THURSDAY, FEBRUARY 19TH

8:00 AM – 8:30 AM  CHECK-IN/REGISTRATION AT GEORGIA CENTER REGISTRATION DESK

8:30 AM – 8:45 AM  WELCOME TO THE GEORGIA CENTER: INTRODUCTIONS
John Walz, Metropolitan Mosquito Control District, St. Paul, MN

8:45 AM – 9:15 AM  STUFF ABOUT THE SIMULIUM GENOME
Charles L. Brockhouse (via teleconference), Department of Biology, Creighton University, Omaha, NE

9:15 AM – 9:45 AM  "YOU'VE GOT TO BE KIDDING MATE! FOSSIL LARVAL SIMULIIDS FROM KOOKWARA!"
Doug Craig, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA

9:45 AM – 10:15 AM SIMULIUM VITTATUM FEEDING BEHAVIOR AND BIOLOGICALLY-BASED LARVICIDES
Joseph P. Iburg, Elmer W. Gray, and Ray Noblet
University of Georgia, Athens, GA

10:15 AM – 10:30 AM  BREAK

10:30 AM – 11:00 AM  CHROMOSOMAL EVIDENCE FOR STABILITY OF COMMUNITY ASSEMBLAGES IN THE BLACK FLY, SIMULIUM ARCTICUM COMPLEX (DIPTERA: SIMULIIDAE)
Gerald F. Shields, Department of Natural Sciences, Carroll College, Helena, MT

11:00 AM – 11:30 AM  PA BLACK FLY SUPPRESSION PROGRAM: UPDATE AND A DEMONSTRATION OF THE PROGRAM DATABASE
Doug Orr, Pennsylvania Department of Environmental Protection, Black Fly Suppression Program, Harrisburg, PA

11:30 AM – 12:00 PM  PHOTOGRAPHY OF BLACK FLIES AND MOSQUITOES
Jena Johnson, Entomology Department, University of Georgia, Athens, GA

12:30 PM – 1:30 PM  LUNCH (PROVIDED AT GEORGIA CENTER)

1:30 PM – 2:00 PM  THE RELATIONSHIP BETWEEN SPECIES RICHNESS AND NICHE BREATH: COMPARISON OF TROPICAL AND TEMPERATURE STREAMS
John McCreddie¹, Peter Adler, Maria Eugen, and Neusa Hamada,
¹University of South Alabama, Mobile, AL
THURSDAY, FEBRUARY 19TH (CONTINUED)

2:00 PM – 2:30 PM  INVESTIGATING A RELATIONSHIP BETWEEN BLACKFLIES (DIPTERA: SIMULIIDAE), ONCHOCERCIASIS AND NODDING SYNDROME. AN ENTOMOLOGICAL PERSPECTIVE
Adam Hendy, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

2:30 PM – 3:00 PM  METROPOLITAN MOSQUITO CONTROL DISTRICT (MMCD) BLACK FLY CONTROL PROGRAM VIDEO AND PROGRAM UPDATE
John Walz, Casey Herrmann and Carey LaMere, Metropolitan Mosquito Control District, St. Paul, MN

3:00 PM – 3:30 PM  BREAK

3:30 PM – 4:00 PM  DIFFERENTIAL DEVELOPMENT OF MALE AND FEMALE LARVAE OF THE SIMULIUM ARCTICUM COMPLEX (DIPTERA: SIMULIIDAE)
Gerald F. Shields, Department of Natural Sciences, Carroll College, Helena, MT

4:00 PM – 4:30 PM  CLARK COUNTY VECTOR CONTROL: HISTORICAL TIMELINE
Chris Bramley, Vector Control, Clark County Dept. of Public Works, Las Vegas, NV

4:30 PM – 5:00 PM  BLACK FLIES DO NOT FEED BIOFILM TO TRICHOMYCETES
Charles E. Beard and Peter H. Adler, Clemson University, Clemson, SC

6:30 PM  BBQ DINNER AT THE UGA BEE LAB (Transportation will be provided)
UPDATE ON BEE LAB RESEARCH TO FOLLOW DINNER

FRIDAY, FEBRUARY 20TH

9:00 AM – 9:30 AM  BLACK FLIES AND WHOOPING CRANES - THE FINAL CHAPTER
Elmer W. Gray, University of Georgia, Athens, GA

9:30 AM – 10:00 AM  DISTRIBUTION AND NUISANCE PATTERNS OF SIMULIUM JENNINGSII IN WESTERN MARYLAND
Rebecca C. Wilson, Alan W. Leslie, Elanor Spadafora, and William O. Lamp, University of Maryland, College Park, MD

(not presented)  TOWARDS A PHYLOGENETIC FRAMEWORK FOR SIMULIUM SENSU LATO: INFERENCES FROM ELONGATOR COMPLEX PROTEIN 1 (ECP1) SEQUENCES
John K. Moulton, University of Tennessee, Knoxville, TN
FRIDAY, FEBRUARY 20TH (CONTINUED)

10:00 AM – 10:30 AM  BREAK

10:30 AM – 10:45 AM  "ZUMSTEIN'S TRACK STREAM, TALES FROM THE PARACNEPHIA FERGUSONI"
Doug Craig, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA

10:45 AM – 11:00 AM  WHERE DO I GO FROM HERE?
Gerald F. Shields, Department of Natural Sciences, Carroll College, Helena, MT

11:00 AM – 11:30 AM  BLACK FLIES AND THE COLORADO RIVER, USA-THE BULLHEAD CITY EXPERIENCE
Elmer W. Gray, University of Georgia, Athens, GA

11:30 PM – 12:00 PM  2016 MEETING PLANNING

LUNCH (ON YOUR OWN)

AFTERNOON  OPTIONAL ACTIVITIES:
TOUR OF RIVERBEND RESEARCH LABS - HOME OF THE BLACK FLY COLONY (Transportation will be provided)

GEORGIA MUSEUM OF ART (Short walk from campus)

STATE BOTANICAL GARDEN (Shuttle available from Georgia Center)

The participants of the North American Black Fly Association would also like to recognize, and thank the Department Head of the University of Georgia's Entomology Department, Dr. Raymond Noblet, and the Metropolitan Mosquito Control District for their continued support of our organization.

And Thanks to the Georgia Center for hosting our NABFA meeting!

PRESIDENT: JOHN WALZ
VICE-PRESIDENT: ELMER GRAY
PROGRAM EDITOR: CAREY LAMERE
T-SHIRT DESIGN: MARTY KIRKMAN/MOLLY NEE

www.nabfa-blackfly.org
BLACK FLIES DO NOT FEED BIOFILM TO TRICHOMYCETES
Charles E. Beard and Peter H. Adler, Clemson University, Clemson, SC

The midguts of larval Simuliidae are commonly colonized by the fungal trichomycete *Harpella melusinae*. We hypothesize that biofilms might be an important source of nutrition for Simuliidae and thus *H. melusinae* in the natural environment. *H. melusinae* is generally considered to have a commensal relationship with its hosts, but little is known of its nutritional relationship with the hosts. Taxonomic resolution of species in the genus has been difficult due to limited and variable morphological features and difficulty extracting DNA for genomic analysis. One way to provide tools for addressing questions about the fungus would be to grow cultures. However, *H. melusinae* has defied efforts to isolate and culture it *in vitro*. We have, therefore, tried *in vivo* assays to shed light on the nutritional sources. By providing different nutritional sources to Simuliidae colonized by *H. melusinae* and observing fungal growth characters, optimal food sources might be determined. Finding components in the stream that provide the best growth for the fungus in the host gut will provide information on nutrition sources for *in vitro* cultures. And also provide information relative to variations in field prevalence based on stream organic content. Typically, laboratory black flies are grown with a fish food diet, which supports *H. melusinae* growth in the larvae, but fish food does not support *in vitro* growth of *H. melusinae*. Therefore, we developed a food recipe which should mimic aspects of biofilms as a nutritional source. Black fly larvae (*Simulium innoxium*) with *H. melusinae* were field collected and placed in feeding containers. Food sources were provided and growth of *H. melusinae* was assayed (spore production and recolonization). The growth of *H. melusinae* did not qualitatively differ based on food source for the host larvae. Since biofilm components did not enhance growth, we reject the hypothesis that biofilms are a better nutritional source. The control, unfed larvae, also supported growth of *H. melusinae*, indicating that *H. melusinae* might be using components intrinsic to the host rather than depending on stream nutritional materials. This result raises the possibility of a mild parasitic relationship. Anecdotal assays support this possibility; larvae collected during periods of reduced host growth sometimes show lower colonization levels.

CLARK COUNTY VECTOR CONTROL: HISTORICAL TIMELINE
Chris Bramley, Vector Control, Clark County Dept. of Public Works, Las Vegas, NV

"YOU'VE GOT TO BE KIDDING MATE! FOSSIL LARVAL SIMULIIDS FROM KOONWARRA!"
Doug Craig, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA

The Early Cretaceous Koonwarra Fossil Bed in Gippsland, SE Victoria, Australia is famous for fish fossils, plus stunningly well-preserved feathers. The later possibly dinosaurian. There are also well preserved aquatic insects, including simulid larvae. Uncovered in the late 1960's the simulid larvae were superficially described in 1986. At that time a brief examination by DAC of eight of these specimens did not allow assignation to any simulid genus and they were put to one side, so-to-speak. New material from the fossil beds was examined recently (2014) and another 30+ specimens were recovered. Relationships are still not known, but the provenance of the fossil bed will be discussed. In the Early Cretaceous, Aptian (116mya), the fossil site was within view of Gondwanan Antarctica and so far south there would have 6 months of winter darkness. Although the Cretaceous was generally a major warm period, with markedly high sea levels, the Mid Aptian was cold enough that snow and ice during winter was possible.
Since description in 1925, Paracnephia fergusoni of Australia has been known formally only from female adults. In 2011 some larvae and pupae from a culvert on Serra Road, Grampians National Park, Victoria, Australia, were unidentifiable until adults were reared. Then the lack of a calcipala and pedisulcus confirmed the identity of the immatures - *fergusoni*. Two years later, that culvert, at the same time of year, was, surprisingly, totally dry and clearly had not run with water for a considerable time. However, a serendipitous find in the stream along Zumstein's Track allowed the full life cycle to be recovered again and, further, provide phenological data. Namely, that inhabiting intermittent streams means *fergusoni* adult females should be flying wide-spread to find other habitats. This hypothesis was tested at Mackenzie Falls where Kevin Moulton netted numbers of *P. fergusoni* females, well removed from apparent suitable habitats. Similarly at Hollow Mountain further north in the Grampians. Two months later, with flow greatly reduced, *P. fergusoni* immatures were gone from Zumstein's, replaced by vast numbers of those of *Simulium ornatisipes*. No adults of any simulid were recovered from there, or Mackenzie Falls, at that time. So? Univoltine??

BLACK FLIES AND WHOOPING CRANES - THE FINAL CHAPTER

Elmer W. Gray, University of Georgia, Athens, GA

No abstract submitted

INVESTIGATING A RELATIONSHIP BETWEEN BLACKFLIES (DIPTERA: SIMULIIDAE), ONCHOCERCIASIS AND NODDING SYNDROME. AN ENTOMOLOGICAL PERSPECTIVE

Adam Hendy, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

More than fifty years after a head-nodding disorder was first described in the Mahenge mountains of Tanzania, a recent interest in nodding syndrome (NS) has prompted a flurry of hypotheses regarding its aetiology. This interest has been spurred by the epidemic occurrence of NS cases in parts of South Sudan and northern Uganda where prevalence estimates are as high as 8.4% in the worst affected areas. Characterized by ‘head-nodding’ seizures, this childhood epileptic disorder can have a devastating effect on individuals and place a disproportionate burden on communities. Case-control studies have repeatedly alluded to an association between NS and onchocerciasis, but a definitive role for the parasite in pathogenesis of the disease has not been established. Recent, but currently unpublished work exploring the possibility of *Onchocerca volvulus* infection triggering an autoimmune response in NS-affected individuals may further strengthen the argument that *O. volvulus* is implicated in NS. However, questions still remain about the relatively isolated distribution of this disease given that onchocerciasis is still widespread throughout much of Sub-Saharan Africa. The occurrence of anomalous blackfly species and behaviours in areas of northern Uganda affected by nodding syndrome may also provide impetus for further entomological research which could help clarify the importance of this perceived NS-*Onchocerca* relationship.

SIMULIUM VITTATUM FEEDING BEHAVIOR AND BIOLOGICALLY-BASED LARVICIDES

Joseph P. Iburg, Elmer W. Gray, and Ray Noblet, University of Georgia, Athens, GA

For many years we have been interested in environmental factors that affect the susceptibility of black fly larvae to *Bti* proteins. Our research revealed that the efficacy of a *Bti* application can depend on the types of suspended materials in the waterway. Clay materials have little to no effect on *Bti* activity; however certain types of algal cells can have a significant effect. Using fluorescent particles and videography we have been able to study the feeding behavior of larvae when confronted with various types of particles. With the exception of diatom frustules, materials with a larger particle size had a greater effect on larval feeding behavior. Larvae flicked their cephalic fans less often when confronted with large particles and spent more time scraping material from them. Diatom frustules caused larvae to retract their fans for long periods of time, most likely due to abrasion. These changes in feeding activity may explain why there has been less-than-optimum control in certain locations over the years.
PHOTOGRAPHY OF BLACK FLIES AND MOSQUITOES

**Jena Johnson**, Entomology Department, University of Georgia, Athens, GA

Photography of small aquatic insects can be particularly challenging. Research published about insects, whether in more traditional print form or online is always enhanced with high quality color photographs that serve to convey a message, illustrate a behavior or educate the viewer. Readers of the popular scientific press have high expectations for compelling and illustrative photographs. There are two approaches to photography of small insects. For extremely small subjects that are 3mm or less a microscope is often necessary. Many subjects larger than 2-3mm can be photographed with a DSLR camera and macro lens. Adequate lighting is essential. Computer software programs that help the photographer to manage, edit and enhance their photos are equally important to create a good insect photograph.

THE RELATIONSHIP BETWEEN SPECIES RICHNESS AND NICHE BREATH: COMPARISON OF TROPICAL AND TEMPERATURE STREAMS

**John McCreadie**, Peter Adler, Maria Eugenia, and Neusa Hamada, University of South Alabama, Mobile, AL

No abstract submitted

TOWARDS A PHYLOGENETIC FRAMEWORK FOR *SIMULIUM SENSU LATO*: INFERENCE FROM ELONGATOR COMPLEX PROTEIN 1 (ECP1) SEQUENCES

**John K. Moulton**, University of Tennessee, Knoxville, TN

Results of phylogenetic analyses of 2,000+ nucleotides from the gene encoding elongator complex protein 1 (ECP1) acquired from all suitably preserved superspecific taxa within the genus *Simulium* Latreille currently available to the author are presented. Approximately 37 subgenera are represented, including several (e.g., Notolepria, Ectemnaspis/Psilopelmia, Thyrsopelmia/Trichodagmia, & Edwardsellum) housing species that serve as vectors of river blindness in the Americas or Africa. Roughly ten species groups within *Simulium sensu stricto* are also included in the analyses. Major findings are as follows: *Hellichia Rivosecchi* is the sister group to all other *Simulium*; most superspecific groups belong to either of putative sister groups comprised of chiefly ornithophilic or mammalophilic taxa; the Neotropical *Simulium* segregates form a clade and are the sister group to a clade containing *Obuchovia*, *Boopthora*, and *Simulium sensu stricto*; primary vectors of human onchocerciasis in Africa versus the Americas are distantly related; and several currently recognized superspecific groups appear untenable, many of which opposed by strong statistical support.

PA BLACK FLY SUPPRESSION PROGRAM: UPDATE AND A DEMONSTRATION OF THE PROGRAM DATABASE

**Doug Orr**, Pennsylvania Department of Environmental Protection, Black Fly Suppression Program, Harrisburg, PA

No abstract submitted

CHROMOSOMAL EVIDENCE FOR STABILITY OF COMMUNITY ASSEMBLAGES IN THE BLACK FLY, *SIMULIUM ARCTICUM* COMPLEX (DIPTERA: SIMULIIDAE)

**Gerald F. Shields**, Dept. of Biological Sciences, Carroll College, Helena, Montana, 59601

I used a 13 year data set of cytogenetic determinations of more than 16,000 larvae of the *Simulium arcticum* complex from 241 collections at 67 sites to assess whether taxon presence and frequency at a specific location one year changed in subsequent years. Such long-term studies within a complex of black flies are nonexistent but could suggest how females choose oviposition sites. At least three outcomes are possible. 1) the same sites could be chromosomally similar from year-to-year, suggesting taxon specific microhabitat selection, 2) the same sites could be chromosomally different from year-to-year, suggesting that gravid females randomly choose oviposition sites or that environmental perturbation has occurred, or 3) geographically distant sites could be ecologically similar suggesting the possibility that females may use similar physical and ecological cues to oviposit. I used Morisita’s Dominance Index of Community Similarity to compare species composition of communities. I also used the Bray-Curtis Polar Ordination Procedure to plot populations along a similarity-dissimilarity matrix. In 30 of 36 comparisons taxa present at a site the first year were also present in subsequent years. Variation among years at the same site always involved small sample sizes. Taxon diversity was also similar at six
sites after five, six, seven, eight and nine years. These observations might suggest that gravid females use similar physical
and ecological cues to select specific microhabitats to oviposit.

DIFFERENTIAL DEVELOPMENT OF MALE AND FEMALE LARVAE OF THE SIMULIUM ARCTICUM COMPLEX
(DIPTERA: SIMULIIDAE)

Gerald F. Shields, Dept. of Biological Sciences, Carroll College, Helena, Montana, 59601

I used a 13-year data set of chromosomally analyzed larvae to determine if sexes differed in development. My analysis
was restricted to the first generation at each site since later collections could potentially confuse analysis. I simply
determined the sex ratio (Feulgen-stained gonads) of early (before March 31), mid-spring (between March 31 and April
15), and late (after April 15) at each site. In 34 of 41 comparisons before March 31 males were more abundant (62%).
Between March 31 and April 15 females outnumbered males (52.5%) but after April 15 sex ratios were about equal.
These observations may be relevant to managers who treat streams with larvicides to control outbreaks of black flies.
Though restricted to the S. arcticum complex, this study might suggest a delay in treatment when females (biters) are most
abundant as potential emergers.

WHERE DO I GO FROM HERE?

Gerald F. Shields, Dept. of Biological Sciences, Carroll College, Helena, Montana, 59601

In collaboration with Drs. Peter Adler of Clemson and Doug Currie and Ida Conflitti of Toronto we have used
cytogenetics, molecular genetics and other features of biology to described nine species and at least 22 cytotypes within
the Simulium arcticum complex (Diptera:Simuliiidae). With the exception of S. negativum, comparisons of DNA
sequences of both mitochondrial and nuclear genes of these taxa suggest non-monophly. That is, taxa described by
morphology of Y chromosomes are not congruent with the molecular data. This has lead us to suggest, contrary to
accepted dogma, that chromosome change, particularly chromosome inversions in putative Y chromosomes, precedes
complete molecular divergence. We have proposed a chromosome model of speciation based on these data. However, it
is possible that our DNA data are not properly tracking molecular divergence. Alternatively, since inverted chromosome
segments may forgo recombination, they themselves may be changing more rapidly than other portions of the genomes,
and thus comparisons of their sequences may best represent the real molecular divergence. Presently, we have no
methodology which allows us to specifically isolate these inverted segments.

METROPOLITAN MOSQUITO CONTROL DISTRICT (MMCD) BLACK FLY CONTROL VIDEO AND PROGRAM UPDATE

John Walz, Casey Herrmann, and Carey LaMere, Metropolitan Mosquito Control District, St. Paul, MN

The goal of the Metropolitan Mosquito Control District’s (MMCD) Black Fly Control Program is to reduce pest
populations of black flies within the MMCD to tolerable levels. The MMCD monitors 171 small stream sites and 28 large
river sites in the 7-county metropolitan area surrounding Minneapolis-St. Paul. An update of the 2014 season will be
discussed along with a short video of control operations.

DISTRIBUTION AND NUISANCE PATTERNS OF SIMULIUM JENNINGSI IN WESTERN MARYLAND

Rebecca C. Wilson, Alan W. Leslie, Elanor Spadafora, and William O. Lamp

University of Maryland, College Park, MD

During the summers of 2013 and 2014, black fly larvae and adults were collected from Washington County, Maryland
and surrounding areas to determine the extent of a reported pest problem. Objectives in this study were to determine the
species, larval source, distribution, and resident perception of the nuisance black flies. Collection kits were given to
residents to sample adult flies. All black flies from these collections were identified as Simulium jenningsi. Larval
specimens were collected from streams of all sizes in the region. S. jenningsi was primarily found in the Potomac River,
with some larvae found in its larger tributaries. To determine the distribution patterns of the adult flies, we sampled using
an aerial net at 125 locations during the summer of 2014. A survey hosted at www.mdblackfly.com was used to assess the
geographic range of nuisance complaints and record how the flies have impacted the lives of residents. Both the aerial net
sampling and survey replies indicated the Pleasant Valley region of Washington County to be an area with high numbers
of adult S. jenningsi.