

THE
B British
S Simuliid
G Group
B Bulletin

Number 25 – January 2006



THE BRITISH SIMULIID GROUP

The British Simuliid Group (BSG) is an informal gathering of scientists of any discipline, from many countries, who have an interest in the Simuliidae. The group's members include entomologists, parasitologists, environmentalists, ecologists and medics, with interests in ecology, bionomics, taxonomy, cytotoxonomy, disease transmission, freshwater biology etc. Our aim is to assemble as diverse a group as possible in order to encourage a wide interchange of ideas and information.

At present the BSG has about 130 members in the UK, Europe, Africa, Australia, New Zealand and the Americas. Membership is FREE - if you're not already a member of the BSG all you have to do is give us your name and postal and e-mail addresses.

Annual meetings have been held at different locations in the UK since 1978. Abstracts of papers presented are published in our Bulletin which is sent free to all members of the group.

The Group also runs an electronic news list with the name "**Simuliidae**" which is now on **JISCMail**. To join "**Simuliidae**" send the following command as one line of text in an e-mail message without subject heading- **join simuliidae your-firstname lastname**
to: **jiscmail@jiscmail.ac.uk**

Membership of "Simuliidae" does not automatically make you a member of the BSG. You have to join each separately. The Simuliidae list owners are the Hon. Secretary and the Editor of the Bulletin.

Recent back numbers of the Bulletin can be viewed on the World Wide Web at URL:
http://www.blackflies.org.uk

*Inquiries about the Group and its activities should be made to John Davies: address inside back cover and e-mail **daviesjb@liverpool.ac.uk***

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The British Simuliid Group Bulletin

The British Simuliid Group Bulletin is an informal publication intended to disseminate information about the Simuliidae. It is published twice each year and is distributed free to all members of the British Simuliid Group.

Content covers papers presented at the Group's Annual Meeting, which is usually held in September, short research notes, notices and accounts of meetings, and articles of anecdotal or general interest that would not normally be found in international journals. Geographical cover is world-wide, and is not restricted to the British Isles. Reports of research carried out by graduates, young scientists and newcomers to the subject are particularly encouraged. It is an ideal medium for offering new ideas and stimulating discussion because of the very short interval between acceptance and publication.

The Bulletin's editor is John B. Davies, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK. E-mail: daviesjb@liverpool.ac.uk

Notes for Contributors

To avoid copy-typing, the editor (address above) would prefer to receive contributions on disc or by e-mail, or typewritten. Details as follows:-

1. Via conventional mail on IBM PC formatted 720Kb or 1.4Mb 3.5 inch diskettes, as unmodified word processor files (most common DOS or Windows word processor formats are acceptable) or as RTF, ASCII or DOS text files (We usually have to change pagination and heading format, anyway). Mark the disc with the format, word processor name and file name(s). Complicated tables and figures can be accepted as separate graphics files (not OLE embedded, please!) but we may ask for a hard copy as a check that all detail has been retained. Remember that figures should have legends and small detail drawn large enough to be visible when reduced to 100mm by 70mm. Diskettes will be returned on request.

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Format for References is flexible. Please refer to the Bulletin for the form appropriate to your article. Scientific Communications should quote the full title and journal name, but Notes and Abstracts may optionally omit titles and show only the abbreviated journal name.

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FROM THE EDITOR

Welcome to Bulletin Number 25. This number is primarily occupied by the report of the 27th Annual Meeting of the Group, held on 2nd. September 2005 in Oxford. Unfortunately, we ran out of time at this meeting and were not able to discuss any plans for the next meeting.

Bearing in mind that we had no meeting in 2004, and the proposed meeting in Novi Sad, 2006, it has been suggested to me that our meeting policy needs to be re-examined. Regrettably the number of active simuliidologists in Britain is dwindling as age takes its toll and the emphasis in research swings away from the old driving force of onchocerciasis vector control. Is there still merit in holding our meetings annually? They are great opportunities to meet old friends, but that could be achieved by holding annual dinners. Would that be attractive enough to entice members to a gathering? Should we meet every two years, or would that lead to a general falling off of interest? What do our members think?

Please let me have your views:-

1. Write by mail to me at 57 North Parade, Hoylake, Wirral,
CH47 3AL,
2. By E-mail to daviesjd@liv.ac.uk
3. Record your views via the web on the Blackfly Wiki at:

<http://blackflies.watersheep.org/phpwiki/index.php/HomePage>
and click on BritSimuliumGrpMeetingsDiscussion.

John Davies

MEETINGS

Announcement

2nd International Simuliidae Symposium

3rd- 6th of September 2006

Novi Sad, Serbia and Montenegro

To be held at the Faculty of Agriculture
Department for Environmental and Plant Protection,
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<http://www.blackflies.org.uk>

The British Simuliid Group 27th Annual Meeting

The 27th Annual Meeting was held on the 2nd September 2005 in the Seminar Room of the Oxford University Museum of Natural History, Parks Road, Oxford.

As is now customary, a dinner was held the previous evening at the Chiang Mai Kitchen Restaurant where 17 members and friends enjoyed an ample Thai dinner.

The meeting was attended by 15 participants including one from Serbia and Montenegro and two from Germany, John Davies took the chair. Proceedings were opened by Dr. Tom Kemp, Senior Curator and Head of Zoology who gave a short description of the origins of the museum. Because it is an interesting story, the following account combines his observations with notes gleaned from the Museum's web site.

Up to the mid-1800's the University's scientific collections of zoological, entomological, palaeontological and mineral specimens, accumulated in the course of the previous three centuries were scattered around the University and the Colleges, and included such important accumulations as the natural history part of the great Ashmolean Museum collections, a large comparative anatomy and physiology collection held at Christ Church, and the Dean Buckland's collection of fossils.

The inspiration and drive to create a single building to house all this material, to display it, and to be the centre of the teaching of natural sciences in Oxford came from Sir Henry Acland, the then Regius Professor of Medicine, supported by John Ruskin.

As a student in Christ Church College in the 1830's, John Ruskin found that academic life in Oxford was still firmly based on the study of Greek and Latin classics. In the 1840's, plans were developed for a new School of Natural Science, but there were no facilities for science.

Under the leadership of Henry Acland, Lee's Reader in Anatomy at Christ Church, who had been a fellow student of Ruskin's at Christ Church, plans for a museum began to take shape. It would be a pioneering building, bringing together the study of Physics, Chemistry, Mineralogy, Geology, Zoology, Anatomy, Physiology, and Medicine, with all the necessary laboratories and offices. It would be full of living knowledge,

a beautiful place where those who cared about the natural world could work and learn together. Acland, like Ruskin, believed that a serious study of science could only promote a fuller understanding of God's dealings with mankind. More conservative forces in the University interpreted change as an attack on religion. Even Oxford's leading scientist, the Reverend William Buckland, Professor of Geology, opposed the new building and any formal teaching of natural history. But the University Commissioners finally gave their support in 1852. After many squabbles over its design the new Museum was finally completed in 1860, and remains a gem of middle Victorian neo-Gothic architecture.

In 1859, just as the building was nearing completion, Charles Darwin published his ground-breaking work on the Origin of Species by Natural Selection. Darwin's evolutionary theory was to drive a decisive wedge between the interests of Victorian science and those of religion.

In one of the many ironies of the Museum's story, its first-floor library, then still empty, became the venue in 1860 for what turned out to be a famous and dramatic meeting of the British Association for the Advancement of Science at which Darwin's theory was debated. Thomas Henry Huxley robustly championed Darwin's work, while Samuel Wilberforce ('Soapy Sam'), the Bishop of Oxford, spoke in fierce opposition. Feelings ran high, and insults were freely traded. The Bishop acidly mocked the idea that a man could have apes for ancestors while Huxley vehemently scorned the Bishop's scientific ignorance. The debate was emblematic of the defeat of ideas about the unity of science and religion that the museum had been intended to express.

The meeting's final talk was a presentation by Aleksandra Cupina of the University of Novi Sad, Serbia and Montenegro, who outlined the plans for the proposed 2006 Simuliidae Symposium, supported by a short video film of the city and conference

facilities. The dates for the meeting were discussed, with a consensus agreeing that the last week of August or the first week of September 2006 would probably be the most suitable.

The meeting was closed with a vote of thanks to Adrian Pont who had negotiated the use of the Seminar Room, booked the restaurant and provided a complimentary supply of Blandford Fly Premium Ale, courtesy of Badger Breweries.

Papers presented at the 27th. Meeting

Laser-assisted microdissection of polytene chromosomes of *Simulium damnosum* s.l.

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Whilst there have been some successes with morphology, isoenzymes and DNA, attempts to find characters for identification of adults of sibling species of the *Simulium damnosum* complex have proved to be generally disappointing over the last 30 years. Because the sibling species have all been defined on the basis of chromosome variation (usually inversions observed in the polytene chromosomes of the larvae) a molecular (DNA) method for scoring the presence/absence of the diagnostic inversions would be as accurate as cytotaxonomy. However, in the absence of a blackfly genome project we have no idea as to the DNA sequences associated with these inversions. We describe a method we have developed to overcome this problem. We have physically dissected and isolated sections of polytene chromosomes using laser microscopy (for example the section containing the proximal breakpoints of inversion 1S-AK which is diagnostic for *Simulium thyolense*) and amplified rDNA from the

nucleolus organising region. This technique enables the development of PCR assays across the breakpoints of diagnostic inversions and should be applicable to all known cytospecies of the *Simulium damnosum* complex (and all other species complexes of blackflies).

Molecular taxonomy of British Simuliidae: A Preliminary Investigation

John C. Day¹, Stephanie J. Hunter¹ & Rory J. Post²

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DNA barcoding is a process whereby short DNA sequences from a pre-determined region of the genome are used for species identification purposes. Compiling a public database of DNA barcodes linked to voucher specimens may, in the future, provide a powerful system for species determination. Furthermore, this method of molecular taxonomy will produce a versatile tool for the establishment of cryptic species within a complex and thereby providing a true measure of biodiversity within the environment.

The blackfly fauna of Britain is currently composed of thirty four reported species; the majority of which can be identified based upon morphological characters (Bass, 1998; Crosskey, 2005). However, it is often the case that species may only be determined based on pupal characters and failing this in some instances a cytotaxonomic approach is necessary to establish species status (Brockhouse, 1985; Leonhardt, 1985).

DNA barcodes have been shown to effectively identify, as well as define, novel sibling species (Herbert *et al.* 2004). Herbert *et al.* used the sequence diversity in a 648-bp region of the mitochondrial gene, cytochrome c oxidase I (COI), to establish

ten new species of skipper butterfly, *Astrartes fulgurator* and proposed this region may provide satisfactory discrimination as a DNA barcode for the identification of other animal species.

Our preliminary investigation presents a small study to test the effectiveness of a COI barcode to discriminate closely related British blackfly species, *Simulium (Wilhelmia) equinum* and *Simulium (Wilhelmia) pseudequinum*. A sequence threshold of 3% was employed, typically encountered between congeneric species pairs recognized by morphological approaches (Herbert *et al.* 2004), to establish the resolving power of this mitochondrial locus to differentiate closely related Simuliidae species. Between *S. equinum* and *S. pseudequinum* a sequence divergence value of greater than 7.6% was seen, two-fold greater than that of the established sequence threshold. To place in context, a sequence comparison was made between *Wilhelmia* species and *Simulium posticatum* in order to establish intra-subgenera sequence variation. Sequence divergence values were 14.9% and 16.9% for *S. pseudequinum* and *S. equinum* respectively indicating high sequence divergence between subgenera.

To investigate the conservation of a barcode within a species and the effect of individual variation nine specimens of *S. equinum* were examined from three different populations in Dorset, Cambridgeshire and Hampshire. Less than 0.5% sequence divergence was found in *S. equinum* from these populations ranging in geographical distance from 62 - 214 km.

Our preliminary investigation into the sequence diversity of COI indicates this locus will be sufficient to differentiate all morphospecies of British blackflies. With a window of 37% sequence diversity, it is also anticipated that cytotaxa will be successfully resolved using DNA barcodes. Due to the high sequence divergence value between our two closely related taxa, the conservation of this sequence in species over 200 km apart and the high amplification success of the COI region in our test samples, this region will become the focus of our further examination of blackfly DNA barcodes in Britain.

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***Simulium (Schoenbaueria) nigrum* Meigen: its developmental cycle and some unresolved questions**

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Both abiotic and biotic factors determine species spectrum and biodiversity within particular habitats. Extensive changes in species composition are frequently the result of human influences on environmental factors, that is to say they drive changes in the biocoenoses. This is particularly clear in aquatic habitats where studies of the European black fly fauna have shown both recent and historical changes in species distribution.

Recent research projects concerned with the occurrence of simuliid pest species have shown that massive populations of *Simulium nigrum* Meigen are present in the river Oder in north east Germany (Werner, 2003). This species belongs to the subgenus *Schoenbaueria*, a subgenus containing 17 supposedly valid species (Crosskey, 2001), the group has many unresolved taxonomic and ecological questions (Peter Adler, pers. comm.).

Simulium nigrum was described by Johann Wilhelm MEIGEN in 1804, with the type locality near Stolberg, a small town close to Aachen in the west of Germany. There are no other records of *nigrum* published from Germany in the 19th century. The Rivers Maas in The Netherlands and River Rhine in Germany could have been the breeding sites for *nigrum*, but the species no longer occurs in any of the rivers around Aachen (Werner, pers. obs.).

Professor Günther ENDERLEIN expanded the Simuliid collection

of the Zoological Museum of Berlin during the first third of the 20th century. His material contains specimens from the subgenus *Schoenbaueria* (Zwick, 1995) and has helped us in our comparison of historical distribution records with the *nigrum* population now occurring in the River Oder (Werner, unpubl.).

For decades the River Oder has been at the centre of conflicts between rival claims of water management, nature conservation, tourism and country border controls. Like other large lowland rivers in Central Europe, it has been characterised by extremely high levels of pollution which have fallen in recent years.

Simulium nigrum and *Simulium reptans* (Linnaeus) are the pest species which occur at massive population levels in the lower section of the river. It is interesting that *S. nigrum* disappears when *Simulium erythrocephalum* (De Geer) occurs, and vice versa. *Simulium nigrum* is able to build up massive populations and displaces other species such as *reptans* in the lower river reaches. *Simulium nigrum* and *reptans* are now the most abundant and aggressive pest species, but the precise details of the developmental cycles remain to be clarified. In 1994, only a few larvae of *nigrum* were found in the Oder, but now 100% of some larval collections are *nigrum*.

It has become apparent that in Central Europe the clean-up and natural recovery of polluted streams and rivers, and general improvements in the water quality, have resulted in massive populations of various insect species, including some that can become serious pests of man and animals because of their mode of life. Fatal injuries to farm stock, including oedema, internal bleeding, and dermal lesions have been caused by mass attacks by *nigrum* and *reptans* near the River Oder. The meadows along the Oder are used for food production and animal husbandry, but in recent years farmers have lost cattle in spring as a result of black fly attacks.

For several years we were not able to observe the mating or

oviposition behaviour or to find the eggs of *nigrum*. After washing and checking shells, sediments, roots, plants and stones, we were finally able to find eggs in the river sediment, and in dry sand along the bank. Laboratory rearing experiments confirmed that these were *nigrum* eggs (Bass & Werner, in press). Our field studies on *nigrum* continued in 2005 but no mating or oviposition behaviour was observed. Sampling of the aquatic life stages established the expanding distribution of *nigrum* along the River Oder and its main tributaries.

Along the lower parts of the river the banks are stabilised by stone breakwaters which extend 20 – 30m out at a right angle to the river bank. They are spaced at regular intervals and some support vegetation and can become partially covered in sediment. These breakwaters play an important role in sediment accumulation and may intercept and slow down the downstream movement of *nigrum* eggs. Currently, we speculate that *nigrum* eggs are laid directly on to the water surface. *Simulium nigrum* eggs recovered from water samples and sediment deposits are unattached to debris and occur as single eggs. This is in contrast to the eggs of *Simulium equinum* (Linnaeus), *Simulium ornatum* Meigen and *reptans*, which are attached in masses to living or non living material in fast flowing water, though sometimes clusters of washed-off eggs were found within the fine sediment.

Eggs extracted from sediment collected at Lebus (January 2005) revealed some clear trends in *nigrum* egg location with most eggs concentrated in the sediment layer between 1.60 and 2.40 metres below the maximum water level. Sampling sediment at various times through the year, at several sites, revealed some aspects of *nigrum* egg distribution. We speculate that *nigrum* eggs drift as the water level changes and eggs are re-distributed and washed deeper into the sediment, which reduces the overall downstream movement and potential loss of eggs from the river.

Our conclusions are:

- ? *Simulium nigrum* definitely produces only one generation per year
- ? its aquatic life stages show relatively high tolerance to water pollution
- ? adults emerge on the River Oder from May to June, when the water temperature is around 8 – 10 °C.
- ? mating and oviposition behaviour is still unknown
- ? males feed on hawthorn blossom in large numbers
- ? we predict that females lay their eggs singly on the water surface
- ? eggs drift down the river and initially settle in areas with low water velocity
- ? in the period 2000-2005 *nigrum* has extended its range more than 200 km upstream on River Oder.

The precise habitat requirements of *Simulium nigrum* and *Simulium reptans* have still to be clarified. However, both species colonise large lowland rivers and are able to attain high populations in suitable environmental conditions, particularly where their predators, parasites and diseases are absent or scarce.

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Evolution of the *Simulium damnosum* complex

A. Krüger *Department Molecular Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

Phylogenetic considerations about the *Simulium damnosum* complex so far mostly relied on the benchmarking cytotaxonomic studies of Dunbar and Vajime dating back to the late 1960s. Nonetheless, some of their data were informally published or incompletely illustrated (in terms of chromosome maps) and were thus hardly reproducible. Over the last 10 years this situation could be improved by re-examining those parts of the original collections, which were in the possession of the late A. McCrae, together with introducing molecular techniques in the examination of these old and many new samples.

By means of parsimonious analysis of chromosome inversion differences Dunbar & Vajime¹ had constructed an un-rooted cytophylogeny of the 20 or so then known cytoforms of the complex including the three taxa *S. mengense* (Mt. Cameroon), ‘*Kibwezi*’ (Kenya/Tanzania) and ‘*Kaku*’ (southwestern Uganda, synonymous to *S. pandanophilum*). Recent cytotaxonomic analyses of these 3 taxa revealed that some basic and apparently ancestral arrangements could be derived from each other, but still there are numerous interspecific differences. This and the disjunct distribution data led to the conclusion that they may represent distantly related relic taxa. However, initially conducted mtDNA sequence analyses² produced some inconsistencies with regard to cytophylogeny and biogeography of certain taxa and disagreed, for instance, with the old relationship of the relic taxa.

In an attempt to overcome these problems the nuclear rDNA ITS2 was analysed. The results widely meet the topology as would be expected from the cytotaxonomic relationships. By inclusion of non-*damnosum* outgroup specimens and by translation of the molecular tree topology into the chromosomal relationships it is now possible to re-construct the first rooted cytophylogenetic tree of the complex. Thus an established hypothetical ancestor substitutes the previously central standards *S. kilibanum* and *S. squamosum*. *Simulium mengense* and *S. pandanophilum* constitute the oldest branch, and 'Kibwezi' together with the Ethiopian *S. kaffaense* and 'Kulfo' appear as a sister clade of the West African branch of taxa.

The greatest diversity of modern subcomplexes can be found in the eastern highlands between Ethiopia, Uganda and Tanzania. However, the relic species at present form a disjunct patchwork, which might be partly filled by *S. juxtadamnosum* and relatives. A major feature of them was the female tarsal claw with an enlarged basal tooth. A similar feature is known from *S. pandanophilum* and *S. mengense*, but not from 'typical' claws in the rest of the complex. Large basal teeth are generally accepted to be associated with ornithophily. In order to measure this feature, a 'claw index' has been introduced. Values for the assumed bird-feeders do not exceed 0.6 (for *S. juxtadamnosum* and relatives this was extracted from figures in the original descriptions), whereas 'typical' claws had ratios of 0.67 to 0.8 (including 'Kibwezi' and *S. kaffaense*).

More evidence for the relationship between *S. mengense* and *S. juxtadamnosum* was recently provided³. The DNA sequence data were also utilized to estimate the divergence times of the major lineages of the complex. The root of the complex dates back to some 2.5 to 3 million years, followed by the appearance of the West and East African lineages about 1.5 million years ago. This time scale roughly corresponds to major changes in African climate, hominid evolution, East African vegetation and the glacial ice volume in the past 3 million years⁴. In some cases, the

mtDNA data, though phylogenetically uninformative, may allow further phylogeographic estimations by considering the possibility of several waves of consecutive colonisation and extinction which could partially also be explained by the ‘pluvial theory’⁵.

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An update of onchocerciasis transmission studies in the Amazonian focus

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Paper published by title only

TRIVIA

Most of us use Word Processors to some extent. Jokes about the vagaries of spell checkers are legion, but your editor was captivated by this anonymous poem.

Ode to a personal computer

Eye have a grate spell chequer
It came with my pea sea
It plainly marques four my revue
Mistakes aye can not sea.

Eye strike a quay and type a word
And weight for it to say
Weather aye am wrong or write
It shows me straight a-weigh.

Whenever a mistake is maid
It shows bee fore to long,
And I can put the error write
It's all most never wrong.

I ran this poem threw it and
I'm sure yore policed to no
It's letter perfect all the weigh,
My chequer tolled me sew.

Sauce unknown.

Hideous, Fanged and Useless Creation – Latest Definition of the Simuliid?

"The blackfly is a hideous, fanged and, most would agree, useless creation that brings misery to everything in its path". So says Mike Gillespie, an editor at the Ottawa Citizen, when reviewing in the Times Colonist (Sunday 3^d July 2005) a mystery thriller novel set in the Algonquin Bay area – *Blackfly Season*, authored by Giles Blunt, published by Random House. Blunt's Detective John Cardinal evidently knows his simuliids, observing that "the worst thing about blackflies – the truly diabolical thing – is that they are absolutely silent. They do not buzz like bees, or drone like horseflies, or emit the high-pitched whine of mosquitoes: there's no warning, no chance for a preemptive smack".

Contributed by Roger Crosskey.

MEMBERSHIP NOTICES

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