrial 16S rRNA gene (Fig. 6), *A. monteiroae* n. sp. male and female formed a clade with several haplotypes of *A. parvitarsum* from Argentina, Chile and Peru, under 99% bootstrap support, with haplotypes of the two species separated in opposite sides of the clade. Two haplotypes of *A. neumanni* branched sister to this clade. The phylogenetic trees inferred by partial sequences of the ITS-2 spacer presented similar topologies by Maximum Likelihood and Bayesian methods, therefore only the Bayesian tree is presented (Fig. 7). The two ITS-2 haplotypes of *A. monteiroae* n. sp. grouped with a haplotype of *A. parvitarsum* from Argentina, which formed a clade with *A. neumanni* with high branch reliability values.

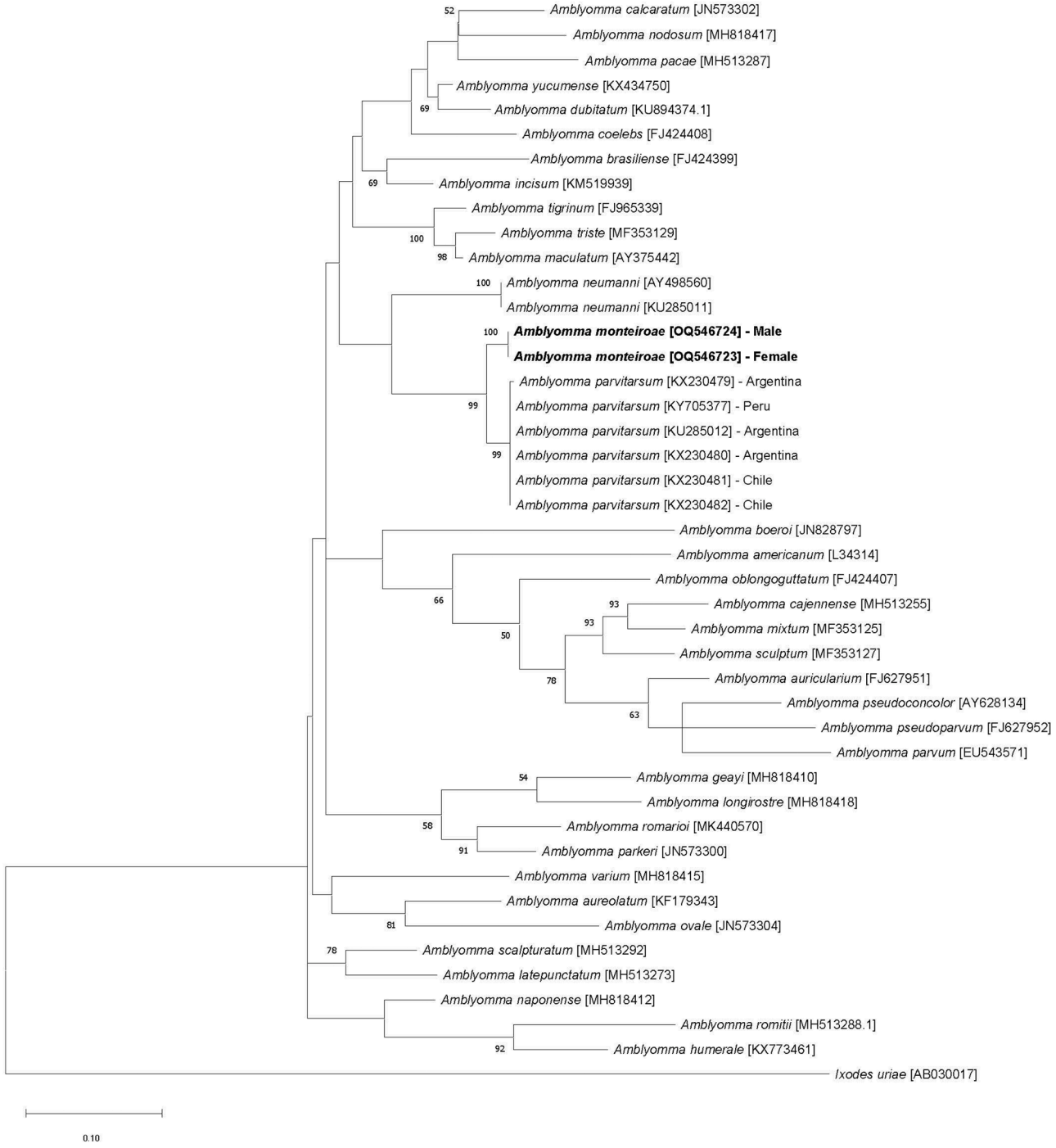

Fig. 6. Maximum likelihood phylogenetic tree of 16S rRNA partial sequences of *Amblyomma monteiroae* n. sp. (sequence names in bold) and closely related Ixodidae. A total of 409 valid positions for each sequence in the final dataset were used. Numbers on the nodes indicate bootstrap values from 1000 replicates. Only bootstrap values >50 are shown. Numbers in brackets are GenBank accession numbers. The corresponding 16S rRNA partial sequences of *Ixodes uriae* [AB030017] was used as outgroup.

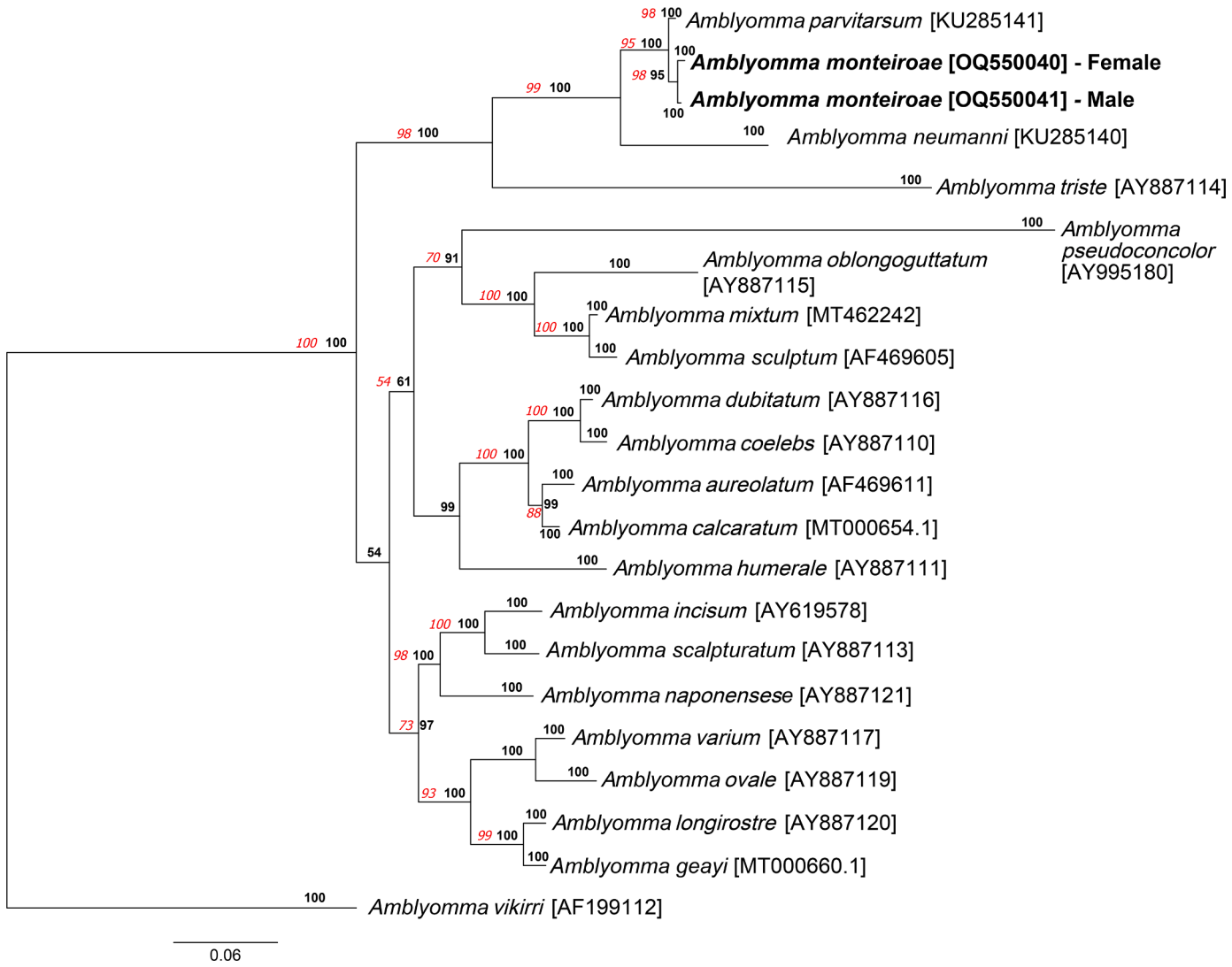

Fig. 7. Bayesian phylogenetic tree of ITS2 partial sequences of *Amblyomma monteiroae* n. sp. (sequence names in bold) and closely related sequences of the genus *Amblyomma*. A total of 1084 valid positions for each sequence in the final dataset were used. Numbers in bold on the nodes indicate support values from 1000 replicates, and in italic and red are the bootstrap values for 1000 replicates. Only bootstrap/support values >50 are shown. Numbers in brackets are GenBank accession numbers. The corresponding ITS2 partial sequences of *Amblyomma vikirri* [AF199112] was used as outgroup.

3.4. Species relationship

Amblyomma monteiroae n. sp. is genetically and morphologically related to *A. parvitarsum*. Both tick species are unique in combining the following morphological characters: scutum extensively ornate with similar arrangement of spots; eyes rounded and bulging; coxa I with two moderate pointed spurs, the external longer than the internal; a single triangular short spur on coxae II-III; presence of two spines on the tibia of legs II-IV; hypostomal dentition 3/3, trochanters without spurs. The males of the two species can be separated by the palpal article II, concave laterally and presenting a pronounced retrograde spur-like dorsal extension in *A. monteiroae* n. sp. (Fig. 8A), versus palpal article II not concave laterally and with just a discrete retrograde spur-like dorsal extension in *A. parvitarsum* (Fig. 8B), and by inconspicuous ventral plates (carena) in the festoons, present in *A. monteiroae* n. sp. (Fig 8C) and absent in *A. parvitarsum* (Fig. 8D). Females of the two species can be separated by coxal I spurs, subequal in *A. monteiroae* n. sp. (Fig. 9A), versus the external spur nearly twice the length of internal spur in *A. parvitarsum* (Fig. 9B), and coxal IV triangular spur, similar to coxae III in *A. monteiroae* n. sp. (Fig. 9C), versus a coxa IV pointed spur at least twice the length of coxa III spur in *A. parvitarsum* (Fig. 9D). Both *A. monteiroae* n. sp. and *A. parvitarsum* are genetically and morphologically related to *A. neumanni*; however, the later can be separated from the formers by the presence of a single spine in tibia II-IV, versus two spines in tibia II-IV of *A. monteiroae* n. sp. and *A. parvitarsum*.

Some authors have referred to the eye of *A. parvitarsum* as orbited (Boero, 1957; Estrada-Peña et al., 2005; Nava et al., 2017), although Neumann (1901) described it as sub-orbited. According to Walker et al. (2003), an "orbit eye" in ticks is defined as "a circular groove surrounding the eye of some ticks, very typical of *Hyalomma* species". Indeed, neither *A. parvitarsum* nor *A. monteiroae* n. sp. has typically orbited eyes as reported for *Hyalomma* ticks. It is possible that the dark-colored areola marked by minute punctuations tended the previous authors to consider the eyes of *A. parvitarsum* as orbited. We conclude that the morphology of the eyes of *A. parvitarsum* and *A. monteiroae* n. sp. are similar (rounded and bulging), differing from the flat eyes present in *A. neumanni*.

The larva of *A. monteiroae* n. sp. can be morphologically distinguished from *A. parvitarsum* only by morphometry, the former species slightly smaller [i.e., length from apex of hypostome to posterior body margin: 0.697–0.774 (0.727 ± 0.022) in *A. monteiroae* n. sp., versus 0.962–0.993 (0.974) in *A. parvitarsum* (Estrada-Peña et al., 2005)]. The

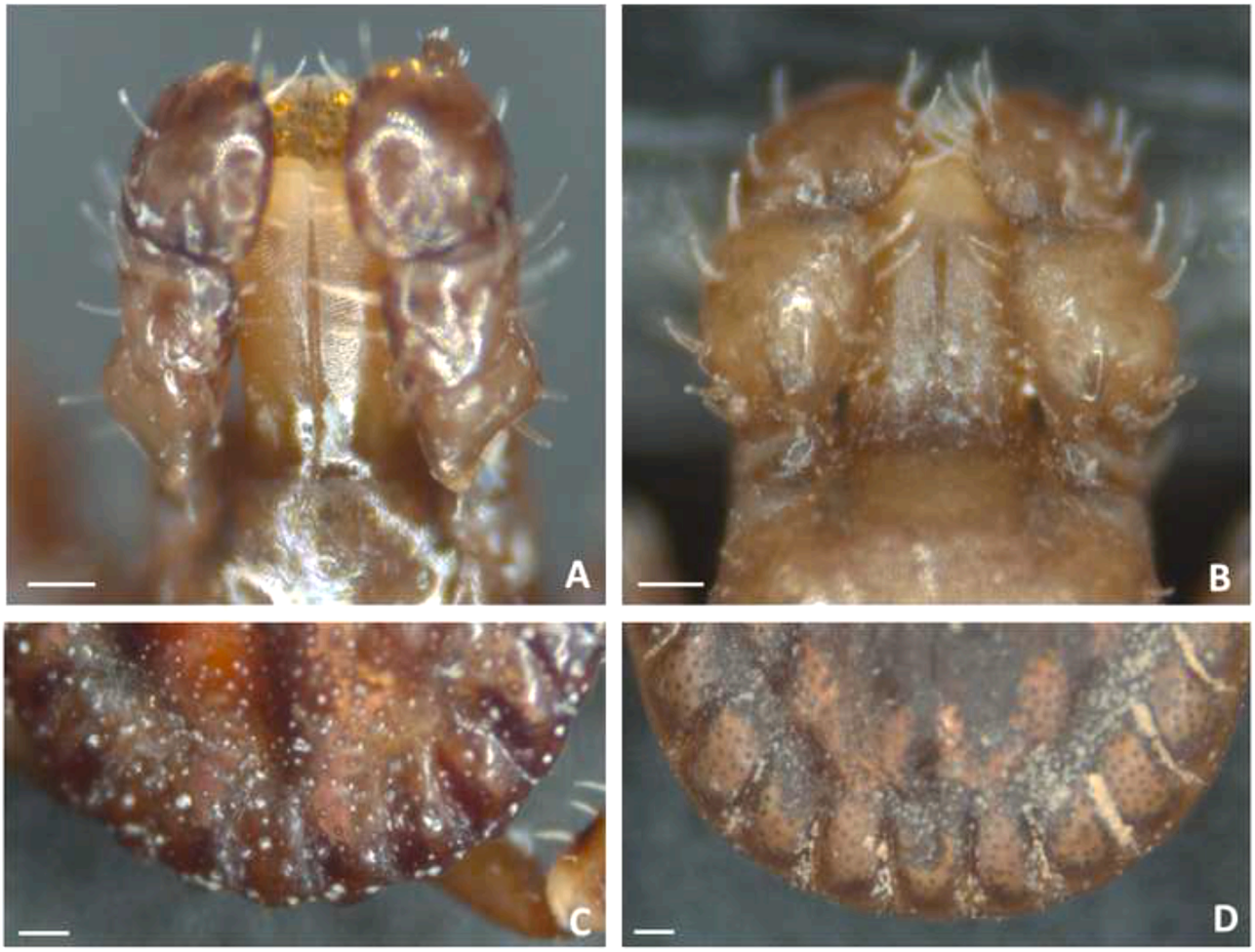

Fig. 8. Morphological differences between the males of *Amblyomma monteiroae* n. sp. and *Amblyomma parvitarsum.* (A) Dorsal view of the palpi of *A. monteiroae* n. sp. showing the article II concave laterally and with a pronounced retrograde spur-like dorsal extension (bar: 0.1 mm). (B) Dorsal view of the palpi of *A. parvitarsum* showing article II not concave laterally and with just a discrete retrograde spur-like dorsal extension (bar: 0.1 mm). (C) Festoons of *A. monteiroae* n. sp. presenting inconspicuous ventral plates (bar: 0.2 mm). (D) Festoons of *A. parvitarsum* without ventral plates (bar: 0.2 mm). Pictures of *A. parvitarsum* were taken from specimens from Argentina (CNC-4588).

larvae of *A. monteiroae* n. sp. and *A. parvitarsum* have a single minute rounded ridge-like spur on coxa I, while the larva of *A. neumanni* has two rounded spurs on coxa I (Estrada-Peña et al., 2005). In the descriptions of the larvae of *A. neumanni* and *A. parvitarsum*, the authors mentioned the presence of eyes without giving further details of the eye morphology (Estrada-Peña et al., 1993; 2005). A more recent study reported that the larva of *A. parvitarsum* has bulging eyes, which also distinguish it from the larva of *A. neumanni* (Nava et al., 2009). Therefore, *A. monteiroae* n. sp. larvae can also be distinguished from *A. neumanni* larvae by the presence of bulging eyes in the former, flat eyes in the latter. Finally, the larva of *A. monteiroae* n. sp. is also morphologically similar to the larva of *Amblyomma boeroi* (Nava et al., 2009); however, they can be distinguished by morphometry [i.e., length from apex of hypostome to posterior body margin: 0.697–0.774 (0.727 $\pm$ 0.022) in *A. monteiroae* n. sp., versus 0.790–0.850 (0.813 $\pm$ 0.006) in *A. boeroi* (Nava et al., 2009)] and by the arrangement of the large wax glands: one dorsal pair (on lateral margin of alloscutum, above the 1st festoon) and five ventral pairs (2 pairs on posteroexternal angles of the 4th and 5th festoons, and one pair posterior to each coxa) in *A. monteiroae* n. sp., versus one dorsal pair (on lateral margin of alloscutum, above the 1st festoon) and four ventral pairs (1 pair on posteroexternal angle of the 5th festoon, and one pair posterior to each coxa) in *A. boeroi* (Nava et al., 2009). Unfortunately, the arrangement of large wax glands of *A. parvitarsum* has not been reported.

3.5. Hosts and distribution

Until now, adult specimens of *A. monteiroae* n. sp. have been collected only from two individuals of the great horned owl, *B. virginianus*, the largest nocturnal wild raptor in Brazil, and one of the largest in America (Sick, 1997; Menq, 2018). The fact that the collected female ticks were engorged and at least two of them laid viable eggs suggests that the great horned owl is a major host for *A. monteiroae* n. sp. Hosts for immature stages of *A. monteiroae* n. sp. remain unknown. Currently, the distribution of *A. monteiroae* n. sp. is restricted to two municipalities in the coastal region (altitude <10 m) of the Rio Grande do Sul (southern Brazil). Because *B. virginianus* has a much larger distribution in the American continent, from Canada to southern South America (Sick, 1997; Konig and Weick, 2008; Menq, 2018), further studies are required to explore a likely broader distribution of *A. monteiroae* n. sp.

4. Discussion

This study provides the first records for the Neotropical region of ticks infesting the great horned owl, *B. virginianus,* which were found infested by adults (males and females) of *A. monteiroae* n. sp. This new species was shown to be morphologically and genetically related to *A. parvitarsum,* and in a lesser extent to *A. neumanni.* The taxon

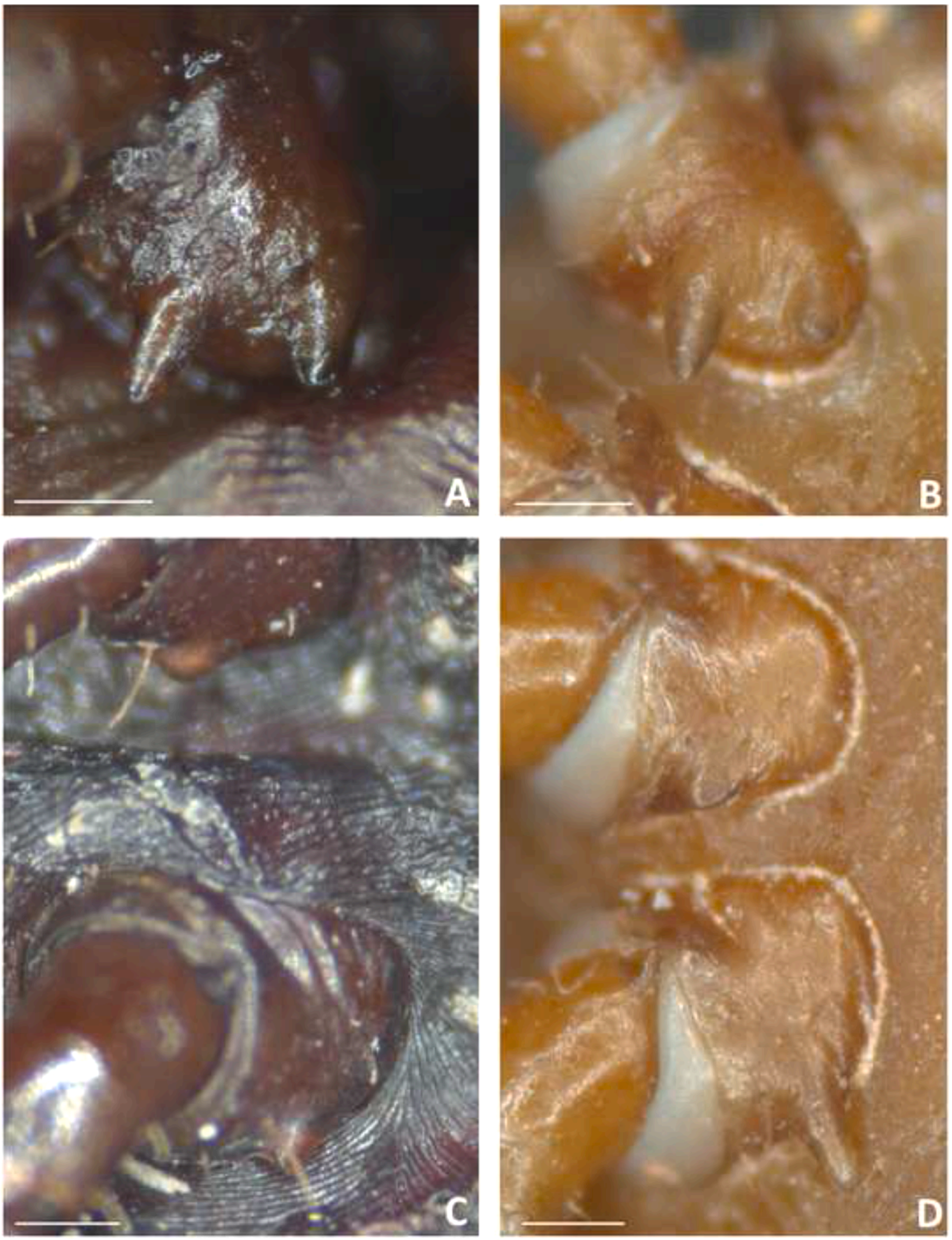

Fig. 9. Morphological differences between the females of *Amblyomma monteiroae* n. sp. and *Amblyomma parvitarsum.* (A) Coxae I of *A. monteiroae* n. sp. showing two subequal spurs. (B) Coxae I of *A. parvitarsum* showing the external spur nearly twice the length of the internal spur. (C) Coxa IV of *A. monteiroae* n. sp. presenting a triangular spur similar in length to coxae III spur. (D) Coxa IV of *A. parvitarsum* presenting a pointed spur at least twice the length of coxa III spur. Bars: 0.2 mm. Pictures of *A. parvitarsum* were taken from specimens from Argentina (CNC-4588).

A. parvitarsum was described based on a female specimen from Bolivia and another one from Patagonia (Argentina), with no host data (Neumann, 1901). Since then, adults of *A. parvitarsum* have been reported mostly as parasites of South American camelids of the genera *Lama* and *Vicugna,* occurring in highlands of Argentina, Bolivia, Chile, Peru, and also in Argentinean Patagonia (Estrada-Peña et al., 2005; Nava et al., 2017; Guglielmone et al., 2021). *Amblyomma neumanni* was described based on a specimen collected in northern Argentina (Ribaga, 1902), from where this species has been widely reported as parasites of large wild and domestic mammals (Nava et al., 2017; Guglielmone et al., 2021). Despite of the morphological and genetic relatedness of *A. monteiroae* n. sp., *A. parvitarsum,* and *A. neumanni,* it seems that they are ecologically very distinct, i.e., *A. monteiroae* n. sp. associated with birds in the coastal region of southern Brazil, *A. parvitarsum* associated with camelid mammals in Andean environments and the Patagonian Plateau, and *A. neumanni* associated with large mammals in the Chaco and Yungas biogeographical regions in northern Argentina.

Neither *A. parvitarsum* nor *A. neumanni* is known to occur in Brazil, except for a single record of a female of *A. parvitarsum* parasitizing an unusual host, a Magellanic penguin [*Spheniscus magellanicus* (Forster, 1781)], in Cassino Beach, municipality of Rio Grande, also in the southern coast of the state of Rio Grande do Sul (Becker et al., 1997), just ≈280 km south to the present localities of *A. monteiroae* n. sp. Indeed, our present description of *A. monteiroae* n. sp. associated with another bird species in the same coastal region of the record of Becker et al. (1997) raises the possibility that the female specimen collected from penguin could be in fact *A. monteiroae* n. sp. Unfortunately, the specimen reported by Becker et al. (1997) was not available for reexamination and morphological comparisons with *A. monteiroae* n. sp. Interesting, there is another record of the adult stage of *A. parvitarsum* on a bird host, which refers to the Darwin's rhea, *Rhea pennata* d'Orbigny, 1837, in Argentina (Boero, 1954); however, this record was from Chubut Province within the Argentinean Patagonia, where *R. pennata* lives with wild camelids within the known distribution range of *A. parvitarsum* (Boero, 1954).

According to Guglielmone and Nava (2014), the only known synonym of the taxon *A. parvitarsum* is *Amblyomma altiplanum* (Dios, 1917), whereas the only known synonym of *A. neumanni* is *Amblyomma furcula* (Dönitz, 1909). Unfortunately, we did not have the chance to examine the types of *A. altiplanum* or *A. furcula*. Although the description of *A. altiplanum* lacks illustrations and sufficient details to distinguish it from *A. monteiroae* n. sp., it was based on adult specimens collected on 'llamas' from the Argentinean-Bolivian highlands (altitude between 1500 and 4000 m) (Dios, 1917), which are typical hosts and biogeographical region for *A. parvitarsum.* The description of *A. furcula* by Dönitz (1909) relied on adult specimens collected from environment in Argentina, with no illustration; however, Robinson (1926) redescribed the male and female of *A. furcula* based on Argentinean specimens that were donated by W. Dönitz in 1909 to the Cambridge collection. In the illustration of the *A. furcula* male provided by Robinson (1926), the tibia IV has only one spine and the coxa I has the external spur more than twice as long as the internal spur, which are characters that separate *A. neumanni* from *A. monteiroae* n. sp. Based on the above statements, we discard the possibility that the present specimens of *A. monteiroae* n. sp. could be *A. altiplanum* or *A. furcula.*

The great horned owl (*B. virginianus*) is the largest species of owl from the New World, with adults weighing over one Kg (Sick, 1997). It occurs from Canada to southern South America (except Argentinean Patagonia and Chile). The great horned owl is known to have resident populations in the state of Rio Grande do Sul (Tomazzoni et al., 2004; Menq, 2018); therefore, our records of *A. monteiroae* n. sp. could represent local populations of this novel tick species, although we cannot discard that the owls became infested in an area far from the sites where they were rescued.

We have considered the females of *A. monteiroae* n. sp. as 'engorged' when collected from an owl. However, we are aware that these females might had not been completely engorged, especially because their mean weight was only 145 mg, considerably lower than the full engorged female mean weight of small species of Neotropical *Amblyomma,* such as *Amblyomma parvum* Aragão, 1908 (424 mg) and *Amblyomma oblongoguttatum* Koch, 1844 (488 mg) (Olegário et al., 2011; Martins et al., 2017). Although the four *A. monteiroae* n. sp. females laid eggs in the laboratory, larvae hatched from only two egg masses, even then with hatching rates <5%. Since no male ticks were found in the female-infested owl, it is possible that these females never copulated with males; thus, the few hatched larvae may have been a product of thelytokous parthenogenesis, a phenomenon not rare among virgin engorged females of metastriata ticks, including some species of the genus *Amblyomma* (Gladney and Dawkins, 1973; Labruna et al., 2011). Finally, the likely parthenogenetic origin of the larvae of *A. monteiroae* n. sp. could explain some morphological abnormalities among some of the unfed larvae, such as missing legs (not shown) and scutal malformation (see the left lateral angle of the scutum of the larva used for SEM in Fig. 5B).

The present description of *A. monteiroae* n. sp. increases to 137 the number of *Amblyomma* species, 136 extant, one extinct (Guglielmone et al., 2015). The Brazilian tick fauna is now composed by 78 species, 53 of the Ixodidae family and 25 of the Argasidae family. Indeed, the genus *Amblyomma* remains the richest in Brazil, now with 34 valid species.

Fig. S1. Engorged female ticks attached to the ventral neck area of a great horned owl (*B. virginianus*) in Tramandaí municipality, state of Rio Grande do Sul, Brazil, on 15 July 2020.

Fig. S2. Male ticks attached to the head region of a great horned owl (*B. virginianus*) at Balneário Pinhal municipality, state of Rio Grande do Sul, Brazil, on 24 July 2020.

Fig. S3. *Amblyomma monteiroae* n. sp., male paratype CNC-4583. Tibia II (A), tibia III (B) and tibia IV (C) showing two spines. Bars: 0.1 mm.

Table S1. Similarity matrix for partial sequences of the 16S rRNA mitochondrial gene of *Amblyomma monteiroae* n. sp., *Amblyomma neumanni* and *Amblyomma parvitarsum.*

Table S2. Similarity matrix for partial sequences of the internal transcribed spacer (ITS2) of *Amblyomma monteiroae* n. sp., *Amblyomma neumanni* and *Amblyomma parvitarsum.*

CRediT authorship contribution statement

João F. Soares: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Marcelo B. Labruna:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Derek B. de Amorim:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **Vinícius Baggio-Souza:** Data curation, Investigation, Methodology, Resources, Writing – review & editing. **Renata Fagundes-Moreira:** Investigation, Methodology, Writing – review & editing. **Aline Girotto-Soares:** Investigation, Methodology, Writing – review & editing. **Barbara Weck:** Investigation, Methodology, Writing – review & editing. **Pablo H. Nunes:** Investigation, Methodology, Writing – review & editing. **Thiago F. Martins:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

The data that has been used is confidential.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102239.

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