



[www.zoo.utoronto.ca/nabfa/NABFA.html](http://www.zoo.utoronto.ca/nabfa/NABFA.html)

## The 4<sup>th</sup> Annual Meeting of the North American Black Fly Association

**Chair: Jay Overmyer**  
**Secretary: Mike Spironello**

---

### **Abstracts from Oral and Poster Presentations**

#### **Grades, clades, and the *Simulium malyschevi* species group: a total evidence approach**

**Mike Spironello<sup>1</sup> and Douglas C. Currie<sup>2</sup>**, <sup>1</sup>Department of Zoology, University of Toronto, <sup>2</sup>Department of Natural History, Royal Ontario Museum

Morphological- and cytological-based approaches to black fly systematics provide a solid framework from which to test evolutionary hypotheses derived from molecular data. Furthermore, the recent proliferation of molecular studies sets the stage for a “total evidence” approach to phylogeny reconstruction in black flies. In this study we performed a total evidence analysis of the *Simulium* (*s.s.*) *malyschevi* species-group using morphological, cytological, and molecular data. Morphological characters were scored from larval, pupal, and adult stages. Molecular data was obtained using three mitochondrial genes (12S, cyt b, and COII), and one nuclear ribosomal RNA gene (ITS). Only a single cytological character was identified in the current study. The total evidence analysis suggests that the monophyly of the *S. malyschevi* species-group, as currently defined, may be questionable with respect to the *S. jenningsi* species-group and *S. reptans* species group. Problems with the current concepts of these species-groups will be discussed.

#### **Screening for the presence of feminizing bacteria in nematode-infected black flies**

**Amy Sharp and Fiona F. Hunter**, Department of Biological Sciences, Brock University

Mermithid nematodes (Nematoda: Mermithidae) parasitize larval, pupal and adult black flies (Diptera: Simuliidae). Such parasitic infections often result in complete or partial feminization of genetic males. Sexual alteration in approximately 80% of all arthropods

has been attributed to interactions with the intracellular alpha-proteobacteria, *Wolbachia*. The observed relationship between black flies and mermithid nematodes affords the possibility that feminization in nematode-infected Simuliidae is the result of a feminizing bacterium. PCR methods were used to screen for the presence of *Wolbachia* in mermithid-infected black fly larvae and adults.

### **Black Fly Larvae and Algae: Assessment of Fan-Flick and Ingestion Rates**

**David R. Rouse, Jay P. Overmyer, and Elmer W. Gray**, University of Georgia

The green alga, *Scenedesmus quadricauda*, has been shown to negatively affect the efficacy of *Bacillus thuringiensis* var. *israelensis* (*Bti*) on larval black flies when present at concentrations > 8,000 cells/ml. However, other species of green algae, such as *Chlorella vulgaris*, have not shown this effect at similar concentrations. In an initial step towards determining the mechanism of reduced *Bti* efficacy by *S. quadricauda*, the flick rate of the cephalic fans on laboratory-reared black fly larvae, *Simulium vittatum* IS-7, and ingestion rate (passage of the algae through the midgut) were assessed. Results showed that the flick rate and ingestion rate of larvae feeding on *S. quadricauda* were significantly faster than larvae feeding on *C. vulgaris*. Potential implications of flick rate and ingestion rate on *Bti* efficacy will be discussed.

### **Esophageal armature: armed for what?**

**Samkyu Kim and Peter H. Adler**, Department of Entomology, Soils and Plant Sciences, Entomology Division, Clemson University 29634-0315

Larval esophageal armature was examined with scanning electron microscopy as well as phase-contrast microscopy. Scanning electron microscopy revealed a wealth of new characters for taxon diagnosis. Esophageal armature is assumed to function in drawing the peritrophic matrix from the cardia posteriorly with muscular activity.

### **Historical biogeography of western Cordilleran black flies (Diptera: Simuliidae) using a phylogeographical framework**

**Julio Rivera<sup>1</sup> and Douglas C. Currie<sup>2</sup>**, <sup>1</sup>Department of Zoology, University of Toronto, <sup>2</sup>Department of Natural History, Royal Ontario Museum

The present-day distribution of North American organisms was greatly influenced by climatic fluctuations that took place during the Pleistocene. During the Wisconsinan glacial maximum, most of northern North America was covered by thick layers of continental ice and organisms were restricted to peripheral refugial areas. When ice began to recede ca. 10,000 yr BP, organisms migrated from their refugia back to the now-unglaciated and available terrain. Some species of black flies are currently widely distributed throughout the western mountains (Cordillera). These populations had an exogenous origin in the northern Cordillera because they now inhabit an area that was unavailable for colonization until recently. Although the refugia from which these

populations originated is unknown, we hypothesize that they were derived from (a) northern refugia (= Beringia), (b) southern refugia (i.e., from the Cordillera south of Wisconsin ice) or (b) from a combination of these two refugial areas. We plan to investigate the origin of western Cordilleran black flies using a phylogeographic framework. Phylogeography is a biogeographic technique that combines phylogenetics and historical biogeography. Phylogeography investigates the genetic relationships and spatial distribution of common mitochondrial DNA (mtDNA) haplotypes, either between closely related species or between individuals from conspecific populations. Since mtDNA is transmitted matrilineally without recombination, this methodology allows us to trace the founding populations of black flies that gave rise to what are now widespread species by examining the geographical distribution of their mtDNA haplotypes. This study will concentrate on widely distributed members of the tribe Prosimuliini, including *Prosimulium travisi*, *P. esselbaughi*, *P. frohnei*, *P. neomacropyga*, *P. fulvum*, *Helodon pleuralis*, *H. onychodactylus*, *H. susanae*.

### **Black flies from the state of São Paulo, Brazil**

**Mateus Pepinelli<sup>1</sup>, Neusa Hamada<sup>2</sup> and Susana Trivinho-Strixino<sup>1</sup>**, <sup>1</sup>Laboratório de Entomologia Aquática, Departamento de Hidrobiologia, Universidade Federal de São Carlos, Caixa Postal 676, 13565-905, São Carlos, SP, Brazil, <sup>2</sup>Coordenação de Pesquisas em Entomologia, Instituto Nacional de Pesquisas da Amazônia, Caixa Postal 478, 69011-970, Manaus, AM, Brazil

Black flies were surveyed in 150 streams distributed over a wide range of stream/river conditions in the state of São Paulo, Brazil. Sixty of the streams are located in the Atlantic Forest mountain region and 90 in agriculture or pasture area (e.g., sugar cane and orange plantation). We collected 47 species; the number of species per site varied from 1 to 10. The total number of species collected was higher in the forested area (38) than in agriculture area (23). Even though many researchers have worked with black flies in the state of São Paulo, new species are still being described and new species records are being reported for this region, since 2003 the number of known species increasing from 42 to 55. High levels of variation have been observed in species of the subgenus *Inaequalium*, a group in which the majority of adults cannot be distinguished. Because of this, we started a cytological and molecular study on selected species of this subgenus.

### **What do black flies have that mosquitoes don't: culturing gut fungi of black flies**

**Charles E. Beard and Peter H. Adler**, Department of Entomology, Soils and Plant Sciences. Entomology Division, Clemson University 29634-0315

The Simuliidae are hosts of the symbiotic fungal genus *Harpella*. This fungus grows in the midgut of black flies, but apparently not in other Diptera such as mosquitoes and chironomids. The major constraint to understanding more about this fungus is that it has defied culturing attempts. Previous workers attempting culturing have not considered the pH of its habitat. Black fly midguts range in pH from 8 to 11 along their length. We have attempted to buffer the pH of culture media upward, which delays death of the cytoplasm,

but does not promote growth. Lipids also might be necessary, as well as enzymes. The midgut reportedly contains alkaline proteases. We have used insect cell-tissue culture media and have seen limited expansion of the fungal cytoplasm, but not normal growth, indicating that insect cells and the fungus have similar nutritive component requirements. Carbonates might be important because they are suspected to be the main natural buffer compounds in the midgut. Consideration is also being given to the use of undefined media, including homogenized larval black flies.

### **Gut Fungi (Trichomycetes) and Their Symbiotic Relationship with Several Dipteran Species**

**Svjetlana Vojvodic and John W. McCreadie**, Department of Biological Sciences,  
University of South Alabama

Trichomycetes (Zygomycota) are a cosmopolitan class of filamentous fungi that live in symbiotic relationship within the digestive tract of various arthropods. Our results indicate that the development of the trichomycete *Smittium culisetae* differs among host and temperature. In addition host and temperature also influences the morphology of *Sm. culisetae*. We also examined the potential for competition of several *Smittium* species using prevalence of thalli as the response variable. No evidence for competition between different species was found.

### **Distribution and life history of *Ectemnia invenusta* (Walker) (Diptera: Simuliidae) in Pennsylvania**

**D. I. Rebeck and M. E. Warfel**, Pennsylvania Department  
Environmental Protection, Division of Vector Management

The primarily northern Nearctic black fly, *Ectemnia invenusta* (Walker), was discovered in Pennsylvania for the first time in December 2001. Subsequent collections to determine the distribution of this black fly in the Commonwealth revealed the presence of the species in two streams in the southcentral region (Clark Creek, Stony Creek) and four streams in the northeast region (Big Bushkill Creek, Brodhead Creek, Little Bushkill Creek, Raymondskill Creek.) Life history of the species was studied in Big Bushkill Creek and Clark Creek through field collections of larvae and pupae and by rearing pupae to adults in the laboratory. Larvae anchored to substrates on silk stalks were collected from early November to mid March at water temperatures often near 0°C. Pupation occurred from early to late March. Adult emergence began in mid March from field-collected pupae reared in the laboratory. Additional Pennsylvania studies are needed to determine the timing of egg hatch in the fall and to identify ornithophilic hosts of the blood-feeding adult females. The discovery of *E. invenusta* in Pennsylvania significantly bridges the geographic gap between known northern and southern populations in eastern North America.

## **Rare species are rarely considered**

**<sup>1</sup>John McCreddie and <sup>2</sup>Peter Adler**, <sup>1</sup>Department of Biological Sciences, University of South Alabama, <sup>2</sup>Department of Entomology, Soils and Plant Sciences. Entomology Division, Clemson University 29634-0315

There are a variety of definition for what constitutes a rare species. For the purposes of this study proposal we will define a species of simuliid within an ecoregion as “rare” if it is found in less than 15% of sites. Rare species are often ignored in ecological studies because it is often assumed because they occur in small numbers, at few sites, or both, that any ecological data they generate can not be rigorously analyzed. We have developed a randomization program that can statistically test (at any desired p-value) if mean distance among sites where a “rare” species is found, is clumped, random or over-dispersed. This program required only the latitude and longitude of each stream site of interest. A clumped distributions would be consistent with poor powers of dispersal whereas an over-dispersed distribution would such a species of species with a very restricted host range. Results from several different areas in both North and South America are given.

## **Stable Isotope Dynamics in Black Flies**

**Jay P. Overmyer and Aaron T. Fisk**, University of Georgia

The use of stable isotopes of carbon ( $\Delta^{14}\text{C}$ ) and nitrogen ( $\Delta^{15}\text{N}$ ) for determining food utilization and trophic status has become popular in recent years. With the wide adoption of stable isotope analysis in food web studies, subsequent laboratory experiments have shown isotope dynamics to be more complex than had been previously recognized and concerns have been raised about the simplistic use of stable isotope analysis in ecology. While the understanding of stable isotope dynamics in higher trophic level organisms is minimal, even less is known about the stable isotope dynamics in aquatic invertebrates. Thus, the objective of this research was to study the dynamics of  $\Delta^{15}\text{N}$  in primary consumers using the black fly, *Simulium vittatum* IS-7. Preliminary results indicate that black flies are depleted in  $\Delta^{15}\text{N}$  relative to their food source and different life stages (larva, pupae and adult) have different  $\Delta^{15}\text{N}$  signatures. These results contradict the assumed  $\Delta^{15}\text{N}$  enrichment with increasing trophic level and might influence results obtained in organisms feeding on different life-stages.

## **Recent Advances in the Systematics of the World's Black Flies**

**Peter H. Adler**, Department of Entomology, Soils and Plant Sciences. Entomology Division, Clemson University 29634-0315

Cytogenetic analyses continue to reveal new species of black flies throughout the world and to provide insight into phylogenetic relationships. The number of known species of black flies in the various zoogeographic regions of the world, including an update on the number known from North America, will be presented, along with highlights of recently

inferred phylogenies of several groups of black flies. Evidence of speciation through hybridization in members of the *Prosimulium macropyga* species group will be discussed. This group of northern black flies includes a disproportionate number of hybridization events that have resulted in the propagation of parthenogenetic, all-female, triploid lineages. The parent species of these hybrid lineages have been revealed. The only other black flies in which hybrid lineages have been found are members of the northern genus *Gymnopais*. The *Prosimulium macropyga* group and *Gymnopais* provide insight into the factors that promote speciation through hybridization.

### **News of Black flies from Brazil**

**Neusa Hamada<sup>1</sup>, Sérgio Luis Bessa Luz<sup>2</sup> & Yamile Benaion Alencar<sup>1</sup>**, <sup>1</sup>Instituto Nacional de Pesquisas da Amazônia, Coordenação de Pesquisas em Entomologia, Manaus, Amazonas, Brazil, <sup>2</sup>Centro de Pesquisa Leônidas e Maria Deane, Fundação Instituto Oswaldo Cruz, Manaus, AM, Brazil

Information on black fly studies done by our research group in Manaus will be presented, including alpha taxonomy, cytotaxonomy and molecular studies. Our work focuses on black flies from the Amazon region, especially the subgenus *Psaroniocompsa*. Preliminary data from a recently started project on microorganisms associated with black fly larvae will also be presented.

### **The Trout Creek Drainage: A Further Test of the *Simulium arcticum* s. s./*S. apricarium* High and Low Elevation Hypothesis**

**Lindee Strizich and G. F. Shields**, Department of Natural Sciences, Carroll College, Helena, Montana

Shields *et al.*, (2005) described elevational differences in emergence sites for *S. arcticum sensu stricto* and *S. apricarium* in four drainages in western Montana. The former emerges at high elevations while the latter emerges at lower elevations. We used conventional methods of polytene chromosome analysis to again test this hypothesis in the previously unstudied Trout Creek Drainage in Lewis and Clark County, Montana. We studied the distribution of larvae of the *arcticum* complex at three sites (mouth, mid-elevation and the headwaters) throughout the summer of 2005. Members of the *arcticum* complex occurred at all three sites but in markedly different frequencies. Among 430 larvae analyzed, *S. apricarium* was the most abundant sibling (35.4% of males); however, it occurred only at the mouth site and, with one exception, only after 5/26. It was also the most prevalent sibling (55.2% of males) at the mouth site. A cytotype new to science, IIL-68 occurred at all three sites and was the next most abundant type in the drainage (29.6%). *S. brevicercum*, *arcticum* IIL-st/st, occurred at all three sites and was the third most abundant sibling (24.2%). *S. arcticum* s. s. was rare (7.1%) but was found at all three sites. These results support the "*S. arcticum* s. s. high/ *S. apricarium* low" hypothesis of Shields *et al.* (2005) in that *apricarium* occurred only at the low elevation site. Though *arcticum* s. s. occurred at intermediate and high elevations, it was the least abundant sibling of the drainage. Discovery of the IIL-68 cytotype (complete linkage to

males in 69 of 69 cases) supports our contention that the Northern Rocky Mountains are a "hot spot" of diversity within the *arcticum* complex.

### **A Longitudinal Analysis of the Distributions of Two Siblings of the *S. arcticum* Complex at Little Prickly Pear Creek, Lewis and Clark County, Montana**

**Greg Clausen and G. F. Shields**, Department of Natural Sciences, Carroll College, Helena, Montana.

The original study site at Little Prickly Pear Creek (LPPC) was the single exception among 14 other sites in five drainages for the "*S. arcticum s. s. High/S. apricarium Low*" hypothesis of Shields *et al.*, (2005). The ratio of *apricarium* to *arcticum s. s.* among 245 larvae analyzed over four years at this site was 0.257:1.0, while that at four other low elevation sites was essentially 1.0: 0. Accordingly, we analyzed the polytene chromosomes of nearly 400 larvae of the *arcticum* complex at four equally spaced sites from the original site to the mouth of the LPPC as it enters the Missouri River. We hypothesized that the proportion of *arcticum s. s.* to *apricarium* would decrease downstream and as temperatures of water increased as summer progressed. Approximately 100 larvae have been analyzed from each of the four sites. *S. arcticum s. s.* and *S. apricarium* were by far the most prevalent taxa, representing 93.4% of the *arcticum* complex at the four sites. *S. brevicercum (arcticum st/st)*, *S. saxosum*, *arcticum* IIL-10, *arcticum* IIL-15 and *arcticum* IIL-18 constituted the remaining 6.6% of taxa present. The proportion of *arcticum s. s.* to *apricarium* remained essentially the same (0.863:0.137) at all four sites from 3/31/05 to 5/6/05. However, by 5/26/05 *arcticum s. s.* had been completely replaced by *apricarium* at the mouth site. It does not appear that this change is temperature influenced however, since water temperature at the mouth site increased only one degree from 3/31/05 to 5/26/05. Heterozygosity for the IIL-20 autosomal inversion was high (0.078). The autosomal polymorphisms: IIL-33, IIL-56, and IIL-71 were found in low frequency. An apparent IIL-intrachromosome transposition was completely linked to the Y chromosome in four males. Six larvae were IIS-11 heterozygotes suggesting the possibility of limited hybridization between *arcticum s. s.* and *apricarium*. Nineteen of the 24 larvae analyzed from green trailing vegetation from the mouth site on 5/26/05 were *S. apricarium* while only five (all females) were *S. arcticum s. s.* Studies of larval distribution, egg deposition patterns and temporal transitions will continue in 2006.

### **Investigations into the Reproductive Status of Seven Siblings and Cytotypes of the *Simulium arcticum* Complex at Rock Creek, Missoula County, Montana**

**Judith Pickens and G. F. Shields**, Department of Natural Sciences, Carroll College, Helena, Montana.

Shields *et al.*, (2005) have documented the presence of three siblings (*S. brevicercum*, *S. arcticum s. s.* and *S. apricarium*) and four cytotypes ( IIL-9, IIL-13, IIL-17 and IIL-19) of the *Simulium arcticum* complex at a single site at Rock Creek, Missoula County, Montana. This speciose site provided the opportunity, not only to determine the

allochronic (temporal) sequence of emergences of these taxa but also, to investigate the reproductive status of each of these types (i. e. are they reproductively isolated). Based on previous analyses of this site we were aware that all seven types were present in early spring (March-April) and therefore, we made careful collections of this site at that time in 2005. We were also aware that certain autosomal polymorphisms were present (particularly, IS-1 and IL-1) among these populations. The presence of these polymorphisms potentially allowed us to determine Hardy-Weinberg equilibrium frequencies, and hence to potentially determine the reproductive status among these taxa. Larvae were collected in abundance, fixed in Carnoy's, stained in Feulgen, and analyzed (Shields and Procunier, 1982) according to sibling/cytotype and genotypic (st/st, st/i, i/i) frequencies. Among the 222 larvae analyzed, taxa were represented in the following proportions: *brevicercum* = 0.013, *arcticum s. s.* = 0.010, *apricarium* = 0.052, IIL-9 = 0.288, IIL-13 = 0.088, IIL-17 = 0.258, and IIL-19 = 0.291. *S. apricarium* appears reproductively isolated from all other taxa of the *arcticum* complex here since it is fixed for the IIS-11 autosomal inversion. *S. arcticum* IIL-9, IIL-13, IIL-17 and IIL-19 are all polymorphic for the IS-1 inversion. IS-1 is in equilibrium within the IIL-19 population;  $0.05 < p < 0.08$ . Equilibrium frequencies could not be accurately be determined for the other taxa since, at this time, our sample sizes are too small. The cytotypes IIL-9 and IIL-17 appear to emerge first at this site (3/5/02), therefore it may be possible to determine equilibrium frequencies for these two taxa by collecting early and in abundance (est. 1000 larvae) in spring of 2006.

### **A Cytogenetic Analysis of the *Simulium arcticum* Complex at the Little Blackfoot River (LBFR), Powell County, Montana**

**Kathryn C. Styren and G. F. Shields**, Department of Natural Science, Carroll College, Helena, Montana.

This two-year study: 1) investigated the distribution of taxa within the *arcticum* complex to test the "*arcticum* high/*apricarium* low" hypothesis of Shields *et al.* (2005), 2) analyzed the status of IIL-st/st males with reference to criteria of species identity for *S. brevicercum* (IIL-st/st) from the type locality in Utah (Adler, *et al.* 2004), 3) attempted to determine the reproductive status of the three siblings (*brevicercum*, *arcticum s. s.* and *apricarium*) and the four cytotypes (IIL-9, IIL-10, IIL-18 and IIL-30) within this drainage, and 4) conducted analysis of a high elevation site in 2005. Regarding objective 1), *S. apricarium* was found only at the Garrison, low elevation site, while *S. arcticum s. s.* was the second most abundant sibling at both Elliston (intermediate elevation) and Kading Campground (high elevation), thereby supporting the aforementioned Shields hypothesis. Regarding objective 2), we found no consistent criteria for identification of classic *S. brevicercum* (loose pairing at the base of IIL, differential expression of the puffing band in section 57, etc.) in this drainage. Thus, IIL-st/st males at LBFR may be a combination of both *brevicercum* and sex-exceptional males. Regarding objective 3) the frequencies of autosomal polymorphisms (IS-1, IL-1) were too infrequent to calculate equilibrium frequencies. Finally, sampling and analysis of the high elevation site in 2005 indicated that *S. arcticum s. s.* did not appear there until 6/27/05 and then in low frequency (of ten males: st/st = 7, *arcticum s. s.* = 2, IIL-18 = 1, and IIL-30 = 1). A



cytotype new to science, IIL-30, was found only in 11 males; this cytotype has also been found in three males in the near-by Trout Creek drainage.

### **Speciation in the *Simulium arcticum* Complex**

**Gerald F. Shields**, Department of Natural Sciences, Carroll College, Helena, Montana.

Nine sibling species and 17 cytotypes have been described for the *S. arcticum* complex in North America. Five of these siblings and 11 of the cytotypes occur in Montana. Since 2000 we have analyzed more than 5000 larvae of the *S. arcticum* complex at 50 sites in Montana and the Pacific Northwest, some sites having been studied throughout some summers and in multiple years. Taken as a whole, these observations have revealed interesting patterns of distribution and abundance of each sibling/cytotype. Siblings broadly distributed elsewhere: *S. brevicercum*, *S. saxosum*, *S. arcticum* s. s., *S. negativum*, *S. apricarium* are also broadly distributed in Montana. However, the 11 cytotypes discovered here have either distributions limited only to several drainages: (e. g. IIL-9, IIL-10, IIL-13, IIL-15, IIL-18, IIL-19, IIL-30) or to single sites (e. g. IIL-17, IIL-21, IIL-23, IIL-68). If extents of distribution are related to rates and extents of divergence, then we may be observing a spectrum of events in the divergence process between old siblings and new cytotypes. Cytotypes found at only one site are always present with numerous other siblings and cytotypes. And although it may be impossible to ever rule out allopatric speciation because we may never be able to describe past distributions accurately, emergence of novel types at unique locations with other siblings/cytotypes argues for the possibility of a sympatric origin. The focus of our current work is two-fold: 1) to investigate the reproductive status of cytotypes through estimates of equilibrium frequencies of autosomal polymorphisms shared by siblings and cytotypes in sympatry and 2) in collaboration with Doug Currie and Mike Spironello to compare DNAs of cytotypically verified individuals to construct a molecular phylogeny for the entire complex. We hypothesize that lineages leading to siblings will have deep roots, while those leading to the apparent new cytotypes will cluster toward the tips of clades. Distributions, chromosomal relationships, and DNA phylogenies will be discussed.

### **Where East meets West: diversity and biogeography of Chukotkan black flies (Diptera: Simuliidae)**

**Douglas C. Currie<sup>1</sup> and Peter H. Adler<sup>2</sup>**, <sup>1</sup>Department of Natural History, Royal Ontario Museum, <sup>2</sup>Department of Entomology, Soils, and Plant Sciences, Clemson University 29634-0315

As little as 10,000 yr BP, the northeastern corner of Siberia was joined to northwestern North America as part of a vast, treeless, landscape called Beringia. Rising sea levels following deglaciation inundated the Bering Land Bridge, isolating the biotas of East Beringia (Alaska and the Yukon Territory) from West Beringia (Eastern Siberia). Previous surveys suggested marked differences in the simuliid assemblages on either side of the Bering Sea, perhaps owing to a lack of detailed comparative studies. Our expedition to Chukotka (the easternmost corner of Asia) during the summer of 2005

revealed that the simuliid faunas of East and West Beringia are much more similar than previously supposed. Of 21 Chukotkan (i.e., West Beringian) morphospecies identified to date, all but one is known from East Beringia. Furthermore, cytological examination reveals no fixed chromosomal differences among any of the species so far examined. The 2005 expedition was hindered by lack of access to upland areas, which resulted in far fewer species than expected. We expect to redress this deficiency in 2006 by undertaking a 17-day rafting expedition along the Enmyvaam and Belaya Rivers between Elgygytgyn Lake and Ust Belaya Village. The results of this study will provide nomenclatural stability to the northern Holarctic Simuliidae, and will also provide grist for ongoing phylogeographical studies.

### **Do Black Flies Play Golf?**

**Kenneth Pruess**, University of Nebraska 68583-0816

Black flies are problems on some Nebraska golf courses. *Simulium johannseni* is an annoying, but rarely if ever biting, early spring species. *Simulium meridionale* is a human biter of minor importance. Both are most common along larger rivers in sparsely populated areas and control is not economically practical. *Simulium luggeri* is of major importance and control is practical in limited circumstances. *Simulium decorum* is important on one Lincoln course. Course managers correctly identify black fly adults as the problem but have little knowledge of black fly biology, and may misidentify midges, caddisflies, or even mayfly nymphs as black fly larvae. Timing of controls is often based on the presence of adults rather than larvae.

### **“Yes Virginia, there are lots of black flies in Vanuatu”**

**Doug Craig**, Department of Biological Sciences, University of Alberta

The number of known species (16) of *Hebridosimulium* from Vanuatu and Fiji is surprising, given that there have been two only recognized for the last 30 odd years. How one goes about dealing with such a plethora of new entities will be succinctly discussed and illustrated with multiple digital images. These will illustrate the taxonomic process of deciding on species and main lineages, and then extracting character states for phylogenetic analysis – the results of which will be shown. There are two well supported main lineages, one callipygous, the other steatopygous. Within the former, there are again, two well supported sub-lineages, one with dorsal tubercles on the larvae, the other without. The steatopygous lineage also has well supported clades. Tubercles and modifications to the posterior larval body appear to be adaptations to deal with fast water and are, in large part, concordant with respective habitats.

## **DNA barcoding for constructive taxonomy and diversity evaluation of black fly populations from Manitoba.**

**Alina Cywinska<sup>1</sup>, Michael Spironello<sup>2</sup>, and Fiona F. Hunter<sup>1</sup>**, <sup>1</sup>Department of Biological Sciences, Brock University, Ontario, Canada, <sup>2</sup>Department of Zoology, University of Toronto, Ontario, Canada.

The technique of DNA barcoding enables rapid collection of the necessary data for effective taxonomy in which the hypothesis of species monophyly can be tested. In this study, we “barcoded” (using a portion of the mitochondrial gene cytochrome oxidase subunit 1) multiple individuals collected from 49 sites throughout the province of Manitoba. Given this province had been heavily sampled in the past (the earliest 20 years ago), we were able to compare patterns of species richness from morphological taxonomy and sequence divergence thresholds. In this case, patterns of species richness were not significantly different. This indicates the reliability of DNA barcoding for species-level identifications.

## **Black fly proctology and mycology in North America**

**Mark P. Nelder<sup>1</sup>, Charles E. Beard<sup>1</sup>, Sam-Kyu Kim<sup>1</sup>, Peter H. Adler<sup>1</sup>, and John W. McCreadie<sup>2</sup>**, <sup>1</sup>Department of Entomology, Soils and Plant Sciences. Entomology Division, Clemson University 29634-0315, <sup>2</sup>Department of Biological Sciences, University of South Alabama

Trichomycetes are obligate, symbiotic fungi that colonize the digestive tracts of arthropods, including black flies. The black fly-trichomycete relationship represents one of the few examples in nature of a symbiotic continuum encompassing commensalism, mutualism, and parasitism. Black flies are model organisms for the study of trichomycete ecology because they are taxonomically well known, allowing precise identifications of trichomycete hosts. Worldwide, black flies are host to 8 genera and 35 species of gut fungi; however, in North America simuliid-trichomycetes are limited to 5 genera and 14 species. Here we report on the distribution and ecology of black fly-trichomycete associations in North America and suggest areas of future research.

## **Overview of black fly suppression operations in the Delaware and Schuylkill River watersheds in eastern PA**

**Benjamin Russell**, Black Fly Suppression Program, Pennsylvania DEP, Southeast Regional Office

The PA DEP Black Fly Suppression Program is a statewide effort to control the emergence of adult *S. jenningsi* complex human pest species. The program originated in central PA in the early 1980's and subsequently spread throughout PA over the next two decades. Control efforts in eastern PA have focused on portions of the Delaware River, Schuylkill River, and several tributary streams. Population control is effected through the

aerial or backpack application of Vectobac 12AS throughout the warm Spring/Summer tourism season.