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Amblyomma hadanii n. sp. (Acari: Ixodidae), a tick from northwestern Argentina previously confused with *Amblyomma coelebs* Neumann, 1899

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Abstract All stages of *Amblyomma hadanii* n. sp. (Acari: Ixodidae) are described from northwestern Argentina. The diagnostic characters for males are a combination of the pattern of scutal ornamentation, *basis capituli* dorsally rectangular with cornua, coxa I with two subequal spurs (the internal wider, the external longer), coxae II-III with a single spur, coxa IV with a single spur not reaching level of anus, ventral plates irregular in shape (larger and sometimes with a small incision on festoons 4, 5 and 6) and hypostome spatulate with dental formula 3/3 in 7–8 rows. The diagnostic characters for the females are a combination of scutal ornamentation, postero-lateral margins of scutum slightly convex, coxa I with two subequal spurs (the internal wider, the external longer), *basis capituli* dorsally rectangular, porose areas rounded, genital aperture U-shaped, and hypostome spatulate with dental formula 3/3 in 7–8 rows. Diagnosis of nymphs can be performed by a combination of *basis capituli* rectangular, scutum with large punctations in the lateral fields and small punctations in the central field, and cervical groove short and ending as a small shallow depression at the eye level. Larvae are diagnosed by the shape of *basis capituli*, scutum with with posterior margin slightly convex, and legs with coxa I with 2 triangular spur (the external longer than the internal), and with coxae II and III each with 1 triangular spur. The hosts recorded for this new tick species are *Tapirus terrestris* (Linnaeus), horse, cattle, dog and humans. Analyses of a 410 bp fragment of the mitochondrial 16S rRNA gene and the complete sequence of the nuclear 18S rRNA gene supported the description of *A. hadanii* as a new species.

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Introduction

The genus *Amblyomma* Koch, 1844 has the highest specific richness in the Neotropical Region, where 64 species range in contrasting habitats and their medical

and veterinary importance as parasites *per se* and vectors of pathogenic organisms is well documented (Guglielmone et al., 2003, 2014; Labruna et al., 2011; Nava et al., 2014). A series of systematic studies on the genus carried out recently in the Neotropics has resulted in the reinstatement of taxa previously considered invalid (*Amblyomma latepunctatum* Tonelli Rondelli, 1939, *Amblyomma romitii* Tonelli Rondelli, 1939, *Amblyomma sculptum* Berlese, 1888, *Amblyomma mixtum* Koch, 1844) and in the description of new species (*Amblyomma boeroi* Nava, Mangold, Mastropaolo, Venzal, Oscherov & Guglielmone, 2009, *Amblyomma tonelliae* Nava, Beati & Labruna, 2014, *Amblyomma patinoi* Labruna, Nava & Beati, 2014, *Amblyomma interandinum* Beati, Nava & Cáceres, 2014) (Labruna et al., 2005; Barros-Battesti et al., 2007; Nava et al., 2009, 2014).

Recent field studies in Yungas Phytogeographic Province *sensu* Cabrera (1994) in the northwestern Argentina resulted in collection of specimens of a new species of *Amblyomma*, and the recognition that it has been misidentified as *Amblyomma coelebs* Neumann, 1899 or determined as *Amblyomma* sp. This paper describes all stages of the new species and presents ecological information for this tick. Analyses of a 410 bp fragment of the mitochondrial 16S rRNA gene and the complete sequence of the nuclear 18S rRNA gene were also performed to infer the phylogenetic position of the new tick species in relation to other Neotropical species of *Amblyomma*.

Materials and methods

Morphological descriptions are based on free-living ticks collected from two localities of Salta and Jujuy provinces, Argentina, belonging to the Yungas Phytogeographic Province as described by Cabrera (1994) (see details in "material examined"). Additionally, specimens deposited in the following five collections were examined: (i) Tick collection of INTA Rafaela, Santa Fe, Argentina (INTA); (ii) Tick collection of Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Santa Fe, Argentina (FAVE); (iii) United States National Tick Collection, Institute for Coastal Plain Science, Georgia Southern University, USA (USNMENT); (iv) Coleção Nacional de Carrapatos of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil (CNC); (v) Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Salto, Uruguay (DPVURU).

Ticks were cleaned for both light and scanning electron microscopy with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1. Ten unfed specimens of each stage were measured and photographed using a Nikon Alphaphot-2 YS2 optical microscope for the descriptions that follow. All measurements are in millimetres and are presented as the range followed by the mean in parentheses. The description of the scutal ornamentation of males follows the terminology of Nava et al. (2014). Scanning electron photomicrographs 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; colour photographs were generated by Lorenza Beati with a BK Plus Lab System (Visionary Digital), stacked with Helicon Focus v. 4.77 in the USNMENT.

DNA was extracted from representative specimens of each stage and processed using a polymerase chain reaction following the methodology described by Mangold et al. (1998a, b). The DNA was then used to amplify a 410 bp fragment of the mitochondrial 16S rRNA gene and the complete sequence of the nuclear 18S rRNA gene, using the primers designed by Mangold et al. (1998a, b). Sequences were aligned together with sequences for the Neotropical *Amblyomma* spp. available in GenBank, using the BioEdit Sequence Alignment Editor (Hall, 1999) with the CLUSTAL W program (Thompson et al., 1994). Phylogenetic relationships were assessed in terms of maximum likelihood method (ML). Best fitting substitution models were determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 5 (Tamura et al., 2011). The number of variable nucleotide positions between sequences was used to calculate pairwise estimates of percent sequence divergence. The best substitution model for the phylogenetic tree constructed with 18S DNA sequences was Kimura 2-parameter (K2) model by using Gamma-distribution with Invariant sites (G+I). The tree made with 16S DNA sequences was generated with the GTR model and a discrete Gamma-distribution (+G). Support for the topologies was tested by bootstrapping over 1,000 replications; *Ixodes luciae* Sénevet, 1940 was chosen as outgroup.

***Amblyomma hadanii* n. sp.**

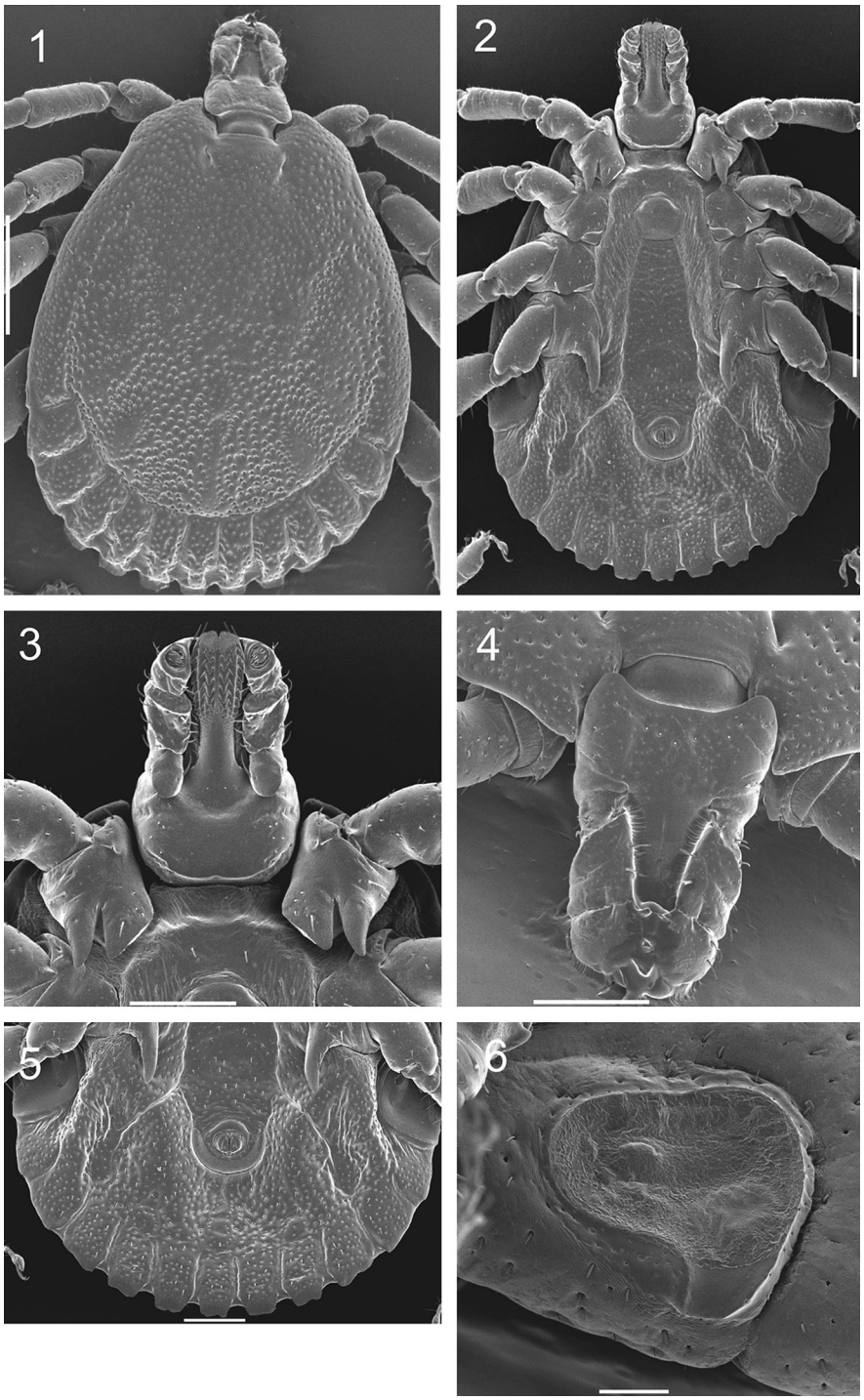
Type-locality: Parque Nacional El Rey (24°41'S, 64°36'W), Salta Province, Argentina.
Type-material: Holotype ♂ and allotype ♀, 1 paratype ♀, 1 paratype nymph ex vegetation, 29.xi.2008, Parque Nacional El Rey, Salta Province, coll. M. Mastropaolo, S. Nava and A.J. Mangold, deposited in USNMENT (Holotype ♂: USNMENT957444; allotype ♀: USNMENT957445; paratype ♀: USNMENT957446; paratype nymph: USNMENT957447); paratypes 10 ♂♂, 10 ♀♀, and 10 nymphs ex vegetation, Parque Nacional El Rey, Salta Province, deposited in INTA (INTA 2238); paratypes 1 ♂, 1 ♀ and 1 nymph ex vegetation, Parque Nacional El Rey, Salta Province, deposited in FAVE (SAER 20b); paratypes 1 ♂, 1 ♀ ex vegetation, Parque Nacional El Rey, Salta Province, deposited in the CNC (CNC 1435); paratypes 5 nymphs ex vegetation, collected in Villamonte (24°18'S, 64°31'W), Jujuy Province, deposited in INTA (INTA 2239); paratypes 10 larvae ex vegetation, Parque Nacional El Rey, Salta Province, deposited in DPVURU (DPVURU 872).
Comparative material: Specimens deposited in INTA, FAVE, CNC and USNMENT: 1 ♂ ex *Tapirus terrestris* (Linnaeus), Parque Nacional El Rey, Salta, Argentina (INTA 1343, identified as *A. coelebs*); 1 ♂ reared from nymph collected on horse, Parque Nacional El Rey, Salta, Argentina (INTA 1880, identified as *A. coelebs*); 3 ♀♀ ex vegetation, Parque Nacional El Rey, Salta, Argentina (INTA 2241); 1 nymph ex cow, Villamonte, Jujuy, Argentina (INTA 2243); 21 ♂♂, 33 ♀♀ ex vegetation, Parque Nacional El Rey, Salta, Argentina (FAVE, SAER 6c, 11a, 17c, 18a, 20b, 43a, 64a, 69a, 70a, 78, 79b, 94, 98, 105a, 115a, 118, 147a, identified as *A. coelebs*); 2 ♀♀ ex horse, Parque Nacional El Rey, Salta, Argentina (FAVE 23c, 150b, identified as *A. coelebs*); 38 nymphs ex vegetation, Parque Nacional El Rey, Salta, Argentina (FAVE, SAER 2b, 5b, 6b, 14b, 16b, 17b, 24b, 26, 39, 44, 46, 51b, 54b, 59b, identified as *Amblyomma* sp.); 4 nymphs ex horse, Parque Nacional El Rey, Salta, Argentina (FAVE, SAER 1b, 40b, 117b, 150c, identified as *Amblyomma* sp.); 1 nymph ex dog, Parque Nacional El Rey, Salta, Argentina (FAVE 116c, identified as *Amblyomma* sp.); 5 nymphs ex human, Parque Nacional El Rey, Salta, Argentina (FAVE, SAER 54b, 59b, 196a, identified as *Amblyomma* sp.); 2 nymphs ex human, Villamonte, Jujuy, Argentina (INTA 2240); 1 ♂, 1 ♀ ex vegetation, Parque Nacional El Rey, Salta, Argentina (USNMENT 117333, identified as "possibly" *A. coelebs*); 10 nymphs ex vegetation, Parque Nacional El Rey, Salta, Argentina (CNC 2319).
Etymology: This species is named for Arieh Hadani, in recognition of his pioneer contribution to the study of ticks and tick-borne diseases of cattle in Argentina.

Diagnosis

Male. Scutal ornamentation: reddish brown in colour; numerous punctations evenly distributed; limiting spots converging posteriorly towards median line forming outline of pseudoscutum; lateral spots, postero-accesory spots and postero-median spot small, barely perceptible; *basis capituli* dorsally rectangular with cornua, coxa I with 2 subequal spurs (internal wider, external longer), coxae II–III with single spur, coxa IV with single spur not reaching level of anus, ventral plates irregular in shape (larger and sometimes with small incision on festoons 4, 5 and 6), hypostome spatulate with dental formula 3/3 in 7–8 rows. *Female*. Scutal ornamentation: central area with patchy enamelled posterior spot, central stripe reaching posterior scutal margin, becoming narrower toward posterior scutal margin, punctations numerous, evenly distributed, postero-lateral margins slightly convex; coxa I with two subequal spurs (internal wider, external longer), *basis capituli* dorsally rectangular, porose areas rounded, genital aperture U-shaped, hypostome spatulate with dental formula 3/3 in 7–8 rows. *Nymph*. *Basis capituli* rectangular, scutum with large punctations in lateral fields and small punctations in central field, cervical groove short, ending as small shallow depression at eye level. *Larva*. *Basis capituli* dorsally subtriangular in shape, laterally extended into moderate points, scutum with posterior margin slightly convex; coxa I with 2 triangular spurs (external longer than internal), coxae II and III each with 1 triangular spur.

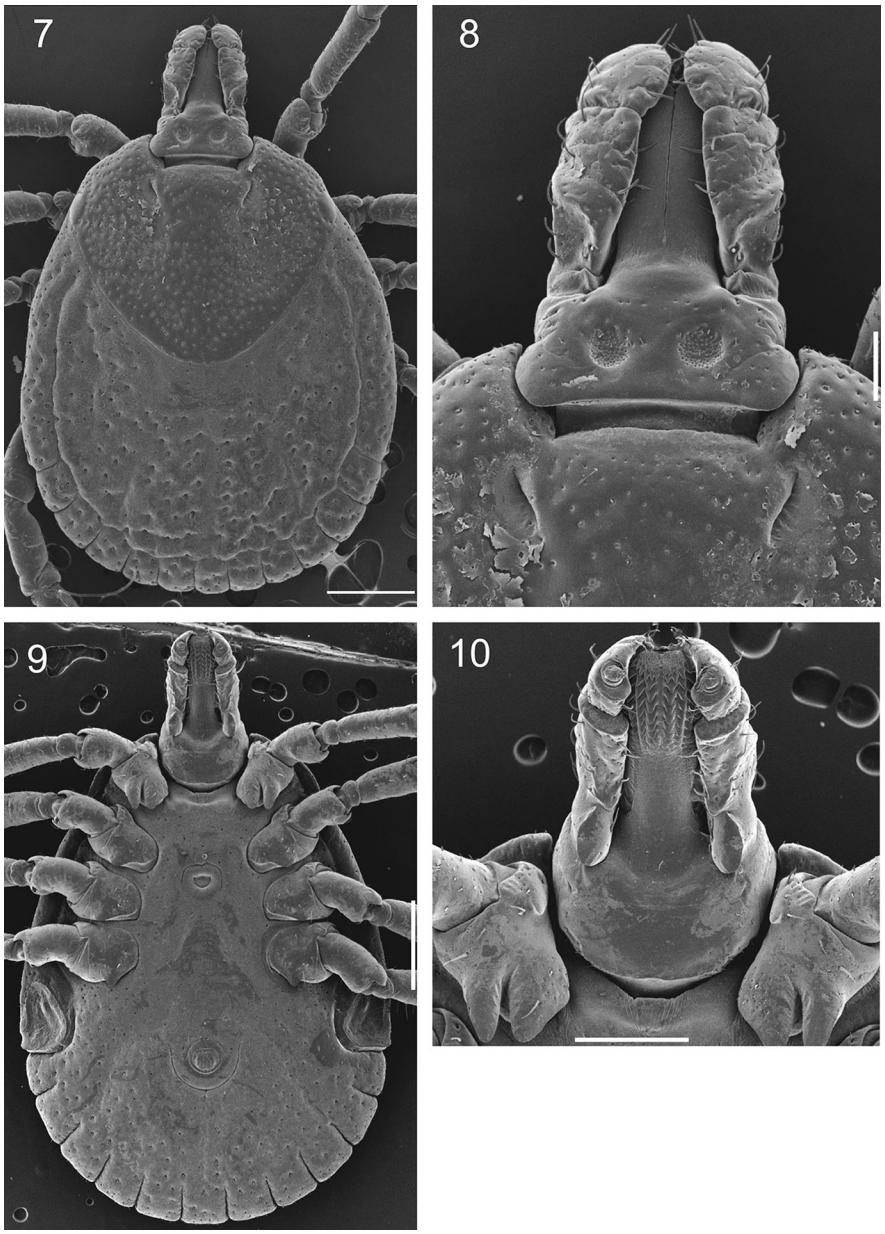
Description

Male (Figs. 1–6, 11)
Body outline oval, broadest at level of legs IV, with small concave margin at level of eyes (Fig. 1); total length 4.82–5.63 (5.12), length from apices of



Figs. 1–6 *Amblyomma hadanii* n. sp. Male. 1, Dorsal view; 2, Ventral view; 3, Hypostome; 4, Capitulum, dorsal view; 5, Ventral plates; 6, Spiracular plate. *Scale-bars*: 1, 2, 1 mm; 3–5, 0.5 mm; 6, 0.2 mm

scapulae to posterior body margin 3.88–4.67 (4.25), breadth 2.95–3.52 (3.20). *Scutum*: ornate, reddish brown, with numerous punctations evenly distributed; limiting spots converging posteriorly towards median line forming outline of pseudoscutum (Figs. 1, 11); lateral spots, postero-accesory spots and postero-



Figs. 7–10 *Amblyomma hadanii* n. sp. Female. 7, Dorsal view; 8. Capitulum, dorsal view; 9, Ventral view; 10, Hypostome. *Scale-bars*: 7, 9, 1 mm; 8, 0.2 mm; 10, 0.5 mm

median spot small, barely perceptible (Fig. 11); eyes pale, flat, scapulae rounded, cervical grooves deep, short, comma-shaped (Fig. 1); marginal groove complete, delimiting all festoons, deep up to level of coxa IV, and then as a line of deep punctations not reaching level of eyes (Fig. 1); festoons longer than wide, with numerous medium-sized punctations; genital aperture U-shaped (Fig. 2). *Capitulum* (Figs. 3, 4): *basis capituli* dorsally rectangular, wider than long, with cornua; length from palpal apices to cornua apices 0.97–1.20 (1.05); *basis capituli* breadth 0.67–0.81 (0.74), length 0.34–0.48 (0.39), length from apex of hypostome to posterior margin 0.84–0.87 (0.85); palpi: total length 0.58–0.74 (0.69), length of segment I 0.09–0.11 (0.09), length of segment II 0.30–0.40 (0.32), length of segment III 0.11–0.20 (0.14); article 1

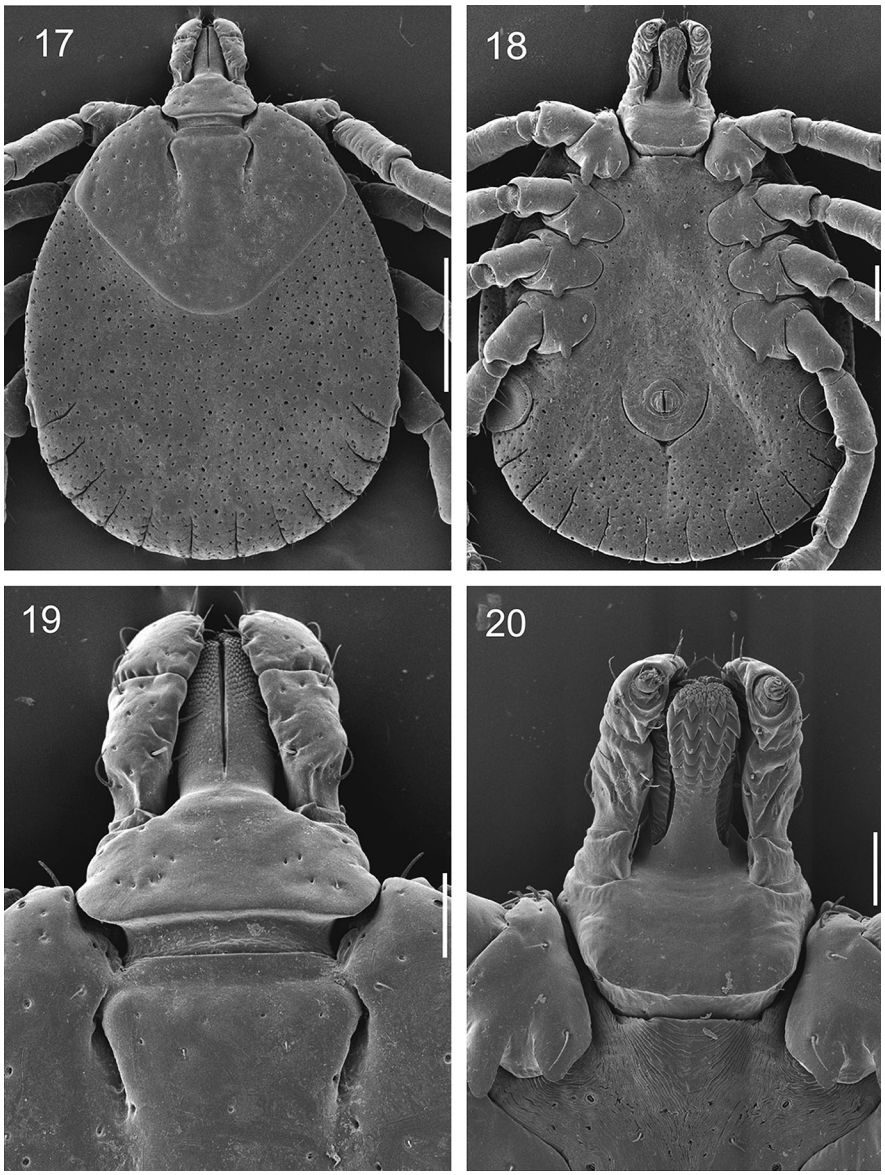
Figs. 11–16 Dorsal view of adult ticks. 11, Male of *Amblyomma hadanii* n. sp.; 12, Male of *Amblyomma coelebs*; 13, Male of *Amblyomma dubitatum*; 14, Female of *Amblyomma hadanii* n. sp.; 15, Female of *Amblyomma coelebs*; 16, Female of *Amblyomma dubitatum*

of palpi with retrograde spur on its ventral surface. *Hypostome* (Fig. 3): apically rounded (spatulate), length from apices to Ph1 setae 0.74–0.81 (0.80), width 0.20–0.30 (0.21), dental formula 3/3 in 7–8 rows; apex with corona of fine denticles. *Legs* (Figs. 2, 3): coxa I with 2 subequal spurs (internal wider, external longer) reaching anterior margin of coxa II; coxae II with single short, rounded spur; coxa III with single short, rounded spur, protruding from ridge-like edge; coxa IV with single spur not reaching level of anus; trochanters with no spurs; *tarsus* I length 0.81–1.0 (0.83) width 0.30–0.35 (0.32). Sclerotised ventral plates (carenas), irregular in shape, larger and sometimes with small incision present on festoons 4, 5 and 6 (Fig. 5). *Spiracular plate* (Fig. 6) broadly pear-shaped, length 0.60–0.72 (0.70) width 0.30–0.40 (0.31).

Principal measurements (in mm) of the male holotype: total length 5.2; length from apices of scapulae to posterior body margin 4.1, breadth 3.1; length from palpal apices to cornua apices 1.0.

Female (Figs. 7–10, 14)

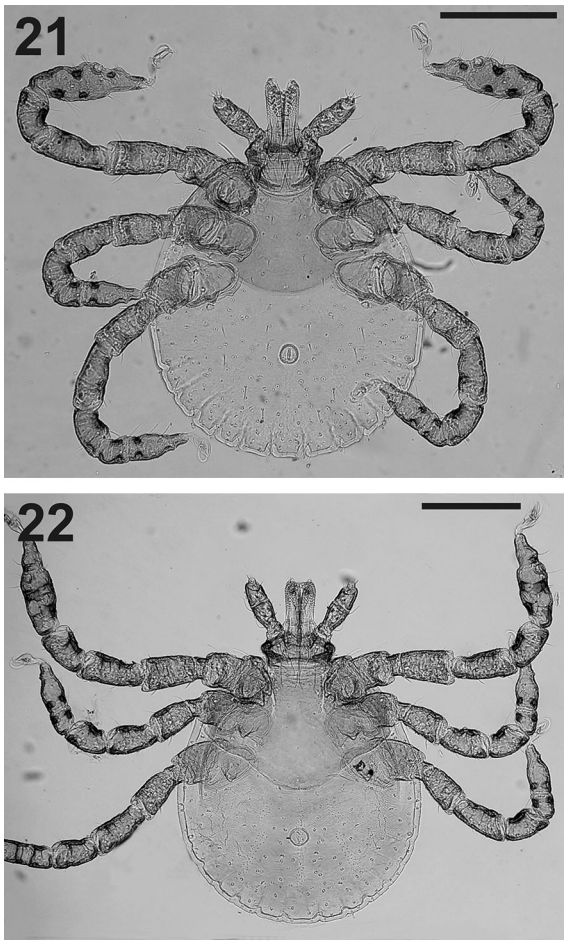
Body outline oval, with 11 festoons (perceptible in unengorged specimens) without tubercles (Fig. 7); total length 4.89–6.17 (5.74); length from apices of scapulae to posterior body margin 3.95–4.96 (4.59); breadth 3.09–3.95 (3.59); eyes pale, flat; scapulae rounded, cervical grooves deep, short, sigmoid, *notum* glabrous (Figs. 7, 8); genital aperture U-shaped, situated between coxae II (Fig. 12). *Scutum*: length 1.85–2.40 (2.17), breadth 2.22–2.74 (2.52), posterior angle broad, postero-lateral margins slightly convex, punctations numerous, evenly distributed (Fig. 7); ornate central area characterised by patchy enamelled posterior spot, with central stripe reaching posterior scutal margin, central stripe becomes narrower toward posterior scutal margin (Fig. 14). *Capitulum* (Figs. 8–10): *basis capituli* dorsally rectangular, wider than long, with cornua; porose areas rounded; length from palpal apices to cornua apices 1.14–1.46 (1.31); *basis capituli* breadth 0.83–1.04 (0.97), length 0.30–0.46 (0.40), distance between porose areas 0.14–0.20 (0.17), diameter of one porose area 0.16–0.21 (0.17); palpi total length 0.76–1.0 (0.89), length of segment I 0.093–0.14 (0.11), length of segment II 0.44–0.58 (0.53), length of segment III 0.24–0.27 (0.24); article 1 of palpi with a retrograde spur on its ventral surface. *Hypostome* (Fig. 10): apically rounded (spatulate), length 0.74–1.07 (0.94), width 0.30–0.34 (0.33), dental formula 3/3 in 7–8 rows; apex with corona of fine denticles. *Legs* (Figs. 9, 10): coxa I with 2 subequal spurs (internal wider, external longer); coxae II–IV with single short, rounded spur; trochanters with no spurs; *tarsus* I length 1.0–1.25 (1.14), width 0.17–0.29 (0.23). *Spiracular plate* broadly pear-shaped, length 0.5–0.7 (0.6), width 0.5–0.7 (0.6).



Figs. 17–20 *Amblyomma hadanii* n. sp. Nymph. 17, Dorsal view; 18, Ventral view; 19, Capitulum, dorsal view; 20, Hypostome. *Scale-bars*: 17, 0.5 mm; 18, 0.2 mm; 19, 20, 0.1 mm

Nymph (Figs. 17–20)

Body outline oval, with 11 festoons (perceptible in unengorged specimens) without tubercles (Fig. 17), length from apices of scapula to posterior body margin 1.76–2.09 (1.92), width 1.43–1.71 (1.58). *Scutum* (Fig. 17): length 0.82–0.88 (0.84), width 1.03–1.17 (1.11), width /length ratio 1.20–1.37 (1.30), inornate, with punctations evenly distributed, larger laterally, smaller centrally; eyes not orbited; cervical grooves deep, short, ending as small shallow depressions at level of posterior margin of eyes (Fig. 17). C*apitulum* (Figs. 19, 20): length from palpal apices to posterior margin 0.35–0.45 (0.40), width 0.37–0.40 (0.37); *basis capituli* dorsally rectangular, posterior margin slightly concave, without cornua; posterior margin convex ventrally, without auriculae; palpi length 0.25–0.30 (0.28), article I with small ventral prolongation, article II 0.15–0.18 (0.17) long, article III



Figs. 21–22 Larvae. 21, *Amblyomma hadanii* n. sp.; 22, *Amblyomma dubitatum*. *Scale-bars*: 0.25 mm

0.07–0.10 (0.08) long. *Hypostome* rounded apically, length 0.28–0.32 (0.30), dentition 2/2 with 7–8 teeth per row (Fig. 20). *Legs* (Fig. 18): coxa I with 2 pointed spurs (internal broader, external longer); coxae II–IV with small triangular spur, emerged from ridge-like medial extension in coxae II and III; trochanters without spur; tarsus I length 0.42–0.52 (0.46), width 0.12–0.13 (0.12). *Spiracular plate* triangular with small dorsal prolongation, length 0.22–0.27 (0.24), width 0.17–0.20 (0.18).

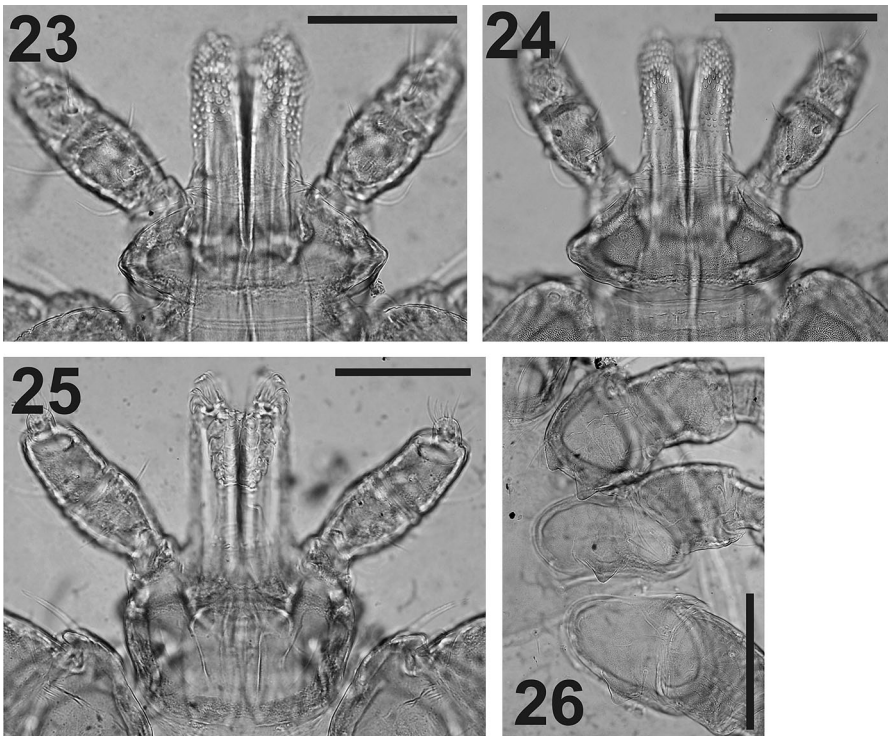
Larva (Figs. 21, 23, 25–26)
Body outline rounded, with 11 festoons (Fig. 21); length from apex of hypostome to posterior body margin 0.85–0.96 (0.89); length from apices of scapulae to posterior body margin 0.68–0.73 (0.71); greatest width 0.63–0.67 (0.65); 10 pairs of dorsal body setae: 2 central and 8 marginal; 15 pairs of ventral setae: 3 sternal, 2 pre-anal, 4 pre-marginal, 5 ventral and 1 on anal valves. *Scutum*: outline as illustrated (Fig. 21) with posterior margin slightly convex, 0.29–0.33 (0.31) length, 0.44–0.53 (0.51) width; 3 pairs of minute setae; eyes flat; cervical grooves deep, parallel. *Capitulum* (Figs. 21, 23, 25): *basis capituli* dorsally subtriangular in shape, laterally extended into moderate points; 1 pair of ventral posthypostomal setae (Ph); *cornua* absent; *basis capituli* 0.12–0.14 (0.13) in length, 0.16–0.18 (0.17) in width; *Palpi* length 0.15–0.17 (0.16); article I 0.02–0.03 (0.02); article II 0.06–0.07 (0.06); article III 0.04–0.06 (0.05); sutures between all articles distinct. *Hypostome* (Fig. 25): rounded apically; length from apices to Ph setae 0.09–0.11 (0.10); width 0.05–0.05 (0.05); dental formula 2/2, with 6 or 7 teeth in 2 files. *Legs* relatively long: coxa I with 2 triangular spurs (external longer than internal) (Fig. 26); coxae II and III each with 1 triangular spur (Fig. 26); *trochanters* lack spurs; *tarsus* I 0.26–0.29 (0.28) length, 0.06–0.08 (0.07) width.

DNA sequences and phylogenetic position

Partial 16S DNA sequences of male, female, nymph and larva of *A. hadanii* n. sp. were identical (GenBank accession numbers: KJ584370–KJ584372). Pairwise differences in 16S rDNA sequences between *A. hadanii* and the remaining *Amblyomma* spp. included in the analysis were larger than 6%. The ML trees derived from both 18S and 16S genes are presented in Fig. 27. *Amblyomma hadanii* formed a clade with *Amblyomma dubitatum* Neumann, 1899 in the phylogenetic tree performed with 18S sequences, but no close association between *A. hadanii* and any other Neotropical species of *Amblyomma* was observed in the tree constructed with 16S sequences. Sequences (18S) for *A. hadanii* n. sp. (GenBank accession number: KJ584368) and *A. dubitatum* (GenBank accession number: FJ464425) differed by 13 nucleotide substitutions.

Discussion

Amblyomma hadanii n. sp. is morphologically similar to *A. coelebs* and *A. dubitatum*. Males of *A. hadanii* can be readily distinguished from *A. coelebs* by the



Figs. 23–26 Capitulum and coxae of larvae. 23, Capitulum of *Amblyomma hadanii* n. sp.; 24, Capitulum of *Amblyomma dubitatum*; 25, Hypostome of *Amblyomma hadanii* n. sp.; 26, Coxae of *Amblyomma hadanii* n. sp. *Scale-bars*: 23, 25, 0.1 mm; 24, 26, 0.15 mm

scutal ornamentation and ventral plates. Males of *A. coelebs* have two bright red-orange patches in the scapular area and the lateral, postero-accessory and postero-median spots are conspicuous whereas males of *A. hadanii* have a pseudoscutum formed by the convergence of the limiting spots, the lateral, postero-accesory and postero-median spots are barely perceptible, and bright red-orange patches in the scapular area are absent (Figs. 11, 12). Ventral plates in *A. hadanii* are larger than in *A. coelebs*. Also, the ventral plates in *A. hadanii* are irregular in shape and in some specimens with a small incision, whereas in *A. coelebs* the ventral plates are similar in shape and not incised. Females of *A. hadanii* and *A. coelebs* are differentiated by the ornamentation of the scutum. Females of *A. hadanii* have a central area with a patchy enamelled posterior spot, and a central stripe which reaches the posterior scutal margin (Fig. 14). Conversely, the scutum of females of *A. coelebs* is characterised by an enamelled posterior spot without central stripe (Fig. 15). Another difference between the females of *A. hadanii* and *A. colebes* comprises the punctations in the central area of the scutum, which are larger in *A. hadanii* than in *A. coelebs*.

Scutal ornamentation, spurs of coxae and spiracular plate are characters that clearly distinguish males of *A. hadanii* and *A. dubitatum*. Ornamentation of *A. hadanii* is characterised by limiting spots converging posteriorly towards the median line forming the outline of a pseudoscutum whereas the limiting spots of *A. dubitatum* are parallel to the scutal midline (Figs. 11, 13). Spiracular plate of *A. hadanii* is pear-shaped while spiracular plate of *A. dubitatum* is oval and larger than in *A. hadanii*, and spurs of coxae I-IV of *A. hadanii* are larger and sharper than in *A. dubitatum*. The females of these two species can be separated using the same characters. Spurs of coxae I–II of *A. hadanii* are larger and sharper than in *A. dubitatum*, and spiracular plate is pear-shaped in *A. hadanii* and oval in *A. dubitatum*. In the posterior scutal margin, the brown central stripe is narrower in *A. hadanii* than in *A. dubitatum* (Figs. 14, 16). Additionally, postero-lateral margins of scutum are slightly convex in *A. hadanii* and sinuous in *A. dubitatum*.

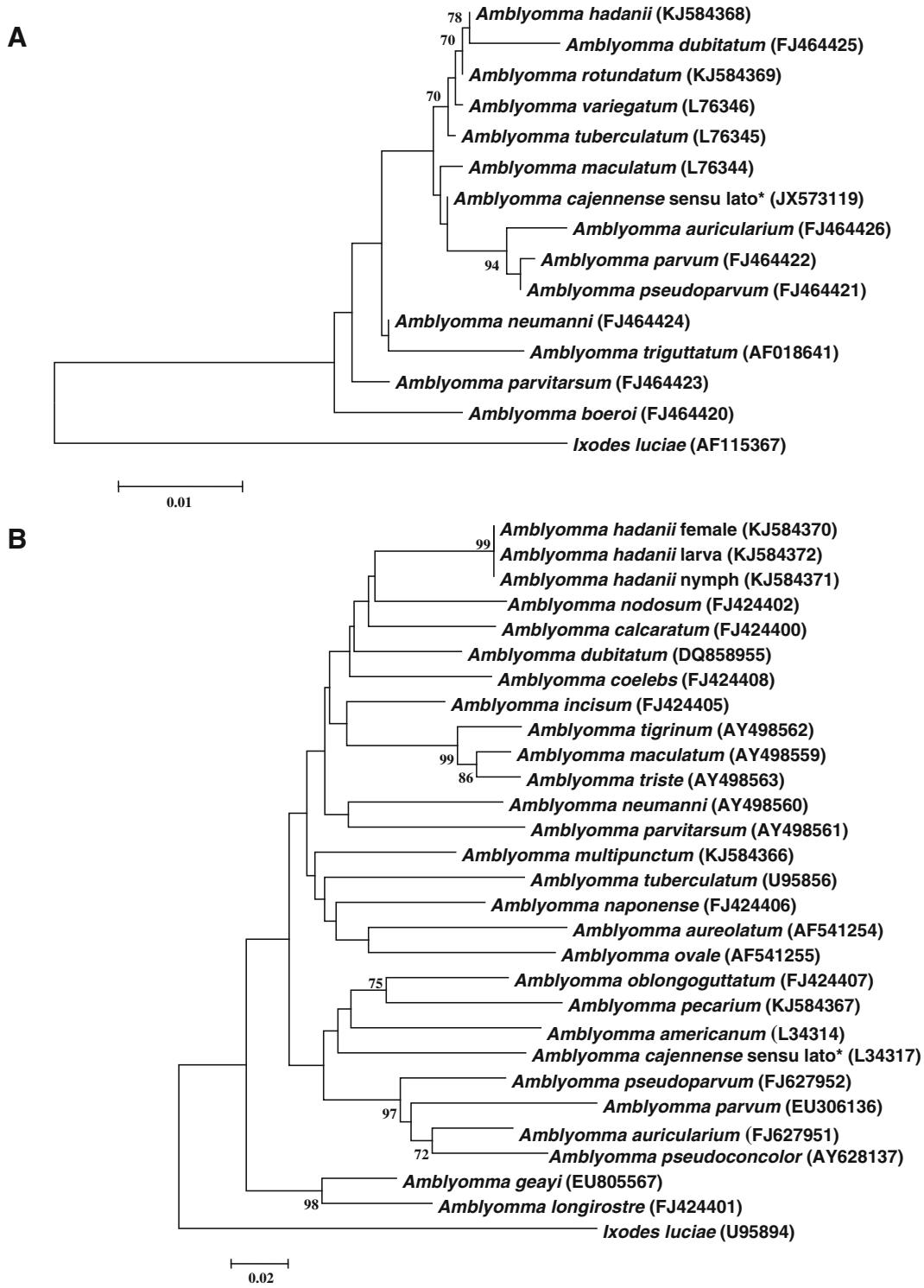


Fig. 27 Maximum likelihood tree constructed from 18S rDNA (A) and 16S rDNA (B) sequences. Numbers represent bootstrap support generated from 1,000 replications. GenBank accession numbers are in parentheses.* Currently it is recognised that the taxon *Amblyomma cajennense* is a complex formed by six species (Nava et al., 2014)

Similarly to the adults, the nymphs of *A. hadanii, A. coelebs* and *A. dubitatum* have a similar morphology. The nymph of *A. hadanii* can be differentiated from that of *A. coelebs* because the former possesses a rectangular dorsal *basis capituli* (*vs* slightly hexagonal) and scutum with large punctations in the lateral fields and small punctations in the central field (*vs* scutum with large and deep punctations in both lateral and central fields). The principal difference among nymphs of *A. hadanii* and *A. dubitatum* is the morphology of the cervical groove (short, ending as a small shallow depression at the eye level in *A. hadanii* (*vs* long, ending posteriorly to the eye level in *A. dubitatum*) and the *basis capituli* (rectangular *A. hadanii* and slightly hexagonal in *A. dubitatum*). In the case of larvae, there are well marked differences between *A. hadanii* and *A. dubitatum* (larva of *A. coelebs* is not described). The posterior margin of the scutum is slightly convex in the larvae of *A. hadanii* (Fig. 21) and sinuous in the larvae of *A. dubitatum* (Fig. 22); further, the lateral projections of *basis capituli* are sharper in the larvae of *A. dubitatum* than in the larvae in *A. hadanii* (Figs. 23, 24). Also, the average body size is larger in the larvae of *A. hadanii* than in the larvae of *A. dubitatum*.

The analyses of both 18S and 16S DNA sequences (Fig. 27) supported the description of *A. hadanii* as a new species distinct from other Neotropical species of *Amblyomma*. Sequences of both genes were markedly different from the sequences of the remaining *Amblyomma* spp. even for the more conserved 18S rRNA gene. In the phylogenetic analysis of the available 16S sequences, the evolutionary relationships between *A. hadanii* and the other Neotropical species of *Amblyomma* remain unresolved. In fact, most of the clades formed in the 16S tree have a low bootstrap support. On the other hand, a close association between *A. hadanii* and *A. dubitatum* was found in the 18S tree, coinciding with the morphological similarity between these two species (unfortunately, there are no 18S sequences of *A. coelebs* available on GenBank). These results are not unexpected because 18S rRNA gene is more conserved than the 16S rRNA gene; this increases the power of phylogenetic estimates based on 18S sequences in inferences of the evolutionary history of the species of genus *Amblyomma*.

Specimens of *Amblyomma hadanii* n. sp. were collected from Argentinean localities in the Provinces of Salta and Jujuy. All records were made between 800 m.a.s.l and 1500 m.a.s.l in areas belonging to the Yungas Phytogeographic Province (Amazonian Dominion). Examination of ticks from Parque Nacional El Rey (Salta, Argentina) deposited in the USNMENT, INTA and FAVE which have been identified as *A. coelebs* has resulted in the identification of these specimens as *A. hadanii*. Consequently, the records of *A. coelebs* in Argentina presented by Beldoménico et al. (2003) and Nava et al. (2004) (summarised in Guglielmone & Nava, 2006) correspond in fact to *A. hadanii*. There is another record of *A. coelebs* in Argentina (Boero, 1957), but the locality and hosts were not specified by this author. The only information presented by Boero (1957) on this finding of *A. coelebs* is "… extremo norte del país. En las zonas del alto Pilcomayo, sobre las fronteras paraguayas y bolivianas". We could not locate the specimens examined by Boero (1957) in any entomological collection in Argentina. Therefore, we consider that the records of *A. coelebs* in Argentina (mentioned in Floch & Fauran, 1958; Jones et al., 1972; Guglielmone et al., 2003; Barros-Battesti et al., 2006) require confirmation.

Adults of *A. hadanii* were collected on *T. terrestris* and horse, and nymphs were found on horse, cattle, dog and human, but the number of host records for *A. hadanii* is too low for any well-grounded conclusion on its host range and host specificity. The findings of *A. hadanii* parasitising humans and domestic mammals indicate that this tick may be relevant from a sanitary perspective. In this sense, it is important to consider that *A. hadanii* was found in an area of Argentina where cases of human rickettsioses were diagnosed (Ripoll et al., 1999; Paddock et al., 2008). Future studies are needed to determine if this new tick species can be involved as vector of tick-borne pathogens.

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