

REVIEW

A guide to the *Simulium damnosum* complex (Diptera: Simuliidae) in Nigeria, with a cytotaxonomic key for the identification of the sibling species

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Although approximately 40% of all the people blinded by *Onchocerca volvulus* are Nigerians, almost nothing was known about the various cytospecies of the blackfly vectors present in Nigeria until 1981. The activation of the Nigerian National Onchocerciasis Control Programme in 1986 (and that programme's initiation of mass distributions of ivermectin in 1991) provided a significant stimulus to understand the biology of the Nigerian vectors but the exploration of any possible differences between the cytospecies has been hampered by a lack of accessible taxonomic information. This review attempts to satisfy that need. There are nine different cytoforms reliably recorded from Nigeria (*Simulium damnosum* s.s. Nile form, *S. damnosum* s.s. Volta form, *S. sirbanum* Sirba form, *S. sirbanum* Sudanense form, *S. soubrense* Beffa form, *S. squamosum* A, *S. squamosum* B, *S. squamosum* C and *S. yahense* typical form), and three more are known from surrounding countries and might be reasonably expected to occur in Nigeria. All of these cytospecies are presumed to be vectors, although there have been almost no identifications of the vectors of *O. volvulus* in Nigeria. The biogeographical distribution of the cytoforms is broadly similar to that known in other parts of West Africa (although many of the cytoforms remain insufficiently studied). The physico-chemical hydrology of the Nigerian breeding sites of the cytospecies does not, however, correspond to that seen elsewhere in West Africa, and it is not clear whether this might be related to differences in the cytoforms. An illustrated cytotaxonomic key is presented to facilitate and encourage future studies.

Human onchocerciasis (river blindness), caused by the filarial parasite *Onchocerca volvulus*, is a debilitating tropical infection that can result in blindness and severe skin disease. Although onchocerciasis is also endemic to tropical America and the Yemen, 99% of all cases occur in Africa,

and 95% of the African cases are infected via the bites of blackflies (Diptera: Simuliidae) of the *Simulium* (*Edwardsellum*) *damnosum* Theobald complex (WHO, 1995). Each year, the filariae transmitted by this complex are responsible for 250,000 cases of blindness, up to 500,000 more cases of severe visual impairment, and the loss of nearly 1 million disability-adjusted life-years (DALY; WHO, 1995; www.who.int/tdr/diseases/oncho/diseaseinfo.html). Compared with their healthy counterparts, individuals with symptomatic

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infection have been found to spend an additional 15% of their annual income on health, infected children have been found to be more likely to drop out of school, and symptomatic farmers have, on average, a third less land under cultivation — all differences that contribute to poverty (Fischer and Büttner, 2002). Although onchocerciasis has been named the third most important cause of preventable blindness in the tropics (Narita and Taylor, 1993), it is skin disease that is responsible for 60% of the DALY lost as the result of onchocercarial infection. There is also some evidence that onchocerciasis may be associated with epilepsy and dwarfism (Basáñez *et al.*, 2006) and the biting nuisance that blackflies represent also has a real but unmeasured effect on economic activities (Hougard *et al.*, 1998).

On the basis of the banding sequences of the polytene chromosomes from the salivary glands of the larvae, *Simulium damnosum* s.l. has been shown to be made up of a complex of sibling species (Dunbar, 1966; Vajime and Dunbar, 1975; Quillévéré, 1975). Sibling species are real species that may differ in aspects of their biology but usually cannot be distinguished by morphology. Such species in the *S. damnosum* complex are often called cytospecies because they have been defined on the basis of their chromosomal variation. Cytotypes, on the other hand, are chromosomally distinctive populations of unknown specific status, although further research will probably show that some of them are actually cytospecies. Cytospecies and cytotypes are known together as cytoforms. There are 55 named cytoforms within the *S. damnosum* complex (Post *et al.*, 2007), making it the largest known sibling species complex of any vector.

It is thought that all the West-African cytoforms of *S. damnosum* s.l. are vectors (although this has not yet been proven for some species, such as *S. mengense*, largely because of problems in identification), albeit of varying importance. As members of the *damnosum* subcomplex have greater longevity

than members of the *squamosum* subcomplex (Millest *et al.*, 1992), they tend to be more effective as vectors. *Simulium damnosum* s.s. and *S. sirbanum* are known to be capable of regular wind-assisted migrations of >400 km, and they can move in such large numbers that they can threaten the success of control efforts in the areas that they reach (Walsh *et al.*, 1981; Baker *et al.*, 1990). In contrast, *S. squamosum* seems to cross shorter distances (up to 125 km; Cheke and Garms, 1983), and *S. yahense* may only travel a few kilometres. The discrimination of the sibling species of *S. damnosum* s.l. was critical to the success of the World Health Organization's Onchocerciasis Control Programme (OCP) in West Africa (Le Berre and Fiasorgbor, 1985; Yaméogo *et al.*, 2004), which, through the insecticide treatment of vector breeding sites between 1974 and 2002, protected 40 million people in 11 countries against onchocerciasis and prevented 600,000 cases of blindness (WHO, 2002).

Nigeria has more people blinded by onchocerciasis than any other country — an estimated 100,000 cases of the 268,000 that occur worldwide — as well as approximately 3.2 million people infected with *O. volvulus* (WHO, 1995). The population of Nigeria exceeds that of all 11 countries covered by the old OCP combined. The Nigerian National Onchocerciasis Control Programme (NOCP) was established in 1982, became active in 1986 and started distribution of ivermectin in 1991 (Jiya, 1998). This programme's aim is to eliminate onchocerciasis as a public-health problem, via annual distributions of ivermectin to communities where the incidence of the disease is high. Ivermectin conveys enormous clinical benefits but it is unclear whether it can normally interrupt transmission by *S. damnosum* s.l. in Africa (Borsboom *et al.*, 2003). The development and application of alternative control strategies in Nigeria will benefit from a better understanding of the epidemiology of the disease, and this should take into account the differences between the vector cytospecies. Unfortunately, the

number of publications on the *S. damnosum* complex in Nigeria remains small. Thirty years ago, Crosskey (1981) listed vector breeding sites throughout the country in relation to onchocerciasis foci but, at the time, the cytotaxonomic identities of the blackfly populations were very poorly known. Since then, although the advent of the NOCP was a significant stimulus to research into the epidemiology and transmission of onchocerciasis in Nigeria (Okonkwo *et al.*, 1991; Adewale *et al.*, 1999; Nwoke and Dozie, 2001; Ubachukwu and Anya, 2001; Oyibo and Fagbenro-Beyioko, 2003; Idowu *et al.*, 2004; Ubachukwu, 2004; Opara *et al.*, 2005), there has only been one direct published indication of which sibling species are vectors in Nigeria (Mafuyai *et al.*, 1997) and this was based on only 14 infective flies [eight *S. squamosum* and six 'savanna' blackflies (i.e. *S. damnosum* s.s. or *S. sirbanum*)]. Even studies on the larval biology of Nigerian *S. damnosum* s.l. (Usip *et al.*, 2003; Opara and Fagbemi, 2005; Ikpeama *et al.*, 2006) have not always included identifications of the cytoforms under investigation.

It is surprising that so little is known about the role and importance of the different cytospecies in the transmission of *O. volvulus* in Nigeria, and the objective of this publication is to facilitate studies on these topics, by reviewing the members of the *S. damnosum* complex that have been reliably recorded from Nigeria, and providing the means for their cytotaxonomic identification. Similar guides have been published for other parts of West Africa (Boakye, 1993) and for East Africa (Krueger, 2006). The identification of the adult female vectors to cytospecies is outside the scope of this review and readers interested in this topic are referred to the publications by Wilson *et al.* (1993, 1994), Mafuyai *et al.* (1996b, 1997), Usip *et al.* (2003), Mank *et al.* (2004), Mustapha *et al.* (2004b), Ibeh *et al.* (2008) and Idowu *et al.* (2008). For an inventory of known cytotypes, with a list of their diagnostic inversions, see Post *et al.* (2007), and for descriptions of cytotaxonomic methods

see, for example, Dunbar (1972), Quillévére (1975), Vajime (1986), Boakye (1988) and Krüger (2003). Descriptions of cytotaxonomic nomenclature have been published by Vajime and Dunbar (1975), Quillévére (1975), Vajime (1986), Boakye (1993) and Post *et al.* (2007).

REVIEW OF CYTOSPECIES OF THE *S. damnosum* COMPLEX IN NIGERIA

Vajime and Dunbar (1975) were the first to identify sibling species of the *S. damnosum* complex in West Africa. They recognised eight sibling species (*S. squamosum*, *S. yahense*, *S. sanctipauli*, *S. soubrense*, *S. damnosum* s.s., *S. sirbanum*, *S. sudanense* and *S. dieguerense*) and published an identification key based on chromosomal features. Quillévére (1975) subsequently criticised the criteria for identifying *S. soubrense* and *S. sanctipauli*, arguing that the inversion 2L-7 was polymorphic within species and not diagnostic. Bedo (1977) also criticised the criteria given by Vajime and Dunbar (1975) for the identification of *S. sirbanum* and *S. sudanense*, for similar reasons. Although Post (1986) showed that *S. sanctipauli* and *S. soubrense* were separate species, he found the old diagnostic inversion 2L-7 [described by Vajime and Dunbar (1975)] to be intraspecific and proposed a new diagnostic inversion which he called 2L-A.

Only two cytospecies (*S. damnosum* s.s. and *S. sirbanum*) were recorded by Vajime and Dunbar (1975) from Nigeria, and the same two sibling species were also identified in Nigeria by Vajime and Quillévére (1978), confirming the earlier work.

The Nigerian cytospecies of the *S. damnosum* complex were first reviewed by Crosskey (1981). Crosskey (1981) noted the presence of *S. squamosum* and *S. sudanense* in Nigeria, but did not cite any published work for these new identifications, referring instead to a personal communication from Dr R. W. Dunbar. Curiously, however, the observations made by Dunbar and Vajime (1981)

partially contradicted Crosskey's report. Dunbar and Vajime (1981) tabulated the distribution of cytospecies found in Africa and recognised four cytospecies from Nigeria (*S. sudanense*, *S. damnosum* s.s., 'Volta form' and *S. sirbanum* but not *S. squamosum*). The separate taxonomic status of *S. damnosum* s.s. and 'Volta form' remains controversial and has not been recognised by many authors. Most authors prefer to consider 'Volta form' as a cytotype within *S. damnosum* s.s. (Boakye, 1993), and this implies that there must also be a 'typical' cytotype. Post *et al.* (2007) referred to these two cytotypes as *S. damnosum* s.s. Volta form and *S. damnosum* s.s. Nile form.

Gregory (1982), in his brief review of the vectors of *O. volvulus* in Nigeria, reported that six cytospecies had been identified in the country: *S. damnosum* s.s., *S. sirbanum*, *S. sudanense*, *S. soubrense*, *S. sanctipauli* and *S. squamosum*. Of these, four had already been reported by Crosskey (1981), but Gregory (1982) was the first to report *S. soubrense* and *S. sanctipauli* from Nigeria. Gregory cited Vajime and Dunbar (1975) to support his statement that *S. soubrense* and *S. sanctipauli* occurred in Nigeria but — although they described eight species from West Africa, including the six cytospecies listed by Gregory — Vajime and Dunbar (1975) only reported two cytospecies (*S. damnosum* s.s. and *S. sirbanum*) from Nigeria (see above). It is unclear where Gregory (1982) obtained his information on Nigerian *S. soubrense* and *S. sanctipauli* but the source was most likely personal communications with Dr R. W. Dunbar and/or Dr C. G. Vajime, who were both working in Nigeria at the time.

Meredith *et al.* (1983) subsequently confirmed the presence of *S. soubrense* in Nigeria, although, at the time of their studies, the species status of *S. soubrense* and *S. sanctipauli* was unclear and the *S. soubrense* was simply called the 'Beffa form'; the 'Beffa form' is now considered to be a cytotype of *S. soubrense* (Post, 1986; Boakye, 1993). Meredith *et al.* (1983)

recorded the Beffa form from the Nigerian border with Benin, in the Okpara River. This finding was significant not only in confirming the presence of the cytospecies in Nigeria but also as a border report. The relevance of identifications on the borders of Nigeria, or from the surrounding countries, is that some members of the *S. damnosum* complex are known to migrate over very long distances (up to, and perhaps exceeding, 400 km) and the risk of such movement has been very important in planning strategies for onchocerciasis control (Garms *et al.*, 1979) because immigrant flies can bring the human-infective larvae of *O. volvulus* with them. Roberts (1985) also reported the Beffa form of *S. soubrense* from Nigeria but did not state the criteria for his identification. It is probable that he was trying to use the morphological criteria described by Meredith *et al.* (1983), since he was not a cytotaxonomist. Outside of the OCP area, however, these morphological criteria should only be applied with great caution, otherwise incorrect identifications can occur. Meredith *et al.* (1983), for example, used the criteria to identify some Liberian blackflies as the Beffa form but this identification was probably incorrect because the Beffa form has never been identified chromosomally from rivers in the study area in Liberia. Post (1986) published the first reliable report of *S. soubrense* Beffa form from sites within Nigeria, identifying the form cytologically by the diagnostic inversion 2S-6b. Although Cheke *et al.* (1987) believed that Meredith *et al.* (1983) had recorded the same form from the Gurara River, near the Nigerian city of Abuja, the identification was morphological and needs to be confirmed cytologically.

Roberts and Irving-Bell (1987, 1996) reported identifications of adult *S. squamosum* and *S. damnosum* s.s. in a Nigerian valley but, again, these identifications were made (Roberts and Irving-Bell, 1987) or were probably made (Roberts and Irving-Bell, 1996) — no relevant detail is provided in the later publication — using the morphotaxonomic keys of Dang and Peterson

(1980). Although these identifications are probably mostly correct [because, according to a personal communication from Dr R. W. Dunbar (Roberts and Irving-Bell, 1987), they correlate with cytotoxic identifications of larvae from the same valley], the morphotaxonomic separation of the adult females of *S. squamosum* and *S. damnosum* s.s. is known to be very difficult (Garms *et al.*, 1982; Garms and Cheke, 1985; Wilson *et al.*, 1993) and not always possible using the keys of Dang and Petersen (1980).

Akoh *et al.* (1987) examined new material from Nigeria and identified it cytotoxicologically, with stated criteria, as five cytospecies: *S. squamosum*, *S. sanctipauli*, *S. damnosum* s.s., *S. sirbanum* and (for the first time in the country) *S. yahense*. The identification of *S. sanctipauli* is surprising because the specimens came from the northern Guinea savannah, which is not typical of *S. sanctipauli* in other countries (Boakye, 1993).

Vajime (1989) synonymized *S. sirbanum* and *S. sudanense* under the name *S. sirbanum* and described them as 'sub-siblings', which was clearly meant to indicate their subspecies status. Vajime (1989) did not, however, designate subspecific trinomials and subsequent authors have treated *S. sirbanum* and *S. sudanense* as synonyms (Boakye, 1993; Adler and Crosskey, 2010) and referred to them as the Sirba form and the Sudanense form of *S. sirbanum* (Post *et al.*, 2007).

Vajime and Gregory (1990) were the first to list eight cytospecies of the *S. damnosum* complex from Nigeria in a single work: *S. damnosum* s.s., Volta form, *S. sirbanum*, *S. sanctipauli*, *S. soubrense*, *S. soubrense* 'Beffa form', *S. squamosum* and *S. yahense*. They did not distinguish the two cytotypes described by Vajime (1989) within *S. sirbanum*. It is questionable if *S. sanctipauli* and *S. soubrense* (typical form) actually exist in Nigeria. This is because the identifications by Vajime and Gregory (1990) were based on inversion 2L-7 (Vajime and Dunbar, 1975), which was already known to be

insufficient for separating *S. soubrense* from *S. sanctipauli* (see above). Post (1986) introduced a new inversion, 2L-A, for this purpose. Any identifications of *S. sanctipauli* and *S. soubrense* (typical form) without reference to 2L-A are questionable and are probably all misidentifications of specimens of the Beffa form of *S. soubrense*.

Mafuyai (1992) identified five cytospecies (*S. damnosum* s.s., *S. yahense*, *S. sirbanum*, *S. sudanense* and *S. squamosum*) in new Nigerian material, but also recognised the occurrence of the Volta form and *S. sanctipauli* in Nigeria. He did not recognise the different cytotypes within *S. sirbanum*, and found that, at two sites, male *S. sirbanum* lacked inversion IS-3, which means that they could not be of the Sirba form, Sudanense form or Type IV (Fiasorgbor and Cheke, 1992). Although this finding might indicate the presence of a fourth cytotype within *S. sirbanum*, this possibility needs further exploration and will not be considered further in this review.

Fiasorgbor and Cheke (1992) found *S. sirbanum* in the Okpara River on the Nigeria-Benin border, where Meredith *et al.* (1983) had already found the Beffa form of *S. soubrense*.

Working with mostly the same material as Mafuyai (1992), Wilson *et al.* (1994) confirmed the earlier findings by reporting four cytospecies (*S. yahense*, *S. squamosum*, *S. damnosum* s.s. and *S. sirbanum*). Mafuyai *et al.* (1996a, 1997) reported *S. damnosum* s.s. (without distinguishing the Volta form), *S. sirbanum* s.l., *S. squamosum*, *S. yahense* and *S. soubrense* (including the Beffa form). Bassey (1998) identified four species and reviewed seven cytospecies. The species she reported from new Nigerian samples were *S. damnosum* s.s. (without distinguishing the Volta form), *S. sirbanum*, *S. squamosum* and *S. yahense*. Boakye *et al.* (1998) summarized the cytotoxic identifications, during the period 1984-1993, for the old OCP area, which includes the whole of Benin and the Okpara River on the border with Nigeria. They confirmed the presence of *S. damnosum*,

S. sirbanum and *S. soubrense* Beffa form in the Okpara River.

Vajime and Dunbar (1975) were the first to notice chromosomally distinct entities within *S. squamosum* but this variation was largely ignored until it was redescribed by Boakye (1993). In Nigeria, Traore-Lamizana *et al.* (2001) were the first to distinguish different named cytotypes within *S. squamosum*, identifying *S. squamosum* A and *S. squamosum* C. Significant new distribution records have been added for south-eastern Nigeria by Ibeh *et al.* (2006) (who also recorded *S. squamosum* B for the first time in Nigeria) and Onyenwe *et al.* (2007), who recorded the Sudanense form of *S. sirbanum*.

The migratory habit noted in blackly sibling species makes cross-border identification very important, with the strong possibility of vector migration into Nigeria from the surrounding countries. Post *et al.* (2007) have listed the cytospecies present in Cameroon, Equatorial Guinea, Niger and Benin. In summary [with the data for countries bordering Nigeria updated from those provided by Post *et al.* (2007)], the cytospecies and cytotypes reliably recorded in Nigeria and her neighbours include:

Nigeria

S. damnosum SUBCOMPLEX

- S. damnosum* s.s. Nile form
- S. damnosum* s.s. Volta form
- S. sirbanum* Sirba form
- S. sirbanum* Sudanense form

S. sanctipauli SUBCOMPLEX

- S. soubrense* Beffa form

S. squamosum SUBCOMPLEX

- S. squamosum* A (typical form)
- S. squamosum* B
- S. squamosum* C
- S. yahense* (typical form)

Cameroon

S. damnosum subcomplex

- S. damnosum* s.s. Nile form

S. damnosum s.s. Volta form

S. sirbanum s.l.

S. squamosum SUBCOMPLEX

- S. squamosum* A (typical)
- S. squamosum* B
- S. squamosum* C
- S. squamosum* D

KIBWEZI SUBCOMPLEX

- S. mengense*

Benin

S. damnosum SUBCOMPLEX

- S. damnosum* s.s. Nile form
- S. damnosum* s.s. Volta form
- S. sirbanum* Sudanense form
- S. sirbanum* Type IV

S. sanctipauli SUBCOMPLEX

- S. soubrense* Beffa form

S. squamosum SUBCOMPLEX

- S. squamosum* C
- S. yahense* (typical form)

Niger

S. damnosum SUBCOMPLEX

- S. damnosum* s.s. Nile form
- S. damnosum* s.s. Volta form
- S. sirbanum* Sirba form
- S. sirbanum* Sudanense form
- S. sirbanum* Type IV

Equatorial Guinea (Bioko)

S. squamosum SUBCOMPLEX

- S. yahense* Bioko form

REVIEW OF DISTRIBUTION OF

THE CYTOSPECIES OF THE

S. damnosum COMPLEX IN NIGERIA,

WITH REFERENCE TO THE

DISTRIBUTION OF

ONCHOCERCIASIS

The distribution of human onchocerciasis in Nigeria was mapped by Crosskey (1981)

from a review of all of the relevant data that had then been recorded. Subsequently (between 1994 and 1996), the disease in the whole country was re-surveyed by the NOCP, using a technique, called REMO (rapid epidemiological mapping of onchocerciasis), that is based upon the prevalence of nodules in selected communities. This yielded preliminary maps (Gemade *et al.*, 1998; Noma *et al.*, 2002) that were then refined and completed to give a detailed working picture of onchocerciasis across Nigeria (WHO, 2004; Basáñez *et al.*, 2006). The maps have been used for the identification of areas of hyper- and meso-endemicity, which are the main targets for community-directed treatment with ivermectin (CDTI; Jija, 1998). The NOCP has established 26 CDTI projects in four primary health zones, with the aim of reaching over 27 million people (WHO, 2004). Although this would not have been possible without the detail provided by REMO, the basic pattern of onchocerciasis described in Nigeria by Crosskey (1981) remains broadly true. Onchocerciasis is now known to be more widely distributed in Nigeria and this has resulted in the filling of gaps in the map that Crosskey (1981) created.

Onchocerciasis is mostly absent from the lowland areas of Nigeria, including the regions that geographers call the Coastlands (in the delta area and the coastal plain), the Niger–Benue Trough (through which the Niger and Benue Rivers flow), the Sokoto Plains (in the north–western corner of Nigeria) and the Chad Basin (in the north–eastern corner) [see figure 3.3 of Iloeje (1981)]. This absence of the disease largely reflects the absence of hard Precambrian rocks; rapids, which form the breeding sites for *S. damnosum* s.l., only generally occur where hard rocks form outcrops in river beds (Crosskey, 1981). The Nigerian lowlands do have some pockets of onchocerciasis, such as a focus near Nguru, in the Chad Basin close to the border with Niger, that

has been mapped by REMO. Crosskey (1981) described a scattered series of small isolated foci associated with the Lower Niger Basin, and these are now known to be wider in extent, with onchocerciasis almost continuously distributed and associated with a number of geographical features, most notably the Udi Plateau and the Scarplands (Onyenwe *et al.*, 2007). In the uplands, the three northern zones of onchocerciasis recognised by Crosskey (1981) — Zones 1, 2 and 3 — have been much better defined and are now known to more-or-less run into each other. Zone 1 (Borgu-Sokoto) is now considered to be more-or-less continuous with Zone 5 (south–western zone, associated with the mostly forested Western Uplands), which itself is continuous across the lower Niger Valley (see above) into Crosskey’s Zone 4 (southern Adamaya, associated with the uplands along the border with Cameroon, including the Oban Hills, Obudu Plateau, Adamawa Plateau, Shebshi Mountains and Alantika Mountains). Crosskey’s (mid–northern) Zone 2 is centred on the network of major rivers that originate as headwater streams around the edges of the Jos Plateau, and is mostly Guinea savanna. Crosskey’s (north–eastern) Zone 3 is mostly Sudan savanna and extends from the Mandara mountains (on the border with Cameroon) and Biu Plateau, in a south–easterly direction, across the Gongola valley and along the northern edge of the Benue Trough to meet the Jos Plateau, where it runs into Zone 2.

Simulium squamosum is not known from Zones 1 (Borgu-Sokoto, Guinea savanna) or 3 (north–eastern, Sudan savanna) but has been reported to be widely spread in all the other zones (mostly Guinea savanna, savanna/forest mosaic, forest and/or montane; Mafuyai *et al.*, 1996a; Bassey, 1998; Traore-Lamizana *et al.*, 2001; Ibeh *et al.*, 2006). It is the only cytospecies to have been confirmed as a vector of *O. volvulus* in Nigeria (Mafuyai *et al.*, 1997), although further studies would be expected

to incriminate additional cytospecies. *Simulium squamosum* C seems to occur throughout Nigeria but is joined by *S. squamosum* A and *S. squamosum* B in the south-east (Traore-Lamizana *et al.*, 2001; Ibeh *et al.*, 2006). *Simulium squamosum* D (Mustapha *et al.*, 2004a) has not been recorded from Nigeria.

Simulium yahense has been reliably recorded from only eight sites in Nigeria, and these are all situated in the south-east (Akoh *et al.*, 1987; Mafuyai *et al.*, 1996; Bassey, 1998; Ibeh *et al.*, 2006), in an onchocerciasis endemic area where Crosskey's Zone 4 is now known to spread eastwards to join his scattered Zone-6 foci. Five of the eight sites are associated with the Udi Plateau (where it is forest or forest/savanna mosaic), one is on the Ibi River in the coastal lowland forest, and the other two are associated with the chain of mountains along the border with Cameroon at Agbokim Falls (on a tributary of Cross River) and the Obudu Plateau. The Bioko form of *S. yahense* (Post *et al.*, 2003) has not been recorded from Nigeria and is probably extinct (Traoré *et al.*, 2009).

It is probable that all records of *S. soubrense* and *S. sanctipauli* in Nigeria refer to the Beffa form of *S. soubrense* (see above). Cytological identifications of *S. soubrense* Beffa form were reported by Meredith *et al.* (1983) from the Okpara River on the border with Benin, in an area of Guinea savanna, where REMO has filled in the gap between Crosskey's Zones 1 (Borgu-Sokoto) and 5. *Simulium soubrense* Beffa form is also known to occur in Zone 5 (south-western rainforest; Post, 1986). It has also been identified cytotaxonomically by Post (1986) and Akoh *et al.* (1987) (as *S. sanctipauli*) around the Jos Plateau in Guinea savanna, in Crosskey's Zone 2. Cheke *et al.* (1987) believed that Meredith *et al.* (1983) had recorded the Beffa form of *S. soubrense* from the Gurara River, near Abuja (also in Zone 2). This identification was morphological and, whilst it needs to be confirmed cytologically, is consistent with the other records from the area.

Simulium damnosum s.s. and *S. sirbanum* have been frequently recorded in Nigeria but without any attempt to distinguish the cytotypes within the two cytospecies (i.e. the Volta form and Nile form of *S. damnosum* s.s., and the Sirba form and Sudanense form of *S. sirbanum*). Published reports for Nigeria show that *S. sirbanum* s.l. has a more northerly distribution than *S. damnosum* s.s. (Mafuyai *et al.*, 1996a; Bassey, 1998; Ibeh *et al.*, 2006; Onyenwe *et al.*, 2007). Of these two cytospecies, only *S. damnosum* s.s. has been recorded from the onchocerciasis focus in the rainforest of south-western Nigeria (Crosskey's Zone 5) (although *S. sirbanum* has been reported from the Okpara River where it forms the border with Benin), and *S. damnosum* s.s. is also common in south-eastern Nigeria (Crosskey's Zones 6 and 4), where *S. sirbanum* has only been recorded from five sites (including forest sites). Both species have been recorded from Crosskey's Zones 1 and 2, which are both situated in the Guinea savanna, but only *S. sirbanum* is known from the north-eastern onchocerciasis zone (Crosskey's Zone 3), which is situated in the Sudan savanna.

Vajime (1989) split *S. sirbanum* into two cytotypes (the Sirba form and Sudanense form) from analysis of material from eight countries, including one site in Nigeria (near Zaria, on the edge of Crosskey's Zone 2) where he found both cytotypes. Onyenwe *et al.* (2007) identified the Sudanense form of *S. sirbanum* breeding at a Guinea-savanna site in south-eastern Nigeria, in an onchocerciasis focus in the Scarplands (Ubachukwu, 2004). Vajime and Dunbar (1975) considered that *S. sudanense* [*sensu* Vajime and Dunbar (1975)] was mostly sympatric with *S. sirbanum* [*sensu* Vajime and Dunbar (1975)], and analysis of the proportions represented by the Sirba and Sudanense forms, using data from Vajime (1989) and Onyenwe *et al.* (2007), showed no correlation with latitude (Spearman's $r_s=0.0838$; $P>0.25$), and hence no evidence for a north-south separation (unpubl. obs.).

There is very little information available concerning the distribution of the Nile and Volta forms of *S. damnosum* s.s.. Dunbar and Vajime (1981) did not present any collection data but simply stated that the Nile form occurred in the rivers draining the Jos Plateau and in the rivers in eastern Nigeria, whereas the Volta form occurred elsewhere in Nigeria. When, however, Vajime and Gregory (1990) reassessed the situation, they found that both forms could be found across the full width of the savanna belt, from Guinea in the west to Uganda in the east. Both forms were common in the Guinea savanna, but the Nile form tended to extend its range into the Sudan savanna and the Volta form into the forest. Vajime and Gregory (1990) presented collection data from 12 sites in Nigeria, which showed the Volta form in sites in the forest onchocerciasis zones of south-eastern and south-western Nigeria and in the Borgu-Sokoto onchocerciasis zone (Guinea savanna). The Nile form was generally much more common and also found in the forest onchocerciasis zones of south-eastern and south-western Nigeria and in the Borgu-Sokoto onchocerciasis zone, as well as in the southern Adamawa onchocerciasis zone (Crosskey's Zone 4) and in the rivers draining the Jos Plateau (Crosskey's Zone 2).

THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE BREEDING SITES

The physico-chemical characteristics of rivers are known to be correlated with the distribution of different species of black-flies (Crosskey, 1990). *Simulium damnosum* s.l. is traditionally considered to be a species of fast-flowing, broken 'white-water' in rapids of rivers, but also medium-sized streams, especially in upland areas (Crosskey, 1990). Grunewald (1976, 1981) noted that members of the *S. damnosum* complex in West Africa have

not been found in rivers with water velocities of <0.4 or >2.4 m/s, nor from rivers with water temperatures lower than 16.8°C or greater than 33°C (most being found in water at 18.3 – 31.8°C in West Africa; Ocran *et al.*, 1982). Members of the complex have also not been found breeding in rivers with high levels of ammonia (>0.5 mg $\text{NH}_4\text{-N/litre}$), a sign of organic pollution, although some other *Simulium* species, such as *S. hargreavesi* and *S. adersi*, are found in such rivers (Grunewald, 1976, 1981). *Simulium damnosum* s.l. only breeds in water with (a wide range of) relatively high oxygen saturations (75% – $>105\%$), whereas other species, such as *S. alcocki* and *S. unicornutum*, have been recorded in Nigerian rivers with only 21.5% oxygen saturation (Grunewald, 1976, 1981). The physico-chemical characteristics of Nigerian rivers occupied by members of the *S. damnosum* complex have been published by Mafuyai *et al.* (1996), Bassey (1998), Nwoke and Uwazie (1991), Opara and Fagbemi (2005), Ikpeama *et al.* (2006), Ibeh *et al.* (2007) and Onyenwe *et al.* (2007) [although Nwoke and Uwazie (1991), Opara and Fagbemi (2005) and Ikpeama *et al.* (2006) did not identify the cytospecies involved]. In Nigeria, members of the complex have been mostly found breeding in water with velocities of 0.55 – 2.2 m/s, but Ibeh *et al.* (2007) reported a mean maximum of 2.8 m/s, and Opara and Fagbemi (2005) reported a range of 0.1 – 5.6 m/s. The latter observation is remarkable because it not only extends both the upper and lower limits for the velocities of water supporting breeding *S. damnosum* s.l., but also the upper limit for Simuliidae as a whole, which was previously recorded at 0.05 – 3.5 m/s (Crosskey, 1990). It is important that these exceptional findings are confirmed. Nigerian breeding sites have been found to have water temperatures of 18 – 33°C and pH values of 5.6 – 8.7 , which are within the limits recorded elsewhere for *S. damnosum* s.l. (Grunewald, 1976, 1981). Opara and Fagbemi (2005) recorded *S.*

damnosum s.l. breeding in rivers with unusually high levels of ammonia in Nigeria (but always <1 mg/litre, except on one occasion when an unexplained measurement of approximately 6 mg/litre was recorded).

With respect to the different sibling species within the *S. damnosum* complex, Quillévéré *et al.* (1977) concluded that the only physico-chemical factor that separated the different cytospecies throughout the whole year was pH. Although Grunewald (1976, 1981) considered that conductivity was also important, dividing the West-African members of the complex into Group I [*S. yahense*, *S. sanctipauli* and *S. mengense*, all supposedly found in acidic waters (pH 5.7–6.2) with low conductivity (<50 µS/m)] and Group II [*S. squamosum*, *S. soubrense*, *S. damnosum* s.s. and *S. sirbanum*, all supposedly found in nearly pH-neutral rivers with higher conductivity (50–150 µS/m)], the results from Nigeria do not support this separation. Mafuyai *et al.* (1996), Bassey (1998) and Opara and Fagbemi (2005), for example, recorded low conductivity (0.5–20 µS/m) at all breeding sites used by members of the *S. damnosum* complex in Nigeria, including those used by sibling species in Grunewald's Group II. Onyenwe *et al.* (2007) also recorded *S. sirbanum* (Sudanense form) breeding throughout the entire year in fairly acidic water (pH 5.6–5.9), although Mafuyai *et al.* (1996) and Bassey (1998) recorded higher pH values (range 6.5–7.9) at all the breeding sites for the *S. damnosum* complex that they investigated in Nigeria. Ibeh *et al.* (2007) found *S. yahense* in neutral to alkaline rivers (pH 6.5–8.6) and *S. squamosum* in water showing an even wider range of pH values (6.4–8.7).

The differences seen in the physico-chemical characteristics of breeding sites used by a particular cytospecies, between the different Nigerian studies and between Nigeria and other parts of West Africa, could be related to cytoform differences. Onyenwe *et al.* (2007), for example, were dealing with the Sudanense form of *S. sirbanum*, and it is not known whether other related studies involved the Sirba form or Type IV. Similarly, it is not clear whether studies of *S. damnosum* s.s. in Nigeria refer to the Nile form, which is probably relatively uncommon to the west of the country (Boakye, 1993). *Simulium squamosum* in Nigeria is represented by *S. squamosum* A, B and C, whereas further west only *S. squamosum* C and E are found.

CYTOTAXONOMIC KEY FOR THE
IDENTIFICATION OF MEMBERS
OF THE *S. damnosum* COMPLEX
IN NIGERIA AND THE
SURROUNDING COUNTRIES, USING
POLYTENE CHROMOSOMES FROM
THE LARVAE

The key is based upon preparation and examination of polytene chromosomes from the larval silk glands (see above), and scoring the banding pattern of the three pairs of chromosomes (Fig. 1). Many of the diagnostic criteria in this key refer to the characteristics of populations rather than individual specimens. For example, the determination of sex linkage of inversion 2L-18 in couplet 3 requires the cytotaxonomic analysis of sufficient numbers of larvae from the population being studied. Taxa known to occur in Nigeria are indicated in **bold** type.

1. 1C expanded (Fig. 2) 1C normal (Figures 1 and 14)	<i>S. mengense</i> Other cytospecies (2)
2. 2L-St/St, St/18 or 18/18 (Figures 3, 4 and 5) 2L-C/C, C/C.8 or C.8/C.8 (Figures 6, 7 and 8) 2L-4.6/4.6 or 4.6.A/4.6.A (Figures 9 and 10)	<i>S. squamosum</i> subcomplex (3) <i>S. damnosum</i> subcomplex (7) <i>S. sanctipauli</i> subcomplex (10)
3. 2L-18 sex linked (Figures 3, 4 and 5) 2L-18 absent or not sex-linked	<i>S. yahense</i> (4) <i>S. squamosum</i> (5)
4. Males 2L-St/18; females 2L-18/18 (Figures 4 and 5) Males 2L-St/18; females 2L-St/St (Figures 3 and 4)	<i>S. yahense</i> typical form <i>S. yahense</i> Bioko form
5. Males with 1C long split (ICa) (Fig. 11) Males with 1C short split (ICb) (Fig. 12) Males without 1Ca or ICb	<i>S. squamosum</i> 'A' <i>S. squamosum</i> 'B' Other <i>S. squamosum</i> cytotypes (6)
6. Males 1S-To/St (Fig. 13) Males without 1S-To (Fig. 14)	<i>S. squamosum</i> 'D' <i>S. squamosum</i> 'C'
7. 2L-C/C or 2L-C/C.8 (Figures 6 and 7) 2L-C.8/C.8 (Fig 8)	<i>S. damnosum</i> s.s. (8) <i>S. sirbanum</i> (9)
8. Males 2L-C/C.8; females 2L-C/C (Figures 6 and 7) Males 2L-C/C; females 2L-C/C (Fig. 6)	<i>S. damnosum</i> s.s. Volta form <i>S. damnosum</i> s.s. Nile form
9. Males 1S-St/3 or St/St; females 1S-St/St (Figures 14 and 15) Males 1S-St/3; females 1S-3/3 (Figures 15 and 16) Males 1S-3/3; females 1S-3/3 (Fig. 16)	<i>S. sirbanum</i> Sirba form <i>S. sirbanum</i> Sudanense form <i>S. sirbanum</i> Type IV
10. 2L-4.6/4.6 (Fig. 9) 2L-4.6.A/4.6.A (Fig. 10)	<i>S. soubrense</i> (11) <i>S. sanctipauli</i>
11. Males 2S-St/St (Fig. 17) Males 2S-St/6b (Fig. 18)	<i>S. soubrense</i> typical form <i>S. soubrense</i> Beffa form

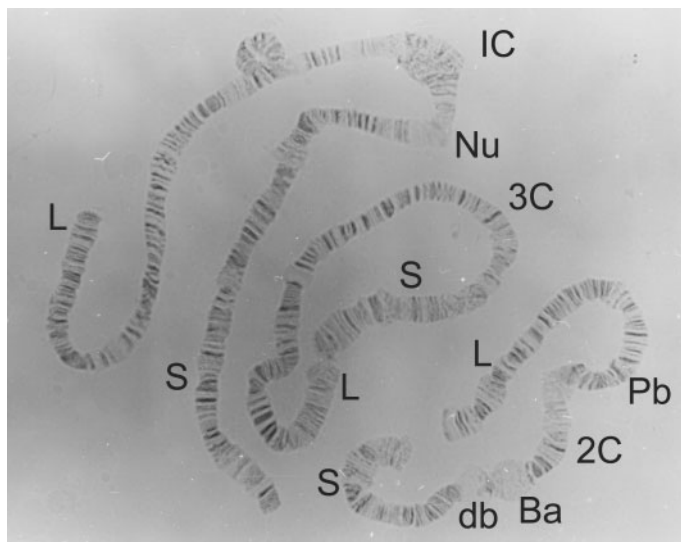


FIG. 1. Full karyotype of *Simulium squamosum*, showing the standard sequence (St/St) in all chromosome arms and normal centromeres. Chromosomes 1, 2 and 3 are labelled at the centromere (as 1C, 2C and 3C, respectively) and the short and long arms are labelled S and L, respectively. Other chromosomal landmarks include the nucleolus organizing region (Nu), the Ring of Balbiani (Ba), the double bubble (db) and the parabalbiani (Pb).

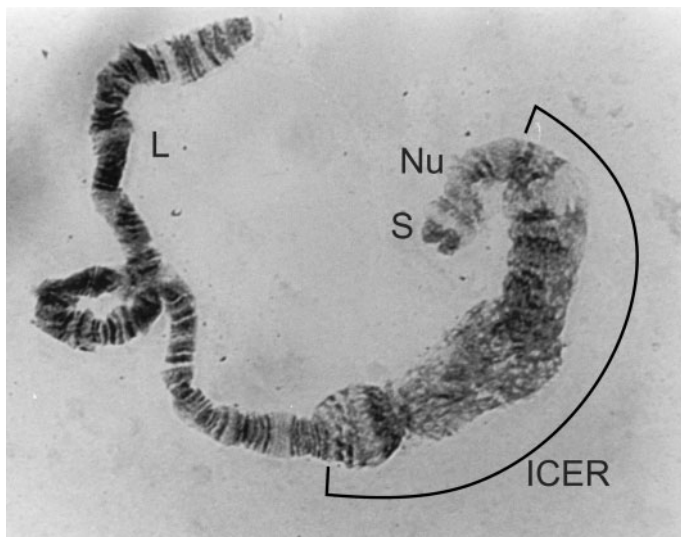


FIG. 2. 1C expanded centromere (ICER) from *Simulium mengense*.

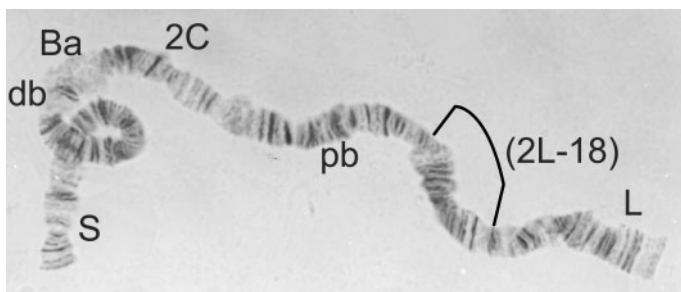


FIG. 3. 2L-St/St from *Simulium squamosum*, showing the relative position of inversion 2L-18.

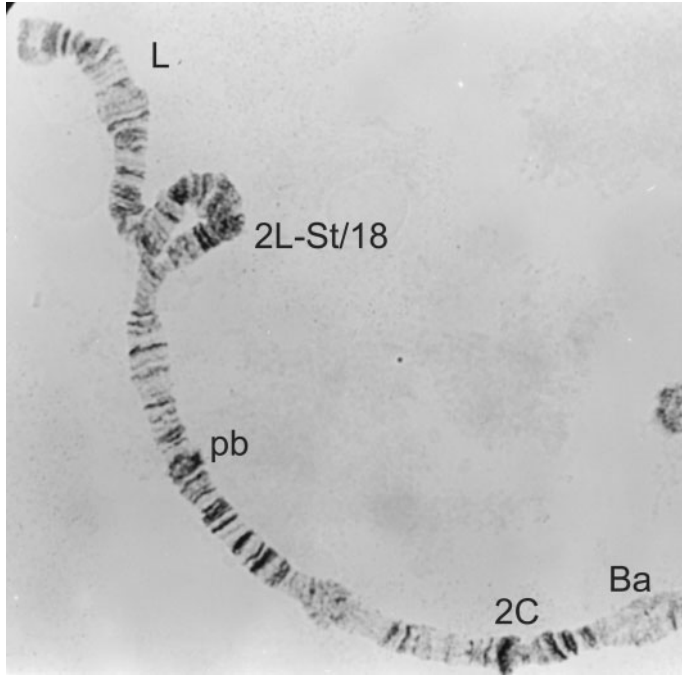


FIG. 4. 2L-St/18 heterozygote from a male *Simulium yahense*.

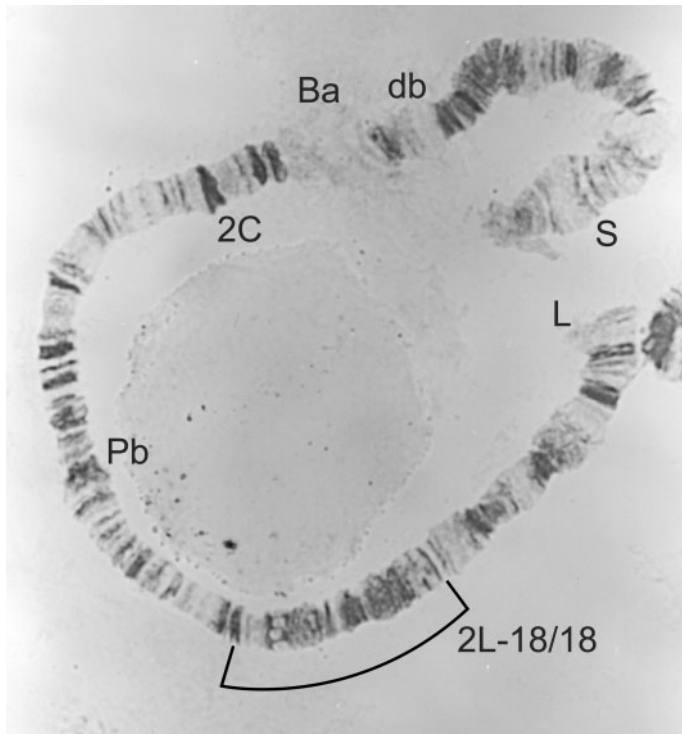


FIG. 5. 2L-18/18 homozygote from a female *Simulium yahense*.

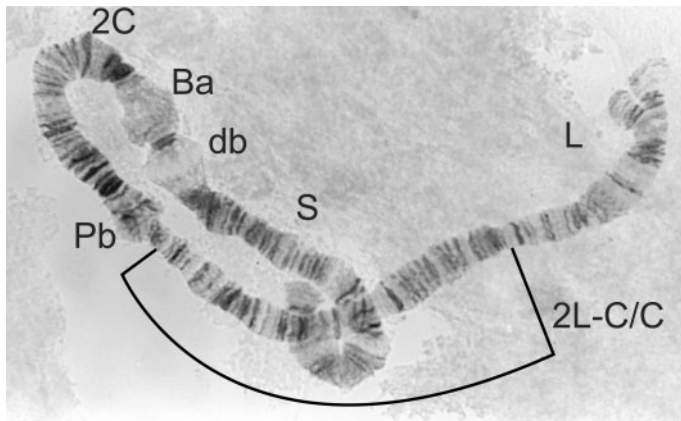


FIG. 6. 2L-C/C homozygote from *Simulium damnosum* s.s.

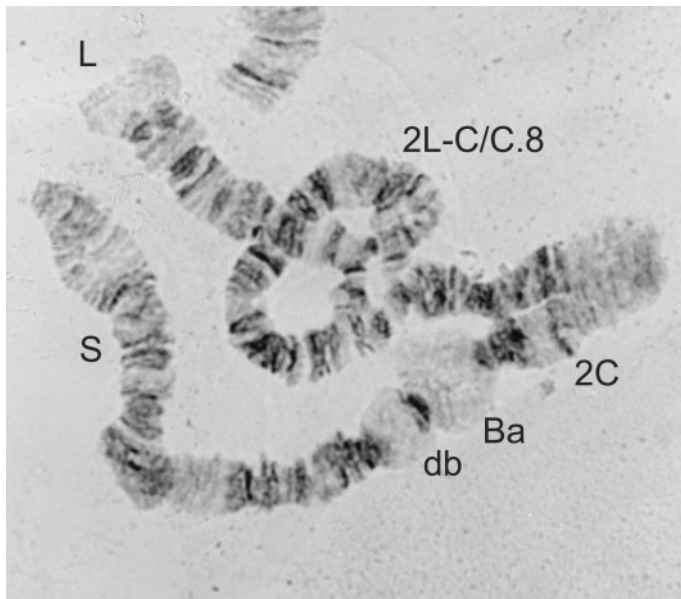


FIG. 7. 2L-C/C.8 heterozygote from a male *Simulium damnosum* s.s.

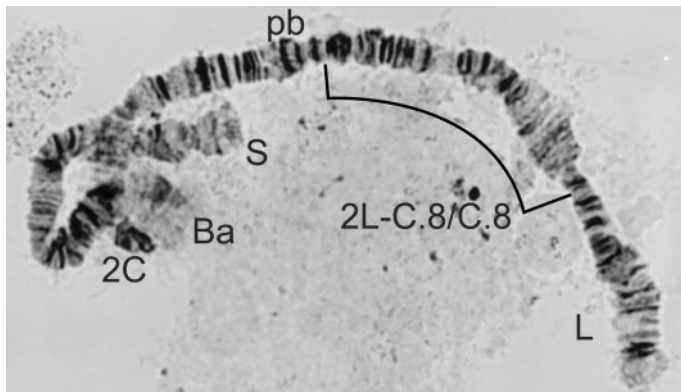


FIG. 8. 2L-C.8/C.8 homozygote from *Simulium sirbanum*.

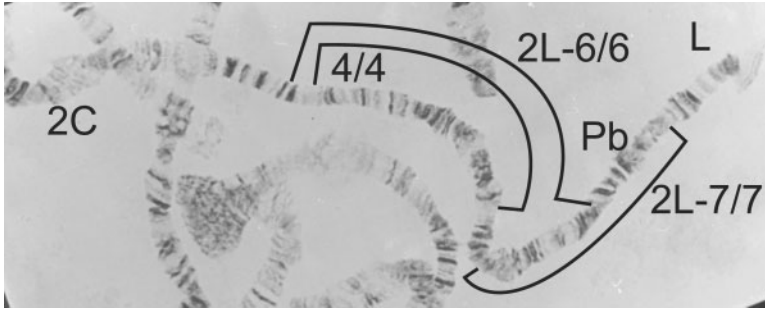


FIG. 9. 2L-4.6/4.6 from *Simulium soubrense* (also homozygous for 2L-7/7).

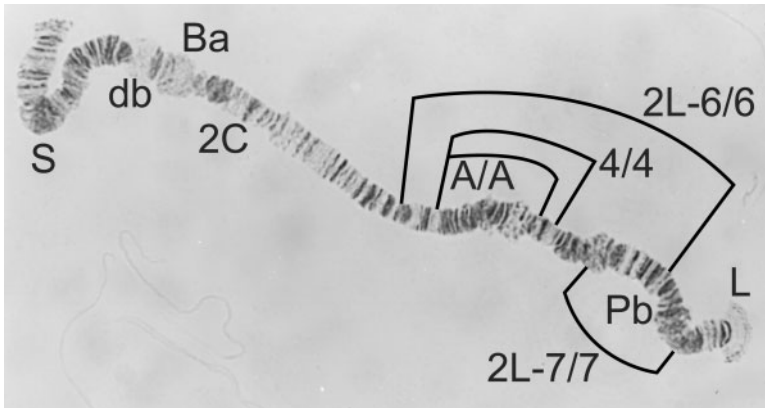


FIG. 10. 2L-4.6.A/4.6.A from *Simulium sanctipauli* (also homozygous for 2L-7/7).

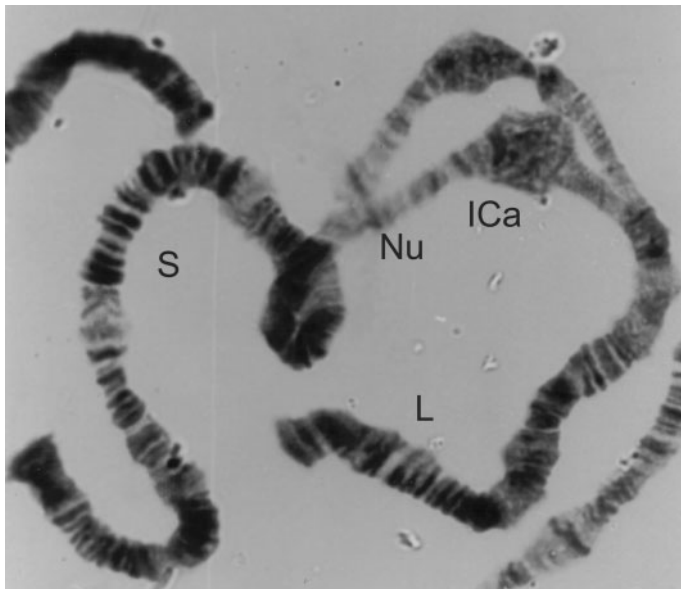


FIG. 11. 1C long split from a male *Simulium squamosum* 'A'.

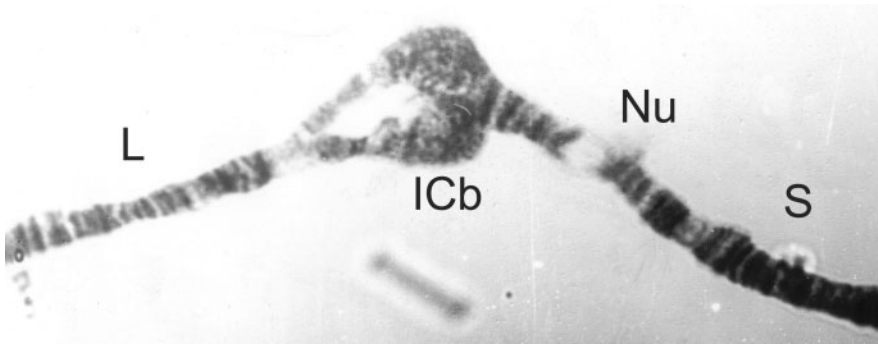


FIG. 12. 1C short split from a male *Simulium squamosum* 'B'.

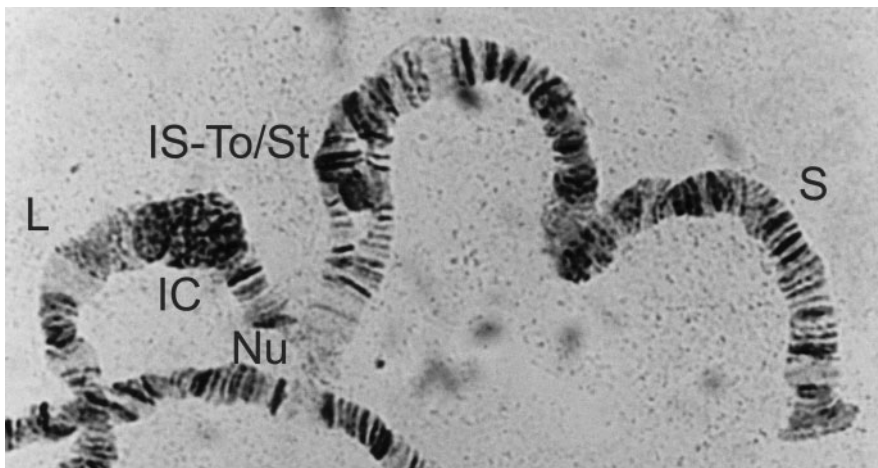


FIG. 13. 1S-St/To from a male *Simulium squamosum* 'D'.

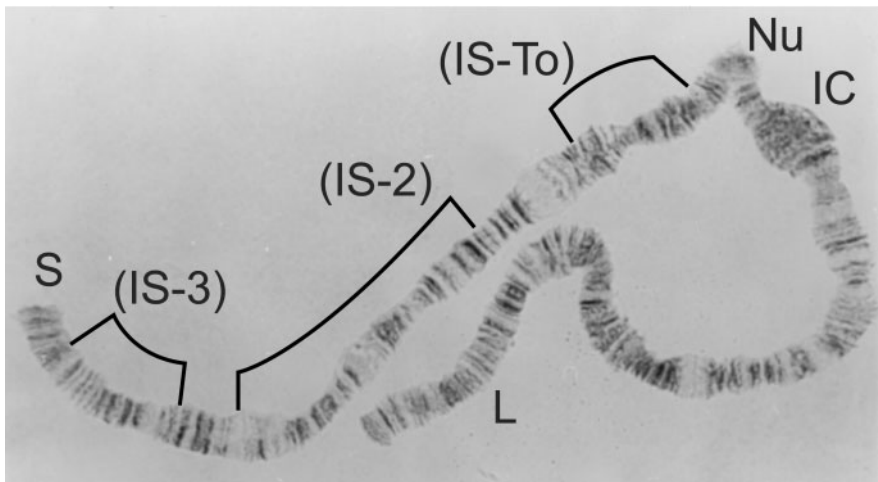


FIG. 14. 1S-St/St and 1C normal from a male *Simulium squamosum* 'C' (also showing the relative position of inversions 1S-To, 1S-2 and 1S-3).

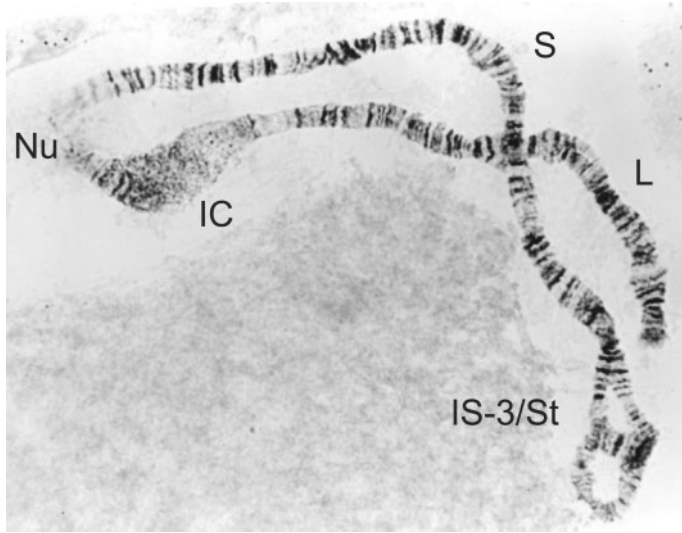


FIG. 15. 1S-St/3 heterozygote from a male *Simulium sirbanum*.

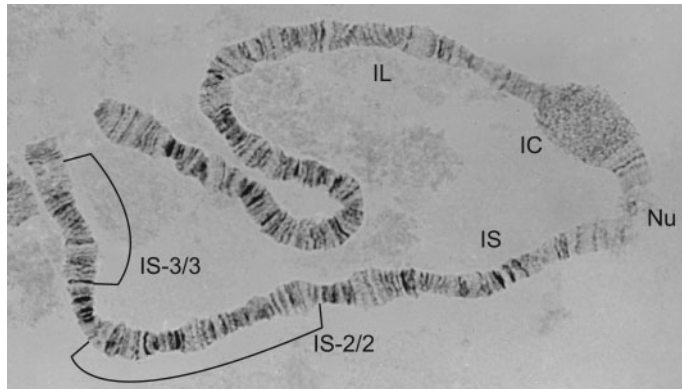


FIG. 16. 1S-3/3 homozygote from a female *Simulium sirbanum* (also homozygous for 1S-2/2).

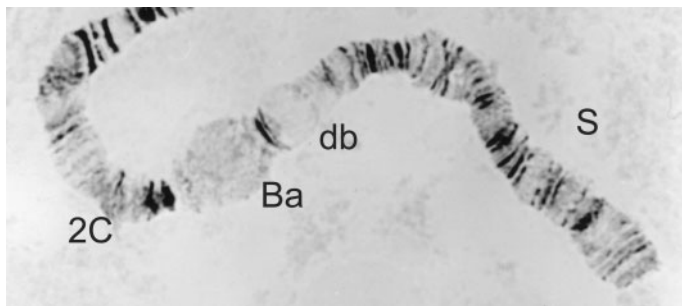


FIG. 17. 2S-St/St homozygote, as seen in a female *Simulium soubrense* of the Beffa form.

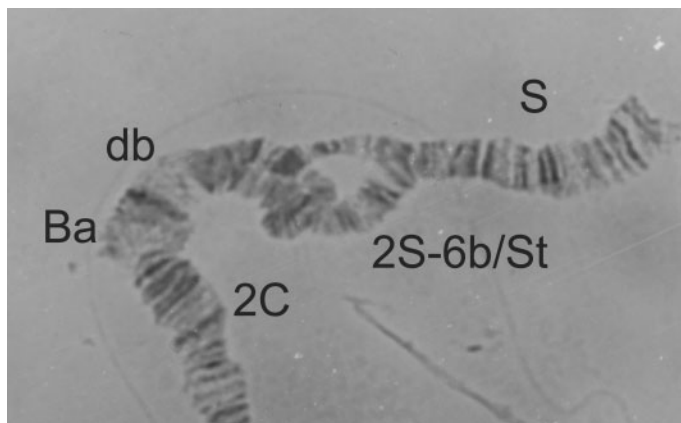


FIG. 18. 2S-St/6b heterozygote from a male *Simulium soubrense* of the Beffa form.

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