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Full Article

Morphological description of *Amblyomma brasiliense* Aragão, 1908 (Acari: Ixodidae) larvae and nymphs

Descrição morfológica de larvas e ninfas de *Amblyomma brasiliense* Aragão, 1908 (Acari: Ixodidae)

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Abstract

The immature stages of Neotropical ticks are poorly known and in many cases have not been described. This work presents a morphological description of *Amblyomma brasiliense* larvae (F1) and a redescription of nymphs (F1). *A. brasiliense* is reported as one of the most aggressive ticks to humans in Brazil. Immature ticks obtained from a laboratory colony initiated from adult specimens collected in the Parque Estadual Intervales (24° 18' S and 48° 24' W), São Paulo, Brazil, were analyzed under scanning electron microscope, and also under light and stereoscopic microscopes. *A. brasiliense* larvae present basis capituli rectangular; short palpi; rounded idiosoma; coxa I with two spurs, the external one being longer than the internal one; and coxae II and III each with one short spur. Nymphs present basis capituli rectangular with a sharp pointed cornua; oval idiosoma with scutum reaching coxa III; coxa I with two evident spurs, the external one being longer than the internal one; coxae II-III each with one short spur; and coxa IV with a very short spur and chitinous tubercles on internal surface of posterior border of idiosoma. These morphological features, in association with chaetotaxy and porotaxy, should make possible the identification of immature *Amblyomma* ticks.

Keywords: *Amblyomma brasiliense*, morphology, description, larva, nymph.

Resumo

Os estágios imaturos dos carrapatos Neotropicais são pouco conhecidos e, em muitos casos, não estão descritos. Este trabalho apresenta uma descrição morfológica das larvas (F1) e redescrição das ninfas (F1) do carrapato *Amblyomma brasiliense*, relatado como um dos ixodídeos mais agressivos ao homem no Brasil. Para este fim, carrapatos imaturos de uma colônia iniciada com espécimes adultos do Parque Estadual de Intervales, São Paulo, Brasil foram analisados sob microscopia eletrônica de varredura, microscopia de luz e lupa estereoscópica. Observou-se que larvas têm a base do capítulo retangular, palpos curtos e idiossoma arredondado, coxa I com dois espinhos, sendo o externo mais longo que o interno e coxas II e III com um espinho. Ninfas têm a base do capítulo retangular com córnua pontiaguda, idiossoma oval, coxa I com dois espinhos evidentes, sendo o externo mais longo que o interno, coxas II e III com um espinho curto em cada uma e coxa IV com um espinho muito pequeno, presença de tubérculos quitinosos na superfície interna da borda posterior do idiosoma. Estas características, associadas à quetotaxia e porotaxia, poderão tornar possível a identificação dos estágios imaturos de carrapatos do gênero *Amblyomma*.

Palavras-chave: *Amblyomma brasiliense*, morfologia, descrição, larva, ninfa.

Introduction

The tick genus *Amblyomma* Koch 1844 is estimated to include 127 species, 57 of which are found in the Neotropical region, with 33 reported in Brazil (BARROS-BATTESTI et al. 2006). However there are few morphological studies about immature stages of this genus, with larvae and nymphs still undescribed for the majority of the species. Unfortunately, most of the existing descriptions were made only under light microscope, and many structures would be better observed with scanning electron microscopy (BARROS-BATTESTI et al. 2005).

The tick *Amblyomma brasiliense* Aragão (1908) is endemic to South America with reports in Argentina, Paraguay, and Brazil (GUGLIELMONE et al. 2003), (Rio de Janeiro, São Paulo, Minas Gerais, Pará and Espírito Santo States) (ARAGÃO 1936; SZABÓ et al. 2006; OGRZEWALSKA et al. 2007). Previous studies reported that larvae and nymphs of *A. brasiliense* are among the

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most aggressive ticks to humans in Brazil (ARAGÃO, 1936), with local reaction to tick attachment varying from almost imperceptible to intense. Itching especially, but hyperemia and swelling as well, were prominent features of the reaction (SZABÓ et al. 2006). Nevertheless, morphological descriptions of the larval stage of this tick are unknown and nymphal stage descriptions are restricted to a study by Aragão (1908) using light microscopy.

Therefore, the present study aims to describe immature stages of *A. brasiliense* by means of light and scanning electron microscopy, thus to enable making fast, accurate identifications of immature stages of this tick.

Materials and Methods

1. Origin of ticks

Larvae and nymphs were obtained from a laboratory colony derived from unfed adult *A. brasiliense* ticks collected in the Intervales State Park (24° 18' S and 48° 24' W), São Paulo State, Brazil, from December 2003 to January 2006 and identified to species using the taxonomic keys proposed by Barros-Battesti et al. (2006). The procedures used to obtain larvae and nymphs have been described elsewhere (SANCHES et al. 2008).

2. Material preparation

A sample of 30 larvae and 30 nymphs originated from different engorged females were kept starving for 30 days, so that the consolidation of exoskeleton took place; afterwards they were preserved in 70% ethanol. Of these, 20 larvae were prepared according to Famadas et al. (1996) to be measured electronically under a MC80DX light microscope coupled with a capture digital camera, using the software Motic Image Plus 2.0 ML. Twenty nymphs were measured under a Leica MZ12 stereomicroscope using the same method.

Five specimens of each immature stage were prepared for scanning electron microscopy. The material was dehydrated for 30 minutes in each of the following concentrations of alcohol: 70, 80, 90, and 100% (three times). They were then kept in acetone until undergoing critical point drying. Micrographs were taken in the Electron Microscopy Laboratory of the Biology Department at the Bioscience Institute, São Paulo State University (UNESP), Rio Claro, São Paulo, and Laboratory of Electron Microscopy, São Paulo State University, Jaboticabal, São Paulo, using a PHILLIPS 505 scanning electron microscope and a JEOL JSM 5410.

The terminology followed Clifford and Anastos (1960) and Hess and Vlimant (1983) for larval idiosomal chaetotaxy, Barbieri et al. (2007) for larval porotaxy and Famadas et al. (1997) for palpal and Haller´s organ terminology.

The larvae and nymphs used for the description and the slide-mounted specimens were deposited at the Department of Biology, Bioscience Institute, São Paulo State University (UNESP), Rio Claro, São Paulo for follow-up studies focusing on cuticular structures.

Results*

1. Larva

Gnathosoma: total length from palpal apices to posterior margin of basis capituli 0.221 ± 0.007 (0.202-0.231). Dorsal basis capituli (Figure 1) width 0.182 ± 0.005 (0.175-0.192), rectangular shape with a lyrifissure pair on median line, postero-lateral angles broad, cornua absent. Ventral basis (Figure 2) with posterior margin slightly convex, lateral angles rounded, auriculae absent. The hypostome length from apices to post hypostomal seta (Ph1) 0.116 ± 0.008 (0.100-0.129), width 0.046 ± 0.004 (0.040-0.054), spatulate and compact, with a dental formula of 2/2. Short palpi with segments well defined, length from apices of tibiotarsal segment to posterior margin of trochanter 0.139 ± 0.007 (0.126-0.148), width 0.049 ± 0.003 (0.043-0.054); palpal trochanter (article I) length 0.012 ± 0.002 (0.011-0.015), width 0.036 ± 0.002 (0.032-0.042); palpal femur (article II) length 0.055 ± 0.004 (0.050-0.061), width 0.047 ± 0.004 (0.040-0.053); and palpal genu (article III) length 0.047 ± 0.003 (0.040-0.054), width 0.040 ± 0.001 (0.038-0.042). Palpal setae (Figures 1 and 2): 12 setae on tibiotarsus; 6 genual setae – 1 antiaxial (Ga1), 3 dorsal (Gd1, Gd2, Gd3), 1 paraxial (Gp1), 1 ventral (Gv1); 6 femoral setae – 1 paraxial (Fp1), 1 dorsal (Fd1, Fd2), 2 antiaxial (Fa1, Fa2), 2 ventral (Fv1, Fv2). Palpal femur with a lyrifissure near the second femoral antiaxial seta (Fa1).

Idiosoma: dorsal surface (Figure 3) rounded, with 11 festoons and a pair of large wax glands near lateral margin of idiosoma, anterior to the festoons. Length from scapular apices to posterior margin of idiosoma 0.598 ± 0.012 (0.584-0.620), greatest width 0.598 ± 0.015 (0.575-0.628). Scutum outline subtriangular, length along median line 0.273 ± 0.007 (0.260-0.289), width 0.448 ± 0.010 (0.435-0.480), irregular integument, few punctuations, superficial cervical grooves, and shallow and flat eyes on lateral scutum surface. Dorsal setae: 3 scutal setae pairs (Sc1-Sc3), 2 central dorsal setae pairs (Cd1-Cd2), and 8 marginal dorsal setae pairs (Md1-Md8). Ventral surface (Figure 4) with a large wax gland under each coxa and on the fifth festoon of each side, 3 sternal setae pairs (St1-St3), 2 preanal setae pairs (Pa1-Pa2), 1 anal setae pair (A), 4 premarginal setae pairs (Pm1-Pm4), 5 marginal setae pairs (Mv1-Mv5).

Legs (Figure 5): Coxa I with two spurs, the external 2.0 times longer than internal and 3 setae, 1 anterior (CIa), 1 posterior (CIp), and 1 paraxial (CIpa). Coxae II and III each with 1 short spur and 2 setae, 1 anterior (CIIa, CIIIa) and 1 posterior (CIIp, CIIIp). Tarsus I (Figure 6) length 0.232 ± 0.009 (0.222-0.252), width 0.080 ± 0.002 (0.076-0.083). Setae: Dorsal – 2 in dorsal I group (dI), 7 dorsal II (dII), 2 dorsal III (dIII), 2 dorsal IV (dIV), 0 dorsal V, 2 dorsal VI (dVI). Ventral - 2 in group 1 (vI), 2 in group 2 (vII), 2 in group IV (vIV); lateral - 1 lateral paraxial in group I (LpI) and 1 lateral antiaxial (LaI), 1 lateral paraxial in group II (LpII) and 1 lateral antiaxial in the same group (LaII).

*All measurements are given in millimeters, with the mean followed by standard deviation and range in brackets.

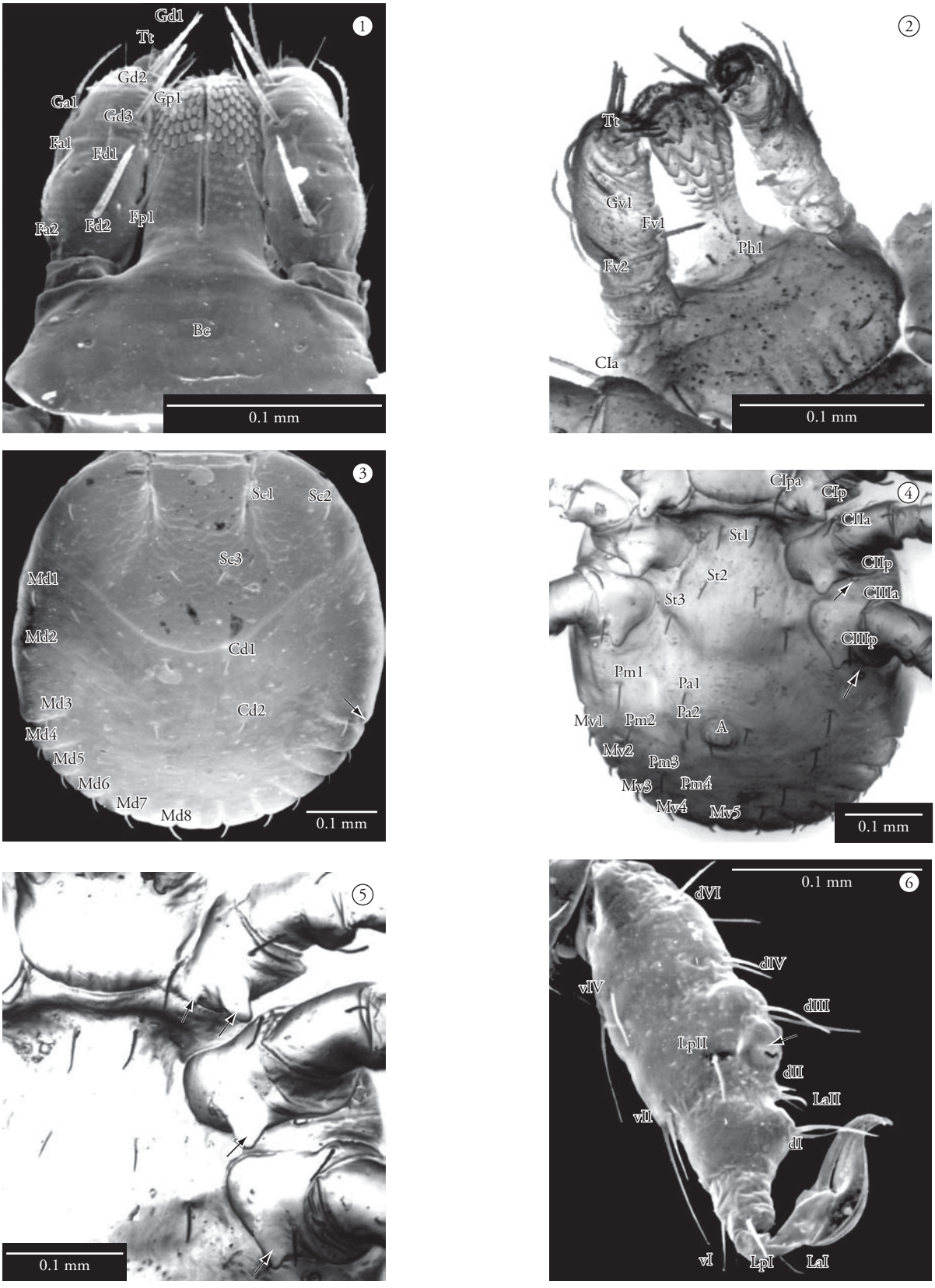


Figure 1-6. *Amblyomma brasiliense* larva. 1. Gnathosoma (dorsal view); 2. Gnathosoma (ventral view); 3. Idiosoma (dorsal view), showing large wax gland (arrowed); 4. Idiosoma (ventral view), showing large wax gland (arrowed); 5. Idiosoma (ventral view), showing coxae I-III spurs (arrowed); 6. Tarsus I, showing Haller´s organ capsule (arrowed). Abbreviations: Bc, basis capituli; F, femoral; G, genual; a, antiaxial; p, paraxial; d, dorsal; v, ventral; L, lateral; m, marginal; Tt, tibiotarsus; Ph, post hypostomal; Sc, scutal; C, central; M, marginal, St, sternal; Pa, preanal; Pm, premarginal; A, anal; CIa, CIIa, CIIIa, anterior setae; CIp, CIIp, CIIIp, posterior setae; CIpa, CIIpa, CIIIpa, paraxial setae.

2. Nymph

Gnathosoma: total length from palpal apices to posterior margin of basis capituli 0.384 ± 0.028 (0.34-0.42). Dorsal basis capituli (Figure 7) width 0.328 ± 0.011 (0.31-0.35), rectangular shape, with a sharp pointed cornua and long emargination. Ventral basis (Figure 8) with posterior margin slightly convex, auriculae short and rounded. Short palpi with segments well defined, length from apices of tibiotarsal segment to posterior margin of trochanter 0.250 ± 0.031 (0.2-0.3), width 0.093 ± 0.005 (0.09-0.1), palpal trochanter (article I) length 0.036 ± 0.002 (0.032-0.042), width 0.054 ± 0.003 (0.052-0.058); palpal femur (article II) length 0.123 ± 0.004 (0.119-0.125), width 0.098 ± 0.009 (0.09-0.111), and palpal genu (article III) length 0.118 ± 0.006 (0.105-0.122), width 0.082 ± 0.007 (0.068-0.085). Palpal setae (Figures 7, 10): 12 setae on tibiotarsus, 5 dorsal genual setae – 1 antiaxial (Ga1), 2 dorsal (Gd1, Gd2), 2 paraxial (Gp1, Gp2); 3 ventral genual setae – 1 ventral (Gv1), 1 genual ventral paraxial (Gp1), 1 genual ventral antiaxial (Ga1); 3 femoral dorsal setae – 1 paraxial (Fp1), 1 dorsal (Fd1), 1 antiaxial (Fa1); 4 femoral ventral setae – 2 ventral (Fv1, Fv2), 2 ventral paraxial (Fp1, Fp2). The hypostome (Figure 9) length from apices to post hypostomal seta (Ph1) 0.220 ± 0.023 (0.190-0.250), width 0.109 ± 0.009 (0.090-0.120), spatulate and compact, with a dental formula of 2/2; both paraxial and antiaxial files with 9 teeth each.

Idiosoma: dorsal surface (Figure 11) oval, with 11 festoons and few small setae dispersed; many large and superficial punctuations. Length from scapular apices to posterior margin of idiosoma 1.52 ± 0.072 (1.38-1.66), greatest width 1.27 ± 0.055 (1.22-1.38). Scutum dark chestnut brown color, outline subtriangular, length along median line 0.273 ± 0.007 (0.260-0.289), width 0.448 ± 0.010 (0.435-0.480), irregular integument, punctuations large and superficial on lateral region of scutum and fine punctuations on scutal central short region and superficial cervical grooves, opaque and flat eyes on lateral scutum surface. Ventral surface (Figure 12) with few small setae dispersed, festoons with small chitinous tubercle on internal surface of posterior border, anal aperture illustrated (Figure 15), anal groove rounded, posterior to anus, 5 anal setae, spiracular plate (Figure 16) oval with dorsal prolongation, eccentric macula.

Legs: moderately long, coxa I with two spurs, the external longer than the internal and 4 setae, 2 anterior (CIa1, CIa2), 1 posterior (CIp) and 1 paraxial (CIpa). Coxae II and III each with 1 short spur and 3 setae, 1 anterior (CIIa, CIIIa), 1 posterior (CIIp, CIIIp), and 1 paraxial (CIIpa, CIIIpa). Coxa IV with a very short spur and 3 setae, 1 anterior (CIVa), 1 posterior (CIVp), and 1 paraxial (CIVpa) (Figures 13-14). Tarsus I (Figure 17) length 0.408 ± 0.024 (0.370-0.450), width 0.151 ± 0.003 (0.148-0.153), with a pair of lyrifissures on lateral posterior region. Haller´s organ capsule aperture mainly transversely slit-like. Tarsus IV (Figure 18) length 0.298 ± 0.026 (0.260-0.340), width 0.102 ± 0.014 (0.084-0.126).

Discussion

Many times, immature stages of ticks are collected from naturally infested animals and to be identified into species are reared in laboratory hosts and molting until the adult stage, given that taxonomic keys to larvae and nymphs of Neotropic are inexistent or incomplete. This procedure was used by Szabó et al. (2006) and Labruna et al. (2007). Nevertheless, considering that some *A. brasiliense*-like tick species have larvae and nymphs with a long pre-molting period and a low molting success in laboratory (SANCHES et al. 2008), it becomes important to be able to make fast, accurate identifications of immature stages by mean of morphological characters.

Some Brazilian studies (SZABÓ et al. 2006; OGRZEWALSKA et al. 2007) show that in this country, *A. brasiliense* has been sympatric mainly with *A. incisum* and *A. naponense* with immature stages until now not described (BARROS-BATTESTI et al. 2006), and *A. ovale* with larvae described by Barbieri et al. (2008a).

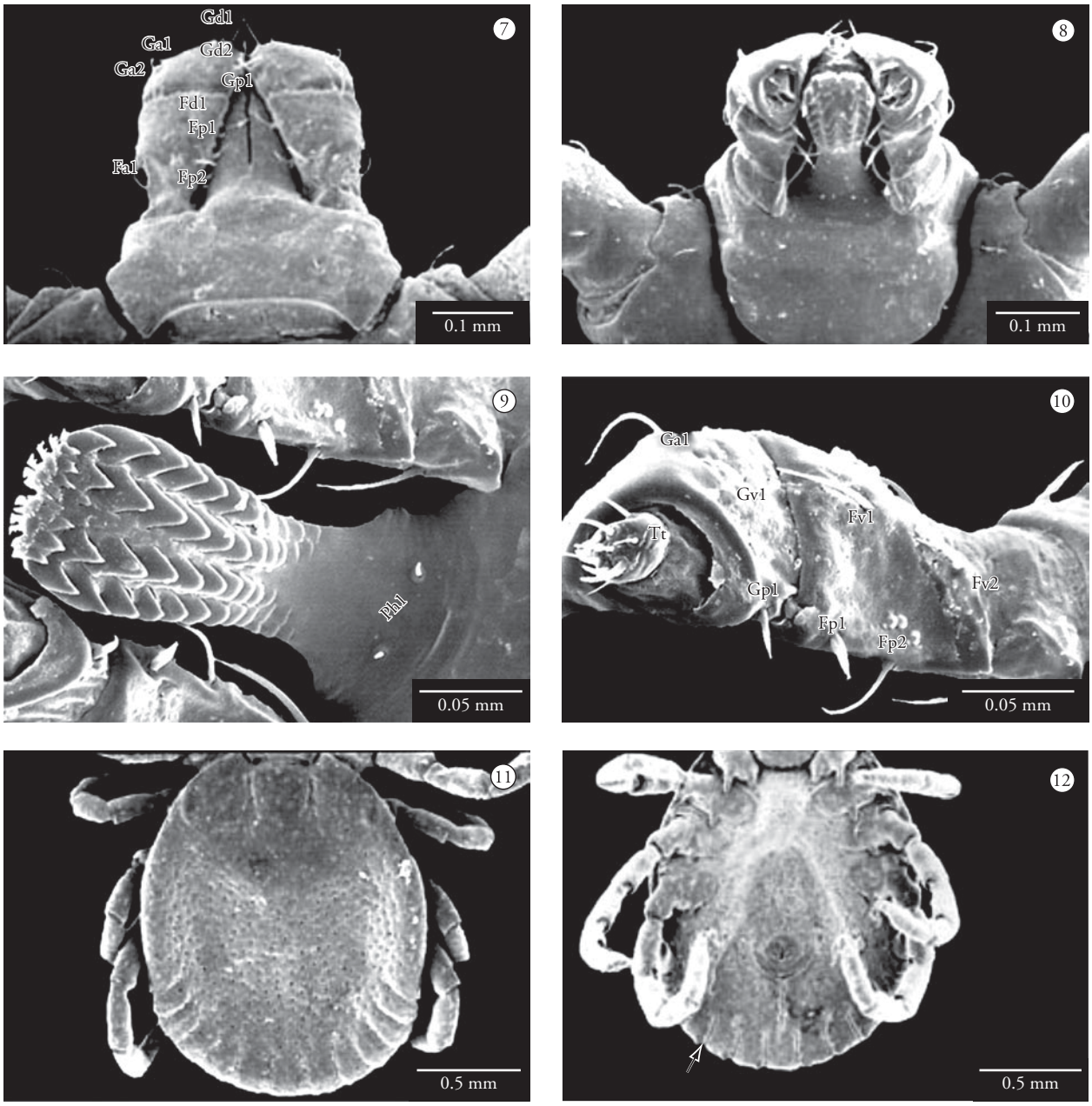
Therefore, morphological characters and measurements of the idiosoma, gnathosoma, and tarsus I of *A. brasiliense* larva were compared with other previously described species of Neotropic such as *A. parvum* and *A. pseudoparvum* (GUGLIELMONE et al. 1990), *A. cajennense* (FAMADAS et al. 1997), *A. longirostre* (BARROS-BATTESTI et al. 2005), *A. triste* (ESTRADA-PEÑA et al. 2002), *A. parvitarsum* (ESTRADA-PEÑA et al. 2005), *A. nodosum* (AMORIM & SERRA-FREIRE 1994), *A. ovale* (BARBIERI et al. 2008a), and *A. pacae* (BARBIERI et al. 2008b).

The basis capituli of *A. brasiliense* larvae is rectangular like in *A. parvitarsum*, with dimensions similar to that tick species, but differs in the length of the palpi – longer in *A. parvitarsum*. On the other hand, *A. parvum* and *A. pseudoparvum* have a basis capituli that is slightly subtriangular or triangular like in *A. pacae* and *A. ovale*. All species used for comparison show a spatulate hypostome with a dental formula of 2/2 and dimensions very close to those in *A. cajennense*, *A. parvum, A. pacae*, and *A. ovale* but shorter than in *A. triste*, *A. longirostre*, and *A. parvitarsum*.

The idiosoma of *A. brasiliense* larvae is rounded, with dimensions similar to *A. parvum,* as measured by Barbieri et al. (2007), and chaetotaxy highly similar and conserved among larvae of *Amblyomma* spp. for idiosoma (exception for tibiotarsus and setae dII on Tarsus I).

Clifford and Anastos (1960) described the presence of four pairs of large wax glands on the idiosoma: one pair located dorsally on the posterior lateral margin of the body, and the others located behind each coxa. Famadas et al. (1997) recorded for the first time a pair of these large wax glands on the fifth festoon of *A. cajennense* larvae and later Barbieri et al. (2007, 2008b) recorded these pairs in larvae of *A. parvum* and *A. rotundatum* and *A. pacae* like in *A. brasiliense* larvae. *A. longirostre* presents these pairs and an additional pair on the fourth festoon like *A. ovale* larvae (BARBIERI et al. 2008a).

A. brasiliense larvae have two spurs on coxa I, with the external one being longer than the internal one. Coxae II and III each have one short spur, as in *A. cajennense*, *A. parvum*, *A. pseudoparvum*,



Figures 7-12. *Amblyomma brasiliense* nymph. 7. Gnathosoma (dorsal view), showing sharp pointed cornua (arrowed); 8. Gnathosoma (ventral view); 9. Hypostome (ventral view), showing a dental formula 2/2 (arrowed); 10. Palpi (ventral view); 11. Idiosoma (dorsal view); 12. Idiosoma (ventral view), showing small chitinous tubercle on internal surface of festoon (arrowed). Abbreviations: F, femoral; G, genual; a, antiaxial; p, paraxial; d, dorsal; v, ventral; Tt, tibiotarsus; Ph, post hypostomal.

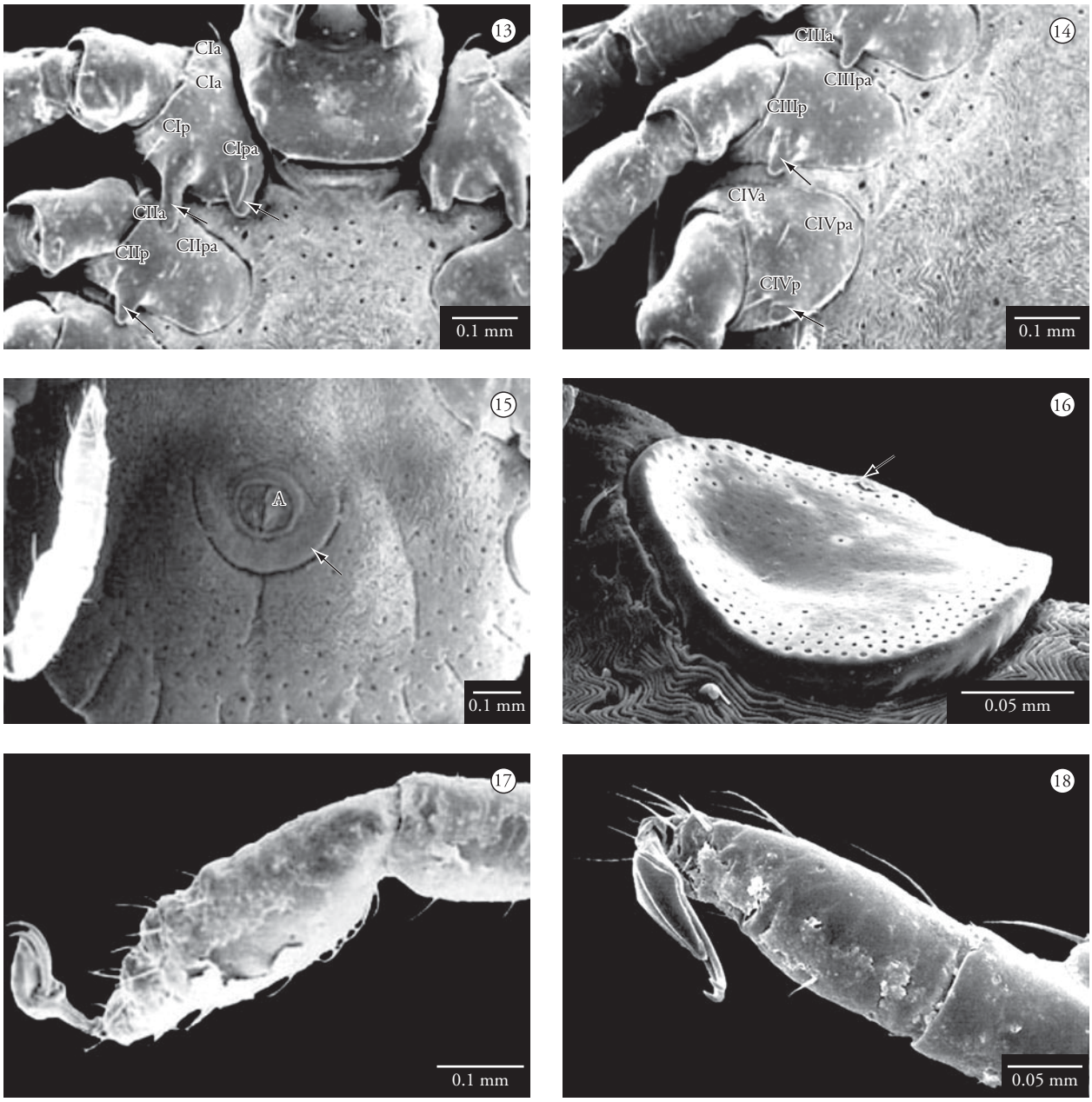
and *A. nodosum*, but in *A. brasiliense*, the external spur is twice as long as the internal one.

According to the formula proposed by Clifford and Anastos (1960) for the genus *Amblyomma* Koch, 1844, the dorsal setae of tarsus I of *A. brasiliense* larvae display the 2:2:2:2:2 arrangement.

Summarizing, the main morphological characteristics of *A. brasiliense* larvae are rectangular basis capituli, short palpi, rounded idiosoma, large wax glands recorded on the fifth festoon, and spurs on coxae and chaetotaxy as discussed.

The current re-description using electron microscope complements the one made by Aragão (1908) of the nymphal stage of *A. brasiliense* using light microscopy.

Morphological characteristics and measurements of the idiosoma, gnathosoma, and tarsus I of *A. brasiliense* nymph were compared with other previously described species such as *A. parvum* and *A. pseudoparvum* (GUGLIELMONE et al. 1990), *A. dubitatum* and *A. triste* (ESTRADA-PEÑA et al. 2002), and with characteristics of *A. neumanni*, *A. tigrinum*, and *A. maculatum* as listed in a taxonomic key (ESTRADA-PEÑA et al. 2005).



Figures 13-18. *Amblyomma brasiliense* nymph. 13. Coxae I-II (ventral view), showing spurs (arrowed); 14. Coxae III-IV (ventral view), showing spurs (arrowed); 15. Anal aperture, showing anal groove (arrowed); 16. Spiracular plate, showing eccentric macula (arrowed); 17. Tarsus I; 18. Tarsus IV. Abbreviations: CIa, CIIa, CIIIa, CIVa, antiaxial setae; CIp, CIIp, CIIIp, CIVp, paraxial setae; CIpa, CIIpa, CIIIpa, CIVpa, paraxial setae; A, anal.

The basis capituli of *A. brasiliense* nymphs is rectangular with sharp pointed cornua, and short rounded auriculae, similar to *A. pseudoparvum*. *Amblyomma neumanii* also presents rectangular basis capituli, but differs in cornua shape (rounded) and in palpi (stronger than in *A. brasiliense*). The hypostome is spatulate, with a dental formula of 2/2 for all *Amblyomma* species used for comparison. These characteristics corroborate Aragão (1908).

Idiosoma of *A. brasiliense* nymphs shows oval dorsal surface, with measurements very close to *A. parvum* but differing in the length of scutum (shorter in *A. brasiliense*). Aragão (1908) reported measurements that are relatively bigger, but it is important to consider that (1) in this study the specimens were collected in different geographic regions, and (2) Aragão did not specify the exact site of starting and ending measurements.

Amblyomma brasiliense nymphs have long legs, coxa I with two evident spurs, the external longer than the internal; coxae II-III each with one short spur; and coxae IV with a very short spur, consistent with Aragão (1908). These characteristics are similar to *A. parvum*, and differ from *A. dubitatum* in the length of spur on coxa IV (shorter in *A. brasiliense*). The same distribution of

spurs is observed in *A. neumanii* but the spurs in *A. brasiliense* are larger. *Amblyomma triste* with triangular external spur on coxa I, *A. tigrinum* with narrow external spur and small internal spur on coxa I, and *A. maculatum* with narrow external spur, all present different numerical and morphological distribution of spurs in comparison with *A. brasiliense*.

Summarizing, the main morphological characteristics of *A. brasiliense* nymphs are rectangular basis capituli with a sharp pointed cornua, oval idiosoma with scutum reaching coxa III, and coxa I with two evident spurs, the external one being longer than the internal one; coxae II-III each with one short spur; and coxae IV with a very short spur and chitinous tubercles on internal surface of posterior border of idiosoma.

The morphological characteristics presented here must be used with caution because larvae of many *Amblyomma* species from the Neotropical region are still unknown. Additional studies of immature stages of other species of this genus are required to allow accurate identification of *Amblyomma* larvae and nymphs.

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