Journal of Entomology (*A*) **46** (1), pp. 53–61, 1 fig., 1971

Two phoretic black-flies (Diptera : Simuliidae) and their associated mayfly host (Ephemeroptera : Heptageniidae) in Cameroon

R. H. L. DISNEY

*Overseas Staff, Medical Research Council, Helminthiasis Research Unit, Kumba, West Cameroon**

SYNOPSIS

Simulium afronuri and *S.lumbwanum* are recorded living in association with nymphs of *Afronurus* in the forest zone of West Cameroon. Observations on infestation rates, attachment sites, larval size distribution and dispersion are recorded. Evidence of niche separation between the two simuliid species is discussed.

Nymphs of the genus *Afronurus* Lestage of the mayfly family Heptageniidae are well known to carry species of phoretic black-flies (e.g. Crosskey, 1965). In West Cameroon the only species associated with *Afronurus* that has been reported up to now is *Simulium rickenbachi* Germain, Grenier & Mouchet (1966), which was discovered in the savanna zone.

In April and May 1969, two species were discovered living attached to *Afronurus* nymphs in the River Wowe (a tributary of the Mungo near Baduma to the north of Kumba) in the forest zone of West Cameroon. One proved to be *S.lumbwanum* de Meillon and the other was an undescribed species, which has been named *S.afronuri* Lewis & Disney (1970).

Several collections of nymphs were made in May, mainly in order to obtain adequate material for taxonomic purposes, both for the black-flies and also for the determination of the species of *Afronurus*.

METHODS

Afronurus live flattened against clean stones in fast running water. The most effective technique for collecting the nymphs was simply to lift the stones from the water for direct examination. After assessing the first collections, it was apparent that a large number of the *Simulium* larvae became detached from the nymphs during transportation back to the laboratory. Consequently the following procedure was adopted. Nymphs were rapidly examined while still on the stone and, if they bore pupae, were transferred

* Present address: Malham Tarn Field Centre, near Settle, Yorks.

to polythene jars containing a little water and a piece of dead leaf. Nymphs with well developed wing pads and no attached *Simulium* were also kept alive for the rearing of adult mayflies. All the rest of the nymphs were transferred direct into individual tubes of alcohol.

The nymphs were examined in the laboratory under a low-power dissecting microscope, and the tube of alcohol was also carefully scrutinised for any dislodged larvae. The positions of all larvae, pupae and cocoons on the nymphs were recorded, and the lengths of the larvae and nymphs were measured to the nearest half mm. (the length of a nymph excluded the tail filaments).

It was apparent that the smallest nymphs were inadequately sampled, partly because even an experienced collector has to concentrate closely if he is to avoid giving inadequate attention to the smallest nymphs while rapidly picking off the medium and large nymphs. The smallest nymphs are not only less easy to see but are also more tricky to pick up.

The procedure for rearing adult *Afronurus* was as follows. Just under 1 cm. of water was put into a 1 litre capacity conical flask, together with a piece of dead leaf and a stick, to allow the emerged adult to rest clear of the water. The nymph was then put in the flask, and the mouth was closed with cotton gauze. The emerged adults together with their associated nymph shucks were transferred to labelled tubes of alcohol, which were sent to Dr M.T.Gillies.

The rearing of adult black-flies was attended by high mortalities of the pupae, but the following procedure proved the most rewarding. The pupae were all found attached to the mesonotum and wing covers. The mesonotum bearing a pupa was carefully teased away from the nymph, and as much adhering tissue was removed as was possible without risking damage to the pupa. The pupa was then rinsed in clean water before being floated on to a strip of filter paper, which was then placed in a glass tube whose mouth was closed with cotton gauze (secured by means of a cork with a hole through the centre). The tubes of pupae were laid on their sides on a damp pad of cotton.

THE MAYFLIES

The numbers of *Afronurus* nymphs examined for attached *Simulium* were as follows: from the Wowe River, 589; from the Menge River (a tributary of the Mungo south of Baduma and north of Ikiliwindi), 119; and from the Bille River (collected at the point where it flows into the Meme River), 122. A few *Compsoneuriella* Ulmer (= *Notonurus* Crass, see Gillies, 1963), an associated Heptageniid, were also examined from the Wowe River.

Twenty-eight adult *Afronurus* were successfully reared: 9 females and 3 males from the Wowe, 3 females and 1 male from the Menge and 9 females and 3 males from the Bille. All these adults emerged by night. One female *Compsoneuriella* was reared.

The examination of these adults and of representative samples of nymphs revealed 2 undescribed species of *Afronurus* and 1 of *Compsoneuriella*. Those collected from the Bille belong to a different species to the *Afronurus* collected from the other rivers. Samples of the mayfly material (including all the adults) have been deposited with Dr M.T.Gillies.

Table 1. Infestation rates of immature *S. afronuri* and *S. lumbwanum* on small, medium and large nymphs of *Afronurus*. Wowe River, May 1969.

		S.afronuri					*S.lumbwanum*				
Nymph size class (mm.)	No. of nymphs examined	No. of larvae	No. of pupae	No. of cocoons	No. of larvae and pupae	Mean no. larvae and pupae	No. of larvae	No. of pupae	No. of cocoons	No. of larvae and pupae	Mean no. larvae and pupae
Small (<4·5)	40	0	0	0	0	0	10	0	0	10	0·25
Medium (4·5–8·0)	379	71	2	0	73	0·19	107	13	3	120	0·32
Large (>8·0)	146	76	9	1	85	0·58	14	0	0	14	0·10

THE SIMULIID SPECIES

Infestation rates

No *Simulium* were found attached to the *Afronurus* nymphs collected from the Bille. One larva of *S.afronuri* and 1 of *S.lumbwanum* were found on the nymphs collected from the Menge River. The 565 nymphs of *Afronurus* collected from the Wowe River in May 1969 (not including 14 nymphs collected in a 6 man-hour search, in which only nymphs bearing pupae were retained), were divided into 3 size groups: small nymphs, 4 mm. or less in length; medium nymphs, 4·5–8·0 mm.; large nymphs, over 8·0 mm. in length. The infestation rates of *S.afronuri* and *S.lumbwanum* on these 3 size-classes of *Afronurus* nymphs are shown in Table 1. The infestation rate is taken as the mean number of larvae plus pupae per nymph.

The results indicate, firstly, that the infestation rate varies with the size of the nymph. Secondly, it is apparent that the peak of infestation with *S.lumbwanum* occurs on medium nymphs, whereas the peak of *S.afronuri* occurs on the large nymphs. The infestation rate of 0·25 *S.lumbwanum* on small nymphs is by no means a negligible fraction of the population of this species. In view of the inadequacy of the sampling of the small nymphs, and the consequent ignorance of the true relative proportions of medium and small nymphs occurring in the *Afronurus* population, it is not possible to make meaningful estimates of the relative abundance of the two *Simulium* species.

The striking difference in the infestation peaks for the two species in relation to the size of the nymphs is of considerable interest, especially when it is realised that *S.lumbwanum*, despite being the larger species, is occurring more frequently on the smaller nymphs (in several cases the length of the *S.lumbwanum* larva is the same as or greater than that of its mayfly host). The larvae of *S.afronuri* are not only smaller but are more structurally modified for a phoretic mode of life than are those of *S.lumbwanum* (Lewis & Disney, 1970).

In 12 instances both species were recorded on the same nymph.

Attachment sites and size distribution of larvae

The attachment sites of immature *S.afronuri* on *Afronurus* nymphs, together with the size distributions of the larvae, are shown in Table 2, and the equivalent data for *S.lumbwanum* are presented in Table 3.

Both species pupate on the mesonotum and wing covers. There is a tendency for the pupae of *S.afronuri* to be situated more posteriorly than those of *S.lumbwanum*. The attachment sites of the larvae of the two species differ: 44·7 per cent. of the *S.lumbwanum* larvae were removed from the mesonotum and wing covers, whereas only 2·6 per cent. of *S.afronuri* were found in this position; 40·0 per cent. of *S.afronuri* were attached to the sides of the second abdominal segment, but only 4·5 per cent. of *S.lumbwanum*.

The smallest larvae of *S.afronuri* were 0·5 mm. first instar larvae (bearing an egg tooth on top of the head), whereas 1·0 mm. larvae were the smallest *S.lumbwanum* encountered. Both species exhibit a peak of 1·5 mm larvae, but the frequency of larvae in the larger size groups present an erratic pattern.

Table 2. Attachment sites of *S.afronuri* to *Afronurus* nymphs.

	Size of *Simulium* larvae (mm.)													
Positions on mayfly nymph	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	Pupae	Empty cocoons	Larvae totals	Percentage
Mesonotum and wing covers	0	0	0	0	0	0	0	2	2	0	11	2	4	2·6
Sides of thorax	0	0	0	1	1	1	0	0	0	0	0	0	3	2·0
Coxae and trochanters	0	1	4	3	3	0	2	0	0	0	0	0	13	8·5
Femora	0	0	0	0	0	0	2	0	0	0	0	0	2	1·3
Abdominal segment														3·3
I—sides	0	0	0	0	0	1	1	0	3	0	0	0	5	
II—sides	0	0	4	3	6	5	16	13	13	1	0	0	61	40·0
III—sides	0	0	2	0	1	2	1	0	0	0	0	0	6	3·9
IV—sides	0	0	0	1	1	0	0	1	0	0	0	0	3	2·0
—tergum	0	0	0	0	0	0	0	0	1	0	0	0	1	0·6
—sternum	0	0	0	0	0	0	0	0	0	0	0	0	1	0·6
V—sides	0	0	1	0	0	0	0	1	0	0	0	0	2	1·3
VI—sides	0	0	1	0	0	0	0	0	0	0	0	0	1	0·6
VII—sides	0	0	1	0	0	0	0	0	0	0	0	0	1	0·6
Dislodged	2	10	20	4	8	1	3	1	1	0	0	0	50	32·7
Totals	2	11	33	12	20	10	26	18	20	1	11	2	153	100
Percentages	1·2	6·7	20·1	7·3	12·2	6·1	15·9	11·0	12·2	0·6	6·7	–	100	

Table 3. Attachment sites of *S.lumbwanum* to *Afronurus* nymphs.

Positions on mayfly nymph	Size of *Simulium* larvae (mm.)															
	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	Pupae	Empty cocoons	Larvae totals	Percentage
Head	0	0	2	0	1	0	0	0	0	0	0	0	0	0	3	2·3
Pronotum	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2	1·5
Mesonotum and wing covers	0	0	7	3	5	6	5	3	9	7	12	2	13	3	59	44·7
Coxae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0·8
Femora	0	1	1	1	0	0	0	0	0	0	0	0	0	0	3	2·3
Tibiae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0·8
Abdominal segment																
I—sides	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	1·5
I—tergum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0·8
II—sides	0	0	3	3	0	0	0	0	0	0	0	0	0	0	6	4·5
II—gills	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0·8
III—sides	0	0	8	2	0	0	0	0	0	0	0	0	0	0	10	7·5
IV—sides	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	1·5
Tail filaments	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	1·5
Dislodged	0	2	29	4	1	1	1	0	1	0	0	0	0	0	39	29·5
Totals	0	4	57	15	8	7	6	3	11	7	12	2	13	3	132	100
Percentages	0	2·8	39·3	10·3	5·5	4·8	4·1	2·1	7·6	4·8	8·3	1·4	9·0	–	100	

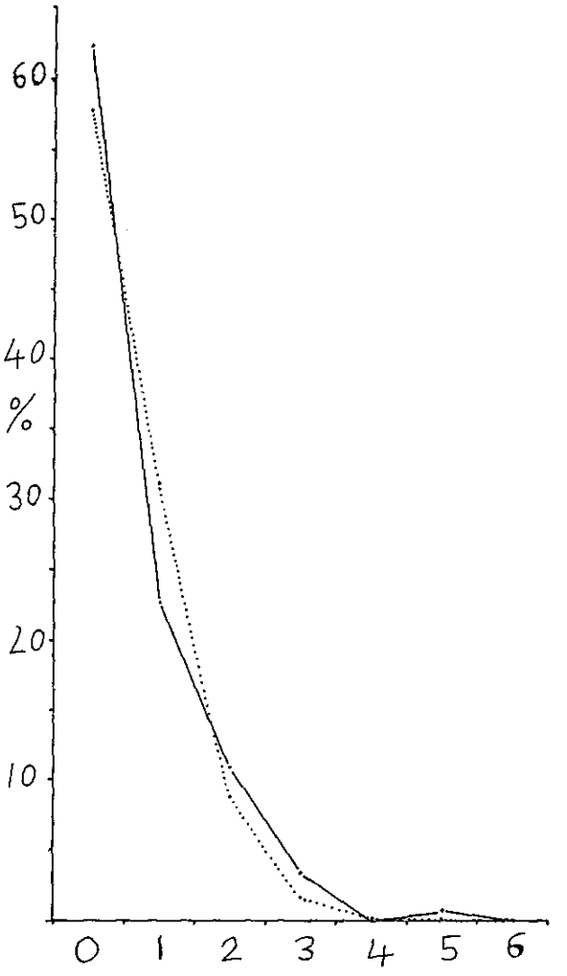

Fig. 1. Dispersion of immature *S.afronuri* on large nymphs of *Afronurus* (Wowe River, May 1969). —— observed dispersion. . . . . Poisson distribution. Vertical axis, percentage occurrence; horizontal axis, number of *Simulium* per nymph.

Dispersion of larvae on nymphs

Freeman & De Meillon (1953), referring to *S.lumbwanum*, suggested it was "possible that more than one larva can live on a single nymph". In the present study, up to five *S.afronuri* and up to three *S.lumbwanum* have been found on single nymphs of *Afronurus*; and once three *S.afronuri* and one *S.lumbwanum* were found on the same nymph.

With the attachment of *S.berneri* Freeman to nymphs of *Elassoneuria* Eaton it has been shown (Disney, 1971*a*) that the dispersion of the larvae on the nymphs (i.e. the frequencies of nymphs bearing 0, 1, 2, 3, etc., larvae) is random. From this it was inferred that the *Simulium* larvae reached their hosts as single larvae, depending on drifting and

chance encounter for success. The dispersion of *S. afronuri* and *S.lumbwanum* on *Afronurus* nymphs appears to be random also. Figure 1 shows the dispersion of immature *S.afronuri* on the large nymphs collected from the Wowe River in May 1969 (i.e. the data used in Column 1 of Table 1) together with the Poisson distribution calculated from the infestation rate. Close coincidence of the two curves indicates a random dispersion.

Emergence of adults

Seven *S.afronuri* and 11 *S.lumbwanum* were successfully reared in the laboratory. All these emerged in the mornings except one female *S.lumbwanum* that emerged in the night and one *S.afronuri* that emerged in the afternoon of the day of collection of the pupa. Van Someren & McMahon (1950) reported that *S.copleyi* Gibbins (which also lives on nymphs of *Afronurus*) emerged by night within an hour or two of the eclosion of the adult mayfly. Such a pattern of emergence is not seen in *S.afronuri* and could not occur with *S.lumbwanum*, in which the pupae collected were all on medium nymphs (i.e. on nymphs too young for eclosion).

DISCUSSION

The presence of larvae of both *S.afronuri* and *S.lumbwanum* on the same *Afronurus* nymph indicates that the species are not mutually exclusive. On the other hand, the differences in the preferred attachment sites of the two species recalls the situation in prawn-phoretic black-flies, in which it has been found (Disney, 1971*b*) that *S.dukei* Lewis, Disney & Crosskey and *S.atyophilum* Lewis & Disney both occur on the prawns *Atya africana* Bouvier and not infrequently together on the same individual prawn. Likewise both species pupate on the same parts of the prawns but exhibit differences in the preferred attachment sites of their larvae; it was suggested that this indicated a niche separation between *S.dukei* and *S.atyophilum*, resulting in reduction of competition. With *S.afronuri* and *S.lumbwanum* such a niche separation leading to reduction of competition seems to have gone a stage further, in that there is also a difference in the preferred size of the nymph for each species (Table 1). It seems probable that the smaller size of *Afronurus* (when compared with *Atya*) has intensified the "need" for niche separation between two phoretic black-flies living on the same host species.

Studies of *S.berneri* (Disney, 1971*a*) indicated that larvae seemed to experience greater difficulty in remaining attached to small *Elassoneuria* nymphs; this, it was suggested, partly accounted for the increase in infestation rate with the size of the nymph. *Afronurus* is smaller than *Elassoneuria*, and so this problem is presumably aggravated. *S.afronuri* is smaller than *S.lumbwanum* and far more obviously modified for a phoretic mode of life (such as the modification of the typical *Simulium* mouth-brush so that it forms a short, flat brush), and it achieves its highest infestation rate on the largest, and therefore presumably the most "suitable", nymphs. *S.lumbwanum* by contrast is larger (*cf.* Tables 2 and 3), and the only obvious structural adaptation for its phoretic mode of life would appear to be the ventral position of the posterior circlet of the abdomen, and yet it attains its highest infestation rate on the medium nymphs.

The above considerations suggest that *S.lumbwanum* has evolved association with *Afronurus* more recently than *S. afronuri*, and that the latter species provided an environmental obstacle to the full exploitation of the *Afronurus* niche by *S.lumbwanum*. It is possible that *S.afronuri* became more restricted in its choice of attachment sites in the

face of the invasion of its niche by *S.lumbwanum*. It would be interesting to compare the infestation rates of *S.lumbwanum* on small, medium and large nymphs in a river from which other *Afronurus*-phoretic species were absent.

SUMMARY

Simulium afronuri Lewis & Disney and *S.lumbwanum* de Meillon live attached to nymphs of an undescribed *Afronurus* near Kumba in the forest zone of West Cameroon. No phoretic *Simulium* were found on nymphs of a different undescribed species of *Afronurus*.

First instar larvae of *S.afronuri* were recorded but not those of *S.lumbwanum*.

The infestation rate varied with the size of the nymph, peak infestation by *S.afronuri* occurring on large nymphs and by *S.lumbwanum* on medium nymphs. The pupae of both species are found on the mesonotum and wing covers of *Afronurus*, but the larvae differ in their choice of attachment sites. The dispersion of the two species on the nymphs appears to be random.

It is suggested that the association of *S.lumbwanum* with *Afronurus* is of more recent origin that that of *S.afronuri* and that the latter has become more restricted in its choice of attachment site because of the invasion of its niche by *S.lumbwanum*.

Mayfly nymphs have been collected by the following members of the Unit's staff: Mr R.E.Oguama, A.Alim, A.Iburu, O.Mbeng, F.Moh and E.Ndifon. Mr P.K.Tatason carried out regular checks on the adult rearing flasks and tubes.

Dr M.T.Gillies (University of Sussex) kindly gave of his time to examine and comment on the mayfly material.

REFERENCES

CROSSKEY R.W. 1965. The identification of African Simuliidae (Diptera) living in phoresis with nymphal Ephemeroptera, with special reference to *Simulium berneri* Freeman. *Proc. R. ent. Soc. Lond.* (A) **40** : 118–24.

DISNEY R.H.L. 1971*a*. *Simulium berneri* Freeman (Diptera : Simuliidae) and its mayfly host (Ephemeroptera: Oligoneuridae). *J. Ent.* (A) **46** : 39–51.

DISNEY R.H.L. 1971*b*. Association between blackflies (Simuliidae) and prawns (Atyidae), with a discussion of the phoretic habit in Simuliids. *J. Anim. Ecol.* **40** : 83–92

FREEMAN P. & DE MEILLON B. 1953. *Simuliidae of the Ethiopian region*. London, British Museum (Nat. Hist.).

GERMAIN M. GRENIER P. & MOUCHET P. 1966. Une Simulie nouvelle du Cameroon Occidental: *Simulium rickenbachi* n. sp. (Diptera, Simuliidae) associée à des larves d'éphémères (*Afronurus*). *Bull. Soc. Path. exot.* **59** : 133–44.

GILLIES M.T. 1963. A list of Ephemeroptera in the National Museum, Bulawayo, Southern Rhodesia. *Entomologist's mon. Mag.* **98** (1962) : 232–33.

LEWIS D.J. & DISNEY R.H.L. 1970. Some Simuliidae (Diptera) from West Cameroon. *Proc. R. ent. Soc. Lond.* (B) **39** : 99–108.

VAN SOMEREN V.D. & MCMAHON J.P. 1950. Phoretic association between *Afronurus* and *Simulium* species and the discovery of the early stages of *Simulium neavei* on freshwater crabs. *Nature, Lond.* **166** : 350–1.

Manuscript received 7th August, 1969