The 3rd International Simuliidae Symposium

Vilnius 2008 09 09-12

including the 29th meeting of the British Simuliid Group, the 7th European Simuliidae Symposium and EMCA Blackfly working group

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ORAL PRESENTATIONS

WORLD PERSPECTIVE OF SIMULIIDAE

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The total number of living, described species of black flies in the world is 2019. Black flies, therefore, represent 0.2% of all described species of insects and 1.3% of all described species of Diptera in the world. The species of black flies are distributed in the six zoogeographic regions of the world as follows: Afrotropical (11%), Australasian (12%), Nearctic (11%), Neotropical (16%), Oriental (17%), and Palearctic (33%). The subgenus *Simulium* consists of 24 species groups representing 439 (22%) of all black fly species worldwide, and includes most of the major pest species. One of my long-term objectives is to provide a phylogeny of the subgenus *Simulium*, based on chromosomal rearrangements. To date, the chromosomal complements of 82 nominal species in 12 of the species groups have been fully resolved, relative to a revised subgeneric standard chromosome map. The chromosomal phylogeny has now been rooted by following critical banding sequences into the subgenera *Boophthora* and *Psilozia*. The resulting cytophylogeny allows an understanding of the evolution of pest status, and provides a basis against which current and future molecular discoveries can be tested. The chromosomes have revealed additional species (i.e., sibling species) within widely accepted nominal species such as *S. bezzii* and *S. ornatum*, and have demonstrated that species, such as *S. ornatum* and *S. tuberosum*, have far more restricted geographical distributions than previously believed. Although most species groups have unique chromosomal markers justifying their recognition, some such as the *S. variegatum* group, do not have any chromosomal markers.

BLACKFLY (DIPTERA: SIMULIIDAE) COMMUNITIES OF THE "WALDVIERTEL" (AUSTRIA) ALONG THE CZECH BORDER

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The northern „Waldviertel" (the southern part of the geographical region „Bohemian Forest"), situated along the border to the Czech Republic, differs from all other Austrian ecoregions in respect of its climate, the geological and the limnological situation.

The climatically rough conditions of the hilly granite and gneiss countryside resemble those of alpine areas. Rivers and brooks appear as acid, intensely brown moor waters. Culicidae communities investigated in the passed years consist mainly of alpine or northern European species.

The purpose of the samples of Simuliidae larvae and pupae from 2007/08 in several running waters of the northern Waldviertel was to investigate, if their composition corresponds to the results known from the Culicidae collections. Early spring fauna is dominated by *S. rostratum* and *S. morsitans*, during the rest of the season mainly larvae and pupae of *S. ornatum*, *S. reptans*, *S. (B.) erythrocephalum*, *S. (N.) vernum* und *S. (N.) carthusiense*-Gr. were found. Collectors were severely bitten by Simuliidae in early spring.

The morphological variety of Simuliidae larvae and pupae of certain species is discussed.

BLACKFLY ASSEMBLAGES (DIPTERA, SIMULIIDAE) OF THE HRON RIVER TRIBUTARIES (SLOVAKIA)

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In total, we have recorded 30 blackfly species in the Hron river basin, which represent 65% of species known to occur in Slovakia. Twenty-five species occurred in the streams belonging to the Carpathicum ecoregion, whereas 14 species in Pannonicum ecoregion. In the upstream and downstream stretches of the basin, there was a relatively large overlap – 50% and 43%, respectively – of occurrence of species common for the Hron river and its tributaries. In contrast, the middle stretch and its tributaries had only 20% of common species because the tributaries drain not only the submontane zone as the Hron river does, but also the mountain parts of the Carpathians.

The blackfly assemblages of upstream Hron river tributaries consisted predominantly of *Prosimulium rufipes, Simulium argyreatum, Simulium monticola, Simulium variegatum* and *Simulium vernum*. The species *Simulium bertrandi, Simulium crenobium, Simulium carpaticum* and *Simulium codreanui* occurred rarely there. The assemblages in the middle stretch tributaries included *Prosimulium hirtipes, Prosimulium tomosvaryi*, *Simulium monticola, Simulium reptans, Simulium variegatum* and *Simulium ornatum.* In the montane zone of the tributaries we recorded *Twinnia hydroides*, a postglacial relict, and *Simulium maximum*. The assemblages of the Hron river downstream stretch were represented by *Simulium ornatum, Simulium erythrocephalum, Simulium reptans, Simulium lineatum* and *Simulium equinum,* while *Simulium angustipes* and *Simulium balcanicum* occurred rarely there.

AERIAL TREATMENT OF THE DANUBE SIMULID BREEDING SITES IN THE REGION OF NOVI SAD (VOJVODINA, SERBIA)

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Highly productive breeding sites of *Simulium erythrocephalum* (De Geer 1776), the most important anthropophilic species, were detected in the length of 20 km of the Danube river course in the region. Immature stages of *S. balcanicum* (Enderlein, 1924), *S. equinum* (Linnaeus, 1758) and *S. reptans* (Linnaeus, 1758) are usually associated with them but present in lower population densities.

It was demonstrated that *S. erythrocephalum* can complete several overlapping generations during the season basically depending on the water level fluctuation. Typical breeding sites were detected close to the river banks, on submerged vegetation (mostly leaves and branches of willow and collapsed poplar trees) and as a rule never recorded in the central section of river bed. Immature stages prefer ecological niches close to the water surface and are usually present at the depth up to 1 m. Occasionally some specimens could be found in the deeper water sections.

Considering the enormous water flow volume of the Danube (app. 3000-6000m^3/s) and the restricted position of the breeding sites, it was necessary to adopt an application method which would provide both low cost and high efficacy. Commonly used simulid spot treatment of entire river bed or multi-spot application of the shore zone were substituted with a fixed wing aerial application flying parallel to river banks. This approach enabled treatment with B.t.i (VectoBac 12AS) targeted exclusively to the larval breeding sites.

A treatment parallel to the shore in targeted bank zones of two river islands was made by an Antonov-2 equipped with size 12 boom and nozzles. The average speed was 160km/h, the width of the swats 30m, the altitude over the water from 3-5 m, over the canopy from 26-36m. The application rate of the product was 214 l/min, which corresponds to the average concentration of 15ppm.

During the treatment of entire marginal zones of the two islands, 2000 l of the product was consumed for total length of 26700 m. High mortality rates (90.48-100%, average 98.51%) were recorded in samples of larvae collected in treated zones, at both superficial layer and in deeper water down to 3.85 m. Carry of the product was related to the comprised streams of B.t.i. from outer and inner islands shores. Carry was evaluated by the mortality rate of larvae exposed at 911m, 1576m and 2008m downstream from the treated zone. At such distances the mortality rate in samples exposed close to the water surface was 100%, while at the depth of 0,7-1m the range was between 76.74% and 95.45% (average 87.64%).

A second trial at a stretch of the Danube without islands (2470m of the shore) was done under similar flight performances and product usage rate. The application rate of the product was 306 l/min, which corresponds to the average concentration of 10ppm. Excellent larval mortalities were obtained within treated zone and carry registered until 634 m downstream (average 99.87% and 98.51% respectively). High treatment efficacy was also recorded at 861 m and 1330m downstream from the treated zone (mortalities 82.16% and 89.66% respectively). Carry effect from 1838 to 2720m downstream was still demonstrated, but in irregular pattern, mortality ranging from 3.53% to 95.8%.

SIMULIIDAE **(D**IPTERA**)** FROM THE **C**ALDERA DE **T**ABURIENTE **N**ATIONAL **P**ARK**, L**A **P**ALMA **(C**ANARY **I**SLANDS**)**

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Data on distribution, abundance and phenology of Simuliidae (Diptera) from the Caldera de Taburiente National Park on La Palma are given, based on systematic Malaise trap and yellow pan trap samples between August 1999 and July 2001. The occurrence of 5 species is proved; all of them were already known from the Canary Islands, but one species (*Simulium tenerificum*) is recorded from La Palma for the first time. The present paper is a result of the project "Inventory and study of the invertebrate fauna of the Caldera de Taburiente National Park".

Keywords: Diptera, Simuliidae, Caldera de Taburiente National Park, La Palma, Canary Islands.

THE IMPACT OF CHEMICAL CONTROL AND SUBSEQUENT RENATURATION ON THE DEVELOPMENT OF MASS POPULATIONS OF BLACK FLIES (DIPTERA: SIMULIIDAE), AS ILLUSTRATED BY THE LOWLAND EUROPEAN RIVERS

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The Simuliidae is a group that has important functions in the aquatic food chain and in the breakdown of organic substances, and is also a group of aggressive ectoparasites. Females of many species are blood feeders and are responsible for various veterinary, medical and economic problems, especially in certain regions where mass emergences of biting adults take place.

Abiotic and biotic factors determine the species spectrum of black flies and therefore also the biodiversity within each habitat. The organic and chemical pollution of the some rivers as well as the introduction and addition of insecticides and pesticides had a strong influence on the development of the aquatic fauna during the period from 1950 to 1990 and have continued to do so since then though to a lesser extent.

Changes in the law and in the guidelines for water management have led to fundamental changes in the conditions of water systems. These changes have resulted in a reduction in the species diversity on the one hand, and in increased possibilities for the dispersal and expansion of the remaining biota on the other hand. The results obtained from surveys of the renatured courses of several rivers will be discussed in relation to this problem.

Changes in the species spectrum and abundance of the Simuliidae, and the harmful effects that these may have, will be demonstrated by means of examples. The current situation in these rivers and its effect on agriculture and animal husbandry, as well as the adverse health effect on the people, who live alongside the lowland rivers and their tributaries, will be shown.

A review of the control methods that have been considered and their influence on the riverine environment in general will also be given. Black flies are attacked in all their life stages by a wide variety of organisms, ranging from birds and fishes at one end of the scale to protozoans and nematodes at the other, and the role of the natural invertebrate predators will also be discussed.

Keywords: Simuliidae, Europe, biodiversity, faunistics, range extensions, pollution

FACTORS AFFECTING THE RIVER HABITATS OF LARVAL BLACKFLIES (DIPTERA: SIMULIIDAE) – PROBABLE CAUSE OF MASS OCCURRENCES OF THE PESTS

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Regular faunal studies on the Simuliidae of Poland have been carried out continuously since the 1950-s (up till the 1990-s) and a map of the pest occurrence of these dipterans was published in 1974. Then the main Polish rivers were too heavy polluted for blackflies and the problem in many regions disappeared. During the last decade the problem of pest occurrences of blackflies has recurred in several regions of Poland but the regions where the pests occurred prior to 1970-s and after 1995 were not always the same. If the only reason for recurrance of simulids was the diminishing water pollution of Polish rivers the regions would be more or less similarly affected by pest occurrence before & after. Thus, there must be several other factors affecting the river habitats which make them favourable for the development of blackfly larvae. Some researches analyzed the problem and pointed to river engeneering – breakwaters and the stabilisation of river bed with rocks and concrete elements - as factors which affect (improve) the living conditions of larval simulids. Such a correlation is visible also in Poland. Also another factor - pollution of rivers with polyethylene bags must be important one in “improvement” of habitats for simulid larvae. On the other hand it seems that there are different effects in water reservoirs – now we observe fewer blackflies close to those reservoirs built in the areas affected earlier by simulids, while there is now a plague occurrence of the pests along 60 km Vistula river down the the huge water reservoir (Włocławek) where previously blackflies caused no problem.

RECENT BLACK FLY (DIPTERA: SIMULIIDAE) CONTROL WITH *B.T.I.* IN SOUTH GERMANY

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The objects of the control programme were the prevention of deaths and severe injuries to cattle and other domestic animals and the decrease of injuries to men inside the affected area. The controlled pest species in the 8 treated rivers and brooks were *Simulium ornatum, S. trifasciatum, S. lineatum, S. equinum and S. noelleri*.

A review of the control measures in the years 2007 and 2008 is given. Surveillance, application technique and time, results and cost-benefit equation are presented.

Keywords: Larval control, *B.t.i.*, South Germany, *S. ornatum, S. trifasciatum, S. noelleri, S. lineatum, S. equinum*

TOWARDS THE ELIMINATION OF THE BIOKO FORM OF *SIMULIUM YAHENSE* FROM BIOKO: PLANNING AND INSECTICIDE TRIALS

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The Bioko form of *Simulium yahense* is a unique cytoform endemic to the island of Bioko (formerly known as Fernando Po), Equatorial Guinea. Field work conducted in 1996 established locations of most of the form's breeding sites and confirmed its vector status, leading to a proposal that the form, and hence onchocerciasis, could be eliminated from Bioko by a judicious programme of vector control conducted during dry seasons when the numbers of breeding sites are restricted. An additional consideration was that no breeding was found above 400m a.s.l. This report describes work executed during 1999-2001 to plan such a campaign, excluding investigations on environmental impact assessment, and the results of trials of ground-based insecticide applications in 2001.

The control operation planning was based on standard procedures established by the WHO Onchocerciasis Control Programme in West Africa (OCP). Blackfly capture points were set up in 1999 at accessible points around the island at Sampaka (River Sampaka), Barleycorn (River Apu), Musola (R. Musola) and Balacha da Riaba (R. Ruma), supplemented from 2001 by one at Riaba (River Ruma). Captures were made at each of these sites at least once per week and processed according to OCP protocols. In addition to training of vector collectors, six locally-based staff were trained in dissection methods to establish pre-control baseline data. Laboratory tests using field-collected larvae showed that the larvae were susceptible to temephos, the insecticide of choice, and to *Bacillus thuringiensis* H-14.

The control trials were predicated on the need for accessibility by road or on foot and thus were unable to include rivers in the south of the island that are difficult to reach. The remaining rivers were prospected for breeding sites. Places where insecticides could be applied from the ground were identified and marked with code numbers and river discharge estimates and recordings of pH and conductivity made. The first round of treatments was conducted during the week beginning 12 February 2001. Berthoud Cosmos 18 Knapsack Sprayers (piston-drawn) were used to dispense a 20% EC formulation of temephos (Abate) to give 0.4 litres m^3s^{-1} of river discharge (i.e. 0.13 ppm for 10 minutes) at a dose of 0.065 ppm when diluted with 50% water, very close to the diagnostic dose of 0.069. As no *Simulium damnosum* had been found breeding in discharges < 0.1 m^3s^{-1}, this was taken as the minimum discharge to be treated in principle, but in practice some rivers with discharges down to 0.05 m^3s^{-1} were treated. The treatments were mostly successful but failures on the Rivers Ruma, Grande and Musola were noticed, attributable to the inaccessibility from the ground of sites upstream of the highest accessible treatment points. Details of the rivers treated and the resulting reductions in flies caught during and after the operations will be presented.

THE ELIMINATION OF THE BIOKO FORM OF *SIMULIUM YAHENSE* FROM BIOKO: THE COUP DE GRACE

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Following extensive surveys of vector breeding sites, assessment of the isolation of the focus, insecticide trials and environmental impact assessment it was concluded that the elimination of the vector of onchocerciasis from Bioko would be feasible and cost effective. In the dry season of 2003 an attempt was made to treat all the vector breeding sites with temephos using both aerial (helicopter) and ground-based delivery. Known breeding sites were wiped out, and biting flies disappeared from most of the island. However, biting flies persisted in a few places, and when the insecticide campaign finished the surviving populations quickly spread out over the whole island. In 2005 the attempt was repeated after identifying the likely refuge breeding sites, and greatly increasing the number of treatment points to ensure full coverage. Known breeding sites were again wiped out and biting flies disappeared from the whole island. The last biting fly was caught in March 2005, and none have been seen since then. It is presumed that the vector is extinct.

SYSTEMATICS OF NEOTROPICAL BLACKFLIES (DIPTERA, SIMULIIDAE)

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The Simuliidae is a family of Diptera containing about 2000 species worldwide (Adler & Crosskey, 2008) of which 12 genera and approximately 359 species are found in the Neotropical Region (see Coscarón & Coscarón-Arias, 2007). The female in the majority of species requires a blood meal for egg maturation and it is this requirement that makes species in this family important as biting pests and in the transmission of parasites of the blood and skin in both man and warm blooded animals (Crosskey, 1990). The fact that all simuliid species breed in running water makes the family also economically important in terms of environmental monitoring of freshwater contamination, particularly because immature stages (larvae and pupae) are highly susceptible to both organic and inorganic pollution. In addition, blackflies are key organisms in the aquatic ecosystems as an important food source for many vertebrates due to the high larval population densities that can reach up to a million individual/m2 (Adler & Currie, 2008). Furthermore, the filter-feeding of the larva plays an important rôle in the streams ecology due to their ability to remove particulates and dissolved organic matter from the water column and egested it as nutritious faecal pellets (Adler & Currie, 2008; Malmquist *et al.*, 2001; 2004).

There are currently various taxonomic problems in Simuliidae in the Neotropical Region. Firstly, several regional faunas, for example, Peruvian Amazon basin, the Guiana Shield, are still poorly known and they are much in need of biodiversity surveys and revisionary studies, where new morphospecies are to be found. Second, the supraspecific classification is currently unstable and problematic; many species or species-groups are placed in the wrong genus, and whether subgenera should be ranked as species groups or genera is still in much debate. And thirdly, species limits remains poorly defined for many taxa because of the presence of species complexes ("sibling species").

This paper highlight the aforementioned problems with some examples from the current research that I am carrying out in collaboration with colleagues in northern Patagonia (Argentina), Brazil (whole country) and Central America (Costa Rica). The two subfamily classification system accepted in the latest World Inventory of Simuliidae (Adler & Crosskey, 2008) is followed. Therefore, all Simuliidae found in the Neotropics are now placed in the subfamily Simuliinae, tribe Simuliini. The validity of several genera, *e.g.*, *Lutzsimulium*, *Pedrowygomyia*, and *Cnesiamima* is debated in relation to their most closely related taxa. Without a doubt, the most controversial classification system is at the subgeneric or species group level within the genus *Simulium*. Here, the status of subgenera such as *Ectemnaspis*, *Inaequalium*, *Shelleyellum*, *Hearlea*, *Hemicnetha*, *Psilopelmia* and *Trichodagmia* is discussed with reference to the latest revision of Coscarón & Coscarón-Arias (2007) for this region. In addition, preliminary results on the application of the bar coding approach to the taxonomy of Neotropical Simuliidae is also given.

A REVISION OF THE TYPE-MATERIAL OF THE GENUS *STEGOPTERNA* ENDERLEIN , 1930 FROM THE COLLECTION OF ZOOLOGICAL INSTITUTE, RUSSIAN ACADEMY OF SCIENSES

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The genus *Stegopterna* has been described by Enderlein in 1930 on the base of 1 species *Stegopterna richteri* Enderlein, 1930, this species name later has been considered as a junior synonym for the species name *Melusina trigonium* Lundström, 1911. The original descriptions of the genus, and of its type-species were very short and did not include all the phases of development. Species, later included in the genus *Stegopterna* earlier were described under several generic names – *Prosimulium, Cnetha, Simulium (Stegopterna), Cnephia* (Malloch, 1914; Enderlein, 1929; Rubzov, 1940; Stone, 1952). Then in the species *S. richteri* (= *trigonium*) several subspecies were described (Rubzov & Carlsson, 1965), for them later the species status hav been suggested (Yankovsky, 2002). Some descriptions and drawings (particularly by early authors) were incomplete.

The material examined from the fund collection of Zoological Institute, RAS, concerning *Stegopterna* Enderlein, includes specimens of 11 species of this genus: males, females, larvae and pupae of *S. dentata* Rubz. & Carlss., 1965 (Komi Rep., Perm. Distr.), *S. duodecimata* (Rubz., 1940) (Northern Europe, Siberia), *S. hamuligera* Yank., 1977 (Khabarovsk Distr.), *S. majalis* Rubz. & Carlss., 1965 (Karelia, Leningrad Oblast), *S. trigonium* (Lundstr., 1911) (Northern Europe, Siberia; also Nearctic); males, females and pupae of *S. byrrangii* Yank., 2000 (Taymyr Pen.); males and females of *S. longicoxa* Rubz., 1971 (Cisbaikalia), *S. tschukotensis* Rubz., 1971 (Tshukotka Reg.); females, larvae and pupae of *S. asema* Rubz., 1956 (Maritime Terr.), *S. decafilis* Rubz., 1971 (Yakutia; also Nearctic); *S. haematophaga* Rubz. & Carlss., 1965 (Perm Distr.). For all these species (excluding *S. trigonium*) the type-series were examined and original drawings were made. In our opinion the morphological differences between the former subspecies investigated are not less, than between acknowledged species, and allow confirming their status as separate species.

Besides, the description of a new species of *Stegopterna* from Yakutia and Tshukotka shall be published in the near future.

The synonymy of *S. tschukotensis* Rubzov, 1971 (as a junior name) and *S. emergens* (Stone, 1952) (Adler et al., 2004) is not confirmed and needs a further studying.

S. tschukotensis sufficiently clearly differs from *S. emergens* by following characters.In males of *S. tschukotensis* gonocoxytes are 3 times as long as gonostyles (while in *S. emergens* the length of gonocoxytes only 1.8-2.0 times surpass the length of gonostyles); in *S. tschukotensis* the length of gonostyles approximately equal to their maximal width (in *S. emergens* gonostyles approximately 2 times as long as wide). Gonopleurites in *S. tschukotensis* are weakly sclerotized and armed by 8-12 short clearly different spines (in *S. emergens* gonopleurites are evidently sclerotized on the lateral edge, distinct spines of gonopleurites are not developed). In *S. tschukotensis* median sclerite is narrow, 7 times as long as maximally wide, distally furked on ½ of its length, branches are long and narrow (while in *S. emergens* median sclerite is short and wide, 3 times as long as maximally wide, branches are short and wide).

In females of *S. tschukotensis* the stem of genital fork is evidently widened in the distal end, branches of the fork are markedly widened distally, posteromedial apodemes of the branches are large, very sclerotized on the posterior margin (while in *S. emergens* the stem of genital fork is not widened in the distal end, branches of the fork are more narrow, posteromedial apodemes of the branches are weakly developed). In *S. tschukotensis* hypogynial valves are very short and weakly sclerotized (0.5 of the length of strongly projected ahead basisternum) (while in *S. emergens* hypogynial valves are evidently sclerotzed jn the medial margin and approximately equal in length to not so projected, as in *S. tschukotensis*, basisternum) In *S. tschukotensis* spermatheca is 1.5 times as wide as long, walls are smooth (in *S. emergens* spermatheca is oval, 1.3-1.4 times as long as its diameter, walls are structurized).

Suffitiently clear differences between the characters of males and females of *S. tschukotensis* and *S. emergens* give the base to consider *S. tschukotensis* Rubzov, 1971 as a separate species. It seems, that regarding this name as a junior synonym to *S. emergens* (Stone, 1952) (Adler et al., 2004) needs further confirmation.

Thus in our opinion the genus *Stegopterna* consists of 19 separate species, and includes, besides 10 Palaearctic and 2 species enumerated above, the Nearctic species: *S. acra* Currie, Adler & Wood, 2004; *S. diplomutata* Currie & Hunter, 2003; *S. emergens* (Stone, 1952); *S. mutata* (Malloch, 1914); *S. permutata* (Dyar & Shannon, 1927); *S. xantha* Currie, Adler & Wood, 2004; and 1 species from Japan *S. nukabirana* Ono, 1977.

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USING MULTIPLE CHARACTER SETS FOR ASSESSING SPECIES STATUS: AN EXAMPLE OF THREE EUROPEAN SPECIES OF THE *SIMULIUM VERNUM* GROUP

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The value of using characters from multiple sources – chromosomes, ecology, gene sequences, and morphology – to evaluate species status of closely related black flies is demonstrated for three European members of the *Simulium vernum* group: *Simulium crenobium*, *S. juxtacrenobium*, and *S. vernum sensu stricto*. *Simulium juxtacrenobium* is a chromosomally, molecularly, and morphologically distinct species that diverged from *S. crenobium* and *S. vernum* about 2 million years ago. It is specialized for intermittent streams and univoltine. In contrast, *S. crenobium*, although confirmed as a distinct species, differs from *S. vernum* by only a few larval and chromosomal characters and a breeding habitat restricted to mountain spring brooks. While all four character sets independently support the specific distinctness of *S. juxtacrenobium* and *S. vernum*, multiple character sets are required to establish the specific validity of *S. crenobium*.

UNDER THE CANOPY: AT WHAT HEIGHTS DO BLACKFLIES SEEK BLOOD HOSTS IN BOREAL FORESTS?

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On seven dates in mid-late June 2007, biting insects were captured using CO2 baited traps at two sites in managed conifer forests (one dominated by Scots pine, the other by Norwegian spruce) near the Vindel River in northern Sweden. Traps were positioned at 1.5, 5 and 10 m above the ground, which allowed a comparison of the vertical distribution of different taxa. More than 36,000 blackflies were captured and identified to the lowest possible taxonomic unit. Fourteen taxa were found with an average of 435 and a range of 0-2301 blackflies per trap and day. The most common taxa, making up 98% of the simuliid catch were *Metacnephia lyra* (47.5%) , *Simulium vernum* group (39.2%) and *Simulium venustum* group (11.1%). Most species were caught in higher numbers in the pine forest and at the lowest height. A notable exception was the *Simulium vernum* group. Members of this group were not only more commonly captured in the spruce forest but also appeared to have a distinct preference for the 10 m traps. These are ornithophilic species whose larvae are common in small streams. The affinity to canopies might explain the disproportionally low encounter rates in most surveys of adult simuliids, surveys being normally carried out near the ground. Adults of *Metacnephia lyra* emerge from large river rapids, where their larvae develop at extraordinary densities. Members of the *Simulium venustum* group, common in traps positioned at a low height, develop in various habitats and have a strong preference for large cervid hosts. We discuss the observed distribution patterns in the light of the availability of blood and sugar sources, distance to breeding habitats, and tree architecture.

BLACKFLIES (SIMULIIDAE) OF NORTHERN EUROPE

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This is a pre-issue presentation of a new CD-ROM on blackflies. Taxonomy and notes on biology and distribution of 74 black fly species that are recorded in Fennoscandia are presented. The species are illustrated in their larval, pupal and adult stages. Identification keys to species of larvae and pupae are provided. Images will be added to the keys in the final version.

The study is based on type material and the authors' collections from Fennoscandia that are deposited in the respective museums of affiliation. Twelve nominal species are hereby sunk in synonymy: *annuliforme* Rubzov, *asema* Rubzov, *carpathicum* Knoz, *curvistylus* Rubzov, *elburnum* Rubzov & Carlsson, *intermedia* Rubzov, *korsakovi* Rubzov, *pitense* Carlsson, *saileri* Stone, *tricrenum* Rubzov & Carlsson, *tumulosum* Rubzov, *usovae* Golini.

The CD-ROM is to be issued spring 2009 by ETI, the Expert Center for Taxonomic Identification, University of Amsterdam.

BLACK FLIES OF THE BALKAN PENINSULA

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The Balkan peninsula covers a significant part of Europe (5-7 % of land area). From the faunistic point of view the region plays an important role as a refugium during glacial periods and as corridor connecting Near East and Europe. It has been also important area in the black fly research – mainly regarding *Simulium colombaschense* (Scopoli, 1780), the type species of the genus *Simulium* and the most important species among the causatives of huge black fly outbreaks and damages in the Iron Gate area. The type locality of 14 black fly species is located in Balkan peninsula, some of them may be endemic to this region. Despite of this, the knowledge on the black fly fauna of the region is poor and heterogeneous. The data on black flies of the Balkan countries is either missing (European part of Turkey, Albania, Moldavia) or the fauna is strongly underrated (Greece, Macedonia, Montenegro, Bosnia, Croatia, Hungary). On the other hand, large numbers of black fly species have been published from Romania and Bulgaria and the reliability of these data is questionable (mainly for Romania), as e.g. several species with nearest known occurrence in Caucasus or Scandinavia have been included. The black fly fauna of Slovenia seems to be moderately underrated. Probably the best known black fly fauna of the region is the Serbian one, however also here additional species are expected and the predominantly more than 30 year old faunistic data have to be updated. Another essential problem of the knowledge of the black fly fauna of Balkan peninsula is the uncertain taxonomic status of many species occurring.

A BIOGEOGRAPHICAL EVALUATION OF TURKISH SIMULIIDAE FAUNA

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Although Turkish Simuliidae fauna is not well known, Crosskey and Zwick (2007) reports 40 identified species from Turkey. We aimed to evaluate the literature on Simuliidae in Turkey and neighboring countries to form a baseline for future studies. In addition to 44 species that have been found up today in Turkey, we pointed the species that exist in the neighboring countries and that are supposed to exist in Turkey. To do this, we used a similarity matrix. The number of the species that possibly exist in Turkey shows that we need an extensive research on Turkish simuliids.

Keywords: Diptera, Simuliidae, blackflies, Turkey.

ESTIMATION OF RELATIONSHIPS BETWEEN THE BLACK FLIES (DIPTERA: SIMULIIDAE) DISTRIBUTION AND HETEROGENEOUS ENVIRONMENT

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Investigations carried out in 2001- 2004 years were focused on black fly taxocenoses of the Sema river, a leftside tributary of the Katun’ river (south Siberia). The aim was to establish relationships between distribution and biodiversity in connection with changing environmental parameters.

The biodiversity included 30 species, which composed about 48 % of specific and 78 % of generic composition of Altai black flies fauna. The longitudinal gradient of the Sema river comprised epyrhithral, metarhithral and hyporhithral zones. The most differences were established between the upper and the lowest points of gradient. The main part of the basin population was formed by the species of metarhithral. In the structure with the statistical analyses were determined 6 classes, which successively disposed on the different altitudes with specific dominant composition. The continual and discrete moments in the distribution of black flies were established. The main factors predicting distribution and structure within the North Altai Mountain region were: altitude as integrating index on which the water temperature depends; periphyton presence, granulometric composition of bottom. The water velocity and river size did not play a definition role in this process. The influence of anthropogenic factors on the running waters were not significant, impoverishment of composition and appearance of super dominant species were not established.

Keywords: Simuliidae, black flies larvae, spatial ecology, distribution, biodiversity, community structure.

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THE EFFECTS OF ALGAE ON *B.T.I.* EFFICACY IN BLACK FLIES

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Products containing the biological control agent, *Bacillus thuringiensis* var. *israelensis (Bti)* are widely used throughout the world to suppress larval black fly populations. Suppression program operators in North America, South Africa and West Africa have report reduced levels of Bti induced efficacy when algal populations increase. As a result, the effects of algae on the efficacy of *Bacillus thuringiensis* var. *israelensis* against larval black flies (*Simulium vittatum* IS-7) have been investigated in laboratory bioassays. The green alga *Scenedesmus quadricauda* has been shown to decrease *Bti* efficacy in laboratory experiments. Two approaches to mitigate these effects include increasing the dose of Bti exposed to the larvae and increasing the length of the exposure period. Additional studies have begun investigating the effects of different algae on ingestion rates and flick rates to try to identify the underlying cause of reduced *Bti* efficacy.

CONTROL OF *SIMULIUM JENNINGSI* GROUP BLACK FLIES WITH *BACILLUS THURINGIENSIS ISRAELENSIS* (BTI) IN PENNSYLVANIA.

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The Pennsylvania Black Fly Suppression Program targets four human-pest black fly species in the *Simulium jenningsi* group in a joint state-county funded control program.

The program goal is to reduce adult black fly populations below pest level (<10 adults per 9 sweeps of a collection net) during the spring through fall tourism and recreational season in participating counties. Program staff collect, identify and analyze more than 10,000 larval, pupal and adult black fly samples per season to determine the need for, timing of, and effectiveness of treatments. Approximately 2,575 kilometers (1,600 miles) of forty-seven rivers and streams in the Delaware, Ohio and Susquehanna River Basins are monitored and treated every seven to fourteen days from April to September. Contracted aerial applicators apply VectoBac 12AS, *Bacillus thuringiensis israelensis* (*Bti*) by helicopter at 11.5 parts per million, in riffle production areas. In 2007, eleven pilots and nine helicopters logged nearly 500 rotor hours on 100 individual spray operations. Treatment operations effectively reduce *Simulium jenningsi* group populations in contract areas. More than three million Pennsylvania residents and tourists benefit from the black fly suppression program each year.

BITING STRATEGIES AND BITING ACTIVITIES BY BLACK FLIES (DIPTERA: SIMULIIDAE)

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Females of many species by Simuliidae are adapted to cut the skin of the host with their mouthparts and to draw blood or body fluids. They are typical pool-feeders and use the life-sustaining proteins to produce their eggs.

In the black fly plague areas in Central Europe, the flies contact their hosts and undertake their biting activities mostly in open areas. Biting activities and strategies have migrated with the wind, and are often close to the breeding source. Observations have been made on *Simulium equinum*, *S. ornatum*, *S. erythrocephalum*, *S. reptans* and *S. nigrum*. For all these species, the seasonality of biting has been recorded and also the influence of climate on development and adult emergence as well as the environmental factors which affect flight activities.

Blood-sucking and feeding experiments have been conducted with a few species and differences in the time, duration and effectiveness of egg development/ maturation/ production will be discussed.

Keywords: Simuliidae, Europe, blood feeding, biting activities, biting strategies

THE *SIMULIUM DAMNOSUM* COMPLEX IN SOUTH TANZANIA – CYTOGENETICS MEETS VECTOR CONTROL

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We investigated the isolation of the South Tanzanian Tukuyu focus of onchocerciasis from possible vector re-invasion. This was achieved by examining the distribution of *S. damnosum* s.l. in- and outside the focus to look for potential re-invasion sources, from 2002 to 2007, including pre-, during- and past-control surveys. Cytotaxonomic identifications of the aquatic stages as well as morphotaxonomic and molecular techniques allowed the incrimination of *Simulium thyolense* as the sole anthropophilic species in both the Tukuyu and the neighbouring Ruvuma foci. Apart from this we detected differences in chromosome inversion frequencies between Tukuyu populations and those breeding in a southwestern river basin and in northern Malawi where there is no man-biting and onchocerciasis, respectively. This suggests that there is not normally a great deal of migration in either direction. However, vector elimination has clearly failed due to a re-invasion from the Ruvuma focus into Tukuyu after temephos application, even though to some degree restricted as indicated by slight differences in chromosome polymorphisms between the two foci. From the accompanying *S. damnosum* s.l. cytoforms composition, e.g. cytoform 'Kasyabone' which is assumed zoophilic, it would have been possible to monitor (subsequent to vector control in the Tukuyu focus) the migratory events since this taxon is exclusively endemic in the two foci. Hence, its re-appearance could only have had Ruvuma as a source. Eventually, 'Kasyabone' displayed a rather unique cytogenetic plasticity with certain geographic and sex linkage as well as rare cases of hybridization. As a result, some individuals with a karyotype analogous to the cytospecies *S. kilibanum* exist, which might represent genetic atavism.

THE BLACK FLY GENOME PROJECT

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We propose a full genome sequencing project for the Simuliidae (Black Flies). The initial focus species is *Simulium squamosum*, a member of the *S. damnosum* complex. The full genome sequence will be an invaluable resource of the insect genomics community, allowing order-wide functional genomic comparative analysis of genomic contents and organization, and for functional analyses of critical parameters such as insect attributes linked to their capacity at transmitting diseases, and basic biological functions. This project aims to make a significant impact in furthering genomic knowledge of vector biology, by promoting comparative research on a disease agent that has close phylogenetic relationships to both mosquitoes (*Anopheles*, *Aedes*) and to non-haematophagous insects. Biological material is supplied by participating laboratories. The Post laboratory has constructed and is characterizing a bacterial artificial chromosome (BAC) library from *S. squamosum* DNA derived from ~3,000 individuals. The Center for Genomics and Bioinformatics (Indiana University, Bloomington) will sequence the BACs, and oversee assembly of the sequence. The database is to be hosted by the VectorBase consortium. We are soliciting the involvement of the simudology and insect genomics research communities for the overall analysis and annotation. The sequencing strategies and progress to date will be presented.

A BAC LIBRARY PREPARED FROM FIELD-CAUGHT *SIMULIUM SQUAMOSUM* ALSO COVERS THE *MERMIS* AND *WOLBACHIA* GENOMES.

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BAC libraries have played a key role in most successful eukaryotic genome projects and been utilised in a variety of capacities ranging from gene characterisation to systematises. When small organisms are used, and insufficient DNA can be taken from a single individual, BAC libraries must be prepared from many individuals, which in the case of insects can be many thousands. Insect BAC libraries are usually prepared from highly-inbred laboratory colonies, which enable genome contamination to be minimised. Despite great effort, attempts to colonise *S. damnosum* s.sl. have thus far been largely unsuccessful and so most work carried out on *S. damnosum* complex is done on field-caught material. Here we describe how positive *S. damnosum* BACs have been identified from contaminating genomic DNA by a single-step hybridisation procedure using 18 distinct families of S. damnosum LINEs and show that some of the remaining non-*S. damnosum* BACs derive from their mermithid parasites, Isomermis Lairdi, and from the intracellular bacteria *Wolbachia*.

WHAT HAPPENS IN A BLACKFLY LARVAL GUT?

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Dissect a blackfly larva and one internal feature dominates - the gut (although the size of the salivary glands is also impressive). Closer inspection reveals a narrow fore-gut, a long and capacious mid-gut and a narrow hind-gut that is formed into a loop. We know something of the function of each of the sections of the gut, but what processes occur within the gut contents and what is their significance?

Food is collected on the cephalic fans using methods that are still unclear and all material that collects is ingested - there is no selection mechanism. Folding of the fans allows cleaning of the fan rays by the mandibles and the accumulated “boluses” are passed into the fore-gut and thence to the mid-gut. Constant additions ensure that the food then passes along the mid-gut, the contents being surrounded by peritrophic membrane to protect the gut wall. This section of the gut is characterised by having a high pH and material passes through it rapidly (less than 1 hour), with no obvious mixing. Evidence suggests that little of the ingested food is assimilated. The final passage of material through the hind-gut results in compaction, followed by the egestion of pelletised faeces. As feeding is a nearly continuous process, large numbers of faeces are produced and these are bound by exopolymers (EPS), not by peritrophic membrane.

I pose the following questions:

(i) What food materials are digested?

(ii) What part does high gut pH play in breaking chemical bonds?

(iii) What is the significance of high proteolytic enzyme activity in the mid-gut and of enzymes that break up α-1,4-glucans and β-1,3-glucans?

(iv) Why are some species autogenous?

(v) What proportion of EPS are collected free from the water and what proportion are secreted within the gut by living organisms?

(vi) What is the role of microbial and algal EPS secreted within the gut?

(vii) Why does the cohesion of the gut contents vary from the anterior mid-gut to the anus?

(viii) What part do faeces from larval blackflies play in running-water ecosystems?

IMPORTANCE OF CHEMICAL STIMULI IN *SIMULIUM LINEATUM* (DIPTERA: SIMULIIDAE) PRE-COPULATORY BEHAVIOUR

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Purpose of the present research was to establish whether black flies possess sex pheromones regulating their precopulatory behaviour.

Bloodsucking stenogamous (ground coupling species) *Simulium lineatum* (Meigen, 1984) was chosen as a model species. For comparative study, a phyllogenetically closest bloodsucking eurygamous (aerial coupling species) *S. equinum* (Linnaeus, 1758) has been chosen.

Mating behaviour of *S. lineatum* was registered both under natural and laboratory conditions. In the nature, *S. lineatum* males demonstrate courtship behaviour towards conspecific blackflies of any sex; however, quantitative characteristics of behavioural interactions differ depending on the sex in contact.

Based on the results of behavioural recordings in nature and under laboratory conditions and after a number of preliminary tests, an original bioassay for search of sex pheromones has been elaborated. A stimulus as a filter paper either with evaporated pure solvent or solvent containing female cuticular chemicals plus a female dummy attached thereon was provided to *S. lineatum* male. Duration of exposition to a stimulus was 1 min. Registered pre-copulatory behaviour elements were the following three: approach, contact (a dummy touch) and attempt to copulate.

The cuticle of *S. lineatum* females contains chemical compounds affecting pre-copulatory behaviour of conspecific males during the final phases of courtship only (touching and attempting to copulate). The effect is dose dependent. The cuticular chemicals are species specific, as those of phylogenetically closely related *S. equinum* females do not affect pre-copulatory behaviour of *S. lineatum* males.

Chromatographic profiles of extracts of *S. lineatum* and *S. equinum* adults (both females and males) differ qualitatively and quantitatively. 27 compounds (10 of them are hydrocarbons with C_{17}, $C_{26,}C_{37}$) are present in *S. lineatum*, and 55 compounds (19 of them are hydrocarbons with C_{11},$_{19}$ and $C_{24,37}$) in *S. equinum* only.

Bioassay confirms presence of sex pheromone in stenogamous *S. lineatum* females. This is the first sex pheromone revealed in the family Simuliidae.

INVESTIGATIONS ON THE ULTRASTRUCTURE OF SENSORY APPARATUS OF THE ADULT FEMALE BLACKFLY, *SIMULIUM CHUTTERI* LEWIS (DIPTERA: SIMULIIDAE)

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In South Africa, blackflies have been implicated in the spread of two pathogens i.e Chlamydia species that cause blindness in sheep and abortion in cattle, and Rift Valley Fever virus. In other parts of the world, the blood-feeding activity of adult female blackflies transmits a variety of pathogens, notably Onchocerca, Leucocytozoon, Mansonella, Trypanosoma and Dirofilaria (Adler, 2005). *Simulium chutteri* Lewis (Diptera: Simuliidae), endemic to Southern Africa, is the most important blackfly pest of livestock in South Africa. A thorough understanding of blackfly behavioural patterns exhibited during host location cannot be fully attained unless information the sensory apparatus of *S. chutteri* responsible for stimuli reception, which elicit host location behaviour, have not yet been studied.

Wild populations of live adult female blackflies, *S. chutteri* were sweep-netted from sheep grazing in open fields along the Vaal River in the Christiana-Bloemhof area, South Africa. Live specimens were anaesthetized with diethyl ether and prepared for scanning electron microscopy (SEM) by fixation in 2.5% gluteraldehyde buffered at 0.075M sodium phosphate at pH of 7.4. After dehydration through a graded ethanol series, the specimens were critical point dried and mounted on SEM stubs with double-sided tape. Conductivity of the specimens was enhanced using RuO4 vapour. Examination and micrography was done on a JEOL JSM 840 SEM, and a JEOL JSM 6000F SEM was used to obtain high resolution micrographs.

Five types of sensilla were found on the antennal flagellum of *S.chutteri*, namely Type 1 sensilla chaetica (T1), Type 2 sensilla chaetica (T2), sensilla trichodea (ST), sensilla basiconica (SB) and the grooved sensilla basiconica (GSB). T1's are uniporous, thick-walled, outwardly curving sensilla protruding and occurring only around the antennal tip, whilst the T2s are thick-walled, grooved spines, outwardly curving, similarly structured as T1's, but shorter and located on the general flagellar surface. ST are thin-walled, porous, evenly distributed over the flagellum and most numerous and inwardly recurving sensilla. SB are thin-walled, consisting of two sub-types, some tapering and some club-like, shorter in the flagellomere ends than mid-region of antennal segments. GSB are thick-walled, multiporous, grooved and sparsely distributed on all flagellomeres mostly associated with SB at distal ends of flagellomeres.

Morphological and structural characters of Type 1 and Type 2 sensilla chaetica have functional adaptations of contact chemoreceptors, whilst the structural adaptability of sensilla trichodea, sensilla basiconica and grooved sensilla basiconica is of olfactory reception.

NEW RESULTS ON DIPTERA PREDATORS IN THE BLACK FLY PLAGUE AREAS OF SOUTH AFRICA

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Black flies have a wide range of natural enemies which attack all their life stages by feeding on them directly. Within the broad context of the “management” of black fly populations, the Diptera predators undoubtedly have a role to play. They do not have such a fundamental effect on black fly populations as do the parasites which infect the larvae, but their role in the regulation of black fly population numbers should not be under-estimated. Numerous predators and scavengers – there is only a fine line between these feeding strategies – from different insect orders are known to feed on black flies and influence the populations in proportion to their own biology, in other words according to the mode of their predatory strategies.

Insects from at least 9 orders are known to feed on black flies and attack all developmental stages. The most important of these are undoubtedly the caddis flies (Trichoptera). Equally important, but under-estimated and certainly under-investigated, are the Diptera. Our field investigations have shown that very many more species than was even recently thought are important obligate predators as larvae or adults or both.

In the course of fieldwork in South Africa two years ago, we were able to show that there are a number of Diptera groups that prey on different black fly species and that many of these are the same groups that are encountered as predators in Europe. This is particularly true of the muscid genus *Limnophora*, in which there is a small group of species in both Europe and South Africa that form a close monophyletic group and which share identical biotopes, habits and predatory behaviour. The endemic Afrotropical muscid genus *Xenomyia* was also studied. Other groups encountered in numbers were the Dolichopodidae and Empididae.

It is clear that there are some very specific associations between certain Diptera predators and black flies, particularly in those regions where black flies occur in plague numbers and thereby offer a rich food resource. Our work has shown that this is a not insignificant role, and further investigations of both larval and adult predators are expected to confirm this and to reveal additional associations.

Key words: Simuliidae, Diptera, South Africa, predation, predators, prey

LANDSCAPE APPROACH TO BLACK FLY PATHOGENS

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Examining larval black flies from a variety of sites over North America, we catalogued the major groups of black fly pathogens. These pathogen groups included the Mermithidae, the chytrid fungus *Coelomycidium simulii*, and the microspordians.The two most commonly encountered microspordians were *Janacekia debaisieux* and *Polydispyrenia simulii*. The overall rate of host infection (with at least one patent pathogen infection) was approximately 6%. We next explored how the occurrence of pathogens varied among larval communities across different regions of North America. For example, in California (Pacific Coast), the overall rate of infections was 1.9%, whereas in South Carolina (Atlantic Coast), prevalence was more than four-fold greater at 8.4 %. Results of regression analysis, using pathogen prevalence as the response variable and latitude and longitude as predictors, are presented. Finally, we examined whether particular groups or species of black flies were more susceptible than others to infection. For example, the majority of larvae harboring mermithids belonged to the *Simulium tuberosum* complex.

POSTERS

THE USAGE OF BIOLOGICAL PREPARATIONS AGAINST BLACKFLIES IN LITHUANIA. ELEVEN YEARS OF EXPERIENCE

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There are known several records about outbreaks of bloodsucking blackflies in Lithuania. The last outbreak of bloodsucking blackflies started in the middle of 1970s in the South-eastern part of Lithuania. Bloodsucking insects caused losses of cattle and domestic birds and annoyed holiday-makers of the Druskininkai health-resort. Data on the fauna of blackflies of Lithuania were very scanty at this time. The main pest species was determined as *Simulium maculatum* Mg. in 1997. Larvae of *S. maculatum* developed in the largest river of Lithuania, the Nemunas River. The first generation of *Simulium maculatum* developed in April – June and the second one developed in the August. The first generation was very abundant and the second generation was not numerous.

The density of *S. maculatum* larvae was increasing each year: 1440 ± 230 larvae/1dm^2 of water plant surface in 1997 and it increased to 2043 ± 282 larvae/1dm^2 of water plant surface in 1999. The bloodsucking activity of blackflies was 356,7 ± 112,6 per 10 min.

Biological preparation against blackflies (Bactoculicid) was used in 1998 in the Nemunas River nearby the Lithuanian – Belarusian border. The preparation is based on the effect of *Bacillus thuringiensis* var. *israelensis*. The effect against blackfly larvae was determined in the segment of the river 19 km downstream from the point of application. This application did not decrease bloodsucking activity of blackflies significantly.

Blackfly control with VectoBac 12AS was started in Lithuania in the Nemunas River in 1999. From 4000 to 8500 kg of preparation VectoBac 12AS was used each year for blackfly control. The discharge of the Nemunas River varied from 152 to 363m^3/s during the application in different years.

The effect on blackfly larvae was detected in distance of more than 150 km. The mortality of blackfly larvae was from 49.8% (in 164 km distance from the point of application) to 95.9% (in 25 km distance from the point of application) on the 7th day after the application.

The density of newborn *S. maculatum* larvae decreased 5 times from 1999 till 2002 (410 ± 108 larvae/1dm^2 of water plant surface). From the year 2002 to 2008 the density of newborn *S. maculatum* larvae fluctuated from 104 ± 11 larvae (the year 2003) to 398 ± 138 larvae/1dm^2 of water plant surface (the year 2008).

The relative abundance of different blackfly species in the Nemunas River have changed. The relative abundance of *Simulium lineatum* (Mg.) and *S. erythrocephalum* (De Geer, 1776) (larvae develop in June – July) increased and the relative abundance of *S. maculatum* and *S. reptans* (L.) (larvae develop in May – June) decreased. *S. maculatum* and *S. reptans* larvae develop together and both of them were affected by biological preparation.

The highest bloodsucking activity of blackflies decreased 13 times from the year 1999. It fluctuated from 4 ± 3 per 10 min. (the year 2006) to 78 ± 41 per 10 min (the year 2007) and depended on the meteorological conditions and on the possibility to treat the segment of the Nemunas River in the neighbour Belarus especially.

ATLAS OF POLYTENE CHROMOSOMES OF 123 SPECIES OF BLACKFLIES FROM THE PALEARCTIC

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Blackflies (Simuliidae) are a difficult object for study. Numbers of mitotic chromosomes are monotonous: 96% species have 2n=6, the others, 2n=4 or 3n=9. The most promising character is polytene chromosomes (PCh), which possess species specific pattern of discs and permit defining a species. Photomaps of PCh and structure of chitinous covers of 123 species of 32 genera of the family are given in the atlas. Conjugation of homologous chromosomes depends on age of species, it can be complete in primitive species, partial or be absent in quite young species. Considerable diversity of centromeres was discovered: they are morphologically absent in primitive species and are distinctly pronounced in the form of strong intensively stained blocks, which sometimes form a chromocentre, in advanced species. Simuliidae are a mononucleolar family. Nucleolus (N) is either situated in chromosome I (*Twinnia*, *Prosimulium*, *Metacnephia*, *Sulciphepia*, *Cnetha*, *Montisimulium*, etc.), or in chromosome III (*Helodon*, *Boophthora*, *Gnus*, *Odagmia*, *Argentisimulium* etc.). Increase of the number of nucleoli and change of their localization is an exceptional phenomenon in blackflies. Puffs and Balbiani rings are localized on the short shoulder of chromosome II (IIS), their number of blackflies is constant. Their localization changes from species to species by means of inversions. Among blackflies there are species with differentiated regions PCh (heterozygous inversion, heterozygous disc, heterozygous N, additional heterzygous block of heterochromatin) determining sex by type XY♂ : XX♀, but there are species with a more complicated sex determining type and with multiple X and Y chromosomes. Polyploidy phenomenon occurs in blackflies, the entire chromosome set (triploids of *P. isos*, *P. pamiricum*, *P. pecticrassum*, etc.) or its part (*O. kiritshenkovi*) can increase. B-chromosomes were discovered in natural populations of blackflies. They are much smaller than A-chromosomes, with distinct disc structure and without it, within the limits of one individual their number is permanent, but their number varies from individual to individual, they are of heterochromatin nature. Structural changes of chromosomes in blackflies include translocation of shoulders of chromosomes and N. As a result of these rearrangements new combinations of shoulders in blackflies appeared, and N was transferred from chromosome I to chromosome III. Deletions, deficiency and small structural rearrangements determine genetic diversity of blackflies. Para- and pericentric inversions occur in blackflies most frequently. There are monomorphic species, e.g. in genera *Twinnia*, *Levitinia*, *Helodon* etc., and there are highly polymorphic species, e.g. in genera *Prosimulium*, *Metacnephia*, *Wilhelmia*, *Boophthora*, *Gnus*, *Argentisimulium*, *Simulium* etc. And eventually, tandem fusion of two chromosomes forming one chromosome played an important role in the evolution of blackflies. This mechanism arose two times independently, in genus *Astega* and in genus *Eusimulium*.

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INVESTIGATION OF THE BLACKFLY FAUNA (DIPTERA: SIMULIIDAE) COLLECTED FROM LOWLAND WATERCOURSES IN NORTH-EAST-HUNGARY

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Blackflies (larvae and pupae) are significant components of lotic systems and are often present in very high densities. Despite the fact that they can colonize almost every type of flowing waters, our present knowledge on the inland blackfly fauna is very poor, so with this study I'd like to proceed to reduce the deficiency. Larvae and pupae were collected from 20 small watercourses and rivers in 2006. Altogether six blackfly species and one species-group (*Simulium (Eusimulium) aureum*-gr.) were found. All taxa were typical in lowland areas, but the occurrence of *Simulium (Simulium) ornatum* at higher elevation (montain and submontain zone) is also not rare. The most frequent species was the *Simulium (Boophthora) erythrocephalum*, which occurred in the 81,5% of the samples followed by the *Simulium (Simulium) ornatum* and *Simulium (Nevermannia) lundstromi* 22,2% and 14,8%, respectively. The *Simulium (Simulium) reptans*, *Simulium (Simulium) noelleri* and *Simulium (Wilhelmia) balcanicus* were the rarest species in this survey. Since blackflies are sensitive to hydromorphological changes, they could be used effectively in the Hungarian ecological quality assessment practices, too.

AQUATIC MACROINVERTEBRATES IN WESTERN GHATS WITH SPECIAL REFERENCE TO BLACKFLY POPULATION

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We analyzed the diversity and distributional patterns of macroinvertebrates and studied the influence of environmental variables on macroinvertebrates with special reference to population of Blackfly assemblages from streams and rivers of Western Ghats. The present study was carried out in 100 streams and rivers of Western Ghats between June 1996 and May 2006. A total of 78,129 individuals belonging to 124 genera, 64 families and 11 orders were collected, of which, *Simulium* sp. (76%) constituted the important taxonomic groupings. The patterns of distribution of dipterans in all sampling sites varied significantly with *Simulium* sp. and constitute 40% collected from stream substrates. Canonical Correspondence Analysis showed that altitudes were highly related with Ephemeroptera, Trichoptera and Plecoptera, stream velocity was related with Diptera and Coleoptera and the substrates were related with all insect orders. The vector species of *Simulium* was highly related with low altitudes and found mainly in the fast flowing stream habitat, basically low altitudes are often associated with anthropogenic impacts and having the risk of transmission of vector borne diseases.

Keywords: community composition, microdistribution, *Simulium*, environmental variables

OBSERVATIONS OF BLACKFLY MATING SWARMS IN FINLAND

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Mating swarms of blackflies have been observed in northern Finland near or above the treeline, and in forest landscape in southern Finland. Records of mating swarms have been made of *Metacnephia* species, and in the *Simulium* subgenera *Simulium* and *Nevermannia*. Most recordes have been made of *Simulium posticatum*, *Simulium ornatum* species group, and *Simulium tuberosum*, respectively, while swarming behaviour among other species has been recorded sporadically. Differing preferences for swarming markers are summarised among species, hill-topping and orientation to trees having been recorded in both south and north.

CONTRIBUTIONS TO THE SIMULIIDAE (INSECTA, DIPTERA) FAUNA OF TURKEY

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The distribution of some Simuliidae species in some running waters in western Turkey was given in relation to some environmental variables. Relationships between Simuliidae species and environmental variables were explored by canonical correspondance analysis.

BLACKFLIES (DIPTERA: SIMULIIDAE) OF UKRAINE

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The detailed historical analysis of faunistic studies of blackflies in Ukraine was presented by A. Panchenko in his monography (2004). Below the specified regular index of species of Simuliidae found in Ukraine is presented, based on classification by Rubzov–Yankovsky (1956, 1988, 2002).

Subfamily **PROSIMULIINAE** Enderlein, 1921

I. Tribe ECTEMNIINI Enderlein 1931. Genus ***Cnephia***: *pallipes* (Fries, 1824); *toptchievi* Yankovsky, 1996.

II. Tribe GYMNOPAIDINI Rubzov, 1955. Genus ***Tvinnia***: *hydroides* (Novak, 1956).

III. Tribe PROSIMULIINI Enderlein, 1921: ***Prosimulium***: *hirtipes* (Fries, 1824), *Pr. latimucro* (Enderlein, 1925), *Pr. nigritum* (Rubzov, 1956), *Pr. rufipes* (Meigen, 1830), *Pr. tomosvaryi* (Enderlein, 1921).

IV. Tribe STEGOPTERNINI Enderlein, 1930: Genus ***Stegopterna***: *trigonia* (Lundström, 1921).

Subfamily **SIMULIINAE** Nevman, 1834

I. Tribe NEVERMANNIINI Enderlein, 1921. Genus ***Boopthora***: *chelevini* Ivashchenko, 1968, *erytrocephala* (De Geer, 1776). Genus ***Byssodon***: *maculatus* (Meigen, 1824). Genus ***Cnetha***: *angustata* (Rubzov, 1956), *bertrandi* (Grenier & Dorier, 1959), *brevidens* (Rubzov, 1956), *carpathica* (Knoz, 1961), *carthusiensis* (Grenier & Dorier, 1959), *chodakovi* Panchenko, 1998, *codreanui* (Serban, 1958), *costata* (Friederichs, 1920), *crenobia* (Knoz, 1961), *cryophila* (Rubzov, 1959), *fontia* (Rubzov, 1955), *geigelense* (Djafarov, 1954), *karajimae* Panchenko, 2003, *lidiae* Semuschin & Ussova, 1983, *silvestris* (Rubzov, 1956), *taurica* (Rubzov, 1956), *verna* (Macquart, 1826). Genus ***Eusimulium***: *angustipes* (Edwards, 1915), *aureum* (Fries, 1824), *krymense* Rubzov, 1956, *securiforme* Rubzov, 1956, *velutinum* (Santos Abreu, 1922). Genus ***Hellichiella***: *latipes* (Meigen, 1804). Genus ***Nevermannia***: *angustitarsis* (Lundström 1911), *latigonia* (Rubzov, 1956), *lundstromi* Enderlein, 1921, *volhynica* Ussova & Suchomlin, 1990. Genus ***Schoenbaueria***: *nigra* (Meigen, 1804), *pusilla* (Fries, 1824), *raastadi* (Usova & Reva, 2000), *subpusilla* (Rubzov, 1940), *suchomlinae* Usova & Reva, 1995.

II. Tribe SIMULIINI Newman, 1834. Genus ***Archesimulium***: *tuberosum* (Lundström, 1911), *vulgare* (Dorog., Rubzov & Vlasov, 1935). Genus ***Argentisimulium***: *dolini* Usova & Suhomlin, 1989; *noelleri* (Friederichs, 1920); Genus ***Cleitosimulium***: *argenteostriatum* (Strobl, 1898). Genus **G*nus***: *ibariense* (Živkovich & Grenier, 1959). Genus ***Obuchovia***: *auricoma* (Meigen, 1818); *brevifilis* Rubzov, 1956; *karasuae* Panchenko, 1998. Genus ***Odagmia***: *acutiphallus* (Rubzov, 1956); *angustimanus* Enderlein, 1921; *argyreata* Meigen, 1838; *baracornis* Baranov, 1926; *caucasica* (Rubzov, 1940); *deserticola* (Rubzov, 1940); *frigida* (Rubzov, 1940); *intermedia* (Roubaud, 1906); *maxima* Knoz, 1961; *monticola* (Friederichs 1920); *ornata* (Meigen 1818); *pontica* (Rubzov 1956); *pratora* (Friederichs, 1921); *rotundata* Rubzov, 1956; *variegata* (Meigen, 1818). Genus ***Paragnus***: *bucovskii* (Rubzov, 1940); *degrangei* (Dorier & Grenier, 1959). Genus ***Simulium***: *abreviatum* Rubzov, 1957; *bergi* Rubzov, 1955; *curvistylus* Rubzov, 1957; *hibernale* Rubzov, 1967; *kachvorjanae* Usova & Zinchenko, 1991; *longipalpe* Beltukova, 1955; *morsitans* Edwards, 1915; *paramorsitans* Rubzov, 1956; *posticatum* Meigen, 1838; *promorsitans*, Rubzov 1956; *reptans* (Linnaeus, 1758); *rubtzovi* Smart, 1945; *schevtschenkovae*, Rubzov 1965; *simulans* Rubzov, 1965; *truncatum* (Lundström, 1911); *voilense* Sherban, 1958; *venustum* Say, 1823; *verecundum* Stone & Jamback, 1955. Genus ***Tetisimulium*: *bezzii*** (Corti 1914).

III. Tribe WILHELMIINI Baranov 1926. Genus ***Wilhelmia*:** *angustifurca* Rubzov, 1956; *balcanica* Enderlein, 1924; *equina* (Linnaeus, 1758); *ivashentzovi* (Rubzov, 1940); *lineata* (Meigen, 1804), *paraequina* (Puri, 1933), *pseudequina* (Seguy, 1921), *salopiensis* (Edwards, 1927) sensu Rubzov, 1956 (Chubareva & all., 2007); *W. turgaica* (Rubzov, 1940); *veltistshevi* (Rubzov, 1940).

Cloning of prokaryotic genes by a universal degenerate primer PCR

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A PCR approach was developed using a hexameric degenerate primer, which reflects the Shine-Dalgarno sequence of prokaryotic transcripts, hitherto named SD-PCR. In standard PCR reactions, the sizes and melting temperatures of the two primers are usually designed to be as equal as possible, while SD-PCR allows the use of a single gene-specific primer pairing with the universal degenerate SD primer. This approach can be applied as an efficient PCR walking approach to clone either the upstream or downstream region of a known DNA fragment just large enough to design a gene-specific primer. We have successfully applied the method to template DNAs of different GC-contents as well as complex mixtures composed of highly contaminating DNA(s).

SPATIAL DISTRIBUTION OF BLACK FLIES (DIPTERA: SIMULIIDAE) IN THE KHEMCHIC RIVER BASIN OF WEST TUVA

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Black fly larvae and pupae were collected in 15 rivers of the Khemchik river basin in mountain-steppe region of West Tuva during first half of summer in 2004 year. The basin borders with Altai Mountains and Hollow of Big Lakes, which is related to Central Asia. The ecological profile in longitudinal gradient from 2200 to 500 m.a.s.l. included mountain tundra, slope-transit forests and dry steppes. Rivers were determined as epyrhithral, metarhithral and hyporhithral. In the West Tuva we established the distribution of 24 species from 9 genera.

The macrodistribution of black fly larvae according to gradient was established in: mountain tundra (1800 – 2200 m), forest taiga (1300 – 1700 m), mountain steppe (900 – 1200 m), plain steppe (500 – 800 m). The aggregation of larvae differed depending on biotope. Basing on black fly community structure those of mountainous part of the profile were classified as monodominant, those of the plain steppe as polydominant. In anthropogenic biotopes population density was very high and one species with wide adaptations to the inhabitant conditions prevailed. Stenobiontic species of mountainous tundra and taiga communities were gradually replaced by eurybionts in plain steppe rivers. Those are more tolerant to the quality of running waters. In the adaptation processes to biotope conditions the definite balance became established between species composition, structure of community and population density.

Keywords: Simuliidae, black flies larvae, spatial distribution, community structure anthropogenic biotopes.

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Contribution to the knowledge of the blackfly fauna of the Baltic States with special focus on the Estonian islands Saaremaa and Hiiumaa

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All over the Baltic States, Estonia, Latvia and Lithuania, a total of 47 sampling sites in running waters with different characteristics were observed with regard to their blackfly communities. The spectrum ranged from small brooks in all three countries to the large rivers Gauja, Venta (Latvia) and Nemunas (Lithuania). Larvae and pupae were collected from stones, submerged plants or wooden debris and preserved in ethanol. As the examination focused on biodiversity, all samplings were conducted qualitatively.

In total, 21 species from two genera (*Prosimulium* and *Simulium*) and nine subgenera, respectively (*Prosimulium*, *Helodon*, *Boophthora*, *Eusimulium*, *Hellichiella*, *Nevermannia*, *Schoenbaueria*, *Simulium* and *Wilhelmia*), were found within the study area.

Simulium (*Nevermannia*) *vernum* (Macquart 1826) was the most frequently found species, having occurred in 45 per cent of all observed creeks, followed by *Simulium* (*Nevermannia*) *cryophilum* (Rubzov 1959) with a steadiness of 32 per cent. Further abundant species that could be found in more than a quarter of all running waters were *Simulium* (*Simulium*) *paramorsitans* (Rubzov 1956) as well as members of the *Simulium* (*Simulium*) *ornatum*-group. In unexceptionally every brook that was influenced by fish ponds, *Simulium* (*Simulium*) *noelleri* (Friedrichs 1920) could be detected in large numbers.

Nine species could only be found in one single sample site each. The rarest of them were *Prosimulium* (*Helodon*) *ferrugineum* (Wahlberg 1844) and *Simulium* (*Hellichiella*) *latipes* (Meigen 1804), both of which were exclusively detected on the Estonian island of Saaremaa. *P.* (*H.*) *ferrugineum* has been reported from Norway, Sweden, Finland and Russia so far, but not from Estonia. The same applies to *Simulium* (*Schoenbaueria*) *pusillum* (Fries 1824), that was also found in Saaremaa and therefore proven for Estonia for the first time.

In Saaremaa a total of ten blackfly species was detected, the most abundant of which were *S.* (*N.*) *vernum* (in 64 per cent of all observed brooks), *S. (S.) paramorsitans* (59 per cent) and *Simulium (Simulium) morsitans* (Edwards 1915) (36 per cent).

On the much smaller Estonian island of Hiiumaa only five species were proven, *S. (N.) vernum* being again the most abundant one.

Furthermore, investigations were carried out in the Latvian Gaujas National Park, where 14 species (or two thirds of all species in the study) could be found. Some of the species proven in the study were exclusively found in this undisturbed area, among them *Prosimulium hirtipes* (Fries 1824), *Prosimulium rufipes* (Meigen 1830), *Simulium (Eusimulium) angustipes* (Edwards 1915) and *Simulium (Nevermannia) costatum* (Friedrichs 1920).

The collected data were finally used to create a dendrogram of the observed blackfly associations.

INVESTIGATIONS OF THE BLACK FLIES (DIPTERA: SIMULIIDAE) IN LATVIA

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The black flies are common in Latvia and have significant veterinary and partly medical importance as bloodsuckers, particularly in the Easter parts of the country. At the same time the scientific researches were limited. In 19th century B.Gimmerthal and F.Sintenis listed some species. In the 20th century only Māris Šternbergs (1940-1996) studied the fauna and ecology of black flies in 1968-1970. He studied mostly fauna with no use of quantitative sampling methods. He stated characteristic species for small streams and brooks, small and large rivers. In total 30-32 Simuliidae species are known in Latvia. *Eusomulium cryophilum* was proposed as protected species and included in the Red Data Book of Latvia. M. Kudule (Misiņa) studied morphological variability, chromosomes and some other aspects. Latvian hydrobiologists (Institute of Biology) widely study the bentic species in the Latvian rivers, but did not identify species. The results of Latvian scientists were neglected by wider scientific community because the articles were written either in Russian or in Latvian (see references).

Numerous popular articles have been written in a case of outbreak of black flies. The last significant outbreak of black flies occurred in May of 2005. Two species *Simulium rostratum* and *S . venustum* were identified. The outbreaks of black flies in the Eastern part of Latvia are periodic with significant impact on animal husbandry. In 2005 about 100 cows have died and numerous need to be treated. After 2005 there was proposed to build a network of Simuliidae monitoring in the Eastern Latvia with Daugavpils University as responsible research institution. But nobody took responsibility. Actually, no Simuliidae specialist is working in Latvia.

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IMPACT OF RIVER REGULATION ON BLACKFLY POPULATIONS AND SURROUNDING TERRESTRIAL ECOSYSTEMS

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The regulation of rivers for the purpose of production of electric energy has a negative impact on blackfly populations. Our studies along seven of the biggest North Swedish rivers suggest that the abundances of adult blackflies had been reduced several fold along regulated versus naturally flowing rivers. However, not much is known how the river regulation and, in turn the amounts of blackflies, might influence terrestrial and water bird populations. On the one hand, positive effects of blackflies can represent an important food resource to insectivorous bird species. On the other hand, negative effects can be expected because of their blood-sucking involving both disturbances (harassment and anemia) and the transmission of avian blood parasites (*Leucocytozoon* sp). Our investigations suggest that the densities of different avian species were affected differentially. More detailed analyses of the breeding of Pied Flycatcher (*Ficedula hypoleuca)* demonstrated higher breeding success along naturally flowing rivers, presumably due to greater food availability.

PHYLOGENY OF BLACK FLIES OF SUBFAMILY SIMULIINAE IN PALEARCTIC

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The subfamily Simuliinae includes tribes: Stegopternini, Estemniini, Nevermanniini, Wilhelmiini, Simuliini. 16 synapomorphic characteristics confirm the ancestry commonality of Simuliinae.

The tribes Stegopternini, Estemniini create the isolated group supported by 5 synapomorphies. The genera *Stegopterna* and *Greniera* are ascribed to the tribe Stegopternini (9 synapomorphies). *Cnephia*, *Metacnephia, Sulcicnephia* are referred to the tribe Estemniini (11). The group *Metacnephia* and *Sulcicnephia* (3) are well isolated in this tribe.

The tribes Nevermanniini, Wilhelmiini, Simuliini have common ancestry what 5 synapomorphies confirm. The tribe Nevermanniini is the lateral, early specialized branch, what is supported by 9 characteristics. It includes the genus *Hellichiella*; the group *Byssodon* and *Psilocnetha* (2); the group *Cnetha*, *Nevermannia*, *Eusimulium*, *Schoenbaueria* (1) and the subgroup from the genera *Cnetha*, *Nevermannia*, *Eusimulium* (2); the group *Gomphostilbia* and *Morops* (3) and the genus *Montisimulium*.

Then the divergence in a subfamily occured by separation of the tribe Wilhelmiini from the Simuliini (3). The tribe Wilhelmiini in Palearctic is represented only by 1 genus *Wilhelmia*.

The isolation of the tribe Simuliini is supported by 9 synapomorphies. In the tribe the group of genera *Boophthora* and *Psilosia* (1), the group *Cleitosimulium* and *Obuchovia* (6), the group *Paragnus* - *Simulium* (2) are clear isolated. The last group is divided into the genus *Paragnus*, the subgroup *Parabyssodon* and *Archesimulium* (3), the genus *Striatosimulium* and the subgroup of genera *Argentisimulium* - *Simulium* (2). The last subgroup is divided into two branches, the first one (2) includes the genus *Argentisimulium* and the group of the genera *Tetisimulium*, *Phoretodagmia*, *Odagmia* (2); the second one (4) contains the genera *Gnus* and *Simulium*.

BLACK FLIES OF THE SOUTHEASTERN PART OF UKRAINE

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50 black fly species belonging to 11 genera have been registered in the south – eastern part of Ukraine:

Subfamily *Simuliinae* Newman, 1834

Cnephia Enderlein, 1921

C. lapponica (Enderlein, 1921)

C. andrei, sp.n.

Hellichiella Rivosecchi et Cardinale, 1975

H. latipes (Meigen, 1804)

subexisum (Edwards, 1915)

H. marinae, sp.n.

Cnetha Enderlein, 1921

C. verna (Macquart,1826)

latipes Meigen sensu Rubzov, 1956 (nec Meigen, 1804)

C. mariae, sp.n.

C. lidiae Semushin et Ussova,1983

Nevermannia Enderlein, 1921

N. angustutarsis (Lundstrom, 1911)

*N. latigonia (*Rubzov,1956)

N. luttae, sp.n.

N. kerteszi Enderlein, 1921

N. lundstromi Enderlein, 1921

Eusimulium Roubaud, 1906

E. aureum (Fries, 1824)

E .angustipes (Edwards, 1915)

E. krymense Rubzov, 1956

E. securiforme Rubzov, 1956

Schoenbaueria Enderlein, 1921

Sch. pusilla (Fries, 1824)

Sch. nigra Meigen, 1804

mathiesseni Enderlein, 1921

Wilhelmia Enderlein, 1921

W. equina (Linnaeus, 1746)

W. tertia Baranov, 1926

W. andreii, sp.n.

W. mediterranea (Puri, 1925)

W. veltistshevi (Rubzov, 1940)

W. lineata (Meigen, 1804)

W. salopiensis (Edwards, 1927)

W. liliae, sp.n.

W. balcanica Enderlein, 1924

Byssodon Enderlein, 1925
B. maculatus (Meigen, 1804)
Boophthora Enderlein, 1921
B. erythrocephala (De Geer, 1776)
sericata (Meigen, 1830)
Odagmia Enderlein, 1921
O. ornata (Meigen, 1818)
O. intermedia (Roubaud, 1906)
O. nitidifrons (Edwards, 1920)
O. pratora (Friederichs, 1922)
O. baracornis (Smart, 1944)
O. caucasica (Rubzov, 1940)
O. frigida (Rubzov, 1940)
O. deserticola (Rubzov, 1940)
O. albifrons (Rubzov, 1964)
Simulium Latreile, 1802
Archesimulium Rubzov et Yankovsky, 1982
S. (Arch.) tuberosum (Lundstrom, 1911)
Argentisimulium Rubzov et Yankovsky, 1982
S. (Arg.) noelleri Fried, 1920
S.(Arg.) dolini Ussova et Suchomlin, 1989
Simulium Latreile, 1802
S. truncatum (Lundstrom, 1911)
S. posticatum Meigen, 1838
austeni Edwards, 1915
S. morsitans Edwards, 1955
S. paramorsitans Rubzov, 1956
S. shevtshenkovae Rubzov, 1965
S. semushini Usova et Zinchenko, 1992
S. kachvorjanae Usova et Zinchenko, 1991
S. verecundum Stone et Jamnback, 1955
S. reptans (Linnaeus, 1758).

The places of preimaginal phase breeding, ways of egg-laying, phenology, wintering, blood-sucking activity and other issues of biology are studied.

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