SCANNING ELECTRON MICROSCOPY OF ECHINOPHOTHIRIUS HORRIDUS (VON OLFFERS), ANTARCTOPHOTHIRUS CALLORHINI (OSBORN), AND PROECHINOPHOTHIRUS FLUCTUS (FERRIS) WITH EMPHASIS ON THE ANTENNAL STRUCTURES (ANOPLURA: ECHINOPHOTHIRIDAE)

Frederick H. Miller, Jr.

Parasitology Section, Division of Microbiology, Department of Pathology and Laboratory Medicine, Nassau County Medical Center, East Meadow, New York 11554

ABSTRACT: The antennal organs of Echinophothirus horridus and Procophothirus fluctus have 2 sensilla basiconica (previously called tuft organs) and 2 pairs of taste organs in the fourth or terminal segment. The sensilla horridus have 11 to 13 hairs protruding from a ring near the apex of the stalk, whereas those of P. fluctus have 7 primary hairs which divide into 2 or 3 branches yielding a total of approximately 17 secondary hairs. Antarctophothirus callorhini has a T-shaped antenna in the adult stage. Six sensilla basiconica and 3 gustatory organs are located on the fifth or terminal segments. The sensilla basiconica have 12 primary hairs of which 3 or 4 usually branch at their midpoint forming a total of 17 to 19 secondary hairs. The number and character of the hairs on the sensilla basiconica may lead to an understanding of the evolution of the family. The tuft organs appear to be associated with the distal sensilla basiconica in each case. The opsinumintrations and variation in the type of organ are also discussed.

Recent studies of the Anoplura by Miller (1967, 1970a, b, 1971) and of the Anoplotoidea by Clar (1970), using the scanning electron microscope (SEM), have shown that variations of the antennal sensilla can be of taxonomic value. It is possible by comparing the variations of the sensilla among groups that the evolution of the sensilla can be deduced. This would ultimately lead to an understanding of the phylogenetic relationship of the. Before undertaking a study of the phylogenetic relationships, or assigning taxonomic significance to the antenna and their sensilla, it is necessary to study the variations found within the order, family, and genus.

The family Echinophothiridae, with some 10 known species (Kim, pers. comm.), is found exclusively upon marine animals of the suborder Pinipedia. The genus Echinophothirus with the single species horridus (von Offiers) occurs on seals of the genera Halichoerus and Phoca. The genus Antarctophothirus contains six species. A. callorhini (Osborn) is found on the fur seals Callorhinus. The genus Procophothirus contains two species. P. fluctus is also found on the fur seals Callorhinus. The antennae of A. callorhini, as with most Anoplura, are incapsulated, whereas those of both E. horridus and P. fluctus are incapsulated.

MATERIALS AND METHODS

This study is based on specimens of E. horridus, 12 specimens of A. callorhini, and 12 specimens of P. fluctus. The 5 males and 7 females of E. horridus were collected from around the mouth of Prince Hugh on Campbell, Alaska, by Dr. Robert L. Branch, who also submitted several specimens to Dr. Ke-Ching Kuo for identification. The 4 males, 8 females, and 2 juveniles of A. callorhini and 4 males, 6 females, and 2 juveniles of P. fluctus were collected from Callorhinus on St. Paul Island, Alaska, and contributed to this study by Dr. Ke-Ching Kuo.

The specimens were cleared in a lactic alcohol solution (50% lactic acid, 50% isopropanol) and stored in 3 changes of 75% ethyl alcohol, air dried, and cemented to metal SEM stubs with aluminum paint. They were then coated with approximately 200 A of gold by means of vacuum evaporation.

Received for publication 3 December 1970.

Figures 1-6. Echinophothirus horridus. 1. Dorsal, dorsal view, showing original tissue, tegument, and accessory sets. 200 x. 2. Anterior, 5. Ventral, and lateral view of branch of theme of second leg. 300 x. 2. Antennal, showing terminal organs of first and second segments. 600 x. 3. Terminal antennal organ showing tuft organ (upper) and two sensilla basiconica (both). 200 x. 5. 6. Sensilla basiconica. 1,100 x.
and studied with a Cambridge Stereoscan Mark II SEM at 20 kv. The specimens are on file at the Parasitology Section, Department of Pathology, and Laboratories, Nassau County Medical Center, East Meadow, New York.

RESULTS

Echinophthirus horticus (van Oeffen)

The general description of E. horticus can be found in the monograph by Ferris (1934). A review of some of the topographical characters reveals the abdominal integument in the form of a smooth, unsegmented, oval-shaped organ. Ferris (1934) describes the abdominal integument for the entire family as membranous or leathery and the genus (Ferris, 1934) Echinophthirus as entirely membranous in both sexes except for the ninth tergum and genital plates. However, he does not indicate whether the integument is smooth or articulated. Scanning electron microscopy reveals that the abdominal integument of E. horticus has a squamous appearance (Fig. 1).

The abdome, thorax, and head are covered with numerous setae of various lengths. The long stout setae appear to be of the same type and texture as the short stout setae. The short stout setae are most common on the head, thorax, and ventral side whereas the longer setae are most common on the abdomen. There are long, slender setae scattered over the thorax and abdomen on the dorsum, similar to those on the region of the gill flaps. Ferris (1934) illustrates the setae at the dorsal side of the larvae opposite the class of each of the legs as being fingerlike and bent. However, these setae are of the short stout type (Fig. 2).

The abdomen is four-segmented and the first and second segments each have a single setae on the cephalic aspect (Fig. 3). There is a circular elevation measuring approximately 2 µm in diameter on the distal end of the first segment on its dorsal aspect which may be a companion organ. There are two squamous baguette and two pore organs on the fourth or terminal segment (Fig. 4). The squamous baguette have 11 to 23 hairs projecting from a ring, near the apex of a stalk (Figs. 5, 6). The two pore organs do not have any spines or plates and appear to be anatomically associated with the distal squamous baguette. There is no evidence of sexual dimorphism of the antennae.

Antennophthirus collarus (Dulabon)

The general description of A. collarus can be found in the monograph by Ferris (1934). A review of some of the topographical characters reveals the abdominal integument to have a squamous appearance. The abdomen is beset with setae of various lengths and sizes (Fig. 7). Scales (Fig. 8), although somewhat variable in form, are for the most part elongated, pointed, and possess irregular serrations near the apex.

The antennae are five-segmented (Fig. 9). There is a circular elevation measuring approximately 2 µm in diameter on the cephalic aspect of the first segment, which may be a companion organ. There are two squamous baguette and two pore organs on the fifth or terminal segment (Fig. 10). The squamous baguette have approximately 13 primary hairs of which at least four branch at their midpoint to form approximately 18 secondary hairs (Fig. 11). Three or four hairs appear to be upright, whereas the remaining hairs lie upon the surface of the opening and surrounding integument. The two depressions considered to be pore organs appear to be anatomically associated with the distal squamous baguette. There is no evidence of sexual dimorphism of the antennae.

Phacophthirus flavus (Ferris)

The general description of P. flavus can be found in the monograph by Ferris (1934). A review of some of the topographical structures reveals a squamous integument on the abdomen and numerous setae (Fig. 12). The legs appear similar, with the tibia and tarsus entirely...
fused (Fig. 13), and a pronounced basal lobe with three distinct pads between the tube and claw (Fig. 14).

The antenna is four-segmented. The same compound-like organ described above is found on the dorsal aspect, near the distal end of the first segment. There are two sensilla basiconica and two pore organs on the fourth (Fig. 15) or terminal segment. The sensilla basiconica have what appear to be approximately seven primary hairs which divide into two or three branches (Figs. 16-18) forming approximately 17 secondary hairs. The two pore organs appear to be associated with the dorsal sensilla basiconica. There is no evidence of sexual dimorphism of the antennae.

**DISCUSSION**

Millberg (1910) describes the ctenida of most Sphingidae (Asphina) as being sculptured. In describing the texture of the abdomen, other authors used terms such as keilhart, nematocercus, or slightly sculptured when referring to the abdomen lacking sclerotization. Fenix (1951) illustrates dental reticulations of Lepidoptera (cuff which was demonstrated by Miller (1956b) to be of squamous texture. The same squamous texture seen on the three species studied here, is found on the abdomen of the Asphina which are described as sculptured, reticulated, and many of these described as keilhart or nematocercus in the previous literature.

Wigglesworth (1941) originally described the sensilla on the fourth and fifth segments of Plectrobus species as tuft organs. He described these same organs on the fifth or terminal segment and one on the fourth. Miller (1951) demonstrated that there was only one such organ on the fifth and one on the fourth antennal segment. The pore organs were located where Wigglesworth described the other two tuft organs. However, the tuft organ was held for most studies of the Asphina. Both and Willis (1951) use the term sensilla basiconica (peg organ) when they describe a similar organ on the antennae of the Tribeniaceae species. Although some studies are needed to confirm this, both Wigglesworth (1941) and Both and Willis (1951) consider the sensilla to be humidity receptors. Therefore it seems practical to establish similar terminology for these sensilla. This would lead to a better understanding of the sensilla in question. I suggest that the tuft organs of the Asphina hereafter be referred to as sensilla basiconica.

Fenix (1951) indicates that the Asphina usually have a ring-like structure on the posterior border of each of the two terminal antennal segments. Studies by Miller (1950, 1951a, b) have shown these ring-like structures to be either sensilla basiconica or sensilla coecocrica. Clay (1950) has demonstrated sensilla coecocrica in a similar region on Amblysera. It has also been shown that a family of pores having sensilla coecocrica on their antennae have a cuticle in the form of the opening, hairs on pegs in the antennae as well as position, texture, and distinctness of pore organs associated with the sensilla (Miller, 1951; Clay, 1950). Those of the same family (Miller, 1950) having sensilla basiconica have a different number of hairs projecting from the staff of the sensilla. The present communication demonstrates this variation in Euchromaphisidae.

Although the Asphina usually have a total of five antennal segments in the adult stages, the nymphal stages usually appear to have less. In these nymphal stages two sensilla basiconica or cockroach are still present, but both are present on the terminal segment.

In these Asphina having a four-segmented antenna it therefore appears understandable that two sensilla basiconica or cockroach, depending on family, would be present on the terminal segments. Of the family Euchromaphisidae, two of the members in this study, E. harviei and P. phaleri, have four-segmented antennae in the adult stages. Two sensilla basiconica are found on each of their fourth or terminal antennal segments. Although the third member, A. caffertii, has a five-segmented antenna, it differs from most Asphina.

**FIGURES 13-18.** Plectrobus phaleri. 13. Foramen and thorns of third leg, 200 x. 14. Three pairs between holes and claws of third leg, 500 x. 15. Antenna showing two sensilla basiconica on terminal segment, 500 x. 16, 17, 18. Sensilla basiconica showing branching of primary hairs, 10,000 x.
in that the two scutella haustoria are found on the fifth antennal segment instead of one on each of the two most distal segments.

The variation of the number of haustoria referred to as pugnose projecting from the stalk of the scutella haustoria on the antennae of the Tribolumen was used by Roth and Wells (1961) to indicate the possible phylogeny of that genus. The hypothesis was based on the evolution of the haustorial scutella from the single form (least number of pugnose). They studied only six of the 16 species of Tribolium and therefore could only indicate the possibility of this hypothesis. A difference in the number of haustoria has been demonstrated on the basiconic scutella of some larvae and this may well lead to the understanding of the evolution of the genus and family. It is also possible, and the author's belief, that the scutellar haustoria evolved from the closing of the arista and elevation of the pug of the scutellar haustoria.

Thus, by use of these scutella, the phylogenetic relationships of the Acrophila and Milliphasa (Phalangida) may be demonstrated, in addition they may prove to be of taxonomic value. Studies to substantiate these possibilities are continuing as specimens become available.

ACKNOWLEDGMENTS

The author is indebted to Dr. B. L. Reischl, of the Department of Health, Education and Welfare, College, Alaska, who so graciously supplied the specimens of E. verrucosa, and to Dr. Ko Chung Kim of the Forensic Entomological Museum, Pennsylvania State University, University Park, Pennsylvania, for providing the specimens of A. calidus and P. floridanus for this study. Appreciation is expressed to Drs. John L. Duffy and Irving Abraham, of New York Medical College, for their discussions and critical review of the manuscript. I am grateful to Miss Elma Fortuna for typing and to Mr. Kenneth Walker, for aid in preparing the graphs.

LITERATURE CITED


Development of Eimeria elipsoidalis from cattle in cultured bovine cells

Hammond, S. D., and Minor (1963). J. Infect. Dis. 114: 764-772. Each cell type was used one or two times (Table 1). One cell of species containing 100,000 to 300,000 sporozoites in a seven-day culture medium was inoculated into each Leighton tube containing medium of one of the five cell types mentioned above. After 8 hr, the medium was removed by aspiration and replaced with 1.7 ml of fresh culture medium, containing 1× fetal calf serum. At 3-day intervals for 3 to 5 days, cell-free Leighton tubes were used in a similar manner. For each of the 20 stained specimens were examined with an oil immersion objective.

One-day-old inoculation, intracellular spores were 3 by 4.5 μm (4.5 to 7.5 μm by 2 to 3.5 μm) and had one to three refractile bodies about 1.5 μm in diameter (Fig. 1). In these, the nucleus was 1.5 μm in diameter and had a small, eccentric nucleus. Tophilophores were first seen at this time (Fig. 2). These were 4.8 by 4.1 μm (4.5 to 5.5 μm by 3.5 to 5.5 μm) and had one to three refractile bodies similar in size to those of the sporozoites. The nucleus and nucleolus were 2 and 0.5 μm, respectively. Some tophilophores had irregular nuclei resembling those of a stage in nucleolus division as described in E. colioporphyrinoid by Spier and Hammond (1967). I. G. R. in 10% in DMK, 1× REF, and 0.2 mg/ml of chloroquine (pH 7.5) were all effective in suppressing the development of E. elipsoidalis. 0.2 mg/ml of chloroquine (pH 7.5) were all effective in suppressing the development of E. elipsoidalis.