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Description of larva of *Amblyomma romitii* (Acari: Ixodidae) by optical and scanning electron microscopy, including porotaxy and phylogenetic analysis

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Abstract The description of the larva of *Amblyomma romitii* Tonelli-Rondelli is based on optical and scanning electron microscopy. Larvae were obtained under laboratory conditions from an engorged female collected on capybara from Rurópolis municipality, State of Pará, Northern Brazil. Several characters are presented including the chaetotaxy of idiosoma, palpi and Haller's organ. The larval porotaxy (topographical and numerical patterns of integumentary structures) was presented and compared to that of the other *Amblyomma* spp. larvae. The mitochondrial 16S rDNA partial sequence of *A. romitii* generated in the present study was aligned with sequences previously determined for other *Amblyomma* species available in Genbank and with some species presently sequenced. The larval morphology of *A. romitii* and other Neotropical *Amblyomma* spp. larvae is discussed as well as the DNA sequence and its phylogenetic position among other species of the genus.

Keywords *Amblyomma romitii* · Larval description · Chaetotaxy · Porotaxy · Phylogeny

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Introduction

The species *Amblyomma romitii* Tonelli-Rondelli was considered a synonym of *Amblyomma extraoculatum* Neumann (Santos Dias 1955) for more than a century. The species was originally described from specimens collected on a capybara from Greats Falls, British Guyana (now Guyana). It was again collected on the same host from Saint Tigre, French Guyana (as *Amblyomma tasquei* Floch & Abonnenc). It was first registered to Brazil in 1957 as *A. tasquei*, a male collected on human from state of Pará, (Aragão and Fonseca 1961). After examination of the types of *A. romitii* and *A. extraoculatum*, the taxon *A. romitii* was validated and redescribed by Barros-Battesti et al. (2007). Since then, the species was again found in the Para State (Sampaio et al. 2010) and it was recorded for the first time to State of Rondonia (Labruna et al. 2010).

Engorged females of *A. romitii* collected with larvae, nymphs and males, on capybara that was killed by hunters from Rurópolis, were sent to Instituto Butantan. One of these females laid eggs under laboratory conditions. Herein we describe the larva of *A. romitii* by means of optical and scanning electron microscopy. The porotaxy (topographical and numerical patterns of integumentary structures) was presented and compared to the other *Amblyomma* spp. larvae. The mitochondrial 16S rDNA partial sequence of *A. romitii* was aligned with Brazilian species of *Amblyomma* presently sequenced and also with those previously determined for other species of the genus available in Genbank.

The larval morphology of *A. romitii* and other Neotropical *Amblyomma* spp. larvae as well as the DNA sequences and its phylogenetic position among other species of the genus are discussed.

Materials and methods

Morphology

Larvae of *A. romitii* were reared from a female collected on capibara, *Hydrochoerus hydrochaeris* (L.), from Rurópolis (04°05′45″S, 54°54′33″W), in the southwest of State of Pará, northern Brazil. The female was maintained at 27 °C, almost 100 % RH, in the dark, and after oviposition it was deposited at the Acari Collection of the Instituto Butantan under the number IBSP10.068. Preoviposition, oviposition, and incubation median periods were 10, 19.2, and 31.3 days, respectively. The median period of eclosion (8.9 days) resulted in more than 3,000 (96 %) emerged larvae.

A sample of 30 unfed larvae was prepared for optical and scanning electron microscopy (SEM) according to Famadas et al. (1996) and to Keirans et al. (1976), respectively. SEM of ventral idiosoma of larva of *Amblyomma humerale* Koch was also prepared in order to compare with *A. romitii*.

For determination of the frequency of integumentary pores, each idiosomal side (left and right) was analyzed independently, according to Klompen et al. (1996). Thus, a total of 20 idiosomal sides were analyzed. Larval chaetotaxic terminology is that of Clifford and Anastos (1960), Hess and Vlimant (1983) and Woolley (1988), while for porotaxy the nomenclature was that proposed by Barbieri et al. (2007).

All measurements are given in micrometers (mean ± SD), and intervals represent ranges of 10 specimens measured with optical microscope Nikon, model Eclipse E200 coupled to NIS-Elements BR 64 Bits measurement system, v 3.33.13.

The remaining life larvae were used to study the life cycle. A part of them was deposited at the collection (IBSP10.661).

Molecular analysis

A sample of 10 adult ticks was processed for DNA extraction by using the DNEasy Tissue kit (Qiagen®), following the manufacturer's recommendations with some modifications according to Desloire et al. (2006). For molecular taxonomic studies, a ~460-bp fragment of the 16S rDNA gene was used, as proposed by Mangold et al. (1998) using primers: 16S + 1 (5′-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3′); 16S − 1 (5′-CCG GTC TGA ACT CAG ATC AAG T- 3′) (Black and Piesman 1994). Fragments of PCR amplified DNA were purified with ExoSAP-IT (USB Corporation) and the products were sequenced using kit Big Dye Terminator (Perkin Elmer).

Phylogenetic trees were inferred by maximum parsimony (MP) method. MP trees were inferred using PAUP* v. 4.0b10 (Swofford 2002) with 1000 replicates of random addition taxa and TBR branch swapping; all positions were equally weight.

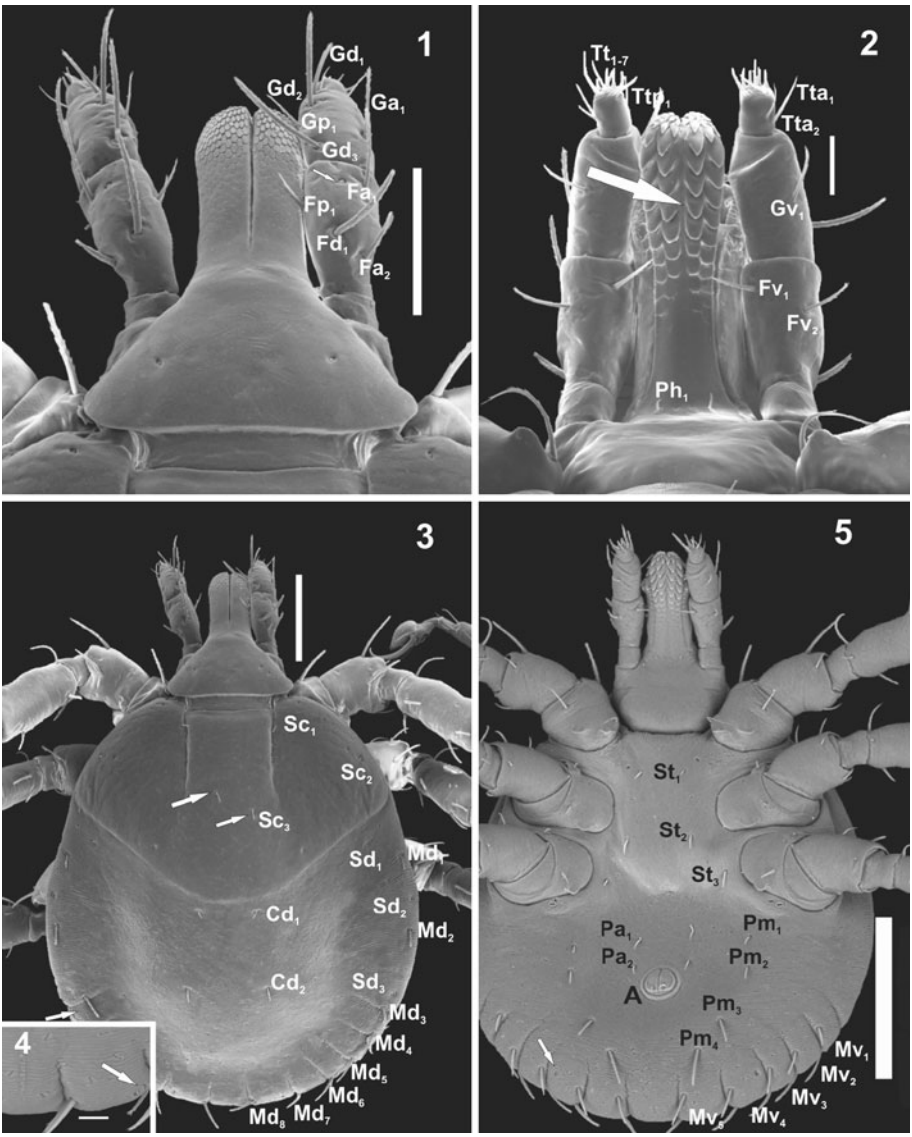
The mitochondrial 16S rDNA partial sequence of *A. romitii* generated in the present study was deposited in Genbank under the number JX141383. It was aligned with sequences of many Brazilian species and with previously determined sequences for *Amblyomma* species available in Genbank. The sequence of *Amblyomma varanense* (Supino) that was deposited as *Aponoma varanense* was used as outgroup.

Results

Description *A. romitii*, larva (Figs. 1, 2, 3, 4, 5, 6, 7, 8)

Gnathosoma dorsal (Fig. 1): *Basis capituli* triangular in outline; length from posterior margin of tibiotarsus to gnathosoma posterior margin 266.57 ± 19.33 (226.31–290.66), width 196.01 ± 7.54 (177.79–204.21). Posterior margin straight, cornua absent. Palpal articles well defined and the tibiotarsus article visible from dorsal view (Fig. 1). *Palpi* length from apices of tibiotarsal article to posterior margin of trochanter 210.34 ± 22.53 (176.08–240.01), width 43.49 ± 4.39 (37.54–49.68); trochanter length 32.62 ± 5.12 (23.18–37.48), width 29.76 ± 3.37 (23.41–34.50); femur length 98.48 ± 8.28 (83.89–109.47), width 42.68 ± 4.80 (35.39–49.68); genu length 71.88 ± 7.73 (64.02–86.09), width 40.58 ± 4.73 (35.32–47.58); tibiotarsus length 20.99 ± 2.61 (17.66–25.41), width 18.64 ± 1.22 (16.56–19.90). Femur with sensillum near seta Fa1 (Fig. 1). Gnathosoma ventral (Fig. 2): Hypostome compact, spatulate, length from apices to posthypostomal setae 171.17 ± 11.43 (157.90–195.36), dental formula 2/2 in file teeth, 3/3 on the apical corona; 1 pair of posthypostomal setae (Ph1) (Fig. 2). Palpal setae (Figs. 1, 2): 11 setae on tibiotarsus, 7 terminal (Ttt1–Ttt7), 2 paraxial (Ttp1, Ttp2) and 2 antiaxial (Tta1, Tta2,); 6 genual setae, 5 dorsal and 1 ventral, 1 paraxial (Gp1), 1 antiaxial (Ga1), 3 dorsal (Gd1–Gd3), and 1 ventral (Gv1); 6 femoral setae, 4 dorsal and 2 ventral, 1 paraxial (Fd1), 2 antiaxial (Fa1, Fa2), 1 dorsal (Fd1), and 2 ventral (Fv1, Fv2); trochanter 0.

Idiosoma dorsal (Fig. 3): Length from apices of scapulae to posterior margin of body 699.55 ± 12.91 (684.50–718.74); greatest width 630.85 ± 20.28 (592.72–653.43); outline oval, with 11 festoons. Dorsal setae: 2 central dorsal pairs (Cd_1, Cd_2), 8 marginal dorsal pairs (Md_1–Md_8). Three large wax glands (Sensilla dorsal, Sd_1–Sd_3) present, Sd_1, Sd_2, and Sd_3 before Md_1, Md_2, and Md_3, respectively. In three specimens it was observed a pair of large wax glands in the borderline of the festoon V dorsally (Fig. 4). Scutum outline subtriangular, length 318.81 ± 15.85 (278.25–333.33); breadth 516.98 ± 21.41



Figs 1–5 Scanning electron microscopy of *Amblyomma romitii*, larva: *1*. Capitulum dorsal view. *2*. Capitulum ventral view, hypostome 2/2 (*arrow*). *3*. Idiosoma dorsal view, *arrow*: third pair of scutal setae. *4*. Large wax gland in focus on the V dorsal festoon. *5*. Idiosoma ventral view, *arrow*: pairs of wax glands on festoons IV and V (segments XII and XIII, respectively). *A* anal, *Cd* dorsal central, *Fa* femoral antiaxial, *Fd* femoral, *Fp* femoral paraxial, *Fv* femoral ventral, *Ga* genual antiaxial, *Gd* genual dorsal, *Gp* genual paraxial, *Gv* genual ventral, *Md* marginal dorsal, *Mv* marginal ventral, *Pa* preanal, *Ph* post hypostomal, *Pm* premarginal, *p* paraxial, *Sc* scutal, *Sd* dorsal sensilla, *St* sternal, *Tt* tibiotarsus, *Tta* tibiotarsus antiaxial, *Ttp* tibiotarsus paraxial, *Scale bars*: 1–80; 2–30; 3–120; 4–25; 5–100 μm

(471.31–542.08) at level of eyes. Integument with irregular hexagonal sculpturing, a few punctations. Setae: 3 scutal pairs (Sc_1–Sc_3). Eyes slightly bulging and shallow; cervical grooves prominent and extending parallel to the proximities of setae Sc_3 pair. Idiosoma

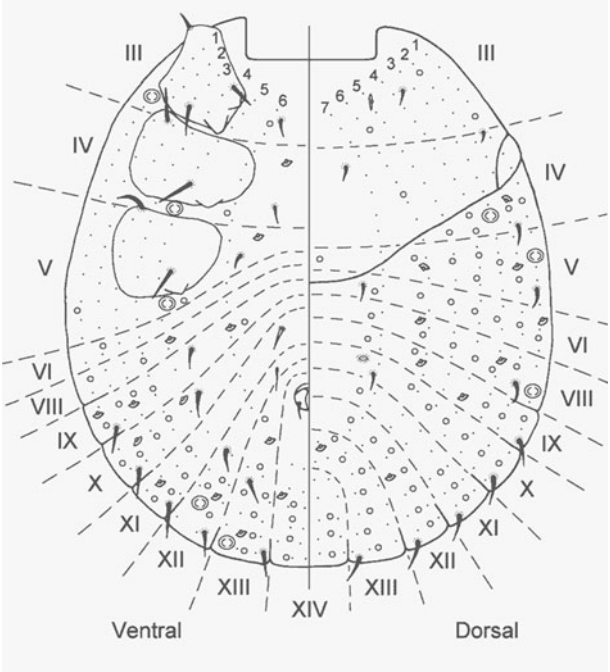
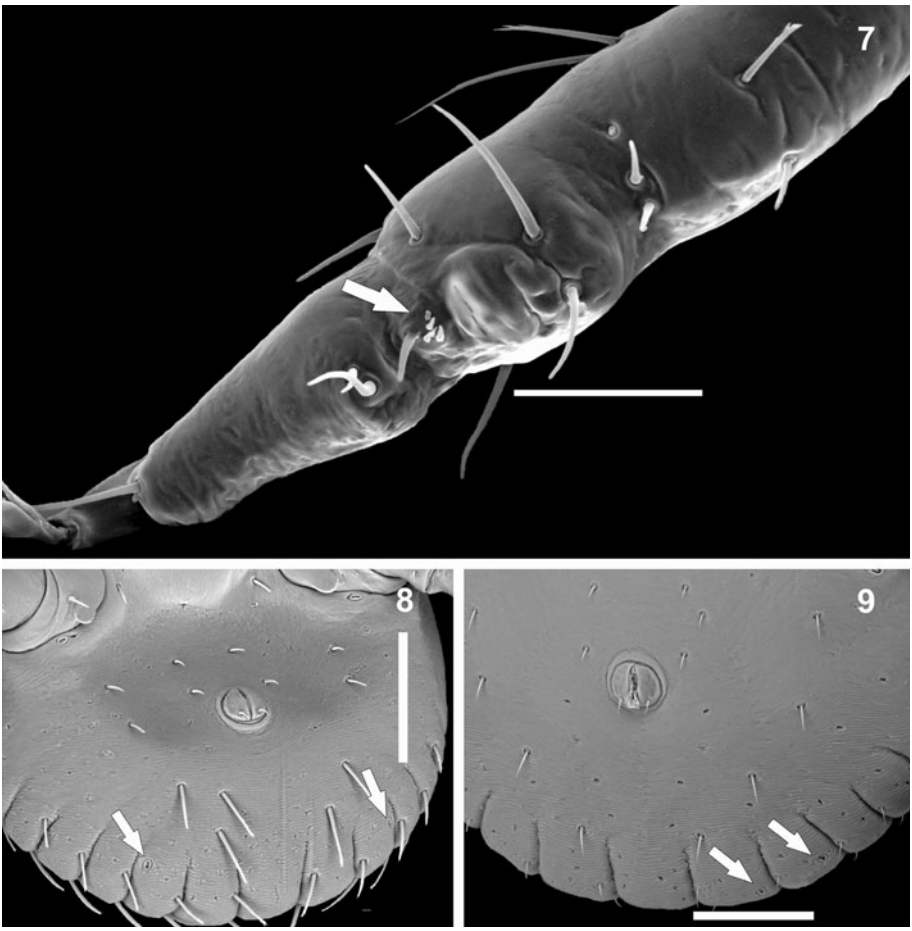


Fig. 6 Porotaxy of the larva of *Amblyomma romitii*. Segmentation model of the idiosoma. Segments are indicated by Roman numerals (III–VI and VIII–XIV) and delimitated by *dashed lines*; series are indicated by Arabic numbers and delimitated by *dotted lines*. Integumentary structures are illustrated

ventral (Fig. 5): Anal aperture on central portion of opisthosoma. Ventral setae: 3 sternal pairs (St_1–St_3), 2 preanal pairs (Pa_1, Pa_2), 4 premarginal pairs (Pm_1–Pm_4) the Pm_4 twice longer, 5 marginal ventral pairs (Mv_1–Mv_5), and 1 pair anal (A). Besides of the three pairs of sensilla, each behind coxae I–III, there is one pair on festoons IV at the position of Mv_5. Legs: Coxa I with 2 triangular spurs (Fig. 7), the external once longer than the internal; coxa II and III each with a single prominent spur. Setae: 3 on coxa I, 1 anterior (CIa), 1 posterior (CIp) and 1 paraxial (CIpa); coxa II and III, each with 2 setae, 1 anterior (CIIa, CIIIa) and 1 posterior (CIIp, CIIIp). Trochanter lacking spur. Tarsus I (Fig. 7) length 274.28 ± 21.19 (302.72–233.94), width 46.57 ± 5.16 (63.07–46.57). Setae dorsal: 2 in dorsal I group (dI1, dI2); 6 dorsal II (dII1–dII6) (Fig. 6); 2 dorsal III (dIII1, dIII2); 2 dorsal IV (dIV1, dIV2); 0 dorsal V; 2 dorsal VI (dVI1, dVI2); setae ventral: 2 ventral I (vI1, vI2), 2 in II group (vII1, vII2) and 2 in III (vIII1, vIII2); lateral anterior, 1 in lateral anterior I group (laI1), and 3 in laII group (laII1–laII3); lateral posterior, 1 in lateral posterior I group (lpI1) and 3 in lpII group (lpII1–lpII3). Haller's Organ with capsule (CHO) aperture transversely slit-like.

Porotaxy dorsal (Fig. 6): 3 pairs of large wax glands (=Sd), located on the lateral margin of the idiosoma, 1 in segment IV, $WdIV_3$ (20/20); 1 in V, WdV_1 (20/20); 1 in VIII, $WdVIII_1$ (20/20). (20/20 = 20 idiosomal sides containing the pore/20 observed idiosomal sides). In the dorsal borderline of the festoon V it was observed 1 pair of large wax glands in three specimens as showed in the Fig. 4. Lyrifissures: 11 pairs—1 par in segment III, $LdIII_5$ (20/20); 1 in IV, $LdIV_2$ (17/20); 2 in V, LdV_2 (20/20) and LdV_6 (20/20); 1 in VI, LdVI (13/20); 2 in VIII, $LdVIII_3$ (19/20) and $LdVIII_6$ (20/20); 2 in IX, $LdIX_4$ (20/20) and



Figs 7–9 Scanning electron microscopy of *Amblyomma romitii* and *A. humerale*. *7*. Tarsus I of *A. romitii*, *arrow*: pre-halleral setae. *8*. *A. romitii*, ventral view, *arrow*: sensilla pair on festoon IV. *9*. *A. humerale*, *arrow*: ventral view, the sensilla pairs on festoons IV and V. *Scale bars*: 7–50; 8–150; 9–200 μm

$LdIX_6$ (18/20); 1 in XII, $LdXII_4$ (19/20); 1 in XIII, $LdXIII_7$ (20/20). Small glands: 60 pairs—4 in scutum, 3 in segment III, $SdIII_1$ (20/20), $SdIII_2$ (20/20) and $SdIII_6$ (20/20); 1 in IV, $SdIV_6$ (20/20); and one additional asymmetric small gland on the posterior central margin of the scutum, SdV_7 (10/10). In alloscutum, 49 pairs on segments: 5 in IV, $SdIV_1$ (17/20), $SdIV_2$ (19/20), $SdIV_4$ (19/20), $SdIV_{4'}$ (9/20), $SdIV_5$ (17/20); 6 in V, SdV_2 (19/20), SdV_3 (20/20), SdV_4 (20/20), $SdV_{4'}$ (6/20), SdV_5 (20/20), SdV_6 (20/20); 7 in VI, $SdVI_1$ (13/20), $SdVI_2$ (15/20), $SdVI_3$ (16/20), $SdVI_4$ (18/20), $SdVI_5$ (16/20), $SdVI_6$ (18/20), $SdVI_7$ (19/20); 6 in VIII, $SdVIII_2$ (16/20), $SdVIII_3$ (13/20), $SdVIII_4$ (14/20), $SdVIII_5$ (15/20), $SdVIII_6$ (16/20), $SdVIII_7$ (20/20); 5 in IX, $SdIX_1$ (14/20), $SdIX_2$ (16/20), $SdIX_3$ (18/20), $SdIX_6$ (14/20), $SdIX_7$ (20/20); 6 in X, SdX_1 (18/20), SdX_2 (15/20), SdX_3 (18/20), SdX_4 (19/20), SdX_5 (20/20), SdX_6 (20/20), and dorsal fovea (dorsal series 7–20/20); 4 in XI, $SdXI_1$ (14/20), $SdXI_2$ (18/20), $SdXI_3$ (11/20), $SdXI_7$ (20/20); 7 in XII, $SdXII_1$ (18/20), $SdXII_2$ (15/20), $SdXII_3$ (15/20), $SdXII_4$ (17/20), $SdXII_5$ (20/20), $SdXII_6$ (18/20), $SdXII_7$ (20/20); 7 in XIII, $SdXIII_1$ (19/20), $SdXIII_2$ (20/30), $SdXIII_3$ (20/20), $SdXIII_4$ (20/20),

$SdXIII_5$ (20/20), $SdXIII_6$ (15/20), $SdXIII_7$ (16/20); 3 in XIV, $SdXIV_1$ (20/20), $SdXIV_2$ (9/20), $SdXIV_3$ (9/20). Porotaxy ventral: 4 pairs of wax glands, one behind each coxa in segments III, IV and V, $WvIII_1$ (20/20), $WvIV_5$ (20/20), WvV_5 (20/20); and 1 in segment XII, $WvXII_2$ (19/20) located in the 4th. festoon. Lyrifissures: 13 pairs on segments—1 in IV, $LvIV_6$ (20/20); 1 in V, LvV_6 (20/20); 1 in VIII, $LvVIII_5$ (20/20); 3 in IX, $LvIX_1$ (20/20), $LvIX_3$ (20/20), $LvIX_6$ (20/20); 2 in X, LvX_2 (20/20), LvX_5 (20/20); 1 in XI, $LvXI_2$ (20/20); 1 in XII, $LvXII_3$ (20/20); 2 in XIII, $LvXIII_2$ (20/20) and $LvXIII_6$ (20/20); 1 in XIV, $LvXIV_4$ (20/20). Small gland: 34 pairs on segments—1 in III, $SvIII_6$ (20/20); 1 in IV, $SvIV_6$, (20/20); 2 in V, SvV_1 (16/20), SvV_5 (20/20); 1 in VI, $SvVI_2$ (20/20); 2 in VIII, $SvVIII_1$ (20/20), $SvVIII_3$ (13/20); 5 in IX, $SvIX_1$ (20/20), $SvIX_2$ (20/20), $SvIX_3$ (20/20), $SvIX_4$ (20/20), $SvIX_6$ (20/20); 4 in X, SvX_1 (20/20), SvX_2 (20/20), SvX_3 (20/20), SvX_4 (20/20); 4 in XI, $SvXI_1$ (20/20), $SvXI_2$ (20/20), $SvXI_3$ (20/20), $SvXI_4$ (20/20); 3 in XII, $SvXII_2$ (17/20), $SvXII_3$ (20/20), $SvXII_4$ (20/20); 7 in XIII, $SvXIII_1$ (20/20), $SvXIII_2$ (20/20), $SvXIII_3$ (20/20), $SvXIII_4$ (16/20), $SvXIII_{4'}$ (10/20), $SvXIII_5$ (16/20), $SvXIII_6$ (20/20); 4 in XIV, $SvXIV_1$ (17/20), $SvXIV_2$ (20/20), $SvXIV_3$ (20/20), $SvXIV_4$ (20/20).

The ventral idiosoma of *A. romitii* (Fig. 8) and *A. humerale* (Fig. 9) shows the large wax gland pairs present in festoons IV, and IV and V, respectively.

Through the phylogenetic relationships based on a partial sequence of the mitochondrial 16S rDNA gene (Fig. 10), *A. romitii* grouped with *A. humerale* within a branch strongly supported (78 % of bootstrap) that also contained the sequences of *A. rotundatum.* The sequence divergence between *A. romitii* and *A. humerale* (Acession Number DQ295780) was 17.5 %.

Discussion

The designation to integumentary structures in the present study was based in Klompen et al. (1996), who used the denominations lyrifissures (Johnston and Moraza 1991), small glands, and large wax glands (Yoder et al. 1993a). Among the products of the large wax gland are included some lipids (Yoder et al. 1993a), but principally, pheromones (Diehl et al. 1991; Yoder 1995) and allomones (Yoder et al. 1993b), substance non-waxy. For this reason, some authors prefer designate these structures as "type 2 glands" following Walker et al. (1996). Considering that both terms are useful because the glands also product wax, and to avoid confusion with previous descriptions we prefer follow the Klompen et al. (1996) until more specific studies about these glands are provided.

Morphologically, the larva of *A. romitii* has chaetotaxy similar to the other larvae of the *Amblyomma* genus. However, the pattern of the porotaxy of this species is unique. It was also found lyrifissures, small and large wax glands distributed throughout idiosoma of the larva of *A. romitii*, except in the scutum where large wax glands were not observed. These structures showed morphological characteristics as reported by Schulze (1942) and Dinnik and Zumpt (1949).

According to Clifford and Anastos (1960) the genus *Amblyomma* larvae have arrangement for large wax glands, being observed four pairs on the ventral surface, three behind each coxa and one in the 5th festoon, and one pair located dorsally on the posterior lateral margin of the body. This arrangement was confirmed for *A. cajennense* (Fabricius) (Famadas et al. 1997; Barbieri et al. 2007); *A. glauerti* Keirans, King & Sharrad, *A. variegatum* (Fabricius), *A. americanum* (L.) (Klompen et al. 1996), *A. parvum* Aragão, *A. rotundatum* Koch (Barbieri et al. 2007), *A. pacae* Aragão (Barbieri et al. 2008b), and *A. brasiliense* Aragão (Sanches et al. 2009).

The pair of the large wax gland on the 5th festoon (segment XIII), was observed in the dorsal surface of *A. romitii*, close to the borderline; however, considering that in other

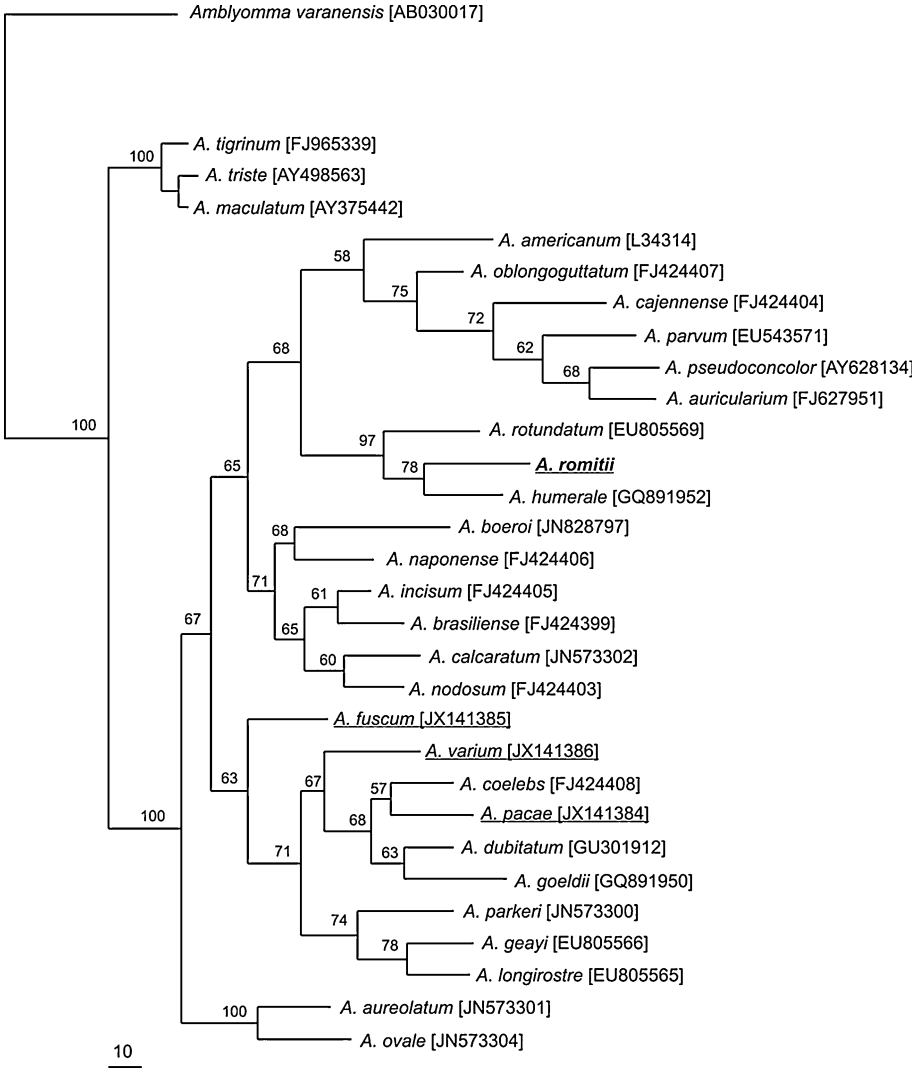


Fig. 10 Phylogenetic tree based on 30 *Amblyomma* ticks with *A. varanense* as *outgroup*. The alignment was made with 631 characters (including gaps) of the 16S rRNA mitochondrial gene used in the analysis. The sequences of *underlined* species were obtained in this study

species the large wax gland of 5th festoon is found on ventral surface, we assume the same to *A. romitii* ($WdXIII_1$). Furthermore, larvae of the *A. romitii* have one additional ventral pair on the 4th festoon (segment XII–$WvXII_2$), also observed in *A. tuberculatum* Marx, *A. geoemydae* (Cantor), *A. babirussae* Schulze (Klompen et al. 1996), *A. aureolatum* (Pallas) (Arzua 2002), *A. longirostre* (Koch) (Barros-Battesti et al. 2005), *A. ovale* Koch (Barbieri et al. 2008a), and in *A. humerale* (unpubl. data). In these species these wax glands are localized next to the lateral margin of idiosoma (serie 1).

Moreover, *A. romitii* have two additional pairs on dorsal surface totalizing 3 pairs, one in segment IV ($WdIV_3$), one in segment V (WdV_1) (Fig. 6), and another in segment VIII ($WdVIII_1$). The presence an additional pair on segment V was verified in the *A. geomydae*

and *A. longirostre* larvae, whereas two additional pairs were observed on segment V and another pair on X in *A. barbirussae*. Therefore, the presence of large wax gland pair on segment IV is characteristic of the larva of *A. romitii*.

The numerical and topographic pattern for lyrifissures on surface dorsal and ventral has been similar among *Amblyomma* species, with 11 and 13 pairs on the dorsal and ventral surfaces of the idiosoma, respectively (Klompen et al. 1996; Barbieri et al. 2007, 2008a, b), except in *A. parvum* that has 12 ventrally (Barbieri et al. 2007). However, the lyrifissure on ventral segment XIV was observed in series 4 ($LvXIV_4$), while in other species has been in the series 2 (Klompen et al. 1996; Barbieri et al. 2007, 2008a, b).

Small glands were the most numerous pores on the idiosoma of the *A. romiti* larva and the less stable. As report for other species (Nawar and Madbouly 1985; Klompen et al. 1996; Barbieri et al. 2007, a, b) the low frequency of small glands may be related to the higher number these structures on the idiosoma (Barbieri et al. 2007). In some segments, both dorsal and ventral surface, we observed two small glands in the same series, the most terminal denominated as (‘), for example, SdV_4 and $SdV_{4'}$ (Fig. 6).

The phylogenetic analysis based on parsimony grouped *A. romitii* and *A. humerale*. However, although the stick together in the same branch, both species present high divergence (17.5 %). This could be explained because few sequences of *Amblyomma* are deposited at the Genbank.

In fact, although both species present some morphological similarities, such as chaetotaxy and hypostomal dentition, the coxal spurs are longer in *A. humerale* and the presence of a pair of large wax gland on segment IV is known only for *A. romitii* until this moment. The proximity of *A. romitii* with tick species that parasitize toads and reptiles suggest the ancient divergence of this group. But, the phylogenetic studies in *Amblyomma* genus remain unclear and the inclusion of new taxa can modify the comparision between the species included in this genus.

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References

Aragão HB, Fonseca F (1961) Notas de ixodologia. VIII. Lista e chave para os representantes da fauna ixodológica brasileira. Mem Inst Oswaldo Cruz 59:115–129

Arzua M (2002) Bioecologia do parasitismo de carrapatos (Acari: Ixodidae) em aves silvestres do Bosque Reinhard Maack, Curitiba, Paraná. Descrição, diagnóstico morfológico e molecular da larva de *Amblyomma aureolatum* (Pallas, 1772). Curitiba, Universidade Federal do Paraná, M.Sc. Dissertation. p 88

Barbieri FS, Chacón SC, Labruna MB, Barros-Battesti DM, Faccini JLH, Famadas KM (2007) Topographical and numerical study of the idiosomal integumental structures of the larva of four Neotropical *Amblyomma* (Acari: Ixodidae) species. Syst Parasitol 68:57–70. doi:10.1007/s11230-006-9078-y

Barbieri FS, Brito LG, Labruna MB, Barros-Battesti DM, Camargo LMA, Famadas KM (2008a) Description of the larva of *Amblyomma ovale* Koch, 1844 (Acari: Ixodidae) by light and scanning electron microscopy. Syst Appl Acarol 13:109–119

Barbieri FS, Brito LG, Labruna MB, Barros-Battesti DM, Camargo LMA, Famadas KM (2008b) Description of the larva of *Amblyomma pacae* Aragão, 1911 (Acari: Ixodidae) by light and scanning electron microscopy. Syst Appl Acarol 13:195–203

Barros-Battesti DM, Arzua M, Rebello VMM, Barbieri FS, Famadas KM (2005) Description of the larva of *Amblyomma longirostre* (Koch 1844) (Acari: Ixodidae) by light and scanning electron microscopy. Rev Bras Parasit Vet 14:51–57

Barros-Battesti DM, Arzua M, Onofrio VC, Labruna MB (2007) Validation and redescription of *Amblyomma romitii* Tonelli-Rondelli, 1939 (Acari: Ixodidae). Syst Parasitol 68:79–86. doi:10.1007/s11230-006-9079-x

Black WC, Piesman J (1994) Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial16S rDNA sequences. Proc Natl Acad Sci USA 91:10034–10038. doi:10.1073/pnas.91.21.10034
Clifford CM, Anastos G (1960) The use of chaetotaxy in the identification of larval ticks (Acarina: Ixodidae). J Parasitol 46:567–578
Desloire S, Valiente-Moro C, Chauve C, Zenner L (2006) Comparision of four methods of extracting DNA from D. gallinae (Acari: Dermanyssidae). Vet Res 37:725–732. doi:10.1051/vetres:2006031
Diehl PA, Guerin P, Vlimant M, Steullet P (1991) Biosynthesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. J Chem Ecol 17:833–847
Dinnik J, Zumpt F (1949) The integumentary sense organs of the larvae of Rhipicephalinae (Acarina). Psyche J Entomol 56:1–17
Famadas KM, Serra-Freire NM, Faccini JLH (1996) A note on slide-mounting technique of unfed immature stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). Mem Inst Oswaldo Cruz 91:139–140
Famadas KM, Serra-Freire NM, Lanfredi RM (1997) Redescription of the larva of *Amblyomma cajennense* (Fabricius) (Acari: Ixodidae) using optical and scanning electron microscopy. Acarologia 38:101–109
Hess E, Vlimant M (1983) The tarsal sensory system of *Amblyomma variegatum* Fabricius Ixodidae: Metastriata). III. Mapping of sensory hairs and evolution of relative importance of sensory modalities during post-embryonic development. Rev Suisse Zool 90:887–897
Johnston DE, Moraza ML (1991). The idiosomal adenotaxy and poroidotaxy of Zerconidae (Mesostigmata: Zerconidae). In: F. Dusbábek, Bukva V (eds), Modern acarology, 2: 349–356. SBP Academic, The Hague
Keirans JE, Clifford CM, Corwin D (1976) Ixodes sigelos n. sp. (Acarina: Ixodidae), a parasite of rodents in Chile, with a method for preparing ticks for examination by scanning electron microscopy. Acarologia 18:217–225
Klompen JSH, Keirans JE, Filippova NA, Oliver JH Jr (1996) Idiosomal lyrifissures, setae, and small glands as taxonomic characters and potential indicators of ancestral segmentation patterns in larval Ixodidae (Acari: Ixodida). Int J Acarol 22:113–134
Labruna MB, Barbieri FS, Martins TF, Brito LG, Ribeiro FDS (2010) New tick records in Rondônia, Western Brazilian Amazon. Rev Bras Parasit Vet 19:192–194
Mangold AJ, Bargues MD, Mas-Coma S (1998) Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitol Res 84:478–484
Nawar MS, Madbouly MH (1985) The possible use of integumentary sense organs as specific diagnostic mean for larvae and nymphs of *Rhipicephalus sanguineus* and *Rhipicephalus turanicus*. J Egypt Soc Parasitol 15:607–621
Sampaio SJ, Martins TF, Labruna MB, Onofrio VC, Faccini JLH, Barros-Battesti DM (2010) A second record of *Amblyomma romitii* Tonelli-Rondelli, 1939 (Acari: Ixodidae) from the State of Pará, Brazil. Syst Appl Acarol 15:184–186
Sanches GS, Bechara GH, Camargo-Mathias MI (2009) Morphological description of *Amblyomma brasiliense* Aragão, 1908 (Acari: Ixodidae) larvae and nymphs. Rev Bras Parasit Vet 18:15–21
Santos Dias JAT (1955) Identidade e sinonímia da espécie *Amblyomma extraoculatum* Neumann, 1899 (Acarina, Ixodoidea). Mem Est Mus Zool Univ Coimbra 229:6
Schulze P (1942) Ueber die hautsinnesorgane der zecke, besonders uber eine bisher unbekannte art von arthropoden-sinnesorgane, die krobylophoren. Zeitsch Morphol Ökol Tiere 39:1–20
Swofford DL (2002) PAUP* phylogenetic analysis using parsimony (* and other methods). Version 4.b10. Sinauer Associates, Sunderland
Walker AR, Lloyd CM, McGuire K, Harrison SJ, Hamilton JGC (1996) Integumental glands of the tick Rhipicephalus appendiculatus (Acari: Ixodidae) as potencial producers of semiochemicals. J Med Entomol 33:743–759
Woolley TA (1988) Acarology. Mites and human welfare. Wiley, New York, p 484
Yoder JA (1995) Allomonal defense secretions of the American dog tick *Dermacentor variabilis* (Acari: Ixodidae) promote clustering. Exp Appl Acarol 19:695–705
Yoder JA, Pollack RJ, Spielman A, Sonenshine DE, Johnston DE (1993a) Secretion of squalene by ticks. J Insect Physiol 39:291–296
Yoder JA, Pollack RJ, Spielman A (1993b) An ant-diversionary secretion of ticks: first demonstration of an ant allomone. J Insect Physiol 39:429–435