MORPHOLOGICAL DESCRIPTION OF TICK LARVAL STAGE (ACARI: IXODIDAE). 5. *ANOCENTOR NITENS* (NEUMANN, 1897).

M. AMORIM[1], G. S. GAZETA[1], L. GUERIM[1] & N. M. SERRA-FREIRE[1].

(1) Laboratório de Morfofisiologia e Patogenia de Carrapatos - LMPC - Departamento de Parasitologia Animal -IB,UFRRJ, CEP: 23.851-970. Laboratório de Ixodides - LI - Departamento de Entomologia IOC / FIOCRUZ, CEP: 21.045-900.

SUMMARY: Teleogina (engorged females) recovered from *Equus caballus* in the Federal Rural University of Rio de Janeiro were identified as *Anocentor nitens*. The eggs were obtained from teleoginae that originated newborn larvae at laboratory conditions. The newborn larvae were sacrificed in hot water (± 70°C), 24 hours after eclosion. They were unfed and were kept in ethanol 70° GL. They were mounted on slides following a routine technique at laboratory of tick morphophisiology and pathogeny (LMPC-DPA-IB-UFRRJ). A hundred larvae were analysed morphologically under light microscope considering characters and attributes. Staining of the newborn larvae and the ornamentation patterns were also analysed using a stereomicroscope, that contributed to the diagnosis of this species.

KEY WORDS: *Anocentor nitens*, morphology, chaetotaxy, larva of tick.

INTRODUCTION

Dermacentor nitens Neumann, 1897, *Anocentor columbianus* Schulze 1937 and *Anocentor nitens* Cooley, 1938 were considered genotipically the same species by ARAGÃO & FONSECA (1953), accepting the genus *Anocentor* Schulze 1937 as characteristic for this ixodid, and *Anocentor nitens* (Neumann, 1897), Schulze, 1937 as its type species. HOOKER *et alii* (1912) described the presence of *A. nitens* in many parts of Central America. YUNKER *et alii* (1986) reported as geographic distribution of *A. nitens* the tropical and sub-tropical areas from the south of United States to the north of Argentina. ARAGÃO (1944) apud FLOUCH & ABONNENC (1945) described for the first time the presence of *A. nitens* in Brazil and ARAGÃO & FONSECA (1953) showed its fast dissemination throughout the country. Actually, *A. nitens* is present in all regions of Brazil.

The non-specificity of *A. nitens* in relation to the host, was characterized by HOOKER *et alii* (1912) who observed the parasitism of this species in equines, asinines, mule, caprines and bovines. This non-specificity was confirmed by COOLEY (1938), DIKMANS (1945), SOUZA LOPES & MACEDO (1950), ARAGÃO & FONSECA (1953), ROCHA *et alii* (1969), SERRA-FREIRE (1982), FOX & LEON (1983), MORENO (1984), FLECHTMANN (1985), FORRESTER *et alii* (1985) and SERRA-FREIRE & MIZIARA (1989) who observed the presence of these ixodids in equines, bovines, felines, swines and dogs or developed the life cycle in rabbits. However, equines are supposed to be the preferential hosts (HOOKER *et alii*, 1912; DUNN, 1915; COOLEY, 1938; DIKMANS, 1945; ROBY & ANTHONY, 1963; DRUMOND *et alii*, 1969) and a preference for the internal face of the ear as a fixation site might occur (HOOKER *et alii*, 1912; COOLEY, 1938; SOUZA LOPES & MACEDO, 1950; MALHEIRO, 1952; FLECHTMANN, 1985). SERRA-FREIRE (1982) working in the State of Rio de Janeiro, found *A. nitens* at the internal face of the coxal, perineal and inguinal regions, of bovines what was confirmed by ROCHA (1986) in bovines of Garanhus city, Pernambuco State. FLECHTMANN (1985) reported the presence of this tick in nasal diverticulum of horses and ROBY & ANTHONY (1963) reported the participation of *A. nitens* in the transmission of the etiologic agent of equine piroplasmosis. FLECHTMANN (1985) showed that the association between instars of *A. nitens*, excrements of this parasite and natural secretion of the host in auricula have originated suppurations and predispose to myiasis and the results are wounds or total damage of auricula in equines as confirmed by SERRA-FREIRE & MIZIARA (1989) in rabbits.

Characterized as presenting monoxenous cycle, DUNN (1915) and DAEMON & SERRA-FREIRE (1987) described

that differences of biological characteristics were due to the origin of the strain of *A. nitens*. HOOKER *et alii* (1912) described that neoginae originated from engorged metanymphs in equines have been fixed in bovines. SERRA-FREIRE & MIZIARA (1989) reported a greater heteroxenous performance using bovines as hosts when compared to rabbit. SERRA-FREIRE *et alii* (1990) observed the presence of larvae and neonymphs in grasses submitted to continuous grazing by bovines. These authors reported to the difficulties related to the specific identification of ticks due to of the lack of bibliography.

Despide the wide geographic distribution, absence of specificity related to the host, the fixation site and its pathogenicity, *A. nitens* is not the most studied species. Therefore few data are known on the anatomic structures, since the original description (Neumann, 1897). This analysis ise restricted to the taxonomic parameters of adults and consider larval and nymph morphology of this species.

MATERIALS AND METHODS

Tick Origin: Teleogina of *Anocentor nitens* were obtained from equines (*Equus caballus* L.) kept at W.O. Neitz Station for Parasitologic Research of Master Science course in Veterinary Parasitology at Federal Rural University of Rio de Janeiro, EPPWON/UFRRJ in Itaguaí, Rio de Janeiro.

Identification of the species: This species was examinated using a stereomicroscope and identified as *A. nitens* following the dichotomic key of ARAGÃO & FONSECA (1961) by professor N. M. SERRA-FREIRE.

Obtention of newborn larvae: Teleoginae were separated and kept individually and identified in plastic flasks with 20 cm^3 and pression cap with a central hole, used for egg posture, incubation and eclosion at room temperature and natural photoperiod. These procedures were carried out at the Laboratory of Tick Morphophisiology and Pathogeny (LMPC), situated in the area of the Animal Sanity Project UFRRJ/ EMBRAPA, "campus" of the UFRRJ. After the incubation period, the newborn larvae, unfed and 24 hours after eclosion were sacrificed in hot water, ± 70°C and preserved in ethanol 70°GL. One hundred larvae were mounted on slides with balsam.

The basic steps for mounting followed the methodology described by AMORIM & SERRA-FREIRE (1994) for *A. nodosum*, *A. varium* and *A. dissimile* and *Amblyomma rotundatum* (AMORIM & SERRA-FREIRE, 1995). All setae were named according to their topographic position: idiosome setae, anal, capitulum basis, palpus, legs, coxa and tarsus. The terminology used for the chaetotaxic study and others morphologic aspects of larva were based on the works of CLIFFORD & ANASTOS (1960), WOOLEY (1988), HESS & VLIMANT (1982, 1983 a,b), MARQUES *et alii* (1992), FAMADAS (1993) and AMORIM & SERRA-FREIRE (1994 a, b, c) and AMORIM & SERRA-FREIRE (1995).

Analysis of parameters: Slides were kept in an incubator at 32°C, dried during at least 72 hours. After drying the mounted specimen was observed under microscope, characterizing the morphological aspects of *A. nitens*. For specific identification were used the following parameters: presence or absence of periocular mark; gnathosoma, scutal and idiosomal shapes; number of hyposthomal tooth row; number of teeth in each file on the hyposthome, number of denticle on the apical crown; number of denticle rows, number of denticles in each file on the cheliceral sheath, number of teeth on the chelicerae digits; number of "sensilla" on the idiosoma, number of spur on coxae I, II and III; number of seta and their localization on the idiosoma, gnathosoma, legs and Haller's organ, relation between palpi, chelicerae and hyposthome length, relation between the size of palpi segments; relation between the size of articulated segments of legs; relation between gnathosoma, podosoma and opisthosoma sizes; palpus, idiosomal, legs and tarsus I chaetotaxy of larva using design tube and phase contrast. Using the estereomicroscope the newborn larvae staining and ornamentation patterns were also analysed. Results of morphometric study are presented in millimetres (mm) and represent the average, standard deviation between parametric values obtained from a sample of 30 specimens.

RESULTS

Diagnosis: small larval size , oval with characteristic subtriangular scutum with fine punctuations: brown, small eyes, pale and flat; capitulum basis quadrangular; hyposthomal dentition 2/2 with heterodont teeth; eight rows of denticles on the chaeliceral margin and eighteen denticles per row; three rows of three denticles on the apical crown (3/3); short palpi; coxae I,II, III with one spur in each one and presence of nine festoons on the opisthosoma.

Description: idiosoma with oval shape (Fig.1,2), longer than wider; length 0,60±0,01 (0,56-0,61), width 0,54±0,01 (0,52-0,56) with nine festoons. A pair of campaniform "sensillum" (SC) on the postero-lateral margin at almost on the line of leg III. Setae: two pairs central dorsal (Cd_1, Cd_2); eight pairs marginal dorsal (Md_1-Md_8), Md_1, Md_2 and Md_3 are situated before the dorsal campaniform "sensilla" and Md_4-Md_8 posterior to the "sensillum" (Fig. 1), with one in each festoon; three pairs external ventral (St_1, St_2, St_3); two pairs pre-anal ventral (Pa_1 and Pa_2); four pairs pre-marginal ventral

(Pm_1-Pm_3); five pairs marginal ventral (Mv_1-Mv_5) (Fig. 2). Ventrally, three pairs of campaniform "sensilla" are present: one pair situated on the lateral margin, external to coxa I, two pairs posterior to coxae II and III, respectivelly.

Presence of parallel and small spur rows (Fig. 1), starting at the podosoma and ending at the opisthosoma, a pair with two rows ending at the third festoon in each side and a sequence with two files ending at the central festoon, reaching the scutal basis, seven spurs on the anterior margin of the second festoon in each side. Podosoma length 0,30±0,01 (0,28-0,33), which is situated the dorsal subtriangular scutum (fig.1), wider than longer, length 0,30±0,01 (0,28±0,31) and width 0,45±0,01 (0,44-0,46), with many regularly distributed fine punctuations small eyes, pale and flat; short cervical grooves lightly marked, three pairs of scutal seta (Sc_1, Sc_2, Sc_3), two pairs of scutal "sensilla" in this region: a pair postero-lateral internal, a pair situated laterally to the scutal setae (Sc_1) and cervical grooves. Presence of others "sensilla" dorsally distributed on the idiosoma, with different shapes, some are small and round and others campaniform type, more specifically situated on the podosoma and opisthosoma and also has a campaniform "sensillum" on the posterior margin. Opisthosoma length 0,29±0,01 (0,27-0,31), where is situated the anal opening in the central region, with an anal seta in each valvule (A_1) (Fig. 2). Ventrally, four pairs of external "sensilla" on the idiosoma: a pair laterally to St_1; a pair between St_1 and St_2 and a pair between St_2 and St_3; and other distributed near setae marginal ventral (Mv), pre-anal and anal.

Gnathosoma: quadrangular basis, length of palpus apex to the posterior margin 0,22±0,01 (0,19-0,23) breadth 0,18±0,01 (0,17-0,19) longer than large. Dorsally with posterior margin in a straight line, absent cornua, absence of dermal "sensilla", presence of a pair of porosae "sensillum" (Fig. 1) situated axially in the capitulum basis; ventrally the capitulum basis has a round shape. Short palpi (fig.3a,b), length from the apex of the tibiotarsal segment to the posterior margin of trochanter 0,14±0,01 (0,12-0,16), trochanter length 0,02±0,005 (0,02-0,03), femur length 0,06±0,005 (0,06-0,07), genus length 0,04±0,004 (0,03-0,05) and the tibiotarsal length 0,02±0,003 (0,02-0,03); the biggest segment is the femural one and the smallest are the trochanter and the tibiotarsal. Number of palpi seta (Fig. 3a,b): eleven on the tibiotarsal segment, of which seven are terminal (Ttt_1-Ttt_7); two antiaxial (Tta_1, Tta_2); two paraxial (Ttp_1, Ttp_2); eight on the genu; three paraxial (Gp_1, Gp_2, Gp_3), one antiaxial (Ga_1) three dorsal (Gd_1, Gd_2, Gd_3), one ventral (Gv_1); six femural: two paraxial (Fp_1, Fp_2), one antiaxial (Fa_1); one dorsal (Fd_1), two ventral (Fv_1, Fv_2), none on the trochanter; We are proposig a denomination for three paraxial setae on the palpi, two on the genu (Gp_2 and Gp_3) and other one on the femur (Fp_3) segments. Chelicerae lenght from the digits apex to the posterior margin of the line of the first palpal articule (trochanter) 0,14±0,01 (0,12-0,16), with eight denticle rows on the cheliceral sheath and eighteen denticles in each file, digits with three teeth (Fig. 4a), one external, fixed and small, one medium and mobile and one internal, fixed and big; spatulated and heterodonte hypostome (Fig. 4b), length from the apex to the line of the post-hyposthomal setae (Ph) (Fig.4b) 0,12±0,01 (0,11-0,13), dentition formula 2/2, with seven teeth on the antiaxial files and six on the paraxial ones (Fig.4b), few denticles on the basal portion and three files with three denticles on the apical crown (3/3); one pair of post-hypostomal setae (Ph_1) (Fig.4b). Palpi and chelicerae of the same size, and bigger than hyposthoma. Podosoma longer than opisthosoma, opisthosoma almost once and a half longer than the gnathosoma (Fig.1,2).

Leg I: (Fig. 6a,b) long, length from the coxa apex to the margin of tarsal claws 0,84±0,02 (0,81-0,86); coxa with a pointed spur and presence of three ventral setae, one big anterior (cIa), one posterior (cIp) and one paraxial (cIpa) (Fig. 6b). Trochanter without spur. Number of setae on the others segments (Fig.6a,b): trochanter: Dorsal: one seta in the group dorsal I ($TrdI_1$). - Ventral: one seta ventral I ($TrvI_1$). - Lateral anterior: one in the group TrlaI ($TrlaI_1$) and two setae in the group lateral posterior ($TrlpI_1$, $TrlpI_2$). Femur: Dorsal - one seta in group dorsal I (FdI_1) and one in group FdII ($FdII_1$) and presence of a femural "sensillum" near FdI_1 (Fig.6a). - Ventral (Fig.6b): two ventral I (FvI_1, FvI_2) and one ventral II ($FvII_1$). - Lateral anterior: two in group FlaI ($FlaI_1$, $FlaI_2$), two in group FlaII ($FlaII_1$, $FlaII_2$). - Lateral posterior: one in group FlpI ($FlpI_1$). Genu: Dorsal (Fig.6a)- presence of a genal "sensillum" on the central portion, two setae in group dorsal I (GdI_1, GdI_2) and one in group II ($GdII_1$). - Ventral: two ventral I (GvI_1, GvI_2). Presence of one seta in lateral anterior group I ($GlaI_1$) and one in lateral posterior group GlpI ($GlpI_1$) and II ($GlpII_1$). Tibia: two dorsal I ($TidI_1$, $TidI_2$) and one dorsal II ($TidII_1$). - Ventral: two ventral I ($TivI_1$, $TivI_2$). Lateral anterior: one in group TilaI ($TilaI_1$). - Lateral posterior: one in group TilpI ($TilpI_1$). Tarsus (Fig. 5a,b) lenght 0,22±0,01 (0,21-0,23) and claws 0,06±0,01 (0,04-0,08). Tarsal seta (Fig. 5a): Dorsal - two in dorsal group I ($TadI_1$, $TadI_2$) one behind the other, five in dorsal group II ($TadII_1$ - $TadII_5$), two in dorsal group III ($TadIII_1$, $TadIII_2$), IV ($TadIV_1$, $TadIV_2$) and VI ($TadVI_1$, $TadVI_2$), absence of seta in dorsal group V. - Ventral: two setae in ventral groups I ($TavI_1$, $TavI_2$), II ($TavII_1$, $TavII_2$) and ventral III ($TavIII_1$, $TavIII_2$). - Lateral anterior: two setae in group TalaI ($TalaI_1$, $TalaI_2$) and three in group TalaII ($TalaII_1$-$TalaII_3$). - Lateral posterior: two in group TalpI ($TalpI_1$, $TalpI_2$) and three in group TalpII ($TalpII_1$-$TalpII_3$). In the distal chamber of Haller's organ (group of setae TadII) was observed the presence of five setae (Fig. 5a) of different sizes. The seta $TadII_1$, was different from the others because was twice longer then the others, one medium size ($TadII_2$) and the others small

(TadII$_3$-TadII$_3$). Inside the Halleral chamber it was also verified the presence of three setae: two antiaxial are long and the other paraxial short (Fig. 5a).

Leg II: (Fig.6c,d) Length 0,72±0,01 (0,70-0,74), coxa with a short spur, round and not pointed and two setae, one anterior (cIIa) and one posterior (cIIp) (Fig. 6d). Trochanter: Dorsal - two setae in dorsal groups I (TrdI$_1$, TrdI$_2$). - Ventral: one ventral seta I (TrvI$_1$). Absence of setae in lateral anterior group and one seta posterior group I (TrlpI$_1$). Femur: one seta in dorsal group I (FdI$_1$) and one in dorsal group II (FdII$_1$). - Ventral: two ventral I (FvI$_1$, FvI$_2$) and one ventral II (FvII$_1$). - Lateral anterior: two in group FlaI (FlaI$_1$, FlaI$_2$) and one in the group FlaII (FlaII$_1$). - Lateral posterior: one serrated seta in groups FlpI (FlpI$_1$) and FlpII (FlpII$_1$). Genu: Dorsal (Fig. 6c) - one seta in dorsal groups I (GdI$_1$) and II (GdII$_1$). - Ventral: two ventral setae I (GvI$_1$, GvI$_2$). - Lateral anterior: two in group GlaI (GlaI$_1$, GlaI$_2$). - Lateral posterior: one serrated seta in groups GlpI (GlpI$_1$) and GlpII (GlpII$_1$). Tibia: dorsal - absence of seta in dorsal group I and only one seta in dorsal group II (TidII$_1$). - Ventral: two ventral I (TivI$_1$, TivI$_2$). - Lateral anterior: two in group TilaI (TilaI$_1$, TilaI$_2$). - Lateral posterior: one in group TilpI (TilpI$_1$). Tarsus (Fig. 6c): Dorsal -absence of setae in groups I, II and IV, one in group III (TadIII$_1$) one dorsal V (TadV$_1$) and one dorsal VI (TadVI$_1$). - Ventral (Fig. 6d): two in groups ventral I (TavI$_1$, TavI$_2$) and II (TavII$_1$, TavII$_2$) and one in groups ventral IV (TavIV$_1$) and V (TavV$_1$). - Lateral anterior: two in lateral group TalaI (TalaI$_1$, TalaI$_2$) and three in group TalaII (TalaII$_1$ - TalaII$_3$). - Lateral posterior: two in group TalpI (TalpI$_1$, TalpI$_2$).

Leg III: (Fig. 6e,f) Length 0,74±0,02 (0,70-0,77), coxa with a short spur, rounded, not pointed and two setae, one anterior (cIIIa) and one posterior (cIIIp) (Fig. 6f). Trochanter: Presence of one setae in group dorsal I (TrdI$_1$) - Ventral: one in groups ventral I (TrvI$_1$) and II (TrvII$_1$). - Lateral anterior: one in group TrlaI (TrlaI$_1$). Femur: one seta in dorsal groups I (FdI$_1$) and II (FdII$_1$) (Fig.6e). - Ventral: two ventral I (FvI$_1$, FvI$_2$) and one in group II (FvII$_1$). - Lateral anterior: two in group FlaI (FlaI$_1$, FlaI$_2$) and one serrated in group FlaII (FlaII$_1$). - Lateral posterior: one in group FlpI (FlpI$_1$) (Fig. 6f). Genu: Dorsal - two in dorsal group I (GdI$_1$, GdI$_2$) and one dorsal II (GdII$_1$). - Ventral: two ventral setae I (GvI$_1$, GvI$_2$). - Lateral anterior: one in group GlaII (GlaII$_1$). - Lateral posterior: two in group GlpI (GlpI$_1$,GlpI$_2$). Tibia: Dorsal - one dorsal I (TidI$_1$) and one II (TidII$_1$). - Ventral (Fig. 6f): two ventral I (TivI$_1$, TivI$_2$). - Lateral anterior: one in group TilaI (TilaI$_1$) - Lateral posterior: one in group TilpI (TilpI$_1$). Tarsus (Fig. 6e,f): absence of setae in dorsal groups I, II, IV and V, one in dorsal groups III (TadIII$_1$) and VI (TadVI$_1$). - Ventral: two ventral I (TavI$_1$, TavI$_2$), II (TavII$_1$, TavII$_2$), two in ventral groups IV (TavIV$_1$, TavIV$_2$). - Lateral anterior: two in group TalaI (TalaI$_1$, TalaI$_2$) and four in group TalaII (TalaII$_1$- TalaII$_4$). - Lateral posterior: two in groups TalpI (TalpI$_1$, TalpI$_2$).

The first leg is longer than the others and leg III longer than leg II (Fig. 6a,c,e).

The chaetotaxy of leg segments related to the number of dorsal and ventral setae were distributed as follows: leg I - 32 dorsal and 30 ventral (Fig. 6a,b); leg II - 22 dorsal and 24 ventral (Fig. 6c,d); leg III - 20 dorsal and 25 ventral (Fig. 6c,f).

The arrangment formula of dorsal setae of tarsus I is 1:1:2:2:2:2 (Fig. 5a).

DISCUSSION

There is a lack in the literature of data related to the tick larvae, but for *Anocentor nitens* larva some parameters obtained were similar to those described for the genera *Dermacentor* and *Amblyomma* by CLIFFORD & ANASTOS (1960), for the species *A. cajennense* by FAMADAS (1993) and *A. nodosum*, *A. dissimile*, *A. varium* by AMORIM & SERRA-FREIRE (1994a-c) and *A. rotundatum* by AMORIM & SERRA-FREIRE (1995).

The idiosoma chaetotaxy in immature forms, was studied as proposed by CLIFFORD & ANASTOS (1960) for larvae of the genus *Amblyomma*, and was verified that in *Anocentor nitens* the number of setae is constant, as was observed by CLIFFORD & ANASTOS (1960) for *Dermacentor variabilis*, FAMADAS (1993) for *A. cajennense*, AMORIM & SERRA-FREIRE (1994a-c) for *A. nodosum*, *A. dissimile*, and *A. varium* and AMORIM & SERRA-FREIRE (1995) for *A. rotundatum*. The difference was related to the position of marginal dorsal setae (Md$_1$, Md$_2$, Md$_3$). The chaetotaxy of legs I, II and III for *A. nitens* larvae was reported for the first time and the same methodology for larvae of five species of the genus *Amblyomma* (AMORIM, 1994), based on the description of HESS & VLIMANT (1982, 1983a,b) was used.

WOOLEY (1988), showed the importance of leg chaetotaxy in the identification and classification of mites species. In larvae of *A. nitens* this parameter was analysed, showing a marked difference related to the larvae of species of the genus *Amblyomma* (AMORIM & SERRA-FREIRE, 1994a-c). This authors have emphasized that this variation helps in the identification of larval species of *Amblyomma* genus.

The chaetotaxic pattern of tarsus I for *A. nitens* larvae was described as suggested by CLIFFORD & ANASTOS (1960) for the family Ixodidae, and the arragement formula of setae on the dorsal surface of tarsus I and its disposition can be expressed by the formula 1:1:2:2:2:2 (Fig. 5a). CLIFFORD & ANASTOS (1960) based on a specimen of

A. nitens (Neumann, 1897) found this formula that was proved through the observation of 100 specimens of *A. nitens*.

Comparing to other species of diferent genera in Ixodidae, it was observed that in *A. cajennence* (FAMADAS, 1993), *A. nodosum*, *A. dissimile* and *A. varium* (AMORIM & SERRA-FREIRE, 1994a-c) .and *A. rotundatum* (AMORIM & SERRA-FREIRE, 1995) the formula is 2:2:2:2:2, in *Dermacentor variabilis* (CLIFFORD & ANASTOS, 1960) is 1:1:2:2:2:2 agreeing with the observed pattern for *A. nitens*; however these setae in the genus *Ixodes*, were also observed by CLIFFORD & ANASTOS (1960), and the formula is 4:2:2. It can be concluded that the setae arrangement on tarsus I can be used as a parameter for generic separation, as well as for specific determination.

Comparing the groups of setae TadI, TadII, TadIII, TadIV and TadVI in *A. nitens* larvae and from other genera, was verified that the chaetotaxic structure in groups of setae TadIII, TadIV and TadVI remained the same, but TadI and TadII were different from one genus to another. In *A. variegatum* one seta was observed in group TadI by HESS & VLIMANT (1983b), similar to what occurred in *A. nitens*, and two setae in this group in *A. cajennense* by FAMADAS (1993), *A. nodosum A. dissimile*, and *A. varium* by AMORIM & SERRA-FREIRE (1994a-c), and *A. rotundatum* by AMORIM & SERRA-FREIRE (1995). However, related to TadII, in *A. cajennense* was observed the presence of seven setae ($TadII_1$-TadII$_7$) in distal cavity (FAMADAS, 1993), in *A. nodosum* (AMORIM & SERRA-FREIRE, 1994a) four setae were observed ($TadII_1$-$TadII_4$), and for *A. nitens* were observed five setae ($TadII_1$-$TadII_5$) in this group similarly what was reported for *A. dissimile* and *A. varium* AMORIM & SERRA-FREIRE, (1994b,c), and *A. rotundatum* AMORIM & SERRA-FREIRE (1995).

Other important chaetotaxic attribute is the palpal setae that show a great variation among the species of this genus, for example the variation of seta number on the tibiotarsal, genu and femur articule. It was observed in *A. nitens* eleven setae on this articule, differently for the genus *Amblyomma* with 12 setae in *A. cajennense* (FAMADAS, 1993), seven setae in *A. rotundatum* (AMORIM & SERRA-FREIRE, 1995) and 10 setae in *A. varium*, *A. dissimile* and *A. nodosum* (AMORIM & SERRA-FREIRE, 1994a-c) and *A. parvum* (GUGLIELMONE *et alii*, 1990). CLIFFORD & ANASTOS (1960) observed that some larval species of the genus *Ixodes* have 12 setae and others 13 setae on the palpus, showing that this variation can help in the identification of species in one genus. Related to the number of post-hyposthomal setae these authors reported that they are important to classify species of larvae of the genus *Ixodes* , what was confirmed by MARQUEZ *et alii*. (1992) also for the genus *Ixodes*. It was observed for *A. nitens* larvae a pair of setae, similarly to *Dermacentor variabilis* (CLIFFORD & ANASTOS, 1960) and to the pattern for the genus *Amblyomma* , based on the studies for *A. cajennense* (FAMADAS, 1993), *A. nodosum*, *A. dissimile*, and *A. varium* (AMORIM & SERRA-FREIRE, 1994a-c), *A. rotundatum* (AMORIM & SERRA-FREIRE, 1995) and *A. parvum* (GUGLIELMONE *et. alii*., 1990).

There is a proposal to denominate two setae on the genu and other one on the femur segments of the palpi. In as much as there is a difference in the setae pattern of *Amblyomma* larvae, reported in *A. cajennense* by FAMADAS (1993), in *A. rotundatum* by AMORIM & SERRA-FREIRE (1995) and *A. nodosum*, *A. varium* and *A. dissimile* by AMORIM & SERRA-FREIRE (1994a-c). This morphological parameter can be used in a taxonomic key for the specific diagnosis in the respective genus.

Examining the Haller's organ of *A. nitens* larva, setae were observed and their position inside the halleral chamber as well as the presence of three setae was verified. AMORIM & SERRA-FREIRE (1994a-c) analysing larvae of the genus *Amblyomma*, have also observed three setae in *A. nodosum*, two in *A. varium*, and *A. dissimile*, two in *A. rotundatum* AMORIM & SERRA-FREIRE (1995) and AMORIM (1994) has verified four setae in *A. cajennense*. This variation can help in the identification of species in the genus, agreeing with AMORIM & SERRA-FREIRE (1994a-c) that have included it as a chaetotaxic parameter for the specific diagnosis of larvae.

Related to the idiosomal chaetotaxy of *A. nitens* larvae it was verified that the number and distribution of setae, were constant agreeing with the results of CLIFFORD & ANASTOS (1960) for *Dermacentor variabilis*, differently for the genus *Amblyomma* in relation to setae distribuition, as dorsal marginal setae (Md_1 and Md_2) are anterior to the campaniform "sensilla" and Md_3 is postero-lateral internal to this "sensillum". This fact was observed by FAMADAS (1993) for *A. cajennense*, AMORIM & SERRA-FREIRE (1994a-c) for *A. nodosum*, *A. dissimile*, and *A. varium*, and AMORIM & SERRA-FREIRE (1995) for *A. rotundatum*. The attributes related to number of denticle rows and number of denticle in a row on the chaeliceral sheath and on the apical crown of *A. nitens*, showed specific particularities that had not been mentioned in the literature yet.

The observation of eight denticle and eighteen denticle in a row on the chaeliceral sheath of immature forms was different to the adult form. SERRA-FREIRE & BARROS (1992) reported that there is a variation related to this parameter for adult forms of *A. nitens*.

AMORIM (1994) observed for *Amblyomma cajennense*, *A. dissimile*, *A. rotundatum* and *A. varium* eight denticle rows and six rows for *A. nodosum*, thought the number of denticles in a row was variable, making possible the diagnostic separation of species in a genus.

Other important factor is related to the apical crown of *A. nitens* larvae, described for the first time: it was verified that the number of rows as well as the number of denticle are constant, agreeing with AMORIM & SERRA-FREIRE (1994a-c) that described four species of the genus *Amblyomma* with three rows with three denticles, except for *A. rotundatum* with two rows with three denticles. The dentition formula 2/2 of ixodids larvae were described by several authors (FONSECA & ARAGÃO, 1952; CLIFFORD & ANASTOS, 1960; GUGLIELMONE *et alii*., 1990; FAMADAS, 1993, AMORIM & SERRA-FREIRE, 1994a-c, AMORIM & SERRA-FREIRE, 1995), though a variable number of teeth on the paraxial and antiaxial rows of *A. nitens*, was recorded agreeing with the number of teeth in a row, in larvae of the genus *Amblyomma*. In *A. dissimile*, *A. nodosum* and *A. varium* AMORIM & SERRA-FREIRE (1994a-c), and *A. rotundatum* AMORIM & SERRA-FREIRE (1995) reported six teeth in the antiaxial row and five in the paraxial row, FAMADAS (1993) observed five teeth in two rows of *A. cajennense* and GUGLIELMONE *et alii*., (1990) described five and six teeth in each row of *A. parvum*. AMORIM & SERRA-FREIRE (1994a-c, 1995) have shown that this parameter and the dentition formula have to be better investigated in relation to other species of ixodid larvae, as CLIFFORD & ANASTOS (1960) and MARQUEZ *et alii* (1992) reported the dentition formula 2/2 and 3/3 for the genus *Ixodes*.

The campaniform "sensilla" have already been mentioned on the dorsal and ventral surface of tick larvae of Ixodidae by CLIFFORD & ANASTOS (1960), FAMADAS (1993) and AMORIM & SERRA-FREIRE (1994a-c, 1995), except for the genus *Ixodes* where they are not present (CLIFFORD & ANASTOS, 1960 and MARQUEZ *et alii*., 1992). In *A. nitens* was observed eight sensorial structures distributed on the idiosoma, similar to that recorded by CLIFFORD & ANASTOS (1960) for *Dermacentor variabilis*, BRINTON *et alii*., (1965) for *D. andersoni*, *D. halli*, *D. occidentalis* and *D. parumapertus*; FAMADAS (1993) observed in *Amblyomma cajennense* eight sensorial structures and one more pair on the fifth festoon in each side. This distribution was also observed by AMORIM & SERRA-FREIRE (1994a-c, 1995) for, making this a pattern for the genus *Amblyomma*.

Another interesting point was the presence of serrated setae on dorsal face in the second and third legs of *A. nitens*. AMORIM & SERRA-FREIRE (1994a-c, 1995) have observed serrated setae on dorsal and ventral surfaces of the legs of *Amblyomma nodosum*, *A. varium*, *A. dissimile* and *A. rotundatum*. This aspect have to be better investigated in relation to its function in ixodids.

Related to the scutum, was used the formula proposed by FONSECA & ARAGÃO (1952) for females of the genus *Amblyomma* and also by AMORIM & SERRA-FREIRE (1994a-c, 1995) for larval species of the genus *Amblyomma*, and the lines reported by these authors to express the length (postero-basal line-PB) and the width (transversal line-TT) of larval scutum of *A. nitens* were studied. It was observed that the shape of dorsal scutum is subtriangular, like the larvae of *Amblyomma nodosum*, *A. dissimile*, *A. varium* and, *A. rotundatum* as was mentioned by AMORIM & SERRA-FREIRE (1994a-c, 1995).

The gnathosoma basis of *A. nitens* larva is quadrangular, differently to the larvae of the genus *Amblyomma* as was observed by AMORIM & SERRA-FREIRE (1994a-c, 1995) for *A. nodosum*, *A. dissimile*, *A. varium* and *A. rotundatum* that have triangular shape.

The presence of a spur parallel to the podosoma at the end of opisthosoma of *A. nitens* as well as seven spurs at the second festoon in each side, called attention. The localization and the number of spurs in each side of festoons change from one genus to another. AMORIM & SERRA-FREIRE (1994a-c) observed five spurs at the first and fourth festoons of *Amblyomma varium* and also five spurs at the fourth festoon on each side of *A. nodosum*. These variations can serve as basis for the generic and specific diagnosis to separate morphologically the larval stages and use them in the key of identification.

The relation between the hypostomal, palpal and chaelicerae length revealed that in *A. nitens* larvae there was a clear tendency that the hypostome was smaller than palpi and chaelicerae.

The relation between podosoma, opisthosoma and gnathosoma length was reported for the first time for *A. nitens*, demonstrating the gnathosoma to be smaller than the other two regions.

Another analysed aspect was the relation between the length of the four palpal segments in *A. nitens*, that was also reported for the first time, showing that the tibiotarsal segment was smaller than the others, agreeing with what was observed for larvae of *Amblyomma nodosum*, *A. rotundatum*, *A. dissimile*, *A. varium* (AMORIM & SERRA-FREIRE, 1994a-c, 1995), and *A. cajennense* (AMORIM, 1994).

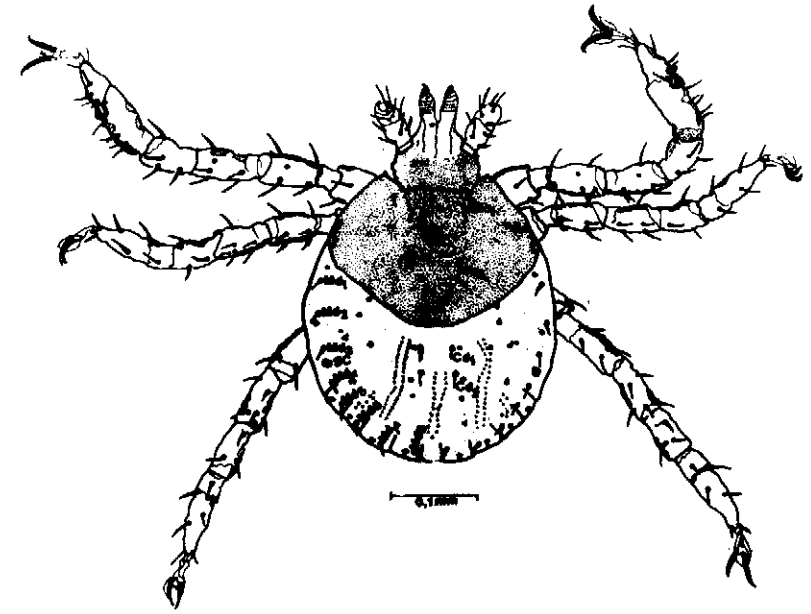
Related to the length of tarsal claws of *A. nitens* larvae, it was observed that this parameter compared to larvae of the genus *Amblyomma* (AMORIM & SERRA-FREIRE, 1994a-c, 1995) shows variation that can help the generic and specific diagnostic was well as the taxonomic key.

Based on the morphological studies of larvae diagnostic characters for *Anocentor nitens* were determined:

- The chaetotaxy of idiosoma, gnathosoma and legs is important for the identification and classification of *A. nitens* larvae.

- The arrangement formula of tarsus I is an attribute that allows the diagnostic of species.

Fig. 1. *Anocentor nitens* larvae: view of dorsal surface.



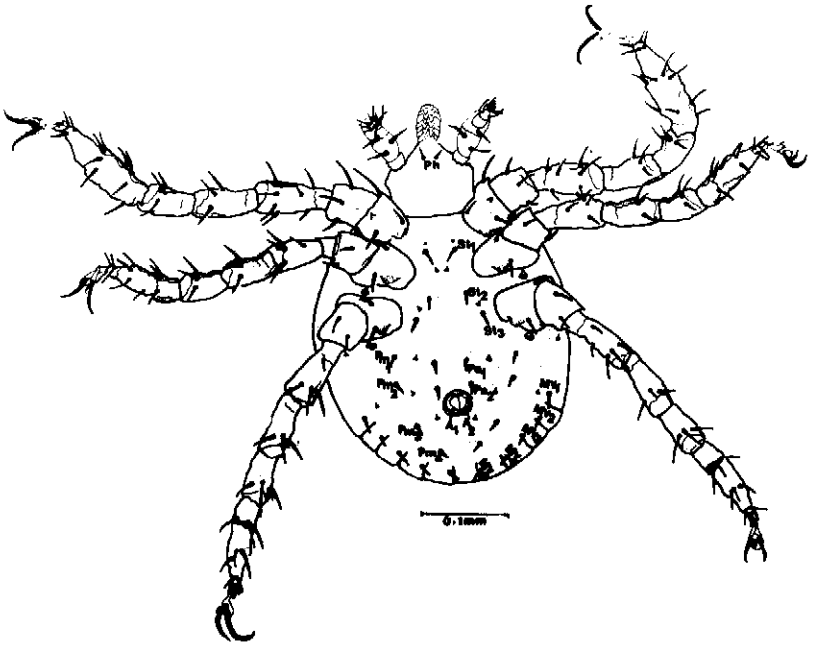


Fig. 2. *Anocentor nitens* larvae: view of ventral surface.

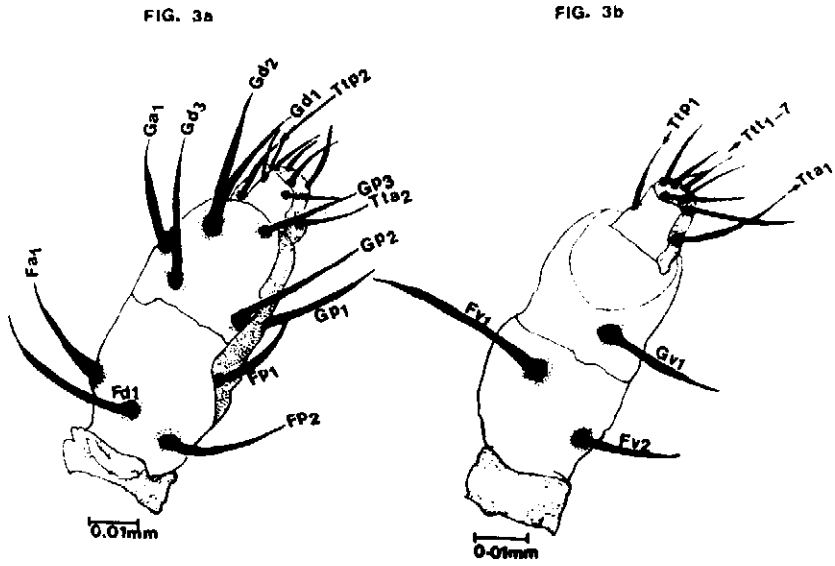


Fig. 3. *Anocentor nitens* larvae: palpi - a : dorsal b : ventral.

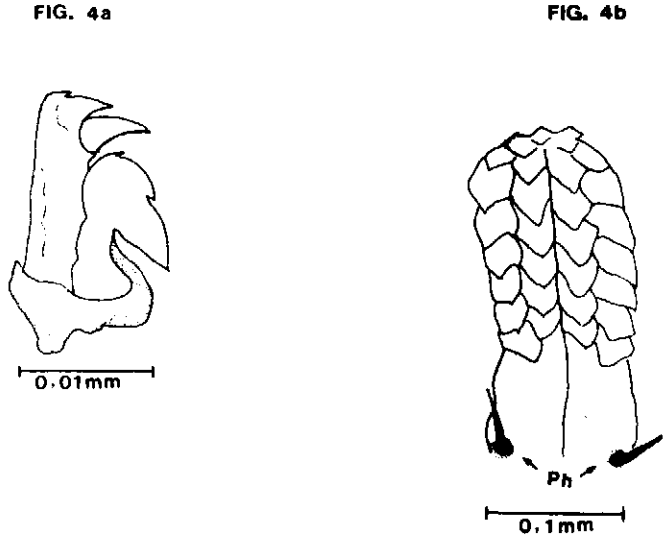
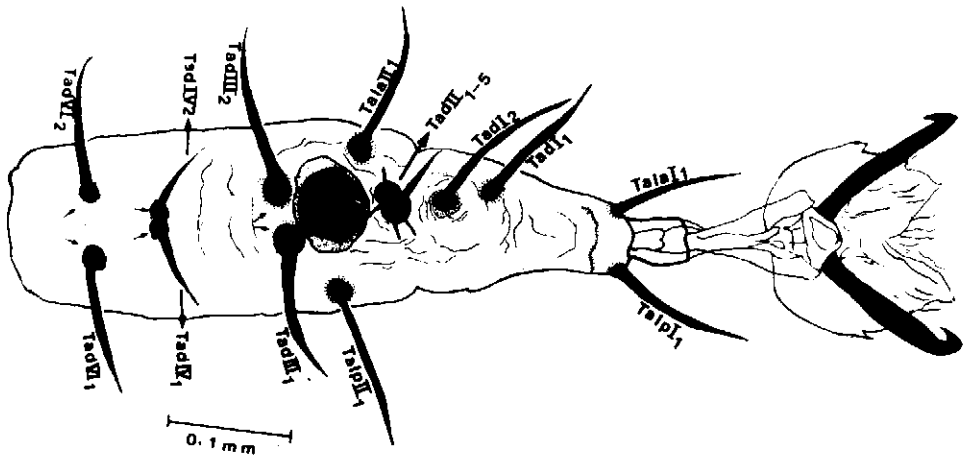


Fig. 4. *Anocentor nitens* larvae: Number of teeth - a: Cholicerae of digits b: Hyposthome

Fig. 5a. *Anocentor nitens* larvae: Tarsus I - dorsal.



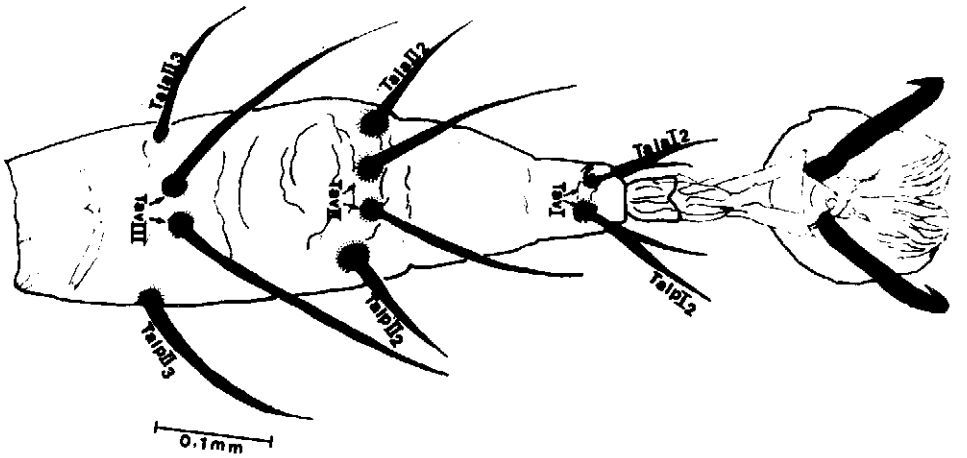
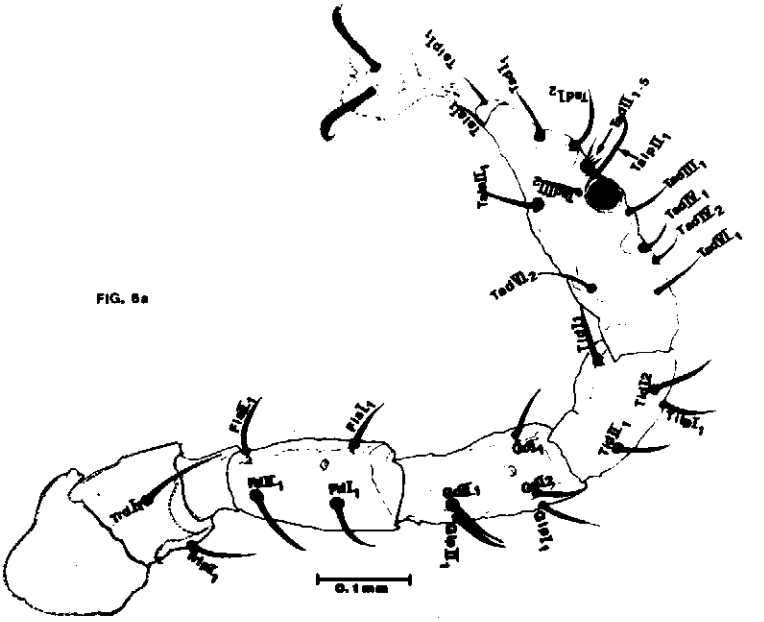


Fig. 5b. *Anocentor nitens* larvae: Tarsus I - ventral.

Fig. 6a. *Anocentor nitens* larvae chaetotaxy: Leg I - Dorsal.



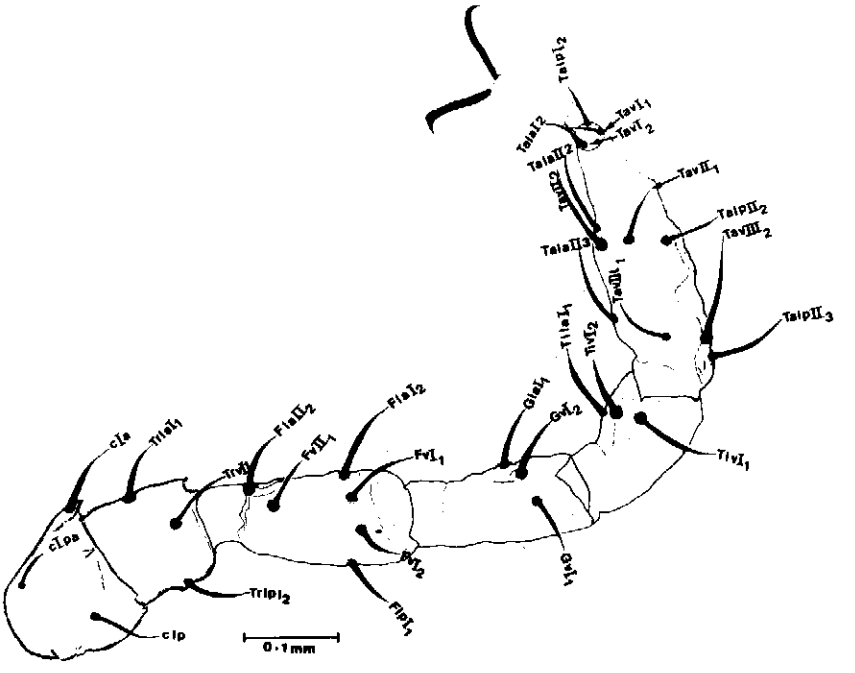
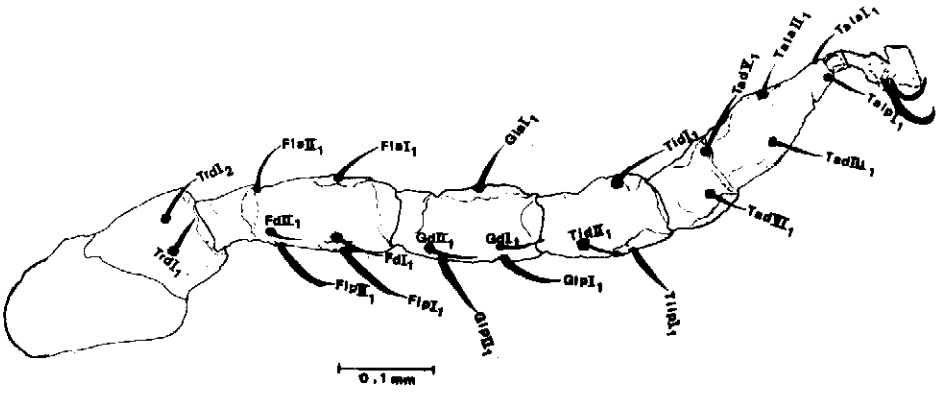


Fig. 6b. *Anocentor nitens* larvae chaetotaxy: Leg I -ventral.

Fig. 6c. *Anocentor nitens* larvae chaetotaxy: Leg II - Dorsal.



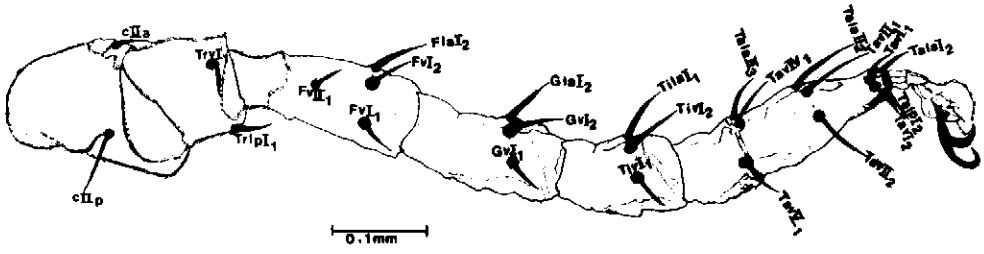
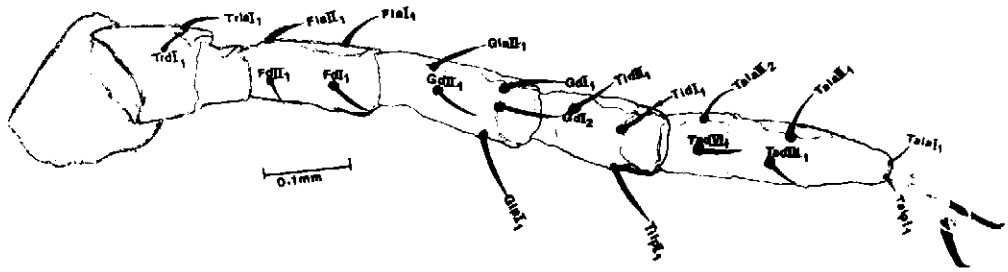


Fig. 6d. *Anocentor nitens* larvae chaetotaxy: Leg II - ventral.

Fig. 6e. *Anocentor nitens* larvae chaetotaxy: Leg III - Dorsal.



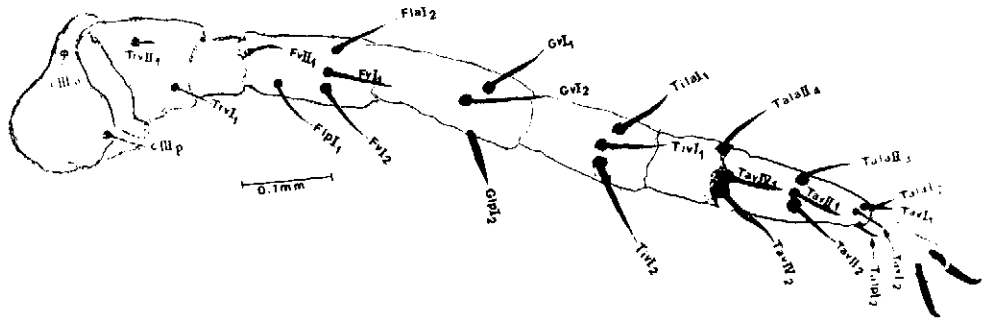


Fig. 6f. *Anocentor nitens* larvae chaetotaxy: Leg III - ventral.

- The gnathosomal basis is a differencial character that can help in the separation of one genus.

- The occurrence of nine festoons on idiosoma and three marginal dorsal setae anterior to "campaniform sensillum" in each side, characterizes the species.

SUMÁRIO

Teleóginas de *Anocentor nitens* foram removidas de *Equus caballus* L. da Universidade Federal Rural do Rio de Janeiro, delas foram obtidas posturas que em condições de laboratório originaram as neolarvas. Estas foram sacrificadas com água aquecida a ± 70°C, 24 horas após a eclosão e ainda em jejum, sendo preservadas em etanol 70° GL. As neolarvas foram processadas segundo técnica de rotina no Laboratório de Morfofisiologia e Patogenia de Carrapatos (LMPC - DPA - IB - UFRRJ) para a preparação em montagem definitiva entre lâmina e lamínula. Cem larvas foram estudadas por microscopia óptica analisando-se caracteres e atributos. Com o auxílio de microscópio estéreoscópico também foram analisados a coloração da neolarva e o padrão de ornamentação que contribuiram na diagnose de larva dessa espécie.
PALAVRAS-CHAVE: *Anocentor nitens*, morfologia, quetotaxia, larva de carrapato.

REFERENCES

AMORIM, M. (1994). Análise Morfológica de Larvas do Gênero *Amblyomma* para Diagnose de Cinco *Espécies*. Tese de Mestrado. Universidade Federal Rural do Rio de Janeiro, Itaguaí, RJ. Brasil. 104pp.

AMORIM, M. & SERRA FREIRE, N. M. (1994a). Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae) 2. *Amblyomma nodosum*, Neumann, 1899. *Revista Brasileira de Parasitologia. Veterinária 3 (2):* 131-142.

AMORIM, M. & SERRA FREIRE, N. M. (1994b). Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae) 4. *Amblyomma dissimile*. Koch, 1844. *Boletim Museu Paraense Emílio Goeldi, série Zoologia 10 (2)*: 273-288.

AMORIM, M. & SERRA FREIRE, N. M. (1994c). Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae) 3. *Amblyomma varium*, Koch, 1844. *Revista Entomologia y Vectores (no prelo)*

AMORIM, M. & SERRA FREIRE, N. M. (1995). Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae) 1. *Amblyomma rotundatum*, Koch 1844. *Revista Parasitologia al Dia 19 (1-2):* 9-19.

ARAGÃO, H. B. (1944). IN FLOUCH, H. & ABONNENC, E. (1945). Ixodidae de La Guaduloupe. Presence de *Dermacentor nitens* Neumann, 1897. *Institut Pasteur de La Guyane et du territoire de L' Imin. Publication ho 118:*1-5.

ARAGÃO, H.B. & FONSECA, F. (1953). Notas de Ixodologia VII. *Otocentor nitens* (Neumann, 1897) versus *Anocentor columbianus* (Schulze, 1937) e comentários sobre a rápida disseminação deste Ixodídeo no Brasil (Acari: Ixodidae). *Memórias do Instituto Oswaldo Cruz 51*: 499-501.

ARAGÃO, H.B. & FONSECA, F. (1961). Notas de Ixodidae VIII. Lista e chave para os representantes da Fauna Ixodológica Brasileira. *Memórias do Instituto Oswaldo Cruz 59(2):* 115-129.

ARAGÃO, H. B. (1944). IN FLOUCH, H. & ABONNENC, E. (1945). Ixodidae de La Guaduloupe. Presence de *Dermacentor nitens* Neumann, 1897. *Institut Pasteur de La Guyane et du territoire de L' Imin. Publication ho 118:*1-5.

ARAGÃO, H.B. & FONSECA, F. (1953). Notas de Ixodologia VII. *Otocentor nitens* (Neumann, 1897) versus *Anocentor columbianus* (Schulze, 1937) e comentários sobre a rápida disseminação deste Ixodídeo no Brasil (Acari: Ixodidae). *Memórias do Instituto Oswaldo Cruz 51*: 499-501.

ARAGÃO, H.B. & FONSECA, F. (1961). Notas de Ixodidae VIII. Lista e chave para os representantes da Fauna Ixodológica Brasileira. *Memórias do Instituto Oswaldo Cruz 59(2):* 115-129.

BRINTON, E.P., BECK, D.E. & ALLRED, D.M. (1965). Identification of the adults, nymphs and larvae of ticks of the genus *Dermacentor* Koch (Ixodidae) in the Western United States. *Brigham Young University Science Bulletin - Biological Series, 5(4): 1-44.*

CLIFFORD, C.M. & ANASTOS, G. (1960). The use of chaetotaxy on the identification of larval ticks (Acarina: Ixodidae). *Journal Parasitology 46:*567-578.

COOLEY, R.H.X. (1938). The Genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States with studies on variations. *National Institut Health Bulletin (171):* 65-68.

DAEMON, E. & SERRA FREIRE, N.M. (1987). Efeitos do parasitismo em bovinos sobre a biologia da fase n_o parasitária de *Anocentor nitens* (Neumann, 1897) (Acarina:Ixodidae). *Revista Brasileira Medicina Veterinária 9 (2)*: 42-47.

DIKMANS, G. (1945). Check list of the internal and external parasites of domestic animals in North America. *American Journal Veterinary Research* 6 (21): 211-241.

DRUMMOND, T.M.; WHETSTONE, T.M.; ERNST, S.E. & GLADNEY, W.J. (1969). Laboratory study of *Anocentor nitens* (Neumann, 1897) (Acarina: Ixodidae), the tropical horse tick. *Journal of Medical Entomology, 6 (2):* 150-154.

DUNN, LH. (1915). Observations on the preoviposition, oviposition and incubation periods of *Dermacentor nitens in Panamá* (Arach., Acar.) *Entomological News, 26:* 214-219.

FAMADAS, K.M. (1993). Morfologia de *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), estágios de larva e adultos: estudos com microscopia óptica e eletrônica de varredura. Tese de Doutorado. Universidade Federal Rural do Rio Janeiro, Itaguaí, RJ. Brasil. 86pp.

FLECHTMANN, C.H.W. (1985). *Ácaros de importância Médico Veterinária*. 3. ed. São Paulo, Nobel, Brasil, 192pp.

FONSECA, F. & ARAGÃO, H.B. (1952). Notas de Ixodologia. II. Uma nova espécie do gênero *Amblyomma* e uma nova espécie do gênero *Ixodes* (Acari: Ixodidae). *Memórias do Instituto Oswaldo Cruz, 50*: 713-729.

FORRESTER, D.; CONTI, J.A. & BELDEN, R.C. (1985). Parasites of the Florida Panter (*Felis concolor coryi*). *Procedings Helminthological Society of Washington, 52 (1):* 95-97.

FOX, I. & LEON, D. DE. (1983). The tropical horse tick and the Southern cattle tick in Puerto Rico. *Journal of Agricultural University of Puerto R.ico. 67 (1):* 57-59.

GUGLIELMONE, A.A.; MANGOLD, J.A. & KEIRANS, E.J. (1990). Redescription of the male and female of *Amblyomma parvum* Aragão, 1908. and description of the nymph and larva. and description of all stages of *Amblyomma pseudoparvum* sp. n. (Acari: Ixodida: Ixodidae). *Acarologia.* t. XXXI, fasc. 2:143-159.

HESS, E. & VLIMANT, M. (1982). The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae. Metastriatta). I. Wall pore and terminal pore sensilla. *Revisté Suisse Zoologie, 89*(3): 713-729.

HESS, E. & VLIMANT, M. (1983a). The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae. Metastriatta). II. No pore sensilla. *Revisté Suisse Zoologie, 90(1):* 157-167.

HESS, E. & VLIMANT, M. (1983b). The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae. Metastriatta). III. Mapping of sensory hairs and evolution of the relative importance of sensory modalities during post-embrionic development. *Revisté Suisse Zoologie., 90(4)*: 887-897.

HOOKER, W.A.; BISHOPP, F.C. & WOOD, H.P. (1912). The life history and bionomics of the some North American ticks. *Bulletin Bureau of Entomology, 6:* 117-123.

MALHEIRO, D.M. (1952). Sobre a ocorrência de *Otocentor nitens* (Neumann, 1897), COOLEY, 1938, Acari - Ixodidae - Ixodidae, em cavalos do Estado de São Paulo, Minas Gerais e Mato Grosso, Brasil, *Revista da Faculdade de Medicina Veterinária, USP, 4* (4): 532-535.

MARQUES, E. J.; MOREL, P. C.; GUIGEN, C. E. T. & BEAUCOURNU, J. C. (1992). Clé dichotomique des ixodidae D'Europe. I. Les larves du genre *Ixodes*. *Acarologia,* XXXIII, 4: 325-330.

MORENO, E. C. (1984). Incidência de ixodideos em bovinos de leite e prevalência em animais domésticos da região metalurgica de Minas Gerais. Tese de Mestrado, Universidade Federal de Minas Gerais, Belo Horinzonte, 105 pp.

ROBY, T. D. & ANTHONY, D. W. (1963). Transmission of equine piroplamosis by *Dermacentor nitens* Neumann. *Journal American Veterinary Medicine Association., 142 (7):* 768-769.

ROCHA, U. F.; SERRA, O. P.; GROCK, R. & SERRA, R. G. (1969). Infestação natural de búfalos- *Bubalus bubalis* L., 1758 - Dos Estados de São Paulo e de Minas Gerais, Brasil, por *Boophilus microplus* (Canestrini, 1887) e por *Anocentor nitens* (Neumann, 1897), Acari, Ixodidae. *Arquivos do Instituto Biológico de São Paulo, 36 (4):* 197-199.

ROCHA, J.M. (1986). Identificação e Incidência dos Ixodideos no município de Garanhus-PE. *Arquivos Brasileiro de Medicina Veterinária e Zootecnia, 38(5):*831-838.

SERRA FREIRE, N.M. (1982). Ixodideos parasitas de bovinos leiteiros na Zona fisiográfica do município de Resende, Estado do Rio de Janeiro. *Revista Brasileira de Medicina Veterinária, 5(3):* 18-20.

SERRA FREIRE, N.M. & BARROS, R.V., (1992). Estudo Morfológico do gnatossoma de adultos de *Anocentor nitens* (Neumann, 1897) Schulze, 1937. *Semina, 13(1):*24-31.

SERRA FREIRE, N.M. & MIZIARA, S.R. (1989). Influência do hospedeiro no ciclo e comprovação do ciclo heteroxeno de *Anocentor nitens* (Neumann, 1897). *Memórias do Instituto Oswaldo Cruz, 84 (4)*: 213-218.

SERRA FREIRE, N.M.; BONILHA, P.C.; CAIAFFA, R.M.; GAZÊTA, G.S. & CAVALCANTI, P.L. (1990). Avaliação da Disponibilidade de estádios Não-Parasitários de Ixodideos em Pastagem Submetidas ao Pastejo Contínuo por Bovinos. *Arquivos da Universidade Federal Rural do Rio de Janeiro, Itaguaí, 13 (1):* 37-43.

SOUZA LOPES, H. & MACEDO, J.M. (1950). Sobre a ocorrência de *"Otocentor nitens"* (Neumann, 1897) no Vale do Rio São Francisco, Brasil (Acarina: Ixodidade). *Revista Brasileira de Biologia, 10 (1):* 59-64.

WOOLEY, T.A. (1988). *Acarology-mites and Human Welfore*. A Wiley Interscience Publication. Wiley, J. & Sons Ed., New York. 484pp.

YUNKER, C.E.; KEIRANS, J.E.; CLIFFORD, C.M. & EASTON, E.R. (1986). *Dermacentor* ticks (Acari: Ixodidea: Ixodidae) of the New World. A Scanning electron microscopy. *Atlas Proceding Entomological Society of Washington, 88 (4):* 609-627.

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