Acarologia is proudly non-profit, with no page charges and free open access

Please help us maintain this system by encouraging your institutes to subscribe to the print version of the journal and by sending us your high quality research on the Acari.

Subscriptions: Year 2021 (Volume 61): 450 €
http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php
Previous volumes (2010-2020): 250 € / year (4 issues)
Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France
ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d’avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

Acarologia is under free license and distributed under the terms of the Creative Commons-BY-NC-ND which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.
REDESCRIPTION OF THE LARVA OF *AMBLYOMMA CAJENNENSE* (FABRICIUS) (ACARI: IXODIDAE) USING OPTICAL AND SCANNING ELECTRON MICROSCOPY

by Kátia Maria FAMADAS¹, Nicolau M. SERRA-FREIRE² and Reinalda M. LANFREDI³

<table>
<thead>
<tr>
<th>LARVA CHAETOTAXY</th>
<th>HALLER’S ORGAN CAMPAÑIFORM SENSILLUM IXODIDAE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LARVA QUETOTAXY</strong></td>
<td><strong>ÓRGÃO DE HALLER SENSILLUM CAMPAÑIFORME IXODIDAE</strong></td>
</tr>
<tr>
<td><strong>LARVE CHAETOTAXIE ORGANE DE HALLER SENSILLUS CAMPAÑIFORME IXODIDAE</strong></td>
<td></td>
</tr>
</tbody>
</table>

**SUMMARY:** The larval stage of *Amblyomma cajennense* is redescribed, using optical and scanning electron microscopy. Larvae were obtained in the laboratory from an engorged *A. cajennense* female which had been collected on a horse in the municipality of Itaguai, State of Rio de Janeiro, Brazil. The chaetotaxy of the idiosoma, palps and Haller’s organ, the campaniform sensillum on the fifth festoon, as well as other structures, are described for the first time. The nomenclature employed in the chaetotaxy of the palps and Haller’s organ is discussed.

**RESUMO:** O estágio de larva de *Amblyomma cajennense* é redescripto, utilizando-se microscopia óptica e eletrônica de varredura. As larvas foram obtidas em laboratório, a partir de uma fêmea ingurgitada de *A. cajennense*, coletada em equino, no município de Itaguai, RJ, Brasil. Foram descritas pela primeira vez a quetotaxia do idiosoma, palpos, órgão de Haller, sensillum campaniforme no quinto festão, além de outras estruturas. A nomenclatura utilizada na quetotaxia dos palpos e órgão de Haller é discutida.

**RÉSUMÉ :** La larve d’*Amblyomma cajennense* est redécrite en microscopie optique et électronique à balayage. Les larves ont été obtenues en laboratoire à partir d’une femelle gorgée récoltée sur un cheval de la municipalité d’Itaguai, dans l’Etat de Rio de Janeiro, au Brésil. La chaetotaxie de l’idiosoma et des palpes, l’organe de Haller, le sensillum campaniforme du cinquième feston, ainsi que d’autres structures, sont décrits pour la première fois. Nous discutons sur la nomenclature employée pour la chaetotaxie des palpes et pour l’organe de Haller.

Among the representatives of the ixodid fauna of the Americas, *Amblyomma cajennense* (Fabricius, 1787) is considered to be one of the most important species, due to high diversity of its hosts, among which are domestic mammals and man (ARAGÃO & FONSECA, 1953). To these hosts it may transmit many pathogens (SMITH, 1975), besides the presence of irritating substances in its saliva, which cause discomfort to the host when injected into the skin (CUNHA, 1978). It also participates, as causative agent, in

1. Laboratório de Ixodídeos, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ Brasil.
3. Programa de Biologia Celular e Parasitologia, Instituto de Biofísica Carlos Chagas Filho, CCS, Universidade Federal do Rio de Janeiro, Ilha do Fundão, RJ, Brasil.

_Acarologia_, t. XXXVIII, fasc. 2, 1997.
ascendant flaccid paralysis in cow, sheep and goat (SERRA-FREIRE, 1983).

The latest studies on the morphology of A. cajennense date from four decades ago and mainly concern the adult forms. As a general rule, the specific determination of larval stage of ticks is a particularly difficult issue, since literature data are as yet inadequate.

CLIFFORD & ANASTOS (1960) attribute the deficiencies in our knowledge of larval tick systematics to the following factors: too few consistent characters for inclusion in keys; inadequate descriptions and drawings; relatively few species reared in the laboratory from identified females; and the virtual impossibility of associating larvae collected in the field with the corresponding adults. Those authors suggest that research should be made on species from several geographic regions.

In order to fill some of these gaps, we here study the morphology of the larva of A. cajennense by optical and scanning electron microscopy.

**MATERIAL AND METHODS**

For a preliminary study, six engorged female A. cajennense specimens were collected from horses maintained under semi-extensive conditions at the Wielhem Otto Nietz Station for Parasitological Research, Rural Federal University of Rio de Janeiro at Itagui, State of Rio de Janeiro. Females oviposited under laboratory conditions (27 ± 1°C, 80 ± 10% RH), and the eggs were individually placed in modified disposable syringes. After all the larvae had hatched, a sample of 100 individuals was kept without food for 15 days, so that the consolidation of the exoskeleton took place. The specimens were subsequently placed in water at 70 ± 10°C and preserved in 70% ethanol.

A single engorged female, from the same group of females as in the preliminary study, produced 50 larvae, 30 of which were prepared for optical microscopy according to FAMADAS' (1993) method. The remaining 20 specimens were processed for scanning electron microscopy according to the method devised by KEIRANS et al. (1976).

The engorged females were identified according to the papers by ROHR (1909), ARAGÃO (1911); ROBINSON (1926), COOLEY & KOHLS (1944), ARAGÃO & FONSECA (1961) and JONES et al. (1972), and the eggs and larvae were kept under laboratory conditions (27 ± 1°C, 80 ± 10% RH).

All measurements are given in millimetres. The average is followed by the standard deviation and the interval represents a sample of 30 specimens measured with a Wild M-11 optical microscope.

A female and four of its larvae are deposited in the Ixodides Collection at the Instituto Oswaldo Cruz (number 034), Rio de Janeiro, Brazil; two larvae are deposited in the U.S. National Tick Collection, Georgia, U.S.A.

**AMBLYOMMA CAJENNENSE, LARVA**

**IDIOSOMA:** Dorsal surface (Figs. 1, 6). Length from apices of scapulae to posterior margin of body 0.510 ± 0.022 (0.481–0.585); greatest width 0.489 ± 0.018 (0.455–0.520); outline oval, with 11 festoons. Lateral margin of alloscutum, nearly reaching coxal III line, with one pair of campaniform sensilla (sensillum campaniform dorsal = SCd). Setae: 2 central dorsal pairs (Cd1, Cd2) (Fig. 7), 8 marginal pairs (Md1–Md8), with Md1, and Md2 pairs before SCd and Md3 pair located in the inner side behind of the sensillum, Md4–Md8 pairs posterior to sensillum, each one in a different festoon. Scutum: outline subtriangular; length 0.201 ± 0.012 (0.180–0.228) along median line (= anteroposterior line = AP; FONSECA & ARAGÃO, 1952); breadth 0.394 ± 0.012 (0.371–0.419) up to the eyes' line (= transversal line = TT; FONSECA & ARAGÃO, 1952). Integument with irregular hexagonal ornamentation, few punctuation (Fig. 9). Eyes slightly bulging and shallow; cervical grooves distinctively extending parallel to the proximities of setae Sc3. Setae: 3 scutal pairs (Sc1, Sc2, Sc3). One pair of pores near Cd1 and Cd2 (Figs. 7, 8).

Ventral surface (Figs. 1, 11) with 3 pairs of campaniform sensilla; 1 pair located on the outer margin of the coxa I, 2 pairs behind coxa II and III, and 2 campaniform sensilla on the 5th festoon (sensillum campaniform of festoon = SCf) (Fig. 13). Central festoon without seta, greatest width 0.063 ± 0.008 (0.052–0.099). Setae: 3 sternal pairs (St1, St2, St3);
2 preanal pairs (Pa₁, Pa₂); 4 premarginal pairs (Pm₁–Pm₄); 5 marginal ventral pairs (Mv₁–Mv₅). Anal aperture on central portion of opisthosoma, with 1 pair of setae on the valva (A₁) (Fig. 12).

**Gnathosoma:** Dorsal (Fig. 14). Basis capituli triangular in outline; length from palpal apices to posterior margin 0.166 ± 0.094 (0.148–0.180), width 0.160 ± 0.059 (0.148–0.175). Posterior margin straight, cornua absent. Basis capituli on median line, with 1 sensillum pair. Palpal grooves segment well-defined. Palpi length from apices of tibiotarsal segment to posterior margin of trochanter 0.117
± 0.067 (0.107–0.143); femur (II) 3.0 times longer than trochanter (III); combined length of femur and genu 0.096 ± 0.005 (0.088–0.113). Femur with sensillum near seta Fd₁ (Fig. 16).

Ventral (Fig. 15). Basis capituli as illustrated (Fig. 15). Hypostome compact, spatulate, length from apices to post hypostomal seta 0.093 ± 0.003 (0.088–0.099), dental formula 2/2 in file teeth, apical corona usually with 9 denticles; 1 pair of post hypostomal setae (Ph₁). Palpal setae (Figs. 2, 3, 14, 15): 12 setae on tibiotarsus, 8 terminal (Ttt₁–Ttt₈), 2 paraxial (Ttp₁, Ttp₂) and 2 antiaxial (Tta₁, Tta₂); 6 genual setae, 1 paraxial (Gp₁), 1 antiaxial (Ga₁), 3 dorsal (Gd₁, Gd₂, Gd₃) and 1 ventral (Gv₁); 6 femoral setae, 1 paraxial (Fp₁), 2 antiaxial (Fa₁, Fa₂), 1 dorsal (Fd₁) and 2 ventral (Fv₁, Fv₂); trochanter 0.

Fig. 2–5: Amblyomma cajennense, larva.

Fig. 6-10: *Amblyomma cajennense*, larva.

6. — Dorsal view. 7. — Detail of the central dorsal setae (cd₁, cd₂) and pores (arrow). 8. — Detail of pores on alloscutum (arrow). 9. — Detail of scutum integument. 10. — Detail of marginal dorsal setae (Md₁, Md₂, Md₃) and campaniform sensillum on lateral margin of alloscutum (Scd = campaniform dorsal sensillum).
FIG. 11-16: Amblyomma cajennense, larva.
LEGS (Figs. 1, 6, 11): Coxa I with 2 triangular spurs, the outer largest and sharp-pointed; coxa II and III each with a single, slightly proeminent 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 0.186 0.006 (0.175–0.201) long. Setae: dorsal (Figs. 4, 17), 2 in dorsal I group (di1, di2) parallel to the longitudinal axis of segment, 7 dorsal II (dII1–dII7) (Fig. 18), 2 dorsal III (dIII1, dIII3), 2 dorsal IV (dIV1, dIV2), 0 dorsal V and 2 dorsal VI (dVI1, dVI2); ventral (Figs. 5, 19), 2 ventral I (vI1, vI2), II (vII1, vII2) and III (vIII1, vIII2); lateral anterior, 1 in lateral anterior I group (laI1) and 3 in la II group (laII1, laII2, laII3) (Figs. 5, 19); lateral posterior (Fig. 5), 1 in lateral posterior I group (lpI1) and 3 in lpII group (lpII1, lpII2, lpII3). Ambulacrum as illustrated (Figs. 20, 21).

DISCUSSION

Since no records in the literature were known on the expression of each character to be analyzed and, consequently, no previous sampling of the offspring of individual A. cajennense females, we carried out a preliminary analysis of a pool of larvae (n=100), descended from six females. In general, we observed that the characters present low percentages of variations, enabling morphological studies based on small samples. This is an important step in character studies, chiefly of those species which are little known, since the trend prevailing among various taxonomic schools is to take up population studies instead of those based on a few specimens.

Based on the samples prepared for optical and scanning electron microscopy, we describe for the first time the chaetotaxic structure of tarsus I of the A. cajennense larva. In order to designate the setae and their position along the tarsus I, the nomenclature proposed by Hess & VLIMANT (1983a) was utilized, also in accordance with that advocated by WOOLLEY (1988), and not with the one proposed by CLIFFORD & ANASTOS (1960), since the former enables a clearer and more practical identification of each setae by a figure, micrograph or map.

According to the observations by CLIFFORD & ANASTOS (1960) on the genus Amblyomma (Koch, 1844), the dorsal setae of the tarsus I of A. cajennense larva also follow the 2:2:2:2:2 arrangement. These authors based this formula on nine species from four continents: North America (Amblyomma americanum (Linné, 1758), A. dissimile Koch, 1844; A. maculatum Koch, 1844; A. tuberculatum (Marx, 1844)); South America (A. cajennense; Africa, A. gemma Dönitz, 1909; A. hebraeum Koch, 1844; A. variegatum (Fabricius, 1787); and Australia (A. triguttatum Koch, 1844). However, Hess & VLIMANT (1983a) studied the ultrastructure of the setae on tarsus I of A. variegatum and found that in the larva, group dl (= pre-Hallerian) is constituted by a single setae and that the no-pore type (np/C), apparently belonging to group dl, represented the anterior lateral setae I1 (IaI1), which was slightly displaced towards the dorsal side.

In our observations on the A. cajennense larva and based on illustrations of the tarsus I of some species of the genus Amblyomma by CLIFFORD & ANASTOS (1960), we were able to observe that the two setae of group dl are laid out in a parallel fashion, rather close to one another, as shown in the electronmicrograph of the tarsus I of an A. variegatum nymph (Hess & VLIMANT, 1983b). These observations corroborate the view of CLIFFORD & ANASTOS (1960), who stress the need for studies of other species of the genus Amblyomma, from different geographic areas, so that a definite chaetotaxic pattern can be established.

The counts of the occurrence and location of each setae on tarsus I of A. cajennense larva yielded an exact figure of 100%. These results are compatible with those of Hess & VLIMANT (1983a), who stated that the number and distribution of setae are intraspecifically constant.

As described by CLIFFORD & ANASTOS (1960), we observed four pairs of sagittiform sensilla (= campa­niform; DASGUPTA & RAY 1955) on the idiosoma and, for the first time, their presence is recorded on the fifth festoon.

WOOLLEY's (1988) methodology for Acari is considered more appropriate than that of CLIFFORD & ANASTOS (1960) for the chaetotaxic structure of the palp of A. cajennense larva, as the former, through the use of codes, enables one to locate the segment.
and the side on which the seta lies. It is also convenient that a nomenclature within this subclass be standardized.

The analysis of the frequency and location of setae on the idiosoma and the gnathosoma also produced percentages of occurrence equal or very close to 100%; only 0.3% of occurrence of accessory setae in the preanal and premarginal groups was observed, confirming chaetotaxy as a good diagnostic character for the larval stage of ticks.

ACKNOWLEDGEMENTS

We are grateful to Dr Sérgio M. Faria, at the Centro Nacional de Pesquisas em Agrobiologia of
the Empresa Brasileira de Pesquisa Agropecuária (CNPA-EMBRAPA) for the use of the scanning electron microscope. Dr João Luiz Horácio Facchin, at the Rural Federal University of Rio de Janeiro (UFRJ), is thanked for his invaluable suggestions.

REFERENCES


