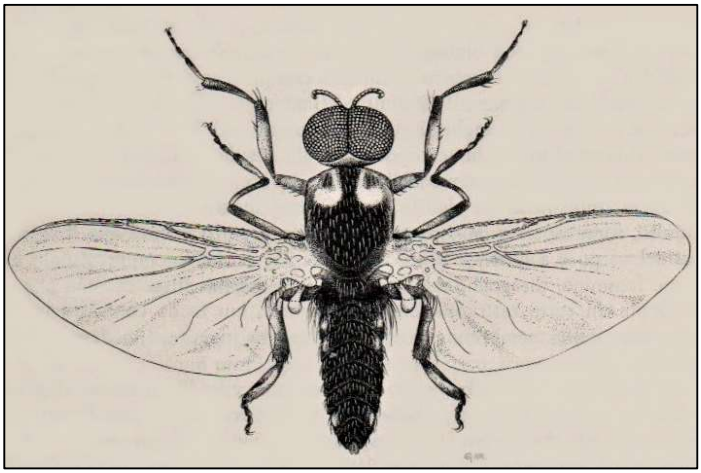
The Simuliid Bulletin

Number 57 January 2022



THE SIMULIID BULLETIN

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From the Editor

It is already two years and four Simuliid Bulletins ago that a virus, which has nothing in common with black flies but affects everything worldwide, started to reduce our in-person contacts. We were informing you mostly about postponed and cancelled meetings. In the beginning, many of us probably did not believe it would last long. Although the practical details on forthcoming events are still missing, there are indications that the situation is finally improving. If the lockdowns and travel restrictions are lifted, we will have the opportunity to meet at the IXth International Simuliidae Symposium in Morocco in September 2022.

Until then, we want to encourage you to share your papers, research ideas, or comments on the pages of the Simuliid Bulletin.

Tatiana Kúdelová, Editor

FORTHCOMING MEETINGS

The IX International Simuliidae Symposium, Chefchaouen, Morocco

Dear friends and colleagues,

If the pandemic situation improves, The IXth International Simuliidae Symposium is planned for

September 2022

You are cordially invited by Boutaïna Belqat and the other members of the organizing committee to **Chefchaouen, Morocco** to meet with colleagues and share your research ideas on black flies.

The exact dates, prices, and further details will be available at the Simuliid Bulletin website.

MEETING REPORTS

10th EMCA Conference: "New insights into mosquito and blackfly control"

David López-Peña
Universitat de València, Institut Cavanilles de Biodiversitat i Biologa Evolutiva,
Laboratory of Entomology and Pest Control,
C/ Catedrático José Beltrán, 2, 46980 Paterna (Valencia), Spain

The most recent European Mosquito Control Association (EMCA) International Conference was held in Vienna, Austria, between the 3rd and 7th of October. It was particularly relevant because it was the first time that blackfly research was included as a central topic of the conference, as indicated in the conference theme: "New insights into mosquito and blackfly control".

The session "Blackflies in Europe: where are we, where do we go?" on Tuesday 5th October consisted of several pertinent talks from blackfly researchers. The keynote was delivered by R. Bernotiene from the Nature Research Centre, Lithuania and A. Ignjatović Ćupina from University of Novi Sad, Serbia, and was titled *Blackfly pest species and their control: experience from Lithuania and Serbia*. Following this were three additional presentations. The first one, *DNA barcoding, identification, and taxonomy of blackflies in Europe,* was based on work by M. Kúdela, T. Kúdelová, B. Bujačková, I. Lužáková and S. Krčmárik from Comenius University in Bratislava, Department of Zoology, Slovak Republic. The second presentation was *Recovering blackfly (Diptera: Simuiidae) sample records for Tormes river basin in Spain* by D. López-Peña[1], J.D. Asís-Pardo[2], M. Portillo-Rubio[2] and R. Jiménez-Peydró[1] from [1]Universitat de València (Estudi General), Institut Cavanilles de Biodiversitat i Biologa Evolutiva, Laboratory of Entomology and Pest Control, Spain, and [2]Universidad de Salamanca, Facultad de Biología, Área de Zoología, Departamento de Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola, Campus Miguel de Unamuno, Spain. The final talk was, *Blackflies (Diptera: Simuliidae) and their parasitic watermites (Acari: Hydrachnidia), eastern Spain* by D. López-Peña[1], R. Gerecke[2] and R. Jiménez-Peydró[1] from [1](see above) and [2]University of Tübingen,

Department of Evolution and Ecology, Tübingen, Germany. Details about all of the mentioned presentations are available at the special Supplement 1 of the Xth International EMCA Conference of the Journal of the European Mosquito Control Association volume 39, ISSN 2054-930X (https://www.wageningenacademic.com/doi/epdf/10.52004/jemca2021.s1).



The attending audience was highly interested in all of the presentations and asked several questions. The attendees also took advantage of the more relaxed atmosphere during the coffee breaks to make conversation with members of the blackfly contingent and ask further questions about their research.

The conference programme also included an excursion, which consisted of two different parts. The first one was a mosquito control demonstration. Attendees witnessed the aerial application of larvicide using a helicopter and a drone. The larvicide consisted of *Bacillus thuringiensis* subspecies *israelensis* (Bti) in granulate format to control the larval populations of mosquito pest species. All of it was performed by Heli-Austria (www.heli-austria.at). The experience was enjoyable and educational because it allowed the attendees to witness where and how the product is loaded, the suitable areas for that purpose, and the way the manned and unmanned aircraft carry out the product application (see the attached picture).

The second part involved a visit to the Palace of Schloss Hof (Schlosshof). The tour guides kindly explained the history of this amazing building. We were shown the astonishing rooms, the cosy and bright interior patios, the geometrically-designed and colourful exterior gardens, and a school farm and petting zoo in which Jezersko-Solčava sheep with four horns and a white baroque donkey stood out among other rare breeds. The guide highlighted that the 'White Baroque Donkey' (*Equus asinus f. africanus* Linnaeus, 1758) is an exceptionally rare breed of which there are only a few hundred individuals left. With a height of 105-120 cm, a weight of approximately 200 kg and a life expectancy of 40 years, the white baroque donkey was originally bred in the 16th century. During that period, the white colour was considered very special. Besides that, they are characterized by having a slight yellow tint called "cremello", as can be seen in the picture.



Eventually, the attendees were welcomed to a living room of great dimensions where the buffet gala dinner took place. This was accompanied by a live concert of Austrian traditional folk music performed by musicians in traditional attire. For those wanting extra information about the palace, visit the following link https://www.schlosshof.at/en/.

SCIENTIFIC PAPERS

Intriguing Genes: Expressed Sequences from the *Simulium vittatum-tribulatum* complex. IV. You are what you eat.

Charles L. Brockhouse*[1], Julia A. Brockhouse[1,2], and Alexie Papanicolaou[3]

1: Marine and Aquatic Genetics Laboratory, Biology Department, Creighton University, Omaha, NE, 68178, USA
2: Parks College of Engineering, Aviation and Technology, St. Louis University, St. Louis, MO, USA
3: Hawkesbury Institute for the Environment, Western Sydney University, Sydney, Australia

*Correspondence to: charlesbrockhouse@creighton.edu

Introduction

The *S. vittatum-tribulatum* transcriptome on which these "Intriguing Genes" reports are based consist of assemblies of approximately 28,000 *S. vittatum* expressed gene sequences ("contigs"), 38,000 *S. tribulatum* contigs, and 39,000 contigs co-assembled from both species. The *vittatum* material was provided by the UGA Entomology colony, while the *tribulatum* was collected from a stream south of Omaha, Nebraska. Each species was further subdivided by sex and developmental stage to allow comparison of the expression levels during development. RNA was extracted from different stages, reversed transcribed to cDNA, and sequenced. The sequence reads were then assembled into longer sequences ("contigs") that represent *expressed* genes, not merely genes present in the organism. The unassembled reads from each stage can then be mapped onto each contig to give a relative measure of how much each contig is expressed in the different stages. More detailed background on the project was given in previous Bulletin issues.

We found 192 contigs expressed during larval stages, but not in adults, while 619 are expressed during all adult stages but not in the larvae. A full analysis of the differentially expressed genes is included in our upcoming transcriptome paper, but we previously presented one particular group in our NABFA and International

Simuliidae Symposium presentations: sequences derived from the diet of the larvae. Blackfly larvae are well established as efficient filter feeders, but also grazers. Here we give genomic evidence of ingestion of rodent tissue by blackfly larvae, uncovered during manual curation of larval-specific genes.

Results:

During manual curation, three larval-specific sequences were found to have no match to Dipteran genomes in standard BLAST searches (Altschul et al., 1990), but had multiple exact matches to mouse sequences, and looser matches against human and other mammalian sequences. Two of the sequences were expressed in both sexes and one was confined to females. Both show a relatively low expression (measured by FPKM value; Fragments Per Kilobase of transcript per Million mapped reads).

1) The mouse lipocalin 2 gene:

The first two contigs matched different portions of a mouse lipocalin gene and shared the same expression profile suggesting that they are sequenced fragments of the same mRNA molecules.

>c170563_g1_i1|m.73248 matches rodent lipocalin 2 gene
CGGTCCAGAAAAAAAACAGAAGGCAGCTTTACGATGTACAGCACCATC
TATGAGCTACAAGAGAACAATAGCTACAATGT
CACCTCCATCCTGGTCAGGGACCAGGACCAGGGCTGTCGCTACTGGAT
CAGAACATTTGTTCCAAGCTCCAGGGCTGGCC
AGTTCACTCTGGGAAATATGCACAGGTATCCTCAGGTACAGAGCTACAA
TGTGCAAGTGGCCACCACGGACTACAACCAG
TTCGCCATGGTATTTTTCCGAAAGACTTCTGAAAACAAGCAATACTTCAA
AATTACCCTGTATGGAAGAACCAAGGAGCT
GTCCCCTGAACTGAAGGAACGTTTCACCCGCTTTGCCAAGTCTCTGGG
CCTCAAGGACGACAACATCATCTTCTCTGTCC
CCACCGACCAATGCATTGACAACTGA

>c178935_g1_i1|m.73902 matches rodent lipocalin 2gene
TTCCGGGGCAGGTGGTACGTTGTGGGCCTGGCAGGCAATGCGGTCCA
GAAAAAAACAGAAGGCAGCTTTACGATGTACAG
CACCATCTATGAGCTACAAGAGAACAATAGCTACAATGTCACCTCCATC
CTGGTCAGGGACCAGGACCAGGGCTGTCGCT
ACTGGATCAGAACATTTGTTCCAAGCTCCAGGGCTGGCCAGTTCACTCT

GGGAAATATGCACAGGTATCCTCAGGTACAG
AGCTACAATGTGCAAGTGGCCACCACGGACTACAACCAGTTCGCCATG
GTATTTTTCCGAAAGACTTCTGAAAACAAGCA
ATACTTCAAAATTACCCTGTATGGAAGAACCAAGGAGCTGTCCCCTGAA
CTGAAGGAACGTTTCACCCGCTTTGCCAAGT
CTCTGGGCCTCAAGGACGACAACATCATCTTCTCTGTCCCCACCGACC
AATGCATTGACAACTGA

FPKM in female larvae: 0.79
FPKM in male larvae: 1.8
FPKM all other stages: 0

Fig. 1. Alignment of a *S. tribulatum* larval expressed sequences with a mouse lipocalin 2 gene. The two sequences are exact matches. (NCBI Megablast output)

2) The mouse aldolase gene:
This mouse sequence was confined to female larvae.

>c40815_g1_i1|m.1135 female larvae only mouse aldolase
AGCATCTGCCAGCAGAATGGCATTGTACCCATTGTGGAGCCTGAAATTC
TCCCTGATGGGGACCATGACTTGAAGCGCTG
CCAGTATGTTACTGAGAAGGTCCTGGCGGCTGTCTACAAGGCTCTGAG
CGACCACCATGTCTATCTGGAAGGCACATTGC

TGAAGCCCAACATGGTCACCCCTGGCCATGCTTGCACCCAGAAATTTTC
CAATGAGGAGATTGCCATGGCAACGGTCACA
GCACTTCGTCGCACAGTGCCCCCTGCTGTCACTGGGGTCACTTTCCTG
TCTGGAGGGCAGAGTGAGGAAGAG

FPKM female larvae: 1.13.
FPKM all other stages: 0

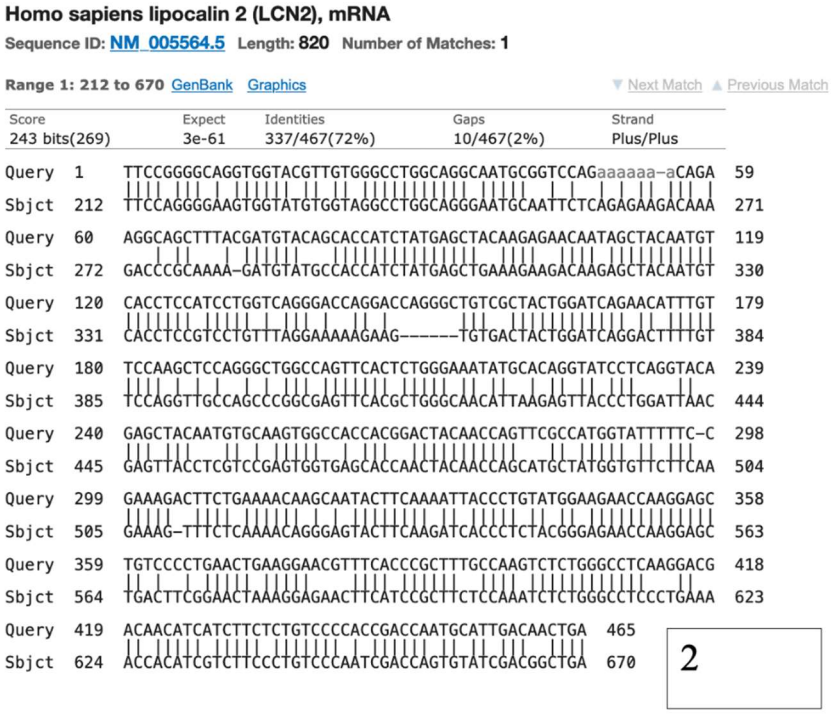

Fig. 2. Alignment of the *S. tribulatum* putative mouse sequence with the human lipocalin 2 gene, showing multiple mismatches. The blackfly sequence does originate from human contamination. (NCBI Megablast output)

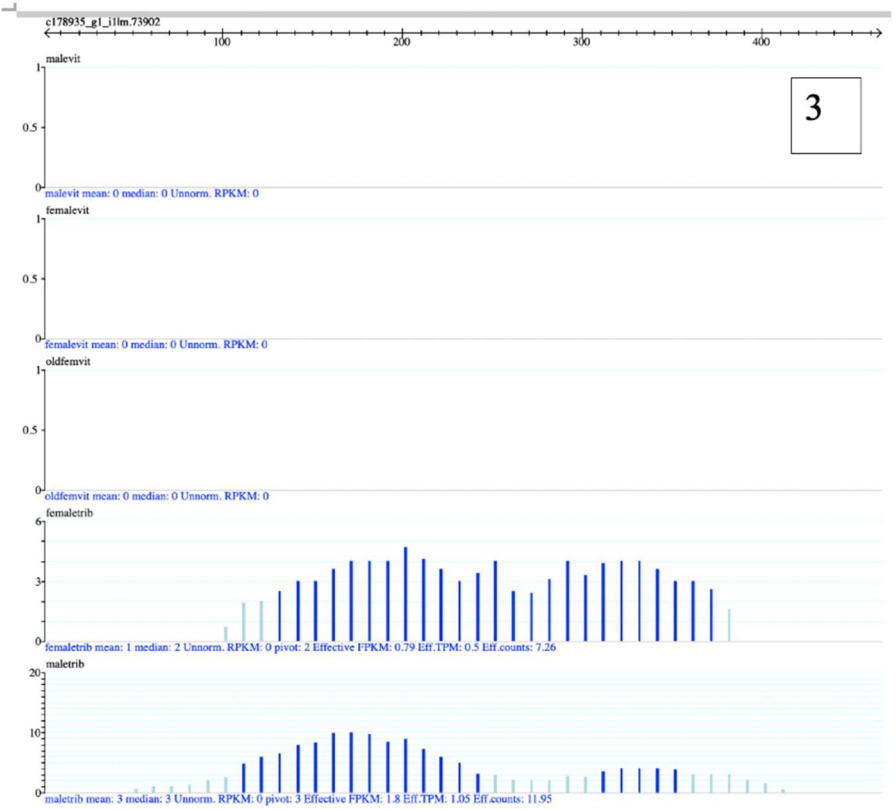

Fig. 3. Expression profiles of 2 fragments of a mouse lipocalin gene. The top 3 panels represent the adult stage; larval females and males are shown in the bottom 2 panels. The mRNA for this sequence was present only in the larval samples.

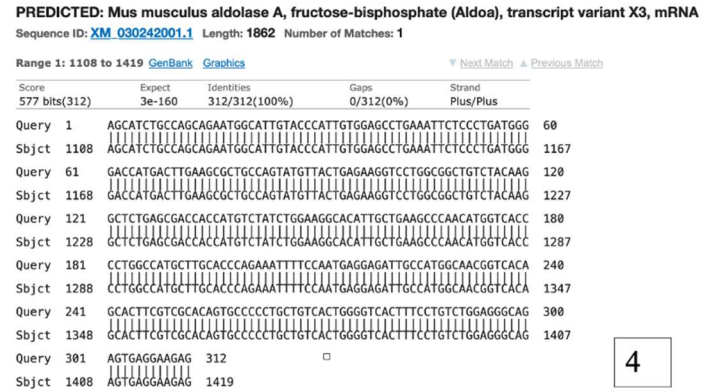

Fig. 4. Sequence alignment between blackfly contig and mouse aldolase sequence (NCBI Megablast output)

Fig. 5. The expression profile of a mouse aldolase gene. The cDNA was present only in female larvae, and absent from all other stages.

Discussion:

The presence of mouse RNA molecules in the Omaha larval, but not the adult colony, samples strongly suggests that the *tribulatum* larvae ingested mouse tissue. The sequences were not simply conserved genes, as they exactly matched the mouse but had multiple mismatches to human, and no hits to dipterans under the same search conditions.

Mouse sequences were present in both sexes, demonstrating that multiple individuals "ate" mouse. The simplest explanation is the presence of a dead mouse at or upstream from the collecting site. We cannot determine if the larvae accidently collected mouse tissue fragments while filter feeding, or if some larvae deliberately grazed from a mouse body.

Methods and References:

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

BLAST searches were performed using NCBI web interface.

□

Notes for Contributors

To avoid copy-typing, the editor (address above) would prefer to receive contributions on disc or by e-mail, or typewritten. Details as follows:-

1. Via conventional mail on CDRom or IBM PC formatted 1.4Mb 3.5 inch diskettes, as unmodified word processor files (most common DOS or Windows word processor formats are acceptable) or as RTF, PDF, ASCII or DOS text files (We usually have to change pagination and heading format, anyway). Mark the disc with the format, word processor name and file name(s). Complicated tables and figures can be accepted as separate graphics files (not OLE embedded, please!) but we may ask for a hard copy as a check that all detail has been retained. Remember that figures should have legends and small detail drawn large enough to be visible when reduced to 100mm by 70mm. Diskettes will be returned on request.
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Content covers scientific papers, short research notes, notices and accounts of meetings, and articles of anecdotal or general interest that would not normally be found in international journals. Geographical cover is world-wide. Reports of research carried out by graduates, young scientists and newcomers to the subject are particularly encouraged. It is an ideal medium for offering new ideas and stimulating discussion because of the very short interval between acceptance and publication. Contributions may be accepted up to two weeks before the publication dates at the end of January or July.

The Simuliid Bulletin conforms to the requirements of the International Code of Zoological Nomenclature.
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