Brazil '19

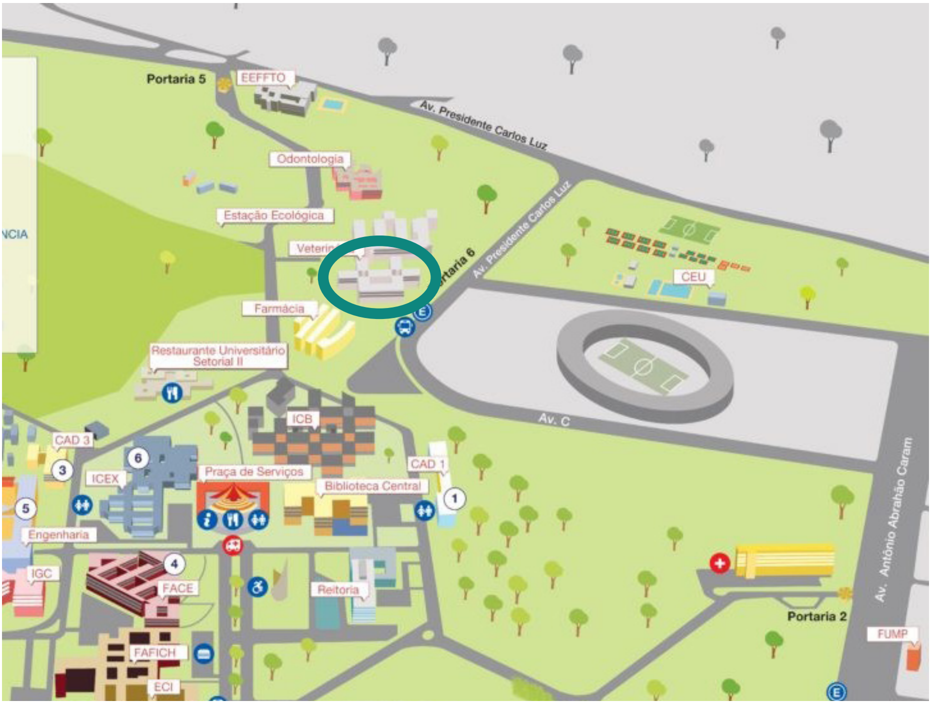
Conference Booklet
4th November 2019

The Pirbright Institute
London School of Hygiene & Tropical Medicine
GCRF Global Challenges Research Fund
BBSRC bioscience for the future

Contents

Location

UFMG Veterinary School, Av. Antônio Carlos 6627, Caixa Postal 567
Campus Pampulha da UFMG, CEP: 31270-901, Belo Horizonte, MG



Welcome from Brazil

Dear colleagues and guests,

On behalf of the local organizing committee and the organizing committee of the Pirbright Institute, it is my great pleasure to welcome you to Belo Horizonte, MG for the Second International Gnatwork Workshop, Brazil ‘19, held on 4 – 8 November 2019 at Escola de Veterinária da UFMG, Belo Horizonte, Brazil. The meeting is sponsored by The Pirbright Institute, UK (TPI) and held in collaboration with the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil.

The Gnatwork workshop brings together an international group of researchers on biting midge, blackfly and sandfly biology. The weeklong workshop will consist of a one-day conference, where 10 researchers will present important scientific data and highlight the current knowledge in the field. At the end of the conference, we invite you to participate in the poster presentation by the early career researchers, which will be a great opportunity to interact with the participants while enjoying a “beer break”.

Following this, a four-day workshop will be held to develop the skills of 30 early career researchers with a basic suite of expertise on these three vector groups. The four-day hands-on training workshop and interactive lectures will cover topics such as vector biology, capture techniques, morphological identification, molecular barcoding, and statistical analysis.

The goals of these International Workshops are to improve the link between researchers from different countries by creating a global community of researchers studying the biology of biting midges, blackflies and sandflies; as well as to offer training and qualification of early career researchers in the capture and identification techniques of the three vector species, as well as in experimental design.

We hope to achieve our goals, creating a worldwide network of scientists working with these three neglected vector groups. We thank each of your for attending our conference and workshop and bringing your expertise to our gathering.

Maria Isabel M. C. Guedes

Dr Maria Isabel Maldonado Coelho Guedes
Local Organizing Committee
Universidade Federal de Minas Gerais

Welcome from the Gnatwork

We are excited to welcome you to Brazil for our second international meeting of the Gnatwork. The Gnatwork was established to bring together workers on blackflies (Simuliidae), sandflies (Psychodidae) and biting midges (Ceratopogonidae) from around the world. We hope that the Gnatwork annual meetings will enable individuals working on these vectors to meet and exchange ideas regarding technical issues that arise when working on small biting flies. Our post-meeting workshops will also teach technical skills to early-career researchers within vector-borne diseases.

We look forward to talking to you all over the course of the meeting and hope that you find the meeting both enjoyable and productive.

Dr Emma Howson
Network Manager of the Gnatwork

Agenda

08:30 - 09:30 Registration

Theme 1: Introduction and vector research within South America
Chair: Dr Simon Carpenter

09:30 - 09:40	Conference welcome Professor Zélia Inês Portela Lobato
09:40 - 10:05	The Gnatwork: membership and opportunites Dr Simon Carpenter
10:05 - 10:30	*Culicoides* as biological vectors of BTV and EHDV in Brazil: what do we know (and don't know) so far Dr Maria Isabel Maldonado Coelho Guedes

Break

10:30 - 11:00	Tea Break

Theme 2: Introduction to the Gnatwork Vectors
Chair: Prof Mary Cameron

11:00 - 11:25	Sandfly biology and their role as vectors Professor Mary Cameron
11:25 – 11:50	The Biology and role of *Culicoides* as vectors Dr Claire Garros
11:50 - 12:15	Biology and vectorial roles of blackflies Professor Robert Cheke
12:15 - 12:40	Aggregation & sex pheromone in the complex *Lutzomyia longipalpis* in Brazil Dr Reginaldo Brazil

Lunch	
12:40 - 14:00	Lunch

Theme 3: Trends in vector research Chair: Dr Karin Darpel	
14:00 - 14:25	Of creatures big and very small – veterinary aspects of vector-borne pathogen transmission Dr Karin Darpel
14:25 – 14:50	Research activities targeted to Visceral leishmaniasis vector (*Phlebotomus argentipes*) control in Bangladesh Rajib Chowdhury
14:50 – 15:15	Taxonomy of phlebotomine sand flies, epidemiology, diagnosis and control of leishmaniasis Dr Edelberto Santos Dias
15:15 – 15:40	Tripartite interaction: *Leishmania*, microbiota and *Lutzomyia longipalpis* Thaís Bonifácio Campolina

Reception and poster session	
16:00 - 17:30	Reception and poster session

Organising Committee

Brazil

Dr Maria Isabel Maldonado Coelho Guedes
Assistant Professor
Universidade Federal de Minas Gerais

UK

Dr Emma Howson
Network Manager
The Pirbirght Institute, UK

Alix Connelly
Network Administrator
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Dr Simon Carpenter
Network Director
The Pirbright Institute, UK

Professor Mary Cameron
Network Co-Director
The London School of Hygiene and Tropical Medicine

Speaker Biographies

Theme 1: Introduction and vector research within South America

Conference welcome
Professor Zélia Inês Portela Lobato

Graduated in Veterinary Medicine from the Universidade Federal de Minas Gerais (UFMG) (1985), Master's degree in Veterinary Medicine from the Veterinary School (1990) and PhD in Animal Science UFMG / Australian Animal Health Laboratory (CSIRO) (1996) in the area of Preventive Veterinary Medicine. She is a Full Professor at the Veterinary School of UFMG and a CNPq productivity fellow. She has experience in Veterinary Virology, acting mainly in the areas of epidemiology and diagnosis of viral diseases, applied immunology, veterinary vaccines, application and testing of biomarkers. Currently conducts research related to the diagnosis and control of the following viruses: bluetongue, parvovirus, circovirus, swine influenza and bovine variola.

The Gnatwork: membership and opportunites
Dr Simon Carpenter

Dr Simon Carpenter has worked for nineteen years on *Culicoides* biting midges and the pathogens that they transmit. During this time, he has published over 70 papers on the biology and ecology of these arbovirus vectors and conducted studies in the field and laboratory in the Mediterranean Basin, India, Brazil and South Africa. Dr Carpenter has also led a previous BBSRC funded network on *Culicoides* in India (IBVNet) and participated in three network-based EU projects on vectors (Medreonet; EDENext and Vectornet). He has recently co-organised the largest ever symposium specifically on *Culicoides* ecology and biology at ICE in Orlando, Florida and acts as a founder member of the OIE policy working group on the genus. Dr Carpenter possesses unrivalled links within the community, both through the pathogen reference laboratories maintained at The Pirbright Institute and his own field- and laboratory-based studies.

Culicoides as biological vectors of BTV and EHDV in Brazil: what do we know (and don't know) so far

Dr Maria Isabel Maldonado Coelho Guedes

Graduated in Veterinary Medicine from the Universidade Fed Master's degree in Veterinary Medicine (Veterinary Pathology and Veterinary Virology) from UFMG, PhD in Veterinary Medicine (Virology, Pathology and Immunology) from the University of Minnesota, and postdoctoral in Veterinary Virology, UFMG and Danish Technical University. She is currently an Associate Professor at the Department of Preventive Veterinary Medicine at Veterinary School, UFMG. She has experience in Veterinary Virology and Veterinary Pathology, with research areas of pathogenesis, immunology, diagnosis and vaccines of viral diseases of farm animals.

Theme 2: Introduction to the Gnatwork vectors

Sandfly biology and their role as vectors

Professor Mary Cameron

Professor Cameron is Professor of Medical Entomology at the London School of Hygiene & Tropical Medicine (LSHTM), UK and has over 30 years of experience in delivering international level field and laboratory research focusing on novel trapping methods for the surveillance and control of a wide range of medically important arthropods, including sandflies. During this time, she has developed a strong collaborative network and is a sought after and leading authority in her field. She is currently involved in consortia and research projects based in India, Brazil and Bangladesh funded by the Bill and Melinda Gates Foundation, EU: Marie Curie – Initial Training Network (ITN), Science Without Borders and the BBSRC. She is also a Member of the WHO Regional Technical Advisory Group to support the Kala-azar elimination programme in the South-East Asia Region. In addition to her research programme, Prof. Cameron enjoys transferring her research skills by training others and disseminating her knowledge. She is the Programme Director for the MSc Medical Entomology for Disease Control and MSc Medical Parasitology at the LSHTM, a Vice-President, Trustee and Fellow of the Royal Entomological Society and is editor-in-chief of the journal Medical and Veterinary Entomology.

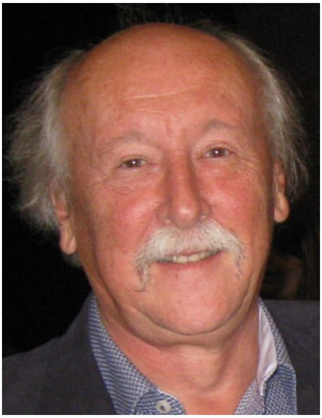
The Biology and role of *Culicoides* as vectors

Dr Claire Garros

Claire Garros is a medical and veterinary entomologist at The French agricultural research and international cooperation organization working for the sustainable development of tropical and Mediterranean regions (Cirad), Montpellier, France. After studying biology and parasitology at the university, she obtained her PhD in Entomology from the University of Montpellier in 2005 with a work on the systematic and taxonomy of anopheline species in Southeast Asia. Then, she joined the University of California, Irvine as a post-doc followed by a second post-doc at the University of Louvain-La-Neuve, Belgium in 2007. When she started at Cirad, Montpellier in 2009 while the bluetongue and Schmallenberg viruses were touching Europe, her focus shifted towards studying the taxonomy and ecology of *Culicoides* biting midges. She also coordinated for 7 years the national surveillance network for *Culicoides* populations. In addition to national activities, she takes part in international networks and research projects related to vector-borne diseases in Europe and in Africa. Since 2016, she is localized in La Réunion Island, French territory in the Indian ocean, where she developed research on mosquitoes and *Culicoides* for the whole south-west Indian ocean region.

Biology and vectorial roles of blackflies

Professor Robert Cheke



Robert A. Cheke is Professor of Tropical Zoology at the Natural Resources Institute of the University of Greenwich, based at its Medway campus in Chatham, UK, and a Visiting Professor at the Department of Infectious Disease Epidemiology at Imperial College London, UK. He has over 40 years of experience of field and laboratory research in Africa focusing on the biology and control of blackflies, vectors of onchocerciasis ("river blindness"), but he has also worked on other vectors of medical and veterinary importance and on agricultural pests such as locusts and quelea birds. From 1979 until 1990 he worked with the WHO Onchocerciasis Control Programme in West Africa studying blackfly migrations, identification methods, larvicide testing and the vector competence of different cytoforms before working with the WHO African Programme for Onchocerciasis Control as part of the team that eliminated onchocerciasis vectors from Bioko, Equatorial Guinea. Latterly he has worked on blackfly projects in Ghana investigating the potential effects of climate change on river blindness and on onchocerciasis transmission dynamics. He is currently involved in a NERC consortium studying mosquitoes in England and working with collaborators in China on models of the transmission of dengue and other diseases. In addition to his research programme, Prof. Cheke contributes to MSc and PhD programmes and other training courses in the UK and Africa.

Aggregation & sex pheromone in the complex *Lutzomyia longipalpis* in Brazil

Dr Reginaldo Brazil

Reginaldo Peçanha Brazil is Senior Researcher in Public Health at Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, Brazil. He obtained his PhD and Post Doctorate from the Liverpool School Of Tropical Medicine (LSTM), UK and has been working in Parasitology and Medical Entomology for the last 30 years. His subject areas of interest are epidemiology of leishmaniases, fauna and chemical ecology of neotropical sandflies. He also has an interest in studing fossil sandflies.

Theme 3: Trends in vector research

Of creatures big and very small – veterinary aspects of vector-borne pathogen transmission

Dr Karin Darpel

Dr Karin Darpel is a veterinarian with more than 10 years' experience in pathogenesis, immunology and virology of *Culicoides*-borne viruses focusing specifically on arboviruses of veterinary importance. Her group at the Pirbright Institute is particularly interested in the mammalian immune response towards insect blood-feeding and/or insect saliva and how the skin-vector interface may influence the dissemination and pathogenesis of transmitted viruses. Ongoing collaborative research projects within her group are funded by BBSRC, DEFRA or EU Horizon 20:20. In addition to her research programme Dr. Darpel contributes to BSc Veterinary Medicine, MSc and PhD programmes and she is a guest lecture in Veterinary Virology at the School of Veterinary Medicine, University of Surrey.

Research on visceral leishmaniasis vector (*Phlebotomus argentipes*) control in Bangladesh

Mr Rajib Chowdhury

My name is Rajib Chowdhury from Dhaka, Bangladesh. I did MSc in Zoology with specialization in Entomology and also MPH in Epidemiology. I work in the International Centre for Diarrheal Disease Research, Bangladesh ('icddr,b') as a consultant and Adjunct Faculty in the Department of Public Health, Independent University of Bangladesh. I have been involved in Neglected Tropical Diseases (NTDs) research, especially visceral leishmaniasis (VL) for more than 17 years. In this long period of work with VL, I focused on generating evidence on epidemiological features, proper diagnosis & management, and effective vector control interventions for Bangladesh as well as the Indian sub-continent. In the meantime, I have worked for the WHO, at the Regional Office for South-East Asia, New Delhi, India as Technical Officer and as a Fellow in the Fellowship programme on Project Management. I was involved in monitoring project activities supported by the WHO/TDR in the South-East Asia Region from 2006 to 2012.

Taxonomy of phlebotomine sand flies, epidemiology, diagnosis and control of leishmaniasis

Dr Edelberto Santos Dias

Graduated in Biological Sciences from the Universidade Federal de Minas Gerais (UFMG) (1978), Master in Parasitology from the UFMG (1982), PhD in Sciences (Concentration area in Entomology) from the UFMG (1989) and Post-Doctorate at the Walter Reed Army Institute of Research, USA. Full Researcher in Public Health at the René Rachou Research Center / FIOCRUZ and a CNPq productivity fellow. Coordinator of FIOCRUZ / MINAS's Flebotominae Vector Competence Reference Center (CRCV). He has experience in Parasitology, with emphasis on Vector Entomology and Leishmaniasis Eco-Epidemiology.

Tripartite interaction: *Leishmania*, microbiota and *Lutzomyia longipalpis*

Thaís Bonifácio Campolina

Thaís is a PhD student in the Postgraduate Program in Health Sciences at the René Rachou Institute. She has a Master's degree in Health Sciences from Fiocruz-MG, with an emphasis on Parasitic Diseases (2016). She graduated in Biological Sciences from the Pontifícia Universidade Católica de Minas Gerais (PUC-MG) (2014). She has experience in Cellular and Molecular Biology, Microbiology and Parasitology (parasitic-vector interaction), in the maintenance of Phlebotomine colony and in the cultivation of *Leishmania* and Virus.

Poster Abstracts

1

DNA Barcodes for Argentinian Phlebotomine fauna

Moya, S.L., Quintana, M.G., Pech-May, A., Liotta, D.J. & Salomón, O.D

Instituto Nacional de Medicina Tropical (INMeT-ANLIS), Argentina

The barcoding approach has been evaluated for phlebotomine fauna from different Latin American countries showing high efficiency with few exceptions generally related to recently divergent species. Our aim is to initiate a DNA barcode library and to evaluate its performance as a complementary tool for the morphological-based identification method for the Argentinian phlebotomine fauna, currently counting 13 genera and 44 species, including 7 proven or putative vectors of visceral and cutaneous leishmaniasis agents. A 527 bp gene fragment of the cytochrome C oxidase I was obtained for 123 specimens belonging to 19 species (primers LCO1490 and HCO2198). Sequences alignment was performed using Clustal in MEGAv.7, as well as the estimation of K2p genetic distances, intra and inter-species, which we analyzed in order to evaluate the presence of a barcode gap. The resulting Neighbor Joining dendrogram identified 18 molecular taxonomic units (MOTUs) with high bootstrap support values (≥99%) consistent with the species morphologically identified, except for Nyssomyia whitmani and Ny. neivai given their low inter-specific genetic distance (2.8%). The last two species belong to a group with evidence of introgression and low congener divergence showing a limit to the effectiveness of the barcode tool. Nevertheless, barcoding helped to solve particular misidentifications and allowed the association between females and males among morphologically similar species of some genera. In conclusion, barcoding made possible discrimination among these phlebotomine species with 95% efficiency. This study also generated the first barcodes for Martinsmyia alphabetica, Ny. neivai, Psathyromyia lanei, Pintomyia pessoai, Cortelezzii complex and Shannoni complex.

2

Simuliofauna of Atalaia Municipal Natural Park, Macaé, Rio De Janeiro: identification, creation in laboratory and development biology studies

Willian Rodrigues da Costa Marinho, Lucas Santos da Cruz, Ruann Guimarães dos Santos, Alessandra da Silva de Alvarenga, Jackson de Souza Menezes, Natalia Martins Feitosa, Ronaldo Figueiró Portella Pereira & Rodrigo Nunes da Fonseca

Federal University of Rio de Janeiro, Brazil

The Black flies have a major impact on public health and the economy of regions. There are currently many ecological studies involving this group, but knowledge of developmental genetics in Dipteros is restricted to fruit fly *Drosophila melanogaster*. Thus, biology studies of the development of simulators are extremely interesting from the point of view of Diptera phylogeny. The objective of the project is to survey *Simuliidae* species in the Municipal Natural Park of Atalaia and investigate the Biology of Development of these Animals, comparing with the development of Most Used Diptera as a *D. Melanogaster*. *Simuliidae* collections are being carried out in Park streams, where they are being used in the laboratory. In the field, abiotic factors related to their development in the natural laboratory environment are measured and mimicked as natural conditions for the development of animals. The eggs collected in the environment are being stored at -20ºC in the laboratory and then nuclear markings are performed with DAPI for developmental biology studies. So far four species of simulators have been found in the Park domains: *Simulium anamariae*, *Simulium pertinax*, *Simulium subpallidum* and *Simulium perflavum*. During laboratory mimics, these organisms survive for 16 days. With the eggs selected in the natural environment, already started as attempts of developmental biology studies with simulators, observing possible situations that seem to be the cephalic fans of the larva and also in some markings it was possible to infer the anterior and posterior region of the embryo.

3

Natural Infection of *Pintomyia fischeri* with *Leishmania infantum* promastigotes in Embu das Artes Municipality, São Paulo state, Brazil

Fredy Galvis Ovallos[1], Adriele Eiko Ueta[1], Gabriella de Oliveira Marques[1], Gabriela Araujo[2], Carmen Sandoval[2], Vânia Mata[2], Márcia Dalastra Laurent[2], Laura Cristina Posada Lopez[1] & Eunice Aparecida Bianchi Galati[4]

[1]Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo.
[2]Laboratório de Patologia de Moléstias Infecciosas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.

Introduction. In the Americas, Visceral leishmaniasis (VL) is a zoonotic disease caused by *Leishmania infantum* and transmitted by the sand fly *Lutzomyia longipalpis*. However, in some regions of São Paulo state autochthonous cases of canine VL were reported in the absence of this vector. By this reason we aimed to investigate the infection by flagellates of *Leishmania* sp in sand flies captured in a transmission area of canine VL of Embu das Artes municipality.

Methods. The specimens were captured two times by month with Shannon trap between 18:00 – 21:00 h. To evaluate the female infection the dissection method was used. Females were anesthetized at 4ºC. After that the wing and leg were removed and the gut was exposed in a drop of saline solution (0,9%) and observed under microscopy at 400X. When parasites were observed in the gut it was separated to get cultures to identify the parasite species.

Results. 364 females were dissected belonging to 12 captures. 87,64% of the females dissected were of the species *Pintomyia fischeri* and 4,40% of *Psychodopygus ayrozai*. Natural infection by flagellates was detected in a female of *Pintomyia fischeri*. The culture of this parasite let us the identification by PCR method the presence of *Le. infantum* in this sand fly species.

Conclusion. The identification of flagellates of the parasite *Le. infantum* in a female of *Pintomyia fischeri*, reinforce the information to the incrimination of this species as vector of CVL in Embu das Artes municipality and demonstrate the circulation of this parasite in this sandfly population that predominate in this region.

4

Microorganism-based larval diets affect mosquito development, size and nutritional reserves in the yellow fever mosquito *Aedes aegypti* (Diptera: *Culicidae*)

Raquel Souza, Flávia Vírginio, Thaís Riback, Lincoln Suesdek, José Barufi & Fernando Genta

Oswaldo Cruz Foundation (FIOCRUZ), Brazil

Mosquito larvae feed on organic detritus from the environment, particularly microorganisms. Little attention has been paid to nutritional studies in *Aedes aegypti* larvae. We investigated the effects of yeast, bacteria and microalgae diets on larval development, adult size, survivorship, lifespan and wing morphology. Microorganisms were offered as the only source of food to recently hatched first instar larvae and their development was followed until the adult stage. The main macronutrients were analyzed in single larvae to correlate energetic reserve accumulation by larva with the developmental rates and nutritional content observed. FITC-labeled microorganisms were offered to fourth instar larvae, and its ingestion was recorded by fluorescence microscopy and quantitation. Larvae developed in all diets, however insects fed with bacteria and microalgae showed a severe delay in development rates and low survivorship. Diets with better nutritional quality resulted in adults with bigger wings. *Asaia sp*. and *Escherichia coli* resulted in better nutrition and developmental parameters and seemed to be the best bacterial candidates to future studies using symbiont-based control. The diet quality was measured and presented different protein and carbohydrate amounts. Bacteria had the lowest protein and carbohydrate rates; yeasts had the highest carbohydrate amount and microalgae showed the highest protein content. Larvae fed with microalgae seem not to be able to process and store these diets properly. Larvae were shown to be able to process yeast cells and store their energetic components efficiently. Our results point that mosquito larvae show high plasticity to feed, being able to develop under different microorganism-based diets.

5

Genetic diversity, phylogeography and molecular clock of the *Lutzomyia longipalpis* complex (Diptera: Psychodidae)

Pech-May A., Ramsey J.M, Giuliani M., Berrozpe P., Raúl E., González Ittig R.E., Quintana M.G. & Salomón O.D

Instituto Nacional de Medicina Tropical (INMeT-ANLIS), Argentina

The *Lutzomyia longipalpis* complex has a wide but discontinuous distribution in Latin America, extending throughout the Neotropical realm between Mexico and northern Argentina and Uruguay. The aim of this study was to analyze the genetic diversity and structure of *Lu. longipalpis* from Argentina, and to integrate these data to reevaluate the phylogeography of the *Lu. longipalpis* complex using mitochondrial markers at a Latin American scale. Genetic diversity was estimated from six sites in Argentina, using a fragment of the ND4 and cyt b genes. Greatest genetic diversity was found in three populations. The Geneland analyses reveal the existence of two genetic clusters in Argentina. Phylogeographic analyses using sequences available in GenBank from diverse geographic sites suggest greater divergence than previously reported. At least eight haplogroups (three of these identified in Argentina), each separated by multiple mutational steps using the ND4, are differentiated across the Neotropical realm. The divergence of the *Lu. longipalpis* complex from its most recent common ancestor was estimated to have occurred 0.70 MYA. This study provides new evidence supporting two *Lu. longipalpis* genetic clusters and three of the total eight haplogroups circulating in Argentina. There was a high level of phylogeographic divergence among the eight haplogroups of the Lu. longipalpis complex across the Neotropical realm. These findings suggest the need to analyze vector competence, among other parameters intrinsic to a zoonosis, according to vector haplogroup, and to consider these in the design and surveillance of vector and transmission control strategies.

6

Multiplex real-time PCR assay for simultaneous detection of dog and human blood and *Leishmania* parasites in sand flies

Kamila Gaudêncio da Silva Sales, Débora Elienai de Oliveira Miranda, Marcelo Henrique Santos Paiva, Luciana Aguiar Figueredo, Domenico Otranto & Filipe Dantas-Torres

Aggeu Magalhães Institute - Oswaldo Cruz Foundation (FIOCRUZ), Brazil

The blood feeding behaviour of female sand flies may increase their likelihood of acquiring and transmitting *Leishmania* parasites. Studies on the host usage by these insects may thus improve our understanding of the *Leishmania* transmission risk in leishmaniases-endemic areas. Here, we developed a fast multiplex real-time PCR assay for simultaneous detection of dog and human blood and *Leishmania* parasites in sand flies. Primers and TaqMan probes targeting the mitochondrially encoded cytochrome c oxidase I and cytochrome b genes of dog and human, respectively, were combined in a multiplex assay, which also includes primers and a TaqMan probe targeting the *Leishmania* kinetoplast DNA. The multiplex assay was 100% specific, with analytical sensitivities of 103 fg/reaction for dog and human and 1 fg for *Leishmania*. By testing field-collected engorged female sand flies (95 *Migonemyia migonei* and two *Nyssomyia intermedia*), 50 *M. migonei* were positive for one or two targets (positivity rates: 45.4% for human, 4.1% for dog and 12.4% for *Leishmania* parasites). This multiplex real-time PCR assay represents a novel fast assay for detecting dog and human blood and *Leishmania* parasites in female sand flies and therefore a tool for assessing the risk of *Leishmania* transmission to these hosts in areas of active transmission.

7

Fiocruz-Colfleb Phlebotomine Collection, History & Services

Carolina Cunha Monteiro, Paloma Helena Fernandes Shimabukuro & José Dilermando Filho

Fiocruz-Minas, Brazil

The Sandflies Collection, COLFLEB, located at René Rachou / Fiocruz Institute in Belo Horizonte, State of Minas Gerais, has about 80,000 copies distributed over 368 sandflies species from the Americas. The sand flies are mounted on the blade and coverslip.

Part of the Collection consists of the “Standard Collection” and the other part is the General Collection. COLFLEB is alphabeticaly organized into sliding bookcases according to each species. The COLFLEB collection is diverse, currently counting 47 amber found in the Dominican Republic, containing 162 sandflies of 9 species; Studies on these amber are underway.

COLFLEB contains 700 vouchers deposited on slides from barcoding DNA studies. The prospects for the sandflies collection are, among others, to further diversify, especially with material exchanges with researchers from other institutions or through specific locations collections, continue to collaborate human resources training. Recently, COLFLEB received as donation part of the collection assembled by the researcher Dr. Ítalo Sherlock (Fiocruz-Bahia), this collection consists of about 15,000 slides that are curated.

COLFLEB is computerized and since 2010 is one of Fiocruz’s biological collections that is integrated with the SpeciesLink network and the Brazilian Biodiversity Information System (SiBBr).

8

Experimental infections and co-infections with *Leishmania braziliensis* and *Leishmania infantum* in two sand fly species, *Lutzomyia migonei* and *Lutzomyia longipalpis*

Joanna Alexandre, Jovana Sadlova, Tereza Lestinova, Barbora Vojtkova, Magdalena Jancarova, Lucie Podesvova, Vyacheslav Yurchenko, Filipe Dantas-Torres, Sinval P. Brandao-Filho & Petr Volf

Aggeu Magalhães Institute/IAM-FIOCRUZ/PE, Brazil

Leishmaniases are neglected tropical diseases and *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Viannia*) *braziliensis* are the most important causative agents of leishmaniases in the New World. These two parasite species may co-circulate in a given endemic area but their interactions in the vector have not been studied yet. We conducted experimental infections using both single infections and co-infections to compare the development of *L.* (*L.*) *infantum* (OGVL/mCherry) and *L.* (*V.*) *braziliensis* (XB29/GFP) in *Lutzomyia longipalpis* and *Lutzomyia migonei*. Parasite labelling by different fluorescein proteins enabled studying interspecific competition and localization of different parasite species during co-infections. Both *Leishmania* species completed their life cycle, producing infective forms in both sand fly species studied. The same happens in the co infections, demonstrating that the two parasites conclude their development and do not compete with each other. However, infections produced by *L.* (*L.*) *infantum* reached higher rates and grew more vigorously, as compared to *L.* (*V.*) *braziliensis*. In late-stage infections, *L.* (*L.*) *infantum* was present in all midgut regions, showing typical suprapylarian type of development, whereas *L.* (*V.*) *braziliensis* was concentrated in the hindgut and the abdominal midgut (peripylarian development). We concluded that both *Lu. migonei* and *Lu. longipalpis* are equally competent vectors for *L.* (*L.*) *infantum*. In relation to *L.* (*V.*) *braziliensis*, *Lu. migonei* appears to be more susceptible to this parasite than *Lu. longipalpis*.

9

Course of infection by *Leishmania* (*Viannia*) *braziliensis* in the presence of salivary gland homogenates from *Migonemyia migonei*

Débora Elienai de Oliveira Miranda, Kamila Gaudêncio da Silva Sales, Luciana Aguiar Figueredo, Virginia Maria Barros de Lorena & Filipe Dantas Torres

Aggeu Magalhães Institute/IAM-FIOCRUZ/PE, Brazil

Leishmaniasis are diseases caused by protozoa of the genus *Leishmania*. Studies on the effect of sandfly saliva on exacerbation or protection against infection *Leishmania* spp. have been developed. The aim of this study is to evaluate the effect of *Migonemyia migonei* saliva on experimental *Leishmania* (*Viannia*) *braziliensis* infection. Salivary glands homogenates from females of *Mg. migonei* were used from the colony established at Charles University, Prague, Czech Republic. Twenty-four mice were divided into four groups, each with six individuals: 1- Mice infected with *L.* (*V.*) *braziliensis* in the presence of *Mg. migonei* salivary gland homogenate; 2- Mice inoculated only with *L.* (*V.*) *braziliensis*; 3- Mice inoculated with *Mg. migonei* salivary gland homogenate only and 4- PBS-inoculated control mice. The experiment will be repeated three times. The clinical evolution of the mice and the immune response were evaluated by the flow cytometry technique using the Cytometric Beads Array (CBA) method. We found that saliva had some immunomodulatory capacity, as evidenced by decreased levels of TNF-alpha in groups 1 and 3 when compared to group 4. In addition, IL-4 and IL-17 levels were negatively modulated after saliva administration in animals of group 1. We hope to know the effect of saliva *Mg. migonei* in the course of *L.* (*V.*) *braziliensis* infection, contributing to the understanding of the role of saliva in modulating the immune response of mice to infection with this parasite by cytokine analysis.

10

Evaluation of the immunological and enzymatic profile of *Aedes aegypti* (Linnaeus) treated with *Metarhizium brunneum* (Metsch.) Sorokin

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Aedes aegypti (Linnaeus, 1762) is a culicid vector responsible for the transmission of several arboviruses which are widely distributed throughout the tropics, such as yellow fever, dengue, Zika and Chikungunya. Currently, physical control is employed as one of the main forms of mosquito control, integrated with chemical control by the use of insecticides. However, the indiscriminate use of these pesticides has generated resistance in diverse populations. In order to avoid selection of resistant vector populations, biological agents are prospected as alternative methods of mosquito control. Widely used for management of agricultural pests, the entomopathogenic fungus *Metarhizium brunneum* (Metchnikoff) Sorokin is a biological agent under study due to its wide infectivity on several arthropods of medical and veterinary importance, as well as the genetic diversity of strains that can determine a trait of pathogenic specificity over such vectors. The following project aims to evaluate, at the enzymatic and molecular levels, the differences in the patterns of synthesis, gene expression and activity of factors linked to the immune and digestive enzyme responses of larvae infected with *M. brunneum* (conidia and blastospores). The humoral components of the immune responses will be evaluated from the detection of antimicrobial activity, expression of antimicrobial peptides, phenoloxidase activity (melanization biochemical cascade) and production profile of reactive nitrogen and oxygen species and detoxifying enzymes. Regarding the characterization of the enzymatic profile from digestive system of infected larvae, enzymatic assays will be performed to quantify the activities of trypsin, chymotrypsin, trehalase and beta-1,3-glucanase enzymes, as well gene expression quantification.

11

Sylvatic Yellow Fever Vectors: Updating the distribution of these culicid species in Argentina (Diptera: Culicidae)

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Yellow fever (YF) is a virus endemic disease in tropical areas of Africa and South America. It is maintained between nonhuman primates and diurnal mosquitoes (*Haemagogus* sp. and *Sabethes* sp.) that breed in sylvatic areas where humans might be incidentally infected, but in urban areas the virus is maintained among *Ae. aegypti* mosquitoes and humans. There is no intermediate cycle known so far in South America, and it is presumed that the dispersion to urban areas would be due to sporadic bites to humans in the sylvatic areas where wild mosquitoes feed on monkeys *Alouatta* and *Cebus* genera, which are the main host/reservoirs of YFV. In Argentina, between 2007 and 2008, epizootics were reported in howler monkeys, causing high mortality in natural populations. This reemergence of YF represents a risk for human populations, and it is necessary to conduct studies on distribution patterns of sylvatic mosquito species that are involved in these epizootics. These species have been reported poorly in the last years, although their historical distribution covers all of Northern Argentina. In this study, we developed a database which gather all the information available in literature. The database includes: Mosquito species, Date of Capture, Method of Capture, Habitat, Etiological Agent, Geographical coordinates. Data is display in a distribution map obtained with QGIS. Once the records were obtained, distribution maps were made through QGIS. Furthermore, field studies will be conducted in a near future to update this species distribution patterns to corroborate their historical distribution.

12

Wolbachia density regulatory factors in Argentine populations of mosquito disease vectors

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Wolbachia is an intracellular bacterium, which naturally infects a number of mosquito species that are important vectors of human diseases, including *Aedes albopictus*, *Culex quinquefasciatus* and *Cx. pipiens*. Recent evidence suggests that *Wolbachia* can induce resistance to pathogens in mosquitoes, modifying their ability to transmit them. It is common for *Wolbachia* densities to vary in different *Wolbachia*: host relationships, and among adult individuals collected in the field from the same host population. It has been observed that this variation in bacterial density correlates positively with the degree of resistance to pathogens. In this work, real-time PCR technique was developed for the determination of the levels of variation of *Wolbachia* density in *Aedes albopictus* according to the tissue in which the infection is lodges, the sex and age of the host insect.

13

Ecological niche modelling of Leishmaniasis vectors and the risk of introduction of visceral leishmaniasis in eastern São Paulo

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Ecological Niche Modeling could be an important tool to understand the behavioral dynamics of Leishmaniasis vectors. This study seeks to reconstruct the distribution pattern of *Lutzomyia longipalpis* and *Nyssomyia intermedia* assessing risk of Leishmaniasis circulation in the Paraiba Valley. Occurrence data were obtained from online databases (Global Biodiversity Information Facility). We used the Maxent software version 3.3.4 for Niche Modelling. Localities for Visceral Leishmaniasis cases detected between 2007-2017 were obtained from DATASUS (http://datasus.saude.gov.br/informacoes-de-saude/tabnet). We selected cases identified at States that are limithrophous to São Paulo State, as well cases detected in São Paulo. The study showed that both species have a wide distribution, with a large overlapping area in Central Brazil. *Lutzomya longipalpis* is more frequently encountered in northeastern Brazil and *Nyssomyia intermedia* in the southeastern region. Our model suggests that the probability of occurrance of the vectors in the Paraiba Valley is higher in *Nyssomyia intermedia* (0.38-0.54) than in *Lutzomyia longipalpis* (0.1-0.15). The association of these vector to the distribution of human cases reveals that the role of *Nyssomyia intermedia* is higher, with a permutation importance of 33,4 against 6,4 for *Lutzomyia longipalpis*, suggesting that *Nyssomyia intermedia* may act as a primary vector in area where *Lutzomyia longiplapis* is less frequently encountered. This research highlights the importance of understanding sandfly ecology and its distribution using predictive ecological models to understand the occurrence of Leishmaniasis.

14

Photoperiod induced effects on female fecundity and egg diapause in *Aedes aegypti* from a temperate region of South America

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Mosquito populations in temperate regions are confronted with unfavorable conditions such as low winter temperatures. These conditions are anticipated by a short photoperiod, which is a major cue to induce changes in the life history of insects to survive these conditions. So far, no effects of photoperiod on the female fecundity or on eggs diapause of *Aedes aegypti* are known, despite the fact that this species is expanding to temperate regions. Thus, the aim of the present study was to assess the effects of photoperiod on the fecundity of females and on their ability to lay diapausing eggs in a population of *Ae. aegypti* from a temperate region (Buenos Aires City, Argentina). To this end, we exposed both the parental generation and the eggs to short-day (SD: 10L: 14D) and long-day (LD: 14L: 10D) photoperiods, and studied the fecundity of females, the hatching and mortality of eggs. Egg hatching and mortality were assessed for different eggs ages (from 14 to 112 days). The individuals from the SD photoperiod showed a lower fecundity and laid eggs with lower hatching response. A trend to higher hatching with increasing egg age was recorded in all treatments. Eggs mortality was also lower in the SD photoperiod. Our results show changes in female fecundity in response to photoperiod. Furthermore, the inhibition of hatching and the lower mortality of SD eggs suggest that they are in diapause, an ability that has not been reported previously in *Ae. aegypti*, which would explain its expansion to temperate regions.

15

Use of semiochemicals for insect control *Culicoides*

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Bluetongue disease (BTV) is an infectious disease caused by the virus of the genus Orbivirus of the family Reoviridae, transmitted by a hematophagous vector of the genus *Culicoides*. This disease can affect domestic and wild ruminants, and is widely disseminated in different regions of Brazil. The BTV disease focus sites are associated with the presence of its main vector, the *Culicoides*. Therefore, the control of this insect becomes effective for the indirect control of BT. Among the different methodologies of parasite control, integrated pest management has been the most suitable for production systems because of their better cost-effectiveness, less invasive for human health and the environment, among others. Alternative methods such as the use of semiochemicals in pest control have presented promising results when combined with other control methodologies. The present research proposes the use of semiochemicals to combat infestation by *Culicoides*, in order to indirectly control the incidence of Bluetongue. Initially, the *Culicoides* will be captured and phenotypic and genetic identification. In addition, the entrainment the OVCs work in sheep, will be identified by gas chromatography coupled to mass spectrometry. Afterwards, tests will be carried out to verify the potential for attracting or repelling OVCs to insects by means of Y-olfactometry bioassays. Subsequently, analogous substances will be developed for the control of parasites at the farm level, aiming to production of chemical baits for the control of *Culicoides*, that is effective and economically viable.

16

Integrated taxonomy to elucidate the diversity and taxonomic limits of biting midges (*Culicoides* and *Leptoconops*, Diptera: Ceratopogonidae) in the Brazilian Amazon

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Biting midges (*Culicoides* and *Leptoconops*, Diptera: Ceratopogonidae) are small hematophagous of the family Ceratopogonidae. Some species of these genera are incriminated as vectors of pathogens associated with the spread of diseases in humans and animals. Species identification and phylogenetic relationships between species are sometimes problematic. In this study, integrated taxonomy is being applied by aggregating morphological, morphometric and genetic characters in order to estimate the diversity of biting midges and to elucidate existing taxonomic limits in some areas of the Brazilian Amazon: Acre, Amazonas, Pará and Rondônia. The Geometric Morphometry is based on the use of anatomical landmarks and superposition methods via Generalized Procrust Analysis. For molecular analysis, DNA is being extracted individually from the specimens and will be subjected to polymerase chain reaction amplification using gender-specific primers, Target-directed LepF / LepR (COI) of mitochondrial DNA, and target-directed PanCulF / PanCulR primers (ITS-1) of ribosomal DNA. To date, 10,137 biting midges specimens have been accounted for, with 35 species identified and new records in the municipalities of Itaituba and Óbidos in the state of Pará, and Presidente Figueiredo and Tefé in the Amazon. The geometric morphometry performed on wings of five species of *Culicoides* of the guttatus group (*C. foxi*, *C. fusipalpis*, *C. insignis*, *C. pseudodiabolicus* and *C. ruizi*) showed significant differences in Mahalanobis and Procrustes distances. In the analysis of canonical variables as in the discriminant function. Therefore geometric morphometry was able to differentiate species within the *guttatus* group. Preliminary testing of the primers amplified the targets.

17

Taxonomic identity and hematophagous activity of black flies (Diptera: Simuliidae) that breed in the Salado River (Province of Buenos Aires, Argentina)

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The quality of life of people living near the upper Salado River basin and tributary canals has been disturbed by the increase in density of black flies. These are known by the expoliative action produced by the adult females' bite, but none transmit disease agents to humans. Moreover, agricultural-livestock activities and local tourism are interfered. The problem began two decades ago, but it was intensified in the recent years. The objective of this study was to know the taxonomic identity of the anthropophilic black flies species and the hours of the day where adult females are most active in the city of Junín, Province of Buenos Aires. Sampling was carried out every 21 days, during the winter and spring seasons of 2017. The adult females were captured by human bait with an entomological aspirator, for two consecutive days between 8 and 20 h, at the Municipal Park. The environmental variables (temperature and relative humidity) were taken from the municipal meteorological service. A total of 1074 individuals were captured and identified. *Simulium* (*Cerqueirellum*) *chaquense* was the only species present with hematophagous activity. During the winter, the greatest activity was recorded between 12 and 16 h. In spring, adult females were present throughout the day, with peaks at sunrise and sunset. The presence of adult black flies is determined by local environmental conditions. Knowing the population dynamics of the species present is essential to carry out effective and, above all, environmentally friendly control actions.

18

Molecular detection of *Wolbachia pipientis* strains in natural populations of *Lutzomyia longipalpis* sandfly vectors (Diptera, Psychodidae, Phlebotominae)

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Sandflies (Diptera, Psychodidae, Phlebotominae) are insects of medical epidemiological importance, involved in the transmission of flagellated protozoa of the genus *Leishmania*, which cause leishmaniasis in Brazil. This zoonosis has a higher occurrence rate with high morbidity rates in the northeast of the country, and control measures for this disease have become ineffective. An alternative is the possibility of using a biological control with the use of the intracellular endosymbionte bacterium *Wolbachia pipientis* (Rickettsiales: Rickettsiaceae), due to the alterations that can cause in the insect vector such as male death, cytoplasmic incompatibility, feminization and parthenogenesis, leading to reduction of insect life span and thus the reduction of pathogen transmission. In this work, 668 specimens of *Lutzomyia longipalpis* from samples collected in extraction. The PCR technique was performed to detect the presence of Wolbachia pipientis. Ninety-eight (14.67%) samples were positive, a percentage higher than that found in similar studies. All positive samples refer to the southern lowlands of the state, which raises the possibility of the occurrence of geographical barriers acting on the distribution of this species and this is the next steps of our study.

19

Ecoepidemiology of Leishmaniasis in the Chaco Region Argentina, with emphasis in Bio-control proposal by Bacteria native strains

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In the Argentinian Chaco Region, Tegumentary Leishmaniasis (TL) is reported since 1950, presenting sporadic outbreaks, with an increasing trend of incidence. Visceral Leishmaniasis human cases are not reported yet in the province, but the records of infected canines is growing, besides the vector was recorded in the area since 2010. In the Chaco province we found 20 out of the 38 Phlebotominae species recorded in Argentina, being *Nysomyia neivai* the most abundant species and considered responsible for the larger TL outbreaks in the country. A high heterogeneity of fauna abundance and distribution was reported at microfocal scales; these differences could be determined by the presence of entomopathogenic fungi, bacteria and protozoa that would act on the Phlebotominae populations, and thereby affecting the transmission of the disease. Based on this hypothesis, a project was started with the aim of isolating and identifying native strains of bacteria with entomopathogenic effect that could act as biocontrollers. Twenty *Bacillus* sp. isolates were obtained from soil samples, and identified by API-50CH and MALDI-TOF. The chitinolytic activity of each strain was determined in a minimal medium containing colloidal chitin and subsequently PCR was performed using 4 pairs of primers designed to detect the presence of coding genes from 4 families of chitinases. Eleven isolates presented ‘in vitro’ chitinase production while enzyme coding genes were detected in twelve of them. The entomopathogenic effect of these isolates is expected to be tested in experimental colonies of phlebotominae with the aim of developing biocontroller systems.

20

Genetic analysis of *Culicoides* paraensis (Goeldi) (Diptera: Ceratopogonidae) populations from six Brazilian biomes and their relationship with Oropouche virus transmission

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Since its first record in Brazil, Oropouche Virus has been responsible for epidemics in urban and wild areas in the Brazilian Amazon region. The virus isolation in wild reservoirs in several states and the recent record of the disease outside the Brazilian Amazon (Bahia) for the first time, reinforce the importance of studying the epidemiology of the disease and the possibility of its expansion to other areas. Until now, only *Culicoides* paraensis (Goeldi) has been incriminated as a vector of the disease. Despite their wide distribution and climate plasticity, little is known about their potential distribution. Furthermore, no molecular analysis has been performed with Brazilian species. Thus, this work aims to characterize molecularly the different populations of *C. paraensis* by analyzing sequences of the COI and EF-alpha genes, evaluating the existence of a species complex. The collections will be made with CDC light trap, in places where were evidenced the presence of disease and/or infestation of the vector, considering six representative states from Brazilian biomes: Amazon, Caatinga, Cerrado, Pantanal, Atlantic Forest and a transition area to the Pampas. Considering the population of *C. paraensis* increases in the summer, the collection will be started in next December. The specimens will be morphologically identified and legs, thorax and abdomen will be separated for DNA extraction. Abiotic data from the collection sites will be recorded to correlate with the results of morphological and molecular analyzes, in order to elucidate the influence of these variables on the distribution of populations.

21

Evaluation of virulence of blastospores and conidia of the entomopathogenic fungus *Metarhizium anisopliae* for biting midge control (Diptera: Ceratopogonidae)

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The biting midges (Diptera: Ceratopogonidae) known as “mosquitos-pólvora maruins and mosquitinhos de mangue” in Brazil are responsible for the spread of arboviruses to cattles and humans, as well as causing intense annoyance to their hosts. The purpose of this study is to use *Metarhizium anisopliae* conidia and blastospores to control immature forms of this culicoid. Insect collection will be performed in a rural area of the city of Anchieta, Espirito Santo State. A BG-Sentinel trap will be used for adult collection. Plant material will also be collected in banana plantations, an area of preference for these insects to lay eggs. Colony establishment and maintenance will be carried out at the Universidade Estadual do Norte Fluminense. *M. anisopliae* isolate LEF 2000 will be used after culturing in PDA medium for the production of conidia. Blastospores will be produced in 250 mL flasks with 3% of corn steep liquor, 4% yeast and glucose, in an orbital shaker for three days. Bioassays will be carried out using different concentrations, ranging from 1 x 104 conidia/mL to 1 x 107 conidia/mL to establish the LD50. Larval mortality will be record daily until the 7th day. Survival analyses will be performed using SPSS 21.

22

Identification of wing patterns of *Culicoides* spp. veterinary and human l interest from Ecuador, with using photos with UV filters and photometry

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The identification of Ceratopogónidos of the genus *Culicoides* spp, known worldwide for its ability to transmit several pathogens of veterinary interest (BTV, EHD) and medical (Oropuche) (Borkent, 2004). The veterinary interest that these insects generate for being the transmitters of deadly diseases in ruminants (Bovines, sheep, goats and deer), makes us think about looking for an effective way to identify species of the genus *Culicoides* spp. (Estrada, and others, 2013). Ultra violet light is a type of electromagnetic radiation, with a shorter wavelength than that of visible light. The ultraviolet photograph is given by recording the amount of energy reflected by the light waves in the ultra violet band, that is to say light that is reflected less than 400 nanometers. (Ariza, 2017). (Pereira, 2015). *Culicoides* insignis has been detected as the biological vector of BTV and EHD in Ecuador, so it leads us to think of a method of morphological identification of the species, in an automated. In this study, we proposed a method to analyze and classify two species of mosquitoes taking into account the morphological characteristics of their wings. Initially, the image acquisition and processing task improved the image quality by the use of UV light to take the picture, accomplishing a correct recognition of particles and areas of interest within the mosquito wing. The proposed method was able to produce features vectors with satisfactory characteristics that help identify both species of mosquitoes with the help of many machine learning techniques (MLCs) as support vector machine (SVM), decision trees (DT) and artificial neural networks (ANN). As proof of concept to validate the quality of the computed features we analyzed around 25 wing samples of *Culicoides insignis* and 23 wing samples of *Culicoides diabolicus*, achieving in the best scenario an accuracy of 84% at the moment of classifying both species.

23

Virulence of entomopathogenic fungus *Metarhizium anisopliae* formulated with the insecticide imidacloprid against adult *Culicoides* paraensis collected from Espirito Santo, State, Brazil

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Insects of the genus *Culicoides* (Diptera: Ceratopogonidae) known as “maruins” in Brazil are of important epidemiological concern, as their bites cause severe skin reactions and the attack on humans makes life in certain regions impracticable. This insect is a vector of *Mansonella* in the Americas and Africa. They also transmit viruses that cause Oropouche, encephalitis and Blue Tongue in cattle. The species causing problems in Espirito Santo is *Culicoides paraensis*. The aim of the present study was to evaluate whether the entomopathogenic fungus *Metarhizium anisopliae* formulated with or without imidacloprid (IMI) would reduce *C. paraenses* survival rates. The insects were collected in the city of Anchieta, Alto Pongal, Espirito Santo State using the BG Sentinel trap. In the laboratory the insects were exposed to filter paper impregnated with the fungus previously left to dry for 16 hours before. The insects were placed in a plastic pot and exposed to the fungus for 48 hours. The survival rates of the insect were monitored daily for 7 days. Two replicates of these experiments were performed. Insects exposed to the fungus had a survival rate of 23.3% (± 4.30). Insects exposed to the fungus formulated with 0.1 ppm IMI had a survival rate of 16.6% (± 4.27). Control treatments using TW or 0.1ppm IMI had an 80% survival rate. Insects exposed to the fungus and fungus + IMI had a mean survival time of 4 and 3 days, respectively. Entomopathogenic fungi have potential for biting midges control.

24

Preliminary studies: new strain/virus of the *Alphavirus* genus detected in *Psorophora* spp. (Diptera: Culicidae) collected in the Chaco province, Argentina

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Arthropod-borne viruses (arboviruses) are important emerging and re-emerging pathogens that cause disease in humans and animals. They are a taxonomic diverse group and some of the members of medical-veterinary importance belong to the genus *Alphavirus* (*Togaviridae*). In the Americas, the members of the genus responsible for outbreaks in humans and epizootics in horses belong to the eastern (EEE), western (WEE) and Venezuelan (VEE) equine encephalitis complex. In Argentina, several epizootics by EEE, WEE and VEE have been reported on the beginning of the 20th century. In the Chaco province, the three complexes have a history of circulation and were isolated and/or detected in humans, mosquitoes and rodents. In the present study, *Psorophora* spp. mosquitoes (API28) were positive to molecular alphavirus detection and the phylogenetic analysis showed that the API28 fragment amplified was grouped with a very low bootstrap in the VEEV complex. Our results show an uncertain position of the API28 within the VEEV complex and this could suggest the possibility of a new strain or virus not currently described. However, is necessary to amplify a larger region for a more detailed phylogenetic analysis to provide information about genetic relatedness of the alphavirus present in mosquitoes collected in Chaco province (Argentina).

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Identification of the transmission cycle of cutaneous leishmaniasis in Alta Verapaz Guatemala

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Cutaneous leishmaniasis (CL), a vector-borne parasitic neglected tropical disease, affects low- and middle-income countries such as Guatemala. In Guatemala, CL lesions are found in poor and remote Mayan populations mainly in Alta Verapaz and Petén, northern Guatemala, that report more than 90% of CL cases of the country. Despite being one of the focuses of CL in Guatemala, eco-epidemiological information on the transmission cycle of CL is not known for Alta Verapaz, as the surveys of CL vectors have not been carried out since 1990. Moreover, intervention by the Guatemalan Ministry of Health targets case treatment without implementing prevention and vector control strategies. In order to improve disease control and to develop a CL prevention strategy, our study aims to identify the transmission cycle of *Leishmania* spp. in CL focus in Alta Verapaz. The genotype of Leishmania species circulating in sandflies and infected human populations in a community with high level of CL endemicity will be established. Along with this, the host-feeding preferences among sandflies will be determined. Sandflies will be collected for one week for 12 months, using Shannon traps and CDC light traps at households where CL has been detected at intradomicile, peridomicile, and surrounding forest in a 1km transect. Human lesions will be aspired. *Leishmania* species will be detected in human samples and sandflies, using RFLP-PCR of the ITS1 region; for the sandflies pooling strategies will be implemented. Blood-source species will be identified with a *cytochrome b* PCR of the samples extracted from the gut of sandflies.

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Phlebotomine sand flies (Diptera: Psychodidae) of the South region of Brazil: mini review

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Around the world there are 1,006 species of sand flies and of these 539 occur in Americas. In Brazil are recorded 277 species distributed in the five Regions. The South region that is composed by the states of Paraná, Santa Catarina and Rio Grande do Sul present the less diversity of sand flies when compared to the others. In the region are recorded 54 sand fly species, that represent 19% of the Brazilian sand fly fauna. This number is very low when compared with North (159) and Central-West (127) regions or same states, such as Minas Gerais where are recorded 93 species. Fifty species have been recorded in Paraná, followed by Rio Grande do Sul with 24 species and Santa Catarina that is composed only by 16 species, representing 93%, 44%, and 30%, respectively, of the species recorded in the South region. Four species present as type-locality some state of the South region, three are endemic and others need more studies. *Pintomyia monticola* have been incriminated as vector of *Leishmania enrietti*, but none study was developed to confirm this role. The 26 records of *Psathyromyia shannoni* are wrong, since that the species does not occur in Brazil. The competence and vectorial capacity of *Lutzomyia gaminarai* to infect and transmite *L. infantum* need to be checked. The real distributions of *Nyssomyia neivai* and *Nyssomyia intermedia* need to evaluated, since missidentification had been registred. Using this informations, the present review discuss about all this aspects to better knowlegment of this fauna.

Notes:

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