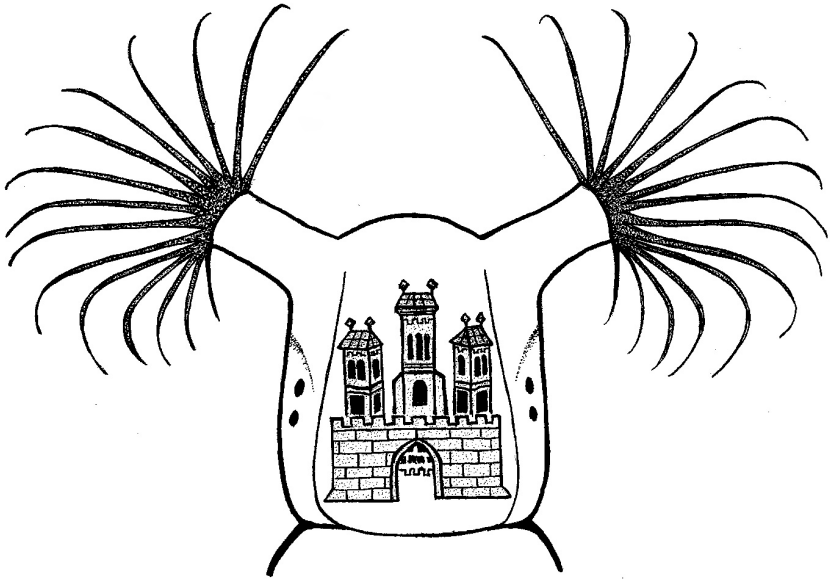
5th International Simuliid Symposium

including the 32th Meeting of the British Simuliid Group

Programme and abstract book



Faculty of Natural Sciences
Comenius University in Bratislava
Slovakia
September 3 – 7, 2012

Organising committee:
Matúš Kúdela, Viera Stloukalová, Tatiana Brúderová

Editors of the book of abstracts:
Matúš Kúdela & Viera Stloukalová

PROGRAMME

Monday 3 September

since 16:00 Registration (botel Fairway)

19:00 – 21:00 Welcome party (botel Fairway)

Tuesday 4 September

8:30 – 9:00 Registration (Faculty of Natural Sciences, Comenius University)

9:00 – 9:30 opening of the Symposium

9:30 – 13:10 presentations

13:10 – 15:00 lunch break

15:00 – 16:00 presentations

16:30 – 22:00 Visit of the Červený kameň castle and

the wine cellar Fuggerov dom in village of Častá, dinner

Wednesday 5 September

whole day field trip

departure of the boat 8:30

arrival with bus cca 20:00

Thursday 6 September

9:00 – 13:10 presentations

13:10 – 15:00 lunch break

15:00 – 18:00 presentations

19:30 gala dinner

ORAL PRESENTATIONS

Tuesday 4 September

9:30 – 10:10 Peter H. Adler: **Biodiversity, ancient DNA barcodes, and symbiotic surprises in the Simuliidae**

10:10 – 10:30 Matúš Kúdela, Ladislav Jedlička, Tatiana Brúderová & Rasa Bernotiene: ***Simulium reptans*, *Simulium galeratum* and similar species in Europe**

10:30 – 10:50 Tatiana Brúderová, Matúš Kúdela: **Morphological and genetic variability of *Simulium colombaschense* – the type species of the genus *Simulium***

10:50 – 11:10 Matúš Kúdela, Ladislav Jedlička & Rasa Bernotiene: **Status of *Simulium* (*Wilhelmia*) *lineatum* and *Simulium* (*Wilhelmia*) *balcanicum* according to analysis of mtDNA COI gene**

11:10 – 11:30 COFFEE BREAK

11:30 – 11:50 Peter H. Adler, Abdullah Inci, Alparslan Yildrim, Gunther Seitz & Onder Duzlu: **Chromosomal insights into the pest status of the subgenus *Wilhelmia* in Turkey**

11:50 – 12:10 Abdullah Inci, Alparslan Yildirim, Onder Duzlu, Peter H. Adler, Zuhal Biskin, Arif Ciloglu, Hakan Yesiloz, Ahmet Demircioglu & Gunther Seitz: **Molecular characterization of blackflies (Diptera: Simuliidae) collected from Kizilirmak River in Nevsehir province of Turkey**

12:10 – 12:30 Alexey Yankovsky, Yerbol Issakayev, Daria Khassanova & Aisulu Tailakova: **New blackfly species in the genus *Montisimulium* from the northeastern Kazakhstan**

12:30 – 12:50 Marija Ivković, Marijana Kesić & Zlatko Mihaljević: **Temporal and spatial variations in phenology patterns of blackflies (Diptera: Simuliidae) and their longitudinal distribution along oligotrophic freshwater system**

12:50 – 13:10 John W. McCreaddie & Peter H. Adler: **A metacommunity view of black fly species assemblages**

13:10 – 15:00 LUNCH BREAK

15:00 – 15:20 Irina Budaeva & Ludmila Khitsova: **The species composition and altitude distribution of black flies (Diptera, Simuliidae) of the North-West Caucasus streams**

15:20 – 15:40 Matúš Kúdela, Aleksandra Ignjatović Ćupina, Tatiana Brúderová & Dušan Petrić: **The blackfly fauna (Diptera, Simuliidae) of the Iron Gate area (eastern Serbia, southwestern Romania) in the past and present**

15:40 – 16:00 Liudmila Petroshitskaya & Vera Rodkina: **Zonal and latitudinal distribution of blackflies (Diptera, Simuliidae) in the West Siberia**

Thursday 6 September

9:00 – 9:55 Rory Post: **Entomology and the elimination of onchocerciasis by community directed treatment with Ivermectin in Africa**

9:55 – 10:15 Poppy H.L. Lamberton, Robert A. Cheke, Osei-Atweneboana, M.Y., Winskill, P., Rory J. Post, Tetteh-Kumah, A., Shew, K.J.S., Wilson, M.D. & Maria-Gloria Basáñez: ***Simulium damnosum* complex geographical distribution and host choice in Ghana where onchocerciasis transmission is under ivermectin control**

10:15 – 10:35 Elmer W. Gray, Joseph P. Iburg, Roger D. Wyatt, Robert A. Fusco & Raymond Noblet: **The effect of seston on larval black fly mortality after exposure to a *Bacillus thuringiensis* subsp. *israelensis* based larvicide**

10:35 – 11:05 **COFFEE BREAK**

11:05 – 11:25 Charles Brockhouse, Soochin Cho, Alexie Papanicolaou, Rory Post, Daniel Boakye, Michael Pfrender & John K. Colbourne (Simulium Genomics Consortium): **Progress in the Simulium Genomics Project**

11:25 – 12:45 Inaki Tirados, Evans Muki, Pierre Baleguel, Graham A. Matthews & Robert A. Cheke: **How do blackflies identify their hosts? Results of a preliminary study of visual and olfactory responses of *Simulium squamosum* B in Southern Cameroon**

11:45 – 12:05 Ruiz-Arrondo I., Martinez E., Kotter H., Figueras L., Muñoz A., Delacour-Estrella S., Alarcón-Elbal P. M., Pinal R. & Lucientes J.: **Blackfly outbreak in Zaragoza in 2011. Spread of blackflies in the Middle Ebro Valley in northeast Spain**

12:05 – 12:25 Rasa Bernotiene & Milda Zygutiene: **The pause in blackfly control in Lithuania**

12:30 – 13:15 **poster session**

13:15 – 15:00 **LUNCH BREAK**

15:00 – 15:20 Robert A. Cheke, Tetteh-Kumah, A., Rory J. Post, Poppy H. L. Lamberton & Maria-Gloria Basáñez: **Compact discs for sampling immature stages of members of the *Simulium damnosum* complex**

15:20 – 15:40 Doreen Werner & Adrian Pont: **New results on Diptera predators in the black fly plague areas of South Africa**

15:40 – 16:00 Aleksandra Ignjatović Ćupina, Dušan Petrić, Elias Papadopoulos, Sokratis Ptochos, Domenico Otranto, Filipe Dantas–Torres, Yasen Mutafchiev & Odile Bain: **Notes on blackfly fauna in Western Thrace (northeastern Greece)**

16:00 – 16:20 Simone Ciadamidaro: **Preliminary notes on black fly fauna in Piedmont region, northern Italy**

16:20 – 16:40 **COFFEE BREAK**

16:40 – 17:00 Csaba Deák & Krisztián Kovács: **First records of *Simulium* (*Hellichiella*) *latipes* (Meigen, 1804) (Diptera: Simuliidae) in Hungary**

17:00 – 17:20 Bruno Maiolini, Sonia Endrizzi & M. Cristina Bruno: **Blackflies as indicators of ecological stress in two Alpine streams with different land use in the catchment**

17:20 – 17:40 Rooschanak Foroutan Saravi & Norbert Becker: **The Simuliidae fauna of South West Germany**

17:40 – 18:00 Rasa Bernotiene, Irina Budaeva, Erbol Issakaev & Liudmila Petrozhitskaya: **Comparison of *Simulium maculatum* Mg. biology in different parts of Palaearctic**

POSTER PRESENTATIONS

Sergej V. Aybulatov: **Blackflies (Diptera: Simuliidae) of Leningradskaya and Vologodskaya regions (Russia)**

Simone Ciadamidaro, Dušan Petrić, Aleksandra Ignjatović-Ćupina & Matúš Kúdela: **Black fly species succession from Alps to lowland rivers in Piedmont, north-western Italy**

Atefeh Khazeni, Zakieh Telmadarrehiy, Mohammad-Ali Oshaqi, Hasan Vatandoost & Seyed Mohammad Abtahi: **A new study on blackflies (Simuliidae)of Iran in central regions**

Gunter Seitz: **The Blackfly Fauna (Diptera: Simuliidae) of the Gesäuse National Park in Austria**

Andrej Štangler: **Typology of running waters according to blackfly communities in conditions of Borská nížina lowland (West Slovakia)**

Hakan Yesiloz, Alparslan Yildirim, Peter H. Adler, Abdullah Inci, Onder Duzlu, Arif Ciloglu & Zuhal Biskin: **Molecular classification of some simuliid larvae collected from Central Basin of Kizilirmak River based on the sequence analyses of mt-COI and ITS-2 gene regions**

ABSTRACTS

Biodiversity, ancient DNA barcodes, and symbiotic surprises in the Simuliidae

Peter H. Adler

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The total number of living, formally described, valid species of black flies in the world currently stands at 2,127. The discovery rate for new species shows no sign of decreasing. Hotspots for new species include the Oriental Region and the South American Andes Mountains. The most powerful approach to species discovery and understanding species limits continues to be the cytogenetic analysis of larval polytene chromosomes—the original DNA barcodes. Several recent discoveries, using this approach, are emphasized: 1) A new species of the subgenus *Rubzovia*, was shown to have a haploid number of two, representing the fourth simuliid taxon to have undergone chromosome reduction from the typical n = 3 condition. 2) Examples of morphospecies claimed to be identical in COI barcodes were shown to differ significantly in their chromosomal banding sequences, corroborating the power of morphological analysis to correctly recognize species. 3) The source area for the remote Okinawa Island black fly *Simulium suzukii* was identified. 4) *Simulium colombaschense*, the type species of the genus *Simulium* and history's most destructive pest of livestock, shows no chromosomal feature uniquely related to pest status; however, clarification of its relationships demonstrated that the evolution of the ability to colonize large rivers in an ancestral species contributed to the monumental pest status of *S. colombaschense*. Relationships of black flies to other organisms are highlighted. The association of black flies and endangered host species is examined, along with the controversial prospects for management of the pest species. At a smaller scale, each individual black fly, whether larva or adult, can be considered an ecosystem, housing a diverse community of symbiotes, some essential to life's processes, others parasitic and lethal. Nearly 200 formally described species of nonbacterial symbiotes, most regarded as parasites, are known from black flies. More than 330 bacterial phylotypes were found among 4 species of North American black flies, with each host species generally having a distinct bacterial profile, most notably differing between males and females within a species. The possibility for novel pest-management approaches might lie in an understanding of the physiological function of the associated bacterial community.

Simulium reptans, *Simulium galeratum* and similar species in Europe

Matúš Kúdela[1], Ladislav Jedlička[1], Tatiana Brúderová[1] & Rasa Bernotiene[2]

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Simulium reptans Linnaeus, 1756 belongs to the first described black flies, but the questions connected with the identity of this species are still not resolved. With more then 20 synonyms its taxonomic position belongs to the most complicated within black flies. The species status of the closely related species, traditionally labeled as *S. galeratum* Edwards, 1920, which was for a long period considered a synonym of *S. reptans*, has been confirmed recently. But since the identity of *S. reptans* was changed with *S. galeratum* by Edwards in the past, *S. galeratm* should be regarded as the synonym of *S. reptans* and for the second species – earlier from Great Britain and central Europe reported as *S. reptans* – the available name *S. reptantoides* Carlsson, 1962 can be used. The other European species, *S. nanum* Zetterstedt, 1838 and *S. zetterstedti* Carlsson, 1962 seem also be synonyms of *S. reptans* but it was not confirmed until recent. We analysed the genetic variability, as well as morphological variability of *S. reptans* and *S. reptantoides*. The basic genetic variability, haplotype nucmber and distribution between species and among populations within species of 44 individuals of *S. reptans* and 36 individuals of *S. reptantoides* were studied. Together 80 sequences of mitochondrial COI were analysed: 38 from Great Britain, 19 from Slovakia, 9 from Lithuania, 2 from Latvia and 12 from Sweden. In 73 individuals a 606 bp section were analysed (long sequences), and in all 80 individuals a fully overlapping 453 bp section (short sequences). The results showed that *S. reptans* and *S. reptantoides* are genetically isolated. The genetic divergence between species is 7.02% in long and 7.46% in short sequences which is about six to nine times higher than within species distance, which is 1.18% in *S. reptans* and 0.83% in *S. reptantoides* in long sequences and 1.38 and 1.05 in short sequences. Haplotype network constructed using TCS and maximum likelihood tree showed that each species consists of two units (A and B). In *S. reptans* the distribution of both forms is not identical – in Slovakia and Baltic area only *S. reptans* B was found, in Great Britain and Sweden both forms are present. In *S. reptantoides* both forms were present in Great Britain and Slovakia, this species is missing in Scandinavia and Baltic area. Studied individuals of *S. reptans* and *S. reptantoides* were significantly different in their morphology and it was possible to distinguish larvae, pupae and adult males. Among the individuals of *S. reptantoides* relatively high variability in the shape of the cocoon (simple cocoons and cocoons with collar) and in the coloration of larval head capsule (positive and negative pattern) was recorded. Variability in morphology of *S. reptantoides* did not correspond to the recorded intraspecific genetic variability (units A and B). In most of the cases this variability was even not depending on the site. The coloration of head capsule was relatively variable also in *S. reptans*, but the shape of the cocoon in this species was uniform. No morphological differences between the genetic units *reptans* A and B were recorded.

Morphological and genetic variability of *Simulium colombaschense* – the type species of the genus *Simulium*

Tatiana Brúderová & Matúš Kúdela

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Simulium colombaschense (Scopoli, 1780), the type species of *Simulium*, was for a long time considered as an endemic species of the Danube around the Iron Gate Gorge. In that area it caused severe losses of livestock and was regarded to be one of the world most dangerous pests among black flies. Therefore also much research was focused on this species, however, the knowledge on its taxonomy and morphology is still insufficient. One of the open taxonomic problems is the difficulty to distinguish between *S. colombaschense* and the closely related *Simulium voilense* Serban, 1960. During our study we have collected individuals of *S. colombaschense* from six sites from five European rivers: Danube (Slovakia and Austria), Belá (Slovakia), Aliakmonas (Greece), Mureş (Romania) and Adige (Italy). Among the 761 individuals of *S. colombaschense* we compared morphological features of larvae (the coloration of head capsule, the shape of postgenal cleft, the number of rays in the large labral fan and the number of rows of hooks in the posterior sucker), pupae (the pupal gill branching and the shape of cocoon) and adults (the shape of male gonosternum). In the shape of postgenal cleft, the number of rays in the large labral fan and rows of hooks in the posterior sucker, and in the shape of gonosternum we recorded differences between *S. colombaschense* and the other two closely related species – *Simulium reptans* (Linnaeus, 1758), and *Simulium reptantoides* Carlsson, 1962. On the other hand, among the individuals of *S. colombaschense* we did not recognized any group of individuals differing significantly, even though the fact that most of the studied characters were highly variable. Subsequent comparison of the genetic variability in the 453 bp section of the COI gene among the 99 individuals of *S. colombaschense*, *S. reptans* and *S. reptantoides* confirmed the results of morphological analysis. All individuals of *S. colombaschense* formed one cluster and the variability among them was lower than the intraspecific variability among the individuals of *S. reptans* and *S. reptantoides*. According to the results of AMOVA the interspecific variability caused by differences among *S. reptans*, *S. reptantoides* and *S. colombaschense* was 82.32%, whereas the variability caused by differences among populations within species was 5.66%. The haplotype network of 17 individuals of *S. colombaschense* did not reveal any clear clade structure within analysed material. According to our results there is no indication that a second species – corresponding to *S. voilense* – is present in the studied material.

Status of *Simulium* (*Wilhelmia*) *lineatum* and *Simulium* (*Wilhelmia*) *balcanicum* according to analysis of mtDNA COI gene

Matúš Kúdela[1], Ladislav Jedlička[1] & Rasa Bernotiene[2]

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[2] Nature Research Centre, Akademijos 2, Vilnius, Lithuania; rasab@ekoi.lt

In present the *equinum* group of the subgenus *Wilhelmia* Enderlein, 1921 contains 25 accepted species, another 50 described names fall into the synonymy of the accepted species.

One not resolved problem is the status of the species *Simulium* (*Wilhelmia*) *lineatum* (Meigen, 1804) and *Simulium* (*Wilhelmia*) *balcanicum* (Enderlein, 1924). The first of them, partly known as the older synonym salopiense, is quite widely distributed in entire Europe (except of some northern countries), in the Near (Lebanon) and Middle East (Pakistan), in Central Asia, as well as China. The second species, described originally from Bulgaria, is known from several European countries, mainly in central and southern Europe and from Anatolia and can be considered as Mediterranean or submediterranean species. Both species can be easily distinguished as pupae, in *S. lineatum* all respiratory filaments arise directly from the basis, whereas in *S. balcanicum* the hind pair of inner filaments arises from a common stalk. However, no reliable identification markers could been found in the larvae and imagoes, neither in male genitalia, not in female spermatheca. Some authors draw to the inference that they do not present two separate two separate species. This opinion was supported also by the study of the type material (females) and after lectotype designation, which did not show significant differences. The unpublished cytotaxonomic results were not unambiguous due to lack of compared material and they needed confirmation. Recent pilot study of COI gene based on material from Lithuania and Romania, thus from different parts of their areas confirmed the presence of two units, probably corresponding to these species. 13 haplotypes were found in *S. balcanicum* and 6 in *S. lineatum*. Both species differ in number of polymorphic sites (31 in *S. balcanicum* $\pi = 0.0160$, 6 in *S. lineatum*, $\pi = 0.0037$), there are 4 fixed nucleotide differences between species with average number of nucleotide differences between populations 13.69. The genetic divergence is 2.56 % which is on the borders between intraspecific and interspecific divergence usually indicated in blackflies. The differences are supported also by the haplotype network where both taxons – except one outlying haplotype – represent clades separated by 9 mutational steps. In the bootstrap consensus tree of evolution history inferred by using the maximum likelihood, the branches are less differentiated (support of 84 % at 1000 replicates) but distinct.

Chromosomal insights into the pest status of the subgenus *Wilhelmia* in Turkey

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The recent emergence of a major pest problem for humans caused by black flies along the Kizilirmak River in central Turkey demanded a deeper understanding of the systematics and genetics of the species involved. Cytogenetic analyses of the polytene chromosomes revealed two species of the subgenus *Wilhelmia*: *Simulium lineatum*, constituting about 90% of the river's black fly population, and *S. balcanicum*. The analysis demonstrated conclusively that *S. lineatum* and *S. balcanicum* are distinct, reproductively isolated species, despite earlier suggestions, based on morphology, that they represent a single species. Chromosomally, these two species have fixed-inversion differences and unique autosomal polymorphisms. No sibling species were present in the Kizilirmak material of either species. Analysis of *S. balcanicum* and *S. lineatum* from the River Rott and the River Isar, respectively, in Germany, suggested that populations of *S. balcanicum* in Germany and Turkey are conspecific, but that the respective populations of *S. lineatum* differ significantly in their chromosomal profiles. The implication is that German and Turkish populations of *S. lineatum* are different cytoforms, possibly representing distinct species; examination of geographically intermediate populations is required to resolve the taxonomic status of the two populations, which are about 2000 km distant from one another. Cytogenetic analysis of *S. lineatum* and *S. balcanicum* revealed more than 80 chromosomal rearrangements, with some larvae carrying as many as 8 heterozygous inversions. The German population of *S. lineatum* is highly polymorphic, whereas the Turkish population is minimally so. Conversely, the Turkish population of *S. balcanicum* is far more polymorphic than is the German population. In light of the cytogenetic insights, the pest status of *S. balcanicum* and *S. lineatum* can be further clarified, particularly by using molecular markers to link pestiferous adult flies with the relevant species, an effort currently underway.

Molecular characterization of blackflies (Diptera: Simuliidae) collected from Kizilirmak River in Nevsehir province of Turkey

Abdullah Inci[1], Alparslan Yildirim[1], Onder Duzlu[1], Peter H. Adler[2], Zuhal Biskin[1], Arif Ciloglu[1], Hakan Yesiloz[1], Ahmet Demircioglu[1] & Gunther Seitz[3]

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[3] District Government of Lower Bavaria, Landshut, Germany; Gunther.Seitz@reg-nb.bayern.de

This study has been carried out on Simuliid (Diptera: Simuliidae) flies which have caused a severe problem in the Central Kizilirmak Basin in Nevsehir province of Turkey and supported by the Scientific and Technological Research Council of Turkey with the project no 111 O 426. Field studies have been conducted in the part of Kizilirmak River which passes through several districts of Nevsehir province and larvae sampling was performed from totally 20 field stations between January and June 2012. Totally 200 simuliid larvae specimens were collected and species identifications were done by morphological and chromosomal analyses. Two species *Simulium lineatum* and *S. balcanicum* have been determined in the research area. PCR analyses were performed on 3 larvae and 1 pupae isolates belong to the *S. balcanicum* and 4 larvae isolates belong to the *S. lineatum* with the primer pairs that amplified mitochondrial partial cytochrome oxidase subunit 1 (mt-COI) gene region. In addition to these isolates three more isolates obtained from Germany [Germany1 (*S. lineatum*), Germany 2 (*S. balcanicum*), Germany 3 (*S. equinum*)] were also included in the molecular analyses. Sequence analyses using the same primer pairs were performed on the obtained amplicons in order to phylogenetic analyses of related gen region. Various nucleotide variations were determined among *Simulium* species and isolates according to the mt-COI gene sequence alignments. The identifications of the species by morphological and chromosomal analyses were confirmed phylogenetically. The percent identities among the *S. balcanicum* isolates and *S. lineatum* isolates were determined as 99.1 – 100.0% and 97.2 – 100.0%, respectively. The identity rates between *S. balcanicum* and *S. lineatum*; *S. balcanicum* and *S. equinum*; *S. lineatum* and *S. equinum* isolates were 97.7 – 98.1%, 87.5 – 88.1%, 87.6 – 88.6%, respectively.

In conclusion, it seems that two main species *S. balcanicum* and *S. lineatum* are prevalent in the Central Kizilirmak Basin in Nevsehir, Turkey. The high genetic variation among *Simulium* species and isolates in this investigation supports the presence of cryptic and/or sibling species in the *Wilhelmia* subgenus.

New blackfly species in the genus *Montisimulium* from the north-eastern Kazakhstan

Alexey Yankovsky, Yerbol Issakayev[1], Daria Khassanova[1] & Aisulu Tailakova[1]

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Male, female and pupa of a new blackfly species in the genus *Montisimulium* (Diptera: Simuliidae) are described from the collections from the north-eastern Kazakhstan (Pavlodar region). Drawings of the structure parts and the differential diagnosis of the new species are given.

Currently, the genus *Montisimulium* includes 52 species, 48 of which are common in the southern part of the Palaearctic (mostly in the mountainous and foothill areas), 4 species – in Indo-Malayan Region. 39 species of the genus *Montisimulium* inhabit in the former Soviet Union. In the north-eastern Kazakhstan is likely to find 9 species, 7 of them, *M. asulcatum*, *M. danijari*, *M. decafile*, *M. longifiliatum*, *M. octofiliatum*, *M. peskovi* and *M. quattuordecimfiliatum*, registered in the south-eastern and southern Kazakhstan, and 2 species, *M. kerzhneri* and *M. schevyakovi* inhabit the adjacent regions as Southern Siberia, Altai and Mongolia. 20 specimen, which by a set of characters belong to the genus *Montisimulium* were found in collections from Pavlodar (Kazakhstan), but clearly distinct from the known species by a number of morphological characteristics. They are described as new species.

This new species differs from the other in the genus *Montisimulium* by the structure of respiratory organ of pupa, which consists of 16 filaments, whereas all other species in the respiratory organ have 6 to 14 (rarely) filaments.

From the species of the genus *Montisimulium*, whose presence is likely in the north-eastern Kazakhstan, the new species differs by the number of filaments in the respiratory organ (*M. octofiliatum* 8, *M. decafile*, *M. peskovi* and *M. schevyakovi* 10, *M. asulcatum* and *M. danijari* 12, *M. longifiliatum* 13 and *M. quattuordecimfiliatum* 14 filamentss), pupae of *M. kerzhneri* are unknown. The imago also differs from these species by the following features. For males – from *M. octofiliatum* it differs by the absence of gonostyles elongated in the long arm of the heel, from *M. kerzhneri* it differed by short and broad gonocoxites, relatively thin gonostyles, less wide body gonosternum (*M. kerzhneri* gonosternum body width is 4.5 – 5.0 times greater than the length); from *M. asulcatum*, *M. longifiliatum*, *M. peskovi* and *M. schevyakovi* it differs by short and broad gonocoxites, relatively thin gonostyles, slightly convex posterior margin of the body gonosternum (in these species it is distinctly concave); from *M. danijari* it differs by relatively thin gonostyles, wider body gonosternum (*M. danijari* gonosternum body width is 1.8 times greater than the length); male *M. decafile* are unknown. For females the new species differs from *M. asulcatum*, *M. longifiliatum*, *M. peskovi* and *M. schevyakovi* by lack of anterolateral sclerotized digitate outgrowths of the branches of genital furca, from *M. danijari*, *M. octofiliatum* and *M. schevyakovi* it differs by presence of peaked posteromedial outgrowths of the branches of genital furca; female *M. decafile* and *M. kerzhneri* are unknown.

Temporal and spatial variations in phenology patterns of blackflies (Diptera: Simuliidae) and their longitudinal distribution along oligotrophic freshwater system

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Black flies are very important component of freshwater lotic habitats and they have high veterinary and medical significance, because the majority of females need a blood meal to produce eggs. This study was done at Plitvice lakes National Park (Croatia) in a wide range of lotic freshwater habitats (spring, streams and tufa barriers-barrage lake outlets). Adults were sampled monthly from March 2007 until March 2008 using pyramid-type emergence traps at 9 locations. A total of 38012 specimens comprising 10 species were collected. The dominated genus was *Simulium*, while the most abundant species was *Simulium angustipes*. A strong relationship was confirmed by MDS analysis between species composition and their habitat type. *Simulium angustipes* was highly related to lake outlets. The blackfly communities at all sites were dominated by species typical for the rhithral zone, but there was a shift in species composition along a longitudinal gradient, from the hypocrenal-epirhithral to the epirhitral-metarhithral zone. All species were univoltine, except *Simulium costatum*, *Simulium angustipes*, *Simulium monticola*, *Simulium trifasciatum* and *Simulium variegatum*. *Simulium costatum* that was present on 8 from 9 studied sites had a multivoltine cycle on sites with constant water temperatures and bi or univoltine cycle on sites with variable water temperature. *Simulium angustipes* had multiple generations per year, while *Simulium monticola*, *Simulium trifasciatum* and *Simulium variegatum* had two generations per year.

A metacommunity view of black fly species assemblages

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We explore the patterns of species assemblages of larval black flies, using two separate, yet complementary, approaches to tease out the influence of biotic, abiotic and dispersal factor on species assemblage. Stream variables and distances (dispersal factors) between sites in the spring collection were correlated significantly with species similarity. Assemblage similarity was significantly correlated with stream conditions only during the summer collections. Results of null model analyses suggested that patterns of species segregation during the spring were consistent with a community structure by competition. In contrast, during the summer months patterns of segregation were consistent with species assemblage influenced by abiotic factors. In summary species co-occurrences of black flies were consistent with assemblages influence by competitive interactions, abiotic conditions, and dispersal. Given that black flies have a significant terrestrial stage, understanding the processes that determine local assemblages in one ecosystems (e.g. streams) will require us to couple the dynamics of different ecosystems (e.g. terrestrial conditions).

The species composition and altitude distribution of black flies (Diptera, Simuliidae) of the North-West Caucasus streams

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The Caucasus region is one of the unique biodiversity centers with high level of endemism. At the same time different geographical provinces of the Greater Caucasus Mountain and the Transcaucasus have a highly specific fauna, which was fully characterized for the black flies of the family Simuliidae.

This research was based on the original material collected at different time of the second half of the 20th century in several parts of the Northern-West Caucasus: Caucasian State Nature Biosphere Reserve (basins of the rivers Kisha and Belaya), Teberdinskiy Nature Biosphere Reserve (basins of the rivers Teberda and Gonchahir) and Sochi National Park (the Blonde and the Svir rivers). Immature stages of black flies were collected in 118 rivers and their tributaries. Tributaries were of two types: high- and turbulent-water streams without vegetation, and small, overgrown, quickly drying up brooks. The bottom of the streams was lined with pebbles and boulders, and there were waterfalls. The water temperature in summer ranged from 6 to 12 °C, depending on the height above sea level. Streams were investigated at height from 100 to 500 m above sea level (Sochi National Park) and at height from 500 to 2500 m above sea level (Caucasian and Teberda Reserves).

27 blackfly species of 11 genera were confirmed, among which the genera *Cnetha* Enderlein, 1921 and *Odagmia* Enderlein, 1921 were dominated (55% of total species composition). Four detected species were endemic of the Caucasus: *Cnetha elata* (Rubzov, 1955), *C. murvanidzei* (Rubzov, 1955), *Nevermannia crassicaulis* (Rubzov, 1955) and *N. gomphocornis* (Rubzov, 1964). Locations of 12 species of black flies previously known for the Caucasus (Armenia, Azerbaijan and Georgia), Uzbekistan and Ukraine (Crimea) were indicated for the Russian Federation for the first time.

The altitude distribution of black flies was characterized by a decrease in the number of species and in the vertical alteration of species composition: from 15 species (*Odagmia* (5), *Cnetha* (4), *Nevermannia* (2), *Prosimulium* (2), *Obuchovia* (1), *Paragnus* (1)) at height 100 – 500 m (zone of beech forests) to 6 species (*Cnetha* (3), *Montisimulium* (1), *Obuchovia* (1), *Prosimulium* (1)) at height 2200 – 2500 m (subalpine zone). *Cnetha* spp. prevailed in all altitude zones, while *Odagmia* spp. were numerous up to a height of 1500 m. In general, the role of highland species enhanced with increasing altitude, while the steppe and Mediterranean-plains species were smoothed over.

Comparisons of the black flies species composition of these areas of the Northern-West Caucasus with Transcaucasus were done. The similarity between the black flies fauna of the Northern-West Caucasus and Azerbaijan is 34% at the species level, and 79% – at the generic level; the similarity between the fauna of the North-Western Caucasus and Armenia is 18% and 90%, respectively, which shows the high degree of isolation of these territories on the one side and a common ways of formation of the local fauna complexes on the other side.

The blackfly fauna (Diptera, Simuliidae) of the Iron Gate area (eastern Serbia, southwestern Romania) in the past and present

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The middle and lower Danube river has the character of a lowland river except the Iron Gate (Djerdap gorge) in eastern Serbia and southwestern Romania, where it flows over a canyon in the Southern Carpathians. This section represented the main breeding site of *Simulium colombaschense* (Scopoli, 1780), the most ferocious blackfly species in Europe, that caused enormous losses of livestock in the past. Due to repeated outbreaks of this species, this region was the targeted study area of several researchers over more then 100 years. Besides establishing the composition of the blackfly fauna in the Danube, at the same time also efforts were done in order to light up the species composition of the Danube tributaries in this area. At the end of 60´s of the last century the river has been dammed here, and significant environmental changes of the blackfly breeding sites occurred. In the first period – during the building of the dams and short after putting them in operation – the blackfly community changes are well documented. The number of blackflies decreased and the ratio of lowland species (*Simulium galeratum* Edwards, 1920 and *S. erythrocephalum* (De Geer, 1776) increased. One species – *Metacnephia danubica* (Rubtsov, 1956) – disappeared completely, the abundance of *S. colombaschense* decreased dramatically and the problems with outbreaks disappeared. In consequence, research of blackflies in this area was abandoned and the data on the present state of the black fly fauna were completely missing over more than 35 years. In 2006 – 2012 we found in the Danube no suitable conditions for blackfly preimaginal stages and the river in the Iron Gate seems not to be breeding site of any blackfly more. We found numerous blackfly breeding sites in the tributaries of the Danube, *S. colombaschense* was not present even here. The closely related S. reptans (Linnaeus, 1758) was dominant in the larger tributaries (Porećka reka, Cerna). We could not confirm the presence of *S. erythrocephalum*, *S. maculatum* (Meigen, 1804), *S. bezzii* (Corti, 1914) and *S. voilense* Sherban, 1960. We found *P. tomosvaryi* (Enderlein, 1921), *P. hirtipes* (Fries, 1824), *P. rufipes* (Meigen, 1830), *S. cryophilum* (Rubtsov, 1959), *S. argenteostriatum* Strobl, 1898, *S. degrangei* Dorier & Grenier, 1960 and *S. monticola* Friederichs, 1920 for the first time here. The taxon labeled as *S. vulgare* Dorogostaisky, Rubtsov & Vlasenko, 1935 in central Europe was found for the first time in Serbia, it was present at many sites investigated. Several of the found species present taxonomically unresolved problems.

Zonal and latitudinal distribution of blackflies (Diptera, Simuliidae) in the West Siberia

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In the West Siberia the classical latitudinal geographical zoning is represented. The Ob – Irtysh water basin stretch from the East Kazakhstan through the Altai Mountains and the West Siberian lowland reaches, the Ob River is the longest and abounding in Siberia. The distribution of blackflies in the landscape-zonal and altitudinal profiles was examined waist with a reliminary reduction of quantitative original and literature data to a single accounting system.

River basins usually are investigated in the context of runoff – from the upper to the lower reaches, for the Ob and Irtysh Rivers this direction is formed from south to north, but that does not coincide with the general approach of studying landscape – zonal distribution from north to south. In order to compare original data we have used the scale of domination structure by Engelmann. Mathematical treatment of the material was done by one of the methods of cluster analysis and non-metric multidimensional scaling.

Five classes were identified in the structure of blackfly communities, species similarity in classes was high – 40 – 60 % and interclass relationships was lower – 18 – 32 %. In the structure of blood-sucking communities the main trends of changes were identified, but there were much fragmentation groups (9 classes) with a high inner similarity (50 – 71 %) and interclass relations were spread from 12 to 40 %. It is probably due to adaptation of females for landscape and environmental conditions and dispersal from breeding sites in search of hosts.

Entomology and the elimination of onchocerciasis by community directed treatment with Ivermectin in Africa

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Recent evidence has indicated that where annual Community Directed Treatment with Ivermectin (CDTI) has been done well in Africa, and reached the target human population, infection by *Onchocerca volvulus* has become undetectable or has been greatly reduced. As a result, in December 2010 the WHO African Programme for Onchocerciasis Control (APOC) was given a new mandate – to eliminate onchocerciasis transmission from the whole of Africa (and in effect drive *Onchocerca volvulus* to extinction). To monitor this APOC have defined a new conceptual and operational framework which includes the definition of independent transmission zones and the evaluation of transmission. Central to this strategy is the application of entomology.

Simulium damnosum complex geographical distribution and host choice in Ghana where onchocerciasis transmission is under ivermectin control

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Variation in geographical distributions and abundances of *Simulium damnosum* s.str. / *S. sirbanum*, *S. squamosum*, *S. yahense*, the Beffa form of *S. soubrense* and *S. sanctipauli* were studied at 7 sites in four regions of Ghana from 2009 to 2011, in both rainy and dry seasons. To investigate their host densities and host choices, adult blackflies (15,466; 85% *Simulium damnosum* s.l.) were collected by host-dependent and host-independent methods, assessed for parity, and stored for molecular and morphological analysis for identification of fly and *Onchocerca* species and bloodmeal origin. Biting rates ranged from 0 to 298 bites/person/day and parity rates from 18 to 27% (wet season) and from 30 to 46% (dry season). Three of the villages had levels of L3 larvae/1,000 parous flies above the World Health Organization's threshold for morbidity and transmission control (range 1.4 to 115.1 L3/1,000 parous flies) despite annual distributions of ivermectin for up to 23 years in one village. In these villages exposure to infective L3 larvae ranged between 0.04 and 3.66 L3/person/day. Flies had fed on a range of hosts, predominantly humans and pigs, but also on sheep, goats, dogs, cattle and some as yet unidentified species. *Onchocerca* spp. other than *O. volvulus* were recorded in some flies. The results confirm that members of the *S. damnosum* complex (1) have multiple blood hosts; (2) may harbour different *Onchocerca* species and (3) are involved in continuing onchocerciasis transmission despite their human hosts receiving annual or biannual ivermectin treatments.

The effect of seston on larval black fly mortality after exposure to a *Bacillus thuringiensis* subsp. *israelensis* based larvicide

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Seston is defined as the organisms and non-living matter suspended in a body of water. Black fly control specialists have been concerned about seston and the turbidity it produces since the development and implementation of *Bacillus thuringiensis* subsp. *israelensis* (Bti) based larvicides in the early 1980's. Recent research has demonstrated that the composition of the seston is critical to larval feeding and Bti induced mortality in larval black flies. Experiments were conducted with water from the North Branch of the Susquehanna River in Lime Ridge, PA USA using the Bti based larvicide, Vectobac 12AS. Results of a controlled current toxicity test (CCTT) using *Simulium vittatum* larvae from The University of Georgia black fly colony demonstrated that the river water, as collected, significantly reduced larval susceptibility to Bti insecticidal proteins. River water was then filtered through a 0.20-µm filter. Recovered seston was resuspended in moderately hard water. Experimentation with the resuspended seston significantly reduced Bti induced mortality. Experiments with the filtrate revealed no effect on Bti induced larval mortality. Multiple analysis were conducted on the river water and seston. Exposure of larvae to selected minerals and nutritive organic material before exposure to a Bti based larvicide resulted in no significant change in mortality. Exposure of larvae to silicon dioxide, cellulose, viable diatoms and purified diatom frustules before exposure to a Bti based larvicide resulted in significant reductions in larval mortality. Exposure of larvae to purified diatom frustules from *Cyclotella meneghiniana* resulted in the most severe impairment of larval mortality. These results demonstrate that suspended particulate material, (seston) in the river water and not dissolved materials are responsible for the decreases in mortality observed in black fly larvae following exposure to a Bti based larvicide. Additional experiments were conducted exposing larvae to a water insoluble pigment. The results of these experiments demonstrated that the larvae ingested less pigment when cellulose and diatom frustules were present in the larval media. It is postulated that altered larval feeding behavior is responsible for the reduced larval mortality.

Progress in the *Simulium* Genomics Project

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The *Simulium* Genomics project was launched in 2006 as a community effort to address a wide range of biological and epidemiological issues using the emerging fields of genomics and bioinformatics. We will review the recent progress of the project, including the construction a whole-body, all life stage, transcriptome of the only colonized blackfly, *Simulium vittatum* Zett., the initiation of NHGRI funded whole genome sequencing of 11 species from 3 continents, the first whole genome sequencing effort of 3 species at 10x coverage, and examples of the integration of genomics, transcriptomics and proteomics to by-pass the traditional barriers to molecular work in blackflies.

How do blackflies identify their hosts? Results of a preliminary study of visual and olfactory responses of *Simulium squamosum* B in Southern Cameroon

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Field studies of visual and olfactory responses of *Simulium squamosum* B were conducted at sites close to the River Sanaga in southern Cameroon (~ 4°26'N – 11°48E'). Black or royal blue targets, made of cloth on metal frames 1.0 m wide x 0.5 m high coated with sticky film (Rentokil FE45, UK) or glue (Ashley, Manchester Hardware, UK), or transparent targets made of polymethyl methacrylate panels were used to assess responses of blackflies to different colours. To assess olfactory responses, black targets were baited with CO_2 or odours from live hosts (one cow or three men) released at 1 litre/min inside a tent, from which the air was drawn out through a tube by a fan and thus expelled at ~2000 litres/min.

Slightly more flies were collected on black targets than on blue targets (1.3x, $P < 0.05$) and almost twice as many as on the transparent targets (1.7 – 1.8x, $P < 0.001$). Cattle-baited targets attracted more flies than those baited with human odour, CO_2 or unbaited controls (1.3x, 1.7x and 2.5x, respectively; $P < 0.001$). Human odour doubled the catch of an unbaited target and increased the catch ~1.3-fold compared to CO_2-baited targets ($P < 0.001$).

The results confirmed that female *S. squamosum* B respond to host kairomones. Although CO_2 plays a role in attraction and/or landing, other host kairomones are also involved. Colour is also a factor in attraction as more blackflies landed on black targets than on blue or transparent ones. This preliminary study suggests that visual and olfactory cues could be used to design new tools for monitoring blackfly densities and, perhaps, control blackflies with community-based vector control or with integrated vector management programmes aimed at killing tsetse flies (*Glossina* spp.) and blackflies simultaneously. For example, traps could be designed according to the observed visual responses and baited with appropriate host kairomones to increase their efficiency.

Blackfly outbreak in Zaragoza in 2011. Spread of blackflies in the Middle Ebro Valley in northeast Spain

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The Ebro is the largest river in Spain. Since 2006 black fly larvae are controlled along the lower Ebro between Amposta and Flix. In 2011 Zaragoza, recorded a significant increase on black fly bites in the month of June. The University of Zaragoza started in cooperation with the Public Health Institute of Zaragoza a survey to assess this new black fly problem and propose a technical solutions for a black fly nuisance control.

Zaragoza the capital of the autonomous region of Aragon is located in northeast Spain. It is the fourth largest municipality in the country. Two of the four streams around Zaragoza have been shown as important simulium breeding sites. The most abundant species are: *S. sergenti*, *S. equinum* and *S. erytrocephalum*. The natural substrate for the immature stages are the macrophytes. In 2011 the Public Health Department of Aragon recorded a dramatic increase of humans with severe allergic reaction on black fly bites. To control the simulium nuisance, larval treatments were conducted by using the *Bacillus thuringiensis* var. *israelensis* Strain AM 65 – 52 based Vectobac ® 12 AS. Trial application for black fly adulticiding with Deltametrin were requested to be carried out in pools in Zaragoza by the local Public Health Institute.

The black fly nuisance in the northeast of Spain was limited in the previous years along rivers in the mountainous areas. In recent years the black flies have colonized new river ecosystems in the Middle Ebro Valley, significantly expanding its presence in the various regions and thus becoming a real nuisance for humans and live stock.

The pause in blackfly control in Lithuania

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The main bloodsucking blackfly species is *Simulium* (*Byssodon*) *maculatum* Mg. in Lithuania. It develops in April – June in large rivers. The Nemunas River was found to be the main development place of this blackfly species in Lithuania. Bloodsucking insects caused losses of cattle and domestic birds and frustrated a recreation in South Lithuania. Biological larvicide based on *Bacillus thuringiensis* var. israelensis was used for bloodsucking blackfly control in Lithuania in 1998 – 2010. Biopreparation was used in the Nemunas River. A sufficient efficacy was achieved in the segment of the river 164 km long every year in spite of the fact that concentration of the preparation as well as the discharge of the river differed each year. The density of *S. maculatum* larvae in the river in spring decreased close to one hundred times during this period. The outbreak of bloodsucking blackflies was successfully stopped. Blackfly control was paused from 2011 because of economical cause. The density of *S. maculatum* larvae in the river increased in 2012. The increase in bloodsucking blackfly activity was detected from 2011.

Some other blackfly species were also detected as pest blackflies in different parts of Lithuania, but their activities were not very high and varied each year. These species were *Simulium reptans* (L.), *S. lineatum* (Mg.) and *S. ornatum* Mg.

Compact discs for sampling immature stages of members of the *Simulium damnosum* complex

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At the 4th International Simuliidae Symposium, Foroutan et al. (2010) showed how compact discs (CDs) could be used for obtaining samples of larvae and pupae of *Simulium* spp. in southwest Germany. Adult females were attracted to shiny CDs trailing from strings in fast-flowing water to lay their eggs and drifting larvae attached themselves to the discs. In order to determine if West African onchocerciasis vectors could also be sampled using CDs, we tested the method at two sites in Ghana: Asubende on the River Pru, where only *S. damnosum* s.str. / *S. sirbanum* was found cytotaxonomically, and Pillar 83 (= Djodji) on the River Wawa (= R. Gban-Houa), where *S. squamosum* predominates. A fourth instar larva of *S. damnosum* / *S. sirbanum* and eggs, larvae and pupae of other species were found on CDs at Asubende but time did not permit a long-term experiment there. However, at Pillar 83 CDs were left trailing in a breeding site long enough for larvae of all stages and pupae (some post-eclosion) of *S. squamosum* to accumulate on them. The results will be presented and discussed as a cheap and easy means of sampling immature stages of onchocerciasis vectors in West Africa.

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.

Insects from at least 9 orders are known to feed on black flies and attack all developmental stages: eggs, larvae, pupae and adults. 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 our fieldwork in South Africa, we have been able to show that there are at least 102 species in 15 families of Diptera that prey on different black fly species. Our studies have been focussed on predators of larval and adult black flies, and in addition to Trichoptera and spiders we have new information on Dolichopodidae, Empididae, Hybotidae and Muscidae. This includes observations on hunting strategies, adaptations to the occurrence of simuliid populations, and also further information and observations on the courtship and mating rituals of predatory flies.

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.

Notes on blackfly fauna in Western Thrace (northeastern Greece)

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The research of blackfly fauna was conducted in the prefectures of Xanthi and Rodopi (northeastern Greece) at the end of June 2011 and at the beginning of July 2012.

Samplings of immature stages were performed in the three main permanent watercourses: Kosynthos river at localities near Xanthi and Amaxades (in 2011 and 2012), Nestos river near Stavroupoli (in 2012) and Kompsatos river near Polyantho (in 2012).

Adult sampling was performed by application of CO2 baited traps (type NS-2) in localities close to the breeding sites (Xanthi and Amaxades in 2011 and 2012; Stavroupoli in 2012). Additionally, in the close vicinity of the Kosynthos river the collection of adults was performed by the use of sweeping net and by sampling from the host (horses, dogs and humans).

High population densities of *Simulium* (*Wilhelmia*) *pseudequinum* Séguy, 1921 and *S.* (*W.*) *balcanicum* (Enderlein, 1924) immature stages were recorded in Kosynthos river in 2011 and 2012. *S.* (*W.*) *balcanicum* was the dominant species in Kompsatos river and *S.* (*Simulium*) *reptans* (Linnaeus, 1758) in Nestos river.

In accordance with the observations of blackfly fauna in the breeding sites of Kosynthos and Nestos rivers, the most frequently captured species in traps were the species of *Wilhelmia* subgenus at the locality of Xanthi and *S. reptans* at the locality of Stavroupoli. Biting activity of adult females of both species of the *Wilhelmia* subgenus was observed on horses and dogs, while *S. reptans* attacked humans as well.

Such results impose the conclusion that those three mammophilic species could be considered as species of medical and veterinary importance in the prospected region. Other identified species (*S.* (*S.*) *ornatum* Meigen, 1818 (complex), *S.* (*S.*) *bezzii* (Corti, 1914) (complex) and *S.* (*E.*) *velutinum* (Santos Abreu, 1922) were recorded in low population densities.

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Preliminary notes on black fly fauna in Piedmont region, northern Italy

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Black fly fauna of Piedmont was first studied by Rubtzov in 1961. He identified 34 taxa among species and varieties in 70 river biotopes of the region and described 4 new species – 3 of which are currently considered synonyms of *Simulium cryophilum* and 1 of *Prosimulium latimucro* – and 4 varieties. Other surveys were performed in the same period by Couvert, who described a single new species (*S. segusina*) and 1 variety. No material concerning these collections was found until now in any regional or national museum, as it is possible for the black fly fauna of several other Italian regions; therefore morphological, cytological and molecular verifications are impossible nowadays. For this reason, a study was started in 2011 aiming at filling this gap and understanding the current distribution of black flies in Piedmont. Preliminary findings are here presented, together with some ecological considerations.

Nearly 50 sites were sampled so far in spring and early summer 2011 – 2012, aiming both at covering the largest possible area of the region and at re-sampling the same sites reported in literature. Therefore, collections were performed on the Alps of Turin and Cuneo (25 sites), in the upper Po valley (15), on the hills of Ivrea (3), in the rice farming area of Vercelli (4) and in the Tanaro Valley (5). Larvae and pupae were collected in the field while adults were obtained in the laboratory from mature pupae.

As a result, 24 species were identified overall through morphological examinations, with *S. variegatum* (29 sites), *S. ornatum* (19), *S. argenteostriatum* (15), *S. reptans* (13), *P. rufipes* (10) and *S. equinum* (10) presenting the highest frequencies. *P. rufipes*, *S. argenteostriatum*, *S. monticola*, *S. argyreatum* and *S. variegatum* were typical of alpine sites, but the last was also collected in lower hill areas, together with *S. reptans* and *S. ornatum*. In hill sites, *S. cryophilum* was often collected, while *S. equinum* and *S. lineatum* were gathered almost only from plane sites. Other species were only sporadically found. A comparison of past and this study's collections is also presented, according with the water course typologies identified by Rubtzov in Piedmont. Nearly 3 species were found on average in each sampled sites, confirming the good biodiversity status of stream biotopes in Piedmont, especially if compared with those that were recently sampled in the area of Rome, which often hosted only 1 species (typically *S. ornatum*) or none at all.

These preliminary collections did not confirm the presence of some of the species found by Rubtzov and Couvert in Piedmont (recognized or not by current taxonomical inventories), but further investigation is ongoing in order to provide a regional museum with a large and representative collection of black fly fauna, together with samples in Carnoy liquid for further analyses.

First records of *Simulium* (*Hellichiella*) *latipes* (Meigen, 1804) (Diptera: Simuliidae) in Hungary

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Larvae and pupae of *Simulium latipes* were collected from six calcareous streams and brooks in the north-western (Által-ér, Bikol-patak, Kardos-ér, Csenke-patak) and eastern (Fülöpi-ér, Villongó-ér) part of Hungary. These were the first occurrences of this species and of the subgenus *Hellichiella* as well. Since this species has already been found in low-land streams of some neighbouring countries (Austria and Lithuania), its appearance in the local fauna could have been expected. By detection of *S. latipes* the Hungarian blackfly fauna contains 26 species and due to the intensive macroinvertebrate assessments of the water inspectorates this number supposedly will increase.

Blackflies as indicators of ecological stress in two Alpine streams with different land use in the catchment

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Two streams in adjacent watersheds were sampled monthly from spring to autumn in 2010 and 2011. The two streams shared similar environmental conditions but one drainage basin was largely used for extensive agriculture and the second was in near to natural conditions. Physical-chemical parameters (mean water velocity, water temperature, dissolved O_2, pH, turbidity and conductivity) were measured in both streams during each sampling occasion. The benthic community was sampled with the standard kick-net technique and all individuals were counted and identified to the lowest possible taxonomical level. Results indicate significative differences between the impacted and reference streams in terms of abundance, composition and seasonality of the benthic communities. The blackly fauna resulted generally more abundant but less diverse in the impacted stream, dominated by more tolerant species.

The Simuliidae fauna of South West Germany

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About 50 Simuliidae species are known from Germany.

Bloodsucking females of the some mammophilic species, such as *Simulium erythrocephalum*, *S. ornatum*, *S. reptans*, *S. noelleri* and *S. trifasciatum* are known as conspicuous pests.

Immature stages of Simuliidae play an important role in aquatic habitats. But some species may represent critical pests and vectors of human and animal pathogens. Considering this fact, the control in the concerned areas is crucial.

The preconditions for an effective control of this special group of Diptera are the knowledge of the species composition in particular study areas, the identification of pest species, their biology and behavior, then the selection and the application of ecologically strategies of control can be followed.

The objective of this research is to classify the blackfly fauna in the streams in South West Germany.

About 60 different sampling stations have been checked during the summer and autumn months 2011 and the spring and summer months 2012. In total 24 of 50 known species have been found in this area. Especially in the summer months, three species appeared dominant; these were *S. lineatum*, *S. ornatum* and *S. erythrocephalum*, representing together about 80 % of the sampled material.

Comparison of *Simulium maculatum* Mg. biology in different parts of Palaearctic

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Simulium (*Byssodon*) *maculatum* is one of the main bloodsucking blackfly species in the East Europe, West Siberia and north regions of East Siberia. It develops only in the large rivers with laminar flow. Once widespread *S. maculatum* is now extinct in many of the large river systems in central Europe. Previously, *S. maculatum* developed abundantly in the Danube, Mogila, Pek and the Juzna Morava Rivers in South Europe. The timing and conditions of *S. maculatum* development was compared from different regions of Palearctic: East Europe (the Nemunas and the Don rivers), Kazakhstan (the Irtysh river) and West Siberia (the Ob river). Discharge of investigated rivers varied from 500 to 12500 $m^3.s^{-1}$ and the current velocity varied from 0,3 to 1 $m.s^{-1}$. In spite of the fact that larvae of this species hatch from eggs from the end of April (East Europe) to the beginning of June (West Siberia) water temperature during larval hatching is similar is all rivers (10 °C). Pupal formation is usually detected from the middle May till the end of June, but the water temperature in all rivers is similar during this period. This species has one or two generations per year, but this can be related with lower water temperatures and longer development respectively.

Some morphological characteristics of *S. maculatum* larvae in the areal and in the gradient of continentality were also compared and discussed.

Blackflies (Diptera: Simuliidae) of Leningradskaya and Vologodskaya regions (Russia)

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In the collection of Zoological Institute of RAN there are specimens of blackflies from Leningradskaya (LR) and Vologodskaya Regions (VR), beginning from 1919. Studies of blackflies in this Institute were carried out at different times by I.A. Rubzov, Z.V. Usova, A.V. Yankovsky and S.V. Aybulatov.

We provide the following list of species of blackflies of the LR and VR, including either found by us in the region for the first time or the ones known from literature: *Prosimulium hirtipes* (Fries, 1824) LR; *P. luganicum* Rubzov, 1956 LR; *P. macropyga* (Lundstrom, 1911) VR; *Stegopterna trigonia* (Lundstrom, 1911) LR; *Cnephia pallipes* (Rivosecchi, 1964) LR; *Metacnephia korsakovi* (Rubzov, 1956) VR; *Wilhelmia equina* (Linnaeus, 1758) LR, VR; *Boreosimulium annae* (Rubzov, 1956) LR; *B. annulus* (Lundstrom, 1911) LR; *Hellichiella tsheburovae* (Rubzov, 1956) VR; *H. latipes* (Meigen, 1804) VR; *Byssodon maculatus* (Meigen, 1804) VR; *Cnetha beltucovae* (Rubzov, 1956) LR, VR; *C. bicornis* (Dorogostajsky, Rubzov et Vlasenko, 1935) LR, VR; *C. cryophila* (Rubzov, 1959) LR; *C. curvans* (Rubzov et Carlsson, 1965) LR; *C. kuznetzovi* (Rubzov, 1940) LR; *C. meigeni* (Rubzov et Carlsson, 1965) LR, VR; *C. silvestris* (Rubzov, 1956) LR, VR; *C. verna* (Macquart, 1826) LR, VR; *Nevermannia angustitarsis* (Lundstrom, 1911) LR, VR; *N. lundstromi* (Enderlein, 1921) LR; *Eusimulium angustipes* (Edwards, 1915) LR; *E. argentipile* Rubzov, 1962 LR; *E. aureum* (Fries, 1824) LR, VR; *E. silvaticum* Rubzov, 1962 LR; *Schoenbaueria nigrum* (Meigen, 1804) VR; *S. pusilla* (Fries, 1824) LR, VR; *S. subpusilla* (Rubzov, 1940) LR, VR; *Boophthora erythrocephala* (De Geer, 1776) LR, VR; *Parabyssodon transiens* (Rubzov, 1940) LR, VR; *Odagmia bronchialis* Rubzov, 1962 LR; *O. frigida* (Rubzov, 1940) LR, VR; *O. fusca* Rubzov, 1963 LR, VR; *O. ornata* (Meigen, 1818) LR, VR; *O. rotundata* Rubzov, 1956 LR; *Archesimulium tuberosum* (Lundstrom, 1911) LR, VR; *A. tumulosum* (Rubzov, 1956) LR, VR; *A. vulgare* (Dorogostajsky, Rubzov et Vlasenko, 1935) LR, VR; *Argentisimulium noelleri* (Friederichs, 1920) LR, VR; *A. palustre* (Rubzov, 1956) LR; *Gnus gabovae* (Rubtsov, 1966) VR; *G. murmanum* Enderlein, 1935 VR; *Simulium abbreviatum* Rubzov, 1957 LR; *S. aemulum* Rubzov, 1940 LR; *S. curvistylus* Rubzov, 1957 LR; *S. curvitarse* Rubzov, 1940 LR; *S. lugense* Yankovsky, 1996 LR; *S. janzeni* Enderlein, 1922 LR, VR; *S. longipalpe* Beltukovae, 1955 LR, VR; *S. morsitans* Edwards, 1915 LR, VR; *S. paramorsitans* Rubzov, 1956 LR, VR; *S. posticatum* Edwards, 1915 LR, VR; *S. promorsitans* Rubzov, 1956 LR, VR; *S. reptans* (Lundstrom, 1911) LR, VR; *S. rostratum* (Lundstrom, 1911) LR, VR; *S. rubzovi* Smart, 1945 LR, VR; *S. simulans* Rubzov, 1956 LR; *S. truncatum* (Lundstrom, 1911) LR, VR; *S. venustum* Say, 1823 LR

Thus, so far only 60 species of blackflies (52 in LR and 37 in VR) were noted in these areas. It must be noted that while studying these regions it is highly probable to detect the following species which have not been found in this territory, but were registered in adjacent territories: *Metacnephia lyra*, *M. saileri*, *Cnetha fontinalis*, *Gnus corbis* and *Helodon ferrugineus*.

Black fly species succession from Alps to lowland rivers in Piedmont, north-western Italy

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The Alps surround Piedmont region in Italy all along its western and northern sides, like a mountain arch dropping down sharply to the Po plane. In this area, rivers and streams can flow from 3000 to 100 m altitude in only 25 – 30 km span. Therefore, a corresponding fast change of ecological conditions can be observed in water-courses: biological communities, such as species assemblages of black flies (Diptera, Simuliidae), mirror the same ecological succession through a definite species shift. The last comprehensive study of black fly species distribution in Piedmont was carried out half a century ago; a preliminary update was performed during 2011 and 2012 by a joint sampling campaign of ENEA (Italy) and the University of Novi Sad (Serbia) and between 2008 and 2012 sampling was performed in Alpi Marittime by the Comenius University in Bratislava (Slovakia). In this study we present the actual distribution of black fly species along the altitude range in Piedmont, from small mountain brooks down to streams and lowland rivers.

Black fly larvae and pupae were collected in nearly 70 sites quite homogeneously distributed from 2000 (Alpine valleys) to 85 metres (Po river close to Alessandria). Adults were obtained from mature pupae in the laboratory.

A total of 24 species were identified, with *S. variegatum* proving to be the most frequent (34 sites); 8 species were collected only in single sites. The study of the altitude range of black fly species showed that *S. variegatum* can be collected from the highest valleys (1630 m) to the large plane rivers (94 m). Other species, such as *P. rufipes* (537 – 1916 m), *S. argyreatum* (537 – 1916 m) and especially *S. maximum* (1100 – 1926 m) were characteristic of mountain sites. *S. cryophilum* and *S. argenteostriatum*, though associated more to cold waters, could still be collected in low hill streams (down to 302 and 385 m respectively). *S. reptans* was collected in low hills and the upper areas of the Po plane (102 – 402 m), but it was not collected in higher hill or lower plane sites, as it happened with *S. ornatum* (82 – 731 m). Finally, *S. velutinum* and *S. equinum* were typical of lowland water courses, the first being preferably collected in brooks and streams and the second in large rivers.

These results, though preliminary, can be useful to depict a present day update of the distribution of black fly species in Piedmont, where Alpine hydroelectric plants and agricultural water needs in the Po plane have strongly altered stream ecosystems during the last 50 years.

A new study on blackflies (Simuliidae)of Iran in central regions

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In what follows we will describe a morphological study on Simuliidae (blackflies) made at 9 lotic breeding sites in central region of Iran in year 2011. In this morphological identification no new species were found. We could identify the specimens by Crosskey key (2002). A brief review is provided of previous works on blackflies in Iran and a checklist is included for the 20 identified species recorded from the country to the present time (2012).

The blackfly fauna (Diptera: Simuliidae) of the Gesäuse National Park in Austria

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The Gesäuse National Park was founded in 2002 and with an area of 110.5 km² is the third largest of the six Austrian National Parks. Located in the area of the Ennstal Alps/Gesäuse it is part of the Northern Limestone Alps with the Dachstein limestone and Ramsau dolomite as most common types of rocks. The highest elevation is called Mt. Hochtor (2,370 m a. s. l.), the altitude difference is 1880 m.

Within the scope of a perennial fountain research project the Simuliidae fauna of 90 sampling points (68 spring outflows, 22 river sections) was evaluated. Hitherto 25 species (including *S.* (*E.*) *petricolum* and *S.* (*N.*) *bavaricum* already reviewed as new) as well as some higher taxa (all in all 7500 individuals) could be found, equivalent to 52 % of the actual Austrian species number. According to the hypocrenal character of most sampling sites, the taxa with the highest constancy belong to the spring species known from literature. The frequency of the remaining 20 species decreases quickly resulting in single findings, the source of which being the small number of samples per biotope, as well as in the general rarity of some of these species (e.g. *T. hydroides*, *S.* (*N.*) *oligotuberculatum*).

In a table showing the occurrence at the maximum altitude all species are ranked. The uppermost findings are those of *P. latimucro* as well as *S.* (*N.*) *beltukovae* and *S.* (*N.*) *bavaricum* at 2020 m and 1984 m respectively; the lowermost those of *S. colombaschense* and *S.* cf. *tuberosum* at 587 m a. s. l. Regarding 6 altitudinal ranges with altitude classes of 250 m each, ranging from 500 to 2000 m a. s. l., the frequency of findings was sorted in 4 frequency groups. According to this, the range from 1500 to 2000 m is the focus of the dominant occurrence of *P. latimucro*, from 1250 to 1500 m that of *S.* (*N.*) *beltukovae* and *S.* (*N.*) *crenobium* and from 1000 to 1250 m a. s. l. that of *P. rufipes*.

Typology of running waters according to blackfly communities in conditions of Borská nížina lowland (West Slovakia)

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The research was carried out during 2006 – 2008 on 30 sites in conditions of Borská nížina lowland and submountain area of Malé Karpaty Mts. Material of preimaginal stages of blackflies was obtained monthly. Larvae and pupae were sampled from individual microhabitats in 15 min. intervals. In total, 27 species of blackflies were identified. Species with highest frequency in submountainous conditions of Malé Karpaty Mts. were *Prosimulium tomosvaryi* Enderlein, 1921, *Simulium argyreatum* Meigen, 1838, *S. ornatum* Meigen 1818 and *S. trifasciatum* Curtis, 1839 while species *Simulium angustitarse* (Lundström, 1911), *S. costatum* (Friedrichs, 1920), *S. degrangei* Dorier et Grenier, 1960 and *S. lineatum* (Meigen, 1804) had lowest frequency in mentioned flows. Borská nížina lowland was characterised with high frequency of species *Simulium erythrocephalum* (DeGeer, 1776), *S. angustipes* Edwards, 1915, *S. noelleri* Friedrichs, 1920, *S. ornatum*, *S. reptans* (Linnaeus, 1758), *S. balcanicum* (Enderlein, 1924) and *S. equinum* (Linnaeus, 1758). According to species composition of blackflies communities it was recognised 7 types of flows: 1. natural foothill spring brook, 2. natural foothill stream, 3. modified foothill stream, 4. natural lowland spring brook, 5. natural lowland stream, 6. modified lowland stream, 7. lowland river. According to TWINSPAN analysis, *Simulium balcanicum* was identified as main indicator species by dividing to particular groups of sites. Presence of *Simulium reptans* together with absence of *Simulium ornatum* characterised lowland rivers. *Simulium noelleri* was recognised as important indicator species of modified lowland and foothill streams. The species was indicator by dividing of sites according to hypsometry.

Molecular classification of some simuliid larvae collected from Central Basin of Kizilirmak River based on the sequence analyses of mt-COI and ITS-2 gene regions

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This study was carried out to determine the molecular characterization of simuliid species which cause a problem in the part of Kizilirmak River localized in Urgup and Gulsehir districts of Nevsehir province, Turkey. This study was financially supported by Erciyes University Research Fund (EUBAP-Project Number TSY-11-3437). Between May and September 2011, totally 150 simuliid larvae were collected from three collection sites. Morphological examination was performed before molecular analyses. Genomic DNA extractions were utilized on 7 larvae selected from the research area and PCR analyses were performed with the primer pairs that amplified mitochondrial partial cytochrome oxidase subunit 1 (mt-COI) and ribosomal complete internal transcript spacer 2 (ITS-2) and partial 28S gene regions. Sequence analyses using the same primer pairs were performed on the obtained amplicons in order to phylogenetic analyses of related gen regions. All of the 150 larvae showed the general characteristics of *Simulium* (*Wilhelmia*) subgenus according to morphological analyses. 4 out of the 7 specimens selected for the molecular analyses were identified as *S.* (*Wilhelmia*) *balcanicum* whereas the remaining 3 were identified as *S.* (*Wilhelmia*) *lineatum* by morphological identification. The percent identities among the *S.* (*Wilhelmia*) *balcanicum* isolates (Kizilirmak 1,2,4,7) and *S.* (*Wilhelmia*) *lineatum* isolates (Kizilirmak 3,5,6) were determined as 98.5 – 100.0% and 99.2 – 99.5% according to the phylogenetic analyses of mt-COI gene region. The identity rates among the *S.* (*Wilhelmia*) *balcanicum* isolates and *S.* (*Wilhelmia*) *lineatum* isolates were determined as 98.1 – 99.7% and 97.6 – 98.7% according to the phylogenetic analyses of ribosomal ITS-2 and 28S gene regions. All of the Kizilirmak isolates were placed in a separate phylogenetic group with the other isolates available in GenBank according to the ribosomal ITS-2 gene region.

In conclusion, the molecular characterization of *S.* (*Wilhelmia*) *balcanicum* and *S.* (*Wilhelmia*) *lineatum* species collected from Central Kizilirmak Basin was firstly revealed in Turkey with this study.

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