The Pulvillus: Cuticular Structure and Function (Acarina: Ixodida)

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ABSTRACT

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The pulvillus in five ixodid ticks is a large, globular structure that has a thick well-sclerotized dorsal cuticle and a thin, flexible and pleated ventral cuticle. Only an endocuticle and a very thin epicuticle are found in the ventral pleated portion of the pulvillus. The pulvillus is similar in morphology and structure in all post-embryonic stages and no distinct sexual dimorphism is apparent in any of the five species. The central lumen of the pulvillus contains lipid and lipid compounds that are secreted on to the surface on which the tick walks. The material probably has adhesive properties that enable ticks to crawl along and up various kinds of surfaces. If the ventral pulvillar surface is coated, ticks are unable to crawl up a surface in order to reach a potential host.

Key words: tick, pulvillus, ultrastructure, cuticle, function, secretion

INTRODUCTION

The pretarsal area on the legs of ixodid ticks is composed of an articulating ambulacrum which has a basal sclerite, a pair of claws and a large, membranous pulvillus. The pulvillus is greatly reduced in most argasid ticks. This portion of the leg has many different functions such as habitat selection and distribution within a particular habitat (Pugh *et al.*, 1981a, b) and phylogenetic inferences on host-parasite evolution in myobiid mites (Paran, 1992). Atyeo (1978) presented a comparative study of the pretarsi of astigmatid mites and concluded that the various well-known divisions of the parasitic astigmatids based on host preferences must be reexamined.

Pretarsal structure is well known in many different groups of insects such as roaches (Roth and Willis, 1952), thrips (Heming, 1971), flies (Bauchhenss and Renner, 1977; Walker *et al.*, 1985) and aphids (Kennedy, 1986; Lees and Hardie, 1988). The chemicals and physical forces that are involved in adhesion to a surface are known for a few species of insects and crustaceans (Yule and Crisp, 1983; Yule and Walker, 1984; Walker and Yule, 1984; Walker *et al.*, 1985; Lees and Hardie, 1988), but very little is known about surface adhesion in Acari. The present study provides ultrastructure and behavioral

information on the functional morphology of pulvillar area of hard ticks (Ixodida: Ixodidae).

MATERIALS AND METHODS

Specimens of the following tick species were used, *Amblyomma americanum* (L.), *Boophilus annulatus* (Say), *Dermacentor variabilis* (Say), *Ixodes scapularis* (Say) and *Rhipicephalus sanguineus* (Latreille).

Transmission Electron Microscopy (TEM). Legs from adult and immatures were fixed in 3% glutaraldehyde and 3% para-formaldehyde in 0.1 M phosphate buffer, pH 7.2 at 4°C for 12 H. Specimens were washed in buffer then post-fixed in 2% osmium tetroxide in the same buffer, pH 7.2 at 4°C for 4 H. After dehydration in a graded series of ethanol, the specimens were infiltrated and embedded in Spurr's low viscosity resin. Thin sections which were placed on formvar coated copper grids, were stained with ethanoic uranyl acetate and lead citrate. One group of thin sections was stained for lipid using the procedure of Seligman *et al.* (1966). The sections were examined with a JEOL CX100-II TEM at 60 kV.

Scanning Electron Microscopy (SEM). The same fixation and dehydration procedures that were used in TEM were utilized in SEM. Specimens were dried in hexamethyldisilazane (HMDS) (Nation 1983) and mounted on aluminum stubs with double sided carbon tape. The legs were coated with gold-palladium and examined with a Cambridge 360S SEM at 20 kV.

Behavioral Experiment. Twenty adult *Amblyomma americanum* from Dorman Lake and Noxubee Wildlife Refuge, Mississippi, USA, were placed at the bottom of a 10 cm plexiglass cylinder with a closed bottom and a white mouse in a screen cage was placed on top. One group did not have painted tarsi with insulating varnish (control), and the other group had painted tarsi (Red GLPT Insulating Varnish, GC Electronics, Rockford, Illinois, USA). The cylinder was examined thirty minutes later and the number of ticks found in the top 2 cm of the cylinder were recorded. This was repeated four times for a total of eighty ticks in each of two groups.

Pulvillar Secretion. Ten acid-cleaned, round (12 mm) coverslips were placed in the bottom of three petri dishes (5 cm in diameter) and five adult ticks (*Amblyomma americanum*) were placed in each dish and then covered. The ticks were allowed to walk on the surface of the coverslips and walls of the petri dishes for one hour. Then the ticks were removed, and coverslips were examined with a phase-contrast microscope to see if the ticks left any kind of material on the surface of the coverslip. These coverslips then were subjected to various solvents such as acetone and hexane which are lipid solvents and two stains, Sudan III and oil red O which are considered as lipid stains.

RESULTS

There are no major differences in the external structure of the claws and pulvillus

that are found on the larval, nymphal and adult stages in a particular species of hard tick (Figs. 1, 2, 3) or between stages of the different species (Figs. 1, 3, 4, 6). The only noticeable difference is the size of the pulvillus and claws that become larger with each successive stage (Figs. 1, 2, 3). There is also slight change in shape of the pulvillus, but this may be due to the fixation and dehydration process that is used in SEM. The claws are situated on the dorsal surface of the pulvillus and have a relatively smooth surface (Figs. 1, 2). They are distinctly hooked in all stages and the basal sclerites and attachment area of the claws are morphologically similar in each stage and in each species studied (Figs. 1, 2, 3). No differences between males and females could be detected.

The dorsal and ventral surfaces of the pulvillus are structurally very distinct (Figs. 1, 3, 4, 6). Two, smooth, cuticular plates which cover the lateral and proximal portions of the dorsal surface function as the attachment site for the claw (Figs. 1, 2). A v-shaped portion of the distal and dorsal surface is composed of flexible cuticle that is deeply plicated (Figs. 1, 2, 3), and the distal edge has secondary pleats that give the distal margin a serrated appearance (Figs. 2, 3). The ventral pulvillar surface consists of the long, cuticular folds that may run the entire length of the pulvillus (Figs. 4, 6). When at rest, the folds or pleats are packed closely together such as in Figure 5 where the pleats range from about $1.4\mu\text{m}$ to $2.0\mu\text{m}$ wide, and the space between the pleats is less than one micron.

The secondary pleating that occurs at the distal end of the pulvillus resembles finger-like projections (Fig. 7). Several ticks, which were vapor-fixed with osmium tetroxide and then air-dried so that the effects conventional processing would be avoided, have a particulate film covering the cuticle of the pleats (Fig. 7). The material is evenly distributed over the ventral surface of the pulvillus.

Internally, the pulvillus consists of a large, central area that is occupied by a substance which contains many lipid droplets (as indicated by the staining method of Seligman *et al.*, 1966, which is a procedure for the detection of lipid and lipid compounds) (Fig. 8). This central region is surrounded ventrally and laterally by the pleated cuticle and dorsally by the cuticle of the pulvillar sclerites (Figs. 8, 9). The sclerite cuticle is $0.7\text{--}1.1\mu\text{m}$ thick (Fig. 9) and has many pore canals, whereas the cuticle of the ventral pleats is $0.3\text{--}0.6\mu\text{m}$ thick (Fig. 8). The cuticle of the anterior distal end of the pulvillus (the secondary distal finger-like projections) is $0.6\text{--}1.0\mu\text{m}$ thick (Fig. 9). The ventral cuticle of the pulvillus does not exhibit any clearly defined divisions such as epicuticle, exocuticle and endocuticle, and numerous distinct canals such as those in the pulvillar sclerite cuticle also could not be located. The cuticle of the tick pulvillus appears to be endocuticle with little or no modifications except for a very thin epicuticle.

In the behavioral experiment the ticks that had their pulvilli painted could not climb up the plexiglass tube to the host animal. After four complete replications (20 ticks/replication) 95% (76 out of 80) of the unpainted ticks could reach the host versus 3.75% (3 out of 80) of the ticks with painted pulvilli. Those ticks with painted pulvilli would drop easily from the wall of the cylinder with the slightest movement of the plexiglass

Fig. 1. Leg I of *Amblyomma americanum* larva, dorsal surface of pulvillus showing the claws (C) sclerites (S), and pleated cuticle (PC). Sclae bar = $2\mu\text{m}$.

Fig. 2. Leg I of *A. americanum* nymph, dorsal surface of pulvillus. Sclae bar = $20\mu\text{m}$.

Fig. 3. Leg I of *A. americanum* female, dorsal surface of pulvillus. Scale bar = $50\mu\text{m}$.

Fig. 4. Leg III of *Boophilus annulatus* female, ventral surface of pulvillus. Scale bar = $50\mu\text{m}$.

Fig. 5. Higher magnification of Fig. 4 showing the pleated ventral cuticle of the pulvillus. Scale bar = $2\mu\text{m}$.

Fig. 6. Leg II of *Ixodes scapularis* larva, ventral surface of the pulvillus. Scale bar = $20\mu\text{m}$.

Fig. 7. Higher magnification of Fig. 6 showing cuticular pleats on the ventral surface of the pulvillus that have a particulate material covering the surface of the pleats (arrow heads) and the distal secondary pleats (SP). Scale bar = $2\mu\text{m}$.

Fig. 8. TEM micrograph of leg I of *Rhipicephalus sanguineus* nymph showing the ventral pleated cuticle (PC) surrounding a large lumen (L) that is filled with secretory substance. Sclae bar= $1.5\mu\text{m}$. Inset: Higher magnification of the cuticle making up the ventral pleats. Scale bar= $0.07\mu\text{m}$.

Fig. 9. TEM micrograph of leg I of *Dermacentor variabilis* female showing the cuticle of the ventral pleats (VP) at the distal edge of the pulvillus, pore canal (PC) and the dorso-lateral sclerites (DS). Scale bar= $1\mu\text{m}$.

tube, and others could not even start up the cylinder.

Several of the coverslips on which the ticks walked had a cluster of oily droplets that varied in size and were insoluble in water. The droplets were easily dissolved in acetone and hexane, and faintly stain with Sudan III and oil red O. The droplets on one of the coverslips did not evaporate after 24 hours of exposure to the air. The droplets also were preserved on the ventral surface of the pulvillus as shown in Fig. 7.

DISCUSSION

The large, pleated pulvillus that is found in all post-embryonic stages of the ticks in the various genera reported in this paper is probably characteristic for all of the hard ticks in Ixodida. Presently, our knowledge of pulvillar structure of each embryonic stage in a given species in any acarine group is deficient, and in those mites that have been studied in detail to date the above mentioned type of pulvillus does not exist (Grandjean, 1941, 1943, 1952; Atyeo, 1978; Pugh *et al.*, 1987a, b; Paran, 1992; Baker, unpublished data). The next step would be to examine the pulvillus of argasid larvae and the post-embryonic states of Nuttalliellidae in order to establish whether this type of pulvillus is characteristic for all of Ixodida. It would also be of interest to look at species in the Holothyrida, which is considered a sister group to the Ixodida.

An increase in size of the pulvillus is the only change that occurs from one post-embryonic stage to the next, which is similar in nymphs and adults of hemimetabolous insects (Slifer, 1950; Roth and Willis, 1952). No distinct sexual dimorphism exists in the species examined, which is also similar in most hemimetabolous insects (Slifer, 1950; Roth and Willis, 1952; Kennedy, 1986).

The ventral surface of the pulvillus in many insects exhibits a very wide variety of surface modifications. It may be smooth (Sarkaria and Patton, 1949; Slifer, 1950; Kennedy, 1986) or possess highly modified setae or microtrichia as found in some Hemiptera, Diptera and Hymenoptera (Grillet and Wigglesworth, 1932; Lewis, 1955; Bauchhenss and Renner, 1977; Walker *et al.*, 1985; Conde-Boytel *et al.*, 1989). The above-mentioned modifications are known to aid in adhesion to a particular surface. Primary and secondary plications probably serve a similar function in ticks especially when they adhere to the surface of different types of vegetation while waiting for a suitable host. The composition of the pleated cuticle which is endocuticle and epicuticle, is similar to what is known about the pulvillar cuticle of insects (Slifer, 1950; Roth and Willis, 1952; Lewis 1955), and this type of cuticle formation would allow for considerable flexibility due to the elasticity of the thick endocuticle.

The tracks or "foot-prints" of the ticks on the coverslips are considered to be a lipid compound because of the following evidence: (1) they dissolved in acetone and hexane which are lipid solvents, (2) faintly stained in two stains which are lipid specific, and (3) did not air dry which indicates they were not water based. When the pulvilli were coated with glypt-paint, the ventral pulvillar pleats with their oily surface material could not come in contact with the cylinder surface and the ticks could not reach the host. It is well documented that a non-volatile oily substance is secreted on to the pulvillar surface of many insects (Roth and Willis, 1952; Hasenfuss, 1978; Walker *et al.*, 1985; Lees and Hardie, 1988). This oily secretion with its adhesive properties plays an important role in the ability of insects and ticks to cling to a surface by maximizing adhesion forces (Walker *et al.*, 1985., Lees and Hardie, 1988).

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