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SCIENTIFIC NOTE

THE PULVILLUS AND EMPODIUM IN *CULEX QUINQUEFASCIATUS*: VISUALIZATION WITH THE LIGHT MICROSCOPE AND A STUDY OF FINE STRUCTURE WITH THE SCANNING ELECTRON MICROSCOPE

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ABSTRACT. The empodia and pulvilli on each postarsus were examined in male and female *Culex quinquefasciatus.* Up to 160× magnification was required to see them clearly under the stereo light microscope but they were more readily visible under the compound light microscope as slide mounts at 100–200× magnification. Scanning electron micrographs of female and male pulvilli showed that they are either well developed (females) or inconspicuous (males) and that they consist of setal tufts composed of both pointed and trumpet-ended setae. The ultrastructure of the pulvilli is described and their possible function in oviposition is discussed.

KEY WORDS *Culex,* pulvillus, empodium, morphology, light microscopy, scanning electron microscopy

Well-developed pulvilli are present only in *Culex* mosquitoes; other genera either do not have them (*Anopheles, Coquilletidia,* and *Mansonia*) or, if present, they are small and inconspicuous (*Aedes* and *Culiseta*) (Harbach and Knight 1980, Service 1990, Harbach and Kitching 1998). The authors of generic keys almost invariably use this character as the most distinctive one for identifying the genus (Edwards 1941, Belkin 1962, Mattingly 1973, Service 1990, Jupp 1997). In his description of the genus *Culex,* Belkin (1962) stated that "pulvilli are usually more or less distinctly developed as densely long-spiculose lobes under each claw." However, pulvilli are difficult to see under the stereo dissecting microscope when identifying mosquitoes.

The 1st purpose of the present study was to assess how readily the empodium and more particularly the pulvilli can be seen with the stereo dissecting and compound light microscopes. Second, the morphology of these characters was studied with the scanning electron microscope on both male and female *Culex quinquefasciatus* Say so as to describe their morphology and to speculate on the function of the pulvilli. The only scanning electron micrographs of the postarsi that we could find in the literature were one of *Anopheles* (Harbach and Knight 1980) to show the absence of pulvilli and another of *Culex* and *Culiseta* to show the well-developed organ and inconspicuous organ, respectively, in these 2 genera (Harbach and Kitching 1998).

The specimens of *Cx. quinquefasciatus* studied came either from field collections or were taken from a laboratory colony. With the stereo dissecting microscope, the postarsi were either examined on the intact female or male mosquito or a leg was cut proximal to the 2nd tarsomere and placed flat but posterior side up in a petri dish. A black background was used and 2 fiber optic lights with focused beams provided incidental lighting. The highest magnification was obtained by attachment of a 2× supplementary lens to the objective.

For viewing postarsi under the compound light microscope 3 categories of female specimens were used: dry pinned specimens, freshly killed mosqui-

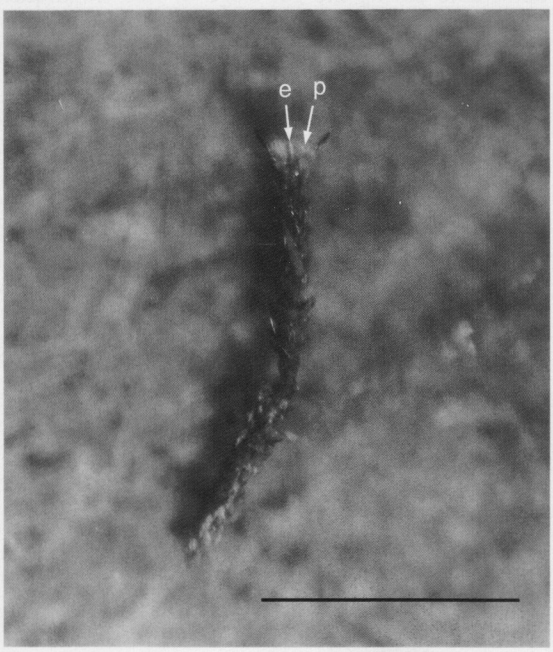

Fig. 1. Light micrograph showing posterior view of part of foretarsus from female *Culex quinquefasciatus* bearing postarsus with pulvillus (p) and empodium (e). Scale bar = 700 μm.

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Fig. 2. Posterior view of postarsus on 5th hindtarsomere from female *Culex quinquefasciatus* showing empodium (e) and pulvillus (p); each pulvillus is composed of setae with either pointed or trumpetlike endings. Particles of adhesive (artefacts) are scattered on specimen. Scale bar = 50 μm.

toes, and those that been stored for 24 years in 70% ethanol with 5% glycerine. Two methods were used to make slide mounts of the postarsi, the polyvinyl lactophenol method and the Euparal method. In the 1st method the mosquitoes were soaked in 99% ethanol for 10–30 min, then rehydrated in 50% ethanol (5 min) and distilled water (5 min) . They were then mounted in polyvinyl lactophenol under a coverslip. In the 2nd method the specimens were soaked in 99% ethanol as above but were then transferred to clove oil where the legs to be examined were excised. Each leg was then transferred to a very small drop of Euparal on a slide that was then sealed with a coverslip.

A series of electron micrographs were made of the postarsi taken from each of the legs of both female and male mosquitoes that had been pinned and stored dry for 1 month. Air-dry specimens were mounted on alloy stubs using double-sided tape. The specimens were coated with gold in a Polaron E500 vacuum coating unit (Premier Technologies, Johannesburg, South Africa) to a thickness of 10

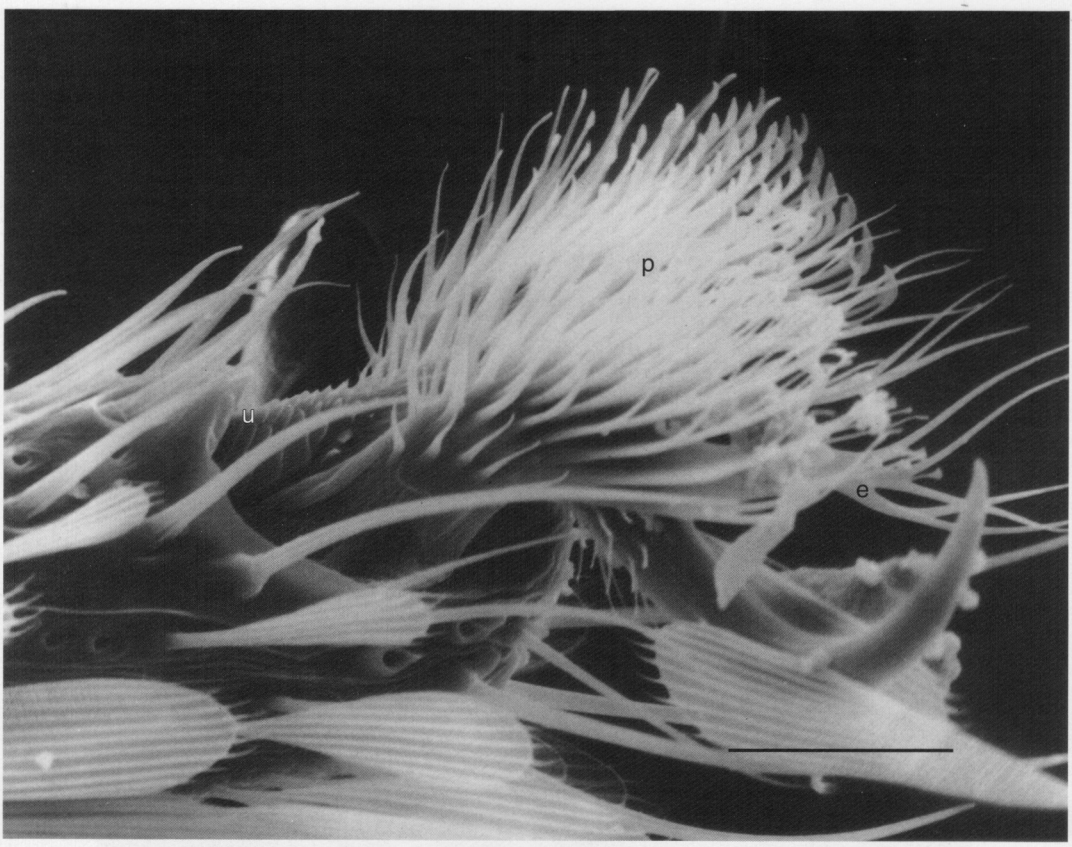

Fig. 3. Lateroposterior view of postarsus on 5th foretarsomere from female *Culex quinquefasciatus* with pulvillus (p) showing its insertion, empodium (e), and unguitractor plate (u). Scale bar = 50 μm.

μm. The specimens were examined and photographed using a JEOL JSM 25S scanning electron microscope (Premier Technologies).

Both freshly killed mosquitoes and those that were killed and pinned 21 years previously were found to be equally suitable for seeing the postarsal structures. The pulvilli and empodium were barely visible at 40× magnification and visible with difficulty at 80×. They became clearly visible at 160× but the supplementary lens attached to the objective lens to obtain this magnification drastically reduced the depth of field as well as the distance between the specimen and the objective lens, making it difficult to illuminate from above. In the female, pulvilli could be seen on all 3 pairs of legs (e.g., the foreleg as shown in Fig. 1), but in the male they could not be seen except on the forelegs, where they were just visible. The pulvilli were most easily seen on the female's forelegs and appeared as a pair of buff-colored setous pads on the posterior side below the claws; the empodium was usually more difficult to discern than the pulvilli. The inconspicuous pulvilli of *Culiseta* could not be seen under the light microscope when pinned specimens of *Culiseta longiareolata* (Macquart) were examined.

The postarsal structures were only visible in 3 of the 10 slides in which tarsomeres were mounted in polyvinyl lactophenol. Pulvilli could be seen at 100× but in order to see the empodium 200× magnification was needed. In the case of 10 slides prepared using the Euparal method, both the pulvilli and empodium were visible in all of them. Again, pulvilli were visible at 100× but 200× was required for the empodium.

Three of the scanning electron micrographs are shown (Figs. 2–4). Each pulvillus is like a brush that consists of a number of setae of varying lengths arising from a thick stalk that is inserted into a hollow at the distal end of the postarsus on each side of the unguitractor plate. On the female hindleg the pulvillus has about 40–45 setae and the posterior aspect of the pulvillus measures about 108 μm long from its point of insertion to the tip of the longest setae and about 49 μm wide at the widest point (Fig. 2). On the female foreleg in lateroposterior view the pulvillus is about 147 μm long and 95 μm high at the highest point (Fig. 3). Two kinds of setae can be seen on each pulvillus, those with pointed tips and those with trumpetlike tips; other setae seem to be intermediate between these 2 forms. Figure 3 shows the insertion of the pulvillus onto the postarsus particularly clearly to the side of

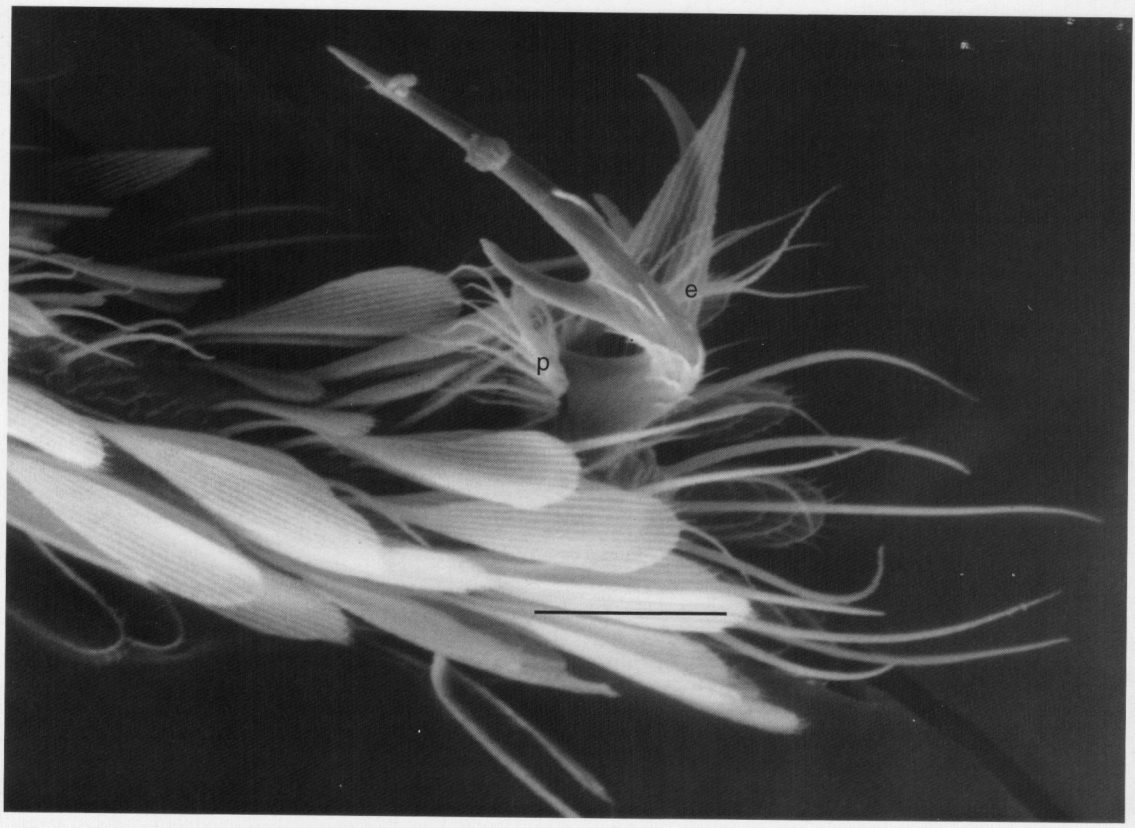

Fig. 4. Lateroposterior view of postarsus on 5th midtarsomere from male *Culex quinquefasciatus* with toothed claw, reduced pulvillus (p), and branched empodium (e). Scale bar = 50 μm.

the unguitractor plate. In the male midleg (Fig. 4), the pulvilli are very much reduced in size, as is the case on all legs of the male. The branched empodium can be readily seen in all 3 micrographs. In Fig. 2 the empodium appears to be inserted into a socket near the tip of the 5th tarsomere but careful examination of both this micrograph and the micrograph showing the lateroposterior view (Fig. 3) indicates that the true situation is as follows. In Fig. 2 an ordinary seta is inserted in the socket on the 5th tarsomere, which happens to be lying exactly above the empodium, which is actually lower down as an extension of the unguitractor plate (Harbach and Knight 1980: 12).

The pulvillus is visible only with difficulty at 80× magnification under the stereo dissecting microscope and the use of a supplementary objective lens to double this magnification is cumbersome. Furthermore, such additional lenses are not always available to the operator. Because of this, in order to identify the subgenus *Culex* it is probably best to make a Euparal slide of the distal tarsomeres from 1 foreleg so that the presence of the pulvillus can be confirmed under the compound light microscope. Noteworthy details in fine structure revealed in the scanning electron micrographs were the insertion of the pulvilli at each side of the unguitractor plate and the 2 different kinds of setae present on each pulvillus.

Because pulvilli are inconspicuous in male *Culex,* the well-developed pulvilli of females of this genus seem likely to have some function in relation to oviposition when the egg raft is deposited on the water surface. However, 2 other genera that are known to deposit their egg rafts on open water either have inconspicuous pulvilli (*Culiseta)* or none at all (*Coquilletidia*) (Service 1990, Harbach and Kitching 1998). Why don't these 2 genera also possess large pulvilli? Perhaps different types of ovipositional behavior among these 3 genera may explain this. *Culex quinquefasciatus, Cx. molestus* Forskal, and *Cx. salinarius* Coquillet stand on the water surface with their hindlegs extended behind the body so that the 5 distal tarsomeres rest on the surface during oviposition (Wallis 1954). On the other hand, *Culiseta inornata* (Williston) crosses the hindlegs at about the 2nd tarsomere to form a "V" into which the eggs are gradually placed as the raft is formed (Pappas and Pappas 1982). No observations apparently have been recorded on oviposition by *Coquilletidia,* but these mosquitoes are known to oviposit near aquatic vegetation, so they possibly stand on floating leaves while placing their rafts onto the water in a manner similar to that of

Mansonia mosquitoes (Lounibos and Linley 1987). Pulvilli probably have a sensory function in relation to placing rafts on open water in the particular behavior pattern of *Culex* and *Culiseta.* Beament and Corbet (1981) observed the final act of oviposition by *Culex pipiens* Linnaeus. When the female had added the last egg to her raft she placed 1 leg on top of the stern end, forcing the last rows of eggs down so that their corollas touched the water and opened. If present, tactile receptors in the pulvillus could be important to allow the mosquito to carry out this action. Other setae in the pulvillus possibly have a chemosensory function similar to that of setae located proximal to the pulvillus on the 5th tarsomere in *Cs. inornata* (Owen 1963) but they possibly respond to chemicals present in the water chosen for egg laying.

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