

REDESCRIPTION OF THE LARVA OF *AMBLYOMMA CAJENNENSE* (FABRICIUS) (ACARI: IXODIDAE) USING OPTICAL AND SCANNING ELECTRON MICROSCOPY

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LARVA
CHAETOTAXY
HALLER'S ORGAN
CAMPANIFORM SENSILLUM
IXODIDAE

LARVA
QUETOTAXIA
ÓRGÃO DE HALLER
SENSILLUM CAMPANIFORME
IXODIDAE

LARVE
CHAETOTAXIE
ORGANE DE HALLER
SENSILLUS CAMPANIFORME
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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 Itaguaí, 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 redescrito, 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 Itaguaí, RJ, Brasil. Foram descritas pela primeira vez a quetotaxia do idiosoma, palpos, órgão de Harller, 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 redérite 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 décrites 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

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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 Itaguaí, State of Rio de Janeiro. Females oviposited under laboratory conditions ($27 \pm 1^\circ\text{C}$, $80 \pm 10\%$ 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 $70 \pm 10^\circ\text{C}$ 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 ($27 \pm 1^\circ\text{C}$, $80 \pm 10\%$ 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 sensillum (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 punctuations (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);

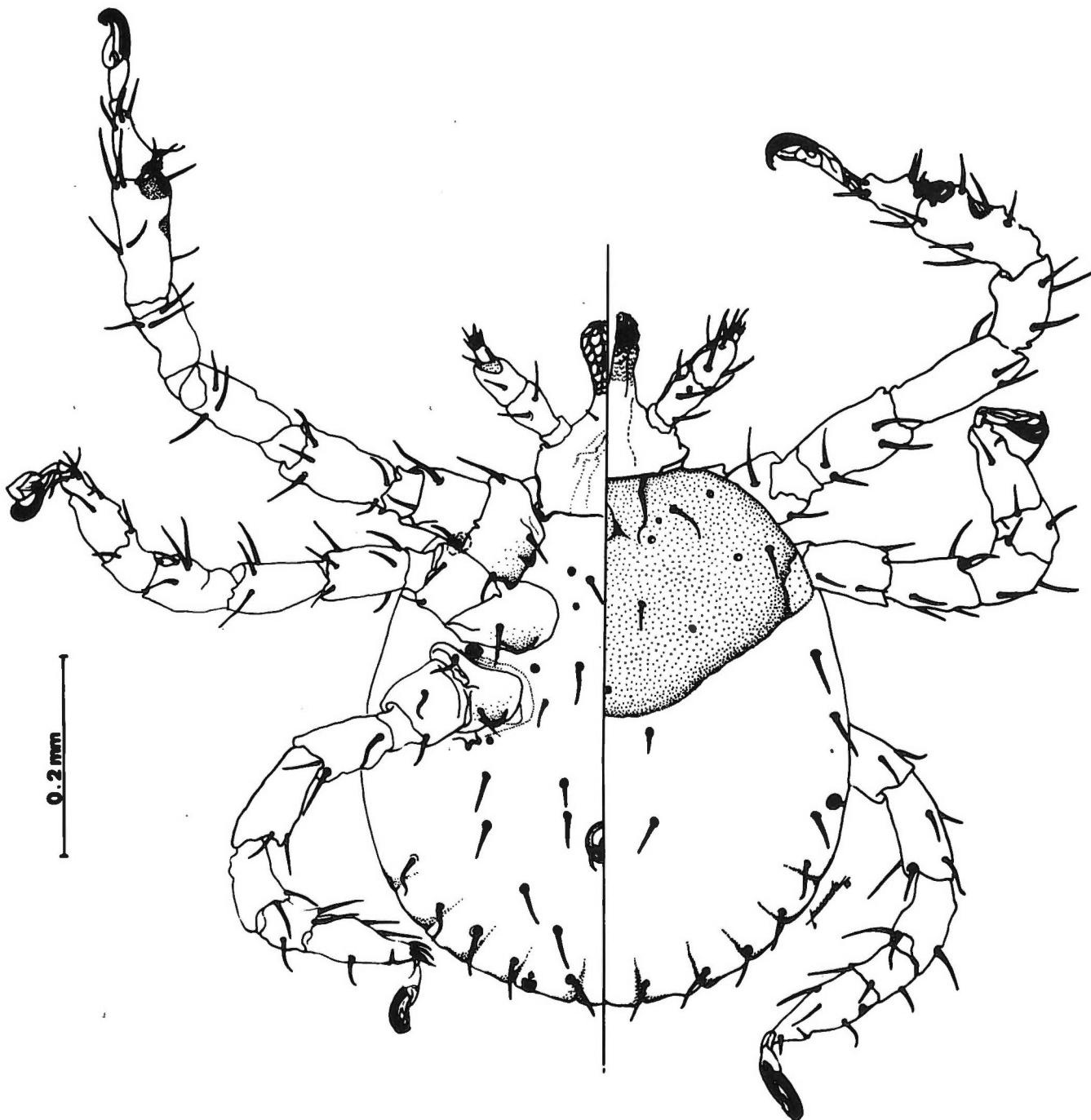


FIG. 1: *Amblyomma cajennense*, larva, dorsal (right) and ventral (left) views.

2 preanal pairs (Pa_1 , Pa_2); 4 premarginal pairs (Pm_1 – Pm_4); 5 marginal ventral pairs (Mv_1 – Mv_5). Anal aperture on central portion of opisthosoma, with 1 pair of setae on the valva (A_1) (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

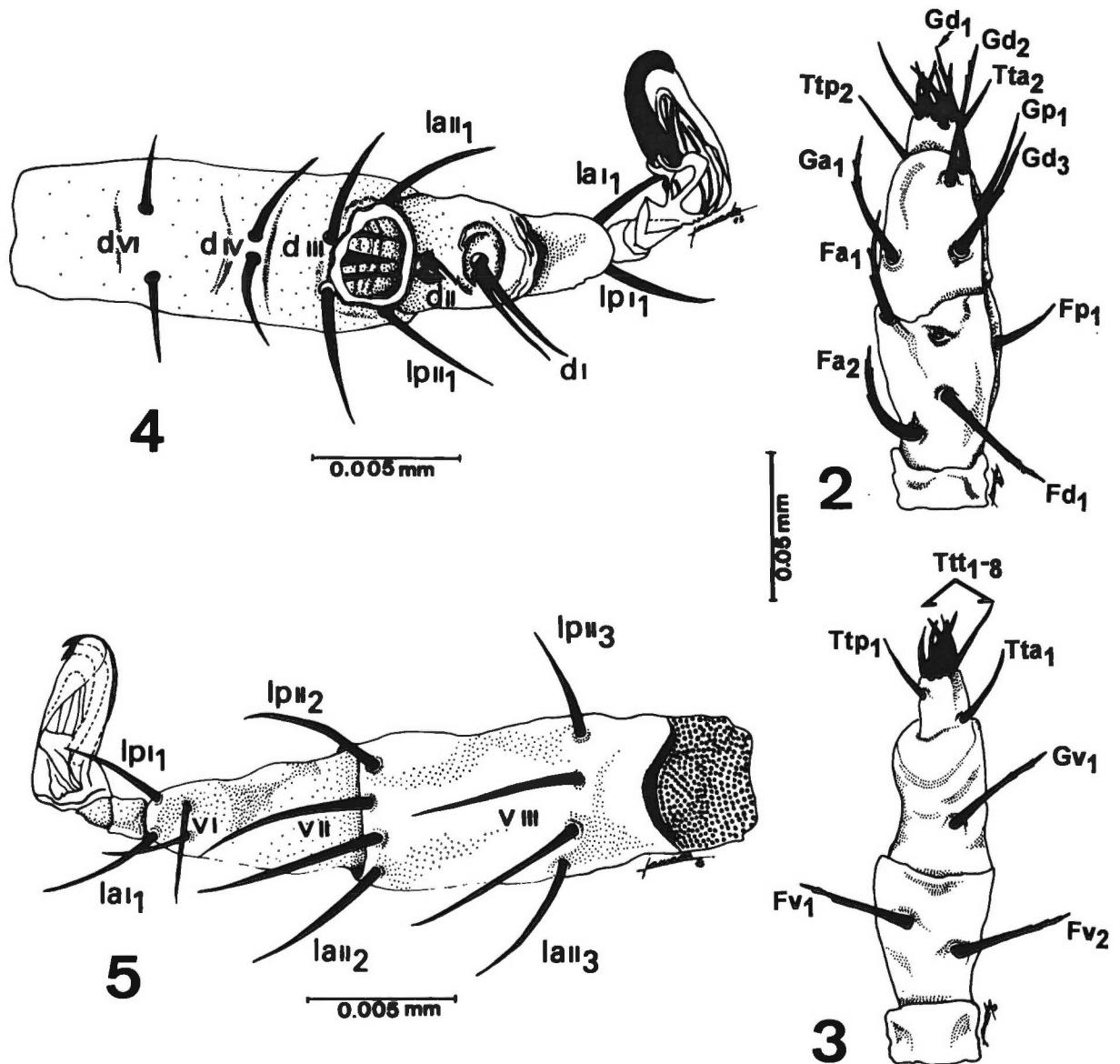


FIG. 2-5: *Amblyomma cajennense*, larva.

2. — Palp, dorsal view. 3. — *Idem*, ventral view. 4. — Tarsus I, dorsal view. 5. — *Idem*, ventral view. Abbreviations: a—antiaxial; F—femur; G—genu; p—paraxial; t—terminal; Tt—tibiotarsus.

± 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 Fd_1 (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 (Ph_1). Palpal setae (Figs. 2, 3, 14, 15): 12 setae on tibiotarsus, 8 terminal (Ttt_1-Ttt_8), 2 paraxial (Ttp_1 , Ttp_2) and 2 antiaxial (Tta_1 , Tta_2); 6 genual setae, 1 paraxial (Gp_1), 1 antiaxial (Ga_1), 3 dorsal (Gd_1 , Gd_2 , Gd_3) and 1 ventral (Gv_1); 6 femoral setae, 1 paraxial (Fp_1), 2 antiaxial (Fa_1 , Fa_2), 1 dorsal (Fd_1) and 2 ventral (Fv_1 , Fv_2); trochanter 0.

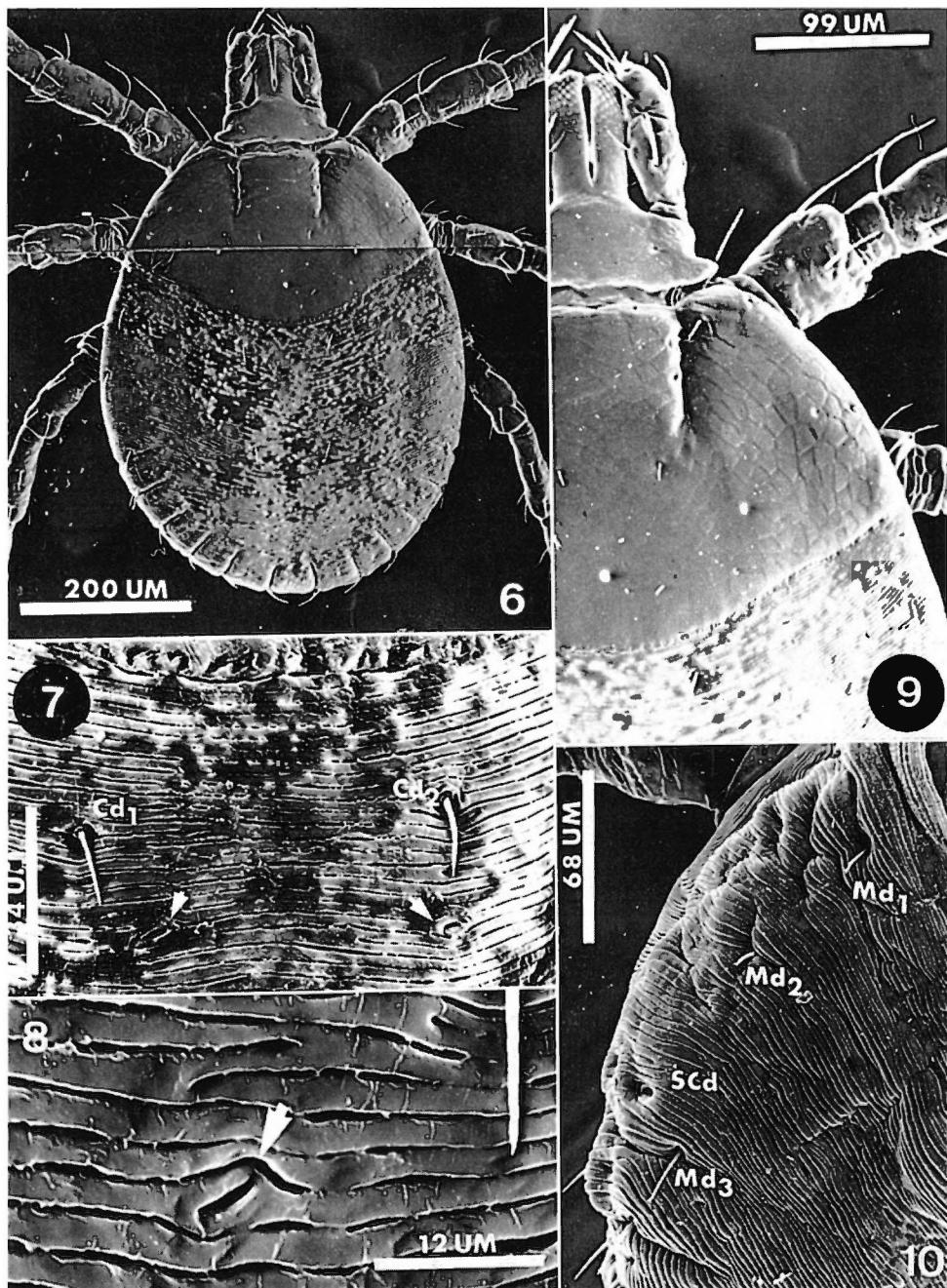


FIG. 6–10: *Amblyomma cajennense*, larva.

6.—Dorsal view. 7.—Detail of the central dorsal setae (Cd_1 , Cd_2) and pores (arrow). 8.—Detail of pores on alloscutum (arrow). 9.—Detail of scutum integument. 10.—Detail of marginal dorsal setae (Md_1 , Md_2 , Md_3) and campaniform sensillum on lateral margin of alloscutum (Scd = campaniform dorsal sensillum).

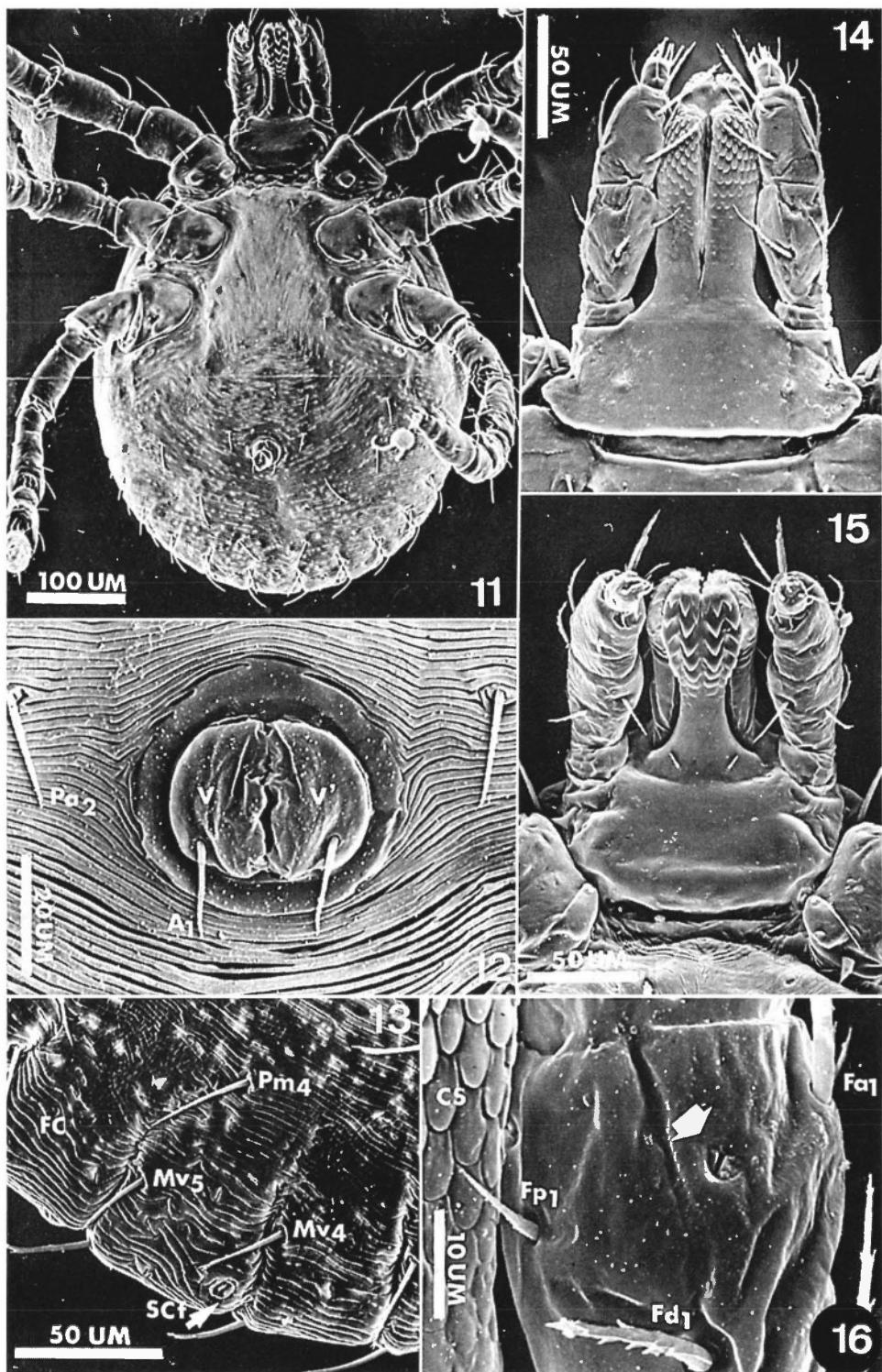


FIG. 11-16: *Amblyomma cajennense*, larva.

11. — Ventral view. 12. — Anal aperture, v-valva, A₁-anal seta, Pa-preanal seta. 13. — Detail of 5th festoon, FC-central festoon, Pm₄-Premarginal seta, Mv₄, Mv₅-Marginal ventral setae, SCf-Campaniform sensillum of the festoon. Gnathosoma (14-15): 14.—dorsal view. 15. — Ventral view. 16. — Detail of sensillum on palpal femur. Fa-antiaxial femoral seta, Fp-paraxial femoral seta, Fd₁-dorsal femoral seta, CS-cheliceral sheath.

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* Dönnitz, 1909; *A. hebraicum* 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 dI (= pre-Halleral) is constituted by a single setae and that the no-pore type (np/C), apparently belonging to group dI, 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 dI 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

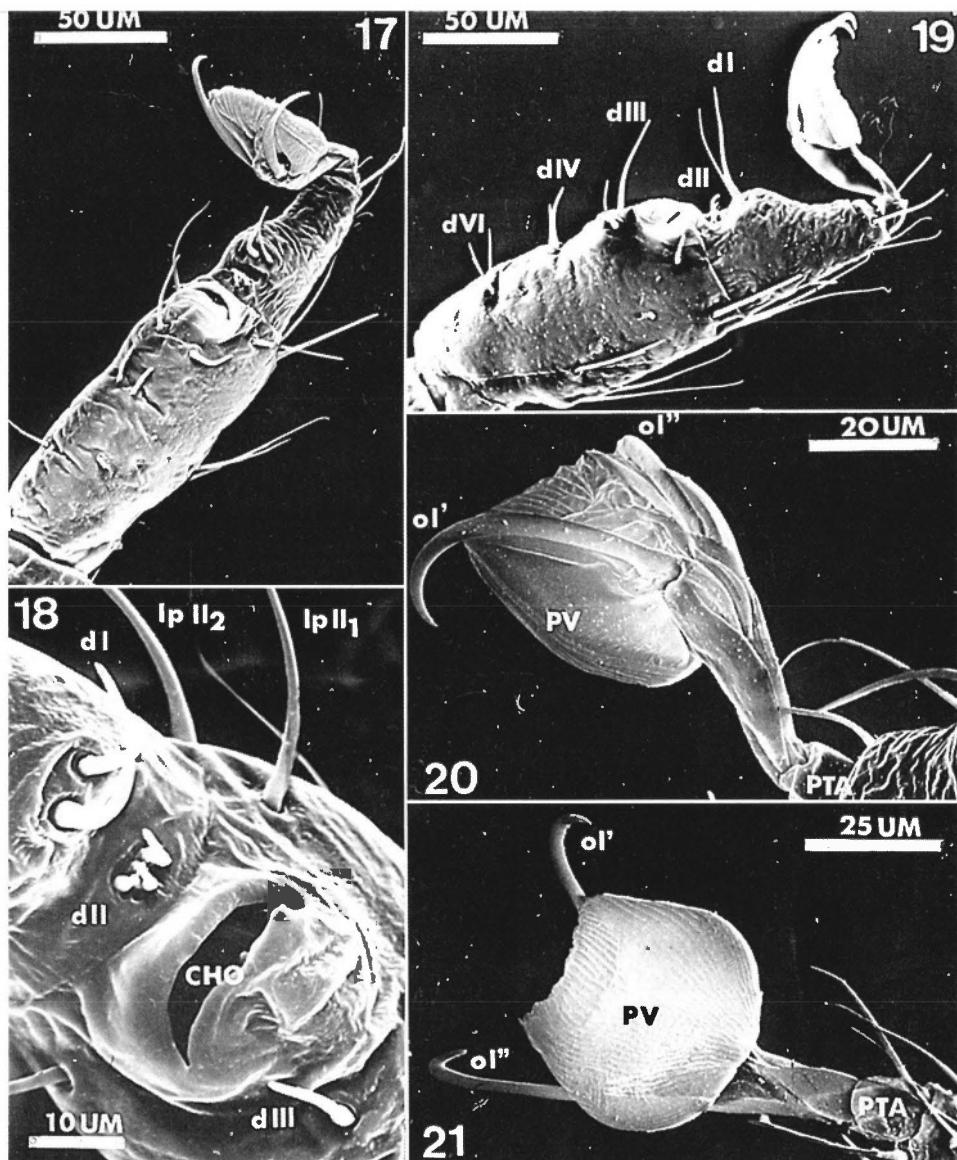


FIG. 17-20: *Amblyomma cajennense*, larva.

Tarsus I (17-19): 17. — Dorsal view. 18. — Detail of Haller's organ. Setae. d—dorsal group (I, II, III), lpII—posterior lateral group (II), CHO—capsule of Haller's organ. 19. — Anterior lateral view. Ambulacrum: (20-21): 20. — Dorsal view. 21. — Ventral view; ol'—anterior lateral unguis, ol''—posterior lateral unguis, PV—pulvillus, PTA—pretarsus.

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.

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