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PROGRAMME

SUNDAY 3RD September

18.00–20.00 Registration and Reception at the Faculty of Agriculture,
Trg Dositeja Obradovića 8

MONDAY 4TH September

08.30–09.30 Registration at the Faculty of Agriculture,
Trg Dositeja Obradovića 8

09.30–10.00 Opening Ceremony
Welcome Address:
· City Major Maja Gojković
· University Chancellor Radmila Marinković-Nedučín
· Faculty Dean Milan Krajinović

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Antonio Paulino Andrade de Luna Dias and Marilza
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The 2nd International Simuliidae Symposium • Novi Sad, September 3rd – 6th, 2006

In association with the British Simuliid Group, the 6th European Simuliidae Symposium and EMCA – Black fly working group

16.30–17.30 Poster session

19.00 Social dinner

WEDNESDAY 6TH September

09.00 Conference Excursion

ABSTRACTS

THE HISTORY OF EUROPEAN SIMULIIDAE SYMPOSIUMS

Manfred Car

Institut für Wissenschaftliche Analyse Brunn am Gebirge, Austria

1. The “Deutschsprachiges Simuliidensymposium”

In 1980 Professor Walter Rühm, having some students working on Simuliidae, started to arrange a meeting of German Simuliidologists at the University of Hamburg. The only “foreigner” was me, Manfred Car, working on a thesis about the Veterinary Importance of Simuliidae in Austria. The symposium was held in German biannually at: Konstanz (1982), Tübingen (1984), Schlitz (1986), Hamburg (1988), Grietherbusch (1990), Essen (1992). After the fall of the “iron curtain” and the unification of Germany the “8th Deutschsprachiges Simuliiden-symposium” was held in Cottbus (former GDR) in 1994. This was the start to give a bigger group of scientists the possibility of participation. The next symposium was held for the first time in a neighbouring country, Austria. Kutzer and Car organised it at the Veterinary University Vienna.

2. The “Mitteleuropäisches / Central European Simuliidensymposium”

In 1996 for the first time scientists of Italy and Norway took part in the Symposium in Vienna. The reasons were losses in cattle in Austria and Italy caused by Simuliidae in spring 1996. It was still called the “9th Deutschsprachiges Simuliidensymposium” but also “1st Mittel-europäisches (Central European) Simuliiden-symposium”. This was the big step to the international community, for the first time talks were also held in English, the next localities were Aarhus/Denmark (1998), Vechta /Germany (2000), Bratislava/Slovakia (2002). This symposium was the last one called “12. Deutschsprachiges/4. Europäisches Simuliidensymposium”.

3. The International Simuliidae-Symposium

The next step was taken by Doreen Werner, 2004, arranging an “International Simuliidae Symposium – 5th European Simuliidae Symposium” in Berlin, lectures being held in English. – Times had changes, the world was becoming smaller – and Prof. Walter Rühm the founder of the symposium idea, unfortunately deceased in 2003. It was held in combination with the 26th annual meeting of British Simuliid Group.

Actually the BSG Meeting started about at the same time as the German meeting, but it was held annually and therefore the 26th one. The Berlin Symposium would have been the 13th according to Rühm's counting.

Like Cottbus had been, in 1994 the first locality outside the borders of Western Germany, to hold a Simuliidae Symposium, in 2006 Novi Sad is the first locality outside the European Union to offer Simuliidologists from all over the world the possibility for exchange of ideas.

Keywords: European Simuliidae Symposiums, history

THE BRITISH SIMULIID GROUP (BSG) – AN HISTORICAL SKETCH

Roger W. Crosskey¹ and John B. Davies²

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To be presented by Manfred Car

The key points in the development of knowledge of Simuliidae in Britain are briefly summarized as background to an account of the British Simuliid Group and its current activities. The formation, purposes, membership, meeting attendance and publications of the Group are briefly described and references provided to its Internet and Web associations. A chart is included showing the numbers of members on the mailing list since the Group started in 1979 up to the present time (2006). Notable points pertaining to the *British Simuliid Group Bulletin*, the Group's hard copy and electronic publication, are highlighted.

Keywords: the British Simuliid Group, history

SYSTEMATICS OF BLACK FLIES IN THE NORTHERN HEMISPHERE

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A tandem cytological-morphological approach to black fly systematics continues to provide insight into new species and phylogenetic relationships, partic-

ularly across the broad geographical expanse of the Holarctic Region. In addition to updating the status of Holarctic simuliid systematics, the evolution of triploidy in black flies will be featured. Examples of sympatric speciation in animals are rare. Among black flies, however, incontrovertible examples of sympatric speciation, revealed macrogenomically, are evidenced by interspecific hybridization with polyploidization that results in parthenogenetic propagation of the instantaneous products over evolutionary time. Only 0.4% of the world's approximately 2,000 simuliid species, all in the Far North, have been formed in this manner. A cytodendrogram presents the formation of six triploid species in the *Prosimulium macropyga* group from clearly identified antecedents. In some cases, the parent species are still extant and have a chromosomal banding pattern identical to one of the chromosome sets of the triploid species. Why this particular mode of speciation is prominent in certain lineages of black flies is not apparent, although a number of ecological correlates will be discussed that might provide insight into the process.

Key words: Holarctic Region, cytogenetics, polytene chromosomes, Simuliidae, speciation, systematics, triploidy

AN OVERVIEW OF THE BLACKFLY SUBGENUS *Inaequalium* (Diptera: Simuliidae) Coscaron & Wygodzinsky IN THE NEOTROPICAL REGION

LUIS MIGUEL HERNÁNDEZ¹, ANTHONY JOHN SHELLEY¹, ANTONIO PAULINO ANDRADE DE LUNA DIAS² & MARILZA MAIA-HERZOG²

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Species of the subgenus *Inaequalium* Coscarón & Wygodzinsky are widely distributed in the Neotropical Region, extending from Central America (Panama) to northeast Argentina. At present, this subgenus includes 20 species, with the vast majority of taxa commonly found in the coastal, mountainous areas of southern Brazil. Some of the species in this subgenus are potentially medically important because of their high anthropophily in northern Brazil and Guyana (e.g. *S. clarki*) and their presence in the endemic focus of pemphigus fo-

liaceus (fogo selvagem) in the state of Mato Grosso, Brazil (*S. inaequale*). In this paper we present an overview of the species of the subgenus *Inaequalium* based on the adults and pupal morphology. The species are placed in two species groups, the *botulibranchium* species group and the *inaequale* species group, based on the morphology of the male and female genitalia. In this subgenus, the most reliable character to identify the species is the configuration of the pupal gill filaments. However, variation in the gill pattern is commonly found in many species. Two species are treated as *species inquirendae* within the *inaequale* species group: *S. lurybayae* and *S. parimaense*, and the position of the recently described species *S. maranguapense* and *S. margaritatum* is discussed. The main taxonomic characters to identify the species in this subgenus are discussed with regards to the closely related subgenus *Psaroniocompsa* Enderlein.

Key words: Simuliidae, black fly, Neotropical region, taxonomy, *Inaequalium*.

CENTRAL-EUROPEAN SPECIES OF *Simulium variegatum* GROUP

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We have studied the taxonomy, morphology and some aspects of the life history of *Simulium variegatum* species group in central Europe. Species of the *S. variegatum* species group in central Europe (*S. variegatum*, *S. monticola*, *S. maximum* and *S. argyreatum*) belong due to their wide distribution and high quantity to the most important blackflies of the area. The last described - *S. maximum* (1961) is almost identical with the characteristics of *S. monticola*, it differs in some characters of larvae, pupae and adults. In the determination keys used in central Europe the pupae of *S. maximum* and *S. monticola* are separated only by a single relevant character - length (presence) of the 3rd common trunk. The differences in the most of the characters that were published are rather small. According to some authors the existence of *S. maximum* was doubtful.

In the material from Western Carpathians a morphological form with different pattern of thoracic tubercles of the pupae was identified (here treated as separate species, provisionally we call it *S. sp. aff. monticola*). Detailed morpho-

metric study of various characters, mainly of pupae showed, that all species differ from each other significantly in many characters and similarly individuals of the new morphological form differ from all other species also.

The found differences in morphometric characters and in the ecology between *S. monticola* and *S. maximum* confirmed the existence of *S. maximum*. Presently, only the identification of pupae is accurate, the identification of other stages is connected with some uncertainty.

The filaments of *S. monticola*, *S. maximum* and *S. sp. aff. monticola* are heavily swollen on the base, and they are directed downwards and then curve forwards. *S. sp. aff. monticola* has a different pattern of tubercles on the thorax and head as *S. monticola* and *S. maximum*. *S. monticola* and *S. maximum* differ in the width and branching of the 5th and 6th respiratory filament and in the length of their common stalks.

The ISSR profiles of the DNA of the species were detected. The profiles were highly variable between individuals of one species, but differences between species were higher, which was demonstrated using cluster analysis (based on Jaccard's similarity index and UPGMA).

S. variegatum, *S. argyreatum* and *S. monticola* are widely distributed in the majority of Europe. The occurrence of *S. maximum* is restricted to the higher mountains of central and southern Europe. *S. sp. aff. monticola* is known only from Slovakia till now.

In Slovakia, *S. variegatum* prefers greater submountain streams in lower altitudes of up to 600 m a.s.l. *S. argyreatum* is widely distributed in Slovakia, it has been found in lowlands as well as in high mountains, mainly in lower montane areas. It prefers altitudes between 400-800 m a.s.l. In the territory of Slovakia *S. maximum* has been found in higher mountains only, but here it is common. It prefers altitudes of 900-1000 m a.s.l. Both, *S. monticola* and *S. sp. aff. monticola* have been found in relatively few mountain areas, they prefer altitudes higher than *S. argyreatum* but lower than *S. maximum*.

S. variegatum, *S. argyreatum*, *S. monticola* and *S. sp. aff. monticola* are typical multivoltine species with 2-3 generations annually. The life cycle of *S. maximum* has never been studied in detail, but the period of pupation indicates a univoltine cycle; the pupae are found mainly in May (63% of all records) and in June, in higher altitudes up to the beginning of August.

Key words: *Simulium variegatum*, *Simulium monticola*, *Simulium maximum*, Central Europe, taxonomy

BLACK FLIES OF THE DANUBE: MISSING INFORMATION AND A BRIGHT FUTURE?

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With a length of 2850 km, the Danube is the second-longest river in Europe. It rises in the Black Forest of Germany close to the European watershed at an altitude of 1078 m a. s. l. (source of the River Breg), and crosses Europe generally in an eastward direction. Its watershed extends from the south-western part of the German Mittelgebirge in the west as far the Wallachian Plain and Moldovan Plateau in the east, and also covers the northern and eastern part of Alps, most of the Carpathians, the Balkans, the Pannonian Plain, the Vienna Basin, etc. This region is of zoogeographic interest, having contacts with the Quaternary refugia and also crossing the postglacial colonization routes.

The black fly fauna of the Danube is inadequately known in general. Very little data are available from both the German and the Hungarian sections and from the lower reaches in Bulgaria and Romania. 15 species have been recorded from the Austrian section, in both the main river channel and its backwaters. The most comprehensive data are from the Serbian Pannonian section of the Danube (10 species in Vojvodina) and from both the Slovak-Hungarian and the Serbian-Romanian sections. Paradoxically, our knowledge of the black fly fauna of two latter areas is a consequence of the damming of the river and the construction of two major hydropower plants (Iron Gate I, II and Gabčíkovo) which have substantially changed the nature of the river and of its fauna and have probably caused the regional extinction of some black fly species. In these two areas, 7 and 18 black fly species have been recorded respectively. The available data on black flies and their longitudinal distribution in the Danube do not enable us to determine the longitudinal and altitudinal zonation of black fly communities or to analyse zoogeographic aspects and the formation of the fauna in terms of the Quaternary climatic processes.

Some taxonomic questions concerning the black flies of the Danube remain unresolved. The most problematic are *Simulium colombaschense* Scopoli, 1780, *Simulium voilense* Serban, 1960, and the whole *reptans* species group; *Wilhelmia* species; *Metacnephia* species (*M. blanci* Grenier & Theodorides, 1953 and *M. danubica* Rubzov, 1956); *Simulium maculatum* (Meigen, 1804) and its form *danubense* Rubtsov, 1956; *Simulium matthiesseni* Enderlein, 1921 and *S. ni-*

grum (Meigen, 1804); and *Simulium baracorne* Smart, 1944. The clarification of these taxonomic questions is essential for resolving problems in faunistics, zoogeography and ecology.

Key words: black flies, Danube River, fauna, taxonomic problems

SIMULIIDAE OF CARPATHIAN MOUNTAINS

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The most complete data on blackflies from Carpathian countries are available from Slovakia. Including adjacent parts of Pannonian basin, recently, 46 blackfly species at morphospecies level are known from Slovakia. According to its distribution areas the species belong to six types: Holarctic, Palaearctic, Eurosiberian, Westpalaearctic, Submediterranean and European sensu lato. The blackfly fauna of Carpathians consists mainly of last group of species, creating about 60 per cent of whole blackfly fauna.

The Ukrainian Carpathians Mountains were covered by the oldest treatment of blackflies from Carpathian countries - in the Fauna of USSR. The Carpathian Mountains with their frontier location within the Soviet Union were marginal for territory of former Soviet Union and data on their blackfly fauna are very scarce.

Fauna of Romania contains numerous data on blackfly distribution, but the taxonomical approach used could not be fully respected.

The distributional data of species published in Fauna of Poland are generalized to higher geographical units; the real sites could be tracked down using the references.

Data on distribution of blackflies from Serbian and Hungarian sections of the Carpathian mountains are missing at all.

Key words: blackflies, Slovakia, Ukraine, Poland, Romania, check-list

TO CONFIRMATION OF THE GENERA NAMES *Boreosimulium* Rubzov et Yankovsky, 1982 and *Taeniopterna* Enderlein, 1925 AND ITS SPECIES CONTENT

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The genus name *Boreosimulium* Rubzov et Yankovsky, 1982 for a long time after its description was considered as a junior synonym of the genus (subgenus) name *Hellichiella* Rivosecchi et Cardinali, 1975. The genus *Boreosimulium* was described by morphological characteristics. Recently the separate position of this group had been confirmed by morphological and cytotaxonomical investigations of several species from Nearctic (including new species) (Adler *et al.*, 2004). Crosskey & Howard (1997) divided the genus *Hellichiella* into 2 groups: *annulus* and *subexcisum*. The species content of these groups resembles the one in the variants proposed later for separate genera (subgenera), but with some changes. Basing on the modern data, our opinion is that the species content of the genus *Boreosimulium* is practically similar to the list of Adler, Currie & Wood (2004) including *B. annuliforme*, *B. annulus* (= *euryadminiculum*), *B. arctium*, *B. baffinense*, *B. balteatum*, *B. canonicolum*, *B. clarkei*, *B. emarginatum*, *B. jocularis*, *B. johannseni* (= *duplex*), *B. parmatum*, *B. quadratum*, *B. rothfelsi* and *B. zephyrus*. Only, from our point of view, in this genus the species *acutum* (Patrusheva, 1971) and *crassum* (Rubzov, 1956) should be included.

The genus name *Taeniopterna* had been proposed by Enderlein (1925) with the type-species *Melusina macropyga* Lundström, 1911. Then the small group of species related to *macropyga* had been separated as a clearly distinguishable species-group *macropyga* in the genus *Prosimulium* Roubaud, 1906 (Rubzov, 1956; Crosskey, Howard, 1997; Adler *et al.*, 2004). Species of this group bear the complex of characters, which are not inherent in other groups of *Prosimulium*: lacinia without teeth, mandible without serrations, very small spermatheca (1/3 of the length of the stem of genital fork) in female, other shape of abdomen in larvae, and other characters. Very important is the triploidy of chromosomes, it connects this group with some

Gymnopais (Wood, 1978; Adler *et al.*, 2004). At least, in our opinion, these differences of characters allow to consider this group as a separate genus in the tribe Prosimuliinae on a level with *Prosimulium*, *Helodon*, *Parahelodon*, *Dis-*

tosimum. From this point of view, the genus *Taenio-pterna* Enderlein, 1925 consists of the following species: *T. arctica* (Rubzov et Carlsson, 1965), *T. erythronotum* (Rubzov, 1956), *T. kolymensis* (Patrusheva, 1975), *T. korshunovi* (Patrusheva, 1975), *T. macropyga* (Lundström, 1911), *T. neomacropyga* (Peterson, 1970), *T. tredecimfistulata* (Rubzov, 1956), *T. ursina* (Edwards, 1935) and *T. zaitzevi* (Rubzov, 1956).

Keywords: Simuliidae, black flies, taxonomy, Boreosimum, Taeniopterna

ECOMORPHOLOGICAL OBSERVATIONS BY COMPARISON OF IMAGES FROM DIGITAL REFERENCE COLLECTIONS OF SIMULIIDAE

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¹Institut für Wissenschaftliche Analyse Brunn am Gebirge, Austria

²Technisches Büro für Biologie, Wien, Austria

The shape of Simuliidae larvae, like that of most aquatic insects, is determined by their habitat. The comparison of images from a virtual reference collection, containing pictures of a great number of species, allows to group species resembling in morphological criteria, which suggest the possibility of adaptations to similar environmental conditions.

The same observations were made in Culicidae and Trichoptera. The images were taken from the electronic keys to Simuliidae (LECHTHALER & CAR, 2005), Culicidae (LECHTHALER, 2005), and Trichoptera (LECHTHALER & STOCKINGER, 2005).

A comparison of **postgenal clefts** of the larval head capsule of Simuliidae living in springs and in the upper reaches of rivers, shows its narrow and flat shape, (e.g. in *angustitarse*, *crenobium*, *lundstromi*, *lamachi*). In contrast, species like *colombaschense*, *erythrocephalum*, *ibariense*, *morsitans*, *noelleri*, *pseud-equinum*, occurring in the river potamal, show a deeper postgenal cleft.

Similar observations were made comparing **hypostoma** and the **microtrichia of filter rays** of these groups of species living in different habitats. The lack of bacteria in clean fast flowing waters and its velocity could lead to the observed wide distance between microtrichia, while high numbers of bacteria in shallow waters make necessary a narrow net of microtrichia to enable feed-

ing on these small particles. It seems logical that these species need short and small hypostomal teeth, species feeding on large particles in fast flowing water show long and slender teeth (upper row of pictures).

In Culicidae the length of the antennae and of the siphon indicate their feeding habits and the type of habitat. Spring species living in temporary ponds often are scrapers showing short antennae and a short siphon.

According to these observations the ecology of new species can be assessed by the shape of body structures as observed in Trichoptera for the recently discovered species *Drusus muelleri* (WARINGER, 2005), which shows the same spinous legs as *Cryptothrix nebulicola*, *Drusus chrysotus* and *Drusus discolor*, which are carnivorous, filtering rough particles (GRAF et al, 2005).

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Keywords: Ecomorphology, larvae, Simuliidae, Culicidae, Trichoptera

THE BRITISH BLACKFLIES DNA BARCODING PROJECT

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In 2005 CEH-Oxford embarked upon a project to DNA barcode all British blackfly species using the mitochondrial COI gene. This approach has been pioneered elsewhere in birds and butterflies, and is being applied to mosquitoes. The British project is expected to provide molecular characters to assist species definition and identification of the British blackfly fauna, and to test the gen-

eral reliability of the method in a morphologically uniform and difficult group. Early results indicate good separation of morphospecies within the subgenera *Eusimulium* and *Wilhelmia*, in contrast to striking uniformity within *Simulium equinum*, for example.

Key Words: Simuliidae, United Kingdom, taxonomy, DNA, barcoding, COI, *Eusimulium*, *Wilhelmia*.

THREATENED SPECIES OR JUST NUISANCE? ASSESSING THE CONSERVATION STATUS OF BLACK FLIES (Diptera: Simuliidae) IN FINLAND

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A national project, funded by the Finnish Ministry of the Environment, aims at improving the knowledge of poorly known taxonomic groups, both flora and fauna, in Finland. One of the main goals is to considerably increase the number of assessed species in the next Red Data Book of Finland, scheduled to be published 2010. One of the poorly known insect families in Finland, along all other dipteran families, is black flies (Diptera: Simuliidae). Due to poor knowledge of trends in the species' occurrence or abundance, the assessment has to be based on the distribution of the species and some general information about their ecology in most insect taxa, following the Criteria of the IUCN. The species list of the Finnish black flies has been updated recently, and new records for Finland are still being made. Also a list of the provincial distribution of all black fly species recorded in Finland has been compiled as a basic information for the assessment of conservation status. Based on the distribution and ecology of the 57 species recorded in Finland, 25 species (44%) can be preliminarily evaluated as non-threatened (Least Concern), four species (7%) as potentially threatened, while 28 species (49%) are so far considered to be too poorly known (Data Deficient) for the conservation status assessment. The non-threatened species are usually wide-spread species of large streams and rivers, while the potentially threatened species are northern species occurring mostly in small streams. The northern part of Finland has been most thoroughly studied, while the diversity and distribution of black fly species in southern Finland is still very poorly known. Hence the present knowledge allows the as-

assessment of the northern species on a fairly good basis, while new provincial records of the southern species are constantly being made while identifying new material. Besides the poor regional coverage of sampling locations in southern Finland, taxonomical problems prevent the assessment of the conservation status of many species. Most of the insufficiently known species are likely to become evaluated as non-threatened when more information can be gathered. The assessment of the conservation status of Finnish black flies is only at the beginning, and knowledge of the species' distribution, ecology and taxonomy is expected to be improved before 2010.

Keywords: Simuliidae, black flies, Finland checklist, conservation status

ZOOPHILY AND ANIMAL FILARIAE IN *Simulium damnosum* s.l.

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Filarial parasites of the genus *Onchocerca* are found in a broad spectrum of ungulate hosts. Only *O. volvulus* is a specifically human parasite causing a disease known as 'river blindness'. The phylogenetic relationships and the bionomics of many of the nearly 30 known species remain dubious. Here, findings of molecular phylogenetic analyses of 11 species representing most major lineages of the genus are presented. Special emphasis is given to an Afrotropical clade containing a yet unassigned specimen from Uganda (*O. sp.* 'Siisa'), which appears to be intermediate between *O. volvulus* and its closest relative, the cattle parasite *O. ochengi*. These three taxa are transmitted by members of the *S. damnosum* complex (i.e. subgenus *Edwardsellum*), with *O. dukei* as a fourth of which species of the subgenus *Metomphalus* are known to be vectors. The two closely related *Simulium* subgenera are also confined to the Afrotropical region. Thus, on the vector-parasite level, there may have occurred a co-speciation. Furthermore, the overall diversity of *Onchocerca* species found in Africa supports an African origin of the genus. The human parasite *O. volvulus*, however, clearly evolved from an ancestral bovine parasite in Africa, but apparently not from *O. ochengi*. Rather, they both shared the same common ancestor together with *O. sp.* 'Siisa'.

The vector of *O. sp.* ‘Siisa’ is the well characterized, zoophilic *S. damnosum* cytoform ‘Nkusi’. The vector of *O. ochengi* in East Africa has not yet been determined, but in Cameroon it is co-transmitted with *O. volvulus* by mammalophilic *S. squamosum* and *S. damnosum* s.str. Neither of these three vector species exhibit any apparent morphological adaptation that may indicate their host preferences. In contrast, females of some populations of West African *S. soubrense/sanctipauli* were found to have enlarged basal teeth of their tarsal claws, indicating ornithophilic behaviour. Indeed, old reports emphasized the abundant presence of unknown filarial larvae in *S. soubrense* from Liberia, and some of these larvae resembled wild bird filariae. A morphological comparison of the tarsal claws of virtually all members of the *S. sanctipauli* subcomplex from across West Africa confirmed that the ‘claw index’ of Liberian *S. soubrense* is particularly pronounced, whereas *S. sanctipauli* s.str. had claws typically seen in the *S. damnosum* complex.

The existence of *O. sp.* ‘Siisa’ (and other unknown taxa) also has epidemiological impact. All previous, onchocerciasis-related differentiation studies may no longer be reliable, if *O. sp.* ‘Siisa’ was widespread but overlooked or undetected in the past. Consequently, the direct PCR amplification and DNA sequencing of mitochondrial genes should be regarded as more reliable typing methods, as opposed to the established marker O-150.

Key words: Onchocerciasis, *Onchocerca spec.*, *Simulium damnosum* complex, phylogeny, *S. soubrense*, tarsal claw, zoophily

ENTOMOLOGIC AND SEROLOGIC ASSESSMENT OF *Onchocerca volvulus* TRANSMISSION IN THE NORTHERN CHIAPAS FOCUS, MEXICO

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One of the Onchocerciasis Elimination Programs’ goals is to reduce transmission below the level that is necessary to maintain the parasite population,

i.e. to reduce transmission below that required to maintain the reproductive rate at 1.0 or above. Unfortunately, the level of transmission necessary to reduce the minimum reproductive rate below 1.0 has not been accurately determined. In the absence of this information, the WHO has developed a series of criteria to certify that an area is free of onchocerciasis. Those criteria focused on entomology are based on demonstrating the 'absence or near absence' of L3 infection rates ('transmission suppression') in *Simulium* black flies for a period of 12 years. In the Northern Chiapas focus where no pre-control entomologic data exist, transmission suppression is defined as an 'absence or near absence' of L3 infection in the vector population. In practical terms, this criterion has been interpreted to mean a prevalence of infectious flies of less than 1/10,000. The Northern focus resulted from the annual seasonal in-migration coffee workers from Southern Chiapas. It has been further hypothesized that onchocerciasis infections in Northern focus are solely a result of this seasonal in-migration, and that no independent transmission of the parasite occurs in this focus. However, there have been no reports of entomological or serological studies to test if independent transmission of *Onchocerca volvulus* is occurring in the Northern focus. Collected *S. ochraceum* s.l. flies (sampling from 07:00 to 16:50 hrs during the dry season of 2001) were screened by PCR-ELISA for *O. volvulus* parasites, and collected serum samples (during 2001 and 2005) were screened by ELISA using a cocktail of three recombinant antigens (Ov16, Ov10, and Ov11) in two communities (El Ámbar and Altagracia) of the Northern focus. The prevalence of infected and infectious flies was estimated using the Katholi's PoolScreen algorithm. The total number of body and head pools screened in Altagracia was 394 (197 each). Five and one PCR positive body and head pools, respectively from Altagracia were found, providing evidence for autochthonous transmission in this community. The prevalence of infected and infectious flies was 5.1/10,000 (95% C.I. surrounding the point estimate = 1.7, and 11.9/10,000), and 1.0/10,000 (95% C.I. = 0.03, and 5.7/10,000), respectively, and the seasonal transmission potential was 0,7 L3s larvae/person. In contrast, no evidence for transmission was found in El Ámbar --where 202 body and head pools were screened (101 each)--, the second community surveyed in the Northern focus. In El Ámbar, the prevalence of infectious flies was zero, with an upper bound of 6.0/10,000 flies. There are at least two possible explanations for this. First, the number of pools examined from El Ámbar was small, and may have been insufficient to detect a low level of transmission. Second, the seasonal biting rate of *S. ochraceum* s.l. in El Ámbar was low relative to that in Altagracia (1,508 bites/person/season versus 4,367 bites/person/season). It is possible that this level of vector-host contact is not sufficient

to maintain transmission in El Ámbar in the face of continuing Mectizan pressure. Of a group of 134 individuals participating in the serologic study, a sentinel cohort of 83, and 51 serologically negative individuals had been followed in El Ámbar and Altagracia since 2001, respectively. After four years (2005), the percentage of individuals becoming positive in this cohort was 27% (23/83), and 43% (22/51), respectively. Likewise, the incidence in children five years and under ($n = 20$) within this sentinel cohort was 40% (4/10), and 20% (2/10), respectively. The antibody serological test do not provide precise estimations of the true exposure infection rates because it is possible that some individuals after contact with the parasite may develop specific antibodies but never get infected. Nevertheless, this limitation does not preclude that a sentinel cohort of negative individuals becoming positive -something that occurred in the Northern focus-, may provide further evidence of circulation of the parasite. This study indicates that, according to WHO criteria and the serologic study, autochthonous transmission exists in this focus. However, the seasonal transmission potential in this focus was extremely low (0,7 L3/person). While the annual transmission potential necessary to maintain reproduction at a level sufficient to sustain the population (the minimum reproductive rate, or R_0) is not known, previous modeling studies using data derived from West Africa have suggested that the ATP necessary to maintain a reproductive rate of 1.0 is approximately 29. If this is correct, this suggests that control efforts in the Northern focus have been more than sufficient to bring transmission below the level necessary to maintain the minimum reproductive rate.

Key words: Helminthiasis-Tissue, Molecular epidemiology, Onchocerciasis, Simulium

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***Simulium-Onchocerca* COMPLEXES: CO-EVOLUTION, SPECIATION AND LONGITUDINAL FOLLOW-UP OF TRANSMISSION DYNAMICS IN NORTH-CAMEROON**

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The origin of the genus *Onchocerca* is supposed to be located in the African savanna, where the species co-evolved with their adult hosts, which are mainly bovidae. Today, in Cameroon, we observe at least 8 *Onchocerca*-species, transmitted by various *Simulium* and *Culicoides* vectors. These filarial parasites are specifically adapted to co-operate both with the immune system of their mammalian hosts and with the internal defence system of their arthropod vectors. However, one same *Simulium* fly may transmit different species of *Onchocerca* infective larvae from various hosts, and one cattle may harbour more than 5 different filarial species. But never, the same vector species transmits two *Onchocerca*-species from the same mammalian host. The stringency of these findings is explained by evolutionary mechanisms of speciation and can be seen as a model for all vector-borne parasitoses.

The dynamics of transmission are highly seasonal in the Sudan savanna environment, where most breeding sites seasonally dry up. As an adaptation, *Onchocerca* parasites show an annual periodicity in their life history and prevail in the skin of their hosts, when and where the biting density of their respective vectors is highest. Furthermore, we observe a yet unexplained seasonal variation in the susceptibility of *Onchocerca-Simulium* complexes, with highest parasite development rates during few months of the year. This is most obvious in the transmission of bovine *Onchocerca ochengi* by *Simulium squamosum*. The physiological basis for this variation in susceptibility is as yet unknown.

Our collection of data on *Simulium* biting rates and *Onchocerca*-transmission started in 1976 and thus provides a good picture on the seasonal and annual variations at different locations in various geographic zones in Cameroon.

The decline in *Simulium* biting densities, which we presently observe, may be due to the ongoing desertification and is therefore likely to continue. The reduction in *Onchocerca volvulus* transmission on the other hand is clearly associated to the mass-distribution of ivermectin. In conjunction with environmental changes and the pronounced dynamics of the vector- and host-populations, these new parameters influence the co-transmission of filarial parasites and thus lead to new equilibria of endemicity: since human and game populations segregate in urban settlements and game reserves respectively they form separate epidemiological unities, divided by an intermediate zone, where livestock farming may provide a zooprophyllactic shelter for the human population.

Keywords: Simulium, Onchocerca, co-evolution, transmission, Cameroon

AN ANNOTATED CHECKLIST OF THE BLACK FLIES (Diptera: Simuliidae) OF TURKEY

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Abstract: A checklist of Simuliidae species of Turkey with annotations is provided as simuliids are important vectors and pests and also are crucial in the animal community dynamics of freshwater ecosystems. Similarities between the simuliid fauna of Turkey and neighbouring countries are discussed. Thus far, Simuliidae research in Turkey has revealed only one subfamily (Simuliinae), with 3 genera, 7 subgenera, and a total of 36 species in various aquatic habitats. Further investigations are needed to compile an accurate list of Simuliidae species in Turkey.

Key Words: Diptera, Simuliidae, blackflies, Turkey.

DEFINING THE THRESHOLD OF PUBLIC TOLERANCE TO BLACK FLIES IN THE REGION OF NOVI SAD

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The interest and necessity for simuliid investigations in the region of Novi Sad derives from numerous objections of inhabitants of settlements near the river Danube and streams on Fruška Gora hills.

The aim of this part of project was to define the threshold of public tolerance to annoyance by black flies. The critical number of flies that is established in this study will be used to assess the right time for control measures.

Public perception was examined through a questionnaire distributed to inhabitants of the settlements close to the river. Ten persons from five localities were questioned in the period from April till August in 2005. Personal impressions or evaluations of black fly presence were divided into four categories and marked as: k1- »no flies«, k2- »few«, k3- »bearable«, k4- »unbearable«. During these five months, total of 129 questionnaires were completed.

At the same time, a dry ice baited trap was placed close to the settlements of chosen people and operated during the night prior to the interview.

As an indicator of relation between the numbers of black flies collected in the trap and perception of annoyance by the public, Spearman's coefficient of rank correlation was used. This analysis determined that the critical number and the threshold of public tolerance for black fly presence were 16 simuliids in the trap during the night.

The capacity of participants who was involved in the evaluation of the threshold level of annoyance caused by the black fly presence was analyzed with Kruskal- Wallis nonparametric test. Analysis showed that the participants could clearly distinguish the situation when there were „no flies“ in the traps with „bearable“ and „unbearable“ as well as between „a few“ versus „bearable“ and „unbearable“. Estimation of differences between „bearable“ and „unbearable“ levels of annoyance as well as between „none“ and „a few“ were not proven to be significant.

For further analysis, the participants were also divided into three categories: „fisherman“ who spent time near the river, „gardeners“ who spent time work-

ing in the gardens or backyards and third group represented by those who spent most of the time indoors.

The results showed that in the case of group „fishermen“ the relation between individual perception and number of simuliids in the trap was the strongest. Spearman’s coefficient (0,735**) was highly significant ($p=0,000$).

Key words: Simuliidae, black flies, nuisance threshold level

MULTIVARIATE ANALYSIS OF SIMULIIDAE COMMUNITIES IN VARIOUS HYDROGRAPHIC NETWORKS IN TURKEY IN RELATION TO ENVIRONMENTAL DATA AND CLIMATE CHANGE IMPACT ON SPECIES COMPOSITION

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The Simuliidae communities broadly reflect environmental conditions and are used as indicators of environmental degradation or restoration.

Simuliidae are widespread and their larvae are important component of benthic fauna in running waters in Turkey. The first evaluation of the detailed faunal structure of Turkish Simuliidae with many new records and their some ecological properties published by Kazancı and Clergue in 1990 and 1992. The zoogeographical analysis indicate that Turkish fauna are essentially dealing with an European palearctic fauna. The oriental faunal element is subgenus *Wilhelmia*. Increases in water temperatures and flow regime as a result of climate change will affect the ecological processes, the geographic distribution of aquatic species, extinction of species and loss of biodiversity. Climate changes will alter hydrologic characteristics and water quality of running waters and will affect species composition and ecosystem functions. There is increasingly clear evidence that climate change impacts on aquatic ecosystems of Turkey. The strong warming trends in averaged annual mean temperature (around 2–3 °C) was determined for whole Turkey. The seasonal shifts would have significant

impacts on benthic communities with Simuliidae species in runningwater ecosystems.

In this study, previously recorded 22 Simuliidae species from 21 sites of the various hydrographic net works e.g. Büyük Menderes, Sakarya, Kızılırmak, Göksu, Fırat, Dicle Rivers were used. Simuliidae composition is related to various physical environmental variables, e.g. riparian vegetation, substratum structure. While several species inhabit various types of habitats, some species are restricted to distinct habitats.

Relationships between species assemblages and environmental variables (altitude, transparency, mean current velocity, water depth, maximum water temperature in summer, runningwater zones, substratum structure, riparian vegetation) were explored by canonical correspondence analysis.

The aims of this study were to determine composition of Simuliidae species and the relationships between their distribution and physical characteristics of collecting sites and use this information for prediction of climate change impact on species.

Key words: CCA, climate change, multivariate technics, Simuliidae, species, Turkey

DESCRIPTION OF THE BLACK FLY LARVAE SAMPLING TECHNIQUE IN THE RIVER SEMOIS (BELGIUM)

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The Walloon part of Belgium has a dense network of rivers that are susceptible to black fly populations. These insects usually do not cause problems except in specific climates that lead to the formation of swarms. In this case, cattle may sometimes be fatally intoxicated by thousands of black fly bites. The use of *Bacillus thuringiensis* (var. *israelensis*) has been considered as a solution for decreasing the number of wintering black fly larvae populations. This was expected to result in reducing the formation of swarms and therefore the risks of cattle death. A sampling technique was developed in order to evaluate the efficiency of *Bacillus thuringiensis* (var. *israelensis*) for the treatments of the black fly larvae populations.

The sampling technique was adapted to the Semois river, which is characterised by a dense population of river water crowfoots (*ranunculus fluitans*). These plants are preferentially colonised by black fly larvae. So, the sampling technique consists of collecting three handfuls of river water crowfoots in such a way that their average size is close to 1 metre long. Each of them is separately and vigorously shaken in a bucket of water in order to separate larvae from the plants. Then, the bucket of water is poured through a sieve and larvae are collected and stored in alcohol. Each handful of river water crowfoots is separately stored for further analysis.

When back at the laboratory, the river water crowfoots are characterised by measuring their weight and counting the number of stems for each handful. Only stems longer than 0.5 meter are considered. The sample of larvae is also characterised. It is first weighed, then a sub sample is collected, weighed and the number of larvae of the sub sample is counted under a binocular microscope. The number of live pupae fixed to river water crowfoots can also be counted per 100 stems.

So, the result of the larvae sampling can be expressed in four different ways: the number of larvae per 100 stems, the weight of larvae per 100 stems, the number of larvae per 100g of stems and the weight of larvae per 100g of stems. The comparison between the number and the weight of larvae allows us to approximately identify the average stage of development of their population.

Key words: black fly, sampling, Semois, larvae, cattle.

Diptera Simuliidae IN TWO NORTHERN ITALY REGIONS

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If compared with other hematophagous Diptera like mosquitoes, sandflies and biting midges, in Italy blackflies rarely raise to the role of pests or vectors, although in the past some cases of massive attacks to livestock have been recorded from Eastern Alps (*Simulium reptans* group), and from coastal and internal plain areas of Central Italy (*S. pseudequinum*) (Rivosecchi, 1978).

We consider useful to give an up-to-date picture on the distribution of this group, exploiting the on going mosquito control projects carried out in Northern Italy, which regularly use CO₂ traps as a standard monitoring tool.

Collected information is therefore to be considered influenced by the monitoring method used as well as by the trap locations.

The fixed monitoring stations we were able to include in our study were as follows:

Piedmont: 2000 (136 stations), '01 (171 stations), '02 (217 stations), '03 (257 stations), '04 (236 stations).

Lombardy: 2002 (20 stations), '03 (25 stations), '04 (30 stations), '05 (25 stations).

Traps were activated during May-September.

Altogether 19,311 specimens were collected of which 11,312 (2.7 individuals/trap/night) in Piedmont and 7,999 (3.9 individuals/trap/night) in Lombardy.

Only three species were collected, belonging to the genus *Simulium*: *S. pseudoequinum* (Séguy, 1921) (50.4%), *S. angustipes* (Edwards, 1915) (48.7%), *S. lineatum* (Meigen, 1804) (0.2%), *Simulium* spp. (0.7%).

While *S. angustipes* is known as an ornitophilic species, *S. pseudoequinum* and *S. lineatum* feed on mammals.

Considering the relatively high population sampled in some of the stations, we were also able to obtain a picture of the seasonal dynamics.

All of these species have been previously recorded from Northern Italy (Boorman et al., 1995. *Diptera Culicomorpha*. In: Minelli A., Ruffo S. & La Posta S, *Checklist delle specie della fauna italiana*, 65. Calderini, Bologna).

Keywords: *Simulium angustipes*, *S. pseudoequinum*, *S. lineatum*, CO₂ trap, monitoring.

OVERVIEW ABOUT POSSIBLE SIMULIIDAE RELATED PROBLEMS IN ALESSANDRIA DISTRICT (PIEMONTE, ITALY)

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In many regions black flies cause problem due to their attacks, both on human and livestock. In tropical Africa, *Simulium damnosum* is the preferential vector of *Onchocerca volvulus*, a human filarial parasite responsible of river blindness disease.

In our country there aren't know human diseases transmitted by Simuliidae, anyway black flies bites are irritant and itching, so that the massive presence of adults can made every human activity near rivers impossible. Black flies are vectors of cattle onchocerciasis and filariosis in other animals (Gnedina 1940, 1949), poultry leucocytozoonosis, tularaemia, myxomatosis, various virus diseases, etc. (Mackerras & Mackerras 1952; Anderson *et al.* 1961; Austin 1967; Zeman 1998; Mead *et al.* 2000). High quantity of bites can also result deadly for animals.

In Italy, some cases of black flies (*reptans* group) massive attack to both cattle and humans occurred in pre-alpine and alpine valleys of Lombardia, Trentino and Veneto (Zanin & Rivosecchi 1975; Rivosecchi & Coluzzi 1962; Rivosecchi & Zanin 1983), resulting in several animal deaths. In Marche (central Italy) *Simulium intermedium* attacked human (Rivosecchi 1997) and recently Contini *et al.* (2001) recorded the first case of massive attack to humans by *S. intermedium* in Sardegna.

In a recent work (Talbalaghi *et al.* 2004), the presence of *Simulium* (*Eusimulium*) *velutinum* (Santos Abreu, 1922), *S. (E) silvaticum* (Rubtsov, 1962), *S. (Wilhelmia) pseudequinum ssp. fluminicola* (Rivosecchi, 1972), *Prosimulium* (*Prosimulium*) *hirtipes* (Fries, 1824) (complex) and *P. (P.) tomosvaryi* (Ender-

lein, 1921) has been recorded in some permanent flow streams of the province of Alessandria (eastern part of the Piemonte region, NW Italy).

With the present study we want to continue the assessment of black flies specimens of the Alessandria province, providing an initial evaluation about the possibilities of an occurrence of black flies attacks, investigating a wide selection of streams of different orders (Scrivia, Borbera, Lemme, Bormida and Belbo catchments) and assessing the presence of larvae, pupae and adults.

Key words: *black flies, Simuliidae, checklist, Piemonte, Alessandria, parasitology.*

METHYLENE BLUE AS A MARKER OF *Simulium ornatum* Meigen 1818 (complex) LARVAE

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Methylene blue has been used in our laboratory in a mixture with larval feed to colour the adults of *Culex pipiens* Complex mosquitoes. The trials with simuliids were conducted in order to follow the downstream movement of *S. ornatum* larvae and to evaluate carry of B.t.i. product in the Danube river. A section of the Danube river in Vojvodina is characterized with huge stream flow (average 2,800 m³/s, maximum 9,290 m³/s) with inconstant flow velocity ought to meandering and configuration of the river banks. Consequently the carry of the B.t.i. could be assessed more accurately if methylene blue is administered during application.

Third to sixth instar larvae of *S. ornatum* were coloured in laboratory and degrees of the intestine and haemolymph coloration were recorded as well as larval mortality. During the trials two dose of vital dye (25 mg/l H₂O and 50 mg/l H₂O) have been used. Coloration degrees of living larvae, larvae treated with hot water and ethanol were assessed both visually and under the dissecting microscope.

Key words: Simuliidae, larval marking, methylene blue

Acknowledgements: This project was supported by Ministry of science and environment protection, Republic of Serbia, Reference grant No. TR-6920B.

SPATIAL ECOLOGY OF LARVAL BLACKFLIES

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Patterns of species distribution can provide insight into the mechanisms that structure species communities. Accordingly, this paper focuses on establishing empirical relationships between the distribution and biodiversity of larval black flies in relationship to changing environmental parameters. The specific topic to be addressed are : i) how does species richness change across different regions of the Americas; ii) what is the influence of local versus regional effects on species richness; iii) what factors best predict distribution within a limited geographic area and; what determine the nature of predictibility. iv) which land classification (e.g., ecoregion, floristic province, soil type) best explains species distributions across North America. Although the completion date for the database is estimated to be 2007, the first manuscript from this project has already been submitted for publication.

Keywords: Simuliidae, black flies larvae, spatial ecology, distribution, biodiversity

THE OUTBREAK OF *Simulium erythrocephalum* (De Geer, 1776) IN THE REGION OF NOVI SAD (SERBIA) IN 2006

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During the past decades *Simulium erythrocephalum* (De Geer, 1776) proved to be an extremely aggressive anthropophilic black fly species in the region of

Vojvodina. The last outbreaks occurred in the province of Vojvodina after severe flooding of the Danube and the Tisa river in the springs of 1965 and 1970, respectively.

Extremely elevated water level of the Danube in early spring 2006 that exceeded the highest value for the last century, causing severe flooding, as well as cold weather at the time created suitable conditions for massive outbreaks of *S. erythrocephalum*. Being situated on the both Danube river banks, city of Novi Sad with numerous villages in the vicinity represents particularly endangered region.

The abundance of adult populations of black flies was recorded in weekly intervals by dry ice baited traps. Positive captures were recorded in 26 out of 28 localities during the season of calamity. Estimation of the biting risk was calculated for each trapping result.

The presence of adult *S. erythrocephalum* females was continually recorded in traps during the season from April to mid July, while periods of frequent cases of elevated biting risks extended from April to the end of June.

Recorded data of clinical cases of patients suffering from the bites were in correlation with the periods of elevated biting risk by *S. erythrocephalum*. Majority of the bites were recorded below the knees that reflect the typical biting habit of the species.

Key words: Simuliidae, black flies, *S. erythrocephalum*, outbreak

Acknowledgements: This project was supported by Ministry of science and environment protection, Republic of Serbia (Reference grant No. TR-6920B) and the Utility Affairs Department, City of Novi Sad

BLACKFLY COMMUNITIES (Diptera, Simuliidae) OF THE HRON RIVER (SLOVAKIA); TYPOLOGICAL CHARACTERISTICS, LONGITUDINAL ZONATION AND EUTROPHICATION

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Investigations carried out in 2003-04 were focused on blackfly taxocenoses of the Hron river, a leftside tributary of the Danube river. Using the distribution patterns of black flies (18 species) from 14 sites, representing a fluent transition from epirhithral up to epipotamal. Multidimensional statistical analyses confirmed continual character of changes in blackfly communities. This continuity is disturbed by anthropogeneous influences, especially eutrofication (phosphorus). The dominance of each species were projected on this longitudinal gradient - ecological position (EP) and each species response was characterized by two typological traits: its typological preferendum (tp) and its typological amplitude (ta), thus creating a synthesis of ecological characteristics. These typological species traits are useful contributions to a database for running waters biomonitoring.

The longitudinal gradient (298 km) of the Hron river comprises epirhithral, metarhithral, upstream stretch of hyporhithral, downstream stretch of hyporhithral and epipotamal. The species *Prosimulium rufipes* (Meigen, 1830), *Simulium argyreatum* Meigen, 1838 and *Simulium monticola* Friederichs, 1920 are typical of the epirhithral. The species *Simulium cryophilum* (Rubtsov, 1959) and *Simulium vernum* (Macquart, 1838) complete this community. Occurrence of *Simulium variegatum* Meigen, 1818, *Simulium argyreatum*, *Simulium monticola* is typical of the metarhithral. The upstream stretch of hyporhithral is characterized first of all by occurrence of *Simulium reptans* (Linnaeus, 1758), *Simulium variegatum* and *Simulium ornatum* Meigen, 1818. The downstream stretch of hyporhithral is characterized by *Simulium lineatum* (Meigen, 1804), *Simulium ornatum* and *Simulium equinum* (Linnaeus, 1758). Species *Simulium erythrocephalum* (De Geer, 1776) and *Simulium lineatum* are typical of the epipotamal. Findings of *Simulium bertrandi* (Grenier et Dorier, 1959) and *Simulium aureum* (Fries, 1824) are interesting from faunistical point of view.

Key words: typology, running waters, blackflies, Carpathians, U-D gradient

THE IMPACT OF CHEMICAL CONTROL AND SUBSEQUENT RENATURATION ON THE DEVELOPMENT OF MASS POPULATIONS OF BLACK FLIES (Diptera: Simuliidae), AS ILLUSTRATED BY THE CENTRAL EUROPEAN RIVER ODER

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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 River Oder as well as the introduction and addition of insecticides and pesticides strongly influenced 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 led to fundamental changes in the conditions of the water system. These changes resulted, on the one hand, in a reduction in the species diversity, and, on the other hand, in increased possibilities for the dispersal and expansion of the biota that remained. The results obtained from surveys of the renatured courses of the River Oder 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 the river and its effects on agriculture and animal husbandry, as well as the adverse health effects on the people who live along the Oder and its 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. The role of the natural invertebrate predators will be discussed. 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. Some act as internal parasites, attacking mainly the larval stages.

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

SOME NOTES ON THE DISTRIBUTION OF BLACK FLY LARVAE IN SMALL RIVERS IN LITHUANIA

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Blackfly larvae and pupae were collected from various aquatic plants and submerged objects in 14 different rivers in Lithuania. The annual water discharge varied from 0,3 to 33 m³/s in study sites. Black flies were collected every month from April till October in 2004. During research some data on physical (water temperature, stream velocity) and chemical indices (ph, total hardness, carbonate hardness, amount of nitrates, nitrites, phosphates, oxygen dissolved in water) were investigated in study sites. Merck compact laboratory for water testing was used.

Nineteen species of black flies were determined. Black flies of *Simulium ornatum* (Mg.) and *S. equinum* (L.) dominated in four rivers out of all rivers investigated, *S. equinum* and *S. morsitans* Edw. dominated in three rivers, *S. ornatum*, *S. noelleri* Fried. and *S. rostratum* (Lundstr.) dominated in three rivers. *S. equinum* and *S. lineatum* (Mg.) dominated in one river, *S. ornatum* and *S. trifasicatum* Curtis dominated in two rivers investigated. We have not found black fly larvae or pupae in one river.

Correlation was proved to exist between the abundance of larvae of some black fly species on the one hand and some physical - chemical indices on the other. To elucidate at least some of the factors that condition the distribution of black flies of different species over water bodies multiple regression and principal components analysis were used.

Key words: black flies, distribution, Lithuania

POTENTIAL OF *Cardiocladius oliffi* (Diptera: Chironomidae) AS A BIOLOGICAL CONTROL AGENT OF *Simulium damnosum* Theobald complex (Diptera: Simuliidae) IN WEST AFRICA

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Immature stages of *Simulium damnosum* sensu lato reared from egg masses collected from the field at Boti Falls and Huhunya (River Pawnpawn) in the laboratory, of *S. damnosum* were observed to be attacked and fed upon by larvae of the chironomid flies *Cardiocladius oliffi* Freeman, 1956 (Diptera: Chironomidae). *C. oliffi* was successfully reared in the rearing system developed for *S. damnosum* and used in control experiments. These predators caused high mortality among *S. damnosum* larvae as a result of disturbances, but the actual predation was on immobile pupae. It was observed that even at a ratio of one *C. oliffi* to five *S. damnosum*, the predators cause significant decrease in the number of adult *S. damnosum* emerging from the systems (treatments) as compared to the control (that had no *C. oliffi* present). However, the study also established that the chironomid flies could successfully complete their development on a fish food diet only.

Keywords: Simuliidae, Chironomidae, biological control, predators.

BLACK FLY REARING AND RESEARCH AT THE UNIVERSITY OF GEORGIA, USA

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The University of Georgia (UGA) Entomology Department is home to an active, innovative and diverse black fly research program. The core of this research program is the world's only known, reproductively isolated colony of black flies, *Simulium vittatum* Zetterstedt cytospecies IS-7. The colony is operated using the Cornell Automated System as developed by Dr. Ed Cupp and his staff at Cornell University. This system incorporates a closed-circulation trough sy-

stem in which water is pumped over a wooden runway creating ideal larval habitat. Each week ~ 200–300,000 larvae are produced.

The colony is operated with associated systems that automatically feed the larvae, capture emerging adults and provide suitable substrates for oviposition. Larval feeding is accomplished by grinding a 1:1 mixture of rabbit chow and soybean meal and washing it through a 53 micron sieve. The resulting food slurry is stored in tanks that are kept in modified refrigerators adjacent to the rearing units. Each tank includes two submersible pumps, one to stir the food solution and one to pump it into the rearing unit. Larval development occurs on the wooden runway (0.3 m. × 1.2 m.).

Upon significant pupation, an emergence hood is placed over the rearing unit to capture emerging adults and initiate the mating process. Adults move towards the light coming through the glass funnel located at the apex of the hood and the associated piece of Tygon® tubing that is attached. Mating occurs in this emergence tube and a smaller mating tube where all adults that have been collected during the day are confined. Adults are provided 10% sucrose and distilled water via cotton pads that are placed on the screened portion of the adult container.

The oviposition process is initiated the following week when the adults are removed from refrigeration and egg development is allowed to resume. After 24 hours, adults are released into insect cages (0.3 m. x 0.3 m. x 0.3 m.) that are situated over discs of cloth (0.15 m. diameter, light green) which are bathed in a film of water pumped from 38 liter aquariums via submersible pumps and a tubing apparatus. The insect cages are covered with black cloth, so the only light available is from below the discs. Females are attracted to the moisture and light, and lay their eggs on the moist, discs of cloth. Egg sheets are pinned to the upper surface of a clean runway and the rearing cycle continues.

Eighteen to twenty day old larvae produced in this system are used in an orbital shaker bioassay for a variety of research purposes. The orbital shaker bioassay consists of 250 ml, flat bottom, extraction flasks filled with test water, larvae and serial dilutions of B.t.i. products. The primary operation of this system is for product development testing and research with the commercial black fly control product, Vectobac® 12AS. Testing has included larval feeding studies, component evaluations, product comparisons and algal/efficacy evaluations. This project is graciously supported by Valent BioSciences.

Key words: *Simulium vittatum*, colony, bioassay, oviposition

GUT FUNGI (Trichomycetes) AND THEIR SYMBIOTIC RELATIONSHIP WITH BLACK FLIES AND MOSQUITOES

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Trichomycetes (Zygomycota) are a cosmopolitan class of filamentous fungi that live in symbiotic relationship within the digestive tract of various arthropods. In a course of two years we have investigated trichomycetes development in black flies and mosquitoes. Our results suggest that *Smittium culisetae* development is different among two dipteran hosts at different temperatures. In addition to that *Sm. culisetae* differ morphologically among two hosts at three temperatures. In the mosquito host we examined the competition of several *Smittium* species, in terms of relative prevalence of thalli and found no signs of competition between different species. When we tested the relative prevalence of trichomycetes within the hindgut we found that trichomycetes are found in rectum in mosquitoes and in the posterior colon in black flies.

Keywords: Simuliidae, black flies, Culicidae, mosquitoes, Trichomycetes, symbiotic relationship

NEW RESULTS ON DIPTERA PREDATORS IN THE BLACK FLY PLAGUE AREAS OF CENTRAL EUROPE AND THE CAUCASUS

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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 strat-

egies - from different insect orders are known to feed on black flies and influence the populations in proportion to their own biology, in other words to their mode of predatory strategies.

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 Germany, England and Armenia during the last two years, we have been able to show that there are at least 102 species in 15 families of Diptera that prey on black flies. 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. So far as larvae are concerned, this is evident in the association between certain Hemerodromiinae (Empididae) and black fly larvae, which in the plague regions of Central Europe are populations of *Simulium equinum*, *S. erythrocephalum*, *S. reptans* and *S. nigrum*. At the time of our fieldwork in Armenia, the principal predators of the pest species *S. equinum* and *S. caucasicum* were Muscidae. 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, food chain, predation, predators, prey

BLACK FLY (Diptera: Simuliidae) CONTROL IN GERMANY WITH B.t.i.

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Since the 1980ies black flies are again responsible for increasing numbers of deaths and injuries to cattle and injuries to men in Germany. After finishing laboratory and field tests, in 1986 first control with B.t.i. started in the states Nordrhein-Westfalen and Baden-Württemberg - and the control is still going on. The most important pest species in the treated river systems are *Simulium ornatum*, *S. trifasciatum*, *S. lineatum*, *S. equinum* and *S. noelleri*.

A review of the control measurements in Germany with B.t.i. from the beginning till today including the treated river systems, application techniques, success and an outlook on future control is given.

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

BLACK FLY CONTROL WITH B.t.i. IN THE USA: APPLICATION METHODOLOGIES AND FORMULATION DEVELOPMENT

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Black fly control with *Bacillus thuringiensis* var. *israelensis* (serotype H-14) (B.t.i.) is now used throughout the United States wherever there is a need to alleviate the nuisance or medical problems on man and/or his domesticated animals. Operational control programs range from very small streams that are less than a meter wide to large rivers that can be 2 km wide. Application equipment varies and is dependent mostly on size of the program and accessibility to treatment sites. Small streams are usually treated by hand using backpack sprayers, watering cans, and etc. whereas boat or aircraft treats large streams and rivers. The early development of these methodologies, especially aerial application techniques and efficacious B.t.i. formulations, which has its origin in the WHO-OCP West Africa Program, will be discussed.

Successful control of black fly larvae requires knowledge of the river system being treated, the black fly species being treated and the proper application technique. Application methodologies vary and are determined by many factors including the size of the stream/river, discharge rate, concentration of algae/other suspended particles in river water, and the river characteristics, in general. Each river system is unique in its morphological/substrate profile, water chemistry, and hydrological conditions. Stream and river dynamics are influenced by a gradient of physical factors formed by the drainage network. Black fly larval distribution is determined by hydrodynamics created by the uniqueness of the river/stream system. It is impossible to make specific recommendations on B.t.i. dose levels and injection time that will provide effective control in all rivers. Breeding sites need to be properly identified and mapped, and pilot B.t.i. studies conducted to ascertain correct downstream distance of larval control (carry).

Keywords: B.t.i., operational control programs, application methodologies, formulation development

THE ECONOMIC IMPACT OF BLACK FLIES (Diptera: Simuliidae) IN SOUTH CAROLINA AND RELATED SURVEILLANCE TECHNIQUES

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The membership of a private golf club in South Carolina's Piedmont Physiographic Region was surveyed to determine perceptions regarding local black fly populations and the effects of these populations on golfing habits. The economic impact of black fly annoyance at this club was estimated to be \$27,202. Adult surveillance indicated that the pest species were members of the *Simulium jenningsi* group; larval surveillance identified the Enoree River, Laurens County, as the primary source of the pest species. A localized suppression program was initiated during the late summer of 1994 and continues to this day. Larvicide applications were conducted on four dates in 1994 and 11 in 1995. In recent years, 13-15 larvicide application sessions have been conducted. All lar-

vicide applications are conducted using the biological insecticide Vectobac® 12AS (*Bacillus thuringiensis* var. *israelensis*). Larval mortalities of 94.5% and 97% were produced during 1994 and 1995, respectively, and consistently high levels of larval mortality have been maintained through the subsequent years. Adult black fly populations were reduced 92 and 88% during 1994 and 1995 respectively, from pre-program populations. As the program has been refined and improved, adult populations have been reduced by as much as 98% from pre-treatment levels.

Adult surveillance is conducted using a standardized monitoring technique. An individual (ideally the same person each time) stands at a location for one minute, after this period, 5 figure-eight sweeps are conducted above the head/shoulders/face with a 38cm diameter, fine mesh, insect net. After the 5 sweeps, a 15 second pause is observed to allow any flies present to regroup. After which, 5 additional sweeps are conducted for a total of 10 sweeps/repetition. Captured flies are anesthetized and counted. Each site is evaluated with 3, 10 sweep repetitions. Monitoring locations are chosen near the typical areas of annoyance with a tendency to target the interface of open and brushy areas.

Larval surveillance is also routinely conducted for this program. Samples are collected by approaching substrates from the downstream side and timing the period spent looking for larvae on quality substrates. Quality substrates would include any materials where larvae would normally be expected to occur in the waterway being evaluated. Larval populations are described as low when <15/minute are observed, moderate when 20-30/minute are found and numerous when >50 larvae/minute are found. Intermediate populations can be scored with a “plus” or “minus” to further refine the evaluation.

Key words: Economic impact, *Simulium jenningsi* group, adult surveillance, larval surveillance

IMPACT OF BIOLARVICIDING ON *Simulium ornatum* Meigen 1818 (complex) POPULATIONS AND RELATED BITING RISK

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Recent studies of black flies in the region of Novi Sad confirmed the dominance of *Simulium ornatum* Meigen 1818 (complex) in small water courses. Highly productive breeding sites of this polivoltine mammophilic species were detected in majority of the streams flowing down from the slopes of the Fruska gora hills, especially in sections running through or in vicinity of populated zones.

Routine treatments of the simuliids breeding sites with biologicals (B.t.i.) were introduced for the first time in the region in 2005. Since, the treatments have been performed on regular basis, following monitoring of larval abundance. The timing of the treatments was chosen carefully for each water course in accordance with the density and age composition of the populations. It has been demonstrated that the optimal moment for larvicide application is the onset of the pupal stage. Application rate and duration of the treatments were calculated and defined depending on the flow and water temperature for each stream. The product dosage and duration of application ranged within the interval of 5 to 25 ppm and 10–20 min respectively. Registered carry of the B.t.i. was 500 m downstream.

Efficiency of the control measures was assessed by comparing the immature population density before and after the treatments. Systematically performed larvicide treatments consequently reflected the number of adult specimens captured in the traps positioned in the zones where humans are exposed to *S. ornatum* (complex) attacks. In given circumstances four larvicide treatments during the spring and summer were sufficient to keep adult population level below critical abundance for humans.

In the situations which are common for the studied sites of the region a treatment performed in autumn which successfully suppress overwintering generation, demonstrates that two to three spring/ summer larvicide treatments were

sufficient to maintain the adult population density permanently under nuisance threshold level.

Key words: Simuliidae, black flies, *S. ornatum*, B.t.i., control

Acknowledgements: This project was supported by Ministry of science and environment protection, Republic of Serbia (Reference grant No. TR-6920B) and the Utility Affairs Department, City of Novi Sad

PROBLEM OF MASS OCCURRENCES OF BLACKFLIES (Diptera: Simuliidae) IN POLAND AND METHODS OF THEIR CONTROL

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There are several regions in Poland where blackflies (Diptera Simuliidae) have always occurred in mass numbers - western regions along Lower Oder, Obra and Warta Rivers, eastern regions of Podlasie, Masuria as well as in Lublin and Suwałki districts. Last 15 years the blackfly nuisance has been reported from more and more new areas, especially along Vistula River. The occurrence of simuliids in mass numbers cause numerous fatal cases in the cattle and horses - only in 1996 at least 809 deaths were reported, then in 1997 next cases were reported and every next year the problem arose every spring. Ornithologists observed that simuliid plagues in new occupied areas cause total nestling mortality in colonial common gulls. Also people suffer from blackfly bites - there are more and more reports about medical cases among people - even among inhabitants of great towns (i.e. Warsaw, Poznań, Zielona Góra, Gorzów Wielkopolski, Włocławek and Toruń). In some regions municipal authorities have decided to control simuliids. Systematic control activities were implemented in several towns along Oder River, especially in Gorzów Wielkopolski. Also in Toruń blackflies have been controlled for 3 years now. Since there is no specialist in biological control of blackflies in Poland, mainly chemical methods for control adult blackflies are used. Therefore there is a need to start a programme for teaching specialised teams and then to implement biological control methods in Poland i.e. with use of bacterial larvicide preparations based on B.t.i..

Key words: blackflies, Simuliidae, Poland, plague occurrences, control

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