The Identification of the Larvae of African Simulium*

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Identification of the larval stages of African Simulium—vectors of onchocerciasis—is becoming increasingly important. In this paper attention is drawn to the principal morphological structures that are of value for identification and illustrations are given to indicate the various characters mentioned. The author provides an identification key to the larvae of the different species-groups of African Simulium and to the larvae of those species of most importance to man, and also presents a tentative key by which a larva may be placed in the probable instar of its development.

With the growing interest in the Simuliidae as the vectors of onchocerciasis it becomes increasingly important to be able to identify the larvae of the African species of Simulium s.l., and to be able to decide not only to what species or species-group any particular larva may belong, but also in which instar of its development it may be. Until recently this has appeared to be a very intractable problem (the monograph of Freeman & de Meillon (1953) deals almost solely with adult flies and pupae), and larval identification has been regarded as almost impossible except in those cases where the larvae were associated with other arthropods or occurred in conspecific masses with the pupae of a single species.

Recent taxonomic study of the larvae of African species (Crosskey, 1960) has, however, shown that their specific recognition, at least in the case of the more commonly collected species, is not always especially difficult, and that many good larval characters exist for their separation into species-groups, corresponding in the main to those based on adult and pupal characters by Freeman & de Meillon (op. cit.). There are, though, in some groups examples of “species-pairs”, in which it is difficult to find really good and constant larval criteria for species separation (e.g., S. unicornatum Pomeroy and S. cervicornutum Pomeroy; S. vorax Pomeroy and S. colas-belcouri Grenier & Ovazza). On the other hand, one or two individual species are clearly recognizable, at any rate in medium-sized or mature larvae, by certain apparently unique characters which they do not share with other African species; for instance, the setae on the proleg in S. damnosum; the combination of fan-shaped scales and simple setae on the abdominal cuticle in S. griseicolle Becker; the straight “cut-off” cephalic fans in S. copleyi Gibbins; and the extraordinary hypostomium in S. berner Freeman. Some groups are better characterized than others, and the ruficorne-group may be instanced as a particularly distinctive group: this group differs from all others in possessing the combination of dark head-spots and ventral abdominal papillae.

The objectives of the present contribution are, first, to draw attention to certain important larval characters through the presentation of an identification key to the larvae of the different species-groups and the most important species, and, secondly, to indicate the lines along which it appears that it might be possible to determine the instar of any larva. The meaning of the terms employed for larval structures is apparent from the accompanying figures.

Most larval characters vary during larval life. The number of rays in the cephalic fans, the number of hooklet rows in the posterior and proleg circlers, the number of antennal segments, and the number of secondary lobules in the anal gills all increase during larval development; after the attainment of the four-segmented condition the antennal segments continue to change their relative lengths. But two characters, the form of the hypostomium and the shape of the postgenal cleft, appear to remain fairly constant during maturation of the larva, although the actual size of these structures naturally increases in successive instars as the head-capsule grows. The shape of the postgenal cleft is perhaps the most valuable single character in the taxonomy of the


African *Simulium* larvae, and may prove useful in determining even the smallest larvae; in combination with hypostomial structure (and perhaps other characters as yet undiscovered), it may ultimately enable us to identify the very young larvae, at least as far as species-group if the actual species cannot be certainly determined. Thus intensive study of young larvae should make it possible to separate those of unimportant harmless species from those of the important species, as can already be done with later-stage or fully grown larvae.

It is especially important to be able to distinguish the very early larvae of the *S. neavei* Roubaud complex from those of other species, since it appears from the recent work of Williams, Hynes & Kershaw,¹ that the earliest instars in *S. neavei* may be non-phoretic. Williams et al. (*op. cit.*) suggest that very young larvae—those less than about one-third grown—in both the *neavei*-complex and the

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**S. copleyi** Gibbins complex (in which there is a phoretic association with mayflies) live attached to stones; hence they could be confused with young larvae of normal “free-living” non-phoretic species. However, from intensive study of very young larvae from stones or other possible substrata in known “neavei streams” it might prove possible to distinguish the very young *neavei*-complex larvae in the non-phoretic stages by means of the hypostomium. As mentioned above, this character appears to remain more constant than most other characters as the larva grows (see, for example, the figures of the hypostomium in each of the seven larval instars of *S. damnosum* given by Grenier & Feraud (1960)), and consequently might be expected to have a similar form in the young non-phoretic *neavei* larvae to that in the larger larvae after attachment to the crabs has occurred. Later-stage larvae of the *neavei*-complex, i.e., those attached to crabs, all possess a hypostomium of characteristic shape and
usually with a rather even row of 13 apical teeth (Fig. 7), and this is true of the smallest larvae which I have been able to find from crabs in the British Museum collection—larvae probably in the third or fourth instar (assuming, as discussed below, that there are six or seven instars in African simuliiid larvae). The *neavei* type of hypostomium is found only in larvae of the *neavei*-complex species, and does not occur in other African Simuliidae; thus its finding in a larva is diagnostic for this complex, and should it occur in very young non-phoretic *neavei* larvae would serve to separate these from other species living in the same habitat.

In order that the larger and fully mature larvae of African *Simulium* can be placed in their species-groups, the following key has been prepared; a key to the larvae of the 21 species occurring in West Africa has been given elsewhere (Crosskey, 1960), and can be used to place most of the common East African species also.

### KEY TO THE LATER-STAGE LARVAE OF THE SPECIES-GROUPS AND PRINCIPAL SPECIES OF AFRICAN *SIMULIUM* 1

1. Hypostomium of unique form, as in Fig. 6
   - Hypostomium not of this form
     - *S. bernerii* Freeman
     - 2

2. Hypostomium with a rather even row of 13 apical teeth and shaped much as in Fig. 7
   - Hypostomium with 9 apical teeth and shaped as in Fig. 4 or 5 (slightly different in some rare *copleyi*-complex species)
     - 3

3. Thorax as well as abdomen with scales or setae
   - Thorax bare, abdomen with or without sparse scales or spines dorsally
     - 4
     - 6

4. Proleg with flattened setae. Anterior abdominal segments with weak or very strong dorso-lateral conical protuberances. Head-capssule with some strong pigmentation and spots. Length of mature larva, 5.0-6.5 mm
   - Proleg bare. Abdominal segments normal. Head-capssule unpigmented. Length of mature larva, 3.75-4.25 mm
     - *S. damnosum* Theobald
     - 5

5. Abdominal cuticle with fan-shaped scales and simple spine-like setae. Postgenal cleft very large and rounded, occupying most of ventral surface of head
   - Abdominal cuticle with one type of flattened scale-like setae. Postgenal cleft smaller and not strongly rounded
     - *S. griseicolle* Becker
     - *S. albibirugulatum*
     - Wanson & Henrard

6. Head-capssule with distinct pattern of dark head-spots and abdomen with ventral papillae
   - Head-capssule with pale head-spots surrounded by variable darker pigmentation, or if with dark head-spots then abdomen without ventral papillae
     - *S. ruficorne-group*
     - 7

7. Ventral papillae present (sometimes weak in *hirsutum-group*). Head-capssule with negative pattern of pale head-spots with at least traces of dark pigmentation around them. Rows of hypostomial setae lying parallel to lateral margins of hypostomium (Fig. 5)
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1 In using the key the following points should be noted: the *hirsutum*-group means the free-living forms and excludes the *neavei* and *copleyi* complexes; *S. schoutedeni* and *S. mcmahoni* are regarded as belonging in the *cervicornutum*-group and their larvae would key out to this group; the *copleyi*-complex has been omitted from this simplified key, but occurs only so far as is known normally on mayfly nymphs.
Ventral papillae absent. Head-capule with positive pattern of dark head-spots, sometimes the dark spots indistinct among general very dark pigmentation (head rarely unpigmented as in S. bovis de Meillon). Rows of hypostomial setae diverging posteriorly from lateral margins of hypostomium (Fig. 4).

8. Abdominal cuticle dorsally with large erect scales. Last abdominal segment with at least traces of a small accessory sclerite on either side in front of posterior circle.

Abdominal cuticle dorsally on the last few segments with branched fan-shaped scales or with a few simple spine-like setae. Last abdominal segment without accessory sclerites.

S. cervicornutum-group

9. Abdominal cuticle with branched fan-shaped scales

Abdominal cuticle with minute simple setae.

S. alcocki-group

S. hirsutum-group

10. Dark brownish head-spots distinct from the creamy ground colour of the head-capule. Abdominal cuticle bare. Postgenal cleft normally small so that the postgenal bridge is longer than the hypostomium.

Dark head-spots usually indistinct among the general dark pigmentation of the head-capule (rarely head-capule unpigmented). Abdominal cuticle with at least a few minute spines on last segments or with scattered scales (as in S. bovis). Postgenal cleft large and postgenal bridge shorter than the hypostomium.

S. dentulosum-group

S. medusaeforme-group

The number of larval instars in the African species of Simulium is in need of investigation, particularly in S. neavei. The number is known with certainty only in S. damnosum, in which there are seven instars including the so-called ”mature” larva with blackened gill-spot (Grenier & Feraud, 1960). In Armenia it has been established by Terterjan (1957) that there are six instars in the larva of Simulium (Wilhelmia) paraequinum Puri, and it seems likely that six is the usual number in most Simuliidae. According to Hinton (1958) the apparent fully mature larva is really a “pharate pupa”, having undergone the larval-pupal moult but not having sloughed off the last larval skin; however, for all practical purposes the pharate pupa has to be regarded as the fully mature larva, and the stage regarded for convenience as the last larval instar. In the African species, presuming that there are normally six or seven instars, moulting must occur at very short intervals if the estimates of the length of larval life are correct (in S. damnosum, for instance, larval life is variously estimated as from 6-13 days). With six or seven instars in a period of one week a larva must undergo an ecdisis about once a day. If the larval life is as short as is generally supposed with tropical African species and there are normally at least six instars, it is curious how infrequently larvae are observed casting their old cuticles and how infrequently cast skins are seen, considering the large masses in which the larvae of many species occur.

The work of Terterjan (1957) and Grenier & Feraud (1960) shows exact agreement in many of the more important findings, such as that the larval antenna first attainsis four-segmented structure at the third instar, is three-segmented in the second instar and two-segmented when first hatched from the egg (in all cases the last segment is minute and in the first instar the antenna appears superficially like a simple shaft). The first instar is always recognizable from the presence of the sclerotized egg-burster on the dorsal surface of the head. In the later instars it is more difficult to be sure what stage has been reached, but the pale buds on the sides of the thorax showing the developing legs, wings, halteres, and pupal respiratory organ appear to give some clue. The following key has been drawn up in an attempt to place any larva in its correct instar; it is based on the presumption of there being at least six instars as a general rule, and must be regarded as only a preliminary guide, which may require considerable modification as more becomes known.
TENTATIVE KEY TO THE INSTARS OF A SIMULIUM LARVA

1. Egg-burster present on the head. Antenna two-segmented ........................................ 1st instar

   Egg-burster absent. Antenna three- or four-segmented ............................................... 2

2. Antenna with three segments .......................................................... 2nd instar

   Antenna with four segments .......................................................... 3

3. Thoracic buds of future legs, wings and pupal respiratory organ undeveloped ........................................ 3rd instar

   At least traces of such buds present .......................................... 4

4. Buds very small and only just differentiated. Cervical sclerites not differentiated from the post-occiput ........................................ 4th instar

   Buds large and more or less easily visible, sometimes very well developed. Cervical sclerites differentiated at the dorsal ends of the post-occiput, but not necessarily separated off ........................................ 5

5. "Gill-spot" whitish, individual filaments not easily distinguishable, leg and wing buds well separated from one another. Cervical sclerites not fully separated from post-occiput ........................................ 5th instar

   "Gill-spot" slightly or much darkened, filaments discernible, leg and wing buds not well separated or more or less meeting one another. Cervical sclerites isolated ........................................ 6th-7th instar

1 Grenier & Feraud (1960) regard the antenna as originally single-segmented in the first instar, two-segmented in the second, and three-segmented when fully formed, but in this key the very small apical cone is regarded as a segment since most authors consider the fully formed antenna as having four segments.

RÉSUMÉ

Le rôle joué par les Simulidés africains dans la transmission de l’onchocercose fait sentir le besoin de disposer d’un moyen de distinguer dès le stade larvaire les différentes espèces et groupes d’espèces. Comme le traité de Freeman et de Meillon (1953) décrit uniquement les formes des adultes et des pupes, il n’existait pas jusqu’à date récente de nomenclature des larves africaines de Simulium. L’auteur du présent article a proposé récemment (1960) une systématisation des larves des espèces de l’Ouest africain qui est en partie valable pour l’ensemble de la faune africaine.

A l’aide de schémas, l’auteur met en évidence et discute les caractéristiques morphologiques qui sont le plus utiles pour l’identification des larves. Un tableau réunit les caractéristiques essentielles et permet de reconnaître au stade larvaire les différents groupes d’espèces et les principales espèces africaines de simulies (réserve faite des larves très jeunes qu’il n’est pas toujours possible de classifier). La configuration de la fente postgénale, et dans une moindre mesure la forme de l’hypopharynx, sont parmi les caractéristiques les plus communément utilisées en taxonomie. Tout en permettant d’identifier les larves du complexe neavei, l’hypopharynx reste un signe de valeur après la fixation des larves aux crabes à un stade avancé de leur développement; il peut contribuer aussi à distinguer des autres larves les minuscules larves du complexe neavei avant cette fixation (étant admis, comme il semble probable, que les œufs du complexe d’espèces neavei ne sont pas déposés sur les crabes et qu’à leurs premiers stades les larves sont incapables de vivre en association phorétique).

Etant donné que les larves jeunes du complexe neavei ne peuvent vivre en association phorétique, il est d’autant plus important d’être en mesure de déterminer à quel stade de leur développement les larves de Simulium acquièrent cette propriété. Les travaux récents conduisent à penser qu’au cours de leur vie larvaire les simulies subissent en principe six mues successives. Il n’en reste pas moins vrai qu’en pratique il est loin d’être toujours facile de décider à quel stade de son évolution est parvenue une larve donnée. Pour tenter de résoudre cette difficulté, l’auteur propose une table d’évaluation qui peut servir à déterminer approximativement le stade de développement de la larve. Ce n’est d’ailleurs là qu’un procédé provisoire qui repose sur l’hypothèse qu’il existerait normalement six mues. Ultérieurement, des modifications devront sans doute être apportées au fur et à mesure que sera mieux connu le développement des variétés africaines de simulies.
REFERENCES

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