



# Proceedings of the 4<sup>th</sup> International Simuliidae Symposium

| 31<sup>st</sup> British Simuliid Group Meeting |  
| 8<sup>th</sup> European Simuliidae Symposium |  
| EMCA Blackfly Working Group |

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# ORAL PRESENTATIONS

## The phenology of Simuliidae in Southwest Germany and their control

Foroutan Saravi R.<sup>1</sup>, Ignjatovic-Cupina A.<sup>2</sup>, Marinkovic D.<sup>2</sup> and Becker N.<sup>1,3</sup>

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In Germany approximately 50 Simuliidae species are described. Especially, the females of the anthropophilic species like *Simulium erythrocephalum*, *S. ornatum*, *S. equinum*, *S. lineatum*, *S. reptans* and *S. trifasciatum* are serious pests.

The intention of our work is to evaluate the species composition in streams in Southwest Germany and to test the biological larvicide, VectoBac 12 AS, based on *Bacillus thuringiensis subsp. israelensis* under laboratory and field conditions. Furthermore, a new sampling method for the standardized assessment of the pre-imaginal stages has been introduced.

Seven Simuliidae species could be identified. Among these species some are known for causing nuisances to the residential neighbourhood like *S. erythrocephalum*, *S. ornatum*, *S. equinum* and *S. reptans*.

Considering the fact that Simuliidae are vectors of human and animal diseases the control of them is crucial but should be carried out without harming the environment. The effectiveness and environmental compatibility of VectoBac 12 AS have been proved for years. For an efficient and reasonable treatment some decisive factors have to be considered. First, an optimal proportion between concentration and time of exposure must be given. According to the larval stage and larval density this proportion varies. The

temperature also effects the development of the larvae. With rising temperatures the larval growth accelerates and the ingestion increases. At the same time, the pupation rate rises.

We have considered these factors in laboratory tests and determined the optimum dosage under our field conditions. The concentration of 20 ppm and a treatment of 15 minutes at 12°C, showed a mortality rate of 91%.

For sampling the larvae and defining the density of the pre-imaginal stage in running water a new method was tested using conventional blank CDs.

In the stream Mörlenbach, the CDs were tied to a cord and put into the running for floating.

The efficiency of the blank CDs was also compared to plastic stripes. The result showed that the density of the pre-imaginal stages was considerably higher on the blank CDs than on the plastic stripes. About 59 % of the larvae preferred the CDs.

**An outbreak of black flies (Diptera, Simuliidae) in some parts of Serbia in 2010, with a first record of *Simulium erythrocephalum* (De Geer, 1776) in south-east Serbia**

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High water levels and the consequent flooding of several rivers during the spring and early summer of 2010 gave rise to a mass population development of *S. erythrocephalum* and *S. reptans* in Serbia. Public attention was attracted by the frequent biting of humans reported from different localities in south-east (Niš) and northern Serbia (Novi Sad and Zrenjanin). The lack of public knowledge of this insect group led to the creation of sensational statements in the daily newspapers, for example that the biting and the nuisance-causing agent was “a mutated mosquito species” or a “completely unknown species of a vampire fly”, whereas some residents in the affected localities correctly linked the phenomenon to the appearance of “Clinton’s flies” (since 1999 the vernacular Serbian name for black flies). According to the photographs of skin reactions to the bites published in newspapers and the general clinical picture described by general practitioners who gave medical assistance to patients, as well as the reports given by the victims of bites, it became clear that black flies were the cause of the attacks on humans.

To elucidate the background of the incidents, adult black flies were sampled by the application of carbon dioxide baited traps positioned in several habitats at localities along the River Danube, from the end of May until the beginning of July 2010. Additionally,



light traps were operated in Zrenjanin and Niš from May to July. Trapping results confirmed the outbreak of the anthropophilic black fly species *S. erythrocephalum* in localities along the River Danube (regions of Novi Sad, Bačka Palanka and Pančevo) and locations close to the River Tisa, and *S. erythrocephalum* and *S. reptans* along the River Nišava (region of Niš).

An increasing number of infested localities, as well as an increasing number of females per trap, were recorded during the period of monitoring along the Danube. An elevated biting risk to humans, as estimated on the basis of the number of *S. erythrocephalum* females captured in the majority of the sampled localities, was in accordance with the frequency of bite case reports from the affected parts of Serbia.

### Density-dependent host choice by Onchocerciasis vectors

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Onchocerciasis (river blindness) is transmitted by *Simulium* spp. and affects 37 million people in Africa, the Yemen and Latin America. The World Health Organization has identified onchocerciasis as a potentially eliminable disease. To achieve this goal, a thorough understanding of transmission dynamics and the effect of control efforts on these dynamics is essential. Mathematical models can help inform and guide such efforts. Novel anti-vectorial intervention may be required in addition to the current ivermectin treatment strategy. Current mathematical models assume that the proportion of bloodmeals taken on humans by blackflies is a fixed parameter resulting in a constant biting rate per vector on humans regardless of vector and host density. This in turn makes the basic reproduction number (the transmission threshold) vary proportionally with vector abundance. The question of whether the proportion of bloodmeals taken on humans by vectors depends on vector and host density, and changes temporally and spatially could therefore affect the ability of models to predict intervention impact at regional and local scales. A nonlinear relationship (i.e density-dependent host choice) would imply that efforts to control onchocerciasis by anti-vectorial measures could have unforeseen effects on the ability of the parasite to invade and persist in host populations. To test this hypothesis, five study sites in three regions of southern Ghana (Ashanti, Volta and Western) have been visited since 2009 in both the rainy and dry seasons, to obtain data on variation in fly and host densities and on host choice by onchocerciasis vectors. It is known that blackflies feed on a large range of hosts; therefore wild bird and mammal surveys (where

possible) in addition to surveys of households and their respective domestic animals were carried out on each sampling occasion. Blackflies were collected at host-independent (oviposition) and host-dependent (host-seeking) sites; counted; identified; dissected for parity status and stored for molecular and further morphological analysis for fly species-, *Onchocerca*- and past bloodmeal-identification. Conurbation sizes varied from 188 to 5,202 inhabitants per village, where numbers of domestic animals ranged from 489 to 11,143. Daily biting rates of blackflies on vector collectors (following Onchocerciasis Control Programme in West Africa protocols) ranged from 0 up to a mean of 237 bites/person/day. In total 2335 flies were collected on Bellec traps, 127 in Monks Wood light traps, 1502 on human and 1065 on cow hosts and a total of 3273 by vector collectors. Line transects were conducted to census bird populations in both dry and wet seasons, along which 136 species were recorded, ranging from 31 to 61 at different sites. The numbers of each species seen or heard will be used to estimate population densities using DISTANCE software once all surveys have been completed. Detailed population censuses and preliminary molecular data on bloodmeal analysis and *Onchocerca* infection prevalence will be presented and discussed in the context of vector and blood host density and possible implications for the quantification of the contact rate in onchocerciasis in particular and vector-borne diseases in general.

**Bioecology of *S. erythrocephalum* (De Geer, 1776) and control strategy in the region of Novi Sad (Vojvodina, Serbia)**

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Productive breeding sites of *S. erythrocephalum* were identified in the Danube river section of the northern lowland part of Serbia (Vojvodina Province). Immature stages of this typically riverine species were also recorded recently close to the river mouth of the Nera river (southeast Vojvodina), left tributary of the Danube and in the Drina river (west Serbia), right tributary of the Sava river. In the region of Novi Sad (south Vojvodina), the main breeding sites were located in the Danube river, where *S. erythrocephalum* represents the dominant black fly species. Rarely, immature stages were also recorded in few small creeks, confluents of the Danube.

In lowland river habitats, where the bottom is sandy/muddy and bare, immature stages were exclusively found attached to submerged bank vegetation, usually on leaves and branches of willow (*Salix* sp.) and poplar trees (*Populus* sp.) or lower vegetation (*Vitis* sp., *Rubus* sp., *Rorippa amphibia* and *Agropyrum* sp.), in the zones where the current velocity was ranging between 0,5 m/s and 1 m/s. As a rule, the highest population density was recorded on substrates not deeper than 1 m below the water surface. Larvae and pupae were rarely sampled down to 2,5 m from the water surface.

In stream habitats immature stages were also found attached to different submerged low vegetation and accumulated plant material, in zones with stony, muddy or combined bottom structure, in conditions of 0,4 m/s to 0,5 m/s current velocity and

water depth of 3 cm to 26 cm. In conditions of prolonged low water level of the Danube and consecutive lack of suitable oviposition substrates, it was observed that females may optionally lay eggs in sections of streams overgrown by dense low vegetation close to the Danube. Furthermore, in such situations the overwintering in stream habitats was also confirmed.

In laboratory, at water temperature that corresponded to outdoor conditions (about 20°C), early-summer generation of *S. erythrocephalum* have had five larval instars. It was also demonstrated that in this part of the season the duration of the period from larval hatching to pupation lasts for 2 to 3 weeks and to the adult emergence 3 to 4 weeks.

Perennial monitoring of both adult and immature stages in the region of Novi Sad demonstrated that this polyvoltine species produces several overlapping generations over the season, reaching high population density in the period from the early spring (beginning of April) to the beginning of summer (beginning of July). During this period, three peaks of adult biting activity could be recorded that clearly correspond to separate generations. In the following part of the season, adults were rarely sampled until the beginning of September.

Outbreaks of this highly aggressive anthropophilic species usually occur in conditions of long-lasting high water level of the Danube river during spring and early summer (in the year 2005 and 2006). The critical value of the Danube water level equal to 450 cm was estimated to trigger outbreaks at the region of Novi Sad. After a gap of 4 years, such situation was recorded again in late spring and early summer of 2010, when frequent bite cases to humans were recorded.

High, very high and extremely high biting risk, corresponding to at least 10, 22 and 41 females captured respectively in dry ice baited trap (type NS-2), was in all three years correlated to the Danube water level above 450 cm lasting at least one month.

Combining the knowledge acquired on biology of the species and hydrological characteristics of the Danube river, adequate control strategy was designed. Bearing in mind that larvae were never found out of the bank zones and that when the Danube water level is high, the water flow reaches enormously high values (from 3500 m<sup>3</sup>/s to 6000 m<sup>3</sup>/s), the conventional cross section spot treatment from one bank to another, covering the entire profile of the Danube, would not be rational solution. Instead, aerial edge treatment, focused on the breeding sites resulted in satisfying mortality rate (over 90%) in both the treated and 1 km carry zone.

**Genetic characterization of *Simulium degrangei* populations from Carpathians and Hellenides**

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*Simulium degrangei* Dorier & Grenier, 1960 has been recorded in southern and central Europe and in Ukraine and Georgia; the distribution seems to have a scattered pattern, usually with high dominance in blackfly communities on some localities. The genetic variability of mtDNA gene CO I has been studied in populations of *S. degrangei* from Western Carpathians (Slovakia) and Northern Hellenides (Greece). Analyzed section has 536 bp. In the material, 21 haplotypes were recorded, twenty of them were private with occurrence only in one mountain range, one haplotype has been found in all studied populations from Western Carpathians. Two haplotypes from Hellenides were private, but are not isolated and fit into the haplotype network from Western Carpathians. The differences between populations analyzed by AMOVA are not statistically significant: variance between populations is only 0.52 % of the overall variance ( $F_{ST} = 0.00515$ ,  $P = 0.40371$ ); in locus by locus AMOVA the differences between populations are 1.17 % of the variance ( $F_{ST} = 0.1166$ ,  $P = 0.2825$ ). The results of the analyses support a hypothesis, that populations of *S. degrangei* in Carpathians and Hellenides are conspecific; the question of the relation to the other two species of the *bukovskii* species group – *Simulium bukovskii* Rubtsov, 1940 a *Simulium vigintifile* (Dinulescu, 1966) – is far not solved.

**The identity and genetic characterization of *Simulium reptans* and *S. galeratum* (Diptera: Simuliidae) from Central Europe**

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The identity and genetic characterization of *Simulium reptans* Linnaeus, 1956 and *S. galeratum* Edwards, 1920 from central Europe has been analysed with respect to basic genetic variability, haplotype number and distribution between species and among populations within species. The study is based on the analyses of the CO1 gene (section between primers LCO, HCO, the analysed section has length of 648bp (=“long sequence”), respectively of a 450bp sequence inside of the previous (=“short sequence”). *S. reptans* and *S. galeratum* are genetic isolated from each other. In *S. reptans* 20 haplotypes in long sequence in pooled material were identified, among them 5 haplotypes in Slovakia and 15 in Britain, in short sequence 5 and 12 haplotypes respectively. In *S. galeratum*, 10 haplotypes in long sequence in pooled material were identified, among them 3 in Slovakia and 7 in Britain. In short sequences in the pooled material 12 haplotypes were found; 2 in Slovakia, 7 in Britain and 4 in Latvia. In both species the populations from Slovakia and Britain are without common haplotypes. In *S. galeratum*, with data also from Latvia, one haplotype is common for Latvia and Slovakia. Results of AMOVA (locus-by-locus) support the isolation of both species, all  $F_{ST}$  between species and populations are statistically significant ( $p < 0.05$ ), inside of populations are non-significant ( $p < 0.05$ ).



**On the different aspects of the ecology of *Simulium lineatum* (Mg.) and *Simulium equinum* (L.)**

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*Simulium lineatum* and *S. equinum* are two species of subgenera *Wilhelmia* widely distributed along the Europe. *S. lineatum* is usual for the Central and South Europe (Crosskey, Adler 2008). The distribution area of *S. equinum* is North of Europe and west of Siberia (Rubtsov, 1956). Morphological differences between *S. equinum* and *S. lineatum* in pupal stage are quite distinct, and identification of other stages is difficult.

This report is based on the material collected in 2002 – 2004 and 2007-2009 in different rivers in Lithuania. Both *Wilhelmia* species are quite common in large rivers and rivers of medium size in Lithuania (river discharge 0.7 – 500 m<sup>3</sup>/s). They can form in average 50,7% of all black flies in the river. *Wilhelmia* black flies were not found in small rivers and springs (river discharge 0.1 - 0.4 m<sup>3</sup>/s, water temperature during the season varied from 10.5 to 14.8°C). In spite of the fact that larvae of both species are found together, some differences in distribution of these two species can be found. *S. lineatum* larvae dominate in rivers of medium size (discharge 4.4 – 50.8 m<sup>3</sup>/s) with warm water (water temperature 12.8 - 15.5°C). Velocity of these rivers varies from 0.66 to 1 m/s, amount of oxygen – 8-9.4 mg/l and amount of organic matter – 7.1 – 8.8 mgO<sup>2</sup>/l). *S. equinum* dominate in smaller rivers (discharge 0.7 – 36.2 m<sup>3</sup>/s) characterized by colder water (temperature 9.6 - 11.4°C), higher amount of oxygen (9 - 9.8 mg/l) and lower amount of organic matter (3.4 – 7.6 mgO<sup>2</sup>/l). Velocity of these rivers varies from 0.55 to 0.86m/s. Larvae and pupae of *Wilhelmia* are found on water plants. Both species are found in large rivers (discharge more than 100m<sup>3</sup>/s) but they do not take the dominant position and make up 4 – 6% (*S. equinum*) and 16-18% (*S. lineatum*) of all black flies.

*S. lineatum* and *S. equinum* have three generations per year in Lithuania. Pupae are found three times per year (April, June and August) and larvae are found almost during all the year. Both species overwinter as larvae. *S. lineatum* females attacking people were determined in June – July, in many localities in Lithuania. Only few *S. equinum* females attacking people or cattle were determined in June.

*S. equinum* and *S. lineatum* differ in behaviour and chemical ecology. Chromatographic profiles of extracts of *S. lineatum* and *S. equinum* adults (both females and males) differ qualitatively and quantitatively in composition: 27 compounds (10 of them are hydrocarbons with 17, 26-37 C-atom chains) are present in *S. lineatum*, 55 compounds (19 of them are hydrocarbons with 11 - 19 and 24 -37 C-atom chains) – in *S. equinum*. The "asymmetry of specificity" of branched hydrocarbons was detected - there were found some the same chemicals in *S. lineatum* females and *S. equinum* males. Obvious differences in chromatographic profiles of female and male extracts of the species may be related to the differences in their mating behaviour. *S. lineatum* black flies are stenogamous (i.e. ground-mating species), their males not swarms, but aggregate on surface near emergence site. While *S. equinum* black flies are eurygamous (i.e. aerial coupling species), their males swarm for mating.

Investigations of genetic diversity according COI gene have shown that pairwise distances between two species were from 11,3 to 13,9%. Different haplotypes of *S. equinum* differ more (1,8%) than different haplotypes of *S. lineatum* (0,8%) in Lithuania. These differences were not related with geographical distribution of black flies.

**Black Fly (Diptera:Simuliidae) Control with the Biological Larvacide *Bacillus thuringiensis* var. *israelensis* in the Middle Kızılırmak River of Cappadocia, Turkey.**

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*Simulium* spp. (Diptera: Simuliidae) populations have recently increased in the Kızılırmak basin of Cappadocia region. Black flies control studies were carried out in the 145 km part of river Kızılırmak, run through Cappadocia. Vectobac 12 AS and Aquabac XT commercial preparats of *Bacillus thuringiensis* var. *israelensis* were used to control for black flies larvae of *Simulium* out break. First application was done in July 2007. In first applications performed in 2007, out break of *Simulium* was prevented as treatment of 3.3 ppm Bti/10 min.dose 2880 l. with 1,2 and 3 km intervals in 30 m<sup>3</sup> /s discharge of the river. In second years at the beginning of the months in 2008, larvacide applications were carried out at 3 km intervals at 3 ppm/10 min. after than the application was adjusted to 1-2 ppm /10 min in July 2008 with the water flowing 50-100 m<sup>3</sup>/s of the river Kızılırmak. According to different commercial preparat and physical properties of the river, the distances between the application points were changed at 3 and 7 km intervals. In 2009 studies, application dose was 1 ppm/10 min. due to the river flows, and the distance of application sides were changed between 3 and 7 km. Control of *Simulium* was realized using 12000 l Bti totally, 1500 l in each application.

**Species with adults and larvae similar. Are the gill filaments of pupae good character to distinguish Neotropical black flies?**

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The gill filaments of black flies are the most characteristic feature of the pupa (Adler et al. 2004). According to Crosskey (1990) the extraordinary diversity in the outward form of the gills is a blessing for taxonomists. Several species has unique gill filaments, being this character very useful to distinguish it. The gill filaments vary in shape, from fine to swollen and in number, from few to hundreds. In the present study all the issues regarding DNA barcoding and morphology-based taxonomy we have found were from closed related species that are only distinguishable by the shape of the gill filaments of the pupae, i.e., you cannot tell those species apart comparing larvae or adults. These unexpected results shed light to further investigations on the use of gill filaments to discriminate black fly species.

The possible problems associated with the use of single-character systems (e.g., pupal gills) occurred with three different pair/ group of species in two genera: *Gigantodax* and *Simulium* (*Inaequalium* and *Psaroniocompsa* subgenera). These results are based in a study of DNA Barcoding of Neotropical Black Flies when successful amplifications of COI barcode gene were obtained for 1040 specimens representing 74 species from four genera: *Gigantodax* Enderlein, *Lutzsimulium* d'Andretta & d'Andretta, *Pedrowyomyia* Coscarón & Miranda Esquivel and *Simulium* Latreille.

The first and more illustrative case of pair of species with DNA Barcoding gene similar is *Gigantodax impossibilis* and *Gigantodax corniculatus*. Both species were described by Wygodzinsky from the same type locality and have very distinct pupa gill filaments and share similar COI sequences. As we have a wide sample of specimens and we collected both species in the same stream,

without intermediate phenotypes, that condition strongly suggests two separate breeding populations.

The pair of species *Simulium stellatum* and *S. auristriatum*, placed in the subgenus *Simulium (Psaroniocompsa)* also shared similar barcode sequences. The later species has swollen filaments, while the other has fine ones. The most important character to distinguish both species is the shape and configuration of gill filaments because the adults and the larvae are morphologically indistinguishable. We have analyzed three populations of *S. stellatum* and one of *S. auristriatum* and they also have closed sequences of COI barcoding gene.

Several species of the subgenus *Simulium (Inaequalium)* that belong to the *inaequale*-species group presented very similar COI sequences. The species within this subgenus are widely distributed in the Neotropical region, and the identification of most species in this subgenus relies on the configuration, type and number of gill filaments, while the adults and larva are impossible to be distinguished because of their morphological homogeneity (Hernández *et al* 2007). However, the configuration, type and number of gill filaments are highly variable in the following species: *Simulium clavibranchium*, *S. rappae*, *S. subnigrum*, *S. diversibranchium*, *S. mariavulcanoae* and *S. travassosi*, being *S. rappae* the species with the mostly remarkable variation in the gill filaments shape (Hernández *et al* 2007). All those species also share similar COI sequences among them.

The discussion about whether or not the differences in the gill filaments are a good character to distinguish Neotropical black flies species is now opened.

**Interactions of Blood-feeding Black Flies and Endangered Wildlife**

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The interactions of black flies that take blood from humans and domesticated animals are well documented. Less is known about the interactions of black flies and wildlife, particularly endangered wildlife. We present case studies in which black flies routinely blood feed, often in great numbers, on endangered birds such as whooping cranes (*Grus americana*). We discuss research methods for monitoring populations of host-seeking black flies and their feeding behaviors, which are sensitive to the special circumstances of federally protected species. We also examine the role of genetics and taxonomy of black flies in understanding patterns of host use for endangered species.

**Paradise Lost? The response of black flies to climate change in northern Canada**

Douglas C. Currie

Royal Ontario Museum, Toronto, Ontario, Canada

The Arctic is among the most fragile ecosystems on Earth; it is also under immense environmental pressure as the effects of global warming are felt most acutely at northern latitudes. The Northern Biodiversity Program (NBP) aims to document changes in Canada's arthropod fauna by repeating the half-century-old Northern Insect Survey — an unprecedented initiative that sampled diversity at 72 arctic and subarctic localities across northern Canada. Biting flies are among the focal taxa, and patterns of Simuliidae distribution will be discussed in view of collections made during the 2010 field season. Preliminary results indicate that 'southern' simuliid species may be migrating northwards in response to warming temperatures. The consequences of this phenomenon — both for humans and other homeothermic animals — will be discussed.

**Distribution, diversity and dynamics of black fly symbiotes.**

John McCreddie

Department of Biology, University of South Alabama, Alabama, USA

This paper will discuss patterns of black fly symbiote distribution and diversity over scales from the stream reach to that of the continent. In addition, a proto-model of how the nature of symbiosis changes from commensalism to parasitism is present



**Effects of land use on black fly assemblages (Diptera, Simuliidae)  
in submontane rivers (West Carpathians, Slovakia)**

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The effects of land use on black fly assemblages were investigated in submontane rivers (the Ľubochnianka river, the Revúca river, upper Váh River catchment) in the Carpathians. We recorded 11 black fly species in the Ľubochnianka River and 10 species in the Revúca River. Land use differs between the catchments; the Ľubochnianka is primarily forested catchment, and the Revúca is markedly deforested catchment. Fifteen environmental variables were analysed. Based on the results provided by CANOCO, phosphorus level and stream slope are the most important variables and have significant influences on the variability of black fly assemblages. In spring, *Prosimulium rufipes* (Meigen, 1830) occurred along the entire lengths of the streams. The black fly assemblage of the Revúca River differs from the assemblage of the Ľubochnianka River mainly by its higher abundances of *Simulium variegatum* and *Simulium ornatum*, by the presence of *Simulium carthusiense*, and by the absence of *Prosimulium hirtipes*.

**Domination structure of Black flies in streams and terrestrial biotope of Middle Russian forest-steppe.**

**I. Budaeva, L. Khitsova**

Voronezh State University, Voronezh, Russia

The structure of domination of 24 species of Black flies is discussed in time aspect in connection with their epidemiological value in the conditions of Central Russian forest-steppe. The structure and number hemipopulations of pre-adult stages in streams and attacking females are various.

**Onchocerciasis Transmission and Anthropogenic Change in Tanzania.**

A Kalinga, O Yasar & RJ Post

Natural History Museum, London, UK

Recent results will be compared with historical data to investigate whether blackfly biting rates and transmission has changed in the Tukuyu and Usambara foci of onchocerciasis which might be related to vector control, deforestation and community directed treatment with ivermectin.

**CONFORMITY OF DATA OBTAINED WITH DIFFER QUANTITATIVE METHODS FOR COUNTING OF BLACKFLIES IMAGO (DIPTERA, SIMULIIDAE)**

**Vera Rodkina, Liudmila Petrozhitzkaya**

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Stable standard methods of account are practiced for bloodsucking dipterans. However, the data cannot be compared of different methods: collecting with the help of Monchadsky's and Berezantsev's bell, Skufin's trap, entomological net, exhauster. All the methods take into account insects in out door and attracting by human without registration the square from which the insects were collected. Account of insects on the transect is practiced in entomological studies of lepidopterous (Yamomoto, 1975; Malkov, 1994; Dubatolov, 2006), it permits to make recalculation of quantitative data per square. We have comparative account data of the bloodsucking blackflies by entomological net «around human» and on the transect, it gives a possibility to calculate the transfer index between different types of collecting, to recount per square. The recalculations give an opportunity to use data of different methods and authors for studies of biocenosis. The correlation of data, obtained by Monchadsky's bell and entomological net «around human», in the southern taiga of the West Siberia is 1,25:1; in the mountains of the East Siberia (Vitimskoye plateau) – 3:1. Between collections «around human» with exposition of 3 min., during which 180 wags are made, and collections on the transect (180 wags with the step 0,85 m in the direction there and back) the correlation compose 1:1,5. It should be noted that collecting of blackflies on the transect permits to account insects that flew out of herbage by attraction.

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**BLACKFLIES (DIPTERA, SIMULIIDAE) OF THE SAYAN-BAIKAL STANOVOI HIGHLAND**

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The Sayan-Baikal Stanovoi Highland belongs to the mountains of the southern Siberia. It is a complex system of ridges, plateaus and depressions. The species composition of blackflies was studied on the base of original and literature data, comparison of the fauna with adjacent territories of the Siberia and Mongolia was made.

The blackflies of the Sayan-Baikal Stanovoi Highland are represented by 68 species of 18 genera. Taxonomic index, including 50 % of the whole composition, consists of genera with the greatest number of species: *Metacnephia* Crosskey (13), *Gnus* Rubts.(8), *Simulium* Latr., *Cnetha* End. and *Prosimulium* Roub. (by 7 species). In the northern district of the Lake Baikal and Stanovoi Highland bloodsucking complex consists mainly of species of the genus *Gnus* Rubts. and *Simulium* Latr.

Faunal similarity of the blackflies in the southern Siberia was assessed using one of the methods of cluster analysis: the highest indexes were observed with the Altai Mts. – 43 % and the north-western territory of the Mongolia – 38 %. Taxonomic characteristics were identified in the spatial distribution of the blackflies on the territory of the South Siberia.

Comparison of the blackfly fauna was made using the basin system of rivers in the Siberia. Severe dependings with the basin systems were not detected in the spatial organization of blackflies.

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**SPECIES COMPOSITION OF BLACKFLIES (DIPTERA, SIMULIIDAE) IN THE TRANSBOUNDARY AREA OF THE RUSSIAN AND MONGOLIAN ALTAI MTS**

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Based on original and literature data, a review on blackfly fauna of the transboundary territory of the Mongolian Altai Mts. and East and South-East parts of the Russian Altai Mts. is presented. Blackflies come from 68 sites located at the altitudes from 1000 m to 2500 m a.s.l.. In the Mongolian Altai Mts., the South-East Altai Mts. and the East Altai Mts., the occurrence of 24, 27 and 18 blackfly species was confirmed. The blackfly fauna of all monitored parts of the Altai Mts. consisted mainly of species of the genera *Prosimulium*, *Metacnephia* and *Simulium* (mainly *malyshevi*-species group). The highest similarity in blackfly species composition was between the South-East Russian Altai Mts. and the Mongolian Altai Mts. It is probably due to similar natural and climatic conditions in the mountain basins which offer favourable conditions for development of species of the *Sulcicnephia* genus and the *Simulium bezzi*-species group.

# POSTER PRESENTATIONS

**Cytogenetic features of six different black fly (Diptera, Simuliidae) species living in Eskişehir City and its near around.**

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Cytogenetics is one of the most useful tools for elucidating sibling species of insect. It is true that there are lots of sibling species in family Simuliidae and cytogenetic analysis of salivary gland polytene chromosome is one of the best methods for taxonomical researches to identify them.

In this study, salivary gland polytene chromosomes of six different black fly species, *Simulium velutinum* (Santos Abreau, 1922), *Simulium variegatum* Meigen, 1818, *Simulium bezzi* (Corti, 1914), *Prosimulium rufipes* Meigen, 1830, *Simulium caucasicum* Rubtsov, 1940 and *Simulium costatum* Friederichs, 1920 in Eskişehir city and its near around were firstly examined. The results were compared with the other researches on the karyological features of these species in Palaearctic region

From examined species, polytene chromosome structures of *Simulium velutinum*, *S. variegatum* and *S. bezzi* are same in numerically and structurally with those of the other populations living in other regions. However, it was observed that chromosomal features of the others three species, *Prosimulium rufipes* Meigen, 1830, *Simulium caucasicum* Rubtsov, 1940 and *Simulium costatum* Friederichs, 1920, were not different in numerically but there were structural inversions in chromosomes of our species.

Keywords: Simuliidae, polytene chromosome, Cytogenetic, Karyotaxonomy, black fly.



**THE EFFICACY OF VECTOBAC 12AS AGAINST BLACK FLIES (DIPTERA: SIMULIIDAE) IN POLAND**

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About 50 Simuliidae species have been recorded in Poland. The predominant antropophilic species recorded in Poland in mass numbers causing a threat to humans and animals are: *Simulium (Boophthora) erythrocephalum* (De Geer), *Simulium (Schoenbaueria) pusillum* (Fries), *Simulium (Schoenbaueria) nigrum* (Meigen), *Simulium (Simulium) morsitans* (Edwards), *Simulium (Simulium) noelleri* (Friederichs), *Simulium (Simulium) ornatum* (Meigen), *Simulium (Simulium) reptans* (L.), *Simulium (Wilhelmia) equinum* (L.). There are several regions in Poland where black flies have always occurred in pest numbers. Since 2001 biorational control strategy, which includes monitoring of mosquito and black fly breeding sites and the use of microbial insecticides based on *Bacillus thuringiensis* var. *israelensis* (*Bti*) have been successfully implemented in Gorzów Wielkopolski, which is located along the Oder and Warta Rivers. However, the program needed to be improved by designing a cost-effective, large-scale control strategy,

using well known protocols to assess the effective dosage of microbial insecticides, as well as selecting the most effective application techniques.

The objective of this study was to determine the efficacy of VectoBac 12 AS ground application against immature stages of simuliids under field conditions in order to make recommendations for routine treatments of black flies in Poland.

The tests were conducted in March, 2009, mostly against *S. erythrocephalum* and *S. morsitans* larvae (L4-L6) at five locations along the Klodawka river in urban area of Gorzów Wielkopolski. The number of larvae was assessed by collecting underwater aquatic macrophytes, occurring at a distance of 10 m along both banks of the river. The percentage reduction in larval black fly densities was calculated in both treated and untreated sites.

A concentration of 2.6 ppm for 15 minutes using Solo<sup>®</sup> back pack (Model: Solo Port 423) application system resulted in sufficient reduction of *S. erythrocephalum* and *S. morsitans* larvae. The average mortality rate ranged from 94.2% (SD: 5.09%) on one day to 100% on day three after applications.

In this study, it was demonstrated that VectoBac<sup>®</sup> 12AS as an aqueous suspension formulation of *Bti* is an excellent control agent for controlling black fly larvae in Poland.

**An universal tool for the analysis of effectiveness of insecticides.**

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Insecticides have leading role in reduction of population of arthropods, especially vectors of infectious diseases. Efficacy of insecticides is usually proved on biological material and described after adjusting the values of parameters in some “theoretical” function to the experimental results. In the standard approach, such function is described by probit regression. However, in some cases reaction of the population on toxine differs from those which could be described by the probit regression curve and the results could not be properly assessed. For such situations we developed other method of estimation, based on nonlinear regression. It was observed that alternative method resulted with better fit to experimental and simulated data. Our solution can be applied in bioassays parameters computation (such as LD50; LT50) in different study designs.

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**Changes in the distribution of *Simulium maculatum* Mg.**

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*Simulium maculatum* black flies develop abundantly in the Nemunas river in Lithuania from the eighth decade of the XX century. It became the main pest species in the South Lithuania and biotechnical means for black fly control were used in the river from the year 1998. This outbreak of bloodsucking black flies has induced investigations of these insects in Lithuania. So, black flies were investigated in the country from the year 1995. Data on black flies of neighboring Latvia did not confirm the existence of *S. maculatum* in the seventh decade of the XX century in Latvia (Sternbergs, 1971).

*S. maculatum* was described by Meigen in 1804 from Germany. This species has been reported from an enormous expanse of the Palaearctic Region breeding in large rivers such as the Danube, the Kolyma, the Ob, the Lena and the Volga. (Adler *et al.*, 2004). It was a transpalaearctic species adapted to the continental climate, larvae of *S. maculatum* developed in large warm rivers rich in organic matter (Yankovsky, 2002). *S. maculatum* once widespread in central Europe was extirpated from many large rivers (Zwick, Crosskey, 1981), from the other hand it is known as species resistant to warm water, low velocity, lack of oxygen and water pollution (Yankovsky, 2002). *S. maculatum* can be found in Poland, Belarus (the Dnepr river), Lithuania, Latvia (the Daugava river), Ukraina, Russia, Kazakhstan (the Irtysh river) and Mongolia.

According to Zhivkovich (1958, Serbia), Kaplich, Skulovec (2000, Belarus) and our data larvae of *S. maculatum* develop in large rivers which can be characterized by water temperature from 15°C to 25°C, oxygen saturation from 68.2-79.9% (Serbia), 75-85% (Belarus) to 80-124% (Lithuania), velocity from 0.3-0.5 (Serbia), 0.4-0.7 (Belarus) to 0.85 (Lithuania). *S. maculatum* has one or two generations per year. The second generation is less abundant than

the first one (Lithuania). The adults of the first generation appear in the middle of May (Serbia), the end of May (Belarus), the beginning of June (Lithuania), July (North of Russia).

**An Outbreak of Black Fly (*Simulium (Wilhelmia) lineatum*)  
(Diptera: Simuliidae) in Central Basin of Kızılırmak River**

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**Abstract:** This study was carried out on Kızılırmak river basin which is lengthen about 150 km from Yamula dam in Yemliha a city of Kayseri to Gülşehir a city of Nevşehir. A severe infestation of adult black flies occurred in Kızılırmak basin of study area after releasing water to basin of river to produce hydro electricity. The fly population peaked during 2006-2007. As a result of this fly infestation, people who live in those areas were unable to continue to do their daily routine works. Hotels in the area had to loose their customers and to cancel their reservations. A toxication or inflammation of cases via vectors of flies was not determined. However, it was observed that these flies placed on head and other parts of body in animals and people when they went out. The black fly species was determined to be *Simulium (Wilhelmia) lineatum* with examination of larvae, pupa, and adult samples that were collected during the study period.

**Keywords:** Central Basen of Kızılırmak River, *Simulium (Wilhelmia) lineatum*.

**CHIRONOMIDAE AND SIMULIIDAE OF SMALL WATER RESERVOIRS  
(INLETS, OUTLETS AND LITTORALS) OF WEST SLOVAKIA**

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In six small water reservoirs of West Slovakia belonging to two catchments (the Váh river and the Morava river catchments) altogether 44 Chironomidae taxa of four subfamilies were recorded: Tanypodinae (6 taxa), Diamesinae (1 taxon), Orthoclaadiinae (14 taxa) and Chironominae (22 taxa). The taxa number in individual reservoirs ranged from 12 to 22. Only *Cladotanytarsus* gr. *mancus* appeared in all six reservoirs. Differences between two catchments were observed. In the Váh river catchment the most abundant were *Cricotopus* (l.) *trifasciatus* (351 ind.m<sup>2</sup>), *Dicrotendipes modestus* (168 ind.m<sup>2</sup>) and *Glyptotendipes pallens* (66 ind.m<sup>2</sup>). In the Morava river catchment *Microtendipes* gr. *pedellus* (410 ind.m<sup>2</sup>), *Endochironomus tendens* (361 ind.m<sup>2</sup>) a *Paratanytarsus* sp. (190 ind.) prevailed.

In inlets and outlets taxa from subfamily Orthoclaadiinae dominated – *Tvetenia* sp. and *Parametriocnemus stylatus* together with filter feeder *Prodiamesa olivacea* (Prodiamesinae).

From Simuliidae, in inlets and outlets together 11 species were detected, 5 species in river Váh catchment and all 11 species in river Morava catchment. The most diverse community in inlets of Lozorno reservoir possessed submountain pollution intolerant 7 species. Ubiquist species *Simulium ornatum* was found in all sampling sites. *Simulium noelleri* which is tolerant for organic pollution prevailed mainly in outlets of reservoirs.

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