7th International Conference on Phthiraptera
Guangzhou, China

10–14th July 2023

Hosted by the Institute of Zoology,
Guangdong Academy of Sciences

Day 1 (10–11 July 2023 in GZ)

GZ Time	Local time		
21:00–21:30		OPENING ADDRESS	
21:45–22:45		Andrew D. Sweet*	**KEYNOTE TALK:** Integrating evolution, biogeography, and host associations of feather lice across time and space
22:45–23:00		BREAK	
23:00–00:00		**SESSION 1: Louse phylogenetics and co-phylogenetics**	
23:00–23:15		Kevin P Johnson*, Jorge Doña	Phylogenomics of Phthiraptera
23:15–23:30		Chunpo Tian*, Lujia Lei, Fasheng Zou, Alexandra A. Grossi, Daniel R. Gustafsson	Co-phylogenetic patterns of two louse groups (*Myrsidea* and *Guimaraesiella* + *Priceiella*) on mixed-species feeding flock birds of South China
23:30–23:45		Tomáš Najer*, Jorge Doña, Aleš Buček, Andrew D. Sweet, Oldřich Sychra, Kevin P. Johnson	Genome sequencing reveals the phylogeny and patterns of mitochondrial fragmentation in Amblycera
23:45–00:00		Mengjiao Ren*, Chunpo Tian, Fasheng Zou, Alexandra A. Grossi, Daniel R. Gustafsson	Cryptic species, cryptic genera: phylogeny of *Philopteroides* Mey, 2004, suggests new, morphologically indistinguishable taxa
00:00–00:15		BREAK	
00:15–01:30		**SESSION 2: Lice of domestic animals**	
00:15–00:30		Muhammad S. Sajid*, Asif Iqbal, Hafiz M. Rizwan	*Haematopinus tuberculatus* (Phthiraptera: Haematopinidae) in *Bubalus bubalis*: epidemiology and therapeutic effectiveness of a synthetic pyrethroid

00:30–00:45	Nidhi Gupta*, Ghazi Khan, Shalini Roy, Surendra Kumar	Licicidal property of the Garlic (*Allium sativum*) extract against 'poultry fluff louse' *Goniocotes gallinae* (Phthiraptera: Ischnocera)
00:45–01:00	Basma Ouarti[1]*, Maureen Laroche, Souad Righi, Mohamed Nadir Meguini, Ahmed Benakhla, Didier Raoult, and Philippe Parola	The emergence of mass spectrometry in the field of medical entomology and the identification of lice
01:00–01:15	Hafiz M. Rizwan*, Muhammad S. Sajid, Haider Abbas, Muhammad Younus	Epizootiology of Louse Infestation in the Livestock Population of District Narowal, Punjab, Pakistan
01:15–01:30	Saima Naz, Rimsha Hafiz, Mohammad Sohail Sajid* and Sajid Siyal	Determinants influencing the distribution of *Haematopinus tuberculatus* (Psocodea: Phthiraptera: Anoplura) infesting Buffalos (*Bubalus bubalis*) in district Hyderabad, Sindh, Pakistan
01:30–01:45	BREAK	
01:45–02:30	**SESSION 3: Community composition**	
01:45–02:00	Mikinley Weaver*, Sydney Brown, David Hyrenbach	An identification of a core Phthirapteran community on Hawaiian Wedge-Tailed Shearwaters
02:00–02:15	Oldrich Sychra*, Vojtech Sychra, Miroslav Capek, Ivan Literák	Chewing lice of hummingbirds
02:15–02:30	Kenza Absi*, Bilal Dik, Tahar-chaouche Souad	The infestation rate of doves (*Streptopelia decaocto* and *S. senegalensis*) by Phthiraptera order of ectoparasites in the Ziban oases (Biskra, Algeria)

Day 2 (11–12 July 2023 in GZ)

GZ time	Local time		
21:00–22:00		Mackenzie L. Kwak*, Skylar R. Hopkins[2]*	**KEYNOTE TALK:** Of Lice and Men: global declines of Phthiraptera and other parasites in the Anthropocene
22:00–22:15		BREAK	
22:15–23:00		**SESSION 4: Parasite conservation**	
22:00–22:15		Abdul Rahman Kazim*, Jamal Houssaini, Dennis Tappe, Chong-Chin Heo	Parasitic lice from domestic and wild mammals in Malaysia: a review of the history, list of species, and the decline of louse studies
22:30–22:45		Alexandra A. Grossi*, Daniel R. Gustafsson	Estimate of true diversity of avian lice (Phthiraptera: Amblycera, Ischnocera) and "preemptive red-listing" of presumed host-specific lice of endangered hosts
22:45–23:00		Daniel R. Gustafsson*, Chunpo Tian, Fasheng Zou	Parasite conservation and host conservation are not necessarily contradictory
23:00–23:15		BREAK	
23:15–00:45		**ROUNDTABLE: Effect of COVID-19 on head louse infestation and policy** Moderator: Ariel C. Toloza	
		F.G. Galassi*, A.C. Toloza, M.I. Picollo, P.G. Audino	COVID-19: Pediculosis decreased during lockdown in the metropolitan area of Buenos Aires
		Kosta Y. Mumcuoglu* and Aysegul Taylan-Ozkan	Effect of COVID-19 on head louse infestations in Israel and Turkey
00:45–01:00		BREAK	

01:00–02:15	**SESSION 5: Parasite symbionts (mainly)**	
01:00–01:15	Juliana Soto-Patiño*, Kimberly K.O. Walden, Jorge Doña, Lorenzo D' Alessio, Sarah E. Bush, Dale H. Clayton, Colin Dale, and Kevin P. Johnson	Replacement of the irreplaceable: Repeated acquisition of endosymbiotic bacteria in feather lice
01:15–01:30	Bret M. Boyd*, Ian N. James, Kevin P. Johnson, Robert Weiss, Sarah E. Bush, Dale Clayton, Colin Dale	Stochasticity, determinism, and contingency shape symbiont genome evolution
01:30–01:45	Ember Clodfelter*, Jorge Doña, Kim Walden, Kevin Johnson	*Wolbachia* in *Penenirmus* spp. Lice (Psocodea: Phthiraptera: Ischnocera)
01:45–02:00	Kelsey Larkin, Ariel C. Toloza, Jose A Gabrie, Carol A. Rodríguez, Maria Mercedes Rueda, Gabriela Matamoros, Oscar Palacio, Shabana Jamani, Gustavo Fontecha and Ana L. Sanchez*	First detection of *Acinetobacter baumannii* in *Pediculus humanus capitis* from Latin America
02:00–02:15	Therese Catanach*, Jason D. Weckstein, Daniel R. Gustafsson, Kimberly K. O. Walden and Kevin P. Johnson	Phylogenomics helps resolve generic boundaries of feather lice in the *Degeeriella*-complex
02:15–02:30	BREAK	
02:30–03:30	**POSTER SESSION**	

Day 3 (12–13 July 2023 in GZ)

GZ time	Local time		
21:00–22:00		**SESSION 6: Phylogenetics and genomics**	
21:00–21:15		Sarah Kelly, Yalun Dong, Wei Wang, Sonja Matthee, Jeanette M. Wentzel, Lance A. Durden, Renfu Shao*	African savanna elephant lice and Asian elephant lice are distinctly different species genetically that co-diverged and co-evolved with their hosts
21:15–21:30		Guo-Hua Liu*	A novel mitochondrial genome fragmentation pattern in the buffalo louse *Haematopinus tuberculatus* (Psocodea: Haematopinidae)
21:30–21:45		Yalun Dong* and Renfu Shao	The contrasting patterns of mitochondrial genome fragmentation among parasitic lice (Phthiraptera)
21:45–22:00		Jorge Doña, Kevin P. Johnson	Introgression dynamics in avian feather lice
22:00–23:30		**ROUNDTABLE 2: Lice as human evolution markers** Moderator: Ariel C. Toloza	
		Marina S. Ascunce*, Ariel C. Toloza, Angélica González-Oliver, and David L. Reed	Demographic and selective processes on the genetic diversity of head lice around the globe
		Niyomi House*	Genome-wide analysis of the human head louse (*Pediculus humanus capitis*) reveals geographically structured genetic populations
		M. Alejandra Perotti*	The secret of their lice
23:30–00:00		BREAK	
00:00–01:00		Maria Soledad Leonardi*	**KEYNOTE TALK:** Aqualice, the extraordinary diving insects
01:00–01:15		BREAK	

01:15–02:15	**SESSION 7: Ecology of lice**	
01:15–01:30	Ghazi Khan*, Shalini Roy, Surendra Kumar and Nidhi Gupta	Haematophagy of Selected Avian Amblycera (Phthiraptera: Insecta)
01:30–01:45	Matthew Waller, Hannah Warr*, Sarah E. Bush, Dale Clayton	Does the avian pectinate claw control ectoparasites?
01:45–02:00	Lajos Rózsa* and József Garay	Parasite or mutualist? Infection-induced harms or benefits at four levels of hierarchical organization
02:00–02:15	Sarah E. Bush*, Matthew M. Waller, Dale H. Clayton	Avian grooming rates vary in time and space and predict survival

Day 4 (13–14 July 2023 in GZ)

GZ time	Local time		
21:00–21:30		**SESSION 8: Human head lice**	
21:00–21:15		Stephen C. Barker* & Dayana Barker	Killing Clothes Lice by Holding Infested Clothes away from Hosts for 10 Days to Control Louse borne Relapsing Fever, Bahir Dah, Ethiopia
21:15–21:30		Hanene Benyahia*	Evolution of MALDI-TOF MS profiles from lice and fleas preserved in alcohol over time
21:30–21:45		BREAK	
21:45–22:30		**SESSION 9: Patterns in distribution**	
21:45–22:00		Wei Wang*, Lance A. Durden and Renfu Shao	The sucking lice (Anoplura) that parasitise Australian endemic rodents: an update
22:00–22:15		Bashar Jarayseh, Maria Amaya, Daniel R. Gustafsson	Overview of the chewing louse fauna of the invasive Common Myna, with new records from Palestine and a redescription of *Brueelia chayanh*

22:15–22:30	Lance A. Durden*	Unparalleled diversity of sucking louse genera associated with sciurid rodents
22:30–22:45	BREAK	
22:45–00:15	ROUNDTABLE 3: **Insecticide resistance on human head louse populations** Moderator: Ariel C. Toloza	
	Niyomi House*	Positive Selection for Target-site Resistance Against Pyrethroids in the Human Head Louse: A Population Genetic Perspective
	Kosta Y. Mumcuoglu*	Resistance of head lice to pediculicides in Africa, Asia and Oceania
	Ian F. Burgess	Resistance to treatments in head lice from Europe and the Americas
00:30–01:15	Assembly of ISoP and ending address	

Oral presentations

July 10 2023

KEYNOTE TALK: Integrating evolution, biogeography, and host associations of feather lice across time and space

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The geographic distributions of organisms often change over time. Incorporating the evolutionary history of a group of organisms is crucial for understanding the biotic and abiotic factors responsible for driving these changes in distributions. For parasitic organisms, it is also important to include information about their hosts' evolution and biogeographic history. In addition to helping clarify patterns of parasite distributions, combining information from parasites and their hosts can provide insights into processes of host-parasite coevolution and the origin of parasite biodiversity. My presentation will focus on integrating evolutionary history and distributions of parasitic feather lice and their avian hosts. Lice are an ideal system for addressing broader questions about parasite evolution because they tend to be host-specific and are obligately associated with their hosts. I will present results from multiple studies that assess the evolutionary history of different groups of lice over multiple geographic and time scales. First, I will discuss the evolutionary history and historical biogeography of a globally-distributed clade of lice from pigeons and doves. Next, I will focus on the phylogeography of lice from specific geographic regions. Finally, I will present how genomic information from an individual louse can provide information on the historical distributions of both the louse species and its host species.

Keywords: Phylogenomics, cophylogenetics, population genetics, coevolution, parasites

SESSION 1: Phylogenomics of Phthiraptera

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Genome sequencing technologies have facilitated considerable advance in the understanding of the phylogenetic relationships within and among major groups of parasitic lice. Phylogenies can now be based on hundreds or thousands of ortholog gene sequences, stabilizing previously uncertain relationships and providing a basis for classification systems less likely to change in the future. Phylogenies of all major groups of lice have now been investigated using these genome sequencing approaches. Within Amblycera, two major clades of mammal lice are recovered, although placement of the genus *Trinoton* makes Menoponidae paraphyletic. Within Phthiraptera more broadly, a larger clade of mammal lice is also recovered with Trichodectera, Rhynchophthirina, and Anoplura forming a single clade. Cophylogenetic reconstruction of this group suggest this major radiation of mammalian lice originated out of Afrotheria (a group containing elephants, hyraxes, and elephant shrews). Within the highly diverse Ischnocera, a large tree containing nearly all genera indicates the backbone of the tree is generally stable, providing a basis for diagnosing families within the group. Phylogenomic data sets have revealed patterns of diversification within several species complexes of related genera. These trees form the basis of comparison with host phylogeny, and these cophylogenetic studies can reveal which processes were dominant in the diversification of parasitic lice.

Keywords: phylogenomics, genomics, Ischnocera, Amblycera, Anoplura

SESSION 1: Co-phylogenetic patterns of two louse groups (*Myrsidea* and *Guimaraesiella* + *Priceiella*) on mixed-species feeding flock birds of South China

<u>Chunpo Tian</u>[1]*, Lujia Lei[2], Fasheng Zou[1], Alexandra A. Grossi[1], Daniel R. Gustafsson[1]

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Host participation in mixed-species feeding flocks (MSFF) has been suggested to have an impact in host associations of chewing lice, as regular, prolonged associations of non-conspecific birds should give rise to more opportunities for successful host switching. Patterns of strict co-speciation are therefore expected to be eroded among lice on birds that participate in MSFF. This erosion may be even more pronounced if lice can take advantage of phoretic transfer between hosts using hippoboscid flies. Here, we examine the co-evolutionary history of two groups of lice occurring on birds that participate in MSFF in South China, often co-occurring on the same bird individual. Lice in the genus *Myrsidea* are typically host-specific and are not known to be capable of phoresy. In contrast, the *Guimaraesiella-Priceiella* group within the *Brueelia*-complex contain both host generalists and host specialists, at least some of which are known to be capable of phoretic transfer. Co-phylogenetic analysis (Jane, eMPRess, ParaFit) suggest that both louse groups have co-radiated with their hosts, but that host switching has been common, including between distantly related host groups. In both data sets, co-speciation events were inferred from both deeper, medium-depth, and near-terminal branches. Host switching was also inferred to be more than twice as common as cospeciation in the *Guimaraesiella*+*Priceiella* data set, but less than twice as common in the *Myrsidea* data set. This suggests that phoretic capability may be a significant factor in structuring louse-bird associations among MSFF birds in South China.

Keywords: Co-phylogenetics, mixed-species feeding flock, *Guimaraesiella*, *Myrsidea*, *Priceiella*

SESSION 1: Genome sequencing reveals the phylogeny and patterns of mitochondrial fragmentation in Amblycera

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Animal mitochondrial genomes (mitogenomes) have a highly conservative structure, likely because of the importance of mitochondria in cell metabolism. In most cases, all the genes are encoded on a single circular chromosome up to 18,000 base pairs long. However, there are exceptions, and some animals have a fragmented mitochondrial genome, with genes spread across multiple smaller chromosomes. Parasitic lice (Insecta: Phthiraptera) are one of the most prominent examples of mitogenome fragmentation. All sucking lice and many feather lice have highly fragmented mitogenomes, in which up to 20 minicircles encode only a few genes on each. Amblycera, on the other hand, appears to show high variability in the extent of mitogenome fragmentation, enabling an exploration of a continuous scale between single-chromosome and highly fragmented mitogenomes. To further understand the process of mitochondrial genome fragmentation in lice, we examined the evolutionary pattern of fragmentation over a phylogenomic tree in one major group of lice, Amblycera. Using whole genome sequencing approaches and a phylogenomic tree derived from these data, we found multiple independent origins of mitochondrial genome fragmentation, across varied timescales. Some instances occur even within genera, showing that mitogenomes might fragment over much shorter times than previously thought. The base composition of the mitochondrial genome, in the form of AT bias of the group, is consistent with other parasitic lice, suggesting that mitogenome fragmentation may be related to genome-wide relaxed selection. We also present the first major higher level phylogeny of Amblycera based on a large set of genes. We provide evidence that lice families Gyropidae, Trimenoponidae, and Menoponidae are paraphyletic, and confirm the monophyly of the other Amblyceran families, Boopidae, Laemobothriidae, and Ricinidae. Analyzing host associations of the respective families, we reveal two likely host-switching events from birds to mammals in the course of amblyceran evolution. Combining phylogenomics and mitochondrial genomics in this group provides an overview of mitogenome evolution across an unprecedented number of animal taxa and represents a substantial advance in understanding the repeated nature of this process in lice.

Keywords: lice, mitogenomes, phylogenomics, Amblycera, fragmentation

SESSION 1: Cryptic species, cryptic genera: phylogeny of *Philopteroides* Mey, 2004, suggests new, morphologically indistinguishable taxa

Mengjiao Ren*, **Chunpo Tian, Fasheng Zou, Alexandra A. Grossi, Daniel R. Gustafsson**

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Lice of the "head louse" ecomorph comprise a diverse assembly of distantly related genera that have convergently evolved similar overall body shape and structure of the preantennal area. The most diverse group of head lice is the *Philopterus*-complex, which includes ~17 genera, most of which are associated with passeriform hosts. Species limits in this group are often poorly known, and the current taxonomy is to a large extent based on host associations. Moreover, abdominal chaetotaxy is often conserved throughout large groups within this complex, and male genitalia may be much reduced, precluding the use of these character sets as good diagnostic characters. Here, we analyze the genus *Philopteroides* Mey, 2004, genetically based on a mixture of mitochondrial and nuclear data. We confirm that this genus divided into at least two cryptic groups, one of which is known only from bulbuls (Pycnonotidae). Morphological differences between *Philopteroides* s. str. and this bulbul-specific group are limited. A new name is proposed for the bulbul-specific group, which shows biogeographical patterns similar to that of their hosts. On the species level, we find cryptic species, separable only on head shape and other minor characters, in both *Philopteroides* and the bulbul-specific genus. We recommend that genetic data should be used in addition to morphological data when describing species in these two groups in the future, as at least the bulbul-specific group contains host generalists that are morphologically indistinguishable from host specialists.

Keywords: *Philopteroides*, phylogeny, biogeography, cryptic species, new genus

SESSION 2: *Haematopinus tuberculatus* (Phthiraptera: Haematopinidae) in *Bubalus bubalis*: epidemiology and therapeutic effectiveness of a synthetic pyrethroid

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Lice are widespread ectoparasites of buffalos (*Bubalis bubalis*), and they cause enormous economic loss. In order to ascertain the incidence of lice infestation, the risk factors associated with it, and the effectiveness of cypermethrin (CYP) against lice infestation in buffalos, an epidemiological survey was carried out in a few selected tehsils (administrative divisions) of the Faisalabad district. For the purpose of evaluating the effectiveness of CYP, 40 buffaloes with head lice were split into two groups: the drug-treated group and the control group (each group contains 20 animals). The medicine cypermethrin was applied as a pour-on at day 0 and lice counts were made from each animal's predilection sites on days 0, 7, 21, 35, 49, and 63. In the Faisalabad district's buffalo population, only one species of sucking louse, *Haematopinus tuberculatus*, was identified. Overall, 37.76% of buffalo reported having a lice infestation. While breed exhibited an insignificant variation in lice infestation, variables like age and sex showed significant (P0.05) variation. Seasons, floor pattern, and feeding method among extrinsic factors showed a significant correlation (P 0.05) with lice infestation, but tehsils and animal keeping as free or tethered showed an insignificant association. Effective results were 100% on day 7, 99.6% on day 21, and 100% on day 35 through day 63. Throughout this trial, there were no clinically significant side reactions, and cypermethrin was proven to be effective. These results back up the assumption that cypermethrin is a successful treatment for head lice.

Keywords: Buffalo, Cypermethrin, Efficacy, *Haematopinus tuberculatus*, Prevalence.

SESSION 2: Licicidal property of the Garlic (*Allium sativum*) extract against 'poultry fluff louse' *Goniocotes gallinae* (Phthiraptera: Ischnocera)

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Poultry lice significantly harm the poultry industry by impairing the health and production of host birds. The organic pesticides commonly used to eradicate ectoparasites not only affect the host's health but also cause environmental hazards. Hence, the efficacy of the plant extracts having potent licicide/ ectoparasiticidal properties deserve evaluation. Garlic (*Allium sativum*) is a species of the plant family Alliaceae and has medicinal as well as repellent properties. The present paper provides information on the Lousicidal properties of Garlic extract against the 'poultry fluff louse' *Goniocotes gallinae*. *Goniocotes gallinae* is the smallest, oval-shaped, yellowish-brown lice that inhabit the plumage of the back and abdominal regions of poultry birds (*Gallus gallus domesticus*). It was found that the mortality caused by different concentrations of Garlic extract (under in vitro and in vivo conditions) remained correlated with its concentration and exposure time. Under in vitro conditions, the maximum mortality recorded at 5%, 7.5% and 10% concentration of Garlic extract was 30%, 52% and 90% respectively in 48 hours of exposure time. While the repellency remained at 17%, 30% and 48% respectively at the same concentration for the same duration of time. On the other hand, more than 50% population of the sample size was eliminated on 3rd day while it required 05 Days to completely eliminate the lice population in vivo conditions (at aforesaid concentrations).

Keywords: *Allium sativum*, *Goniocotes gallinae*, Licicidal property, Poultry fluff louse

SESSION 2: The emergence of mass spectrometry in the field of medical entomology and the identification of lice

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The emergence of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), rapid, low-cost and accurate tool for arthropod identification has revolutionized medical entomology. In the present study, this proteomics tool was used to identify lice collected from livestock and poultry in Algeria. MS spectra of 408 adult specimens were measured for 14 species, including *Bovicola bovis*, *B. ovis*, *B. caprae*, *Haematopinus eurysternus*, *Linognathus africanus*, *Linognathus vituli* and *Solenopotes capillatus*. *Menacanthus stramineus, Menopon gallinae, Chelopistes meleagridis, Goniocotes gallinae, Goniodes gigas, Lipeurus caponis* and *Pediculus humanus corporis* raised in the laboratory. A total of 305 MS profiles were of good quality. Spectral analysis revealed intra-species reproducibility and inter-species specificity that were consistent with morphological and molecular classification. A blind test of 248 specimens from species included in a spectral database confirmed that MALDI-TOFMS is an effective tool for discriminating lice species with high identification rates ranging from 76 % to 100% and high identification scores (mean LSV : 2.115). These results indicate that MALDI-TOF/MS is a reliable tool for the lice identification.

Keywords: MALDI-TOF MS, Lice, Phthiraptera, Anoplura, Ischnocera, Amblycera

SESSION 2: Epizootiology of Louse Infestation in the Livestock Population of District Narowal, Punjab, Pakistan

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In livestock, pediculosis results in severe irritation, scratching, and stress, which frequently leads to skin damage and poor coat condition. In district Narowal, Punjab, Pakistan, the current study was carried out to determine the level of louse infection among the sheep, goats, cattle, and buffalo population. Of the 384 animals examined for this investigation, 31 (8.07%) showed lice infestation. Four species of lice, *Haematopinus eurysternus* (cattle), *Haematopinus tuberculatus* (buffalo), *Linognathus pedalis* (sheep), and *Linognathus stenopsis* (goat), were identified in the current study. The intrinsic variables like age and sex showed significant ($P<0.05$), while animal species showed insignificant ($P>0.05$) association with louse infestation. Only feeding style among extrinsic factors demonstrated a significant association ($P<0.05$) with louse infestation, whereas housing and tehsils showed i ($P > 0.05$). The findings of the current study give new knowledge regarding the various species of lice and the frequency distribution of lice infestation in livestock population of the Narowal district. The reported total prevalence is typically quite high, which could lead to significant economic losses due to lower production and productivity, skin damage, and animal deaths. These issues need to be addressed right away by professionals and the government.

Keywords: Livestock, Louse infestation, Intrinsic factors, Extrinsic factors, Prevalence.

SESSION 2: Determinants influencing the distribution of *Haematopinus tuberculatus* (Psocodea: Phthiraptera: Anoplura) infesting Buffalos (*Bubalus bubalis*) in district Hyderabad, Sindh, Pakistan

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More than 95% of the World's buffalo (*Bubalus bubalis*) population is found in Asia. These animals are considered as the "Black Gold of Pakistan". Sind contributes 25.27% of Pakistan's buffalo population; however, lacks research on the epidemiology of parasitological fauna which might be attributing a major set-back in their productivity. The present study focused on the distribution of sucking louse; *Haematopinus tuberculatus* (Psocodea: Phthiraptera: Anoplura) and its correlation with the extrinsic (type and formation of yard flooring and season), and intrinsic (age, gender, and area of body) determinants in the in the climatically warm and semi-dry Hyderabad district of Sind, Pakistan for one calendar year. The two types of yards *viz*., (a) cemented, and (b) Muddy were found in peri-urban and rural areas of the study district within the four tehsils (administrative divisions) including Hyderabad Rural, Hyderabad City, Qasimabad, and Latifabad. The lice collected, preserved, and transported to the lab were identified as *Haematopinus tuberculatus* (Burmeister, 1838) using standard parasitological procedures. The prevalence of lice was higher in muddy floors (54.47%), with a frequency of lice (68.88%) and mean intensity (111.37±4.38), showed a high significance (p<0.05) and tested with T-test (0.005) to analyze statistically. Among the four tehsils, the maximum abundance and mean intensity of lice were recorded higher in all mud-floored yards in Hyderabad rural (2,012; 111.78±6.12), followed in order by Latifabad (1,972; 131.46±12.34), Qasimabad (1,967; 109.27±7.089) and Hyderabad city (1,511; 94.43±8.02). Overall prevalence of louse infestation was highest in buffalos of Latifabad (54%) and lowest in those of Qasimabad (46.7%) with statistically significant population (p<0.05) in Hyderabad rural, Hyderabad city and Latifabad (t-test =0.03, 0.04, 0.04 respectively) due to humid and warm climate whereas it is non-significant in Qasimabad (0.07). The yard-type-wise prevalence was recorded maximum in buffalos reared on the muddy floors (57.69%) in Latifabad. In cemented yards, it was observed 50% each in buffalos of Latifabad and Hyderabad city. The mean intensity of louse infestation at 95% confidence interval was higher in muddy yards (131.46±12.34) in Latifabad; whereas, it was higher in cemented yards (84.31±6.6) in Hyderabad city. Age-wise analysis revealed the calves (>1-1.5 years) were the most vulnerable to *H. tuberculatus* (73.33-90.91%) than older age buffalos (1.6-10years) (55.17-34.62%) showing a negative correlation with the prevalence of louse infestation (Pearson correlation = –0.93). The gender-wise prevalence of infestation was higher in she buffalos (55.79%) and bulls (44.08%). The temporal distribution of lice was found highest in the winter season (17.06% with p-value 0.02) irrespective of the floor types, showed a high significance in population. The mean intensity of distribution of lice was found significantly higher on buffalos reared on muddy floors than those on the cemented ones on their head-and-

neck (including dewlap) region (57.36±2.18 and 34.67±1.7, respectively) compared statistically using Fisher Test at $CI_{95\%}$ (F=0.4; $p<0.05$). The results of the present study are maiden attempt to correlate the determinants including the floor type, age, gender and season with the *H. tuberculatus* infestation in the dairy buffalos of district Hyderabad, Sindh, Pakistan.

SESSION 3: An identification of a core Phthirapteran community on Hawaiian Wedge-Tailed Shearwaters

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Wedge-Tailed Shearwaters, *Ardenna pacifica*, were examined for ectoparasites, including chicks and post-fledging fallout birds. Ectoparasites were collected from 32 live chicks in October and November 2022 using a fumigant and plastic bag collection method. Seventy-eight post-fledging birds were collected between the fallout seasons of 2015 and 2022 when they were found and provided to us after they were deceased. The birds were frozen and later had the ectoparasites brushed off them. Four species of Phthiraptera, *Halipeurus* (*Halipeurus*) *mirabilis* Thompson, 1940, *Trabeculus hexakon* Waterson, 1914, *Austromenopon paululum* (Kellogg and Chapman, 1899), and *Naubates* (*Naubates*) *harrisoni* Bedford, 1930 were found on the fallout birds, while only H. mirabilis was found on the chicks pre-fledging. Comparisons with previous records reveals that these species likely represent the core ectoparasite community – a previously undescribed community that did not significantly vary. A significant increase in lice abundance during 2015 coincided with a major El Niño event, suggesting that seabird ectoparasite loads may be affected by external conditions such as weather or temperature. There was also a significant difference in lice prevalence between chicks and post-fledging juveniles, which likely coincides with the lice hatching and maturing.

Keywords: Seabirds, Core Community, Ontogenetic shift, Hawai'i

SESSION 3: Chewing lice of hummingbirds

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We examined hummingbirds for presence of chewing lice during rainy season (July-September) 2004-2014 in 19 locations in Costa Rica, Brazil, Honduras, Paraguay and Peru. We used fumigation chamber method with chloroform. A total of 579 hummingbirds of 49 species have been examined in locations with different elevations: lowlands (0–550 m a.s.l.; 12 locations; 252 birds), highlands (700–1500 m a.s.l.; 4 locations; 176 birds), and mountains (2000–3000 m a.s.l.; 3 locations; 151 birds). Lice and/or louse eggs have been found on a total of 80 (13.8%) birds of 22 (44.9%) species. Neither louse nor louse eggs have been found on 27 species of hummingbirds. A total of 267 lice of four genera have been collected, with a total mean intensity 4.6 and mean abundance 0.5. Dominance of louse genera were: *Trochiloecetes* (81.6%), *Trochiliphagus* (15.4%), *Myrsidea* (2.6%) and *Leremenopon* (0.4%). A total sex ratio was female-biased with 34.5% of males. Age ratio was adult-biased with 66.3% of adults. Most birds (90%, n=59) was parasitized with 1-10 chewing lice. No difference in prevalences between dimorphic and monomorphic species (14.7 vs. 12.4); significantly higher prevalence in males than in females of dimorphic species (19.2 vs. 8.4); and significantly higher prevalence in hummingbirds from mountains than in lowlands and highlands (24.5 vs. 9.5 and 10.8) have been recorded. In addition, we revised available material of hummingbird lice, including type material borrowed from several world museums. A total of 787 permanent slides with 1165 individuals of lice from four genera were used: *Trochiloecetes* (dominance 73%), *Trochiliphagus* (20%), *Leremenopon* (5%) and *Myrsidea* (2%). In adults of the genus Trochiliphagus, 9–10 basic dimensions were measured, which were then related to the size of the hosts. The correlation analysis confirmed the so-called Harrison's rule, a statistically significant positive correlation. The SHAPE program was used for morphometric analysis. By evaluating the variability of the shape of the head, prothorax and entire body of lice, the existence of three groups of forms corresponding to three different species groups of the genus *Trochiliphagus* was confirmed.
This work was supported by the University of Veterinary Sciences, Brno, Czechia (Grant number 2023ITA22).

Keywords: hummingbirds, *Trochiloecetes, Trochiliphagus*

SESSION 3: The infestation rate of doves (*Streptopelia decaocto* and *S. senegalensis*) by Phthiraptera order of ectoparasites in the Ziban oases (Biskra, Algeria)

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This study was carried out in the oases of Biskra for two years (2017 and 2018), on 450 doves consisting of three species, *Streptopelia senegalensis* (Linné, 1766), *Streptopelia decaocto* (Frivaldszky, 1838) and *Streptopelia turtur arenicola* Linné (1758). A total of 1290 ectoparasite specimens were identified. On *Streptopelia decaocto*, five lice (Phthiraptera order contains three families and five species of ectoparasites) species recorded from the first time in Biskra (Algeria) were detected and the population was dominated by *Columbicola bacillus* (82.67% ± 3.92) followed by *Colocerus piageti* (22% ± 3.35), *Hohorstiella* spp (9.33% ± 2.93), *Hohorstiella modesta* (8% ± 2.66), and *Bonomiella concii* (3.33% ± 3.12). On *S. senegalensis*, two lice species were recorded (Phthiraptera order contains two families) *Columbicola bacillus* (87.33 ± 2.76) and *Coloceras chinensis* (31.33 ± 4.14). Concerning *S. turtur*, two species of lice were recorded *Columbicola bacillus* with (85.47% ± 2.76) and *Coloceras britannicum* with (28.67% ± 4.07). Higher prevalence of single infestation was observed on *S. decaocto* (50%), compared with double (36.66%), triple (9.33%) and quadruple (1.33%). However, the difference of single and mixed infestation between male and female was statistically significant ($p < 0.05$), on the other hand, a non-significant difference ($p = 0.738$) in the prevalence of single and mixed ectoparasites infestations between the two sexes of the host species *S. senegalensis* is recorded. The different types of infestations are almost similar in both sexes of *S. senegalensis* had higher prevalence of single infestations (63.33%), compared with double (26%) and triple infestation (5.33%), and quadruple (2%).

Keywords: Oases of Biskra, *Streptopelia decaocto*, *S. senegalensis*, Phthiraptera, ectoparasites

July 11 2023

KEYNOTE TALK: Of Lice and Men: global declines of Phthiraptera and other parasites in the Anthropocene

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Lice are species rich, cosmopolitan, and often host-specific. This has made them incredibly vulnerable to co-extinction when their host species decline. Yet despite this, conservation efforts directed at co-threatened lice remain largely unrealised. Dr. Hopkins and Dr. Kwak co-chair the newly established IUCN parasite specialist group which aims to support and expand efforts to assess and conserve the planet's parasites, including lice. Dr. Kwak will open with an examination of modern louse extinctions (and recoveries) and an overview of the present state of louse conservation biology. Dr. Hopkins will then detail the pair's recent efforts to assess the extinction risk of lice and other ectoparasites in the Antarctic region and to understand the drivers of louse declines in the region. They will then close with an overview of the short and long-term goals of the IUCN parasite specialist group, which aims to conserve lice and all other metazoan parasites of vertebrates.

Keywords: parasite conservation, IUCN, Antarctic, co-extinction, and ectoparasite.

SESSION 4: Parasitic lice from domestic and wild mammals in Malaysia: a review of the history, list of species, and the decline of louse studies

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Literature records of parasitic lice (Phthiraptera: Anoplura, Amblycera, Ischnocera, Rhynchophthirina) from Malaysian domestic and wild mammals were reviewed, resulting in a list of 48 established species belonging to 19 genera (*Ancistroplax, Atopophthirus, Bovicola, Felicola, Enderleinellus, Gliricola, Gyropus, Haematomyzus, Haematopinus, Hamophthirius, Heterodoxus, Hoplopleura, Linognathus, Lorisicola, Neohaematopinus, Pedicinus, Polyplax, Sathrax* and *Trichodectes*). Other louse species that are dubious or possibly occurring in Malaysia, are listed in this paper. Furthermore, pathogens known to be associated with the sucking lice are listed. The alarming conservation status of some Malaysian mammalian host species and their parasitic lice warrant more research and imminent action to preserve the biodiversity of the country. This paper presents the complete checklist of mammalian lice from both Peninsular Malaysia and Malaysian Borneo, while discussing the history and decline of louse studies in the country.

Keywords: Louse-host association; mammal hosts; checklist; pathogens; Malaysia

SESSION 4: Estimate of true diversity of avian lice (Phthiraptera: Amblycera, Ischnocera) and "preemptive red-listing" of presumed host-specific lice of endangered hosts

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As a general rule, most avian hosts are parasitized by 3–5 different species of lice, with exceptions mainly being larger-bodied birds like gamefowl (Galliformes) and tinamous (Tinamiformes). This suggests that the approximate true diversity of lice would be 3–5 times the number of bird species, for a total of ~30000–50000 species of lice. However, several large groups of birds are parasitized by numerous host generalists, which would artificially inflate the true diversity in a simple multiplication. For instance, *Menacanthus eurysternus* is known from over 200 host species, most of which are not parasitized by an additional host-specific *Menacanthus* species. To estimate the true diversity of lice and take into account variations in host specificity between different louse taxa, we calculated the Index of Specificity (IS) for each louse genus and each host family separately. This host-family specific IS was then multiplied by the number of known birds in each family. The resulting estimate of louse diversity is 47113 species, which is likely an overestimation, due to our patchy knowledge of louse-host associations in the Passeriformes. By comparing IS values over 0.51, 0.75, and 1.00 with host species listed as Extinct in the Wild, Critically Endangered, or Endangered in the IUCN redlist, we arrive at a list of possible associations of lice that have not yet been described but which are likely host specific to threatened birds. We argue that these louse species should be preemptively redlisted, so that any species of lice specific to threatened birds do not get eradicated in future breeding and reintroduction programs.

Keywords: IUCN redlist, Index of Specificity, diversity estimate, conservation of parasites.

SESSION 4: Parasite conservation and host conservation are not necessarily contradictory

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Parasites have been implicated in the failure of breeding or reintroduction campaigns of endangered animals, and are often routinely medicated against or otherwise removed during conservation efforts. This methodology runs the risk of driving one or several species to extinction in order to save a single, more charismatic, species. Moreover, parasites form a part of both the evolutionary history and the ecological interactions network of these hosts, and it could therefore be argued that the species that comes out of the breeding program is not the same as the one that went into it. Here, we present data on an unintentional parasite conservation success story, that of the crested ibis (*Nipponia nippon*). The global population size of this bird has through a successful breeding program increased from seven to several thousand individuals. However, this was done without removing parasites from the original seven individuals. During a survey of 23 ibises within the breeding program, we found three host-specific louse species and one host-specific mite species. This indicates that at least four of the five louse and mite species known from this host before its catastrophic decline have survived within the breeding program, without jeopardizing its success. Following proposed modifications of the IUCN redlist criteria to accommodate parasites, two of these species (*Ardeicola nippon* and *Colpocephalum nipponi*) should likely be considered Endangered, whereas the third louse species (*Ibidoecus meinertzhageni*) may be Critically Endangered; as only one mite specimen was found, this species may also be Critically Endangered. However, additional surveys of head lice during 2023 suggest that *I. meinertzhageni* may have been overlooked during the initial survey. The success of this breeding program despite the presence of at least four ectoparasites indicates that conservation of hosts and low-impact parasites are not contradictory.

Keywords: IUCN redlist, Crested ibis, parasite conservation, breeding programs

ROUNDTABLE 1: Effect of COVID-19 on head louse infestation and policy

Chair: Dr. Ariel C. Toloza

Participants: Dr. Federico Galassi, Dr. Kosta Mumcuoglu

In March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak as a pandemic. COVID-19 is caused by SARS-CoV-2, a variant of the coronavirus. Therefore, in March 2020, the Argentinean and Israeli governments established mandatory isolation for an indefinite period. This obligatory isolation interrupted regular classes avoiding direct contact between children, thus affecting the dispersal route of individuals and the evolution of head louse populations. Here, we show the results obtained through an online survey or sales of pediculicides of how confinement affected the prevalence of lice during lockdown compared to the situation prior to confinement. These works highlight the impact of social distance in the population dynamics of head lice and how it could affect the control strategies in the future.

ROUNDTABLE 1: COVID-19: Pediculosis decreased during lockdown in the metropolitan area of Buenos Aires

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Pediculosis is a worldwide disease affecting school-aged children produced by the presence of the head louse, Pediculus humanus capitis De Geer, an obligate ectoparasite on the human scalp feeding exclusively on blood. Transmission occurs primarily through direct physical head-to-head contact. In March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak as a pandemic. COVID-19 is caused by SARS-CoV-2, a variant of the coronavirus. Therefore, on March 18, 2020, the Argentinean government established mandatory isolation for an indefinite period. This obligatory isolation interrupted regular classes avoiding direct contact between children, thus affecting the dispersal route of individuals and the evolution of head louse populations. In this study, we evaluated through an online survey how confinement affected the prevalence of lice during lockdown compared to the situation prior to confinement. The survey allowed to discriminate the different control strategies, the number of treatments, and the amount of insects recorded by parents. Data of 1118 children obtained from 627 surveys were analyzed. As the main result, it was observed that prevalence of lice decreased significantly from before (69.6%) to during (43.9%) COVID-19 lockdown. Moreover, head lice infestation was more effectively controlled in households with up to 2 children in comparison to households with 3 or more children. This is the first study that analyzed the prevalence of head lice during COVID-19 pandemic. In addition, this work demonstrated the impact of social distance in the population dynamics of head lice and how it could affect the control strategies in the future.

Keywords: Covid-19, Pediculosis, Head lice, Lockdown, Buenos Aires

ROUNDTABLE 1: Effect of COVID-19 on head louse infestations in Israel and Turkey

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In March 2020, WHO declared the COVID-19 outbreak as a pandemic. Soon thereafter mandatory isolation for extensive periods including closure of school systems for very long time was implemented in many countries. The resulting social distancing naturally reduced direct contact between children, thus potentially affecting transmission of head lice. Studies conducted in Argentina, France, Poland, Turkey and Israel showed that there was a decrease in the number of infested children and sold pediculicides. In Israel, during the period 2010–2019 a significant increase of pediculicide sales was observed, the highest number being from 2019. In 2020, a significant drop was observed compared to previous years and to 2019. The results of this study suggest that head louse infestations are increasing in the last years in the country, while the extended isolation of children due to COVID-19 influenced significantly the infestation rate of children with head lice. In Turkey, during the period 2015-2022, a slight decrease of pediculicide sales was observed in the years 2019 and 2020. During those years a steady increase in sales of dimethicone-based products was observed while the consumption of pharmacologically active pediculicides such as permethrin, pyrethrin, sumithrin decreases over the years. These facts together with studies conducted in the country, show that local lice developed resistance to pyrethroid-based pediculicides. In addition to the isolation of the children, the use of ivermectin to control Covid-19 infection might have an additional effect on the prevalence of head louse infestations where this medication was largely used.

SESSION 5: Replacement of the irreplaceable: Repeated acquisition of endosymbiotic bacteria in feather lice

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Symbiotic relationships between organisms play a central role in shaping novel ecological niches and facilitating specialized lifestyles. Insects, across various taxa, host a wide diversity of heritable and intracellular bacteria (endosymbionts) that confer numerous benefits, including protection against pathogens, digestive mutualism, and nutritional provisioning. While these associations often persist over long periods, evidence of endosymbiont replacement has been observed in various insect groups throughout their evolutionary history. A notable example occurs within the louse genus *Columbicola*, where it appears that each species has independently acquired its endosymbiont relatively recently in evolutionary time, often from the bacterial lineage *Sodalis*. Given their parasitic lifestyle and specialized diet, feather lice (Ischnocera) provide an ideal system for gaining deeper insights into endosymbiont evolution and the phenomena of symbiont replacement. By using a novel genome-resolved metagenomic approach, we analyzed whole genome sequence data throughout the diversity of feather lice to detect the presence of the *Sodalis* bacterial lineage and reconstruct the phylogenetic relationships of these endosymbionts. From over 1,000 samples, representing nearly all feather louse genera, we discovered a widespread presence of *Sodalis* (found in 229 samples from 58 louse genera), with high prevalence noted in specific louse genera or clades. The phylogeny of feather louse *Sodalis* exhibits a star-like shape with almost no bootstrap support, yet very closely related louse species sometimes harbor closely related *Sodalis* with high support. However, there were instances where closely related lice had distantly related Sodalis. A comparison of the *Sodalis* phylogeny with the louse phylogeny reveals minimal congruence, except for very closely related species. These results indicate that the Sodalis bacterial lineage is a widespread, albeit not universal, endosymbiont across a diversity of feather lice, suggesting multiple instances of endosymbiont replacement throughout the evolutionary history of this insect group. These results provide new avenues to further understand aspects of evolution and functional genomics of symbiosis in nature.

Keywords: Ischnocera, metagenomics, *Sodalis*, symbiosis

SESSION 5: Stochasticity, determinism, and contingency shape symbiont genome evolution

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Many insects harbor heritable bacterial endosymbionts with some of the smallest known bacterial genomes. The transition towards obligate host-association leads to a reduction of genome size. While the genomes of many endosymbionts have been examined, mutualistic endosymbionts and their hosts are phylogenetically and ecologically diverse, limiting our ability to distinguish stochastic and deterministic components of genome degeneration. We examined endosymbiotic bacteria from feather-feeding lice (Phthiraptera: *Columbicola*) that have been repeatedly acquired from a progenitor in the genus Sodalis. Inspection of endosymbiont gene inventories revealed that redundant gene functions are subject to loss, driven initially by stochastic mutational processes. These initial losses then shape subsequent additional losses as a consequence of contingency, leading to outcomes in which endosymbionts achieve similar mutualistic capabilities, but maintain distinct functional gene inventories. We also found evidence that rates of mutation and genome reduction are not consistent among symbiont lineages. These differences are not explained by variation in the efficiency of natural selection, but potentially by differential retention of genes influencing DNA repair and replication fidelity. Collectively, our results shed important new light on the enigmatic process of microbial genome degeneration, a process potentiated by obligate host-association which then proceeds over millions of years.

Keywords: louse, endosymbiont, genome degeneration

SESSION 5: *Wolbachia* in *Penenirmus* spp. Lice (Psocodea: Phthiraptera: Ischnocera)

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Insects with specialized, nutrient poor diets commonly have endosymbionts that help them to reach their nutritional needs when their diets do not. This has been shown to be the case in the clade Phthiraptera (order Psocodea) which contains feather- and blood-feeding lice, both nutritionally poor diets. Bacteria from the genus *Wolbachia* are present in a wide range of invertebrate taxa and are often characterized as reproductive parasites causing male-killing, cytoplasmic incompatibility, and feminization of genetic males. In some nematodes the *Wolbachia* are thought to be reproductive symbionts. In the bedbug, *Cimex lectularius*, it is thought that its *Wolbachia* is a nutritional symbiont and provides B vitamins. It is clear *Wolbachia* can take on many roles inside their hosts. Here, we examine the presence and phylogeny of *Wolbachia* in the monophyletic louse genus *Penenirmus* in the suborder Ischnocera (chewing lice). We used two metagenomic analysis techniques, MinYS (a reference based genome assembler) and metaWRAP (a de novo assembler). *Wolbachia* was found in almost every sample we examined. There was evidence of multiple supergroups in the phylogenetic tree generated, and there was even evidence of dual infections in some samples. And most interesting, the *Wolbachia* phylogenetic tree does not line up with the louse phylogeny generated from the same samples.

Keywords: *Wolbachia*, Phylogenetics, Endosymbiont, Ischnocera

SESSION 5: First detection of *Acinetobacter baumannii* in *Pediculus humanus capitis* from Latin America

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Several studies have documented the presence of *Acinetobacter baumannii*, a known multi-drug resistant pathogen in the human head louse, *Pediculus humanus capitis*. Since no reports from countries in Latin America have been published, the aim of the present study was to determine whether *A. baumannii* was present in head lice specimens collected in this geographic region. Head lice specimens from Argentina, Colombia, and Honduras were analyzed. PCR assays were performed to confirm the specimens' species, and to investigate whether the DNA of *A. baumannii* was present. The products of the latter were sequenced to confirm bacterial identity. Altogether, 122 pools of head lice were analyzed, of which two (1.64%) were positive for A. *baumannii*'s DNA. The positive head lice had been collected in the poorest study site in Honduras. The remaining specimens were negative. This study is the first to report the presence of *A. baumannii* in human head lice from Latin America. Further investigations are required to elucidate whether these ectoparasites can effectively transmit *A. baumannii* to humans.

Keywords: *Pediculus humanus capitis*; *Acinetobacter baumannii*; Head lice; Argentina; Colombia; Honduras; Latin America

SESSION 5: Phylogenomics helps resolve generic boundaries of feather lice in the *Degeeriella*-complex

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The *Degeeriella*-complex contains 16 louse genera parasitizing around 25 bird families including raptors, woodpeckers, gamebirds, and toucans. A morphologically similar genetic group, the *Otidoecus*-complex, contains an additional three genera. Using Sanger Sequencing data previous studies suggested these two complexes are not monophyletic with respect to each other and also found several genera were not monophyletic. However, these studies lacked statistical support for critical nodes in the phylogeny and had limited taxon sampling. Using a targeted assembly method, we assembled approximately 1,000 genes from 61 members representing all major lineages of both the *Degeeriella* and *Otidoecus* complexes. The resulting phylogeny confirmed that members of *Otidoecus*-complex were embedded within the *Degeeriella*-complex, however we found the three genera traditionally placed within the *Otidoecus*-complex did form a monophyletic lineage, a finding in conflict with previous studies. Additionally, our results indicate that many currently recognized genera within the complex are not monophyletic, suggesting an extensive history of intraordinal host-switching.

Keywords: Phylogeny, *Degeeriella*-complex, host-switching, genus limits

12 July 2023

SESSION 6: African savanna elephant lice and Asian elephant lice are distinctly different species genetically that co-diverged and co-evolved with their hosts

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The African savanna elephant lice and the Asian elephant lice are currently recognised as the same species, *Haematomyzus elephantis* (Piaget, 1869), based on morphology despite the fact that their elephant hosts diverged 8.4 million years ago and are classified into different genera, *Loxodonta* and *Elephas*. In a recent study, we sequenced the partial mitochondrial (mt) genome of African savanna elephant lice collected in Manyeleti Nature Reserve, South Africa. We compared the sequences of 23 mt genes between African savanna elephant lice and Asian elephant lice. Our comparisons revealed >23% divergence for the 23 mt genes as a whole and ~17% divergence for cox1 gene between African savanna elephant lice and Asian elephant lice, which are far higher than that expected within an animal species, thus strongly rejected the current recognition of these lice as being conspecific. Furthermore, the mt gene sequence divergences between these lice are 3.4 to 4.6 times higher than that between their hosts, the African savanna elephants and the Asian elephants, which are expected for the co-divergence and co-evolution between lice and their elephant hosts. We conclude that African savanna elephant lice and Asian elephant lice are distinctly different species genetically that co-diverged and co-evolved with their hosts. The taxonomy and classification of elephant lice need to be reviewed and updated in the light of the genetic evidence produced in our study.

Keywords: elephant lice, *Haematomyzus elephantis*, co-divergence, co-evolution

SESSION 6: A novel mitochondrial genome fragmentation pattern in the buffalo louse *Haematopinus tuberculatus* (Psocodea: Haematopinidae)

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Sucking lice are obligate ectoparasites of mammalian hosts, causing serious public health problems and economic losses worldwide. It is well known that sucking lice have fragmented mitochondrial (mt) genomes, but many remain undetermined. To better understand patterns of mt genome fragmentation in the sucking lice, we sequenced the mt genome of the buffalo louse *Haematopinus tuberculatus* using next - generation sequencing (NGS). The mt genome of *H. tuberculatus* has ten circular minichromosomes containing a total of 37 genes. Each minichromosome is 2.9–5.0 kb long and carries one to eight genes plus one large non-coding region. The number of mt minichromosomes of *H. tuberculatus* (ten) is different from those of congeneric species (horse louse *H. asini*, domestic pig louse *H. suis* and wild pig louse *H. apri*) and other sucking lice. Two events (gene translocation and merger of mt minichromosome) are observed in *Haematopinus*. Compared to other studies, our phylogeny generated from mt genome datasets showed a different topology, suggesting that inclusion of data other than mt genomes would be required to resolve phylogeny of sucking lice. To our knowledge, this is the first report of a ten mt minichromosomes genome in sucking lice, which opens new outlook into unexplored mt genome fragmentation pattern in sucking lice.

SESSION 6: The contrasting patterns of mitochondrial genome fragmentation among parasitic lice (Phthiraptera)

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Mitochondrial (mt) genome fragmentation has been discovered in all five parvorders of parasitic lice (infraorder Phthiraptera). All 26 species studied to date in Anoplura, Rhynchophthirina and Trichodectera have fragmented mt genomes with the number of mt minichromosomes ranging from 9 to 20 in each species. Multiple phylogenetic studies have concluded that these three parvorders form a monophyletic group to the exclusion of the other two parvorders (Amblycera and Ischnocera). Thus, it is plausible to suggest that mt genome fragmentation occurred only once in the most recent common ancestor of Anoplura, Rhynchophthirina and Trichodectera. In Amblycera and Ischnocera, however, both fragmented and the typical single-chromosome mt genomes have been found. Of the 28 species of Amblycera studied to date, 8 species have fragmented mt genomes with 2 to 6 minichromosomes in each species. Of the 30 species of Ischnocera studied to date, 17 species have fragmented mt genomes with 3 to 17 minichromosomes in each species. Furthermore, evidence available to date indicates that mt genome fragmentation occurred at least 5 times independently in Amblycera and at least 9 times independently in Ischnocera. It is clear that mt genome fragmentation as a general feature does not unite all the parasitic lice that have this feature. However, each independent mt genome fragmentation event does produce unique derived minichromosomal characters that can be informative for phylogenetic studies of parasitic lice at different taxonomic levels.

Keywords: mitochondrial genome fragmentation, minichromosomal characters, phylogeny, parasitic lice

SESSION 6: Introgression dynamics in avian feather lice

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The highly specialized relationship between avian feather lice and their hosts has made it an iconic system for understanding coevolutionary dynamics. Recent studies have found evidence that opportunities for interspecific gene flow in host-symbiont systems may be higher than previously thought. On the other hand, genomic studies on plant and animal patterns of introgression have found that introgression can increase effective population size (Ne) and can be particularly useful in changing or novel conditions, allowing populations to cross fitness valleys and evolve and persist on new adaptive peaks. To further evaluate these processes, we explored the extent of introgression in feather lice, based on whole-genome data for over 700 louse species. We evaluated the amount of introgression among closely related species and uncovered how the amount of introgression relates to the timeframe when hybridization may have occurred. Additionally, we investigated how introgression relates to macroevolutionary events, such as host switching. Integrating hybridization into the study of coevolutionary dynamics opens many avenues for understanding the complex processes that shape the diversity of host-symbiont systems.

Keywords: Avian feather lice, Introgression dynamics, Coevolutionary dynamics, Host-symbiont systems, Hybridization events

ROUNDTABLE 2: Lice as human evolution markers

Chair: Dr. Ariel C. Toloza

Participants: Dr. Marina Ascunce, Dr. Niyomi House, Dr. Alejandra Perotti

Head lice have co-evolved with humans for millennia and can be used to study human history and evolution. From the analysis of the genetic variation in human lice by using both nuclear microsatellite loci and female-inherited mitochondrial DNA sequences, head lice populations can be divided into two major genetic clusters named I and II. Cluster I was found to be more common in Africa, while Cluster II was found to be more common in Europe and the Americas. Then, high-quality DNA from 1,500-2,000-year-old nits from ancient Argentinian human remains revealed that the mummies' ancestors had migrated from northwest Amazonia to the Andes of central-west Argentina. The cement also preserved ancient environmental DNA, including the earliest recorded case of Merkel cell polyomavirus. Animal-lice nits found on the hair of ~5000-year-old human remains support a very close interaction with camelids and foxes, proposing early events of animal domestication. Finally, whole genome sequences of human head lice from 43 countries showed that head lice populations are highly structured geographically, with five major genetic clusters corresponding to the five major continents with the highest genetic diversity detected in sub-Saharan Africa.

ROUNDTABLE 2: Demographic and selective processes on the genetic diversity of head lice around the globe

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The human louse, *Pediculus humanus*, is an obligate blood-sucking ectoparasite that has coevolved with humans for millennia. Because of the intimate relationship between this parasite and the human host, the study of human lice has the potential to shed light on aspects of human evolution that are difficult to interpret using other biological evidence. In this study, we analyzed the genetic variation in 274 human lice from 25 geographic sites around the world by using nuclear microsatellite loci and female-inherited mitochondrial DNA sequences. Nuclear genetic diversity analysis revealed the presence of two distinct genetic clusters I and II, which are subdivided into subclusters: Ia-Ib and IIa-IIb, respectively. Among these samples, we observed the presence of the two most common louse mitochondrial haplogroups: A and B that were found in both nuclear Clusters I and II. Evidence of nuclear admixture was uncommon (33 lice) and was predominately found in the New World potentially mirroring the history of colonization in the Americas. These findings were supported by novel DIYABC simulations that were built using both host and parasite data to define parameters and models suggesting that admixture between cI and cII was very recent. This pattern could also be the result of a reproductive barrier between these two nuclear genetic clusters. The microsatellite data set was also used in a global analysis for outlier detection using BayeScan. Few outlier loci were detected and will be mapped in the reference genome to further assess their closeness to genes that could have potential roles in insecticide resistance.

ROUNDTABLE 2: Genome-wide analysis of the human head louse (*Pediculus humanus capitis*) reveals geographically structured genetic populations

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The human head louse (*Pediculus humanus capitis*) is an obligate ectoparasite of humans and has the potential to uncover aspects of human history that cannot be directly inferred from genetic data derived from humans. Previous studies have shown that global louse populations exhibit restricted patterns of genetic variation. With the aim of capturing the genetic diversity of head louse populations from around the world, we generated whole genome sequences of human head lice from 43 countries, spanning five continents and Oceania, to determine if louse nuclear diversity mirrors its mitochondrial haplotypes or if population genetic structure, genetic diversity, and population connectivity are associated with geographical regions or host behavior. We discovered five nuclear genetic clusters that are associated with large geographical regions, either at continental or intercontinental levels. High genetic variation was found between African and non-African individuals and the highest genetic diversity was found in samples from sub-Saharan Africa, similar to that of humans. Unlike the mitochondrial clades examined in previous studies, nuclear genetic clusters of lice examined here are highly structured based on geography (continentally and major regions within continents). Results from our genome analyses revealed that host-mediated global dispersal as the likely primary process in shaping diversity and maintaining genetic population boundaries within the nuclear genome of the human head louse.

ROUNDTABLE 2: The secret of their lice

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Most ancient humans carried at least one louse species on their body. Dried lice might look like debris. Lice and/or nits are commonly overlooked on archaeological material. They can be gathered from human and animal remains, from textiles or from animal skins housed in museums. Studies of ancient lice and nits on their hosts also allow understanding the interaction of their hosts with environmental factors before and at the time of death, and perhaps revealing the cause of death. Ancient ectoparasites are a window of information into their hosts past, accurately revealing circumstances of the life, of the genetic background of their hosts and their ancient movements or migrations. In a recent study on host DNA gathered from the cement that glues nits to hair, the isolated aDNA was of the highest quality helping decipher the genetic affinities of a number of ancient humans from South America, as well as informing of the occurrence of human pathogens, even after 2,000 years. Analysing the location of nits found glued onto hair in three ancient human baby remains from South America, from ~ 4,500 to ~ 600 Years Before Present, allowed, among other things, the estimation of the age of the babies at the moment of their death. Identification of the handful of nits glued to their head hair showed the presence of two different lice species nesting on their hair in each of two of the babies. The remains and nits were very fragile, thus, we were not allowed to extract material and the identification and measurements were carried out in situ. One of the oldest individuals (~ 4, 500 YBP), who looks a stillborn, carried just one nit of a wild-mammal louse species at about 2 mm of the scalp skin, suggesting it was just born. The other individual, of a nearby human population ~ 4,500 YBP, had just five nits, two of a wild-mammal louse species and three of *Pediculus* sp. Their location from the scalp suggests an age of 2 months at the time of death. While the third baby of a different population ~ 600 YBP, carried fourteen Pediculus sp. nits, allowing an estimation of age of 4 months. The presence of nits of wild-mammal lice nesting on human head hair demonstrates a very close interaction between ancient humans and wild mammals, like they were living together, suggesting isolated early events of animal domestication unknown until present in South America.

Keywords: ancient humans, ancient lice, domestication, ancient human migrations

13 July 2023

KEYNOTE TALK: Aqualice, the extraordinary diving insects

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Insects are considered the most ecologically and evolutionarily successful group of animals, surpassing all others in biomass, diversity, and ability to colonize almost any environment. However, while these statements are true for terrestrial environments, insects are virtually absent from the oceans, the largest habitat on Earth. Remarkably, a group of sucking lice are ectoparasites of amphibious hosts, such as pinnipeds (seals, sea lions, and the walrus) and the northern river otter. During the evolutionary transition of pinnipeds from land to sea, sucking lice had to manage the amphibian biology of their hosts, some of which can spend more than 80% of their time submerged and perform extreme dives to over 2000 m below the surface. These obligate and permanent ectoparasites have adapted to tolerate hypoxia, high salinity, low temperature and extremely high hydrostatic pressure. A major question that has been debated for decades is how seal lice survive during the long diving excursions of their hosts in the open ocean. For most of the year, lice are exposed to daily dives that in some cases can exceed depths of 2000 meters. In addition, they are unable to breathe atmospheric air during feeding periods that can last for months. In this talk I will discuss the major ecological, behavioral and physiological adaptations of seal lice to these conditions and how they are able to survive where no other insect can, in the depths of the ocean.

Keywords: adaptations, diving, seal lice.

SESSION 7: Haematophagy of Selected Avian Amblycera (Phthiraptera: Insecta)

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Birds lice are known to exhibit diverse feeding habits (*i.e*., feather feeder, partly haematophagous or exclusively haematophagous). The haematophagous nature of avian Amblycera is of great concern, as they not only affect the vitality and productivity of their host's birds but are often involved in transmitting pathogens (*i.e*., bacteria, viruses, filarial worms and even cestodes). Present paper deals with the degree/ extent of haematophagy of sixteen avian Amblycera (Black kite louse, Laemobothrion maximum; Black partridge louse, *Menacanthus kalatitar*; Blue rock pigeon louse, *Colpocephalum turbinatum* and *Hohorstiella lata*; Cattle egret louse, *Ciconiphilus decimfasciatus*; Common quail louse, *Amyrsidea fulvomaculata* and *Menacanthus abdominalis*; Common mynah louse, *Menacanthus eurysternus* and *Myrsidea invadens*; Domestic fowl louse, *Menacanthus cornutus*, *Menopon gallinae* and *Menacanthus stramineus*; Indian grey hornbill louse, *Chapinia clayae*; House crow louse, *Myrsidea bakttitar* and *Menacanthus gonophaeus* Mallard duck louse, *Holomenopon maxbeiri*). The presence of host blood was not detected in the crop of three amblyceran lice (*i.e., Colpocephalum turbinatum, Myrsidea bakttitar, Myrsidea invadens*) while the remaining thirteen species exhibited varying degrees of haematophagy (35% to 89%), recorded on the basis of analysis of their crop contents. Sex-related (male-female) and stage-related (adult-nymph and 1st, 2nd and 3rd instar nymph) differences in the degree of haematophagy of thirteen haematophagous species have also been recorded. The nature of the ventral spine of seven species has also been noted by performing SEM (Scanning Electron Microscopy) studies of the ventral side of heads.

Keywords: Avian Amblycera, Degree of Haematophagy, Phthiraptera

SESSION 7: Does the avian pectinate claw control ectoparasites?

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Grooming is the main behavioral defense of birds against ectoparasites. Grooming consists of preening the body with the beak, and scratching the head and neck with the claws of the feet. Preening and scratching are both very effective in combatting ectoparasites, such as feather lice (Insecta: Phthiraptera). Birds in 17 families have a pectinate claw, which is a serrated comb-like structure. This claw is strikingly similar to louse-combs that are used to remove lice from humans and other mammals, yet the effectiveness of the avian pectinate claw for ectoparasite removal has never been tested. Other hypothesized functions of the pectinate claw include general feather maintenance and removal of stale powder down. We conducted an experiment using wild-caught cattle egrets (*Bubulcus ibis*) to test whether the pectinate claw contributes to ectoparasite control. The egrets in our study were naturally infested with two species of feather lice: *Ardeicola expallidus* and *Ciconiphilus decimfasciatus*. The claw was harmlessly removed with a Dremel tool from half the egrets, chosen at random. The claws of birds in the other half of the experiment (controls) were sham dremeled. During the course of the experiment, which lasted four months, we quantified the time birds spent grooming, as well as the quality and general condition of feathers on the head. At the end of the experiment, we quantified the abundance of both species of lice. Although birds scratched frequently, the parasite loads of birds with and without pectinate claws did not differ at the end of the experiment, indicating that the claw does not play an important role in parasite control.

Keywords: Avian lice, anti-parasite behavior

SESSION 7: Parasite or mutualist? Infection-induced harms or benefits at four levels of hierarchical organization

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Parasitism is a long-lasting relationship between individuals of different species harming the hosts. However, how to define "harm" (a.k.a. costs, expenses, losses, or damages) caused by infections is equivocal. The same infection can cause opposing effects (harm versus benefit) in parallel at different hierarchical levels of biological organization, defining the relationship as parasitism or mutualism. We show that the same infections' effects on the survival and reproduction of host cells, host individuals, host lineages, and host populations can be opposing. Therefore, the idea that reducing the infected host individuals' survival, reproduction, or both necessarily exert selection pressure for antiparasitic avoidance and defense may not always hold. Especially, infection-induced shortening of host longevity is often misunderstood; it is traditionally interpreted as a cost of infection, although – all else being equal – it can benefit the infected/infested host lineages. Cases of infections' opposing effects at different levels of the organizational hierarchy are rare, however (except for the cellular level compared to higher levels). For the parasitic lice (Phthiraptera), the only published case is the suspected past mutualist role of human head lice. Therefore, we would welcome any new examples proposed by fellow phthirapterists.

Keywords: definition of parasitism, costs of parasitism, host population growth, multilevel selection, levels of biological organization

SESSION 7: Avian grooming rates vary in time and space and predict survival

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Birds have evolved a variety of behavioral adaptations to care for their plumage, such as preening and scratching. These grooming behaviors keep the plumage clean, parasite-free, and properly arranged. Despite extensive research on the grooming of in animals, including birds, mammals and arthropods, the degree to which grooming responds to geographic and seasonal variation remains largely unexplored. We investigated geographic variation in the grooming behavior of American kestrels (Falco sparverius) in the sub-tropical Bahamas and temperate Utah in both summer and winter. Bahama kestrels groomed significantly more than Utah kestrels and had significantly greater parasite pressure from feather lice (Insecta: Phthiraptera). Within each region, however, birds with more lice did not groom more. This pattern is consistent with geographic differences in programmed grooming over evolutionary time, rather than stimulus-driven grooming in ecological time. We also investigated the fitness consequences of variation in grooming time using a population of marked kestrels on San Salvador Island, Bahamas, where resighting probability is high. We found a strong quadratic relationship between grooming time and long-term survival, consistent with stabilizing natural selection on grooming time.

Keywords: Behavioral Ecology, Avian parasites

SESSION 8: Killing Clothes Lice by Holding Infested Clothes away from Hosts for 10 Days to Control Louse borne Relapsing Fever, Bahir Dah, Ethiopia

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Louse borne relapsing fever (LBRF) was once a cosmopolitan disease but it now occurs only in the Horn of Africa. Recent cases in refugees to Europe made LBRF topical again. Crowded boarding houses and church dwellings in Ethiopia are analogous to the crowded air-raid shelters of World War II. Thus, we might learn from experiments the London School of Tropical Hygiene and Medicine conducted during World War II. When the vector of *Borrelia recurrentis* (*Pediculus humanus* lice) were held away from the host for 10 days, 100% of nymphal and adult lice starved to death and 100% of eggs did not hatch. We hypothesize that holding infested clothes away from hosts in plastic shopping bags will kill enough lice to control LBRF in Ethiopia. Owning 2 sets of clothes might be useful; 1 set might be held in a plastic shopping bag for 10 days to kill lice and their eggs.

Keywords: *Pediculus humanus, Borrelia recurrentis,* Ethiopia

SESSION 8: Evolution of MALDI-TOF MS profiles from lice and fleas preserved in alcohol over time

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MALDI-TOF is now considered as a relevant tool for the identification of arthropods, including lice and fleas. However, the duration and conditions of storage, such as in ethanol, which is frequently used to preserve these both ectoparasites, could impede their classification. The purpose of the present study was to assess the stability of MS profiles from *Pediculus humanus corporis* lice and *Ctenocephalides felis* fleas preserved in alcohol from one to four years and kinetically submitted to MALDI-TOF MS. A total of 469 cephalothoraxes from lice (n=170) and fleas (n=299) were tested. The reproducibility of the MS profiles was estimated based on the log score values (LSVs) obtained for query profiles compared to the reference profiles included in the MS database. Only MS spectra from *P. humanus corporis* and *Ct. felis* stored in alcohol for less than one year were included in the reference MS database. Approximately 75% of MS spectra from lice (75.2%, 94/125) and fleas (74.4%, 122/164) specimens stored in alcohol during 12 to 48 months, queried against the reference MS database, obtained a relevant identification. An accurate analysis revealed a significant decrease in the proportion of identification for both species stored for more than 22 months in alcohol. It was hypothesized that incomplete drying was responsible of MS spectra variations. Then, 45 lice and 60 fleas were subjected to longer drying periods from 12 to 24 hours. The increase in the drying period improved the proportion of relevant identification for lice (95%) and fleas (80%). This study highlighted that a correct rate of identification by MS could be obtained for lice and fleas preserved in alcohol for up to four years, on the condition that the drying period was sufficiently long for accurate identification.

Keywords: lice, fleas, MALDI-TOF MS, ethanol, duration of storing, species identification

SESSION 9: The sucking lice (Anoplura) that parasitise Australian endemic rodents: an update

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The sucking lice that parasitise endemic Australian rodents have been under-studied for decades. Sixty-three species of native rodents in 14 genera have been recorded in Australia. However, only 11 species of sucking lice, all in the genus *Hoplopleura*, have been recorded from 12 species of endemic Australian rodents in 8 genera. To understand the diversity of sucking lice that parasitise Australian endemic rodents, we examined 989 rodent specimens of 54 species in seven museums across Australia between 2017 and 2019. We collected 2,111 adult sucking lice and 1,064 nymphal sucking lice. Based on morphological features, we described 12 new species of sucking lice, all in the genus *Hoplopleura*. These new species increase the number of sucking louse species on endemic Australian rodents from 12 to 24 and extends the records of sucking lice to all of the 14 genera of endemic rodents in Australia. Our results show that sucking lice are much more diverse among rodents in Australia than previously known. The *Hoplopleura* species in Australia show a highly host-specificity with each Hoplopleura species found only on a single host species except for *H. irritans* and *H. melomydis*, each of which is found on two host species. We updated the dichotomous key for identifying Australian *Hoplopleura* species. In addition to the new Hoplopleura species, we also investigated two introduced *Polyplax* species (*P. spinulosa* and *P. serrata*) among Australian endemic rodent species based on both morphological and molecular analysis. We found that *Polyplax spinulosa* has expanded its host range in Australia to include at least six endemic rodent species. *Polyplax serrata*, however, failed to expand to any endemic rodent species in Australia. The expansion of *P. spinulosa* has occurred in the time frame of 200 to 400 years, which is remarkably rapid compared to its host expansion to eight rat species in Eurasia over approximately ~13.1 million years since its divergence from *P. serrata*.

Keywords: Anoplura, *Hoplopleura, Polyplax spinulosa,* endemic Australian rodents

SESSION 9: Overview of the chewing louse fauna of the invasive Common Myna, with new records from Palestine and a redescription of *Brueelia chayanh*

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The State of Palestine is home to diverse habitats and a rich avifauna, but invasive bird species, such as the common myna, pose a threat to native species and ecosystems. The common myna (*Acridotheres tristis*) has successfully established breeding populations in various regions, including Palestine, and exhibits aggressive behavior towards other birds. However, studies on the prevalence and community structure of chewing lice on the common myna are limited. We surveyed the chewing lice species found on the invasive Common Myna *Acridotheres tristis* in Bethlehem district. Forty-five birds of this species were examined, and all ectoparasites present were collected and slide mounted. The 1004 specimens of chewing lice were identified as only two species: *Menacanthus eurysternus* (73.3% of samples) and *Brueelia chayanh* (26.7% of samples). Other species of chewing lice known from this host species in its native range were not found in our samples. This indicates a possible sorting event in the founding population of Common Myna in the region. In addition, we found high overall prevalence of lice (100%), and a high lice load per bird (22.3) compared to other studies on the Common Myna. Research on lice parasitizing common myna in Palestine revealed a reduced louse fauna compared to their native range. It is unclear whether the missing species of lice were ever present in the Palestinian myna populations or if they drowned upon arrival. Further surveys are needed in areas where mynas are more recent colonizers to investigate whether losses of louse associations are primarily due to missing the boat or drowning on arrival events. To aid in future research on sorting events of parasites on invasive species, we provide an overview of the known louse fauna of Common Myna worldwide, redescribe *Brueelia chayanh*, and propose a new synonymy in the genus *Sturnidoecus*.

Keywords: Invasive species, Common Mynah, *Brueelia*, *Myrsidea*, sorting events

SESSION 9: Unparalleled diversity of sucking louse genera associated with sciurid rodents

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Sciurid rodents (squirrels, chipmunks, susliks, marmots, etc.) are collectively parasitized by members of 12 different genera of sucking lice (Anoplura), which far exceeds the number of genera associated with any other family of mammals. Sciurid-infesting sucking louse genera belong to three families, namely the Enderleinellidae - *Atopophthirus* (two species), *Enderleinellus* (47 species), *Microphthirus* (one species), *Phthirunculus* (one species), *Werneckia* (five species) and a new genus (one species), the Hoplopleuridae - *Hoplopleura* (19 species) and *Paradoxophthirus* (one species), and the Polyplacidae - *Johnsonpthirus* (five species), *Linognathoides* (11 species), *Neohaematopinus* (30 species) and *Polyplax* (one species). As a group, flying squirrels (subfamily Petauristinae) have an especially diverse sucking louse fauna both taxonomically and morphologically. However, members of many genera and species of flying squirrels have been inadequately or never sampled for ectoparasites which suggests that additional undescribed genera and species of Anoplura might parasitize these highly specialized squirrels. Some sciurid species can be concurrently infested by at least three different species of sucking lice belonging to different genera, for example *Enderleinellus* or *Microphthirus* with *Hoplopleura* and *Neohaematopinus* on Nearctic squirrels, *Enderleinellus*, *Werneckia* and *Johnsonpthirus* on Afrotropical squirrels and *Atopophthirus*, *Phthirunculus* or *Enderleinellus* with *Hoplopleura* and *Neohaematopinus* on Southeast Asian squirrels. The tiny tibiotarsal claws of enderleinellid lice can only grasp the small underfur host hairs whereas the larger claws of members of other genera such as Hoplopleura and Neohaematopinus grasp thicker pelage or guard hairs, permitting some spatial segregation on the host. However, other factors could be involved in the coexistence of sucking lice belonging to different genera on the same host. Some squirrel-associated lice have been implicated as vectors of pathogens. Flying squirrel lice can transmit *Rickettsia prowazekii* strains in North America, which can cause sporadic epidemic typhus in humans, and squirrel lice in Britain have been stated to be involved in the transmission of squirrelpox virus.

Keywords: Anoplura, sucking lice, Sciuridae, squirrels

ROUNDTABLE 3: Insecticide resistance on human head louse populations

Chair: Dr. Ariel C. Toloza

Participants: Dr. Niyomi House, Dr. Kosta Mumcuoglu, Dr. Ian Burgess

Insecticide resistance on human head louse populations is a growing problem worldwide and makes it more difficult to treat head lice that can lead to an increase in the number of head lice infestations. This is due to the excessive use of insecticides, which has led to the development of resistance in head lice. Here, we discuss the resistance levels, consequences and most frequently used actives of several regions like Africa, Asia, Oceania, Europe and the Americas. Finally, population genetic pathways are undergoing strong selection pressure exerted by the use of pediculicides by its host, identified by several signatures of positive selection. Also, genetic variants found on VSSC revealed a unique origin of kdr-type mutations.

ROUNDTABLE 3: Positive Selection for Target-site Resistance Against Pyrethroids in the Human Head Louse: A Population Genetic Perspective

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Infestations of lice on humans, pediculosis, can be a social, economic and an epidemiological concern because of the negative impacts brought on by these infestations. In efforts to control it, chemical treatments containing pyrethroids that target the Voltage Sensitive Sodium Channel (VSSC) are most widely used. In populations with high pyrethroid insecticide use, head lice have acquired resistance by a mechanism known as the knockdown resistance (*kdr*). Primarily, three mutations (namely M815I, T917I, and L920F) in the alpha subunit gene of the VSSC have led human head louse populations to acquire resistance to pyrethroids. We examined a global population of human head lice to detect population genetic signatures of undergoing strong selection pressure excreted by the use of pediculicides by its host and identified several signatures of positive selection. First, the genetic diversity within louse individuals that possess known *kdr* mutations were comparatively low, and the genetic differentiation between susceptible and resistant lice were much higher indicating the effect of strong selection pressure on lice with resistant phenotype. Based on the VSSC gene, the population structure of global louse populations showed two genetic populations: one containing resistant lice and the other containing susceptible lice. Genetic variants found on VSSC revealed Europe as the epicenter for genetic exchange. Furthermore, we detected signals of positive selection that spanned beyond the VSSC gene and into the nuclear genome of lice that indicated genomic consequences of acquiring resistant phenotypes that may have shaped the worldwide genetic structure of human head lice.

ROUNDTABLE 3: Resistance of head lice to pediculicides in Africa, Asia and Oceania

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Resistance to pyrethroids such as permethrin and d-phenothrin, as well as to ivermectin has been reported from several countries worldwide. In Africa, permethrin resistance was reported from Egypt and Madagascar, in Asia from Israel, Turkey, Iran, Jakarta, Korea, Japan, and Thailand, and in Oceania from Papua Neu Guinea and Australia. In the majority of studies knock-down-resistance mechanisms were reported. Brought usage of permethrin-based pediculicides, exposure to this insecticide via impregnated bed nets used for malaria control, the residual effects of permethrin on treated hair and bed nets, and cross-resistance with DDT and other pyrethroids most probably permitted the relatively quick appearance of resistance to this insecticide. It is estimated that more than half of human head lice are resistant to pyrethroid insecticides, with the highest prevalence of resistance to permethrin. Resistance to ivermectin was first reported in head lice from Senegal by testing it on infested individuals and molecular techniques. In tropical countries such as Senegal, ivermectin is being used for the control of parasitic nematode ailments such as onchocerciasis and strongyloidiasis, as well as in the treatment of arthropod ectoparasite infestations such as scabies.

ROUNDTABLE 3: Resistance to treatments in head lice from Europe and the Americas

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Resistance to insecticide head louse treatments is widespread in Europe and the Americas. Mainly this affects the pyrethroids permethrin and phenothrin and is most obviously mediated through knockdown resistance (*kdr*) as a result of mutations to the voltage-sensitive sodium channel of nerve membranes. To date at least three common mutations have been identified. However, do these entirely prevent successful treatment or do they simply make treatment harder? There is some evidence of the latter. Alongside *kdr*-mediated pyrethroid resistance, resistance to organophosphates like malathion arose more or less concurrently in European states where that insecticide is used. That resistance was initially quite specific to malathion but subsequently lice acquired a more general loss of sensitivity mediated through non-specific esterase enzymes that sequester and degrade other insecticide entities, including permethrin. In parallel, a trend for using terpenoids derived from essential oils (EOs) initially proved effective but overuse, especially in other consumer products, resulted in loss of activity, especially as some EOs are degraded by the same enzymes as insecticides. As a result, from around 2003, there has been increasing use of physically acting treatments based mainly on synthetic oils like dimethicone, isopropyl myristate, and oligodecene, as well as some active surfactants, initially showing high levels of activity against lice that had developed tolerance and resistance to neurotoxic insecticides. In many countries only physically acting products are now used. As with insecticides, consumer use can be less thorough than ideal and during the past 3-4 years cases of infestation have been identified that survive exposure to the occlusive oils. It is now the time to investigate new methods for treatment before this resistance also becomes widespread.

Poster presentations

Microscale genetic diversity of human head lice from Buenos Aires and its potential effect on the spread of pyrethroid resistance

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Every year millions of children in both developed and developing countries are infested with head lice. To control them, pediculicides containing pyrethroids are being intensively used leading to the development of insecticide resistant among louse populations worldwide. Pyrethroids act on the louse nervous system affecting voltage-sensitive sodium channels (VSSC). Three-point mutations at the corresponding amino acid sequence positions M815I, T917I and L920F in the voltage-gated sodium channel gene are responsible for contributing to the knockdown resistance (*kdr*). Despite its medical importance, little is known about the movement patterns and the effects of insecticide treatments on the genetic structure of the insect populations. In this study, we analyzed the genetic variation of 46 head lice collected from four elementary schools in the city of Buenos Aires, Argentina. All lice were phenotypically tested to assess the resistance degree to permethrin. The same lice were subjected to DNA extraction, and we obtained their genotypes at the VSSC gene typed as: RR, RS and SS, as well as their genotypes for 15 microsatellite loci. All populations survived the permethrin treatment and showed resistant degree (RD) values from 33 to 76. The T917I mutation was found in high frequency (around 80%) in all the sites suggesting that is almost fixed. There was no strict correlation between the RD and the percentage of VSSC genotypes. The microsatellite allelic richness ranged from 2.23 to 3.17 and the genetic diversity varied from 0.38 to 0.54. Microsatellite genotypes clustered in two groups according to the non-parametric discriminant analysis of principal components (DAPC). The estimation of global differentiation among sites was high (FST = 0.125) however gene flow was also high (NeM=1.38). Both high gene flow associated to high genetic structure could facilitated the fixation of pyrethroid resistance genes among louse populations. Knowing the processes that shape the genetic structure of parasite populations is critical to understand how insecticide resistance evolves and for the design of effective control methods.

Keywords: Head lice, Population genetic, Insecticide resistance

Epidemiology of pediculosis in the school-going children of district Hyderabad, Sindh, Pakistan

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The human head louse is a hematophagous obligate ectoparasite that causes dermatitis and hair loss problems in humans especially in children. In the present survey, the data was collected from school-going children in urban and rural areas of district Hyderabad, Sindh, Pakistan from August 2018 to August 2019. A total of 1235 children were examined including 641 boys and 594 girls. The children were divided into three groups, 4-8 years old, 9-12 years old, and 13-16 years old. Overall prevalence was recorded at 25.11% for boys and 53.36% for girls. The highest prevalence of pediculosis was found in the 9-12 years old group with 34% in boys and 70% in girls. Region-wise prevalence of lice was higher in girls (51.6%) in rural Hyderabad than boys (13.5%) in the Qasimabad region, whereas the prevalence of nits was higher in girls (80%) in rural Hyderabad than boys (54%) in Hyderabad city. The Fisher's Exact Test was used to compare the association between the conditions prevailing pediculosis and the rate of infestation in all school children, which showed higher infestation in non-dandruff (0.29), those who shared beds and combs (0.53) and those who wash hairs twice a week (0.28). A high rate of infestation was observed in girls.

Keywords: Epidemiology, Pediculosis, Human head louse, school going children, Pakistan

The Study of Chemical Communication in Head Lice as Control Strategy Against Pediculosis

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Pediculus humanus capitis is an obligate bloodsucking ectoparasite of the human scalp. Pediculosis affects mainly kids at scholar age. Chemical cues are involved in the head lice-human interaction. Recent studies of our laboratory demonstrated that adult head lice responded to host chemical stimuli (odor). Moreover, these studies demonstrated that head lice were highly attracted to a human scalp compound extract. More recently, we demonstrated that head lice recognize and prefer the whole odor (highly and moderately volatile emanations) of the human head over the whole odor of other parts of the human body. In this work, we identified the main components from human scalp extract by CG-MS analysis. The major components were squalene (also presented in other parts of the human skin), tetradecanoic acid, palmitoleic acid, palmitic acid and oleic acid. Attraction to these individual and mixed components at natural concentrations was tested in an experimental arena, composed by 55 mm diameter discs of Whatman #1 filter paper divided in two arenas (control and treated). The arena was enclosed with a 5 cm circular glass ring to prevent insect escapes and kept at 21 lux light and 30°C temperature. A 1.5 cm2 filter paper square impregnated with the test sample was placed on one half of the arena and an untreated filter paper on the other. One insect was gently placed in the center of the arena and allowed to walk freely for 180 seconds. Its movements were observed by an infrared camera and recorded by a digital video. The control arena consisted of the divided circular filter paper with one untreated filter paper square on each side. No attraction of lice was found by the isolated components neither a diluted mixture (1/5) of the five components. Otherwise, a mixture of these 5 components at their natural concentrations showed significant attraction to head lice ($p<0,05$). This work found that blends from human scalp compounds are attractive to human head lice at natural concentrations. These results suggest that the mixture of compounds from the human scalp can orient the insects to their habitat: the human head. This work contributes to our understanding of the attractivity or not of some individuals, and could explain the difference in attractivity between young children and adults, and even among susceptible and non-susceptible children.

Keywords: head scalp compounds, insect attraction

Interaction between fungi communities and *Pediculus humanus capitis*

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Pediculus humanus capitis De Geer is known as head louse, it belongs to the family Pediculidae, order Phthiraptera. The head louse is a cosmopolitan human ectoparasite, it causes pediculosis and is one of the most prevalent parasitic diseases of humans. Lice have a clear attraction towards volatile compounds from head odor and human scalp, as well as intraspecific attraction among individuals of *P. humanus capitis*. Recent work from our lab supports that volatile active substances present in the feces of head lice are connected with their aggregation behavior. These results suggest the hypothesis that the associated mycobiota could produce these volatile compounds. The objective is to study the fungal community associated with *P. humanus capitis*. The first approach of this study involved the isolation of fungi from the cuticle and gut of live head lice. We collected lice from children of scholar age, lice were transported in sterilized boxes and they were examined under biosafety laminar flow. First, lice were inoculated in saline buffers (PBS) to extract fungi from cuticles, the lice guts were dissected under magnifying glass and inoculated in PBS. Both homogenates are transferred to Petri dishes containing Sabouraud culture media and incubated at 28 degrees and darkness per 7 days approximately. The strains were identified by evaluation of the macro morphological aspects of the colonies as well as by the micromorphological characters observed under a light microscope. Our results determined at least 15 different morphotypes, the strains most abundant were 5. A total of 348 strains were extracted from cuticles and 599 from guts. The most abundant strain presents a relative frequency of 33% in cuticle and 23% in gut, and the second relative frequency was 12% in cuticles and 18% in guts. These fungi are different species of the genus *Penicillium*, *Alternaria* and *Rhodotorula*. These strains will be submitted to comportamental analysis to examine the lice responses towards the fungal volatile compounds. These results represent the first analysis of the fungi community of *P. humanus capitis*.

Keywords: lice, fungi, communities, species

Pediculicidal activity of binary mixed monoterpenoids on head lice

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Essential oils are a special group of chemical compounds belonging to the natural products, which are in general highly volatile substances obtained from a wide variety of sources. The different components of essential oils have different physicochemical properties and possess an extraordinary bioactivity against head lice. The combination of monoterpenoids with an adjuvant can enhance efficiency and be environmentally friendly. In Argentina, pediculosis infestation is around 30% and most of the head lice are resistant to permethrin. The aim of this study was to study the pediculicidal activity of nanoformulations containing mixed monoterpenoids in comparison with pure compounds. Adult head lice were obtained from infested children of elementary schools from Buenos Aires. The monoterpenes used were eugenol, 1,8-cineol, linalool and thymol, prepared by stirring poloxamer at variable concentrations. The binary mixtures were: eugenol-linalool (1), 1,8-cineol-linalool (2), and eugenol-thymol (3) in proportions 3:1, 1:1, 1:3. We evaluated the insecticidal activity by the immersion bioassay of the different nanoformulations and the base formulation consisted of 5wt% polaxamer 407 aqueous solution was used as control. The knockdown time that affects 50% of the individuals (KT_{50}) expressed in hours was determined. To establish the type of interaction between each mixture, the combination index (IC) was estimated using the CompuSyn software. The KT_{50} values obtained from the pure compounds were linalool 6.07; 1,8-cineol 8.83; eugenol 7.17 and thymol 27.23h. All the binary mixtures (1) had an effectiveness less or equal to the nanoformulations of the pure compounds with values of 13.33, 8.16 and 6.71h in the proportions 3:1, 1:1, 1:3. The binary mixtures (2) had similar values: 10.02; 8.16 and 6.71h. In addition, the 1:1 and 1:3 ratios of the binary mixtures (3) presented values of 9.40 and 32.93h, while the 3:1 ratio showed the greatest effectiveness with a value of 4.42h. The IC determined for the mixture (1) was >1 showing an antagonistic interaction, and the combination 3:1 had the strongest antagonistic interaction. The binary mixtures (2) had $IC_S \simeq 1$, suggesting an additive interaction for all the mixes. For the binary mixtures (3) an IC< 1 was determined for the combinations 3:1 and 1:1 indicating a synergistic interaction, while the 1:3 combination of the same mix did not show any interaction. This study suggests that eugenol stabilizes linalool by decreasing its effectiveness, while 1,8-cineol does not significantly affect linalol. On the other hand, thymol acts as a synergist, enhancing the activity of eugenol. These results are an important contribution in the development of pediculicidal nanoformulations based on botanical compounds.

Keywords: Essential oils, head lice, pediculicidal, nanoformulations, monoterpenoids

Morphometry of chewing lice of the genus *Colpocephalum* (Phthiraptera: Amblycera) from wild birds

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In this work microscopic slides were processed from parts of two collections from Dr. Karel Pfleger, which are deposited in the Slovak National Museum in Bratislava and National Museum in Prague. In these collections we performed and determined the slides, on which individuals of chewing lice from genus *Colpocephalum* occurred. Further, we performed morphometric measurements and evaluation of Harrison's rule, whether larger lice parasitize on larger hosts. In the collections there were 1 183 individuals, 989 individuals (454 males, 535 females) were used for morphometric measurements, with the remainder being larvae. There were 11 species of Amblycera parasitizing on 14 species of birds from 6 orders of birds (Ciconiiformes, Accipitriformes, Columbiformes, Passeriformes, Falconiformes, Piciformes). Based on morphometric measurements, statistical evaluation was performed using the correlation method, which compared the morphometrically measured values of chewing lice with the body length and also with the weights of avian hosts. The result of all correlations was statistically insignificant, so the functionality of Harrison's rule was refuted within the genus *Colpocephalum*. Species determination of chewing lice, as important ectoparasites of birds, is necessary for the evaluation of parasite-host association, and thus the possible assessment of their effect on the host, therefore the applicability of individual determinants was further evaluated. The morphological features listed in available keys for the genus *Colpocephalum* are relatively useful, but may be affected by the number and quality of available preparations and therefore require some experience.

Keywords: *Colpocephalum*, morphometry, wild birds

Chewing lice of starlings (*Sturnus vulgaris*)

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We examined wild starlings (*Sturnus vulgaris*) in three locations: 44 starlings in Gbelce, Slovakia in 2008, 2016, 2019; 5 starlings in Lake Volvi, Greece in 2013 and 48 starlings in Mišník, Czechia in 2021-2022. Chewing lice have been found on a total of 82 (84.5%) birds. A total of 759 lice of four species have been determined, with a total mean intensity 13.1 and mean abundance 7.8. Dominance, prevalence, mean intensity and abundance, sex ratio and age ratio of particular louse species were: *Myrsidea cucullaris* (39.1%; 35.8%; 12.4; 3.1; 59% of males; 39% of nymphs), *Brueelia nebulosa* (34.7%; 59.7%; 6.7; 2.7; 46% of males; 29% of nymphs), *Menacanthus eurysternus* (22.7%; 35.1%; 5.9; 1.8; 22% of males; 60% of nymphs), and *Sturnidoecus sturni* (3.6%; 10.4%; 3.9; 0.3; 89% of males; 25% of nymphs). Most birds (66%, n=58) was parasitized with 1-10 chewing lice, with highest intensity of 51 *Brueelia nebulosa* on one bird. Half of birds have been parasitized by two species of lice – mostly by a species of ischnoceran lice and an amblyceran one (*Brueelia+Menacanthus*, *Brueelia+Myrsidea* or *Sturnidoecus+Menacanthus*; 17 cases). Occurrence of three species of lice per host have been found on a total of 8 birds (*Brueelia+Menacanthus+Myrsidea*, 6 cases; *Brueelia+Sturnidoecus+Menacanthus/Myrsidea*, 2 cases). Haematophagous behaviour is well-know for *Menacanthus eurysternus*. We confirm that almost all adult *Menacanthus* had blood in their digestive tract. On the other hand we observed blood also in *Myrsidea cucullaris*, with prevalence 28% (n=142) and with equal occurrence of digested blood in nymphs (29%; n=45) and adults (27%; 97).

This work was supported by the University of Veterinary Sciences, Brno, Czechia (Grant number 2023ITA22).

Keywords: *Sturnus vulgaris, Brueelia, Menacanthus, Myrsidea, Sturnidoecus*

Chewing lice of the genus *Neopsittaconirmus* (Phthiraptera: Ischnocera: Philopteridae) from parrots of Pakistan and evaluation of its distribution on captive parrots around the world

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The genus *Neopsittaconirmus* Conci, 1942 is a host-specific genus, found on both wild and captive parrots and love birds (order Psittaciformes). Two species of this genus: *N. lybartota* (Ansari, 1947) and *N. chandabani* (Ansari, 1947) have been previously reported from the Punjab province, Pakistan. We recorded *N. lybartota* from *Psittacula krameri borealis* and *Psittacula eupatria nipalensis*. Record on *P. eupatria nipalensis* represents a new host association for this louse species. We present also a new record of *Neopsittaconirmus vendulae* from a captive cockatiel in Pakistan. We also summarized and discussed all previous records of *Neopsittaconirmus* lice on captive parrots around the world. It shows that despite long-standing systematic veterinary care, some *Neopsittaconirmus* lice have a cosmopolitan distribution and also that they are able to successfully survive and reproduce in captivity with their hosts, and even colonize novel hosts.

Keywords: *Neopsittaconirmus*, Psittaciformes, captive parrots, new records, Pakistan.

Population genomics among mainland and island chewing lice (*Physconelloides eurysema*) from Common ground doves (*Columbina passerina*).

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Despite the importance of parasites for ecosystems and diseases, relatively little is known about the dispersal patterns of parasite between fragmented habitats, such as oceanic islands. *Physconelloides eurysema*, a body-specific feather louse from Common ground doves (*Columbina passerina*), are a model system to compare the influence of oceanic islands on parasite evolutionary and ecological patterns. The overarching goal of this study is to highlight *P. eurysema* phylogeography and population genetics among several island and mainland populations. We sampled feather lice from mainland and island populations, including from the U.S. (Texas), Ecuador, Brazil, Mexico, Bahamas, Jamaica, and Cayman Islands. This data was acquired through existing specimens and samples accessible on the National Center for Biotechnology Information (NCBI) SRA database. We extracted DNA from available samples using a Qiagen QIAamp DNA Micro kit and sequenced whole genomes using paired-end Illumina reads. We aligned these reads to an existing reference genome to obtain single nucleotide polymorphisms (SNPs) for population genetic analysis. We then used these SNPs to test for population genetic structure among the samples of lice. We also used a partial Mantel test to test for isolation by distance. We expect to find strong genetic structure between mainland and island populations with an isolated by distance pattern. This is because of the limited parasite dispersal among hosts from populations separated by large oceanic distances. We also expect island populations to have reduced genetic diversity due to the founder's effect on islands. This project will further our understanding of parasite-host relationships, dispersal pathways, and divergence patterns among species and populations of oceanic island taxa.

Keywords: population genetics, island dispersal, *Columbina passerina*

Prevalence and diversity of Ectoparasites of domestic pigeon (*Columba livia*) in Boufarik, Algeria

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The bird species of greatest concern and potential risk are those that live close to residents, such as the rock pigeon. The latter come into contact with birds from different regions with health statuses that are sometimes poorly known, and this can result in the circulation of pathogens that are potentially dangerous for human health. For this reason, we have chosen the *Columba livia* species to study. The aim is to identify the ectoparasites it harbours, so as to be able to assess a possible risk of contamination for its inhabitants. Our sample consisted of ectoparasites collected from pigeons (*Columba livia*) captured in Boufarik (36°34′ N., 2°54′ E) between January 2020 and September 2021 using the dropping or moulting box trapping method with food bait. These were transported in cages to the Zoology Laboratory at the National Veterinary School in El Alia for examination. The ectoparasites were collected after examination of the feathers under a binocular magnifying glass. All the ectoparasites collected were preserved in 70° ethyl alcohol for identification. 1329 individuals of ectoparasites were collected from 13 pigeons spread over 5 insects. The species, *Columbicola columbae* and *Campanulotes compar* dominated with (P=100%) followed by *Hohorstiella lata* with (P=53.85%) followed by *Colpocephalum turbinatum* and *Bonomiella columbi* with (P=23.8%).

Keywords: Pigeon, Ectoparasites, lice