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Agenda and Abstracts

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Meeting Agenda

Thursday June 21.

Nicholson Building, Nettlefold Room^[1]_{SEP}.

9:00 -9:15. Registration continued. Morning coffee.

9:15: Welcome and opening remarks (John Colbourne and John Davies)

9:30: Special Tributes to Roger Crosskey. I.

A Tribute to Roger Ward Crosskey (1930–2017) and His Contributions to Simuliidology. Peter H. Adler.

10:00-10:30 Ecology and Distribution. I. Chair: Charles Brockhouse
Simulid Control In The River Cinca, Alcanadre And Flumen At Its Pass Through The Regions Of Cinca And Monegros. P. Valle.

Comparative notes on the bioecology of *Simulium erythrocephalum* (De Geer, 1776) in some European countries. Rasa Bernotiene.

10:30-10:45: Coffee Break and Posters. The Refectory^[1]_{SEP}

10:45-11:45. Ecology and Distribution II. Chair: John McCreadie.

Biodiversity of Mesoamerican Blackflies (Diptera: Simuliidae): The blackflies of Costa Rica. Luis M. Hernández-Triana.

Genetic and morphological variability in black flies inhabiting big rivers in Europe. Tatiana Kúdelová.

12:30-13:30. Buffet Lunch. The Refectory.

13:45-14:15. Special Presentation by Gnatworks.

The Gnatwork Network. Emma Howson and Simon Carpenter.

14:30-15:30. Control of Blackflies and Applied Research. I.

Updates on the most important pest species of blackflies in some European countries: the situation in Germany, Lithuania, Serbia and Spain. Aleksandra Ignjatović Čupina.

Study of blackfly outbreaks analyzing the medical assistance through the OMI-AP application in Primary Care in Zaragoza (Spain) in the period 2009-2015. I. Ruiz-Arrondo.

15:30-16:00: Coffee break. The Refectory.

16:00-17:00. Control of Blackflies and Applied Research. II.


Mosquito magnet traps for monitoring host-seeking *Simulium*. R. A. Cheke.

Spanish blackfly (Diptera: Simuliidae) history, situation from past to present.
David López-Peña and Ricardo Jiménez-Peydró

Could the species richness of black fly (Diptera: Simuliidae) be affected by the altitudinal profile of rivers of the Valencian Autonomous region (Spain)? David López-Peña and Ricardo Jiménez-Peydró

19:00-21:00 Dinner in Lucas House's Park Restaurant.

Friday June 22. Nicholson Building, Nettlefold Room

9:00-9:15. Morning coffee. 

9:15-9:30. Special Tributes to Roger Crosskey. II. Chair: Charles Brockhouse

History of the Blackflies (Diptera : Simuliidae) in Morocco: (1999-2002), a period key for the knowledge of the fauna with the grand master Professor Roger Crosskey. Boutaina Belqat, *read in absentia by Peter.H. Adler*.

9:30-10:30. Regional Reports and Blackfly Fauna. I. Chair: Luis M. Hernández-Triana

Black flies (Diptera : Simuliidae) of the Central Sahara, Southern Algeria. M. Cherairia and P. H. Adler.

Diversity of black flies of the genus *Prosimulium* in Europe. Matúš Kúdela.

10.30-11:00. Coffee break and Posters. The Refectory.

11:00-12:00. Regional Reports (continued).

Abstract 14: Blackfly control in Lithuania. Can we stop it? R. Bernotienė.

Onchocerciasis vectors in northern Uganda. R. Post.

12:00-12:30. Simulium Genomics Project. Chair: J.K. Colbourne.

A novel approach to metagenomic analysis of lotic habitats. Max Hoeffel and Cameron Kell.

12:30-13:30. Buffet Lunch. The Refectory.

13:45- 14:45. Simulium Genome Project (continued). Chair: Charles Brockhouse

Introduction to Genomes. John Colbourne

Gene models for the *Simulium vittatum* complex. Romain Derelle. (Abstract pending).

Finding gene function in the *Simulium vittatum* complex. Charles Brockhouse

15:45. General Wrap-Up Discussion. Where do we go from here

- Special thanks to John Davies
- Simulium Bulletin Editorship
- Venue of the next Simuliidae Symposium -proposal for Morocco
- Future Funding: Crowd Funding?
- GCRF funding: how do we tackle this available research fund?
- Remaining Issues and Priorities

The meeting's formal sessions concluded with a vote of thanks to John Colbourne and Charles Brockhouse.

19:00- Optional Conference Dinner. Birmingham Botanical Gardens

Saturday June 23. Departure.

Abstracts of Oral Presentations

1 . Spatial Distribution and Dispersal Ability in Simuliidae.

John McCreddie

Biological Sciences, University of South Alabama.

We examined whether differences in commonness and rarity in an actively dispersing group of winged insects could be explained by differences in dispersal ability using 12 ecoregion-level larval data sets ($n = 476$ sites) from North America. The patterns of spatial distributions of common and rare species did not show any marked difference. Correlations between species frequency and mean distance among sites typically was not significant. Species classified as common, rare, or vagrant in one ecoregion data set were often classified differently in other data sets. Our results suggest that if dispersal is important in local assemblages of black flies, it does not markedly affect rare species more than common species. Given the high dispersal ability of many black flies, dispersal-related processes might have limited influence on local assemblages, regardless of whether species are common or rare.

2. A Tribute to Roger Ward Crosskey (1930–2017) and His Contributions to Simuliidology

Peter H. Adler
Clemson University

Abstract

The life of Roger W. Crosskey and his contributions to simuliidology are presented, from his early interests in natural history through his entomological career of 65 years. He began in 1951 as a medical entomologist with the Colonial Service in Northern Nigeria, working initially with tsetse and then the onchocerciasis problem. He returned to London in 1959 and officially began studies of dipteran taxonomy, first with the Tachinidae and continuing with the Simuliidae, particularly while a scientific officer with The Natural History Museum in London. His legendary contributions to the fight against onchocerciasis and to the study of the Simuliidae are highlighted.

3. History of the Blackflies (Diptera : Simuliidae) in Morocco (1999-2002), a period key for the knowledge of the fauna with the grand master Professor Roger Crosskey

Boutaïna BELQAT

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I acquired my first experiences, all my knowledge in blackflies from the grand masters, Professor Roger Crosskey (1999-2002) and Professor Peter Adler since 1998 till now.

When, in 1997, I began the study on Simuliids, I could not imagine the difficulty and complexity of the Family. I started the work with gran interest, eager to do a research work, but I could not imagine what a gulf I was sinking into!

My first contact with him was by e-mail, in early 1999, since that time, he introduced me to the world of taxonomy, on the basis of the list of the 16 species of Simuliidae known at that time in the Rif, he facilitated me. Since then, he gave me constant advices and encouraging discussions.

On 2000, I travelled through London to visit Pr Roger, who gave me a warm welcome at the Natural History Museum in London, where I was able to benefit at all times from his great knowledge on taxonomy. He generously provided me with

all the necessary comparison material and introduced me to the value of taxonomy.

Three years of rich correspondence, by hand-letters and e-mails, crowned in 2002 by a doctoral thesis: Systematic, Ecological and Caryologic study of the Black flies (Diptera: Simuliidae) of Morocco: particular case of the Rif.

The total of 16 species known became 28 for the Rif mountains and 42 species of Simuliidae were recorded in Morocco. An identification key to the simuliids of Morocco was included for the first time.

Key words: caryology, key, Morocco, Natural History Museum, Rif, Simuliidae, taxonomy.

4. Comparative notes on the bioecology of *Simulium erythrocephalum* (De Geer, 1776) in some European countries

Rasa Bernotiene¹, Aleksandra Ignjatović Čupina², Ignacio Ruiz Arrondo³, Mihaela Kavran², Dušan Petrić², Helge Kampen⁴, Doreen Walther⁵

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Simulium (*Boophthora*) *erythrocephalum* is a widespread European blackfly species, which usually develops in large and medium size rivers. Adult females are known as aggressive bloodsuckers creating problems to humans and animals in some parts of Europe. It represents the main pest species in Germany, Serbia and Spain, while in Lithuania it is not considered a pest species, although it periodically may cause some nuisance problems in humans. The objective of this study was to compare the bioecological characteristics of this species in four European countries: Lithuania, Germany, Serbia and Spain.

In Lithuania, the main breeding sites are located in the two largest low-land rivers (the Nemunas and the Neris). The females lay the eggs on objects close to the water surface, in the zones close to the river banks and across the river profile (e.g. floating leaves of aquatic plants, leaves of submerged branches of

trees). Pupae of the first generation, that usually is not abundant, are found in late April at a water temperature of about 10°C, while pupae of the second generation are found in late June when the water temperature is around 18°C. In Lithuania, this species has three to four generations per year and overwinters in the larval stage.

Depending on the seasonal temperatures, three to five generations per year can also be expected for Germany. Although the species is known as a late swarmer, the first generation is due in late February or early March. Larvae of the first generation are always attached to dead plant substrates hanging into the water. They can mainly be found in medium-sized lowland rivers such as Spree, Havel and Rur or the first section part of the Danube. Only the second and following generations may cause problems to cattle and humans.

In Serbia, *S. erythrocephalum* also typically breeds in large lowland rivers, such as the Danube and the Tisa rivers, but it occasionally also breeds in small water courses. In river habitats, the females exclusively lay eggs on objects close to the water surface (e.g. floating leaves on submerged branches of poplar, willow trees and other plants or plant fragments) in the zones close to the river-side. Therefore, the number of generations and the population density strongly depend on the water level. As demonstrated in the Danube river, abundant populations are usually produced at high water levels (above 450 cm in the region of Novi Sad) and at water temperatures ranging between 11°C and 27,5°C during spring and summer time (April to late June or early July). In the following part of the season, possible increases in water levels do not evoke the emergence of new generations of adults. In Serbia, the species most probably produces up to four overlapping generations annually and overwinters in the larval stage.

In Spain, *S. erythrocephalum* inhabits the middle and lower stretches of medium and large rivers, such as the Ebro, the Turia and the Henares. The substrates for immature stages vary according to the type of river but it has been observed that macrophytes and especially *Potamogeton pectinatus* represent excellent attachment substrates all across the river profile. The submerged vegetation along the riverside also represents a suitable breeding substrate. The number of generations and the population density of *S. erythrocephalum* in Spain also depend on the hydrology of the rivers, but prolonged high flows during the winter and the beginning of spring seem to negatively affect the breeding sites, as demonstrated for the Ebro river in the region of Zaragoza. In Spain, the species most probably produces up to five overlapping generations annually and it also overwinters in the larval stage.

The research was supported by the Ministry of education, science and technological development of the Republic of Serbia (projects III43007, TR31084).

5. Updates on the most important pest species of blackflies in some European countries: the situation in Germany, Lithuania, Serbia and Spain

Aleksandra Ignjatović Ćupina¹, Rasa Bernotienė², Ignacio Ruiz Arrondo³, Mihaela Kavran¹, Dušan Petrić¹, Helge Kampen⁴, Doreen Walther⁵

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The medical importance of blackflies in Europe is primarily attributed to nuisance and their biting activity. Blackfly bites usually cause dermatological problems to humans, and massive blackfly attacks may affect animal welfare and livestock production, ultimately causing death of animals, as reported in different parts of Europe during the past. The current situation about the pest species of blackflies and bite-related problems in some particularly affected European countries (Germany, Lithuania, Serbia and Spain) are elaborated in this study.

A major pest species of medium German lowland rivers, such as Oder, Spree, Havel and Rur, *Simulium erythrocephalum* has long been considered as emerging only in May/June in Germany. However, this nuisance-causing generation is already the second of the species in the year. The first generation develops in late February/early March from the overwintering larvae and remains inconspicuous. By contrast, other important German pest species, such as *Simulium equinum* (Linnaeus, 1758), *Simulium lineatum* (Meigen, 1804), *S. nigrum* (Meigen, 1804), *S. ornatum* complex, and *Simulium reptans* (Linnaeus, 1758) breed in the large lowland rivers Oder, Elbe, Donau and Rhine and occur in May/June with their first generation. Generally, problems with simuliids bothering humans and livestock in

Germany are registered early in the year and are due to the first generation. Few problems occur in summer.

Simulium maculatum (Meigen, 1804) is the main pest species in Lithuania. It develops in the Nemunas, the largest river in the country and produces two generations per year. The first generation, emerging in May and June, is usually abundant, while the population density of the second generation, emerging in August, is regularly low. *Simulium reptans* is also known as a pest species in Lithuania which usually bites cattle. It also develops in large rivers during April - May. *Simulium ornatum* complex and *S. lineatum* were also registered as pest species in some years and in some localities, but these cases were very local.

Due to the repeated outbreaks of few blackfly pest species (*Simulium colombaschense* (Scopoli, 1780), *S. maculatum* and *S. erythrocephalum*) and consecutive economical losses and health problems, Serbia was considered as the most threatened European country in the past. The most important mammalophilic and anthropophilic pest species of Serbia in recent times is *S. erythrocephalum*. Its most productive breeding sites were detected in large lowland rivers in northern parts of Serbia (the Danube and the Tisa), where it represents the dominant blackfly species, but it was also detected in some medium-size rivers in the hilly regions of central Serbia (the Drina and Nišava rivers), as well as in some small confluent streams, periodically. The bite-related problems in both humans and animals usually occur from April to July, with the highest peaks of activity at the beginning of April, in mid-May and mid-June, especially after high water levels and flooding of the rivers.

Simulium ornatum complex is also known as a pest species in Serbia, with a high preference for mammals including humans. *Simulium ornatum* usually inhabits small water courses, and where present, produces abundant populations and represents the dominant blackfly species in the habitat. Immature stages of *S. ornatum* may breed in water contaminated with high level of organic pollution, where other blackfly species are not able to survive. In the streams of the Fruška Gora mountain (region of Novi Sad, northern Serbia), *S. ornatum* produces four overlapping generations of adults during the season, and the larvae of the fifth generation overwinter. The highest peaks of adult activity usually occur in March/April, May/June and July.

Simulium erythrocephalum is the main human-biting species in Spain. This anthropophilic species causes nuisances in many regions of the country, such as Aragón, Cataluña, Madrid and Comunidad Valenciana. Every summer since 2011, thousands of people require medical assistance for allergic reactions and dermatitis caused by the bites in Zaragoza. In this city, a high biting activity of *S. erythrocephalum* is recorded between April and October. While the peak of annoyance is in late May and early June, the number of bites later descend considerably.

In the middle Ebro valley (Aragón, Spain), in zones near riverbanks, simuliids also cause problems to livestock. Besides *S. erythrocephalum*, species involved in nuisance and health problems to domestic animals in Spain belong to the subgenus *Wilhemia*: *Simulium sergenti* Edwards, 1923, *S. equinum*, *Simulium pseudequinum* Séguy, 1921 and *S. lineatum*. The discomforts to the herds in this area have a negative economic impact on the farmers. In flocks of sheep, a loss of the general state of health of the animals is observed as a result of anorexia and restlessness. In the case of grazing animals, farmers are forced to increase their food supply to try to compensate for the loss of body condition of their animals, with the extra economic costs implied, due to the absence of effective registered repellent products. On other occasions, farmers have chosen to change the grazing schedules, taking the animals to the field at night while staying closed in semi-open shelters during the day. Both options do not improve the discomfort that the animals suffer from by blackfly bites. Some sheep owners move their flocks to insect-free areas with the additional expense of both moving and leasing new pastures. Equids also suffer acutely from blackfly bites in the Ebro valley. As a result of the continuous bites into the ears and in the lower abdomen, the animals are restless and constantly nod, which makes it difficult to manage them, and the owners are forced to move their horses to riding schools in areas not affected by blackfly nuisance and bites.

The research was partly supported by the Ministry of Education, Science and technological development of the Republic of Serbia (projects III43007 and TR31084) and by the Research Council of Lithuania (S-MIP-17-27).

6 A Gene Annotation and Expression Study System for Simuliidae.

Charles Brockhouse¹, Anna Wattles¹, Kelli Mans¹ and Alexi Papanicolaou²

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Blackfly larvae are nearly ubiquitous in lotic habitats. Because of their extraordinary ability to filter-feed on a wide-range of substances from DOM to particulate matter and to graze from substrate they are heavily exposed to anything suspended in the water column. And because they can be precisely identified to biological species using polytene chromosome analysis, they are prime candidates to serve as tools for environmental monitoring.

Using a combination of developmentally stage and sex sorted adults from the University of Georgia (Athens) colony, and sex sorted larvae from a natural population near Omaha, NE, we constructed a series of cDNA libraries were sequenced, co-assembled, and annotated using the JAMp pipeline (Papanicolaou, unpublished. <http://jamps.sourceforge.net>). JAMp allows the user to query the transcriptomes using Gene Ontogeny terms, and then to examine the expression profiles of selected sequences.

To demonstrate the use of JAMp we chose “sex determination” as the GO term, and randomly selected a specific sequence for expression analysis. The sequence turned out to be a homologue of the scute-achaete complex, with expression limited to larval stages, and differential expression between males and females.

The second GO term selected was “response to insecticide”. The sequence chosen from that list displayed putative differential splicing among developmental stages, and was identified as a gustatory receptor cDNA. In other species, these genes have extensive alternate splicing.

Our expression system is useful for species other than *S. vittatum* and *S. tribulatum*. It can be used to select candidate genes of interest in other species. We have successfully tested this for selecting genes and designing PCR primers for the *S. damnosum* species complex.

7. BLACKFLY CONTROL IN LITHUANIA. CAN WE STOP IT?

R. Bernotienė

Nature Research Centre, Lithuania

The outbreak of bloodsucking blackflies had become a serious problem in the South-eastern part of Lithuania in the end of the XX century. The main pest species was determined as *Simulium maculatum* Mg. Larvae of this species develop in the largest river of Lithuania, the Nemunas River. The species had two generations per year: the first one could be detected in May – June and the second one was not numerous and developed in August. Blackfly control was started in Lithuania in 1999 and was persisted until 2010, afterward was stopped for five years and was reactivated in 2016. The biological preparation VectoBac 12AS, based on the activity of *Bacillus thuringiensis* var. israelensis, was used for the blackfly control each year.

As a consequence of blackfly control the density of *S. maculatum* larvae was decreasing each year after the 1999 and decreased more than 100 times until 2011. The highest bloodsucking activity of blackflies was $356,7 \pm 112,6$ per 10 min in 1999 but during 2000 – 2011 the rate reduced to $3,9 \pm 1,9$ per 10 min. From the year 2011, when blackfly control was stopped, the density of blackfly larvae started to grow in the river. It increased almost three times during the first year. The density of *S. maculatum* larvae in 2014 was 14.6 fold higher comparing with the density in 2011. The bloodsucking blackfly activity remained low during first two years after the stop of blackfly control, but it increased until the 2015. Blackfly control was reactivated in the Nemunas river in 2016.

8. Study of blackfly outbreaks analyzing the medical assistance through the OMI-AP application in Primary Care in Zaragoza (Spain) in the period 2009-2015

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The city of Zaragoza (Aragon, Spain), with 700,000 inhabitants, has become in the last years the paradigm of annoyances by blackflies at European level. The bite of *Simulium erythrocephalum*, a species responsible for blackfly outbreaks in the city, sometimes causes allergic reactions that require medical assistance. Since 2011, problems due to arthropod bites have increased at the region's health centers.

Electronic health records are used in all public health centers in Aragón, and they are collected and managed through the OMI-AP application of the Government of Aragon, where health interventions are coded according to the International Classification of Primary Care (ICPC-1).

The aim of this work was to evaluate the medical consultations due to arthropod bites in Aragón in the period 2011-2015. For this purpose, the medical consultations were analyzed in Hospital Emergency Services at Aragon level and in Primary Care through the OMI-AP application in Zaragoza city.

The OMI-AP system has confirmed that the increase of medical assistance due to arthropod bites in Primary Care and Hospital Emergency Services in the city of Zaragoza since 2011 is because of the bites of simuliids. In addition, it has been demonstrated that the systematic analysis of the records from the OMI-AP application can be a very useful tool for evaluating the population dynamics of the blackflies and early detection of the onset of a public health problem at regional level.

9. Genetic and morphological variability in black flies inhabiting big rivers in Europe

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The black fly species or species complexes in European big rivers – *S. reptans*, *S. reptantoides*, *S. colombaschense*, *S. balcanicum* and *S. lineatum* show different distribution patterns of genetic variability. Genetic markers including the barcoding region of the mitochondrial COI gene and fragments of COII and 18S/ITS1 genes have been studied in populations of these species from significant parts of their areas. The species of *S. reptans* group could be recognized based on the

morphological as well as molecular data. The molecular structure of *S. reptans* shows two distinct lineages, differing morphologically and probably corresponding to different species – one of them with broad distribution reaching from the Balkan Peninsula up to northern Scandinavia, and the other restricted to northern Europe. In *S. reptantoides* the two molecular lineages do not correspond to the morphology or geographical distribution. The five cytoforms of *S. colomba-schense* seem to be bound to the particular big rivers with restricted dispersal between different river systems. The analysis of COI, COII and 18S/ITS1 genes, as well as morphological characters confirmed this cytoform pattern. The closely related species of the subgenus *Wilhelmia* – *S. lineatum* and *S. balcanicum* differ only in small morphological details. Morphological analysis of material from large parts of central and southern Europe did not reveal new characters suitable for species discrimination. Statistically significant differences were recorded in the length of the third pupal gill, present at every studied site, and in the number of rays in the labral fans and number of hook rows of the posterior sucker of larvae, found at the majority of the sites. According to the genetic analysis of the COI gene, *S. lineatum* was paraphyletic, and only one part of the haplotypes formed a well supported monophyletic clade, whereas the other part of *S. lineatum* haplotypes formed a poorly supported clade, more closely related to *S. balcanicum*. *S. balcanicum* formed a monophyletic clade, but without sufficient support. The intraspecific and interspecific K2P genetic distances were overlapping. Syntopic occurrence of *S. lineatum* and *S. balcanicum* was recorded only sporadically, in such cases one of the species was clearly dominant.

10. Spanish blackfly (diptera: simuliidae) history, situation from past to present

David López-Peña and Ricardo Jiménez-Peydró

Institut Cavanilles de Biodiversitat i Biologia Evolutiva (ICBiBE), Laboratory of Zoology and Pest Control, University of Valencia, Valencia, Spain.

These days Spain is having the same trending topic news every spring and summer seasons since some years ago. Spanish media are launching information about the cities, towns and villages whose citizens, feed up to be pestered by Simuliidae bites, make complains about these annoyance to their town halls. These protests are increasing year after year. In addition, hospitals and health centers use to be collapsed by people who go to these places to have their bites checked since some of them suffer important allergic reactions, intense itch

as well as bleeding wounds and edema in the swollen area of the bite, which even can promote being infected by bacteria or virus and endure illnesses. Besides, chemists affirm that lately their stock of antihistaminic creams use to run out.

Assuming the fact that some people and headlines of newspapers, radio and TV presenters say that there is a lack of knowledge of this Diptera. We wanted to verify how much truth there is in this statement. As a consequence of this worry, this study was promoted.

The main objective of this project was to do a deep search of published bibliography as well as participations to congresses, in order to clarify what type of information there is available and what topics were treated in them.

As a consequence, we provide a useful and updated information about the situation of blackfly in Spain addressing the timeline of publications and congress communications. Afterwards, we tried to discover if there has been or not any exhaustive university dissertation about this arthropod, and see what was the main interesting point. Last but not least, we make tribute to foreigners and national distinguished authors who contributed hugely to the knowledge of simuliids of this country. We thought that it can be very useful for scientific community, government institutions and pest control enterprises.

11. Biodiversity of Mesoamerican Blackflies (Diptera: Simuliidae): The blackflies of Costa Rica.

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Abstract. Certain black flies are of medical concern because they transmit nematodes that cause onchocerciasis and mansonelliasis and perhaps also a pathogen that causes nodding syndrome and epilepsy without nodding. In the present study, we report the results on a countrywide survey in Costa Rica with regards to blackflies in combination with the use of COI DNA barcoding region for the identification of species in the country. In total we collected 232 rivers and streams from different habitats in Costa Rica. We found nineteen out of the 23 species recorded in the country belonging to the genera *Gigantodax*, *Simulium* and *Tlalocomyia*. The Neighbour Joining tree (NJ) derived from the DNA barcodes grouped most specimens according to species or species groups recognized by morphotaxonomic studies. Intraspecific sequence divergences within morphologically distinct species ranged from 0.07% to 1.65%, while higher divergences (2.05% - 6.13%) in species complexes suggested the presence of cryptic diversity. The existence of well-defined groups within *S. callidum*, *S. quadrivittatum*, and *S. samboni* revealed the likely inclusion of cryptic species within these taxa. In addition, the suspected presence of sibling species within *S. paynei* and *S. tarsatum* was supported. DNA barcodes also showed that specimens of species that are difficult to delimit morphologically such as *S. callidum*, *S. ochraceum* s.l., *S. pseudocallidum*, *S. travisi*, relatives of the species complexes such as *S. metallicum* s.l. (e.g. *S. horacioi*, *S. jobbinsi*, and *S. puigi*), and *S. virgatum* s.l. (e.g. *S. paynei* and *S. tarsatum*) grouped together in the NJ analysis, suggesting they represent valid species. DNA barcoding combined with a sound morphotaxonomic framework provided an effective approach for the identification of medically important black flies species in Mesoamerica and for the discovery of hidden diversity within this group.

Key Words. DNA barcoding – COI – Simuliidae – medically important black flies – Mesoamerica

12. Diversity of black flies of the genus *Prosimulium* in Europe

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Black flies of the genus *Prosimulium* have a distribution limited to the Holarctic Region. The majority of the 79 species belong to the *P. hirtipes* group. In Europe, 12 species of the *P. hirtipes* group occur over the entire continent and the species diversity increases from the north to the south. Integrated taxonomic study of *P. hirtipes* group over large parts of Europe showed that virtually all species constitute complexes of species and cytoforms. Molecular and cytological analysis of *Prosimulium latimucro* (Enderlein, 1925) revealed the presence of at least five different taxa and several of them show considerable internal genetic variability indicating presence of additional cryptic species or cytoforms. The genetic divergence based on Kimura-2 parameter between the lineages is 4.4–6.5 % and suggests long-term isolation. Comparison of the shape of the hypostoma in larvae and the branching pattern of the pupal gill filaments also revealed differences among the studied cytoforms, with cytoform A being the most distinct. Molecular data indicated that *P. albense* is closely related to cytoform B of *P. latimucro*, known in the southwest of the Alps and in the Apennines. The interspecific genetic divergence between *P. albense* and *P. latimucro* B is 1.4 %, whereas the divergence between *P. albense* and *P. latimucro* A is 6.3 %. According to the banding pattern of the larval polytene chromosomes, *P. albense* differs from all known cytoforms of *P. latimucro* by only one fixed inversion, which is unique to *P. albense*. Other inversions indicate the closest relationship of *P. albense* is to *P. latimucro* C. Molecular and cytological analysis revealed deep genetic divergence between *P. italicum* from Italy and *P. hirtipes* from northern and central Europe and confirmed the species status of *P. italicum*. All analyzed populations of *P. hirtipes* (Slovakia, Sweden, England, and Scotland) carried fixed inversion IS-9, whereas populations of *P. italicum* either lack this inversion or carry it as an X-chromosome polymorphism. The average K2P genetic distance was 3.7% between *P. italicum* and *P. hirtipes* from Sweden and 4.3 % between *P. italicum* and *P. hirtipes* from Slovakia. Cytogenetic analysis showed the presence of two cytoforms of *P. hirtipes* and two cytoforms of *P. italicum* differing in their sex chromosomes and autosomal polymorphisms, suggesting that *P. hirtipes* and *P. italicum* might each be a complex of cryptic species. Cytological analysis confirmed the species status of *Prosimulium* sp. affinity 3 over a large territory reaching from the western Alps, over the Carpathians and Balkan mountains up to the Hellenides, consisting also of additional cytoforms. Specimens of three samples from the Iberian peninsula, the Alps and Greece could not be attributed to the known species and suggest that the true diversity of *Prosimulium* in southern Europe has not been described satisfactorily.

13. MOSQUITO MAGNET TRAPS FOR MONITORING HOST-SEEKING *SIMULIUM***R. A. Cheke^{1*}**, F.M. Hawkes¹, G. I. Gibson¹, T. G. Acott²

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Keywords: Mosquito Magnet trap, *Simulium equinum*, Seasonality, Environmental variables

Mosquito Magnet® Executive Mosquito traps were deployed from May to October 2017 in different habitats in England as part of a project surveying mosquitoes (WetlandLife project of the NERC-sponsored Valuing Nature programme). The traps, which use carbon dioxide and octenol (MosquitoMagnet, Lititz, Pennsylvania, USA; <http://www.mosquitomagnet.com/>) to attract host-seeking insects were run for 5 days every fortnight. At two sites at each of Bedford Country Park (52° 7' 46"N, 0° 25' 47"W) and Northward Hill Nature Reserve (51° 23' 47"N, 0°42' 36"E), the by-catches were examined revealing numerous adult female *Simulium* spp. at the former, but only 6 at the latter. Nearly all of the blackflies were provisionally identified as *S. equinum* (Linnaeus 1758), an infrequent man-biter but a nuisance pest of veterinary importance that attacks horses and cattle. Occasionally their bites may lead to the allergic condition known as "sweet itch" in horses, ponies and donkeys. The seasonality of the catches, which were maximal in May with one catch of 1236, will be presented and discussed. It is suggested that Mosquito Magnet traps could be useful for monitoring, and perhaps controlling, haematophagous blackflies.

14. A NOVEL APPROACH TO METAGENOMIC ANALYSIS OF LOTIC HABITATS

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The analysis of environmental DNA (eDNA) is emerging as a crucial tool in the understanding and protection of natural systems. DNA sequences from lakes, soil, and ponds can be extracted and sequenced with great success. It is difficult to produce similar results in lotic habitats (rivers and streams), because the environment is constantly “moving away”, preventing the build-up of usable concentrations of eDNA. The Simulium Genomics Consortium has developed a novel technique to gather eDNA from moving water using the gut contents of blackfly larvae. Larvae can collect both particulate and dissolved organic material. By isolating and sequencing DNA from the stomach contents, an overview of what lived in and near the water source is created. We present microbe sequences collected from both Nebraskan and other lotic habitats, and discuss the problems with data analysis and identification confidence.

15. BLACK FLIES (DIPTERA: SIMULIIDAE) OF THE CENTRAL SAHARA, SOUTHERN ALGERIA

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The Ahaggar National Park is located in the heart of the largest sandy desert in the world, "The Sahara", in the extreme southern part of Algeria. It was listed by the United Nations in the national parks list and protected areas and contains a cultural heritage that is a testament to the civilizations that have existed there since the birth of humanity.

Although black flies are economically important pests and vectors of disease

agents to animals and humans, the number of formally described species in Algeria is only 1.4% of the total number of the world's nominal species. A dearth of studies have been published about this group to date in the country, particularly in the southern region. The early papers devoted to the black flies of the central Sahara were first published in the 1950s and 1960s. The current presentation is intended to provide a faunistic assessment and update on the composition and distribution of the Simuliidae fauna in the Hoggar Mountains (The Ahaggar National Park), which represent the largest mountain massif in Algeria.

In this survey, samplings were conducted during 2017 in 16 watercourses in the Ahaggar National Park at elevations ranging from 1132 m.a.s.l to 1975 m.a.s.l. Black fly immature stages were collected at 15 of these sites. It is expected that the ongoing survey will improve the knowledge of this family of Diptera in Algeria and provide a more complete understanding of the simuliid fauna of North Africa.

Keywords: *Algerian Sahara, The Ahaggar National Park, Biodiversity, Simuliidae.*

16. Could the species richness of black fly (Diptera: Simuliidae) be affected by the altitudinal profile rivers of Valencian Autonomous Region (Spain)?

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For the last years, blackflies (Diptera: Simuliidae), have been receiving diffusion on different Spanish media owing to the annoyance occasioned to citizens, as a consequence of their hematophagic character. Even though in European latitudes simuliid species are still not reported to behave as worrying vectors of pathogen agents causing disease, it is very important to be aware of their dispersion patterns in order to predict what human settlements could endure their painful bites and the derivative symptomatic reactions.

Although the information about this group in affected areas around the world is relatively extensive and detailed, there are other places where they have been superficially studied because of the absence of damaging, a clear example of this is the East Mediterranean coast of Spain, particularly in Valencia

Autonomous Region. Valencia is one of the regions afflicted by Simuliidae, and even there are some works about this Diptera, there is still not enough knowledge of this annoying arthropod. In order to gain knowledge about their bioecology, the next study has been carried out.

The main aim is providing information about what species there are in the rivers of the Valencian Autonomous Region, as well as seeing which of them are important for human beings, and also for animals. Moreover, the second objective is based on discovering if there is a connection between altitude and the distribution pattern of blackfly species.

The results show that there are 21 different species of blackflies, of which *Simulium angustitarse*, *S. equinum*, *S. erythrocephalum*, *S. lineatum*, *S. ornatum*, *S. pseudequinum* and *S. reptans* are of sanitary and veterinary interest. In addition, there does exist a clear and positive correlation between species richness and the altitude variable in rivers studied in the Valencia autonomous region. Consequently, we can infer that the difference of altitude affect the composition of blackfly community. Moreover, it has been confirmed that some of these species show preference to occupy certain stretches of the rivers, while others do not show any preference.

Posters

Updates on the most important pest species of blackflies in some European countries: the situation in Germany, Lithuania, Serbia and Spain

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The medical importance of blackflies in Europe is primarily attributed to nuisance and their biting activity. Blackfly bites usually cause dermatological problems to humans, and massive blackfly attacks may affect animal welfare and livestock production, ultimately causing death of animals, as reported in different parts of Europe during the past. The current situation about the pest species of blackflies and bite-related problems in some particularly affected European countries (Germany, Lithuania, Serbia and Spain) are elaborated in this study.

A major pest species of medium German lowland rivers, such as Oder, Spree, Havel and Rur, *Simulium erythrocephalum* has long been considered as emerging only in May/June in Germany. However, this nuisance-causing generation is already the second of the species in the year. The first generation develops in late February/early March from the overwintering larvae and remains inconspicuous. By contrast, other important German pest species, such as *Simulium equinum* (Linnaeus, 1758), *Simulium lineatum* (Meigen, 1804), *S. nigrum* (Meigen, 1804), *S. ornatum* complex, and *Simulium reptans* (Linnaeus, 1758) breed in the large lowland rivers Oder, Elbe, Donau and Rhine and occur in May/June with their first generation. Generally, problems with simuliids bothering humans and livestock in Germany are registered early in the year and are due to the first generation. Few problems occur in summer.

Simulium maculatum (Meigen, 1804) is the main pest species in Lithuania. It develops in the Nemunas, the largest river in the country and produces two generations per year. The first generation, emerging in May and June, is usually abundant, while the population density of the second generation, emerging in August, is regularly low. *Simulium reptans* is also known as a pest species in Lithuania which usually bites cattle. It also develops in large rivers during April - May. *Simulium ornatum* complex and *S. lineatum* were also registered as pest species in some years and in some localities, but these cases were very local.

Due to the repeated outbreaks of few blackfly pest species (*Simulium colombarhense* (Scopoli, 1780), *S. maculatum* and *S. erythrocephalum*) and consecutive economical losses and health problems, Serbia was considered as the most threatened European country in the past. The most important mammalophilic and anthropophilic pest species of Serbia in recent times is *S. erythrocephalum*. Its most productive breeding sites were detected in large lowland rivers in northern parts of Serbia (the Danube and the Tisa), where it represents the dominant blackfly species, but it was also detected in some medium-size rivers in the hilly

regions of central Serbia (the Drina and Nišava rivers), as well as in some small confluent streams, periodically. The bite-related problems in both humans and animals usually occur from April to July, with the highest peaks of activity at the beginning of April, in mid-May and mid-June, especially after high water levels and flooding of the rivers.

Simulium ornatum complex is also known as a pest species in Serbia, with a high preference for mammals including humans. *Simulium ornatum* usually inhabits small water courses, and where present, produces abundant populations and represents the dominant blackfly species in the habitat. Immature stages of *S. ornatum* may breed in water contaminated with high level of organic pollution, where other blackfly species are not able to survive. In the streams of the Fruška Gora mountain (region of Novi Sad, northern Serbia), *S. ornatum* produces four overlapping generations of adults during the season, and the larvae of the fifth generation overwinter. The highest peaks of adult activity usually occur in March/April, May/June and July.

Simulium erythrocephalum is the main human-biting species in Spain. This anthropophilic species causes nuisances in many regions of the country, such as Aragón, Cataluña, Madrid and Comunidad Valenciana. Every summer since 2011, thousands of people require medical assistance for allergic reactions and dermatitis caused by the bites in Zaragoza. In this city, a high biting activity of *S. erythrocephalum* is recorded between April and October. While the peak of annoyance is in late May and early June, the number of bites later descend considerably.

In the middle Ebro valley (Aragón, Spain), in zones near riverbanks, simuliids also cause problems to livestock. Besides *S. erythrocephalum*, species involved in nuisance and health problems to domestic animals in Spain belong to the subgenus *Wilhemia*: *Simulium sergenti* Edwards, 1923, *S. equinum*, *Simulium pseudequinum* Séguy, 1921 and *S. lineatum*. The discomforts to the herds in this area have a negative economic impact on the farmers. In flocks of sheep, a loss of the general state of health of the animals is observed as a result of anorexia and restlessness. In the case of grazing animals, farmers are forced to increase their food supply to try to compensate for the loss of body condition of their animals, with the extra economic costs implied, due to the absence of effective registered repellent products. On other occasions, farmers have chosen to change the grazing schedules, taking the animals to the field at night while staying closed in semi-open shelters during the day. Both options do not improve the discomfort that the animals suffer from by blackfly bites. Some sheep owners move their flocks to insect-free areas with the additional expense of both moving and leasing new

pastures. Equids also suffer acutely from blackfly bites in the Ebro valley. As a result of the continuous bites into the ears and in the lower abdomen, the animals are restless and constantly nod, which makes it difficult to manage them, and the owners are forced to move their horses to riding schools in areas not affected by blackfly nuisance and bites.

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The Simuliidae of Talassemtane National Park in North Morocco with a preliminary list of the accompanying Diptera (Insecta)

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A checklist of blackflies species recorded from the Talassemtane National Park (North Morocco) is based on material newly collected in the area. Sixteen species from two genera and five subgenera have been collected and are recognized.

A preliminary inventory of the Diptera of Talassemtane National Park is presented, based on a study of 6818 specimens, collected from 44 sampling sites during excursions made mainly during 2003-2007.

The faunal list is rich and diverse reflecting an important diversity of habitats. Forty three families of Diptera were obtained, including the collecting of larvae, pupa and adults in the park, assembling a total of 308 species.

Larvae of Simuliidae are a major component of the macroinvertebrate fauna in streams and rivers. The preimaginal stages of Simuliidae were collected in all types of running water, from imperceptible seepages to large rivers.

Key words: Diptera, biodiversity, National Park of Talassemtane, Morocco, Simuliidae.

IMPACT OF PHYSICOCHEMICAL QUALITY OF WATERCOURSES OF EL ABIOD BASIN ON THE BLACKFLIES DISTRIBUTION

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Keywords: Black flies, distribution, canonical correspondence analysis, El Abiod river basin, Algeria.

Blackflies in the El Abiod River Basin in southern Aurès of Algeria were sampled at 6 sites along the main river from January 2008 to December 2009, between 125 and 1360 m. Blackflies were collected monthly sampling, using a surber sampler, based on eight samples per site. Four taxa were identified belonging to *Simulium* Latreille genera among 4118 specimens. The species are: *Simulium* group *aureum*, *Simulium ruficorne* Macquart, *Simulium* group *ornatum* and *Simulium pseudequinum* Ségué. *S. gr. ornatum* and *S. gr. aureum* were the most abundant and ubiquitous taxa, representing respectively 45,7% and 41,6% of the total fauna, occupying all the sampling sites.

In order to describe the typology of this river and the relationship between species abundance and environmental parameters, a canonical correspondence analysis (CCA) was carried out. The data analysis allows a fairly significant organization of the river typology. Our taxa were classified as follows thermophilic taxa that prefer temperate and mineralised waters in low altitudes like *Simulium* group

aureum and *S. ruficorne*, eurytopic taxa that have a wide distribution (*Simulium pseudequinum*) and rheophilic taxa that prefer fresh waters at high altitudes : *Simulium* gr. *ornatum*.

River biodiversity in a changing world: Italian Simuliidae tell us three emblematic stories and a peculiar one

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River systems are the areas of the world presenting the highest biological diversity and the most intense human exploitation (Dungeon et al., 2006). Actually, freshwater constitutes only 0.01% of the planet's water and approximately 0.8% of its surface; yet this small fraction of global water hosts 6% of all described species. Therefore, inland waters and freshwater biodiversity constitute a valuable natural resource in scientific, economic and cultural terms (MAE, 2005). Nevertheless, fresh waters are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems, with alarming trends in human demands for water and, at the same time, in species loss. Threats to global freshwater biodiversity derive from 5 principal causes: overexploitation, water pollution, flow modification, destruction or degradation of habitat and invasion by alien species (Tockner et al., 2009). Their combined and interacting influences have resulted in population declines and range reduction of freshwater biodiversity worldwide (Butchart et al. 2010; IUCN, 2009). Conservation of river biodiversity is complicated by the landscape position of water courses and wetlands as collectors of land-use effluents, and the problems posed by high endemism percentage and thus non-substitutability.

Blackflies (Diptera, Simuliidae) are key organisms in both river and terrestrial ecosystems (Malmqvist et al., 2004). They are studied in depth worldwide both for their ecological and medical importance (Crosskey, 1990). 2,015 living nominal species of this insect family are recognized as valid in 2018 (Adler & Crosskey, 2018). The Palearctic Region has by far the largest number of described species (33% of the total; Currie and Adler, 2008). Considering only currently recognized valid species, 69 were recorded in Italy, 19 of which were firstly described from specimens collected in the Country and 12 being Italian endemic species (Rivosecchi, 2015). Four species' research activity and current conservation status are presented to describe the effect of changes in river systems on Simuliidae and

river fauna biodiversity in general. 1 - The blackfly from the Garigliano river and water pollution. *Simulium liriense* Rivosecchi, 1961, a Central Italy endemic species of the “reptans group”, was massively abundant in the lower basin of the Liri, Sacco and Garigliano rivers. Industrial pollution of the Sacco valley has affected the river water quality since the early '70s. Four recent research campaigns carried out from 2008 to 2017 did not make it possible to collect the species again, except for a single dubious pupa; the survival of the taxon could be granted by some relict populations, also in close basins. 2 - The blackfly from Susa valley, flow modification and climate change. *Simulium (Trichodagmia) segusina*¹ (Couvert, 1968) was collected and described from two Alpine brooks in north-western Italy. It was described as a reophilous species with two generations in May and August. Two recent attempts to collect this endemic species of Piemonte region (in 2012 and 2014) resulted unsuccessful: the water withdrawal for drinking and the increasingly earlier melting of the snow in south-western Alps dry the holotype's collection stream in August, while in May only other more common species were collected. Further survey is thus required. 3 - The blackfly from the Pontina plain (Lazio), land cover change and habitat destruction. *Simulium pontinum* Rivosecchi, 1960 was discovered in a creek flowing from a sulphur spring in southern Lazio, and subsequently in other similar environmental conditions in marshy areas of Central Italy. The reclamation of swamp land for agriculture and residential use, the channelisation of streams and the captation of springs strongly reduced the species range: a single population could be recorded in a large sampling campaign during 2008-2009. 4 - *Simulium bezzii* and the Marche earthquake. Neither holotype nor paratypes are available for *Simulium bezzii* (Corti, 1914) (complex). In Corti's collection in Pavia there are only some specimens collected by Kertesc in Moncenisio (Alps), but probably Corti examined also one female collected by Bezzi from the Sibillini mountains, Apennines (Rivosecchi, 2015). Actually, only minimal anthropogenic alterations affected the Monti Sibillini National Park, yet natural environmental changes were undoubtedly negatively influential on our blackfly collection success: researches in Marche region planned for the past year, aimed at establishing a neotype, turned out to be impractical due to the recent earthquakes, that caused landslides and inaccessibility of large areas, (hopefully) temporarily isolating the narrow valley that leads to the type locality.

¹ as reported in the World Blackflies Inventory; proposed species name: *Simulium (Trichodagmia) segusinum* (Couvert, 1968), to respect genus' gender concordance

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