





# Conference venue: Arbanashki han hotel

(http://www.arbanashkihan.com)



#### Organizers

Prof. Pavel Zehtindjiev Dr. Mihaela Ilieva Dr. Dimitar Dimitrov Dr. Aneliya Bobeva Mr. Martin Marinov Ms. Karina Ivanova Mr. Strahil Peev Institute of Biodiversity and Ecosystem Research Bulgarian Academy of Sciences

Prof. Abdullah Inci Prof. Alparslan Yildirim Mr. Arif Ciloglu Erciyes University Vectors and Vector Borne Diseases Implementation and Research Center Kayseri, Turkey

#### **Scientific Committee**

Prof. Staffan Bensch Department of Biology Lund University, Sweden

Prof. Pavel Zehtindjiev Institute of Biodiversity and Ecosystem Research Bulgarian Academy of Sciences

Prof. Gediminas Valkiūnas Dr. Vaidas Palinauskas Nature Research Centre, Vilnius, Lithuania

Dr. Ravinder Sehgal Department of Biology San Francisco State University, USA

# Program

# Tuesday 27 September 2016

9.30-10.00	Welcome and introduction	
Theme 1: Parasite – vector interactions		
10.00-10.40	Invited speaker Gediminas Valkiūnas: Studies of sexual development of haemosporidian parasites: Completing the cycle	
10.40-11.00	Rita Žiegytė: Culicoides impunctatus (Ceratopogonidae) is markedly susceptible to avian haemoproteids	
11.00-11.30	Coffee	
11.30-12.10	Invited speaker Ana Rivero: Heterogeneity of infection outcomes in malaria-infected mosquitoes	
12.10-12.30	Nubia Estella Matta: The big challenge: to implicate an avian Haemosporida vector in Colombia	
12.30-14.00	Lunch	
14.00-14.20	Dovilė Bukauskaitė: Culicoides nubeculosus is an effective vector of avian haemoproteids	
Theme 2: Ecol	ogy, Conservation and distribution of parasites	
14.20-15.00	Invited speaker Carter Atkinson: Vector-borne parasites and conservation of Pacific island forest birds	
15.00-15.20	Paulo Cesar Pulgarin Restrepo: Distributional ecology of avian malaria parasites and their hosts in Papua New Guinea	
15.20-15.40	Coffee	
15.40-18.40	Microscope workshop - Posters.	
19.00-	Dinner	

# Wednesday 28 September 2016

Theme 2 continued: Ecology, Conservation and distribution of parasites		
9.00-9.40	Invited speaker Robert Ricklefs: Impacts of haemosporidian parasites on the distribution and abundance of their hosts	
	Invited speaker Ravinder Sehgal:	
9.40-10.20	Avian haemosporidians: from DNA to Deforestation	
	Farah Ishtiaq: Prevalence and diversity of avian haemosporidians across migrant and resident western	
10.20-10.40	Himalayan birds in India	
10.40-11.10	Coffee	
11.10-11.30	Erika Braga: Habitat modification and secondary succession influence avian haemosporidian distributions in southeastern Brazil	
11.30-11.50	Nayden Chakarov: Leucocytozoon-raptor interactions in space and time	
11 50 12 10	Leonardo Chapa Vargas: Haemosporidian prevalence and parasitemia in the American kestrel (Falco	
11.50-12.10	spurvenusjin centrul iviexico	

	Leticia Soares: Temporal dynamics of avian haemosporidian parasite assemblages in the West Indies
12.10-12.30	archipelago
12.30-13.40	Lunch
Theme 3: Par	asite impact on hosts
	Vaidas Palinauskas: Characterisation of virulent Plasmodium elongatum strain (lineage pERIRUB01), with
13.40-14.00	remarks on its diagnostics
	André Dhondt: Apparent effect of chronic Plasmodium infections on disease severity caused by experimental
14.00-14.20	infections with Mycoplasma gallisepticum in house finches
14.20-14.40	Mikas Ilgūnas: Plasmodium (Giovannolaia) homocircumflexum kills birds
	Olaf Hallanan. Canatia diversity of avian malarin, within and between infections
14.40-15.00	Olot Heligren: Genetic alversity of avian malaria, within and between infections
15.00-15.20	Helena Westerdahl: To study key host genes in host-pathogen interactions
15.20-15.50	Coffee
15.50-16.10	Alfonso Marzal: Volume and antimicrobial activity of uropygial gland secretions are correlated with malaria infection and survival in birds
Theme 4: Pare	asite host specificity
16.10-16.30	Maria Svensson Coelho: Resource predictability and specialization in avian malaria parasites
	Michaël Moens: Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood
16.30-16.50	parasites of Andean birds
16.50-17.10	Xi Huang: Choosy parasites: infection pattern of a generalist parasite in a multi-host community
	Jenny Dunn: High diversity of generalist blood parasites in UK columbids and evidence for parasite transmission
17.10-17.30	in UK and Africa
	Pavel Munclinger: Rare disease specialists: parasite-host associations of avian blood parasites in a tropical
17.30-17.50	forest on the Mount Cameroon
18.30-19.30	Dinner
20.00-	Entertainment: Light and sound Show

# Thursday 29 September 2016

08.00-10:30	Excursion – Local birds and culture in the surroundings of Arbanasi	
10.45-11.00	Coffee	
Theme 5: Molecular and experimental methods		
	Invited speaker Staffan Bensch:	
11.00-11.40	Beyond the genome of Haemoproteus tartakovskyi	
11 40-12 00	Janus Borner: Nuclear gene markers for phylogenetic analyses of Haemosporida	
11.40-12.00		
	Karina Ivanova: A discrepancy between morphological and molecular analyses of a haemosporidian parasite in	
12.00-12.20	little bitterns (Ixobrychus minutus) from Bulgaria	
12.20-13.40	Lunch	
12 40 14 00	Rasa Bernotiene: Detection of mixed haemosporidian infections: how to solve the problem?	
13.40-14.00		
14.00 14.20	Dimitar Dimitrov: Single sampling underestimate haemosporidian' prevalence: an experimental study of	
14.00-14.20	natural relapses	

14.20-14.40	Jennifer Stockdale: Use of multiple primer pairs reveals high levels of cryptic co-infection in UK Columbiformes
14.40-15.00	Pavel Zehtindjiev: Experimental approach in haemosporidian studies: past, present and future in Bulgaria
15.00-15.40	Final discussion. Next conference – Funding for network?
15.40-16.00	Coffee
	End of Conference. Concluding remarks

# List of Posters (abstracts pages 25-33)

Aneliya Bobeva	What is hidden in the bloodmeal?		
Daliborka Stanković	Diversity of haemosporidian parasites in wild birds in Serbia		
Elsa Ziegler	A bioinformatics approach to generate genomic contigs of the haemosporidian parasite Polychromophilus sp.		
Karina Monzalvo	Haemoparasites in passerine birds from semi-arid areas of Mexico: effect of mine-induced metal pollution		
Maria Svensson Coelho	Turnover in avian haemosporidian assemblages: assessing the relative effects of host evolution, habitat, and geographic distance		
Martin Marinov	Do mixed haemosporidian infections exert additive effects on the behaviour of a passerine host?		
Mihaela Ilieva	Tracking the parasites: Inferring the transmission areas of haemosporidian parasites by tracking their hosts		
Mizue Inumaru	Prevalence of avian haemosporidia in injured wild birds rescued in Tokyo and surrounding areas of Japan		
Nubia Estella Matta	GERPH: A biological collection of haemoparasites of wildlife		
Peter Shurulinkov	On the haemoparasites of Ravens (Corvus corax)		
Strahil Peev	New data on diversity, host specificity and transmission areas of haemosporidian parasites in Palearctic-African bird migratory system		

### Abstracts - Oral Presentations (sorted by the surname of the presenting scientist)

### **Vector-Borne Parasites and Conservation of Pacific Island Forest Birds**

Carter T. Atkinson

U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawaii, 96718, USA

Endemic and indigenous forest birds in the Pacific Basin are believed to be vulnerable to invasive diseases and disease vectors, but the parasite fauna of the region is poorly explored and little baseline information is available about indigenous vector-borne parasites. While introduced Culex mosquitoes, avian malaria (Plasmodium relictum) and Avipoxvirus spp. have had significant effects on Hawaiian forest birds and threaten the Galapagos avifauna, their distribution and impacts on other archipelagoes and isolated islands are less well known. We sampled forest bird populations along an east-west transect across the southwestern (SW) Pacific, collecting blood samples from endemic, indigenous, and introduced species from New Caledonia, Vanuatu, Fiji, Tonga, Wallis and Futuna, Independent Samoa, American Samoa, and the Cook Islands. We detected an unexpected diversity of Plasmodium and Haemoproteus lineages, filarial worms, avian trypanosomes, and haemoccoccidians that decreased with both land area and distance from Australia and New Guinea. Several of these appear to be undescribed species based on both morphology and cytb sequence data and may even be endemic to specific archipelagoes. We detected the Hawaiian lineage of P. relictum (GRW4) in a small number of samples from New Caledonia (3), Fiji (1) and Independent Samoa (1), but GRW4 was conspicuously absent from a large collection of blood samples from American Samoa that spanned a 10 year period between 2001-2011. Avipoxvirus infections were also not detected in any of the passerine birds we handled, suggesting that it may be absent or rare in the central Pacific. Unlike more isolated archipelagoes in the north central and eastern Pacific, the SW Pacific has a relatively diverse fauna of indigenous mosquitoes, ceratopogonid flies, and black flies that extends as far east as French Polynesia. In spite of this, there were unexpected gaps in the distribution of Haemoproteus and no definitive detections of Leucocytozoon across the region. Unlike Hawaii and the Galapagos Islands, SW Pacific avifaunas appear to be similar to continental areas in terms of hematozoan diversity.

#### Beyond the genome of Haemoproteus tartakovskyi

#### S. <u>Bensch</u>

Department of Biology, Lund University, Sweden

Avian haemosporidians of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* count a few hundred described species and perhaps more than ten times as many cryptic or undescribed species. Over the past 15 years, a region of the parasites' cytochrome *b* (cyt *b*) gene has become the standard for barcoding, and since 2009, this information is compiled and made available in the database MalAvi

(http://mbio-serv2.mbioekol.lu.se/Malavi/). Thanks to joint efforts of many laboratories world-wide screening haemosporidians, the database contains host- and geographic data for >2000 unique cyt b lineages. Most of these are either host specific or rare, as they so far have been found in single host species, but a smaller proportion are broad generalists with findings in >100 host species. To understand the evolution of variation in host specificity requires sequences from nuclear loci to 1) obtain better supported phylogenies than what can be constructed from the cyt b gene, 2) precisely define species limits based on recombination analyses, 3) examine population genetic structure of proposed generalists and 4) design primers for studies of genes involved in the host evasion path-way. The publication of the genome of *Haemoproteus tartakovskyi* and the first transcriptomes from bird *Plasmodium* parasites now makes these questions tractable on a large scale. I will present ongoing work based on selective whole genome amplification and sequence capture to obtain the genomic data necessary for exploring these questions.

### Detection of mixed haemosporidian infections: how to solve the problem?

R. Bernotienė, V. Palinauskas, T. A. Iezhova, G. Valkiūnas

Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania

Polymerase chain reaction (PCR)-based detection methods are widely used in wildlife haemosporidian studies. Applications of these diagnostic tools have revealed a remarkable genetic diversity of haemosporidian parasites. However, it has been shown that these methods are often insensitive in reading mixed haemosporidian infections, which can be overlooked if solely PCR-based diagnostic tools are used. Determination of mixed infections is important because they predominate in wildlife and often are associated with high virulence. In the present study, five different PCR assays were applied and their sensitivity in detection of experimentally designed mixed infections of Haemoproteus and Plasmodium parasites was compared. Three of these PCR assays used primer sets that amplify fragments of mitochondrial genome cytochrome b gene and one of cytochrome oxidase subunit I gene, and one primer set targeted the apicoplast genome. Results of the study show that the use of a single PCR assay markedly underestimates the biodiversity of haemosporidian parasites. Only from 7 to 53% of investigated mixed infections was detected using a single PCR assay. The application of at least 3 PCR assays in parallel detected the majority (up to 90%), but still not all genetic lineages present in mixed infections. The preferences of different PCR assays in the detection of parasites belonging to Plasmodium or Haemoproteus genera during mixed infections were determined and combinations of widely used PCR assays, which can be sensitive in reading many mixed infections were identified. We call for more careful research and data analysis of mixed haemosporidian infections in wildlife through the application of microscopic and several PCR-based assays, which should be applied in parallel. The study was partly supported by the Research Council of Lithuania (MIP038/2015).

### Nuclear gene markers for phylogenetic analyses of Haemosporida

J. <u>Borner</u><sup>1</sup>, I. Bruchhaus<sup>2</sup>, T. Burmester<sup>1</sup>

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Our understanding of the evolutionary history of Haemosporida and the acquisition of novel life-history traits by these parasites depends on a solid phylogenetic back-bone. Over the past decade, the datasets used for inference of the haemosporidian tree of life have improved considerably in terms of capturing the enormous biological diversity of this parasite order by extended taxon sampling. However, due to the challenges involved in developing nuclear markers for this diverse group of parasites, studies were limited to a small set of genes mostly of mitochondrial or apicoplast origin. These genes are not well suited for the inclusion of the more distantly related apicomplexan outgroups. Additionally, the phylogenetic signal contained in the relatively short gene fragments might not be sufficient to resolve the deeper nodes of the tree. Using a bioinformatic approach, we generated a set of 21 nuclear gene markers which are capable of amplifying gene fragments from most major haemosporidian lineages. These primers allowed us to obtain sequences from parasites of the taxa *Leucocytozoon, Haemoproteus, Parahaemoproteus, Polychromophilus* and *Plasmodium*. Phylogenetic analyses resulted in a well-resolved phylogeny that is robust to the choice of outgroup and the method of tree inference, thus proving the utility of the gene markers for deep-level tree inference.

# Habitat modification and secondary succession influence avian haemosporidian distributions in southeastern Brazil

Francisco C. Ferreira Junior, Vincenzo A. Ellis, Raquel A. Rodrigues, Lemuel O. Leite, Magno A. Z. Borges, Érika M. <u>Braga</u>

Land conversion can alter vector-borne diseases, and these factors together can cause loss of biodiversity and increase risks of infectious diseases to humans and animals. However, natural forest recovery through secondary succession can counteract habitat loss by improving vegetation complexity and increasing fauna diversity. Our objective was to test the effects of secondary succession on the communities of vertebrate hosts and their haemosporidian parasites. We mist-netted 461 birds in four different successional stages in areas that constituted Seasonally Dry Tropical Forest fragments: pasture areas, early, intermediate and late stages. The first three stages consisted in pasture areas abandoned 8, 12 and 30 years ago, and the late stage has no reports of human intervention for more than 50 years. Plasmodium and Haemoproteus were detected by a screening PCR from the collected samples, and positive samples were submitted to a nested-PCR and subsequent sequencing of a fragment from the parasites' cytochrome b gene. Overall prevalence differed across successional stages, being higher in pasture as compared to any other areas. Haemosporidian prevalence was also higher in pasture than in non-pasture areas when only common bird species in both areas were considered. Parasite communities were similar between successional stages. Total haemosporidian prevalence did not vary between the end of the rainy season and the middle of the dry season, but it did increase at the end of the dry season and remained at the same level at peak of the following rainy season. This increase in prevalence was

detected at the onset of the breeding season in southeastern Brazil, so it may be related to seasonal changes in the reproductive period of the sampled birds. Our results show that the recovery of disturbed areas into more complex forests change avian communities, benefitting the ones related to advanced successional stages with lower prevalence of pathogenic parasites. Understanding the effects of forest recovery on the dispersal of parasites may help to formulate conservation strategies to improve environmental and animal health. We also found effects of seasonality in parasite prevalence and in lineages dominance at community and individual levels. These results warrants further long-term investigations in tropical forests to understand temporal drivers of parasite ecology.

### Culicoides nubeculosus is an effective vector of avian haemoproteids

Dovilė <u>Bukauskaitė</u><sup>1</sup>, Rita Žiegytė<sup>1</sup>, Vaidas Palinauskas<sup>1</sup>, Dimitar Dimitrov<sup>2</sup>, Mikas Ilgūnas<sup>1</sup>, Tatjana A. Iezhova<sup>1</sup>, Rasa Bernotienė<sup>1</sup>, Mikhail YU. Markovets<sup>3</sup>, Gediminas Valkiūnas<sup>1</sup>

1 Institute of Ecology, Nature Research Centre, Akademijos 2, Vilnius 21, LT-08412, Lithuania 2 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciencies, 2 Gagarin Street, Sofia 1113, Bulgaria

3 Biological Station Rybachy of the Zoological Institute, Russian Academy of Sciences, Rybachy 238535, Kaliningrad Region, Russia.

Haemoproteus parasites (Haemosporida, Haemoproteidae) are widespread, and some species cause disease both in birds and blood-sucking insects. These infections are transmitted by Ceratopogonidae and Hippoboscidae insects, but vector species remain unknown for many of these parasites and their genetic lineages. The aim of this study was to investigate sporogonic development of 5 widespread Haemoproteus species in experimentally infected biting midges Culicoides nubeculosus (Ceratopogonidae). Laboratory reared *C. nubeculosus* flies were infected experimentally by allowing them to take blood meals on owls (Asio otus and Strix aluco) and passeriform birds (Luscinia luscinia, Motacilla alba and Sturnus vulgaris) naturally infected with Haemoproteus noctuae (lineage hCIRCUM01), H. syrnii (hCULCIBO01), H. balmorali (hROBIN1), H. motacillae (hYWT2) and H. pastoris (hLAMPUR01), respectively. The engorged insects were maintained in the laboratory at 16-19 °C, and dissected at intervals in order to follow the development of sporogonic stages. The presence of corresponding lineages of parasite in infected insects was confirmed by polymerase chain reaction (PCR). Phylogenetic analysis was used to determine phylogenetic relationships among these and other closely related haemosporidians. All these parasite species completed sporogony in C. nubeculosus, and ookinetes, oocysts and sporozoites were reported. In accordance with sporogony data, the phylogenetic analysis placed lineages of these parasites in a clade with other *Haemoproteus* (*Parahaemoproteus*) parasites transmitted by Ceratopogonidae species. *Culicoides nubeculosus* is highly susceptible to Haemoproteus (Parahaemoproteus) parasites, with numerous sporozoites reported in salivary glands. These biting midges are likely the natural vector. Importantly, C. nubeculosus is easy to cultivate in laboratory conditions, and we recommend it to use in experimental research with avian haemoproteids. This study provides additional evidence that phylogenies based on cytochrome b gene indicate parasitevector relationships and can be used for prediction of haemoproteid vectors.

### Leucocytozoon-raptor interactions in space and time

<sup>1</sup>N Chakarov, <sup>2</sup>O Krüger, <sup>1</sup>S Bensch

<sup>1</sup> Molecular Ecology and Evolution Lab, Lund University, Sweden <sup>2</sup> Animal Behaviour, Bielefeld University, Germany

The host-parasite arms race is dependent on the genetic diversity of both parties and the transmission dynamics in and among host populations. Therefore, these interactions should be explored in a metapopulation framework. For many generalist avian malaria parasites this would be possible only with strong restrictions on sample size and/or genetic detail. However, for more specialized parasites such as raptor-specific *Leucocytozoon buteonis* and *Leucocytozoon mathisi* the restricted host community allows the study in greater detail of the molecular interactions between host and parasite populations. We studied the within-lineage genetic diversity of *Leucocytozoon* in and among populations of their diverse raptor hosts in Europe. Our main focus was on candidate genes involved in antigenic variation for the parasites and genes involved in different defense arms of the immune system of the hosts. Our sample provides full coverage of all available individuals of the main hosts in some populations and the full range of available host species. This gives us a unique view on the metapopulation dynamics of haemosporidian transmission and adaptation. First results will be presented.

# Haemosporidian prevalence and parasitemia in the American kestrel (Falco sparverius) in Central Mexico

L. <u>Chapa-Vargas<sup>1</sup></u> and R. Tinajero-Hernández<sup>1</sup>

<sup>1</sup>División de Ciencias Ambientales. Instituto Potosino de Investigación Científica y Tecnológica A.C. Mexico.

We investigated factors influencing haemosporidian prevalence and parasitemia in the American Kestrel at a semi-arid region in Central Mexico. Factors investigated included habitat type (natural scrublands vs agriculture vs villages), sex, age (juveniles vs adults), and season (reproductive vs non-reproductive). Parasite prevalence and parasitemia were quantified through microscopy analysis of blood samples from 56 individual kestrels. The data were analyzed through logistic regression for prevalence, and generalized linear models for parasitemia. Models explaining variation in these two response variables were compared using the Akaike Information Criterion (AIC). The results suggested that the most important variables explaining variation in prevalence were habitat type and sex, with the lowest prevalence in natural scrublands, intermediate prevalence in agricultural fields, and highest prevalence in villages, and with higher prevalence in males than in females. Parasitemia was best explained by habitat type, and by kestrel sex and age; the lowest parasitemia was recorded in natural scrublands, whereas the highest parasitemia was found in villages. Finally, in terms of sex and age, males and adult kestrels showed the highest parasitemia.

# Apparent effect of chronic *Plasmodium* infections on disease severity caused by experimental infections with *Mycoplasma gallisepticum* in house finches

André A. Dhondt (Lab of Ornithology) and Keila V. Dhondt (College of Veterinary Sciences),

#### Cornell University, Ithaca, NY 14850, USA

To test if house finches with chronic haemosporidian infections would respond differently to coinfection with the bacterial pathogen *Mycoplasma gallisepticum* (MG) we created groups of wild caught juvenile house finches half of which were naturally infected with *Plasmodium* and in half of which no *Plasmodium* was detected using PCR. We will report on the time to infection, and on the severity and duration of disease in the two sets of birds using three strains of MG that differed in virulence.

# Single sampling underestimate haemosporidian' prevalence: an experimental study of natural relapses

Dimitar <u>Dimitrov</u><sup>1</sup>, Steffen Hahn<sup>2</sup>, Karina Ivanova<sup>1</sup>, Tamara Emmenegger<sup>2</sup>, Martin P. Marinov<sup>1</sup>, Strahil Peev<sup>1</sup>, and Pavel Zehtindjiev<sup>1</sup>

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To complete their life cycle, avian malaria and related haemosporidian parasites synchronize their reproductive phase with the occurrence of both their invertebrate vectors and their vertebrate hosts. In temperate-breeding birds evidence of higher prevalence usually occurs during the breeding season of the host species. This period is associated with abundance of haemosporidian vectors, but also the availability of a new generation of immunologically naive avian hosts. What is not known, however, is the extent to which the increased prevalence of haemosporidian infections during the breeding season is due to new infections versus natural relapses. We address the potential contribution of relapses to haemosporidian prevalence in Great Reed Warbler (Acrocepahlus arundinaceus) by serially sampling birds during pre-breeding, breeding and pre-migratory periods. Blood samples were collected from freeliving birds when first captured and the uninfected were selected and maintained in vector-proof aviaries for 7-60 days. The second blood sample was taken at least 7 days after catching. Blood sampling of birds which were assessed as uninfected and kept in captivity was conducted on each 3th day to estimate haemosporidian prevalence using a combination of microscopic and PCR-based methods. We found that single versus serial sampling significantly underestimated the haemosporidian prevalence in Great Reed Warblers at their breeding sites. Relapses of malarial infections (Plasmodium spp.) were much more common than haemoproteid infections (*Haemoproteus* spp.). Additionally, we found that microscopic examination is more sensitive to detect relapsed haemosporidian infections in wild birds than PCR-based detection methods. We conclude that the assessment of total haemosporidian prevalence should be used cautiously even if the sampling has been done during avian host breeding season, and examined with microscopic and PCR-based methods.

# High diversity of generalist blood parasites in UK columbids and evidence for parasite transmission in UK and Africa

<sup>1,2</sup>J.C. <u>Dunn</u>, <sup>2</sup>J.E. Stockdale, <sup>1</sup>AJ. Morris, <sup>3</sup>PV. Grice, <sup>4</sup>KC. Hamer.

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PCR techniques have dramatically increased knowledge of the diversity and ecology of blood parasite strains infecting birds worldwide. Here we screen blood samples collected from the European Turtle dove Streptopelia turtur, a trans-Saharan migrant, and from three resident heterospecifics from UK breeding grounds: Collared doves S. decaocto, Stock doves Columba oenas and Woodpigeons C. palumbus. We screened 168 samples collected during 2011-2014 at sites across south-east England, UK, using multiple primer pair combinations targeting the entire cytochrome b (cytb) gene region of Plasmodium, Haemoproteus and Leucocytozoon haemoparasites. We identified 15 strains of Leucocytozoon and Haemoproteus within our dataset where our sequences fully overlapped the section of cytb covered by the MalAvi database and an additional 31 distinct strains with partial overlap which matched 13 existing parasite lineages with 92-100% identity. Our 15 strains with full MalAvi overlap matched seven existing parasite lineages (5 Haemoproteus and 2 Leucocytozoon) with 99-100% identity. One Haemoproteus strain was isolated from adult and nestling resident Woodpigeons across multiple sites, having initially been identified as infecting a resident adult columbid in Malawi, Africa. This suggests transmission of this parasite both in the UK and in Africa, which may imply spread via migratory species although we did not isolate this strain from turtle doves. We found one generalist Leucocytozoon strain previously isolated from Falconiformes, Gruiformes, Piciformes and Charadriiformes across Europe, Russia and the Philippines in a juvenile Woodpigeon, although we could not confirm patent infection from blood smears. Our finding of multiple lineages showing 100% identity match to the same characterised strain in MalAvi highlights the need to examine a larger amplicon from the cytb region, or additional genes, in order to reliably identify parasite lineages.

### Genetic diversity of avian malaria, within and between infections

O. <u>Hellgren</u>

#### Department of Biology, Lund University

In the field of avian malaria we have gone from determining the parasite diversity based on morphology to using *cyt*. b diversity and lately several nuclear genes. At each step we have gained new information about host-specificity, population structures and transmission ranges. Here, I present preliminary data obtained from the transcriptome sequencing of *Plasmodium relictum* (SGS1) that reveals a new level of genetic diversity. Using transcriptome sequencing we can now start to investigate parts of the genome that show high or low levels of genetic variation between different strains of the parasite. Moreover, transcriptomics data also show that there is a lot of genetic diversity within each infection episode even

if the infection started with the same donor strain. This haplotype diversity found within hosts could have originated from allelic variation during the diploid phase in the mosquito or through acquired mutations. The frequency of these different haplotype seems to vary, not only between infected individuals but also over the course of infection within individuals. The shifts in haplotype frequencies would either be a result of random genetic drift or through selection imposed by the individual hosts, which could be a piece in the puzzle of understanding individual variation in infection outcomes.

# Choosy parasites: infection pattern of a generalist parasite in a multi-host community

Xi Huang, Staffan Bensch

#### Department of Biology, Lund University, Sweden

Avian malaria parasites exhibit considerable variation in host specificity, geographical range, and effects on host individuals. For generalist parasites, which can infect a large variety of different host species, prevalence and parasitemia may vary among those host species, e.g. due to the time allowed for coevolution. In some non-typical host species, parasitemia level might be extremely low as the parasite may not be able to develop into mature gametocytes. *Haemoproteus majoris* is a generalist avian malaria parasite, infecting a wide range of host species and is prevalent worldwide. Among its five mitochondrial lineages that have been morphologically described, WW2 was chosen as the study organism because of its wide host range. In this study, we developed a lineage specific q-PCR protocol based on a nuclear gene, circumsporozoite- and TRAP-related protein (CTRP), and detected the infection pattern of WW2 lineage among different species in a wild bird community in the forests around Krankesjön, Sweden.

### Plasmodium (Giovannolaia) homocircumflexum kills birds

M. <u>Ilgūnas<sup>1</sup></u>, D. Bukauskaitė<sup>1</sup>, V. Palinauskas<sup>1</sup>, T. Iezhova<sup>1</sup>, N. Dinhopl<sup>2</sup>, N. Nedorost<sup>2</sup>, C. Weissenbacher-Lang<sup>2</sup>, H. Weissenböck<sup>2</sup>, G. Valkiūnas<sup>1</sup>

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Avian malaria parasites (*Plasmodium*) are widespread, but their virulence has been insufficiently investigated. The aim of this study was to investigate the pathology caused by *Plasmodium* (*Giovannolaia*) homocircumflexum (lineage pCOLL4) in three species of experimentally infected common European birds – Eurasian siskin Carduelis spinus, common crossbill *Loxia curvirostra,* common starling *Sturnus vulgaris* – and also in domestic canary *Serinus canaria*. These bird species were infected by subinoculation of infected blood, developed malaria infection, survived the peak of parasitemia, but suddenly died between 28-38 days post exposure. Numerous secondary exoerythrocytic meronts (phanerozoites) were visible in histological sections of all organs in all birds, but they were easier recognisable in the same samples after application of chromogenic in situ hybridization (ISH) assay.

Blockage of brain capillaries with phanerozoites led to cerebral ischemia caused cerebral paralysis. That is most likely the main reason of the sudden death of all infected birds. Mortality was reported when parasitemia decreased or even turned into chronic stage, indicating that the light parasitemia is not always indication of improved health during avian malaria. In other words, solely testing of blood samples is an insufficient method to understand avian malaria virulence, which might be underestimated in many *Plasmodium* spp. due to lack of information about their exoerythrocytic development. Several cycles of tissue merogony occur during bird malaria, and many *Plasmodium* spp. produce phanerozoites, which are induced by merozoites developing in erythrocytic meronts. Phanerozoites damage organs, but remain insufficiently investigated in the majority of described *Plasmodium* spp. We recommend application of traditional histological and ISH methods in parallel in investigations of exoerythrocytic development of avian malaria parasites. Application of both these tools in parallel speeds up the search for tissue meronts and also provides information about morphological characters of the parasites and their host cells. This study was funded by the Research Council of Lithuania (MIP-045/2015).

# Prevalence and diversity of avian haemosporidians across migrant and resident western Himalayan birds in India

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Avian malaria (*Plasmodium* spp.) and other haemosporidians (*Haemoproteus* and *Leucocytozoon* spp., Phylum Apicomplexa, class Haemosporida) are a diverse group of vector-transmitted blood parasites which are globally distributed and found in many avian species [1, 2]. The ultimate goal of current research is to determine the influence of ecological variability, such as changes in temperature, vector community, host migration patterns and habitat, on the epidemiology of avian malaria and spread of disease in high-altitude malaria-free zones. A total of 2000 birds were sampled in breeding seasons (April-May) in 2014-2015. Using molecular and microscopy methods, 14% of birds were found infected with *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.. Of the total positives, 105 cyt b gene sequences representing unique parasite lineages were isolated. High proportion of infections of Haemoproteus spp., representing 63 lineages whereas Plasmodium spp. had 17 lineages and Leucocytozoon spp. had 25 lineages. Low altitude birds exhibited high parasite prevalence with diverse parasite fauna. The parasite prevalence was higher in migrant host species than resident hosts in highaltitude. The high parasite prevalence in high-altitude sites is mainly driven by migrants or hosts which show regular seasonal movements. In addition, vector sampling across different altitudes revealed five culicine mosquitoes spp. and seven ceratopogonids spp. and many unidentified black flies spp., potential vectors for Plasmodium, Haemoproteus and Leucocytozoon, respectively. These preliminary results highlight that cost of migration for migrant birds and the potential of transmission to immunologically naïve birds in high-altitude breeding ground.

# A discrepancy between morphological and molecular analyses of a haemosporidian parasite in little bitterns (*Ixobrychus minutus*) from Bulgaria.

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The study of haemosporidians has greatly improved with the development of molecular techniques, discovering higher diversity of parasites than previously known from the traditional morphology. The application of both molecular and microscopic approaches has developed barcodes for previously described morphospecies and has allowed the identification of cryptic species which is essential in respect to understanding parasite diversity and evolution. Even though haemosporidians are globally distributed and occur in most bird species, the knowledge on parasite diversity is quite uneven and concentrated on passerine bird species. In the present study, we investigate haemosporidian parasites of a non-passerine bird – the little bittern (Ixobrychus minutus). We sampled 63 little bitterns during their breeding season in northeast Bulgaria. Microscopic examination of blood smears showed high prevalence of haemosporidians over 70%. On the basis of morphological characters we were able to identify the species Haemoproteus herodiadis, a common parasite among birds of the Ciconiiformes. However, molecular analyses of mitochondrial cyt b gene using 2 different primer pairs of samples infected with *H. herodiadis* failed to amplify a *Haemoproteus* parasite. Instead all obtained sequences were belonging to *Plasmodium* sp. During smear analysis meronts were absent on most samples and when present, assumed to be the result of a mixed infection of a *Plasmodium* sp. parasite. Our preliminary results based on long mitochondrial regions (full COI and cyt b genes) and qPCR confirmed that amplification and parasitaemia correspond to a *Plasmodium* parasite. Thus, we conclude that the parasite gametocytes we observed are very similar to *H. herodiadis* and can be easily misidentified. Considering the preliminary molecular results, the observed gametocytes (and rare meronts) might instead belong to a *Plasmodium* species with synchronized development and reduced asexual replication in the blood of the host.

# Volume and antimicrobial activity of uropygial gland secretions are correlated with malaria infection and survival in birds

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Animals have developed a wide range of defensive mechanisms against parasites to reduce the likelihood of infection and its negative fitness costs. The uropygial gland is an exocrine gland that produces antimicrobial and antifungal secretions with properties used as a defensive barrier on skin and

plumage. This secretion has been proposed to affect the interaction between avian hosts and their ectoparasites. Because uropygial secretions may constitute a defense mechanism against ectoparasites, this may result in a reduction in prevalence of blood parasites that are transmitted by ectoparasitic vectors. Furthermore, other studies pointed out that vectors could be attracted by uropygial secretions and hence increase the probability of becoming infected. In a first study we explored the relationship between uropygial gland size, antimicrobial activity of uropygial secretions and malaria infection in house sparrows *Passer domesticus*. We found that uninfected house sparrows had larger uropygial glands and higher antimicrobial activity in uropygial secretions than malaria-infected individuals. We found a positive association between uropygial gland size and scaled body mass index, but only in uninfected sparrows. Female house sparrows had larger uropygial glands and higher antimicrobial activity in a second study we analysed the relationship between uropygial gland volume, malaria infection and survival. Preliminary results showed that uropygial gland volume explained variation in survival, where individuals with higher gland volume had the highest chance of survival. These findings suggest that uropygial gland secretions may play an important role as a defensive mechanism against malaria infection and other deadly pathogens.

### The big challenge: to implicate an avian haemosporida vector in Colombia

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Approximately only 10 % of the avian haemosporida species known have a detailed life cycle description. Studies looking to determine vector competence and incrimination of avian haemosporida in the Neotropical countries are scarce and represent a real challenge. For that reason, since several years, it has been our objective to track vectors from different experimental models. First: surveillances for adult blood-sucking insects (Simuliidae, Ceratopogonidae and Culicidae) were carried out from different localities previously confirmed for avian haemosporida transmission; on this material the morphological determination, blood source, and parasites amplifications were done, to establish the spatio-temporal relationships between parasites, hosts and possible vectors. Then, to corroborate the parasite development on selected blood-sucking insects, a series of experimental studies were developed: (1.) Colombian Highlands: Experimental Model (EM): Birds natural infected with Leucocytozoon-Simuliids. (2.) Campus Universidad Nacional, Bogotá-Colombia: (EM): Serinus canaria inoculated with Plasmodium (N.) unalis or P.(H.) lutzi- Culex quinquefasciatus. (3.) Campus Universidad Nacional, Bogotá-Colombia: (EM): Columba livia natural infected with Haemoproteus columbae-Culicoides venezuelensis. (4.) Campus Universidad Nacional, Bogotá-Colombia (EM): Zonotrichia capensis infected with Haemoproteus coatneyi or H. erytrhogravidus-Culicoides venezuelensis. The research involved several approaches: 1) to know which are the species present in the study area, 2) to explore different traps in order to improve vector capture, 3) to analyse their blood source, 4) to design our own experimental models, 5) to overpass the hassle of not having vector colonies (simuliids or culicoides),

and 6) to follow experimental infections for the period of time that is necessary to develop infective parasite stages: oocysts and sporozoites. As of yet, new considerable knowledge has been produced but vectors have not been incriminated.

# Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood parasites of Andean birds.

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Understanding how parasites fill their ecological niches requires information on the processes involved in the colonization and exploitation of unique host species. Switching to hosts with atypical attributes may favor generalists broadening their niches, or may promote specialization and parasite diversification as the consequence. We analyzed which blood parasites have successfully colonized hummingbirds, and how they have evolved to exploit such a unique habitat. We specifically asked (i) if the assemblage of *Haemoproteus* parasites of hummingbirds is the result of single or multiple colonization events, (ii) to what extent these parasites are specialized in hummingbirds or shared with other birds, and (iii) how hummingbirds contribute to sustain the populations of these parasites, in terms of prevalence and infection intensity. We analyzed 169 hummingbirds and 736 other birds of 19 and 112 species respectively along an elevation gradient in Southern Ecuador with microscopy and molecular techniques. Hummingbirds hosted a phylogenetically diverse assemblage of generalist Haemoproteus lineages shared with other host orders. Among these parasites, Haemoproteus witti stood out as the most generalist. Interestingly, we found that infection intensities of this parasite were extremely low in passerines (with no detectable gametocytes) but very high in hummingbirds, with many gametocytes seen. Moreover, infection intensities of H. witti were positively correlated with prevalence across host species. Our results show that hummingbirds have been colonized by generalist Haemoproteus lineages in multiple occasions. However, one of these generalist parasites (H. witti) seems to be highly dependent on hummingbirds, which arise as the most relevant reservoirs in terms of both prevalence and gametocytaemia. From this perspective, this generalist parasite may be viewed as a hummingbird specialist. This challenges the current paradigm of how to measure host specialization in these parasites, which has important implications to understand disease ecology.

# Rare disease specialists: parasite-host associations of avian blood parasites in a tropical forest on the Mount Cameroon

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The pattern of parasite-host associations ranks among the central topics of ecology. Theory predicts that parasite specialization should be highly favoured and host range should narrow in the course of evolution. Differences in specificity have been assumed between temperate and tropical regions. Higher species richness in tropics usually results in lower abundance of hosts. Hence single host might not serve as an easily exploitable resource and more generalist parasite strategy should be favoured. On the other hand, highly specialised interaction are expected to evolve in tropics due to stable conditions. Empirical studies of host specificity in tropics show rather inconsistent results; however. Haemosporidian blood parasites of birds offer splendid system for studying specificity of parasite-host associations. We studied host associations of avian haemosporidian blood parasites in a tropical forest on the Mount Cameroon. We used network analysis approach to estimate reciprocal specialization of parasites and host and we also assessed the effect of host traits on the probability of infection. Contrary to previous haemosporidian studies in Western Africa we aimed at dense sampling of hosts (1044 individuals of 76 species) on a restricted geographic area. Our study plot represents unique system due to relatively low abundances of insect vectors. Despite extensive precipitations, volcanic tuff layers at the Mt. Cameroon absorb water so quickly that streams and water reservoirs necessary for avian malaria insect vectors development are scarce. Hence we assumed low prevalence of haemosporidian parasites which should result in lower specificity of parasite-host interactions. Surprisingly only the first assumption was met. Haemosporidian parasites occurred in low prevalence but they showed extremely narrow host breadth. Concerning the host traits, the host abundance and time spent foraging on ground influenced parasite prevalence, while parasite lineage richness was significantly influenced only by host abundance.

# Characterisation of virulent *Plasmodium elongatum* strain (lineage pERIRUB01), with remarks on its diagnostics

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*Plasmodium elongatum* is one of the most dangerous agents of avian malaria. This parasite develops and causes lethal disease not only in natural hosts, but also in non-adapted endemic birds. According to phylogenetic analysis based on mitochondrial cytochrome *b* gene, there are several closely related lineages that may belong to *P. elongatum*. However, these lineages might have differences in virulence and their ability to develop in certain mosquitoes and birds. This experimental study provides information about the molecular and morphological characterisation of the virulent *P. elongatum* strain (lineage pERIRUB01) isolated from a naturally infected European robin *Erithacus rubecula* and discusses diagnostics of disease caused by this infection. According to phylogenetic analysis the lineage pERIRUB01 is closely related to the lineage pGRW6 of *P. elongatum*. Morphologically indistinguishable blood stages and patterns of development in mature and polychromatic erythrocytes indicate that they belong to the same species. Both pathogens are virulent causing death of their vertebrate hosts. Massive infestation of bone marrow cells is the main reason of mortality. This study shows that light parasitemia, which is commonly observed in wild birds, is not always a measure of bird health because infection may have detrimental effects on bird fitness due to the interruption of the erythropoiesis. We demonstrate that syncytium-like remnants of tissue meronts slip out from bone marrow to the peripheral blood and may serve as a template for PCR amplification. This finding contributes to better understanding positive PCR amplifications in birds when parasitemia is undetectable. Sporogonic development of *P. elongatum* (pERIRUB01) completes in *Culex pipiens pipiens* form *molestus* and *Culex quinquefasciatus* mosquitoes. However, these mosquitoes exhibit fractional susceptibility. The obtained information is important for better understanding the epidemiology of *P. elongatum* transmission and diagnostics of avian malaria infections. The study was partly supported by the Research Council of Lithuania (MIP038/2015).

# Distributional ecology of avian malaria parasites and their hosts in Papua New Guinea

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Tropical mountains serve as natural laboratories for understanding the ecology and evolution of montane organisms. The distributions of birds and other taxa often show strong species turnover across steep environmental gradients. However, the drivers of sharp replacements across altitudinal belts remain poorly understood. We studied the ecology and evolution of bird-parasite interactions to evaluate the possible role of parasites in maintaining range boundaries in tropical montane birds along a gradient in the YUS Conservation Area in Papua New Guinea ranging from 230 to 2940 m. Specifically, we studied: 1) community-wide distribution and diversity of avian malaria parasites (Plasmodium and Haemoproteus) spanning a full range of elevations, and 2) the patterns of prevalence, richness, and lineage distribution in several groups of closely related bird species with abutting range boundaries (parapatric elevational distributions). We examined 2268 individuals from 66 species and 16 families. Using molecular methods, we detected a total of 1049 infections (overall prevalence = 46%) and obtained over 800 mtDNA parasite sequences. Infection prevalence was highest below 2200 m, but infected individuals were present throughout the entire elevational range. We found a strong phylogenetic signal in prevalence: some families exhibited infection prevalence exceeding 85% (e.g., Melanocharitidae - berrypeckers and longbills - and Pretroicidae - Australasian robins), while prevalence was lower than 5% in other families (e.g., Rhipiduridae - Fantails). We detected a great diversity of mtDNA malaria haplotypes across bird species, with higher Haemoproteus diversity relative to *Plasmodium* diversity, in agreement with previous studies. Haplotypes were often, though not always, species-specific. Three species of Melanocharis berrypeckers restricted to different elevational zones are each parasitized by different avian malaria lineages, a pattern consistent with a model in which mutual intolerance of specialized pathogens may establish sharp distributional boundaries.

# Impacts of haemosporidian parasites on the distribution and abundance of their hosts

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Haemosporidian infections of birds have been shown to reduce individual fitness in several experimental studies, and populations of some species in isolated archipelagoes have been devastated by introduced pathogens. However, the population consequences of endemic infections are poorly documented owing to the difficulty of conducting population level experiments and of comparing independent populations of the same host species. In an analysis of the relative abundance of host populations and prevalence of haemosporidian parasites across 13 islands in the Lesser Antilles, we found significant negative associations across islands between populations of several host species and particular lineages of parasite, which were not necessarily common parasites of the affected species (*J. Biogeogr.* 43(7):1277-1286). Comparative analyses generally fail to find such patterns in continental avifaunas, but also reveal dramatic short-term dynamics and geographic heterogeneity in avian haemosporidian communities. Large-scale surveys, together with studies employing experimental infections, will be required to understand the temporal dynamics and geographic variation of these host-pathogen systems.

### Heterogeneity of infection outcomes in malaria-infected mosquitoes

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Avian malaria is the oldest experimental system for investigating the biology and transmission of Plasmodium parasites. It was the model used by Ross in 1898 to demonstrate that malaria was transmitted through the bite of infected mosquitoes, but fell out of fashion when rodent malaria was discovered in the 1950's. Avian malaria has recently come back to the research scene as a unique animal model to understand the ecology and evolution of the disease, both in the field and in the laboratory. I will take stock of our most recent work on avian malaria, which has focused on the interactions between Plasmodium relictum SGS1, the most prevalent avian malaria lineage in Europe, and its natural vector, the mosquito Culex pipiens. Mosquito-Plasmodium interactions are complex and there are often heterogeneous outcomes in mosquito infection success, load, virulence and transmission, which in turn can have important consequences for the evolution and epidemiology of the disease. Our lab is working on identifying the sources of this heterogeneity: while feeding on infected hosts, why are some mosquitoes not infected while others attain extremely high infection rates? Why is the infection practically avirulent for some mosquitoes while highly virulent for others? I will give a whistle stop tour of some of the results we have recently obtained in our lab where we have tried to understand to what extent this heterogeneity in infection rates and infected mosquito fitness is driven by the bird host, the parasite or the mosquito vector.

# Avian haemosporidians: from DNA to Deforestation

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The effects of rapid global environmental changes on parasite distributions are diverse and despite potential consequences to ecosystem health, large-scale studies involving wildlife have been scarce. Here we present data of the effects of deforestation and global climate change on the prevalence and diversity of blood parasites in birds ranging from the tropics to the arctic. Using complementary techniques of blood smear analysis and molecular biology, mosquitoes and avian blood samples are assayed for haemosporidian parasites. We have obtained results regarding the host-specificity, prevalence and lineage diversity of these parasites in several communities of birds from Africa, Central and South America, California and Alaska. In Africa we have initiated a long-term study of mosquitoes, birds and avian malaria to determine how deforestation for the cultivation of palm oil plantations affects parasite transmission. We find that habitat degradation leads to altered patterns of parasite prevalence and disruptions in parasite species dominance. We also present data on how biogeography may affect the evolution of lineage diversity and specialist vs. generalist strategies in avian malaria. Our work incorporates satellite imagery and bioclimatic data to quantify differences among collection sites, and predict how microhabitat changes may affect the spread of infections. We have also initiated studies on genes involved in host specificity, with the characterization of the transcriptome of the chicken parasite P. gallinaceum. With our long-term agenda to discern the interplay between habitat, vector ecology, and genetics on the host-specificity of parasites, we emphasize that influences of land use changes on parasite prevalence are complex, and will require the detailed study of the vector ecology, and the further quantification of fine-scale habitat effects. Through time, our multidisciplinary approach will aid in predicting how habitat changes will influence future scenarios of host-parasite interactions.

# Temporal dynamics of avian haemosporidian parasite assemblages in the West Indies archipelago

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We investigate how rapidly haemosporidian parasite assemblages change by comparing parasite assemblages on avian populations that have been isolated for different periods of time. In the West Indies archipelago, lowered sea levels during past glaciations in the Pleistocene exposed land bridges, connecting islands that were previously isolated. Five pairs of islands that were connected up until 2.5 kyr ago provided a natural isolation experiment for avian host populations. We assumed that before islands were isolated, birds could freely move across land bridges, homogenizing parasite assemblages. We then quantified the extent to which haemosporidian assemblages have differentiated since island isolation, compared to differences between islands that have always been separated by deep water. We accounted for possible assemblage dissimilarity generated during short-time intervals by comparing

assemblages separated by similar distances within islands and by one or two decades within the same location. These comparisons of insular parasite assemblages constitute three assemblage isolation groups (the experiment) and one control group. We analyzed ca. 2,000 haemosporidian lineages determined by sequencing parasite cytochrome *b* regions from ca. 10,000 avian blood samples collected across 21 islands in the West Indies. We calculated the Chao-Sorensen dissimilarity index for each pairwise comparison, and compared that value to a distribution of dissimilarity estimates based on assemblage randomizations. We found that pair-wise comparisons between haemosporidian assemblages present a pattern of increasing dissimilarity in direct relation to time since island separation. Assemblages on islands that have been connected, but have been separated for ca. 2 kyr, are as differentiated as haemosporidian assemblages that have never been connected. Temporal turnover in haemosporidian parasite assemblages probably reflects biological and biogeographic processes, including evolution of host- resistance, as well as parasite colonization and extinction.

## Use of multiple primer pairs reveals high levels of cryptic co-infection in UK Columbiformes

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The diversity of haemoparasite DNA lineages greatly surpasses those of morphologically described species and potentially equals the number of avian hosts. Typically, in haemoparasite DNA studies, two universal primer pairs are used to screen for the presence or absence of either Leucocytozoon, or Haemoproteus and Plasmodium haemoparasites, followed by sequencing of the resulting amplicon to identify strains. Here, we test multiple primer pairs in order to detect cryptic co-infections in resident and migratory UK columbids. We collected blood samples from 167 individuals of four species of columbid across multiple sites in the UK. Universal primers did not reliably amplify parasites within our samples, so we tested 11 primer pairs in order to detect and molecularly characterise parasites present within adults and nestlings. We did not detect *Plasmodium* in any of our samples, but found a high prevalence of *Haemoproteus* and *Leucocytozoon* infection within adult woodpigeons (n=25) and turtle doves (n=20), with 92% and 85% respectively infected with at least one parasite. Infection rates in adult collared doves (n=11), feral pigeons (n=11) and stock doves (n=5) was lower, at 55%, 27% and 40% respectively infected with at least one parasite. We found nestlings to have unexpectedly high infection prevalence, with stock doves (n=9, sampled at 10 days old), turtle doves (n=56, sampled at 7 days old) and woodpigeons (n=29, sampled at 10 – 14 days old) showing 33%, 29% and 69% infection rates respectively. The use of multiple primer pairs revealed up to four co-infections within a single bird, with 31% of infected birds showing multiple infections, and 26% of infected birds showing potentially cryptic infections with multiple lineages present from the same parasite genus. Whilst co-infection by a single strain each of Leucocytozoon sp. and Haemoproteus sp. would be detected by universal primers, our data suggest that coinfections with multiple strains from the same parasite genus may regularly be missed.

### Resource predictability and specialization in avian malaria parasites

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Parasite population persistence depends on the transmission potential  $(R_0)$ —the number of new infections per infected host individual—being at least 1.0. In general, this poses a challenge for parasites that are specialized on rare hosts, leading to low contagion, compared to parasites specialized on a single common host or able to infect many host species. Specialization might reflect adaptive responses to qualities of hosts, as well as interactions with other parasite species. If so, more general hypotheses for ecological specialization might draw our attention to correlates of specialization. We tested whether avian haemosporidian (malaria) parasites specialize on hosts that can be characterized as predictable resources at a site in Amazonian Ecuador. We incorporated phylogenetic relationships and frequency distribution among hosts in assessing parasite specialization, and we examined associations between parasite specialization and three host characteristics—abundance, mass, and longevity—using quantile regression, phylogenetic logistic regression, and t-tests. Hosts of specialist malaria parasite lineages were on average more abundant than hosts of generalized parasite lineages, but the relationship between host abundance and parasite specialization was not consistent across analyses. We also found some support for a positive association between parasite specialization and host longevity, but this relationship also was not consistent across analyses. Despite inconclusive support, our findings suggest that the predictability of a host resource may play a role in the evolution of specialization. Alternative explanations to the variable degrees of specialization in avian haemosporidia include: (1) that interspecific interactions among the parasites themselves might constrain some parasites to a specialist strategy, and (2) that frequent encounters with multiple host species, mediated by blood-sucking insects, might promote generalization within this system.

# Studies of sexual development of haemosporidian parasites: Completing the cycle

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Numerous recent studies have addressed various aspects of the distribution, ecology and evolutionary relationships of wildlife haemosporidian parasites (Haemosporida). However, relatively few deal with host parasite-interactions during their sporogonic development. We designed several experiments addressing patterns of *in vitro* and *in vivo* sporogonic development of widespread species of avian *Haemoproteus* and *Plasmodium*. PCR-based and microscopic methods were applied in parallel. It was shown that 1) the same species of biting midges (*Culicoides*) can transmit numerous species of avian *Haemoproteus* and phylogenies based on partial cytochrome *b* sequences readily indicate vectors of these infections; 2) simultaneous sexual process of two genetically distant lineages of haemosporidians might increase the efficiency of reproductive cells, resulting in the development of a greater number of

ookinetes and possible beneficiary effects of simultaneous sporogony of different lineages; 3) the viability of *Haemoproteus* gametocytes might change dramatically within 1-2 days during the course of parasitemia, indicating that the presence of mature gametocytes in the circulation does not necessarily indicate their ability to exflagellate and produce ookinetes; 4) *Plasmodium relictum* (pGRW4) completes sporogony in *Culex pipiens* mosquitoes at a mean laboratory temperature of 19 °C, indicating that ecological factors other than availability of vectors or temperature restrict spread of this invasive malarial parasite in Europe; 5) intense *Haemoproteus* infections kill bird-biting dipteran insects, indicating that low intensity infections (≤1%) are particularly important for transmission of these parasites; 6) PCR-based diagnostics should be carefully used in studies of haemosporidian vectors because they detect parasites for several weeks after initial infection, but do not distinguish abortive parasite development. The marriage of molecular, microscopic and experimental approaches deserves more attention among haemosporidian researchers, particularly in wildlife studies, because integration of these methods provides a better understanding of the biology of these parasites. This requires close multidisciplinary collaboration among researchers with common interests. This study was partly funded by the Research Council of Lithuania (MIP-045/2015).

### To Study Key Host Genes in Host-Pathogen Interactions

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In host-pathogen interactions the host and pathogen have very different agendas; the pathogen benefits from multiplying and spreading to new hosts whereas the host strives to clear the infection. Arms-race and co-evolution are two mechanisms by which host-pathogen interactions may evolve and here genetic components of both the host and pathogen are of importance. The Major Histocompatibility Complex (MHC) genes encode proteins that play a significant role in the immune system of all vertebrate hosts and MHC has been used as an indicator of the diversity of host immunity in many studies of wild birds. Although, specific MHC alleles have been associated with the infection status of several different avian malaria infections across a wide range of bird species, high MHC diversity (many gene copies) causes major statistical difficulties when searching for such associations. Songbirds have particularly high MHC diversity and we set out to test the hypothesis that different MHC genes play different roles in host immunity. We tested the putative importance of different MHC genes in immunity by measuring their relative expression in blood in birds from the Passer clade; house sparrows (Passer domesticus), Spanish sparrows (P. hispaniolensis), and tree sparrows (P. montanus). Analyses of expression showed that only a minority of the MHC genes (probably a single gene) is highly expressed. The divergent functions of different MHC-I genes in sparrows, and likely in other songbirds, should therefore be considered in future studies of associations between for example MHC alleles and resistance to avian malaria infections. Such approach would not only enable testing genes of key importance but also enable testing effects of MHC in host-pathogen interactions with much better statistical power.

# Experimental approach in haemosporidian studies: past, present and future in Bulgaria

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In Bulgaria, the first study on experimental inoculation of haemosporidians from one bird to another was published in 1936 by Dr. Angel K. Drenovsky, director of the Research Station for Control of Malaria in Plovdiv (S Bulgaria). For long time, this study has not been in the focus of the Bulgarian avian blood parasites research group working in the field of diversity, systematics and ecology of haemosporidians. That publication was unknown for us when we started our "first in the country" experiments with inoculation of *Plasmodium* in Great Reed Warblers at the Kalimok Experimental Station in 2007. Looking back into haemosporidian research history in Bulgaria is a new source of inspiration for us. In this report, we present comparison of our experiments and the results obtained by Drenovsky (1936). His drawings representing positive development of *Haemoproteus* after experimental inoculation of blood from one infected bird to another bird are presented. The aim of this provocative comparison is to start a discussion on examples of "occasional" from evolutionary point of view and therefore probably abortive development of unspecific parasites in new bird hosts as well as on their effects for bird populations in nature. We are planning, 80 years after their publication, to repeat the experiments of Drenovsky in order to check the result obtained by him and to discuss their evolutionary and ecological importance.

# *Culicoides impunctatus* (Ceratopogonidae) is markedly susceptible to avian haemoproteids

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*Haemoproteus* parasites are widespread and cause diseases in wild and domestic birds. However, vectors remain unknown for many avian haemoproteids. This information is crucial for better understanding the biology of all haemosporidians in wildlife. We investigated sporogonic development of 4 widespread *Haemoproteus* species in experimentally infected *Culicoides impunctatus* biting midges. Wild-caught insects were infected by allowing them to take a blood meal from one individual of the following birds species: European pied flycatcher *Ficedula hypoleuca*, blue tit *Parus caeruleus*, yellow wagtail *Motacilla flava* and great reed warbler *Acrocephalus arundinaceus*. These birds were naturally infected with *Haemoproteus pallidus* (lineage hPFC1), *H. majoris* (hPARUS1), *H. motacillae* (hYWT1) and *H. nucleocondensus* (hGRW08), respectively. Infected females were collected, maintained in the laboratory and dissected in order to obtain the development of ookinetes, oocysts and sporozoites. The microscopic examination of insect preparations was used to detect parasites and PCR-based methods were applied to determine their genetic lineages. We show that all these parasites completed sporogony in *C. impunctatus*, which likely is a natural vector. Sporogonic stages of these parasites were reported and described for the first time. Formerly, *C. impunctatus* has been considered to be mainly mammalophilic, but it willingly takes blood meal on birds and is known to be a competent vector for

several other *Haemoproteus* parasites. These data show low specificity of *Haemoproteus* spp. to biting midges. *Culicoides impunctatus* is widespread in Europe, and its maximum biting activity coincides with bird breeding season when infection is transmitted to offspring. Interestingly, *H. pallidus*, and *H. nucleocondensus* have been found only in adult birds at our study site, indicating possible ecological isolation of their avian hosts from *C. impunctatus*. We conclude that *C. impunctatus* is markedly susceptible to avian *Haemoproteus* parasites and ecological factors likely play crucial role preventing transmission of some parasites. The study was partly supported by the Research Council of Lithuania (MIP038/2015).

### Abstracts - Poster Presentations (sorted by the surname of the presenting scientist)

### What is hidden in the bloodmeal?

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Many biting midges of the genus *Culicoides* are competent vectors of a diverse number of pathogens with medical and veterinary importance, such as bluetongue virus (BTV), African horse sickness, avian trypanosomes and haemosporidians. The identification of their feeding behaviour and of vector-host associations is essential for understanding their transmission capacity. Here we investigated the blood hosts of six biting midge species – C. circumscriptus, C. festivipennis, C. punctatus, C. pictipennis, C. alazanicus and C. griseidorsum, as well as the occurrence of haemosporidian parasites in the engorged insect individuals. All haemosporidians isolated from blood-fed biting midges correspond to species identification of bloodmeals found in the same insect individuals, thus revealing high degree of association between haemoproteids (Haemoproteus spp.) and their hosts, both vertebrate and potential invertebrate. Two *Haemoproteus* lineages were detected in more than one species of biting midges. These are H\_CIRCUM01 found in blood-fed individuals of C. alazanicus (N=1), C. circumscriptus (N=1) and C. festivipennis (N=3), and H CIRCUM03 recorded from single blood-fed individuals of C. alazanicus and C. festivipennis. Both haemosporidian lineages have been isolated for the first time from C. circumscriptus and have not been recorded in vertebrate hosts yet. The present results demonstrate that all five individual insects harbouring H\_CIRCUM01 also contain DNA of long-eared owl (Asio otus) in their bloodmeals. DNA of magpie (Pica pica) is present in the abdomens of the two insects harbouring H\_CIRCUM03. These observations suggest that these parasite lineages might be typical for the corresponding avian hosts, and the reason they have not been found in birds before may due to the fact that the haemosporidians of both vertebrate species are poorly studied. Furthermore, the magpie and the long-eared owl are sedentary avian species in the studied area, strongly suggesting that both parasite lineages are transmitted at the study site.

# Tracking the parasites: Inferring the transmission areas of haemosporidian parasites by tracking their hosts

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Migratory birds often travel thousands of kilometres to distant areas on the globe where they encounter a great variety of parasites absent in their breeding grounds. The advance in tracking technologies gives us an opportunity to find the specific areas where the birds are wintering or stopover. Often the parasites in these areas are poorly studied and tracking of birds' movements can give us hints where parasite transmission takes place. Here we investigated the patterns of haemosporidian infections (Plasmodium and Haemoproteus) in two Great Reed Warbler (Acrocephalus arundinaceus) populations, from the Czech Republic and Bulgaria, wintering in two different parts of Sub-Saharan Africa. We obtained information about the stopover and wintering areas of 27 birds using lightweight geolocators and checked them for presence of haemosporidian parasites. In addition, we analysed >100 individuals from each population for haemosporidian infections using PCR-based methods and a large part of them also by microscopic examination. Using geographic positions obtained from geolocator data and the available information on haemosporidian parasites in this host species from the MalAvi database, we estimated the location of population-specific transmission areas. A total of four cytochrome b lineages of Haemoproteus and nine lineages of Plasmodium were recorded, with the majority of the lineages transmitted in Sub-Saharan Africa. Some of the lineages differed in their prevalence between the studied populations or were recorded in only one of them. Several lineages can potentially serve as geographical markers when migrating birds with unknown breeding and wintering areas are sampled.

# Prevalence of avian haemosporidia in injured wild birds rescued in Tokyo and surrounding areas of Japan

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Avian haemosporidian parasites have been found from various birds in Japan. Virulence is especially of concern in endangered and captive zoo bird species such as rock ptarmigan (*Lagopus muta*) and penguins, respectively. Compared to free flying wild birds, it is easier to obtain blood samples from rescued birds. There are several rehabilitation centers and vet clinics in Japan where those injured wild

birds are treated and taken care of. We investigated the prevalence of haemosporidia in those injured wild birds rescued in Tokyo and surrounding areas. A total of 475 birds of 80 species were sampled from the four facilities (two rehabilitation centers in Chiba and Kanagawa Prefecture and two private vet clinics in Tokyo and Chiba) from 2013 to 2016 for demonstrating avian haemosporida by PCR and light microscopy. Overall infection rate was 21.3%. Prevalence in wintering birds, migratory breeders, and sedentary birds were 44.2%, 19.3%, 18.5% respectively. Wintering birds showed a significantly higher prevalence. Some detected lineages were found to be derived solely from Japan, suggesting unique differentiation within Japan. In addition, some lineages are closely related to those from captive zoo birds, showing that transmission between wild birds and captive birds might occur. Aspects regarding infection of haemosporidia to the host birds remain unclear in various parts of Japan. Rescued birds would help to get enough samples for examination of the prevalence of avian haemosporidia in areas difficult to access to wild birds such as Tokyo. By investigating rescued birds continuously, we can find how and what lineages are distributed within Japan. Also, we still lack data on the distribution and migration status of many birds. However, our ongoing investigation to track migratory route such as gulls by using geolocators would provide more data on how birds migrate through Japan.

# Do mixed haemosporidian infections exert additive effects on the behaviour of a passerine host?

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Blood parasite infections have been shown to influence behavioural traits of their avian hosts, in particular activity level and boldness. Following the hypothesis that a mixed infection by different parasite species should have noticeably higher effects than single-species infections, we analysed activity and boldness in wild-caught yellow wagtails (*Motacilla flava*), a trans-Saharan migrant, during the energetically demanding spring return migration. Eighty-five percent of the birds were chronically infected with haemosporidian blood parasites of the genera *Haemoproteus* or *Plasmodium* (Haemosporida), and 27% with both. No differences in activity were found among uninfected, single and mixed infection groups. However, birds with mixed infections showed a more fearful behaviour in their first experience to a keeping cage, and a tendency to show higher latencies to approach a novel object than uninfected birds, with birds with single infections at intermediate levels.

### **GERPH:** A biological collection of haemoparasites of wildlife

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With biological collections it is possible to know biodiversity, to understand the ecological and evolutionary processes that may exist in natural ecosystems because they may give us information about hosts, evolution and trophic relationships. Advances in molecular biology have promoted the haemoparasites trace and the number of lineages reported from them. However, classical morphological determination remains as gold standard for diagnosis, because it provides information about parasite morphology, co-infections, several haematological parameters and discarding abortive infections. The biological collection GERPH: Grupo de Estudio Relación Parásito-Hospedero. http://ciencias.bogota.unal.edu.co/gruposdeinvestigacion/gerph/biological-collection-gerph/, represents the first collection of wildlife haemoparasites in Colombia and one of the few in Latin-America that are functional. This collection has blood smears from 3880 birds of 350 species, captured in several ecosystems such as Paramo, Andean forest, gallery forest, savannah, wetlands, swamp and urban environments. Furthermore, some birds have been sampled in captivity in zoos and rescue units; also the collection has samples from both migratory and resident birds. There are deposited 5 hapantotypes from: Haemoproteus erythrogravidus, Haemoproteus macrovacuolatus, Leucocytozoon pterotenuis, Leucocytozoon quynzae, Plasmodium unalis. Additionally, GERPH collection has representative material of: Babesia, Haemoproteus, Hepatozoon, Leucocytozoon, Plasmodium, Trypanosoma; and microfilaries. This collection also has (in minor numbers) blood smears from: amphibians, reptiles, and mammals. We designed a gallery with selected parasite found in our country, where each one shows data about main parasite taxonomic characters, type of sample deposited, parasite distribution, host/s, and references. Information about parasites deposited in our collection, has been uploaded in databases such us in Genbank, MalAvi and Bold, and currently the collection is implementing the inclusion in the national database SiB (Sistema de Información sobre Biodiversidad), which automatically migrates the information to GBIF (Global Biodiversity Information Facility), promoting the share of biodiversity information freely.

### Haemoparasites in passerine birds from semi-arid areas of Mexico: effect of mine-induced metal pollution

K. <u>Monzalvo Santos</u><sup>1</sup>, M. C. Alfaro de la Torre<sup>2</sup>, L. Chapa Vargas<sup>3</sup>, J. Castro Larragoitia<sup>4</sup>, R. Rodríguez Estrella<sup>5</sup>

<sup>1</sup>Multidisciplinary Graduate Programs in Environmental Sciences, Autonomus University of San Luis Potosí, <sup>2</sup>Faculty of Chemical Sciences, Autonomus University of San Luis Potosí, <sup>3</sup>Division of Environmental Sciences, Institute for Scientific and Technological Research of San Luis Potosí, <sup>4</sup>Faculty of Engineering, Institute of Geology, Autonomus University of San Luis Potosí, <sup>5</sup>Northwestern Center for Biological Research, Division for Terrestrial Biology, La Paz, México. For the last 500 years, intensive mining exploitation for the extraction of metals of economic has dumped metal residues into ecosystems. The effects of these pollutants on organisms have not been studied deeply. We evaluated potential relationships between heavy metal (Arsenic, Lead, and Antimonium) concentrations on bird feathers, bird immune system as assessed through the heterophyl:lymphocyte (H:L) ratio, and haemoparasite prevalence and parasitemia in three bird species that are abundant in this region. Based on 91 bird samples, we found that feather concentrations of all three heavy metals were higher in mining sites than in those unexposed to mines (P<0.05). H:L ratios were larger in mining sites for Campylorhynchus brunneicapillus (P=0.008) and Melozone fusca (P=0.02), but not for Toxostoma curvirostre (P=0.29). Haemoparasite prevalence was 30.3% (0% for Campylorhynchus brunneicapillus, 38.1% for M. fusca, and 42.4% for T. curvirostre). Prevalence, however, did not differ between polluted and unpolluted sites for either *M.fusca* (Fisher test, *P*=0.75) or T. curvirostre (P=0.42). Parasitemia correlated negatively with feather antimonium and arsenic contents for M. fusca (P=0.002, and 0.03, respectively). In T. curvirostre, parasitemia also correlated negatively with antimonium contents in feathers (P=0.03). These results differ to some degree from our expectation of negative effects of pollutants on all aspects of bird ecology, thus suggesting that heavy metals could possibly have a stronger negatively effect on haemoparasites than on birds. However, because different bird species showed different responses, more study is needed to increase understanding of this interesting topic.

# New data on diversity, host specificity and transmission areas of haemosporidian parasites in Palearctic-African Bird Migratory System

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Studying diversity and distribution of haemosporidians is of high importance in respect to climate change, wildlife conservation and better understanding of host-parasite interactions. The rapid development and application of molecular tools in haemosporidian studies has contributed great amount of knowledge on host specificity, diversity and evolution. However, little information is given about the successful transmission of haemosporidian parasites supported by presence of gametocytes in the blood. By the present study, we aim to add information and summarize the published data in order to assess possible transmission areas of lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) found in Palearctic-African Bird Migratory System. To investigate parasite's areas of transmission, we are focused at haemosporidian lineages infecting resident bird species in tropical Africa and at lineages found in juvenile individuals of migratory bird species sampled in Bulgaria. For estimation of the prevalence and intensity of the infections, we apply both molecular and morphological methods. We present new data on the haemosporidian diversity found in variety of resident bird species in Gabon and Ethiopia as well as in young individuals from migratory bird species sampled during their first autumn migration in Bulgaria.

# On the haemoparasites of Ravens (Corvus corax)

Peter Shurulinkov, Nayden Chakarov, Lachezar Spasov, Georgi Stoyanov

A total of 110 Ravens were studied for blood parasites -64 adults in Bulgaria (Dolno Ozirovo, Vratsa district) and 46 nestlings in Germany. For the detection of the blood parasites we used molecular methods and light microscopy. Among the adults we found *Plasmodium, Leucocytozoon* and *Haemoproteus* infections with prevalence of 40.6%, 29.7% and 1.5% respectively. The total prevalence of haematozoan infections was 57.8%. A total of 10 haematozoan lineages were detected (three *Plasmodium*, six *Leucocytozoon* and one *Haemoproteus*). Three of the *Leucocytozoon* lineages are new and belong to a clade of corvid parasites. Only three of the studied nestlings showed haematozoan infection with *Leucocytozoon* (prevalence 6.5%). Preliminary data on seasonal dymamics of prevalence of the blood parasites is discussed.

# Diversity of Haemosporidian parasites in wild birds in Serbia

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Prevalence and morphology of Haemosporidian blood microorganisms in wild birds are rather well studied across Western Europe from Sweden to Spain, while little has been known about the distribution and the ecology of avian blood parasites across the Balkan Peninsula. Recent data were published just for Bulgaria but research on avian malaria have never been done before in Serbia. We used a nested PCR protocol to examine the diversity of cytochrome b lineages from blood parasites of genera *Plasmodium, Haemoproteus* and *Leucocytozoon*. Birds were caught in the breeding season in 2011 at five different localities. In total 85 birds of 27 species and 9 families were examined for the presence of parasites. The overall prevalence of haemosporidians was 33.75%, estimated by blood smear screening. The most common parasite genus was *Haemoproteus*. No differences between juvenile were found, but there was a difference between males and females (18 males from seven species were infected in regards to six females from six species). The composition of parasites varied geographically.

# Turnover in avian haemosporidian assemblages: assessing the relative effects of host evolution, habitat, and geographic distance

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Surveys of avian haemosporidia ("malaria") have revealed tremendous variation in assemblage structure at regional scales. Several factors may underlie this variation; for example, geographic distance, climate and habitat variation, and host-parasite coevolution. We studied haemosporidian assemblages in two suboscine antbird sister species (Pyriglena spp., Thamnophilidae) in the Atlantic Forest of South America to determine how parasite beta diversity (dissimilarity in species identity and relative abundances among sites) is related to host evolution, climate, habitat, and geography. We predicted haemosporidian beta diversity to relate positively to host genetic distance, climate differences (annual rainfall and mean temperature), geographic distance, and habitat differences (normalized difference vegetation index, NDVI). We found a weak but significant relationship in the predicted direction between beta diversity and host genetic distance, and a similarly weak but significant relationship in the opposite direction of what we predicted between beta diversity and climate differences. That beta diversity increases with increasing host genetic distance can be attributed either to a direct coevolutionary relationship or to shared regional history, depending on parasite specialization. That more similar parasite assemblages are situated in localities with different climate profiles may result from the many factors influencing assemblage structure simultaneously; for example, some of these similar assemblages with different climate profiles are geographically close. Although other factors ought to be examined for a complete understanding of avian haemosporidian assemblage structure, our study demonstrates that one cannot ignore host evolution.

# A bioinformatics approach to generate genomic contigs of the haemosporidian parasite *Polychromophilus* sp.

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Whole-genome analyses provide the greatest potential for resolving the evolutionary relationships among haemosporidian parasites. With the availability of *faster* and *cheaper* next-generation sequencing technologies, a growing number of complete haemosporidian genomes, almost exclusively of the genus *Plasmodium*, have been produced. Due to the relatively small size (~25-30 Mb), the genomes of other key species for deciphering the evolutionary history should easily be obtained. However, the success of high-quality genome sequencing depends largely on sufficiently uncontaminated genetic material that is free from host DNA. Even though the problem of obtaining sufficient quantity and quality of haemosporidian DNA has been addressed through the development of a number of different techniques, host contamination has remained a big challenge, especially in samples with low parasitaemia. The presence of only few nucleated host cells results in a disparity that completely inundates the sequence data with host genomic sequences. These contaminations severely

affect the data quality, causing missassembly of sequence contigs and incorrect conclusions in downstream analyses. Here, we used a bioinformatics approach to separate genome sequences of the malaria-like blood parasite *Polychromophilus* sp. from the sequences of its host *Myotis daubentonii*. We performed two paired-end sequencing runs using Illumina DNA sequencing technology and obtained approximately 138.8 million reads. The paired reads were mapped against three *Myotis* genomes, using a 90% match threshold. Reads that mapped to the reference assemblies were discarded with their mate pairs from the read set. Remaining reads (28.7%) were assembled into contigs that were extended or merged by iterative mapping the original read set back to the contigs. BLAST analyses were then used to identify contigs with homology to apicomplexan sequences, providing direct evidence of the haemosporidian-derived origin of the filtered reads.

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