ULTRASTRUCTURAL STUDIES ON OLIGONEURIIDAE - TAXONOMIC APPLICATIONS

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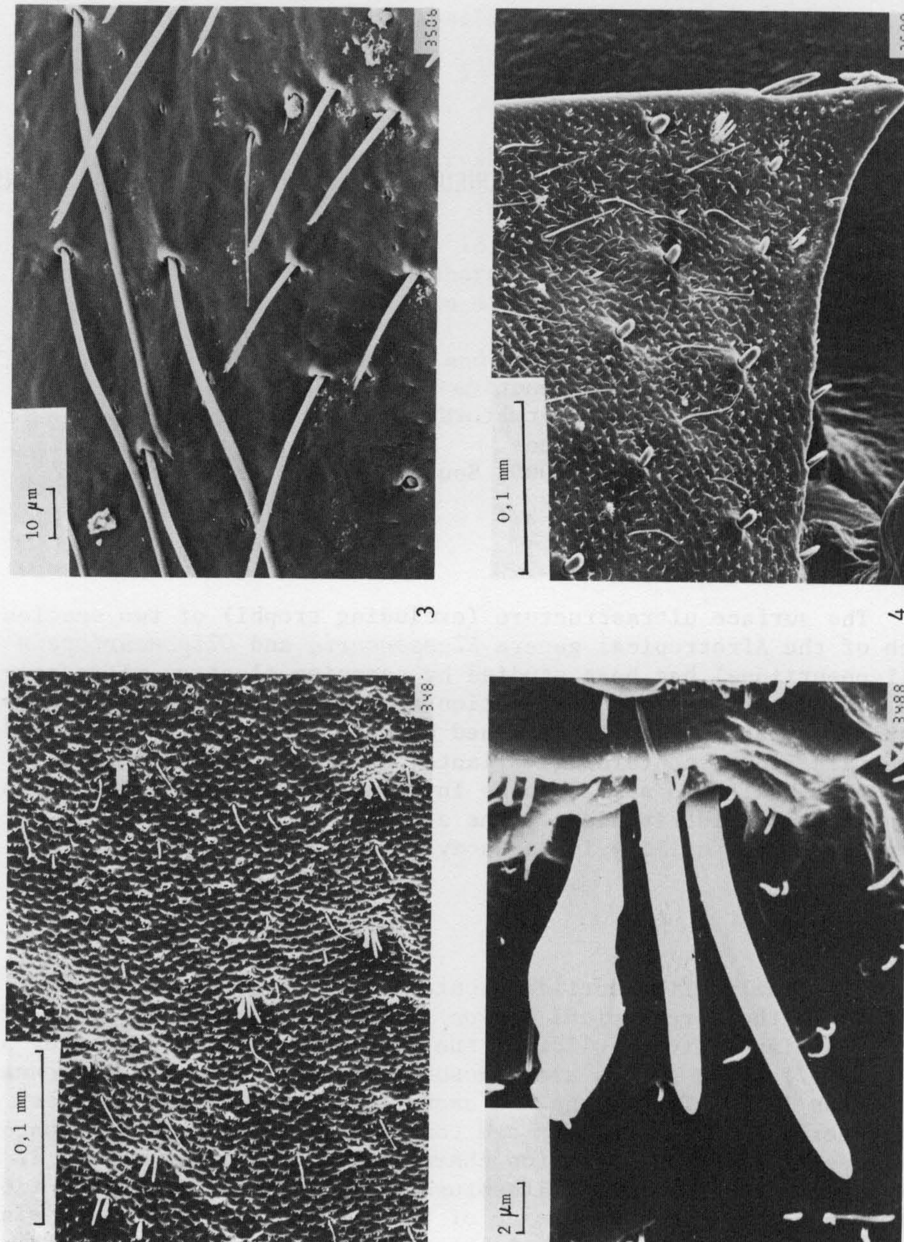
ABSTRACT

The surface ultrastructure (excluding trophi) of two species each of the Afrotropical genera *Elassoneuria* and *Oligoneuriopsis* (Oligoneuriidae) has been studied by scanning electron microscopy. New methods of specimen preparation are described; these give improved results compared to those obtained by methods currently advocated. Five main kinds, containing variants, of micro- and macrotrichia are described; some appear to be intermediate or connecting types. The taxonomic distribution of the structures within the study material is given; their utility in taxonomy is discussed.

INTRODUCTION

The family Oligoneuriidae contains, *inter alia*, two genera endemic to the Afrotropical Region (this term is used in place of the old Ethiopian Region, following the recommendations of Crosskey and White 1977). The genera are *Elassoneuria* Eaton 1881 and *Oligoneuriopsis* Crass 1947. The nymphs are among the larger mayfly species encountered in the area, and are confined to swiftly flowing waters. The general trophic adaptation characteristic of these nymphs is the development of elaborate filterbrushes on the fore femora and tibiae. Although no study has been made of the feeding mechanism in living specimens, it is probable that material (presumably particulate organic matter) is strained from the current by these brushes and is transferred by the fringed maxillae. It is also possible that the brushes are used as rakes on the substrate.



1 2 3 4

Plate 1

Other adaptations common to members of this family are the development of accessory gills of the fibrilliform or tuft type at the base of the maxillae, and protection of the lateral abdominal gill tufts by covering plates or lamellae. These plates articulate with, and seem to be derived from, the general body integument since they have the same basic surface ultrastructure as the latter, in both genera studied. This supports the old view that the gills are homologous with abdominal appendages which have been modified. Snodgrass (1935) states that there is no doubt that the gills arise from limblike rudiments of the embryo, and that their structure and musculature suggest that they are parts, at least, of true abdominal appendages.

Nymphs of the two genera may be distinguished by *Oligoneuriopsis* having a rounded frontoclypeus with no carina whereas *Elassoneuria* has a carina extending from between the antennal bases to the front of the head. The other differences are: in the adult, *Oligoneuriopsis* has 3-jointed ♂ forceps and 4 convex longitudinal veins in the forewing; *Elassoneuria* has 4-jointed forceps (sometimes with a minute extra joint) and 3 main veins. *Oligoneuriopsis* has been regarded as the more primitive of the two on adult features (Crass 1947) and is close to the European *Oligoneuriella* Ulmer, as noted by Edmunds (1961). In fact, the distinguishing feature between the last two genera in the nymphs, namely presence or absence of a lamella on the ventral gill of the first abdominal segment, seems no longer valid. I have found a lamella to be present on all mature nymphs from various parts of southern Africa, including those ascribable to the type species *O. lawrencei* Crass.

On the other hand, all *Oligoneuriopsis* nymphs have a well-developed tuft of hairs in the midline, toward the posterior border of abdominal sternites 2-4 or 2-5 inclusive. These are much better developed than those figured by Sowa (1973) for the European species *Oligoneuriella keffermuellerae* Sowa, *O. mikulskii* Sowa, *O. pallida* (Hagen) and *O. rhenana* (Imh.). The function of this tuft is undoubtedly to assist in attachment to the substrate under near-torrential conditions. In central and southern Africa, nymphs of

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Plate 1. Figures 1-4. *Oligoneuriopsis lawrencei* Crass (GEN 596A. 24 Feb. 1960. Natal: Royal Natal National Park. 28°58'E, 28°42'S)

1. Fronto-clypeal area, dorsal view.
2. Detail of 1.
3. Lateral abdominal projection, under gill lamella, dorsal view.
4. Posterolateral projection of abdominal tergum, dorsal view.

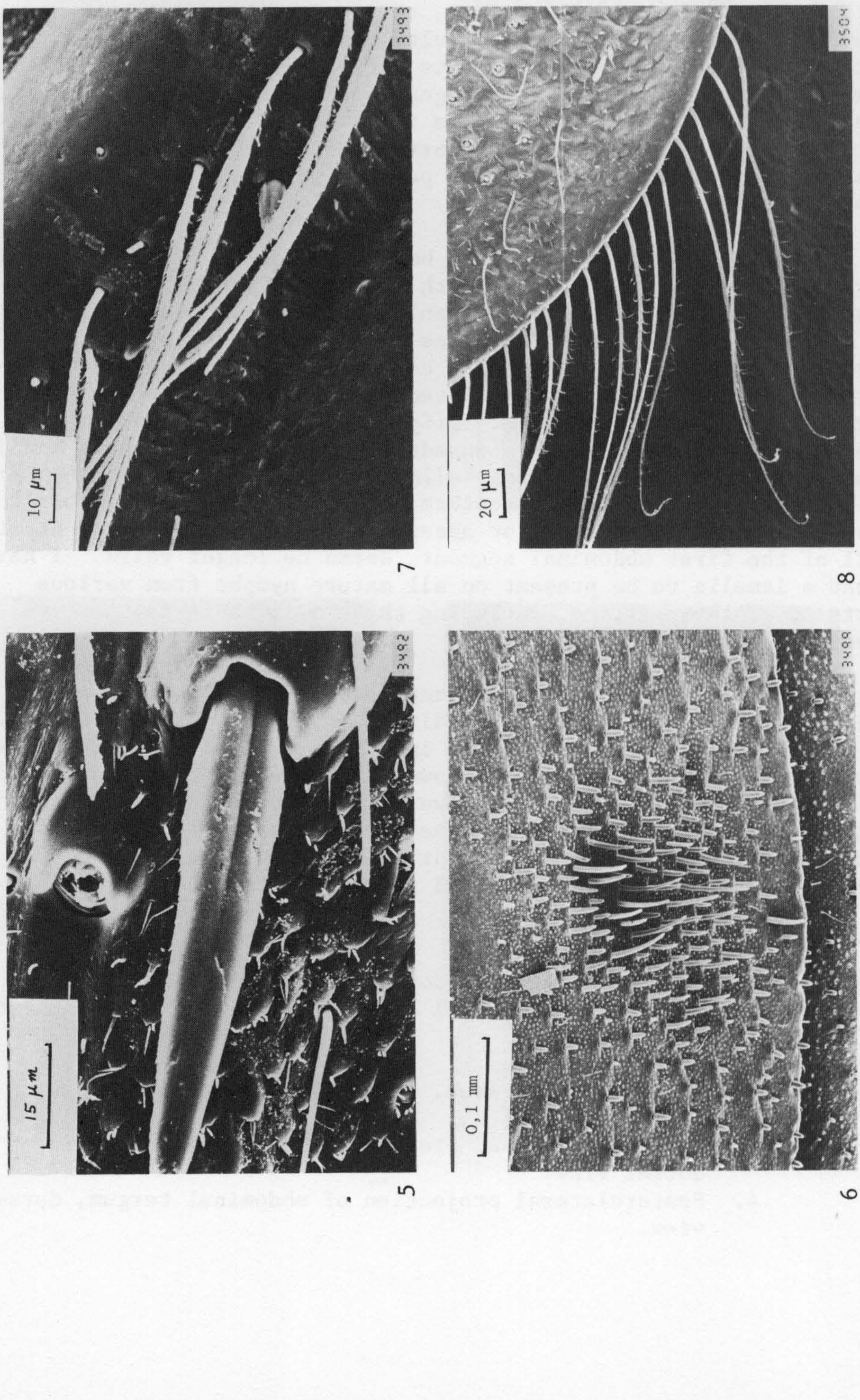

Plate 2

Oligoneuriopsis are cool-adapted, and found only at fairly high altitudes whereas those of *Elassoneuria* occur in the lower lying tropical and subtropical rivers. The requirements for cool conditions mean that the higher zones with their concomitant high current speeds must be chosen, hence the adaptive significance of the structures mentioned.

MATERIAL

The taxonomy of the nymphs, that is species discrimination, has been based largely on details of the gill lamellae. During routine examination of these structures using phase optics, it became clear that a wealth of further detail existed, which would require resolution higher than that offered by an optical system, for adequate study.

A fairly large sample of oligoneuriid nymphs belonging to both genera and collected at various localities throughout Southern Africa has been under study and these results will be reported elsewhere. Large numbers of nymphs of some species were collected and it was thus possible to prepare representative specimens for scanning electron microscopy (SEM). Where only a few individuals of a particular species were available, it was not feasible to do this because of the risk of damaging the specimen during preparation and viewing. The storage of coated specimens also presents difficulties due to their fragility, and the fact that they must be kept dry and dust-free if they are to retain their usefulness, means that one must usually discard preparations after study.

The catalogue numbers of specimens are those used by the hydrobiological survey teams of the National Institute for Water Research of the C.S.I.R. Two species of *Oligoneuriopsis*, namely *O. lawrencei* Crass and *O. elisabethae* Agnew and two as yet unnamed species of *Elassoneuria* (distinguished by their catalogue numbers) have been studied in this paper.

Plate 2. Figures 5-8. *O. lawrencei* Crass (GEN 596A. 24 Feb. 1960. Natal: Royal Natal National Park. 28°58'E, 28°42'S)

5. Detail of spination - posterior border of fore femur, dorsal view.
6. Abdominal sternite 5, posterior median area showing brush.
7. Fore femur, posterior border dorsal view.
8. Detail of inner border of gill lamella.

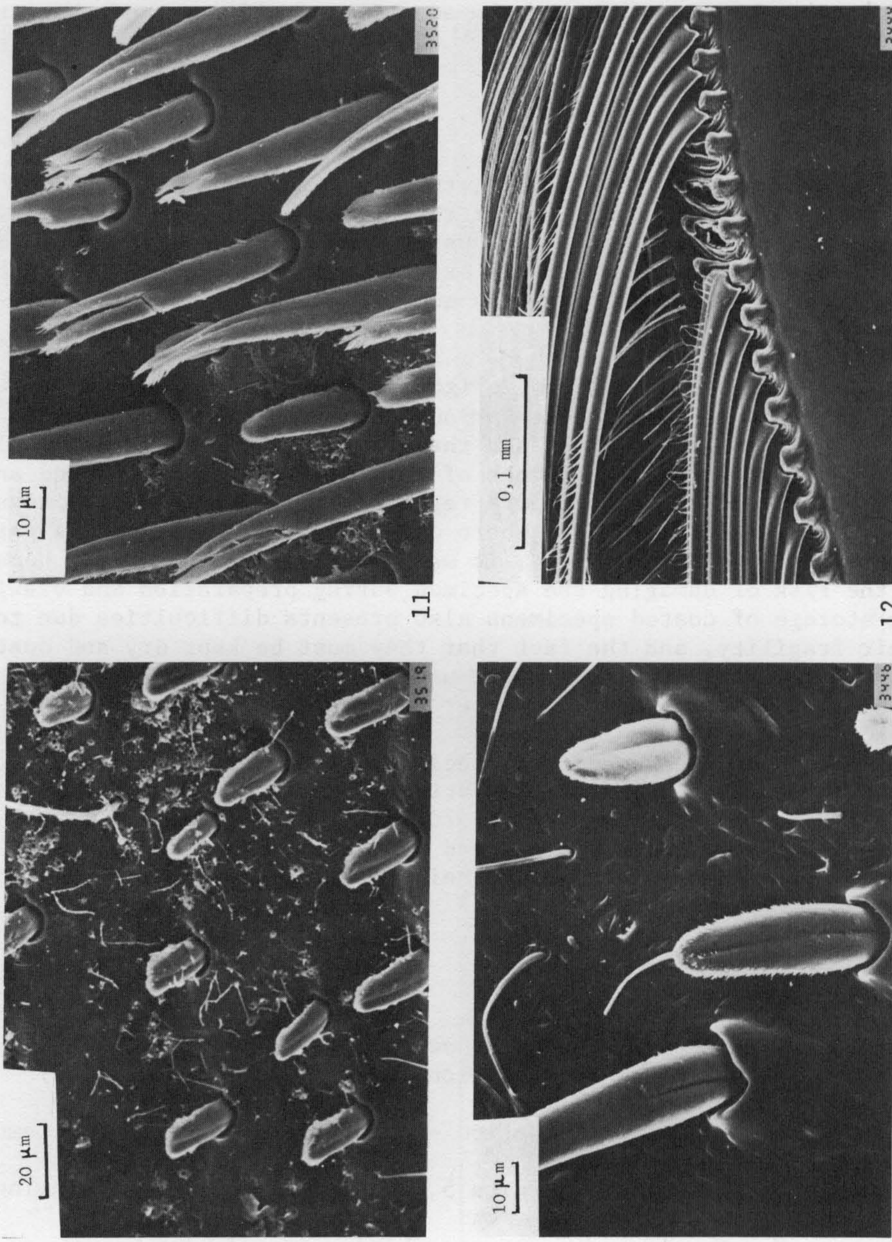

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Plate 3

METHODS

Two severe problems encountered in specimen preparation were: (1) cleaning the specimens thoroughly since they are always covered with fine debris etc. and (2) dehydrating specimens without distortion, prior to coating. A search of the literature showed that many different methods have been advocated for overcoming these difficulties; in practice most of them are less than satisfactory. Regarding the cleaning of specimens, the best method was found to be the use of an ultrasonic tissue disintegrator or homogenizer (an MSE model was used) the probe of which is inserted into a beaker of 80% alcohol containing the specimen. With the amplitude of the probe set at a fairly low setting (4-6 μm), a few minutes gave adequate cleaning without damage to the specimen. Too high a setting caused parts of the specimen to detach.

With regard to specimen dehydration, air drying and critical-freeze-point drying after passage through a graded alcohol-amylacetate series were tried. Air drying tends to bring about collapse of the specimen which results in distortion, especially with large specimens such as Oligoneuriidae. Critical freeze-point drying is a lengthy process and yields a fragile specimen which disintegrates easily on handling and coating.

Good results were obtained using a method suggested by Professor H.E.H. Paterson. After cleaning, specimens are dehydrated through an alcohol series, then brought into xylene using one or two intermediate baths. Times used depend on specimen size. The xylene-hardened specimens retain their original shape after drying for a day in a desiccator. They may be affixed to the carrier stub using collidal graphite (I used DAG-580) suspended in absolute alcohol.

Specimens were coated with Au-Pd and viewed in a JEOL JSM-T-20 machine operating at 20 Kv.

Plate 3. Figures 9-12. *Oligoneuriopsis elisabethae* Agnew (HRG 30B. 16 Mar. 1965. Transvaal: Vaal River on farm Klipbank 104. 30°06'E, 26°42½'S)

9. Bristles next to tuft on sternite 2.
10. Detail of spines near base of fore femur.
11. Details of long bristles of brush; ventral abdominal sternite 2.
12. Details of brush on inner margin of fore tibia.

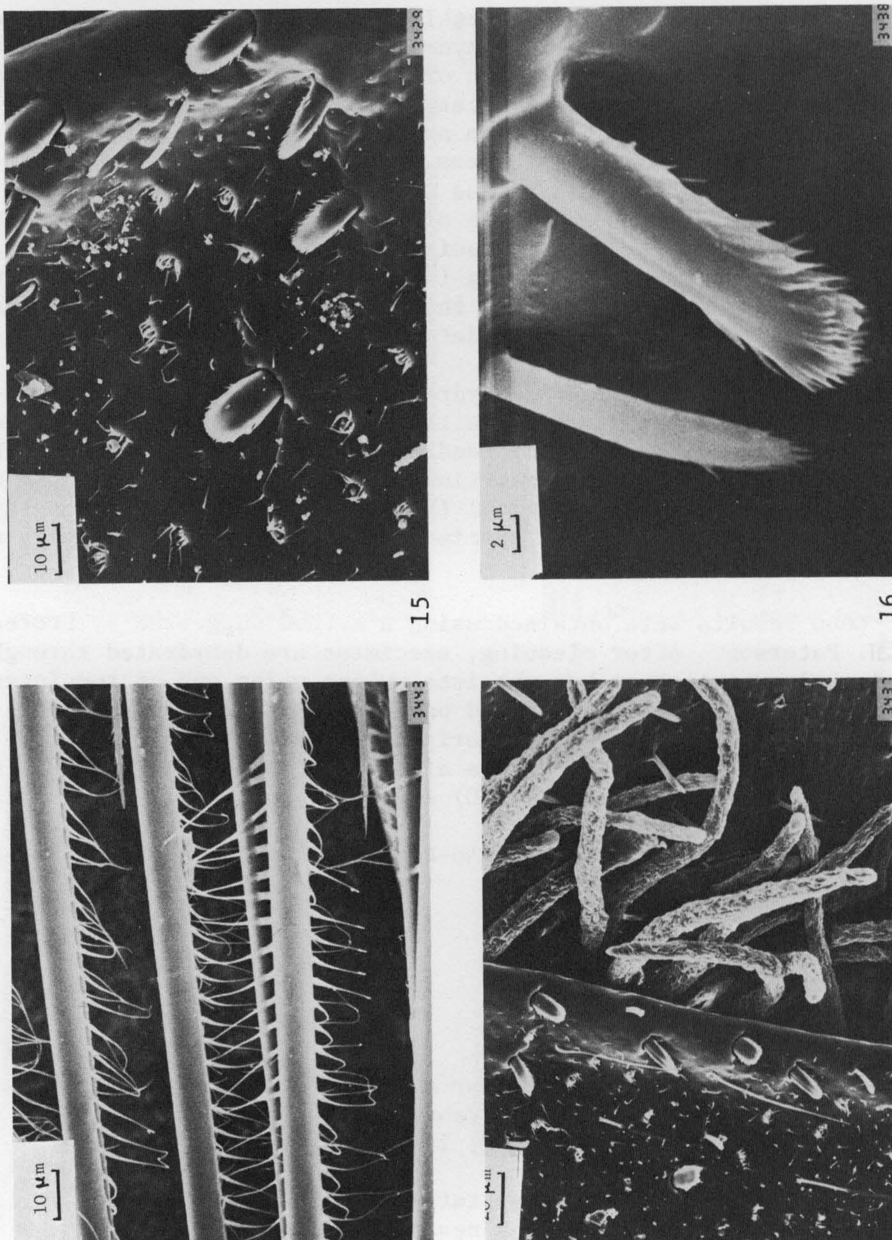

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Plate 4

RESULTS

It is not possible to classify all structures found into clear cut types because of considerable overlapping in size. In fact it is likely that in evolution most of the types may have been derived one from the other or from one or more ancestral types. This matter can obviously only be resolved by comparative and/or developmental studies using a wide spectrum of representative material including fossil material.

Nevertheless, with the material at hand, it is possible to recognize the following main types:

(a) cuticular points: these are 1-5 μm in length and range in number from 2-4 per 'cell' in *Oligoneuriopsis* (Fig. 1) and 2-6, but sometimes up to 8 per 'cell', in *Elassoneuria* (background in Figs. 21 and 22). They are found in all parts of the surface with minor variations.

(b) hairs: these are scattered over most parts of the body surface. The simple kinds are 30-50 μm long in *Oligoneuriopsis* (Fig. 4) and up to 100 μm long in *Elassoneuria* (Fig. 20). Both genera have longer ones, up to 130 μm in length on the lateral parts of the abdominal terga, underlying the lamellae (Fig. 3); these are non-tapering with split ends and in addition are finely digitate along their length in both genera. Another type of hair may be termed the tinsel hair and this is virtually non-tapering and is found along the borders of the femora (rear edge) and gill lamellae (Figs. 7 and 8, *Oligoneuriopsis*; Fig. 24, *Elassoneuria*).

(c) clusters are distributed in varying degrees of density all over the general body surface. (Being found also on the antennal segments, gill lamella, appendages and caudal filaments. Clusters generally show up pure white in the e.m. photographs). They are fundamentally different in the two genera. In *Oligoneuriopsis* each cluster consists of three elements: an outer spine (the longest) 16 μm in length, which curves gently, a middle straight spine 12 μm in length and an outer, club-shaped spine, 12 μm in length (Fig. 2). In *Elassoneuria* the clusters consist of four elements, most pictures

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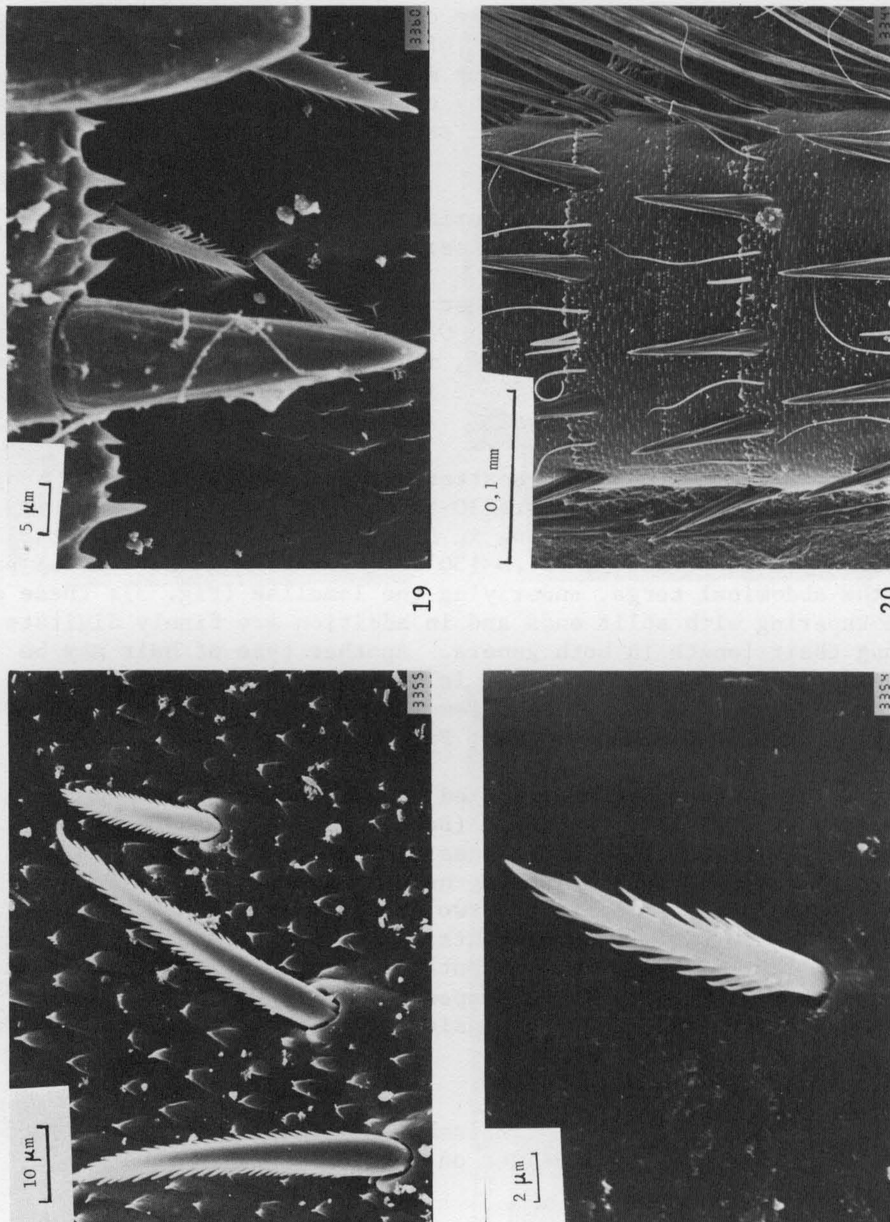
Plate 4. Figures 13-16. *O. elisabethae* (HRG 30B. 16 Mar. 1965. Transvaal: Vaal River on farm Klipbank 104. 30°06'E, 26°42½'S)

13. Detail of filter brush on fore femur.
14. Inner margin of gill lamella, dorsal view.
15. Gill lamella, dorsal view of mine posterior area.
16. Detail of bristles on gill lamella.



17 18 19 20

Plate 5

showing only three but it is possible that four are always present since the elements are packed in a circular arrangement, and sometimes one appears to be obscured. All elements are the same here, each consisting of a very gently tapered smooth spine about 40 μm in length, the ends coming together (Figs. 21 and 24). This does not occur in *Oligoneuriopsis*.

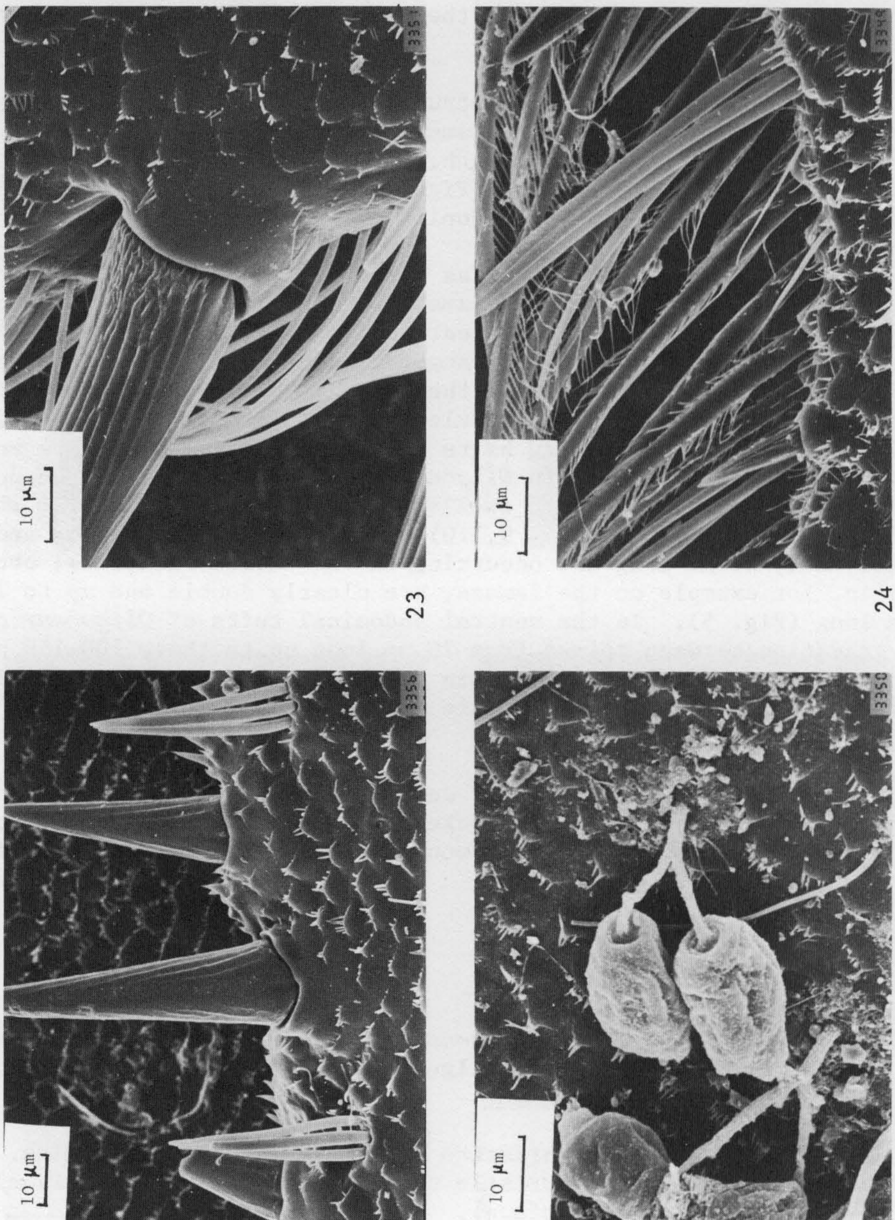
(d) feathers are single structures, found only on the proximal, mesial parts of the gill lamellae. In *Elassoneuria* (Figs. 17, 18, 19) they are ∿20 μm in length, tapering and digitate on either side while in *Oligoneuriopsis* (Figs. 14, 15, 16) they are more peg-like and split around the tips only.

(e) spines of various sizes and shapes are found all over the general body surface and gill lamellae. In *Elassoneuria* they vary in length from 35-90 μm and appear to be of multiple formation since they show a slightly spiral arrangement along their tapering length (Figs. 20, 21, 23). If indeed they are actually multiple in origin it is likely that the long bristles of the filtering brushes (Figs. 12 and 13) and the swimming hairs of the caudal filaments are modified from these spines. In *Oligoneuriopsis* the spines may be peg-like, about 10 μm long with swollen middles and most pictures show them to be double (Figs. 4, 9, 10); others are subtriangular and flattened, 20 μm long and occurring on the caudal filaments; others again, for example on the femora, are clearly double and up to 70-100 μm long (Fig. 5). In the ventral abdominal tufts of *Oligoneuriopsis* a gradation between spines from 20 μm long up to those 100-150 μm in length may be found, and again they all appear to be double in structure (Figs. 6, 9, 11). This appears to be a clear case of adaptive modification.

Morphologically, the types described above would fall into microtrichia i.e. without a basal socket and these would be the cuticular points, whereas all the others would be types of macrotrichia since they have sockets.

Plate 5. Figures 17-20. *Elassoneuria* sp. (HRBA34A. 19 Mar. 1965 Orange Free State: Wilge River at Frankfort. 28°29'E, 27°18'S)

17. Detail of bristles on lateral abdominal tergite.
18. Detail of bristle on posterior area of gill lamella (dorsal surface).
19. Gill lamella 4, inner posterior area of dorsal surface.
20. Detail of median filament.



21 22 23 24

Plate 6

DISCUSSION

A discussion of these results is bedevilled by the fact that the functional significance of the types described is largely unknown. While the function of filtering and swimming paddle bristles is fairly clear, it is not certain what significance must be attached to the other types. What, for example, is the real function of clusters and feathers?

In the descriptive section it was suggested that some of the spine types may have given rise to others in evolution. Some counts of spines on the gill lamellae gave coefficients of variation of 10-15 percent, suggesting that these structures are under multifactorial control. This would form a genetic basis for natural selection to modify the structures adaptively for various purposes.

Whatever the physiological meaning and evolutionary relationships, it is seen that the two genera possess homologous yet recognizably different types of trichia and that their distribution confirms the formal taxonomic distinction. Studies of this kind would undoubtedly be important in the interpretation of fragmentary fossil material and in stimulating further studies on the adaptive significance of the structures which are at the interface of the animal and its external environment.

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Plate 6. Figures 21-24. *Elassoneuria* sp. (HRGA34A. 19 Mar. 1965 Orange Free State: Wilge River at Frankfort. 28°29'E, 27°18'S)

21. Posterior border of tergite 9.
22. Gill lamella surface with attached stalked protozoa.
23. Gill lamella surface - anterior border.
24. Gill lamella, border just posterior to apex of lamella.

RESUME

La ultrastructure de surface (à l'exclusion des trophi) de deux espèces des deux genres afrotropicaux *Elassoneuria* et *Oligoneuriopsis* (Oligoneuriidae) a été etudiée au moyen de la microscopie par scintigramme électronique. De nouvelles méthodes de préparation d'échantillons y sont décrites qui donnent de meilleurs résultats que ceux que l'on obtient par les méthodes que l'on préconise actuellement. Cinq espèces prinicipales de micro- et de macrotrichies y sont examinées avec leurs variantes, certaines paraissant être de type intermédiaire ou faisant figure de trait d'union. La matière étudiée comprend la répartition taxonomique des structures et l'on y traite également de l'utilité de ces structures en taxonomie.

ZUSSAMENFASSUNG

Mit Hilfe von "scanning electron" Mikroskopie wurde die Oberflächen Ultrastruktur (Mundpartien ausgenommen) je zweier Arten der afrotropischen Gattungen *Elassoneuria* und *Oligoneuriopsis* untersucht. Neue Methoden der Präparierung von Specimina werden beschrieben, wobei sich im Vergleich zu bisher befürworteten Methoden weitaus verbesserte Resultate ergeben. Fünf Hauptarten, die Varianten von Mikro- und Makrotrichiasis enthalten, werden dargestellt. Einige davon scheinen Zwischen- oder Koppeltypen zu sein. Die Arbeit befaßt sich weiterhin mit der taxonomischen Verteilung von Strukturen innerhalb des Studienmaterials und erörtert ihren Nutzen in der Taxonomie.

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