2019 ASP Meeting

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Book of Abstracts
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Waiter, There’s a Fly in my Eye, or...Myiasis: Maggots Muchin’ on Man
"Oh! That looks fun too!" Or, how to cobble together a career when everything sounds fun

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Parasites are my first love, and my personal favorite title is Adjunct Assistant Professor at Rice University. But I also love science communication, and recently co-authored a pop sci book that hit the NYTimes Bestseller list. And making people laugh is great too! So I run a science-themed comedy event called BAHFest. At the moment, I’m using money from book writing and event organizing to build a small ecology research station in rural Virginia. In this presentation, I'll attempt to extract some lessons from the tortuous path I've trod on the way to my career.

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2018 Survey of Ticks and Their Pathogens in Southwest South Dakota

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Ticks vector a variety of diseases, including Lyme disease, Ehrlichiosis, Anaplasmosis, and Rocky Mountain Spotted Fever (RMSF). The last few decades have seen an increase 1) in the number of reported tick-borne diseases, 2) in the geographic range of many tick species, and 3) in the number of newly recognized tick-borne diseases. Thus, there is a burgeoning necessity for adequate data on ticks to help health officials accurately diagnose tick-borne diseases. This is especially true in parts of the United States least able to afford extensive tick surveys. To address this, we surveyed ticks in Pine Ridge Reservation located in Ogallala Lakota County, South Dakota. We collected 196 adult ticks from eleven sites during the period of May 16 – 31, 2018. Based on morphology, all ticks were identified as the American dog tick (Dermacentor variabilis). Of these, we identified 103 males and 93 females. PCR analysis of total DNA isolated from individual ticks was performed to confirm tick identifications and test for the presence of Rickettsia rickettsii the causative agent of RMSF, and Ehrlichia sp. the causative agents of Ehrlichiosis. We have currently analyzed the DNA of 83 ticks from this survey and found twenty ticks carrying R. rickettsii and seven ticks carrying Ehrlichia sp. We present recent results from a tick survey of one of the poorest counties in the United States and consider its ramifications on the reservation community.

A Twisted Path in Parasitology

Susan Perkins

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As a parasitologist who works on malaria at the American Museum of Natural History, I’m often asked about the path that I took that led me to my “alternative” career in parasitology – a curator of microbiology at a natural history museum. The truth is that this was not necessarily something that I planned or took careful steps in order to achieve, but rather the result of fortuitous circumstances that took me from an interest in animals and their behavior to being enraptured by parasites to diving into evolutionary theory and genomics. In this talk, I’ll attempt to recap this journey and share some of the things that I did that in retrospect were good decisions, as well as some mistakes that I made along the way and what I learned from them. I’ll do my best to try to provide some sound advice – including the best advice of all – there is no one straight path to success.

A cryptic species of Myxobolus (Myxozoa: Myxobolidae) parasitizing the central nervous system of native brook trout (Salvelinus fontinalis) in western North Carolina

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We used microscopy, molecular sequence data, and histopathology to characterize Myxobolus sp. infecting the central nervous system (CNS) of brook trout (Salvelinus fontinalis). This parasite was discovered while performing the standard pepsin-trypsin digest and microscopic examination for the detection of the exotic, invasive trout pathogen Myxobolus cerebralis (causative agent of whirling disease). We morphologically characterized these specimens as: myxospore pyriform, 12.0–16.0 μm (mean ± SD = 13.9 ± 0.9; N = 83) long, 8.0–12.0 (9.6 ± 0.8; 74) wide, 7.0–10.0 (8.1 ± 0.7; 17) thick; valve having 0–4 (2.8 ± 0.8; 69) sutural markings; polar capsules 2 in number, 6.0–10.0 (7.8 ± 0.8; 153) long, 3.0–5.0 (3.5 ± 0.5; 159) wide, having 7–10 polar filament coils; intracapsular process 1.0–3.0 (2.3 ± 0.5; 124) long, 1.0–2.0 (1.5 ± 0.5; 124) wide; sporoplasm containing iodinophilic vacuole and 2 nuclei; mucous envelope prominent on rounded posterior margin, 2.0 μm (2.0 ± 0.0; 3) thick. This species resembles Myxobolus arcticus, another CNS-infecting myxozoan of salmonids (Oncorhynchus spp. and Salvelinus spp.) in Russia and the Pacific Northwest (US) but differs from it by having sutural markings and a mucous envelope. Sequences of the small subunit ribosomal DNA (18S) of Myxobolus sp. differed from M. arcticus by 2.7%, supporting the morphology-based assertion of a cryptic species of Myxobolus. Extracted DNA failed to amplify using the primers and nested PCR reaction for M. cerebralis. Histological sections revealed intercellular myxospore aggregates that lacked evidence of a plasmodium. The parasite exhibits host specificity and tissue tropism: only the medulla oblongata and nerve cord of 23 of 29 (79%) brook trout were infected, and 13 rainbow trout (Oncorhynchus mykiss) and 20 brown trout (Salmo trutta) were not infected. Morphonologically differentiating this new species from M. cerebralis is critically important in the context of hatchery checks and biosecurity because it superficially could be misdiagnosed as the causative agent of whirling disease.

A long noncoding RNA promotes epithelial defense against Cryptosporidium parvum

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Cryptosporidium parvum is an intestinal parasite that is a common surface water contaminant and major cause of diarrheal illness, particularly in developing countries and young children. Additionally, C. parvum remains an important AIDS-related opportunistic pathogen, with no fully effective treatment. Long noncoding RNAs (lncRNAs) can act as key modulators of diverse cellular processes through their interactions with DNA, RNA, and proteins. LncRNAs have been identified that play a role in the innate inflammatory response, however, the functional mechanism has been elucidated only in a select few. A microarray performed in a mouse intestinal epithelium cell line, IEC4.1, infected with C. parvum Iowa strain identified 1,385 significantly differentially expressed lncRNA. My current focus is on one lncRNA referred to as lncRNA-25B, which was induced 2.3 fold after C. parvum infection compared to uninfected control. Expression of lncRNA-25B after C. parvum infection was also verified ex vivo in a mouse enteroid model (2.1 fold), and in vivo, using a well-established cryptosporidiosis infection model in neonatal mice (2.8 fold). Further, the infection burden of C. parvum after overexpression of lncRNA-25B was significantly reduced compared to empty vector control, while knockdown of lncRNA-25B significantly increased the infection burden compared to a scrambled siRNA control. To understand how lncRNA-25B may impact the infection burden, RNA-seq was performed to identify genes whose expression may be modulated by lncRNA-25B knockdown. Many defense genes identified which are suppressed by lncRNA-25B knockdown are NF-κB target genes, suggesting a potential promotion of NF-κB signaling by lncRNA-25B. Using RNA immunoprecipitation, I demonstrated that lncRNA-25B physically interacts with NF-κB p65, and that the interaction is increased 2.7 fold following C. parvum infection. Future directions will investigate how lncRNA-25B may facilitate NF-κB signaling and promote epithelial antimicrobial defense.

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A mosquito larval infection differentially impacts the adult response to a bacterial and malaria infection

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Often, a mosquito acquires multiple infections throughout its lifetime, and within the adult life stage a prior infection can impact the immune response mounted against a subsequent infection. Whether an infection acquired during the larval stage affects the response to an infection acquired during the adult stage was unexplored, and thus, we tested this in the African malaria mosquito, Anopheles gambiae. We found that a larval infection enhances the antibacterial response of adults, and that this enhanced response correlates with a higher number of hemocytes (immune cells), a higher phagocytic activity by these hemocytes, and a higher expression of some immunity genes. However, a larval infection does not have a meaningful effect on the ability of adult mosquitoes to survive a bacterial infection, and increases their susceptibility to the malaria parasite, Plasmodium yoelii. In summary, a larval infection induces transstadial immune activation, but this activation results in both costs and benefits to adult mosquitoes.

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A new dedicated pipeline to annotate the cestode mitogenome

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Nucleotide sequences of the mtDNA genome (mitogenome) are used as molecular markers in a great variety of studies, including in phylogenetic systematics. Despite their undeniable informational content, whole mitogenomes are still relatively underexplored in phylogenetic studies for a multitude of taxa, especially those without commercial relevance, such many groups of cestodes. The advent of high-throughput DNA sequencing technologies has reduced the cost and effort required to obtain mitogenomic data. Still, assembling and annotating mtDNA sequences of non-model organisms remains challenging, mainly due to the scarcity of reference sequences and dedicated genetic databases for homology-based annotation. Well-established tools such as MITOS2 Web Server, a pipeline designed to compute consistent de novo annotation of the mitogenomic sequences, can fail when analyzing data from cestodes. This is evident when comparing original annotations of six NCBI’s RefSeq cestode mitogenomes to the annotation provided by MITOS. We observed that the rate of false positives varied from 33 to 47%, possibly due to the lack of lineage-specific training data or problems related to the implementation of distinct parameters, such as translation tables. These limitations reduce the efficiency of the annotations for cestode mitogenomes, resulting in some erroneous initial or final positions, or even failure in detecting some genes. The correct annotation via manual curation is time-consuming, undermining the reproducibility of annotations. We propose a new and time effective dedicated pipeline to annotate cestode mitogenomes. The Cestode Mitogenome Annotator (CMA) consists of Python scripts that rely on open source programs and on curated translation tables and databases that are specific to cestodes. This pipeline increases the precision and accuracy of annotation, and is already available as an online tool to the scientific community.

A species of Achtheres (Siphonostomatoida: Lernaeopodidae) from buccal cavity of striped bass (Morone saxatilis) in Lake Lanier, Georgia

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Achtheres (Siphonostomatoida: Lernaeopodidae) comprises 4 species reported from the buccal cavity and gill of a diversity of freshwater fishes representing 5 orders (Clupeiformes; Gadiformes; Perciformes; Salmonidae; Siluriformes), 6 families (Centrarchidae; Ictaluridae; Lotidae; Percidae; Salmonidae; Moronidae), 12 genera (Ambloplites; Clupea; Coregonus; Enneacanthus; Ictalurus; Leptomis; Lota; Micropterus; Morone; Perca; Pylodictus; Sander), and approximately 22 species. Two European species, Achtheres percarum (ex. Perca fluviatilis) and A. sandrae (ex. Sander lucioperca), both inadequately described, were only recently morphologically differentiated as host-specific cognates. Descriptions of A. lacae and A. pimelodi (both regarded by us herein as species inquirendae) from North America are also incomplete; type material is lost; and some host-parasite records are doubtful. We used light and scanning electron microscopy to provide novel observations of the appendages of Achtheres sp. infecting buccal cavity of striped bass (reported host for A. lacae [type host is a species of Perca]) collected from Lake Lanier, Georgia. The adult female differs from that of A. percarum by having 1) an antennule with 3 segments, the terminal segment having 7 setae (vs. indistinct segmentation and 6 setae), 2) an antenna terminal endopodal segment lacking an elongate ventral process (vs. present), 3) and a maxillule with a dorsal papilla (vs. ventral papilla) having the longest seta. The adult female differs from that of A. sandrae by having 1) an antenna terminal endopodal segment with a flattened (vs. rounded) ventral spinulose pad and 2) a maxilliped subchela claw with a single (vs. 2) accessory denticle. This work comprises the first record of a species of Achtheres from Georgia and is a critical first step to systematically revise Achtheres.
A systemic evaluation of Toxoplasma gondii infection on a farm through the examination of soil contamination and animal infection

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The environmental contamination of Toxoplasma gondii contributes to infection in humans and animals. However, T. gondii environmental contamination is largely uninvestigated due to the lack of a comprehensive evaluation system. In this study, we assessed the environmental contamination of T. gondii by examining soil oocyst contamination and infection in wild and domestic animal on a dairy farm in East Tennessee from May 2016 to April 2017. Twenty-two soil samples were collected from two cat habitats on the farm. Oocysts in the soil were sucrose-floated and subjected to DNA extraction and PCR-RFLP. Three samples (13.6%) were PCR-RFLP positive; however, T. gondii DNA was not consistently detected from these three samples on repeat testing. Rodents and meso-mammals were trapped on the farm from which blood and or tissue samples were obtained. Serological tests via Modified Agglutination Test revealed that at the cutoff of 1:25, 18.5% (5/27) of house mice, 40% (2/5) of wood mice, 70.6% (12/17) of raccoons, and 50% (1/2) of farm cats, were seropositive for T. gondii antibodies. No antibodies were found in one brown rat, six cotton rats, one mole, 16 opossums, and two skunks. Twenty-nine tissues samples from rodents were subjected to PCR detection. T. gondii was not found in any of the tissues of twenty-nine rodents via PCR-RFLP; however, one house mouse was positive for Hammondia hammondi and another house mouse for Sarcocystis spp. Sera from 30 heifers from the same farm were seronegative using MAT. In summary, due to low oocyst concentration in the environment, soil samples may not be sufficient to determine T. gondii contamination. Furthermore, our results indicated that meso-mammals such as raccoons and cats are better sentinels of T. gondii infection.

Absence of Babesia spp. in a population of Carolina Wrens, despite high tick burdens

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Parasites of the genus Babesia are transmitted through the bite of an infected Ixodes tick and infect hosts’ red blood cells. Despite the emerging importance of Babesia as a zoonotic pathogen, little is known about its ecology and host specificity. Carolina Wrens Thryothorus ludovicianus (hereafter: CW) commonly host Ixodes spp. ticks in the Eastern U.S.. We predicted that ticks infecting CW likely exposed them to Babesia spp. parasites. Juvenile birds have been found to host more ticks than adults, so we hypothesized that if ticks transmit Babesia to CW hosts, then infections would be more prevalent in juvenile CW. We captured birds using mist-nets during the months of June and July, 2018. From each bird captured, we collected a blood sample to prepare a thin blood smear. Captured birds were inspected for the presence of ticks, which we enumerated and classified by life stage (larva or nymph) when possible. We captured 75 birds, 27 of which were CW. These birds hosted 148 ticks total, 128 of which were on CW. Juvenile CW hosted a comparable number of ticks (t = -1.7, d.f. = 16.7, p = 0.09, Nticks = 85; Njuveniles = 13 ; x̅_juveniles = 6.5; s.d. = 6.3 ) to adults (Nticks = 43; Nadults = 14; x̅_adults = 3.07, s.d. = 2.9). We found 38 larvae (x̅ = 3.4; s.d. = 5.0) and 25 nymphs on (x̅ = 2.2; s.d. = 2.3) juvenile CW, whereas adults hosted 24 larvae (x̅ = 2.0; s.d. = 2.7) and 7 nymphs (x̅ = 0.6; s.d. = 0.9). The number of larvae on juvenile versus adult CW was comparable.
(p = 0.4), but juveniles hosted more nymphs than adult CW (p = 0.03). We looked for parasites in the blood smears of 11 CW individuals (6 adults and 5 juveniles) by analyzing 100 fields under 100x magnification using an Olympus CX31 microscope. We did not find Babesia sp. parasites infecting red blood cells of CW. It is likely that both larvae ticks and juvenile CW in our sample had not been previously exposed to Babesia parasites. We also suggest that CW might be refractory to Babesia infections, aborting parasite development even if transmission takes place.

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Adding reality to the folklore of the iconic host manipulating lancet fluke: hard data on clonal transmission in infected ants from southern Alberta, Canada

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Over 40 years ago, kin selection was invoked as a means of driving the evolution of the famous host-manipulating behavior of the lancet fluke Dicrocoelium dentriticum. Specifically, cotransmission of clonemates (genetically identical individuals of the same clone) was thought to mitigate the ultimate cost of the self-sacrificing metacercaria that infects an ant's suboesophageal ganglion. However, the perpetuated idea that asexual reproduction in the first host should lead to a high abundance of clonemates in ants remains untested. Moreover, high clonal diversity (proportion of unique clones to genotyped individuals) estimates of > 90% from several trematode species actually suggest there could be a low abundance of clonemates. We used microsatellite loci and population genetic analyses to test for clonemate cotransmission of metacercariae collected from 18 ants in southern Alberta, Canada. We found an unprecedented low level of clonal diversity of 19.9% with just 54 unique clones (i.e., progenitor miracidia) among the 272 genotyped metacercariae. There was a strong signature of clonemate cotransmission where the percentage of clonemate dyads within hosts, 54%, was much greater than the 4% expected by chance alone. At least one pair of clonemates occurred in 17 of the 18 hosts and 5 ants had a single clone. In 6 of 11 ants where a brain fluke was genotyped, there were clonemates of the brain fluke encysted in the abdomen. The mean number of equally frequent clones within ants was 2.9. The latter result supports existing theory that indicates the altruistic behavior can evolve even in the presence of multiple clones within the same ant host. The lancet fluke stands, thus far, in stark contrast to other trematodes in having a high abundance of clonemates. Importantly, our study provides empirical data showing conclusive clonemate cotransmission and as such, we find support for the potential of kin selection to drive the evolution and maintenance of this iconic host manipulation.

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Additional morphological features and molecular analysis of Paracamallanus cyathopharynx (Baylis, 1923) Yorke et Mapleton, 1926 (Nematode: Camallanidae) infecting Clarias gariepinus (Burchell, 1822) in Kenya

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This study provides additional taxonomic features based on scanning electron microscopy (SEM), molecular data and anatomy of the sclerotized buccal capsule structures following digestion of soft tissues of Paracamallanus cyathopharynx. The parasite was collected from the intestines of the African catfish, Clarias gariepinus from Kibos fish farm, Kisumu, Kenya. Additional features include: four long equal length digital processes on the caudal end of the female; on the male, four caudal processes (2 smaller and 2 larger), right spicule with a sharp claw-like structure at the mid with a sharply pointed distal tip. Detail about the tridents and sclerotized plate extending laterally on the buccal capsule, and the narrow isthmus separating the anterior portion of the buccal capsule from posterior were observed. The 18S rDNA fragments were amplified, sequenced and compared to other Camallanid taxa. The 18S data set confirmed the identity as P. cyathopharynx. This provides the first geographical record of P. cyathopharynx in Kenya.

Keywords: Buccal capsule, digestion, Kibos, Kisumu, scanning electron microscopy

Additional species diversity of the rhinebothriidean genus Stillabothrium

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The rhinebothriidean genus Stillabothrium was erected by Reyda et al. (2015) for seven cestode species parasitic in stingrays and guitarfish. The genus now houses nine species, with others identified via molecular analysis, but not yet formally described. Collection of rays from the Pacific and Indian Oceans allowed for continued molecular prospecting for members of this genus. Thus far, 28S rDNA has been sequenced for samples from the following host species: Maculobatis astra (NT-26) from Australia, Maculobatis cf gerrardi 6 (MZ-16) from Mozambique, and Rhinobatos schlegeli (TW-16) from Japan. Thus far, sequence analysis has revealed the following: M. astra hosts two morphotypes of Stillabothrium, one species allied with S. jeanfortiae and one allied with S. cadanati. Two specimens of Stillabothrium sequenced thus far from M. cf gerrardi were also found in the clade with S. jeanfortiae. The rhinebothriidean cestodes collected from R. schlegeli are consistent in morphology with Phyllobothrium biacetabulum, originally described by Yamaguti (1934). However, morphological study of specimens reveals the species consistent with other species of Stillabothrium, and analysis of ribosomal sequence place the species within the clade containing other Stillabothrium species.

Age-Related Resistance of Biomphalaria glabrata to Infection with Schistosoma mansoni

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Whereas most natural populations of Biomphalaria glabrata snails are susceptible to infection by compatible strains of Schistosoma mansoni, the BS-90 laboratory strain of B. glabrata is refractory to infection with all tested strains of S. mansoni. However, as first reported in the 1950s, neonatal BS-90 snails are susceptible to infection, becoming nonsusceptible as juveniles. Results of the present study show that 1 mm (shell diameter) BS-90 snails are nearly 100% susceptible, whereas
4-mm snails are refractory to infection. In addition to declining infection prevalence between 1 and 4 mm, histological data show that the proportion of hemocyte-encapsulated sporocysts, numbers of layers of encapsulating hemocytes around each sporocyst, and the mitotic response of the snail hematopoietic tissue increase at 48 hours post infection as a function of size. Furthermore, the number of germinal cells/sporocyst decreases in larger snails. These results suggest that size-associated nonsusceptibility in growing BS-90 snails results from maturation of the snail’s internal defense system (IDS) and consequent immune killing of the parasite (i.e., immunological resistance). Neonatal susceptibility in resistant snails provides a novel model system to study mechanisms of snail-schistosome incompatibility, inasmuch as resistance results from altered gene expression in a single strain during development, rather than allelic differences between snails from susceptible and resistant strains. Moreover, an investigation of neonatal susceptibility in resistant snails will provide information on mechanisms of transmission and potential control strategies. Very small snails, due to their large numbers in nature, can potentially make a significant contribution to cercarial density, and consequently any future attempt to control transmission by introduction of genetically resistant snails, as has been proposed, could be compromised by susceptible neonates. This work was supported by the Fletcher Jones Foundation.

An unnamed *Eimeria* species causing clinical coccidiosis in chukar partridge (*Alectoris chukar*): Characteristics and prospects for control by vaccination.

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A local chukar partridge (*Alectoris chukar*) producer has had recurrent issues with frequent clinical coccidiosis and high flock mortalities. Identification of this pathogenic agent was determined to later explore vaccination methods for the management of coccidiosis in commercial chukar partridge operations. To characterize the causative *Eimeria* species, morphological and biological characterization of the life cycle and sequence-based genotyping methods were employed. Morphometrics of oocysts and sporocysts were measured using light microscopy with computerized image analysis software. Experimental infections with coccidia free chukar partridges were used to describe the complete endogenous development and daily fecal collection post-inoculation was used to determine the prepatent period and duration of shedding. Endogenous development was determined histologically from samples collected at 8 locations along the intestinal tract every 8 hours throughout prepatency. The parasite had 5 asexual generations prior to oocyst formation over its 120 hour prepatent period; oocyst shedding persisted until 10 days post-inoculation. To complement observations on the parasite’s life history, the complete mitochondrial genome and partial nuclear 18S rDNA were sequenced. Molecular and biological observations suggest that this parasite has not been previously reported to infect partridges and other galliform birds, and will need formal description. Following biological characterization of this new parasite, two vaccination methodologies will be explored to elicit protective immunity: 1) live oocyst vaccination followed by a carefully monitored 2-step partial house brooding; and, 2) a ‘bioshuttle’ (vaccination/anticoccidial combination). Effective coccidiosis control will enhance flock health and improve commercial chukar operations as a whole.

Antigenic cross-reactivity between *Schistosoma mansoni* and the house dust mite *Dermatophagoides farinae*: a role for cross-reactive carbohydrate determinants (CCDs) and implications for the hygiene hypothesis.
In recent decades, in countries with advanced health systems, there has been a marked increase in diseases attributed to immunological disorders such as asthma and allergies. However, people infected with parasitic helminths including schistosomes have been found to suffer less from allergy. This has led authors to formulate a helminth parasite variant of the so-called ‘hygiene’ or ‘old friends’ hypotheses. Previous studies have found that rabbit IgG antibodies raised against \textit{Schistosoma mansoni} egg antigens cross-react with allergens such as peanut, grass pollen and natural rubber latex. In this work, we describe how rabbit IgG antibodies raised against \textit{Schistosoma mansoni} soluble egg antigens (SmSEA) are cross-reactive with molecules in house dust mite (HDM) \textit{Dermatophagoides farinae} somatic extracts. A cross-reactive molecule from HDM with approximate mass of 98 kDa was identified by tandem mass spectrometric (TMS) analysis to be the allergen Der f 15. Rabbit anti-schistosome IgG antibodies eluted from the HDM molecule reacted with the three major \textit{S. mansoni} egg glycosylated antigens IPSE/alpha-1, omega-1 and kappa-5. Moreover, anti-\textit{S. mansoni} egg antibodies that had been eluted from the HDM cross-reactive antigen also reacted with antigenic constituents of a variety of plants which are known to be allergenic in humans. This extensive cross-reactivity was ablated by sodium metaperiodate treatment of the film carrying the plant antigens, indicating it was due to cross-reactive carbohydrate determinant (CCDs). In this work, we have also used the humanized Rat Basophilic Leukemia RS-ATL8 reporter system which is used to detect allergen specific IgE. RS-ATL8 cells were sensitized overnight with high dilution of sera of the patient with allergy to HDM and stimulated with HDM allergen the next day, we found the range from 10 ng/ml to 100 pg/ml represent the optimal concentrations. These findings are novel, and provide a possible explanation for the hygiene hypothesis and a potential starting point for improved allergen-specific immunotherapy.

Assessing the antiplasmodial activity of Sica acuta leaf extract and its effect on liver function of albino rats infected with Plasmodium berghei.

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This work assessed the efficacy of ethanol leaf extract of Sida acuta on malaria parasite load and its effect on the liver of albino rats infected with Plasmodium berghei. The experimental design consists of two distinct protocols that required a total of fifty rats, twenty five for each protocol. Protocol 1 involved the four day suppressive test employed 25 rats which were grouped into five groups of five rats each. Group one was not infected with malaria parasite(normal control), group two was infected but not treated with the drugs(negative control), and group three was infected and treated with 30mg/kgbdwt of chloroquine (positive control). The fourth and fifth groups were also infected and treated with 400mg/kgbdwt and 800mg/kgbdwt of Sida acuta respectively. Thereafter, three hours post – infection treatment commenced. On the fourth day after administration of extracts, blood samples were collected via ocular purtture and thick smears were made to determine the parasite density. Protocol 2 which is the curative test, grouping was done as above. Treatment commenced seven days post – infection and thick smears of the collected blood were made four days and eleven days after extract administration to determine parasitaemia level. Blood was also collected from the rats and separated to obtain serum used to determine the level of liver enzymes. The result demonstrates that the administration of the extract reduced parasite density in the different treatment groups. The positive control had a great reduction in the parasite density when
compared to other groups (p<0.05). Also, group 5 with the high dose (800mg/kgbdwt) showed a
significant reduction in parasite density when compared to group 4 with low dose (400mg/kgbdwt)
(p<0.05). The level of parasite reduction in this group was significantly similar with the group that
received standard drug, chloroquine (p<0.05). The ALT activity increased in all groups while AST,
ALP and the total bilibirin levels were slightly decreased in groups treated with chloroquine and
400mg/kgbdwt of the extract. This study shows that the antiplamodial activity of Sida acuta was
higher at the dosage of 800mg/kgbdwt and there was a fluctuation in the level of liver enzymes
which may not be in accordance to the treatment dose.

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Attempts At Biological Control Of Proliferative Gill Disease In Catfish Aquaculture

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Proliferative gill disease (PGD) in channel (Ictalurus punctatus) and hybrid (I. punctatus x I. furcatus) catfish is attributed to the myxozoan parasite Henneguya ictaluri. Outbreaks typically occur in the spring and to a lesser extent in the fall, when environmental conditions are conducive to prolifera-
tion of the oligochaete host. In channel and hybrid catfish, continuous exposure to the waterborne
actinospore stage triggers a severe inflammatory response at the gills. This inflammatory response
and associated gill damage results in impaired osmoregulatory and respiratory function, leading to
reduced feeding activity and mortality. There are no effective control measures and attempts to eradi-
cate the oligochaete host through chemotherapeutic pond treatments have been unsuccessful. Re-
searchers have explored avenues to mitigate impacts of PGD through biological control. Co-stocking
with a benthic detritivore (Ictiobus bubalus) to reduce oligochaete populations did not have a mea-
surable effect on PGD incidence, parasite density, or overall catfish production. Similarly, attempts
were made to exploit non-specific cues that stimulate polar filament discharge and sporoplasm emis-
sion from actinospores by co-stocking with non-target fish species. During controlled infectivity
trials, the presence of Gambusia affinis did not significantly reduce H. ictaluri transmission to channel
catfish. Lastly, research has demonstrated H. ictaluri myxospore development is arrested in
hybrid catfish. While hybrids demonstrate an inflammatory response comparable to channels during
acute stages of infection, significantly less H. ictaluri DNA is present in hybrid tissues across the
developmental timeline and mature Henneguya spp. myxospores are almost non-existent. To
evaluate the impacts of these findings at the pond level ponds were stocked with channels or hy-
brids and maintained as monoculture systems for 3 years. Water samples were collected monthly
for eDNA analysis. In addition, sentinel fish exposures were performed in April and May to esti-
mate PGD severity in naïve fish stocked into these systems. No differences were observed in the
first year, but H. ictaluri DNA and lesion scores in hybrid systems were significantly reduced in the
2nd year. While these results are promising, outbreaks of PGD are still reported from commercial
hybrid culture systems, even those devoted to hybrid culture for > 3 years.

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Behavioral syndromes in fish: do parasites matter?

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Behavioral syndromes are correlated traits, such as boldness, aggressiveness, and exploratory behavior, that are consistent among contexts but vary among individuals. Variation in these behaviors may be affected by parasites, especially trophically transmitted ones. The trematode, *Posthodiplostomum* sp. is transmitted when the second intermediate fish hosts are preyed on by the definitive host, herons and egrets. We conducted an experiment to determine if the infection of *Gambusia affinis* (western mosquitofish) by *Posthodiplostomum* sp. affects host boldness, aggressiveness, and activity. Fish were captive-raised and *Posthodiplostomum*-naïve. To test boldness, fish were placed in a tank with a PVC pipe as a potential refuge, and a predator attack was simulated by striking the water surface with a wooden heron head. Boldness was measured as the time to emerge from the refuge. For aggressiveness, a mirror was placed in the tank, and we recorded the duration of time that the fish attacked its reflection (a proxy for a conspecific). Activity was determined by the amount of time the fish moved around the tank for five minutes. Two trials were conducted pre-infection. To infect fish, first intermediate *Physa* hosts were collected throughout July and August 2017 and screened for *Posthodiplostomum*. Fish were placed in individual containers, and 100-300 cercaria were added. Fish were maintained in the lab for thirty days for parasite development. Post-infection trials were completed in the same manner as pre-infection trials. All fish were necropsied to determine infection success and intensity. Only 7 of 60 fish were infected. Analysis of these 7 individuals suggests that infection by *Posthodiplostomum* may cause an increase in boldness. Once infected, fish emerged from the refuge faster after a predator attack. Aggressiveness and activity did not differ pre- and post-infection. Failed infections may be due specificity in fish hosts. Regardless, we hypothesize that *Posthodiplostomum* sp. increases boldness in *G. affinis* hosts, which could lead to a greater transmission rate. 

Biodiversity in a Changing Ocean: Salinity Alters the Effects of Host Diversity and Density on Oyster Parasite Prevalence

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Host community diversity, composition, and density can be strong drivers of parasite transmission and prevalence. Perhaps most notably, a dilution effect has been described in multiple systems, whereby increased host diversity reduces infection risk. However, this effect is not universal, and increased host diversity can also amplify infection. Here, we examine the effects of the host community on multiple parasites (Polypedora websteri, Perkinsus spp., and bacterial pathogens) of the eastern oyster, *Crassostrea virginica*. All of these parasites are affected by changes in temperature and salinity, and are therefore likely to be influenced by climate change. Most notably, growth and transmission of *P. marinus*, which primarily infects *C. virginica*, only occurs in higher salinity waters, whereas *P. chesapeaki*, which preferentially infects *Macoma balthica*, survives in lower salinities. We set up a mesocosm experiment manipulating host density and diversity using water from the Rhode river, a tributary of the Chesapeake Bay. To test the effect of density, we placed either 30 (low) or 90 (high) organisms in a mesocosm. We created a low diversity treatment containing only *C. virginica* and a high diversity treatment combining *C. virginica* with the mussel *Ischadium recurvum* and clam *Macoma balthica*. The experiment ran from June-October 2018. At the conclusion, a subset of organisms were weighed and measured, presence of *P. websteri* was recorded, and tissue samples were taken for 1) RFTM analysis to determine the abundance of Perkinsus spp. and 2) 16S sequencing to screen for bacterial pathogens. The experiment coincided with a low-salinity year in the bay, which significantly reduced the prevalence of Perkinsus spp. in oysters. We observed high intensity infections of *P. chesapeaki* in *M. balthica* and, for the first time, potential transmission from *M. balthica* to *C. virginica* within a mesocosm. We found slight effects of host diversity on the prevalence of *Polypedora* and identified multiple putative bacterial pathogens of both oysters and humans.
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Camallanid nematodes from freshwater turtles and their phylogenetic relationships with representatives of Camallanidae from other hosts.

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Nematodes of the family Camallanidae are globally distributed group parasitising the digestive tract of aquatic poikilothermic vertebrates. Several species of these nematodes found in freshwater turtles were assigned, based on their morphology, either to the genus Serpinema Yeh, 1960 (from North and South Americas, Europe and Asia) or Camallanus Railliet et Henry, 1915 (primarily from Africa and Australia). The value of morphological differences between Serpinema and Camallanus is still being discussed and considered as intergeneric, intersubgeneric or intrageneric by different authors. Recently published studies including molecular analysis based on partial 28S rDNA gene alignments showed distant relationships between S. octorugatum (Baylis, 1933) from Thailand and five species of Camallanus from Australian turtles. Studying helminthological material from turtles, we found three species of nematodes of which two are clearly differed from all previously known species: C. chelonius Baker, 1983 from Pelusios sinuatus (Smith, 1838) and Camallanus sp.1 from Pelomedusa sp. from South Africa, and Serpinema sp.1 from Rhinocollemmys punctularia (Daudin, 1801) from French Guiana. Nucleotide alignments of nuclear (18S and 28S rDNA) and mitochondrial (COI) genes were obtained for all three species. The Bayesian Interference and Maximum Likelihood analyses were performed using available data downloaded from GenBank. A phylogenetic tree based on the 28S alignments showed that Camallanus from Australian turtles form a well-supported clade separate to the clade of Camallanus from South Africa and to two species of Serpinema (not grouping together). Since only partial 28S sequences of turtle-parasitising camallanids are available in GenBank, our analyses of COI and 18S rDNA could only confirm the distant relationships between found species and Camallanus spp. parasitising fishes and frogs.

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Can a horse (hairworm) lead a cricket to water? A new method to evaluate host manipulation of the cricket, Acheta domesticus infected with a horsehair worm, Paragordius varius: is humidity the key?

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The study of host manipulation by parasites, recently named neural parasitology, continues to be an area of growing interest to both scientists and the general public. The main reason that this topic leads to attention, is the implication that brains are much like computers, able to be manipulated and controlled by outside forces, and thus challenges the idea of free will. Two important key points of neural parasitology remain largely unexplored. First, it has been hard to demonstrate that changes in host behavior benefit the parasite’s fitness. Second, the exact mechanism used to influence and control the host’s behavior have remained elusive. One main factor having hampered the study of neural parasitology is a lack of easily-manipulated laboratory model systems, in which the manipulation
can be easily recognized and studied in detail. Horse hairworms (Nematomorpha: Gordioidea) have been recognized for over a century as a host manipulator of crickets (Insecta: Orthoptera). When worms are mature, crickets, which are terrestrial, are known to seek out and jump into water, releasing worms. Only one detailed behavioral study exits using the hairworm system. In this study, use of exclusively wild caught crickets and of a y-maze lacking proper environmental control, especially humidity, produced confounding results regarding the extent and type of manipulation present. For the past 20 years, a model laboratory model has been used to study the interaction of the hairworm *Paragordius varius* in *Acheta domesticus* crickets. However, the presence of manipulation within this system has not been studied. We set out to test whether manipulation exists in this laboratory model, and whether a more effective laboratory behavior test (modified controlled y-maze) could produce more clarity in the extent and types of behaviors being manipulated. We built a y-maze with a 45-degree angle out of clear PVC piping, each arm being 2.13m long. Boxes were then placed at the end of each arm that the pipes led into as flush as possible. A wet arm was established by placing a finger bowl of water and wet paper towels into one of the boxes. The other arm was designated as dry by and contained an empty finger bowl and dry paper towels. Each box was connected to an air pump by aquarium hosing. Hygrometers were placed in each arm to measure the difference in humidity. The entrance arm was covered with a cap with holes in it to allow for air flow and the establishment of a humidity gradient. Using infected and possibly manipulated crickets within this novel experimental set-up, we hope to be able to test whether hosts are 1) drawn towards water and 2) the type of manipulation that occurs once the infected host is near water. A better understanding of the manipulation of crickets by hairworms will eventually allow us to elucidate the underlying mechanisms used by the parasite to alter its host’s behavior.

**Can you tell the difference? Recognizing whether larval echinostomes are cryptic**

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Increased taxonomic resolution of helminth parasite diversity requires the use of genetics, morphology, and host use. By not recognizing parasite diversity within ecosystems, estimates of biodiversity, geographic distributions, and host specificity are compromised. Among echinostome trematodes, species diversity may be underestimated due to cryptic species. There are several definitions of crypsis including “sensu stricto” (no morphological differences, only genetic differences) and “functional” (as defined by the systematist). Our objective was to determine if either type of crypsis was present in samples of larval echinostomes from freshwater snails. *Helisoma trivolvis* and *Lymnaea elodes* echinostome-infected snails were collected from Minnesota and Manitoba. Partial ND1 gene sequences revealed one species (*Echinostoma trivolvis* lineage a) in *H. trivolvis* and two species (*Echinoparyphium* sp. and *Echinostoma revolutum* sensu lato) in *L. elodes*. Morphological analysis of stained specimens showed that the two genera differed in spine count (37 vs 45), and in the presence of penetration glands and paraesophageal glands. These features were present in *E. trivolvis* lineage a and absent in *Echinoparyphium* sp. In contrast, multivariate analysis of 12 other features from live cercariae showed no differences between the genera. Measurements from *E. revolutum* sensu lato cercariae are ongoing and will be discussed. Preliminary results show no support for “sensu stricto” crypsis between the genera. Further, we identified a set of characteristics that could improve the taxonomic resolution of functionally cryptic groups. These features could be included in dichotomous keys to aid in species identification. Overall, our results will improve the taxonomy of echinostomes from North America and promote accurate estimates of larval echinostome diversity.

**Canine Heartworm Infection in the Cumberland Gap Region of TN, KY, and VA**
Canine heartworm (CHW), *Dirofilaria immitis*, is a mosquito-transmitted disease facilitated by the obligate relationship between infected dogs and vector competent mosquitoes. It is a significant veterinary health issue for pet dogs and an emotional and economic burden for their caretakers. This study compared the prevalence of CHW in pet dogs and prophylactic use by their caretakers with the local availability of mosquito vectors responsible for its transmission and maintenance in the population. Pet dogs from the Cumberland Gap region in Tennessee, USA were serologically tested for CHW and their caretakers interviewed about monthly prophylaxis use and perceptions regarding CHW knowledge and prevention by pedestrian neighborhood survey. CHW prevalence was 2% (3/125) in the tested population and non-use of monthly prophylaxis was present in 42% of surveyed pet caretakers. Significant (p < 0.05) predictors of prophylaxis use identified by a logistic regression model include household income, use of veterinary services, altered reproductive status (spay/neuter), knowledge of CHW, and confinement when they are outdoors. Mosquito vectors collected with CO2 baited traps were identified morphologically, and assayed in pools of like-species by PCR for *D. immitis* specific DNA. As of this submission, canine heartworm DNA has been detected in 1 of 289 assayed mosquito pools. Our ongoing research continues to focus on identification of principal mosquito vector species and their association with environmental effects such as temperature, elevation, and habitat segregation on their temporal and spatial distribution in the region.

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Cestodes invoke "The Meg"

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The tapeworm genus *Clistobothrium* was originally established for a species reported from the spiral intestine of a great white shark off California. Since then, 2 additional species have been assigned to the genus—a second species from the great white shark and a species from the shortfin mako shark. In isolation, these reports suggest this genus parasitizes lamniform sharks of the family Lamnidae. New collections confirm these associations. Two new species were found in the longfin mako shark in Taiwan and 1 new species was found parasitizing the porbeagle shark off Massachusetts. However, collections from Ecuador led to the discovery of 2 additional new species from the crocodile shark—formally expanding the host associations of the genus to include the lamniform family *Pseudocarcharidae*. Data for the 28S rDNA gene were generated for adults of these 5 new species and *Clistobothrium tumidum* from the great white shark to complement published data available for 2 of the described species. These data reveal unique molecular signatures for all 8 species. Comparable data generated for larvae found parasitizing sockeye salmon, an oarfish, and a diversity of small squaliform sharks yielded 3 distinct molecular signatures, 2 of which match known species of *Clistobothrium*. The exception was the larval form from sockeye salmon. Given the diet of the salmon shark, we predict these larvae will ultimately be found to be conspecific with adults of an undescribed species of *Clistobothrium* found parasitizing this shark species. The most puzzling aspect of this system is that our work did not serve to resolve a long standing issue surrounding the identity of 3 larval forms that are routinely reported parasitizing marine mammals, and especially cetaceans. These larvae were originally provisionally assigned to *Monorygma* or *Phyllobothrium*, but 28S rDNA data revealed them to likely represent species of *Clistobothrium*. Generation of comparable data for representatives of both of the former genera confirms this placement. But somewhat unexpectedly, none of the 8 adult species of *Clistobothrium* for which 28S rDNA data are now available appear to be conspecific with any of these larval forms. Given that cestodes are trophically transmitted between hosts in their life-cycle, and *Clistobothrium* appears to be restricted to lamniform sharks, the obvious place to look for the adult counterparts of these larvae would be in the 2 large species of
Characterization of Glycogen Branching Enzyme from the Parasitic Protist Trichomonas vaginalis

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The parasitic protist Trichomonas vaginalis is the causative organism of trichomoniasis, which is the most common non-viral sexually transmitted infection worldwide. Like many other organisms, T. vaginalis accumulates glycogen, a branched polymer of glucose, as a store of carbon and energy. Indeed, the dry weight of T. vaginalis is up to 15% glycogen, implying that this compound plays an important part in the life cycle of the organism. The synthesis of glycogen requires the activity of at least two enzymes. Glycogen synthase catalyzes the synthesis of α1,4 linked chains of glucose residues and the α1,6 branches, characteristic of mature glycogen, are introduced by a glycosyltransferase referred to as branching enzyme. Glycogen degradation requires the concerted action of glycogen phosphorylase, which removes glucose as glucose-1-phosphate from α1,4 linked glucose chains, and a debranching enzyme, which removes the α1,6 branch points. Previously, we have characterized glycogen synthase and glycogen phosphorylase from T. vaginalis. Currently, we are studying an open reading frame, TVAG_276310, that is similar to branching enzyme from other species. We used PCR to amplify this open reading frame from T. vaginalis genomic DNA. We then expressed it in E. coli and found that the purified, recombinant protein had branching enzyme activity. Currently, we are attempting to complement a yeast branching enzyme mutant through expression of the T. vaginalis open reading frame. Our overarching goal is to characterize the enzymes required to make and degrade glycogen in T. vaginalis. We expect that a better understanding of basic metabolic processes in the organism might open up new avenues of therapy for trichomoniasis.

Characterization of Sex Pheromones in Three Species of Nematomorphs

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The understudied Phylum Nematomorpha is closely related to the Phylum Nematoda, which partly consists of human and plant parasitic worms. Instead of mating inside humans and plants, Nematomorphs mate outside their terrestrial and aquatic arthropod hosts, making it easier to observe their mating behavior in contrast to Nematodes. Research on Nematode sex pheromones have indicated that asacarosides serve as the major sex pheromones. To date, no one has characterized the sex pheromones of the closely related Nematomorphs. Our study characterizes the sex pheromones of three Nematomorphs: dioecious Paragordius varius and Chordodes morgani and the parthenogenetic...
Paragordius obamai. *P. varius* and *C. morgani* will be reared in their respective terrestrial hosts, crickets and wood roaches. As the Nematomorphs emerge from their respective terrestrial hosts, they will be stored, by sex, in RO water. This water sample will be collected and freeze dried for residues of sex pheromones. Residues will be prepared and dissolved in Folch’s solution for characterization by gas chromatography and mass spectrometry. Preliminary data found differences in male and female residues of *P. varius*. We expect that the sex pheromones will contain fatty acids and monosaccharides, which is typical of ascarosides found in Nematodes. We further expect that the sex pheromones of *P. varius* and *C. morgani* will be ascarosides while the parthogenetic Paragordius obamai will lack ascarosides. This research will help create synthetic versions of the sex pheromones, which can be used in behavioral trials to test mating behavior of *P. varius* and *C. morgani*. It may also lead to drug treatments that could disrupt nematode mating.

### Characterization of Spermatozoon Ultrastructure in Tetragonocephalum sp. (Cestoda: Lecanicephalidea) from the whipray, *Urogymnus asperrimus* 1

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Variation in sperm ultrastructure has long been studied among platyhelminths. Previous authors have suggested its utility in determining phylogenetic relationships. Of the 19 cestode lineages, some major groups of cestodes have received considerable attention in regards to their spermatozoon ultrastructure (e.g., Bothriocephalidea). Other groups such as Lecanicephalidea have received little attention. The purpose of this research is to describe key spermatozoon characteristics for a member of the Lecanicephalidea, representing the monogeneric family Tetragonocephalidae. Spermatozoon ultrastructure has only been investigated for one other lecanicephalidean: *Adelobothrium* sp., a member of the family Cephalobothriidae. The present study will begin to allow us to understand the variation of spermatozoon ultrastructure among lecanicephalidean families. Furthermore, it sheds light on the diversity of spermatozoon characters across tapeworm orders. Specimens of *Tetragonocephalum* sp. were collected from *Urogymnus asperrimus* 1 from the Solomon Islands. Individual worms were fixed for transmission electron microscopy (TEM), as well as light microscopy. Two mature proglottids were embedded and processed for TEM. Additional specimens have been stained and mounted as whole specimens or sectioned using traditional histological techniques. Results from whole mounted specimens show that mature spermatozoa are housed in the vas deferens. TEM images show that spermatozoa are long filiform cells, tapered at the anterior end, becoming greatly expanded posterior to the midline, and then tapered in the extreme posterior region. Characterization of the spermatozoon has identified five regions from anterior to posterior. Each region is distinguished by a suite of characters, such as the presence of a single axoneme, the crested body, electron-dense granules or of the nucleus. The results presented in this study differ from those observations made for *Adelobothrium*, which suggests spermatozoon ultrastructure may not be conserved among lecanicephalidean families.

### Chilean ducks and their haemosporidian parasites: The importance of biogeography and non-passerine hosts

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Biogeography is known to have shaped the evolutionary history of avian haemosporidian parasites across the Neotropics. However, a paucity of information exists for the temperate Neotropics and especially from non-passerine hosts. To understand the effect of biogeography in the temperate Neotropics on haemosporidians and their non-passerine hosts we screened ducks (Anseriformes) from central Chile for the presence of these parasites. In June of 2015 tissue samples from 42 individuals comprising four species, *Anas cyanoptera* (*n*=4), *A. flavirostris* (*n*=1), *A. georgica* (*n*=34), and *A. sibilatrix* (*n*=3), were collected and assessed for haemosporidians infection. *Haemoproteus* and *Plasmodium* were identified in only two host species, *A. cyanoptera* and *A. georgica*, with no *Leucocytozoon* positive samples identified. Overall haemosporidian prevalence was low (14.2%), with the prevalence of *Plasmodium* (11.9%) greater than *Haemoproteus* (4.8%). Six haemosporidian cytochrome *b* lineages were recovered, two *Haemoproteus* and four *Plasmodium*, with both *Haemoproteus* and three *Plasmodium* lineages identified for the first time. In phylogenetic reconstruction the Chilean *Plasmodium* lineages were more closely aligned with South American passerine lineages than to known anseriform lineages. Both *Haemoproteus* lineages were located within the clade of the subgenus *Haemoproteus*, which never before has been identified from any anseriform host. The results of phylogenetic reconstruction demonstrate a unique evolutionary history of these Chilean parasites, differing from what is known for this host group. The unique isolating geographic features of Chile would present opportunities for parasite diversification and further work is needed to investigate how strong biogeographical isolation has shaped the haemosporidian parasites of this area. Our results add to the growing body of evidence that non-passerine hosts support novel haemosporidian parasites, while also demonstrating the importance of biogeography on haemosporidian parasites from the temperate Neotropics.

Choleoeimeria taggarti: A novel coccidian parasite from Antechinus flavipes (yellow-footed antechinus) that challenges several aspects of coccidian phylogeny

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The genus *Eimeria* is polyphyletic, with members grouping in several separate clades among the *Isospora*, *Cyclospora*, and *Caryospora* species. *Eimeria taggarti* was described from the prostate of a single *Antechinus flavipes* (yellow-footed antechinus). We completed the sequence for the nuclear 18S rDNA and sequenced the complete mitochondrial (mt) genome de novo. Phylogenetic analysis resolved *E. taggarti* in a well-supported clade with *Choleoeimeria* and *Acroeimeria* species. Our identification of bivalvate sporocysts, in addition to the phylogenetic position of *E. taggarti*, supported the reassignment of *E. taggarti* to the genus *Choleoeimeria*. The mt genome organization of *C. taggarti* is identical to that of the only *Choleoeimeria* sp. for which that sequence is available, an unnamed *Choleoeimeria* sp. infecting the gall bladder of a prairie kingsnake; both mt genomes include two inverted regions when compared to Stieda-body possessing eimeriid coccidia. *Choleoeimeria taggarti* is unique in the genus for infecting the prostate of a homeotherm host; all previously-identified *Choleoeimeria* species infect the gallbladder of herptiles. These unique features of *C. taggarti* and its host and tissue tropisms expand our understanding of the biologies of *Choleoeimeria* spp. and generate a range of questions on the evolutionary history of this parasite (host switching?) and the intrinsic and extrinsic factors contributing to morphological features currently considered diagnostic of this basal group of coccidia.
Clinical manifestations of lymphatic filariasis and current practices in morbidity management in Kogi State, Nigeria.

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Community participation in identifying clinical manifestations of lymphatic filariasis is an important component of mobilizing for compliance in the mass administration of albendazole. We undertook a systematic search for hydrocoele and lymphoedema as rapid assessment procedures to ascertain lymphatic filariasis prevalence. Quantitative and qualitative research approaches were also utilized to investigate current management practices in endemic communities in Kogi State, Nigeria. A total of 731 adults were randomly examined in seven communities with 8.1% having various degrees of hydrocoele and 17.7% having severe lymphoedema. Other lymphatic filariasis-related skin manifestations were documented in 12.7% of adult population. Clinical manifestations varied significantly across communities ($X^2 P < 0.05$). A total of 1,536 residents participated in the morbidity management exercise and 33.7% attributed causes of the disease to superstitious beliefs ($X^2 9.7, P < 0.05$). Only 16.5% of adults correctly identified mosquitoes as vectors of filariasis. A significant 67.4% of individuals with severe filariasis-related swelling resorted to traditional healers as first line of treatment seeking option. Prevailing community superstitious beliefs influence morbidity management and posed formidable obstacle for drug administration compliance campaign. The communities’ capacity to protect themselves is hindered by a lack of understanding of the causes, symptoms, transmission route, prevention and treatment of the disease. The challenge for the Nigerian Lymphatic Filariasis Elimination Programme is to translate this information into practical ways of promoting and improving lymphatic filariasis prevention, control and management for individuals and communities.

Clowns to the left jokers to the right: paired antennal gland infection patterns in the fluke Alloglossidium renale

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Infection patterns between paired organs can have consequences for both hosts and parasites. There are 4 possible outcomes for infections of paired organs: parasites could be randomly distributed, uniformly distributed, biased to one particular organ (e.g., left vs. right), or inconsistently biased (aggregated, but does not favor one side). We tested for these patterns in the trematode Alloglossidium renale, which as adults infect the paired antennal glands of grass shrimp. Prior studies have shown that morphological asymmetries in hosts can lead to biased infections of paired organs. There is no apparent anatomical asymmetry between the antennal glands of grass shrimp which alone leads to the hypothesis that there would be no bias for one particular organ. However, A. renale is hermaphroditic so avoidance of inbreeding could lead to inconsistent bias. We collected grass shrimp from one location at two time periods and another location at one time point. In total, 137 infected grass shrimp were examined (mean intensity = 2.2, range: 1-9). Using GLMM, intensity per gland was not different between the left and right glands and the covariables host sex and host body size were also not significant. To test for inconsistent bias and uniform infections, we used a unique Monte Carlo simulation to account for the probabilities of different infection patterns across the observed infection intensities. Using the index of dispersion as a test statistic, we found that A. renale infected the paired glands in a more uniform pattern than expected by chance. Future studies will examine if unbalanced infections affect host swimming ability. The same simulation also showed no evidence of inbreeding avoidance as the occurrence of singly infected glands (in multiply infected hosts) was as expected by random chance. Interestingly, 41% of flukes occur as single infections. Future population genetic studies will examine if this lower bound selfing rate corresponds to the level of population inbreeding. Work supported by NSF DEB 1655147, REU 1916069.
Comparative genomics and decrypting the novel morphology of an enigmatic shark tapeworm

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This study has been examining the evolution of novel morphology within the monogeneric cestode order Litobothriidea, members of which infect lamniform sharks. Our previous work has demonstrated that the newest member of this order, Litobothrium aenigmaticum, lacks all the characters typically demonstrated by members of this group, yet it robustly nests within the genus with the barcoding gene 28S rDNA (D1-D3). Furthermore, examination of the internal anatomy of L. aenigmaticum revealed the presence of 11 unique cell types within the scolex; nothing similar to this structure has been observed in any other cestode. Our current work is investigating the potential mechanisms that may have allowed for this novel morphology to evolve using comparative genomic and transcriptomic techniques. To date, we have assembled and annotated genomes for three litobothriidean species, L. aenigmaticum, L. daileyi, and L. amplifica. The genomes were estimated to be 400–430 Mb in size and our assembled genomes are 296–355Mb size with BUSCO scores of approximately 60%. The gene space annotations were completed using a combination of the MAKER and Braker annotation pipelines. The annotation process resulted in 21,742 gene models with a BUSCO score of 79.8% for L. aenigmaticum, 19,084 gene models with a BUSCO score of 79.6% for L. daileyi, and 8,358 gene models with a BUSCO score of 42.9% for L. amplifica. Gene family evolution is currently being assessed with the program CAFE and the synteny between L. aenigmaticum and L. daileyi is being examined with the program SynMap. Preliminary synteny comparisons between L. aenigmaticum and L. daileyi suggest that there have been over 2,000 translocations, 60 inversions, and 130 relocations within the L. aenigmaticum genome relative to that of L. daileyi. We have also assembled 14 transcriptomes for three litobothriidean species, L. aenigmaticum (5 transcriptomes), L. daileyi (3 transcriptomes), and L. nickoli (6 transcriptomes), from two different host individuals. The transcriptomes range in size from 13–20Mb, with that of L. aenigmaticum being nearly twice the size of those of L. daileyi and L. nickoli. Raw reads were mapped to the individual transcriptomes with HISAT2 and differential expression analyses were performed on the read count data with the R package DESeq2. These analyses revealed that 136 of 7,588 tested transcripts are significantly differentially expressed. These 136 transcripts are currently being functionally annotated with the program EnTAP and the R package GOSeq will be used to identify over represented GO terms. Overall, our current results indicate that differences in coding regions may be the most prominent mechanism driving the evolution of novelty within the litobothriidean tapeworms.

Comparative transcriptomics as a holistic approach to understanding host-parasite relationships: responses of Biomphalaria glabrata to the trematodes Schistosoma mansoni and Echinostoma paraensei and to the nematode Daubaylia potomaca

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Biomphalaria glabrata is an important vector snail in the transmission of Schistosoma mansoni, one of the causative agents of human schistosomiasis infecting over 200 million people. This research
aims to reveal a broad spectrum of the transcriptional repertoire of B. glabrata when challenged with infections by two related trematodes Schistosoma mansoni and Echinostoma paraensei and a nematode, Daubaylia potomaca. One of our goals is to understand how the same host responds to parasites with similar or very different evolutionary backgrounds. M-line strain snails were individually exposed to each parasite and at 2, 8 and 40 days post exposure (dpe), 7-8 snails/group were collected. Unexposed control snails (matched at 2 and 40 dpe) were also sampled. Snails were extracted for RNA, PCR assays run to check for parasite presence, and cDNA libraries (3 snails/group/time point) were paired-end sequenced on an Illumina NextSeq500 instrument. Bioinformatics tools were used for differential expression gene analysis and Gene Ontology term enrichment analysis. Each parasite provoked a distinctive overall pattern of responses, but in general the responses provoked by the two trematodes, the sporocyst-producing S. mansoni and rediae-producing E. paraensei, were more similar to each other (persistent patterns of overall down-regulation) than what was noted with the nematode (early down-regulation followed by dramatic late up-regulation). The results are consistent with the need for trematodes to establish stable long-term infections in which progeny are continually produced, relative to the nematode which overwhelms the snails and is transmitted only when the snail is about to die. Noteworthy snail genes specifically or generally responsive to the parasites will be discussed. We also note that parasite genes deployed at different stages of the relationship can also parse out an examined with this transcriptional approach. This study was supported by NIH grants P20GM103452 and R37AI101438.

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Comparing infection rates of Chordodes morgani in two species of wood roaches.

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Little is known about the life cycle of the hairworm, Chordodes morgani, which can be found in some creeks near Lincoln, NE. In the summer of 2017, we found evidence that its terrestrial insect host is the wood cockroach (Parcoblatta pennsylvanica) and confirmed this in the lab by an experimental infection. In the summer of 2018, we found evidence that flat-headed mayflies (Heptageniidae) serve as an aquatic insect host and wish to confirm this in the lab. To test this, we collected aquatic snails and mayfly larvae from a creek known to harbor C. morgani adults and froze them at -80 C. We later confirmed the presence of C. morgani cysts before exposing them to two different species of lab-reared roaches (P. pennsylvanica and P. fulvescens). For P. fulvescens, 21 were exposed to crushed snails and 24 were exposed to crushed mayflies. For P. pennsylvanica, 10 were exposed to crushed snails and 11 were exposed to crushed mayflies. We expect to find differences between how quickly the worms develop and how many worms emerge between the two species of roaches. We also expect to find differences between the roaches exposed to snails and those exposed to mayflies. Results from this study will help researchers rear this horsehair worm in the lab. This will allow researchers conduct comparative experiments with the more commonly reared hairworm, Paragordius varius.

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Comparison of Three Methods for Detecting Cyclospora cayetanensis during 2018 in the United States

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*Cyclospora cayetanensis*, a coccidian protozoan that causes diarrhea in humans, is a cause of food-borne outbreaks in the United States. Transmission occurs via contaminated food or water and is often linked to fresh produce from Latin America. Several states experienced outbreaks associated with salads from a fast-food chain, and vegetable trays and pasta salad sold at Midwestern grocery/convenience stores during June and July 2018. We examined the detection rates of 3 different test methods performed at a large international reference laboratory, Mayo Clinic Laboratories (MCL), from specimens received the 2018 outbreaks. MCL offers 3 stool tests to detect *C. cayetanensis*: traditional ova and parasite (O&P) exam, modified safranin stain (comparable to acid fast stain), and multiplex PCR (Biofire® gastrointestinal panel). The O&P uses light microscopy wet mount and trichrome stain in which oocysts appear colorless. In contrast, the modified safranin stain is a microscopic method in which oocysts stain bright pink-red. Lastly, the multiplex PCR can detect 22 bacterial, viral and parasitic GI pathogens, including *C. cayetanensis*. During June 1-July 31, 2018, MCL saw a 13.1% increase in O&P test volumes, a 145.3% increase in the safranin stain test volumes, and a 7.6% decrease in the GI multiplex PCR panel, suggesting ideal test utilization by ordering providers when *C. cayetanensis* was strongly suspected. During these months, 70/6244 (1.1%) O&P exams, 75/416 (20.4%) safranin stains, and 25/1425 (1.75%) PCR tests were positive for *C. cayetanensis*. 75 specimens were tested by both O&P and the safranin stain; 12/75 (16.0%) were positive by the safranin stain, while 3/75 (4.0%) were positive by O&P exam. 131 patients were tested by microscopy (O&P and/or safranin) and the multiplex PCR; 6/131 (4.6%) were positive by PCR, while 2/131 (1.5%) were positive by microscopy. While the O&P is useful in detecting *C. cayetanensis*, the safranin stain is the preferred microscopic method as it offers increased sensitivity at a decreased cost. PCR offers increased sensitivity over that of microscopy but a multiplex PCR panel may not be needed if only *C. cayetanensis* infection is suspected.

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**304 Consequences of parasite infection on gut microbiome function in an herbivorous rodent**

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Parasite infection can alter the host microbiota, but there has been little investigation into how these changes impact host physiology and microbiome function. The gut microbiome plays a critical role in digestion and toxin metabolism, and these functions might be particularly sensitive to infection-induced changes in the bacterial community. White-throated woodrats (*Neotoma albigula*) provide an ideal natural system in which to examine how parasites impact digestion and toxin metabolism. These rodents feed primarily on oxalate-rich *Opuntia* cactus, and rely on their gut microbiota to ferment plant fiber and metabolize oxalate toxins. To examine how infection alters digestive function, we conducted parasite surveys and feeding trials to measure feeding rate, oxalate degradation, and fiber digestibility in *N. albigula* in Castle Valley, Utah. Here, we present results from field parasite surveys and captive feeding trials, providing insights into how infection impacts gut function.

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**371 Continued taxonomic investigations on cestode species of the Anthocephaliidae**

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The rhinebothriidea genus *Anthocephalum* was erected by Linton (1890) for a single species from *Bathytoshia centroura* and as of the present houses 23 species. Ruhnke and Seaman (2009) predicted 60-80 additional *Anthocephalum* species above the nine known at the time. Collection of rays from the Pacific and Indian Oceans allowed for continued molecular prospecting for members of this genus. Thus far, 28S rDNA has been sequenced for cestode samples from the following host species: *Anthocephalum gracile* (type species) and *Phyllobothrium foliatum* from *B. centroura*, three *Anthocephalum* species from *Maculobatis astra* (CM03-82 and NT-100) and *Pateobatis fai* (NT-33) from Australia, in addition to two *Anthocephalum*-like species taken from *Hemitrygon benneti* (TW-18) from Taiwan, and *Taeniura lymna 1* (BO-131) from Borneo. Thus far, sequence analysis has revealed the following that the species identified as *Anthocephalum* do in fact group with other species of that genus. *Phyllobothrium foliatum* grouped with the *Anthocephalum*-like species from *H. benneti*. The other *Anthocephalum*-like sample was basal to the clade containing *Anthocephalum*.

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**Copepod phylogenomics reveals surprising relationships in the broader Crustacea: insights, intrigue, and patterns of genome size evolution**

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There are about 12,000 described species of copepods, and about 6,000 of these are symbionts with other metazoans. To establish a preliminary phylogenetic framework for copepods, we incorporated all published interspecific molecular phylogenetic studies on copepods into the Open Tree of Life, combined these data with taxonomic information from WoRMS for all 12,000 copepod species, and generated a synthetic phylogeny for all described copepods. We then mapped parasitic life style onto the resulting phylogeny and estimate that copepods have evolved a parasitic lifestyle at least 11 times. The diversity of parasitic clades ranges from a few species in some clades to over 2,000 species in others. We then set out to survey copepod genome sizes across a subset of these transitions to parasitism. We find that parasitic copepods generally have smaller genomes than free-living species. We also discover the smallest copepod genome to date, a parasitic species with a genome of only 90MB. While a robust phylogeny for the Copepoda remains elusive, our phylogenomic study of copepods has produced hundreds of new molecular markers for copepod phylogenetics. The incorporation of a broad diversity of crustacean taxa as outgroups in our phylogenomic analysis unexpectedly revealed novel crustacean relationships, particularly in the Malacostraca. These findings led us to further expand our taxon sampling in order to explore relationships across the Tetraconata (i.e., Crustacea + Hexapoda).

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**Cost-Effectiveness Analysis of LAMP Assay for Molecular Diagnosis of Human Schistosomes**

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Schistosomiasis in Africa is an ongoing public health problem which in recent times has attracted a major campaign to control the disease. It is caused by two major species, *Schistosoma mansoni* and *S. haematobium*, which often cause concurrent infections in the human population. Due to control efforts, the issue of diagnostic sensitivity has become more critical in the assessment of program effectiveness and the World Health Organization has drawn attention to the need for field-applicable tests with high specificity and sensitivity. To address that, we have evaluated the amplification of *S. mansoni* and *S. haematobium* by loop-mediated isothermal amplification (LAMP) from field-collected filtered urine samples collected from school children in Zambia. We have used four DNA extraction techniques (Qiagen, LAMP-PURE (LP), Chelex, and heating) to determine their impact on LAMP sensitivity and specificity along with cost analysis and person-time involvement for each approach. DNA extraction by LP is the fastest (average 20 min.) compared to the other three methods, although it is the most expensive including amplification (nearly twice the cost of heating extraction and amplification). Chelex extraction is slower and simpler than LP and detected 20% more positive infection than heating. Heating extraction is very fast, inexpensive, and simple to perform. However, LAMP amplification for heating-extracted samples resulted in false-negatives, possibly indicating the presence of inhibitor(s). Qiagen and LP extraction both detected all positive infections, but Qiagen extraction is more cost-effective than LP. We have demonstrated the sensitivity, cost-effectiveness and time requirement of LAMP for detection of dual schistosome parasites from field collected urine samples. LAMP can be used as a point-of-care (POC) test for surveillance and assessing success of control intervention in Zambia as part of their ongoing local schistosomiasis control program.

Creepy Dreadful Wonderful Parasites: Knowledge Translation, Engagement and Impact of an International Educational Case-Based Blog

Bobbi Pritt

Creepy Dreadful Wonderful Parasites (http://parasitewonders.blogspot.com) is an educational blog providing weekly parasite cases to an international audience since 2007. It is hosted on a freely-available platform (Blogger®) and receives approximately 25,000 page views/month. A new case is posted each Monday, with the corresponding answer posted the following Friday. Participants are notified of new postings via email, society listserv, Twitter, Facebook, and LinkedIn. A 3-question survey was disseminated via email and social media avenues to assess when cases were accessed by participants (upon receipt or later) and how cases were used for educational purposes. Additional metrics were collected through the blog platform. 94 participants responded, comprising 50 doctoral level scientists/physicians, 7 residents/fellows, 17 lab technologists, 2 nurses, 2 general public, 1 graduate student, and 15 with unknown occupation. Most (72%) viewed cases upon receipt of notification. All used the blog for self-education and 55% also used the blog for education of others. Email distribution was the most common means of sharing cases (42%), followed by incorporation of content in lectures (25%), and lab rounds (21%). Two participants incorporated content into graded examinations, and one assigned cases to students as real-time weekly quizzes. Page views originated from >30 countries, with the USA being the primary source, followed by the UK, Canada, Australia, Brazil and India. In 2018, 44% of participants used mobile devices to access blog content. Facebook was the primary referring site. Despite geographical diversity, most survey respondents have a science background; however, responses from individuals with limited medical knowledge indicate an opportunity for audience expansion. A significant majority viewed cases as they became available, indicating a preference for real-time education. Further, nearly half of the participants accessed the blog from a mobile device, emphasizing the importance of displaying content in a compatible manner. Social media sites were the primary source of referrals compared to search engine referrals, providing evidence of a community of practice around parasite education. Finally, there is evidence that free blog content is incorporated into traditional curricula. These data may be helpful to other medical professionals wishing to start a similar educational blog.
Dactylosomatids of anurans: Past, present, and future

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Haemogregarine (Apicomplexa: Adeleiorina) blood parasites are commonly reported from anuran hosts. Dactylosomatidae (Jakowska and Nigrelli, 1955) is a small group of haemogregarines comprising the genera Dactylosoma Labbé, 1894 and Babesiosoma Jakowska and Nigrelli, 1956. Currently the genera Dactylosoma and Babesiosoma contain five recognised species each. In the current study, a total of 643 anurans, comprising 38 species, 20 genera and 13 families were collected and their blood screened for the presence of dactylosomatid parasites, in South Africa (n = 618) and Belgium (n = 25). Three species of anurans were found infected namely, Ptychadena anchietae (Bocage, 1868) and Sclerophrys gutturalis (Power, 1927) from South Africa, and Pelophylax lessonae (Camerano, 1882) from Belgium. Based on morphological characteristics, morphometrics and molecular findings a new dactylosomatid, Dactylosoma sp. 1 is described from Pty. anchietae and Scl. gutturalis. The species of Dactylosoma isolated from Pel. lessonae conforms morphologically with Dactylosoma splendens Labbé 1894, thus questioning the validity of D. splendens from synonymy with D. ranarum (Kruse, 1890). Phylogenetic analysis shows species of anuran Dactylosoma as a monophyletic group separate from the other haemogregarine groups. Additionally, the mosquitoes Uranotaenia (Pseudoficalbia) mashonaensis Theobald, 1901 and U. (Pf.) montana Ingram and De Meillon, 1927 were observed feeding on Scl. gutturalis in situ and their role as potential vectors for Dactylosoma sp. 1 is discussed. This study is the first to describe a dactylosomatid parasite based on morphological and molecular data as well as to provide evidence of a dipteran as a potential vector for these parasites.

Description of a new polystome genus and three new species (Monogenea, Polystomatidae) from rhacophorid tree frogs (Anurans, Rhacophoridae) of Southeast of Asia

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Monogeneans are mainly ectoparasitic on fish, but the family Polystomatidae is the single family which radiated onto tetrapods. Polystomes and can be found on the skin and gills of the Australian lungfish, in the urinary bladder of adult frogs, gills and skin of salamanders, cloaca and phalodeum of caecilians, on the eye, in the nostrils, mouth, cloaca or urinary bladder of freshwater turtles, and on the eye of the hippopotamus. These host organisms are ecologically related through their association with freshwater habitats that favour parasite transmission. Prior to this study, less than 30 polystome species from five genera (Diplorchis, Eupolystoma, Neoriojatrema, Polystoma and Sundapolystoma) were formally described from Asia. Molecular phylogenetics showed from a sampling including polystomes of the main genera of the Polystomatidae that Polystoma was not monophyletic.
Eupolystoma and their closest relatives Madagascan Kankana and Madapolystoma are indeed nested within Polystoma, with Indian Polystoma being the most basal taxon. Based on molecular and morphological examination of new material from Japan and China, one new genus and an additional three new species are proposed from congeneric tree frogs of the family Rhacophoridae. Besides, seven already described species of Polystoma are reassigned to this genus. Morphologically, all these species are characterized by having an haptor small relative to the body size (haptor length : body length ratio <0.2), pair of similar hamuli without a prominent notch and marginal hooklet C1 with a prominent thick blade.

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Detecting Wildlife Plasmodium Infections in Anolis sabanus

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The malaria parasites (Plasmodium spp.) are a polyphyletic group within the haemosporidian genera, which infect a range of hosts including humans, other mammals (bats, rodents, apes), and sauropsids (birds, lizards). Recent phylogeny work indicates that mammalian Plasmodium resulted from a divergence from a sauropsid malaria ancestor, which was followed by a switch back to the sauropsid hosts. Studying sauropsid Plasmodium parasites is advantageous in that it can tell us more about the biological method of infection in wild hosts, but still holds relevance to human infective malaria due to the evolutionary relationship between mammalian and sauropsid Plasmodium. Plasmodium floridense, P. azurophlium, and P. leucocytica infect the lizard Anolis sabanus on Saba island in the eastern Caribbean. These three parasites are ideal study organisms due to their unique biological mechanisms and niche partitioning within the host. P. floridense infects red blood cells and produces hemozoin as a byproduct of hemoglobin digestion, both of which are typical features of Plasmodium species. Conversely, P. azurophlium infects red blood cells but does not produce hemozoin, and P. leucocytica infects white blood cells (and, therefore, does not produce hemozoin). Approximately half of the lizards on Saba are infected with low parasitemia levels and many of these lizards are coinfected with two Plasmodium species. Accurate detection of infections is therefore necessary before any future studies, such as whole transcriptome comparisons, can be completed. Due to the limit of detection when parasitemia is counted by eye and the nature of the wildlife infections (low parasitemia levels), a qPCR method can be used to increase sensitivity and accurate detection. DNA was extracted from dried blood spots on filter paper, which were collected from infected lizards on Saba. Whole mitochondrial genomes were sequenced using Sanger sequencing for each parasite in order to design species-specific primers.

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Detection of Cyclospora cayetanensis in strawberries, raspberries, blueberries, blackberries and mixed berries by a real time PCR assay: A comparative study

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Cyclospora cayetanensis is a major cause of diarrheal illness and outbreaks in the US. Raspberries have been linked to Cyclospora outbreaks in the US in recent years. Other berries could also be implicated; therefore, it is necessary to have a reliable method for detection of Cyclospora in various kinds of berries. This study evaluated the performance of the FDA validated method for detection of C. cayetanensis in raspberries, to determine its efficacy in other berries, including strawberries, blueberries, blackberries and mixed berries, and compare the results. Each type of berry compared in this study - raspberries, strawberries, blueberries, blackberries, and mixed berries - was divided into 50 g subsamples and seeded with either 5 (n=10), 10 (n=10), or 200 (n=7-8) C. cayetanensis oocysts per sub for each berry type. Unseeded subsamples were used as negative controls. The method included washing the produce, extracting DNA from wash pellets, and performing qPCR using a dual Taq-Man assay targeting the 18S rRNA gene of C. cayetanensis and an internal control (IAC). As few as 5 oocysts were detected in every type of berry analyzed with detection rates ranging from 70% in blackberries and strawberries to 100% in mixed berries. All berry samples seeded with 200 oocysts (n=38) were positive and all unseeded berry samples (n=14) were negative. Comparatively, CT values of blackberry samples seeded with 200 oocysts (CT = 31.4±0.5) were significantly lower (indicating a higher rate of detection) compared to strawberries (CT = 32.7±0.6), blueberries (CT =32.7±0.4) and mixed berries CT = (33.0±0.4) (p=0.002). Additionally, C. cayetanensis 18S rRNA gene copy numbers in samples seeded with 200 oocysts were significantly higher in blackberries versus mixed berries (p=0.02). In conclusion, the method was able to detect very low levels of C. cayetanensis oocysts in several types of berries. Differences in detection amongst berry types highlight the importance of validating the C. cayetanensis detection method in different food matrices prior to outbreaks in those food types.

Determining Parasitic Co-infection Prevalence in Pregnant Women from Ghana

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In sub-Saharan Africa, a significant proportion of the population is exposed to malaria, schistosomiasis and soil transmitted helminths (STHs). Most importantly, about 40 million pregnant women are infected with STHs and Schistosome spp. along with malaria. The consequences include intrauterine growth retardation, low birth weight, pre-term delivery and neonatal mortality. When parasitic diseases overlap in distribution, high rates of co-infection occur and there is a shortage of data about co-infection prevalence in pregnant women in Ghana. We have detected single, dual and multiple infections (malaria, schistosomiasis, and Strongyloides) among pregnant women by amplifying cell-free repeat DNA (CFRD) fragments via polymerase chain reaction (PCR) from single filtered urine sample collected from two districts (Addidome and Battor) of Ghana. In addition, sensitivity and specificity of PCR was evaluated against parasitological tests based on stool, urine and blood. Out of 163 samples, Schistosoma haematobium had the highest prevalence (47%) by PCR and then 37% for S. mansoni. The prevalence of malaria infection for Addidome district was 18% by rapid diagnostic test (RDT), whereas no infection was detected in Battor by RDT. Also, 10 positive infections for Strongyloides stercoralis were detected by PCR. We found malaria co-infection with schistosomes and with Strongyloides, when RDT and PCR were compared. Detection of CFRD by PCR from single urine sample is more cost-effective than individual parasitological tests and definitely more sensitive. The study addressed the weaknesses in the current diagnostic techniques available for malaria, schistosomes and Strongyloides. Our approach and method can be optimized to the use in the clinical set up in endemic countries to determine the multiple infection prevalence and for surveillance for this vulnerable group of population.

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Differences in Drosophila’s Demeanour: Behavioural Consequences
Parasites by their nature cause damage to their hosts, thus hosts typically employ multiple forms of anti-parasite defence. Behavioural defences can vary depending on intrinsic and extrinsic factors, such as current infection status. While extensive research has examined different types of behavioural defences, only a few have examined the impact of current infection on the efficacy of host defences against future parasite attack. We hypothesized that because of the energetic costs of infection, parasitized hosts will be less able to mount an effective behavioural defence and hence be more susceptible to future infection. To test our hypothesis we used the *Drosophila nigrospiracula-Macrocheles subbadius* host-parasite system. We predicted that increasing mite load would increase susceptibility to future mite attachment. We also predicted that the increase in susceptibility would be mediated by a parasite-induced reduction in fly defensive behaviours. We used laboratory experiments and an activity monitor in order to: (1) produce a range of infection intensities to determine the relationship between parasite density and host susceptibility and (2) examine the effect of infection intensity on a host’s overall level of activity when exposed to another parasite. Our results indicate that host susceptibility to future infection increased with higher current infection intensity. Interestingly, the overall activity of individuals differed by sex and infection intensity, but not in the ways we predicted. Together these data indicate that *M. subbadius* parasitism and density of attachment has repercussions on insect host susceptibility to future infection.

**Differential changes in shell form induced by Nematode and Trematode infections in invasive apple snails (Pomacea maculata) from Alabama, USA.**

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Although the effects of parasitism on shell form are widely reported in the literature, few address differential changes induced by two disparate lineages of worms in a single snail species. The invasion of apple snails (*Ampullaridae: Pomacea maculata, native to South America*) into a pond in ThreeMile Creek, Mobile, AL, was first observed in the 2000s but no surveys to determine if these snails harbor helminth pathogens were conducted until our study in 2017 and 2018. These snails were infected with high a prevalence of Nematodes and Trematodes. This survey resulted in the first record of Angiostrongylus cantonensis (rat lung worm) in Alabama and the first record of trematodes infecting invasive apple snails in North America. During the 2017 and 2018 surveys snails were photographed, weighed, and dissected to determine parasite presence. Prior to dissection, each snail was photographed with a digital camera. Next, the intestinal tissues of snails were dissected and tissue smears were visually examined under a compound microscope to determine parasite presence. In total, 150 individuals including uninfected, infected with trematodes, infected with nematodes, and co-infected snails were included in the geomorphometric analysis which is a technique to quantitatively analyze shell morphology and identify differences within and between groups. About 20 homologous landmarks for each individual snail were assessed via Procrustes analysis in MorphoJ. Statistical analyses showed that snails infected with trematodes had smaller apertures with larger body whorls than both uninfected and nematode infected individuals. While individuals infected with nematodes had smaller body whorls and larger apertures than uninfected and trematode infected individuals. The differential changes in snail host morphology may be a result of different
infection strategies used by each parasite. Clearly there is a need to understand host/infection dynamics, including morphological changes to snails to determine if targeted strategies to detect and monitor infected snails is possible.

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Distribution and Phylogeny of the Eye-flukes Infecting a Native Fish in the Southern Alps of New Zealand.

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_Tyloleophys darbyi_ is a recently described, and the only identified, diplostomid trematode in New Zealand. As metacercariae, _T. darbyi_ reside in the eyes of _Gobiomorphus cotidium_, the most widespread freshwater fish in New Zealand, and can reach near 100% prevalence in Lake Hayes. The definitive host is the threatened Australasian crested grebe _Podiceps cristatus australis_, which in New Zealand is only found on alpine lakes of the South Island. To date, _T. darbyi_ has only been recorded as metacercariae from Lake Hayes and as adults from Lake Wanaka on the South Island. In addition to _T. darbyi_, other eye-flukes were observed in the eyes of _G. cotidium_ from Lake Wanaka in October 2017. So, our objectives were 1) to determine the distribution, prevalence (P), and mean intensity (I) of _T. darbyi_, and the other eye-flukes, in alpine lakes in the Otago and Canterbury regions, and 2) determine the phylogenetic relationships among species and localities. We sampled 10 lakes across three distinct areas, the Ashburton Lakes, the Mackenzie Basin, and the Otago Lakes, in January 2019. _Tyloleophys darbyi_ was found in both the Ashburton and Otago Lakes but not the Mackenzie Basin, and two undescribed eye-fluke taxa were found in all three. This study also provided two new locality records for _T. darbyi_ in lakes Heron (P = 70.0%, I = 11.3) and Hawea (P = 14.3%, I = 5.0). Phylogenetic analysis is currently underway for three taxa across all sampling locations.

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Distribution of oocysts of two neogregarines (Mattesia sp. and Ophryocystis elektroscirrha), which infect the hypodermis of fire ants, Solenopsidini, and milkweed butterflies, Danaini.

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Protozoa in the order Neogregarinorida (Gregarinina: Apicomplexa) infect the fat body, hypodermis, intestine or Malpighian tubules of insect hosts. All known _Mattesia_ species infect the fat body or Malpighian tubules of beetles, moths, and fleas, with the exception of 2 species, which develop in the hypodermis of ants. Additionally, all species of _Ophryocystis_ infect the Malpighian tubules of beetles, except for _O. elektroscirrha_, which develops in the hypodermis of Danaid butterflies. In this study we examined the distribution of oocysts and their associate pathology of an undescribed _Mattesia_ sp., in adult red imported fire ants, _Solenopsis invicta_ and _O. elektroscirrha_ in adult monarch, _Danaus plexippus_ and queen, _Danaus gilippus_, butterflies using histological and SEM techniques. Our results indicate that oocysts of the _Mattesia_ sp. were always located within the hemocoel of their ant hosts. Oocysts were distributed throughout the head, thorax and abdomen, and thousands of oocysts were observed surrounding the brain of infected ants. Additionally, infected ants showed an atypical yellow-orange colored head. In contrast, oocysts of _O. elektroscirrha_ were distributed
on the surface of the abdomen, inside the aedeagus of male and within the vulva, ductus bursary and ovipore of female monarch and queen butterflies. Importantly, we observed differences in the number of oocysts embedded in the cuticle of monarch and queen butterflies. No oocysts were ever embedded in the cuticle of queen butterflies. However, oocysts were commonly embedded in the cuticle of female monarch butterflies; forming small pits on the abdominal surface. These observations may be important as previous studies indicate that female monarchs lose weight at a faster rate than uninfected monarchs; suggesting *O. elektroscirrha* infections may increase the rate of water loss through the cuticle. It is currently unclear what effects, if any, the occurrence of *Mattesia* sp. oocysts surrounding the brain of infected fire ants may have on ant fitness or behavior.

### Diversity in Mitochondrial DNA of Cervid Piroplasm Species for Sequence-Based Genotyping

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Cervid piroplasms (Apicomplexa Piroplasmida) belong to one of two genera, Babesia or Theileria, transmitted through blood-feeding by tick definitive host. In dead-end cervid hosts (e.g. reindeer, elk), piroplasms can cause hemolytic anemia and death. Although frequently used for species identifications, nuclear 18S ribosomal DNA (nu rDNA) sequences may lack sufficient species-level diversity to provide reliable identifications of closely related piroplasms. In contrast, piroplasm mitochondrial (mt) DNA exhibits species-level divergence that can be exploited for sequence-based genotyping. We used DNA samples from three reindeers confirmed to have died of a Babesia odocoilei infection and one elk confirmed to have died of a Theileria cervi infection. Polymerase Chain Reaction (PCR) amplifications used primers targeting conserved regions of piroplasm mt genomes based on available complete mt genomes of related piroplasms (e.g. Babesia gibsoni, Babesia bigemina, Babesia bovis, Babesia caballi). PCR amplicons were purified and Sanger sequenced. A substantial majority (>5.3 kilo base pairs) of the *B*. *odocoilei* mt genome was generated; it has the same components and arrangement as other related piroplasms including three protein-coding regions (CDS; i.e. cytochrome c oxidase [COI], cytochrome c oxidase III [COIII], and cytochrome B [CytB]) and numerous fragmented rDNA. Unlike the nu 18S rDNA, the mt CDS had considerable genetic divergence among these piroplasms. For example, pairwise sequence identities of partial COI sequences ranged from 76 to 82% among the related Babesia species. A variant of *B*. *odocoilei* that exhibited minor pairwise differences in the COI region compared to other *B*. *odocoilei* sequences was found in one of the reindeer samples; we confirmed that minor pairwise differences also existed within the fragmented mt rDNA. These new *B*. *odocoilei* mt sequences will provide targets for more reliable species delimitation than using nu 18S rDNA and, perhaps, practical species-specific primer sets suitable for diagnostic PCR of piroplasms in cervid hosts.

### Diversity, including cryptic diversity, of trematodes infecting freshwater snails in Vermont

**Allison Neal**

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Trematode parasites display remarkable complexity in their life cycles, requiring up to four different hosts to complete development. Despite this complexity, almost all pass through a larval stage in snails. As a result, much of the diversity of trematodes in an area can be evaluated by looking at their diversity in snails. Exploring this diversity can help us better understand the distribution of different trematode species, uncover potential threats to human or veterinary health, and identify interesting directions for more in-depth study. There are few records of trematode diversity in Vermont, so our goal was to describe this diversity using both morphology and genetic analysis. We collected snails from twelve lakes and ponds throughout central Vermont over the summer months (June – August). We collected over 4,000 snails representing six different snail families, of which around 300 were infected with trematodes. After classifying each infection based on the cercarial (larval) morphology, we also collected a sample of cercariae (larvae) for genetic analysis. DNA was extracted from these samples, PCRed at two loci within the mitochondrial COI gene, and sequenced. Sequences were compared against each other and against the NCBI database to provide species delination and, when possible, identification. Results indicate that Vermont freshwater snails are infected by at least 25 trematode morphotypes, some of which represent multiple cryptic species based on sequencing results. These results provide much needed information on the distribution of these trematode species in the northeastern United States and also provide a foundation for future research in the area.

Do helminth parasites affect the body condition of hosts or vice versa?

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Assessing body condition is one way to monitor the health of wildlife populations. In Manitoba, wildlife managers recently observed that spring migrants of lesser snow geese (Chen caerulescens) were in poorer body condition than expected. It was predicted that these birds would increase their protein levels prior to reproduction. Surprisingly, the geese had less protein and were not gaining protein prior to laying eggs. We necropsied the geese to determine the relationship between body condition and parasitism. A total of 30 geese were necropsied with 10 each in poor, fair and good body condition. Three levels of body condition were determined apriori using size-adjusted protein and fat indices. Three structural measurements were used to scale the protein and fat reserves, so that the indices reflected an individual’s body condition relative to the rest of the sample and were also corrected for the influence of structural size. One hypothesis is that there would be a negative relationship suggesting that more parasites contribute to a reduction in host body condition. Alternatively, there could be less parasitism in hosts in worse body condition if changes in diet have occurred. Many goose parasites are transmitted through invertebrate intermediate hosts that would be dietary sources of protein for goose definitive hosts. If some geese are ingesting less protein, they may be consuming fewer infected intermediate hosts resulting in fewer parasites. The results of our necropsies and statistical analysis will be reported and discussed. By testing these hypotheses, we can better understand host-parasite interactions and their impacts on wildlife populations.

Does classification reflect the molecular phylogeny of the leech genus Theromyzon (Hirudinea: Rhynchobdellidae: Glossiphoniidae)?

Maddy Foote1
Theromyzon is a genus of blood-feeding, waterfowl-parasitizing leech in the family Glossiphoniidae with a confusing classification history in North America. Since its discovery in North America by Baird in 1869, five Theromyzon species have been named in the continent. There have been a number of redescriptions of species and refutations of observed diagnostic morphology due predominantly to difficulty in observing diagnostic characters without specialized imaging. Given the confusing taxonomy of this genus and the inability to easily identify species, my questions for this project are as follows: how many species of Theromyzon are there in North America? Do the current diagnostic morphological traits line up with molecular genetic classification? How are the North American species related? I address these questions using a concatenated molecular phylogeny combining two mitochondrial loci and one nuclear locus to observe species relationships in a sample set of 47 North American specimens from the Royal Ontario Museum collections. Additionally, I observe diagnostic morphology through CT scanning imagery of 20 representative specimens taken from distinct clades within the phylogeny. Preliminary results indicate there may be more than the five named species of Theromyzon in North America.

Ecological significance of oxylipins in host-parasite interactions

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Many parasites alter the behaviour of their hosts to increase the likelihood of transmission, but the mechanisms underlying this interaction are poorly understood. Some hosts and parasites release signalling molecules that may affect transmission, but the limited taxonomic and contextual scope of these studies constrains our ability to understand the role of signalling molecules in parasite-modified behaviour. For example, oxylipins (oxidized fatty acids) in hosts can be affected by diet as well as parasitism. Thus, we characterized oxylipins in two species of freshwater snails that are commonly infected with trematode parasites. We tested for differences in the diversity and amounts of oxylipins based on host species, host diet (field diet versus lab diet), infection status, and parasite activity (presence or absence of emerging cercariae). Snail-conditioned water samples were created by placing five snails into 50ml of artificial spring water for four hours. Oxylipins were extracted from the samples and quantified using high performance liquid chromatography-tandem mass spectrometry. Preliminary results indicate that the diversity and amounts of specific oxylipins differ between host species and according to infection status. In contrast, parasite activity did not affect most oxylipins. Freshwater snails are required hosts in the life cycles of many trematode parasites. By determining the factors that affect oxylipins, we will better understand their function in this essential host-parasite relationship. As parasite behavioural modification can play an important part in freshwater ecosystem structure and function, further understanding of the mechanisms behind these interactions is crucial.

Eimeria innocua: Innocuous or Injurious?

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Eimeria meleagrimitis and E. adenoeides have been recognized as highly pathogenic to turkeys. Recently, the 4 remaining Eimeria spp. infecting turkeys (i.e., E. dispersa, E. gallopavonis, E. innocua and E. meleagridis) were detected commonly in commercial turkey flocks. Detection of these ‘minor’ species has become possible through application of a nested, species specific PCR assay that differentiates all 6 species in fecal samples, including E. innocua, the last species added to this assay. Eimeria innocua has been considered non-pathogenic; however, our observations suggest this may not be the case. To describe the pathogenic potential of E. innocua, an in vivo infection trial was conducted. Poults were reared coccidia-free and infected by oral gavage at 14, 23, 30 or 40 days-of-age with 100 to 1,000,000 oocysts per bird. Five days post-inoculation, poults were necropsied for description of macroscopic lesions at the various challenge doses. Tissue samples were taken from each bird at fixed positions along the GI tract for histopathology. Body weights of each bird were taken immediately before inoculation and again at necropsy. Eimeria innocua produced macroscopic pathological changes that, at higher doses, extended from the duodenal loop into the ileum (20cm or more beyond Meckel’s). Bleaching, ballooning, and thinning of the intestinal mucosa were evident. Both lesion severity and length of intestinal tract with lesions increased with higher doses of oocysts. However, dose-dependent increases in lesion severity were inconsistent for the younger poults; maturation of the gut may be a previously unrecognized mitigating factor affecting the impact of E. innocua infections that could confound interpretations of challenge experiments. Body weight gains during infections with the highest doses were up to 30% less than uninfected sham controls. The pathogenic potential of E. innocua and its likely impact on infected poults may have been grossly underappreciated in the literature.


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Coccidiosis, caused by Eimeria spp. parasites, is the primary biological threat to the poultry industry. Parasite replication damages enterocytes, producing disease ranging in severity from simple unthriftiness to death. The production losses caused by coccidiosis equate to billions of dollars in losses for farmers every year. Application of live vaccines has become an increasingly popular method of coccidiosis control in recent years. This strategy leverages the biology of Eimeria spp. for immunological control of coccidiosis, relying on the immunogenicity and self-limiting lifecycles of these parasites. Vaccination requires establishment of infection with sufficiently numerous viable oocysts to stimulate development of immunity, but few enough to avoid disease. Dosage that falls within these parameters additionally promotes effective vaccine cycling throughout the barn, which ensures solid protective immunity across the flock. Knowledge of actual oocyst viability is therefore required for determination of optimal dosage. Unfortunately, no rapid and accurate method for determination of oocyst viability exists; the current gold-standard is via live infection trials, which are expensive, time-consuming, and only semi-quantitative, at best. We have demonstrated an in vitro assay that uses the ability to induce transcriptional activity as a proxy for viability of Eimeria spp. oocyst viability. We have investigated a range of specific assay targets, with early data showing a strong correlation between abundance of these targets and oocyst viability. An ongoing hunt for the most-appropriate mRNA targets, and optimization of transcriptional stimulation and transcript quantification protocols, aim to increase assay performance. The optimized assay will ensure maximum vaccine efficacy, helping to improve the sustainability of the poultry industry. Other potential assay applications include use as an epidemiological tracking tool or validation of strategies for environmental control of Eimeria and related parasites.
Elucidating complex trematode life cycles in catfish aquaculture systems in the southeastern USA

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Farm-raised catfish remain the most extensively cultured freshwater food fish in the United States, with most of their production concentrated in the southeastern states. These intensively managed systems are plagued by avian depredation pressures and are ideal environments for the successful completion of trematode life cycles. Of these trematodes, Bolbophorus damnificus is responsible for direct mortality and insidious production deficits due to parasite-induced anorexia. While most research has focused on B. damnificus, recent works have highlighted the diversity of trematodes in these systems. The avian definitive hosts primarily responsible for catfish depredation and introduction of trematodes into these systems are the American white pelican Pelecanus erythrorhynchos, the double-crested cormorant Phalacrocorax auritus, the great egret Ardea alba, and the great blue heron Ardea herodias. Suitable snail intermediate hosts for trematodes include Planorbella trivolvis, Biomphalaria havanensis, and Physa gyrina. Using classical morphological, experimental, and molecular techniques, we have elucidated complete and partial life cycles of >5 trematode species. At least two Austrodiplostomum spp. have been identified in B. havanensis and from the eyes and brain of catfish and other forage fishes commonly inhabiting catfish ponds. The two clinostomids Clinostomum marginatum and C. album infect the great egret, P. trivolvis and forage fish within catfish ponds. The echinostomatid Drepanocephaulus spathans infects the double-crested cormorant, both planorbid snails, and catfish and is speculated to be associated with subtle production losses. In addition to the trematodes infecting cultured ictalurids, the snail hosts also released cercariae of trematodes that utilize amphibians as intermediate hosts. Recent work investigating their impacts on amphibian host health will be discussed. The identification of the hosts in these life cycles expands our understanding of trematode geographic distribution and systematics and is critical to their control and prevention.

Elucidating cryptic diversity in Alloglossidium spp. from Ictalurid fishes

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The digenean genus Alloglossidium has been advocated as a potential model for the evolution of complex life cycles, but, the usefulness of the Alloglossidium system hinges on establishing the correct taxonomy. Prior to 2011, only 3 of 14 species were reported from catfish hosts (A. corti, A. geminum, and A. progeneticum). However, the broad host and geographic ranges and subtle morphological differences of these flukes suggested the high potential for cryptic species. Indeed, subsequent molecular investigations supported the description of 2 new species (A. fonti and A. floridense) and the resurrection of a third (A. kenti). This study focused on further elucidating the cryptic morphology of Alloglossidium species from fishes through molecular identification with an emphasis on delineating the diagnostic morphological characters of the group. Specimens were obtained from broad-scale surveys of the eastern two-thirds of the United States and southern Canada as well as type and voucher specimens previously deposited in the US National Parasite Collection. Sequence
mtDNA and nuclear rDNA) and morphometric (27 traits) data were tied to type specimens or host localities when possible. In this talk, we present a comprehensive suite of 9 morphological traits that appear to be the most useful with regards to identifying *Alloglossidium* in fishes. As a result of a combined molecular-morphological reassessment of these species, we also present 3 new species and discuss the necessity of providing formal redescriptions to assist with proper identification of *Alloglossidium* from fish hosts. Finally, our findings highlight the pivotal role genetic data can play in corroborating observed morphological differences.

Engagement of host vascular responses by the anthelmintic praziquantel

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Broad spectrum chemotherapy for the neglected tropical disease schistosomiasis, as well as other parasitic flatworm infections, relies on the drug praziquantel (±PZQ). Although the molecular target(s) of ±PZQ in parasitic flatworms remain unknown, there has been considerable recent progress in resolving the molecular targets for PZQ enantiomers in the human host [1, 2]. We recently demonstrated that the anti-schistosomal enantiomer R-PZQ (the eutomer) acts as a partial agonist at the human serotoninergic 5-HT2B receptor, causing vasoconstriction of host mesenteric vessels where the adult worms reside [1]. S-PZQ (the distomer) acts as a partial agonist of human transient receptor potential melastatin 8 channel (TRPM8, [3]). Understanding the molecular poise and commonality between these targets will likely aid identification of relevant parasite target(s) for PZQ.

Here, we address the issue of endogenous tissue expression of PZQ-sensitive targets in the host vasculature by profiling PZQ enantiomer activity against a variety of cell lines, and tissue strips isolated from mouse mesenteric vasculature. In calcium imaging experiments, ±PZQ was found to evoked endogenous responses in primary smooth muscle cells as well as immortalized smooth muscle cell lines that were blocked (10µM, 5 mins) by preincubation with the 5-HT2BR antagonist SB204741. High speed calcium imaging of mouse mesenteric artery strips allowed direct visualization of PZQ-evoked calcium signals in smooth muscle cells. These data suggest a direct action of PZQ on host vascular smooth muscle, which may be significant for flushing paralyzed schistosome worms to the liver for elimination.


Entomopathogenic Xenorhabdus Bacteria: The Swiss Army Knife Symbionts

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The mutualistic association between *Xenorhabdus* bacteria and *Steinernema* nematodes enables these organisms to infect, kill, digest, and reproduce within insects. *Xenorhabdus* bacteria contribute key activities to this life cycle including insect immune suppression, virulence factors, defense of the
cadaver, and nutritional support for nematode reproduction. We have investigated the mechanisms by which *X. nematophila* bacteria regulate gene expression through the life cycle such that mutualistic or pathogenic traits are optimally expressed at the appropriate stage of the life cycle. Bacterial populations vary phenotypically to adapt to changing environments. Environmental parameters like nutrient availability can affect phenotypic switching and cause population heterogeneity with respect to a given trait. Our work has established that the *X. nematophila* global transcription factor Lrp controls expression of symbiotic traits at each stage of the life cycle and controls phenotypic variation between mutualistic and pathogenic behaviors. *X. nematophila* cells with high levels of Lrp are optimal for the mutualistic traits of nematode colonization and supporting nematode reproduction but are attenuated for pathogenic traits of immune suppression and virulence. *X. nematophila* cells expressing low levels of Lrp have the inverse phenotype: they are optimized for virulence but attenuated for mutualism. We are using green-fluorescent-protein reporters of Lrp-dependent gene expression to monitor the cellular and population dynamics of Lrp regulation as *X. nematophila* progresses through its mutualistic and pathogenic phases. Our results suggest that nutrient availability influences Lrp-dependent phenotypic switching, which in turn serves as a pre-adaptive mechanism for bacteria to transition from mutualism to pathogenesis in anticipation of insect infection.

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**Entomopathogenic nematodes contribute to pathogenesis through their secreted proteins**

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Entomopathogenic nematodes (EPNs) are parasites of insects that participate in a tripartite interaction between symbiotically associated bacteria, nematodes, and their insect hosts. EPNs are used in biological control because of their ability to rapidly kill insect hosts. The infective juvenile (IJ), which is the only free-living stage, is a developmentally arrested stage that seeks out insect hosts to infect in soil and epigeal habitats. IJs are associated with insect-pathogenic bacteria, which, upon infection are released into the host along with an arsenal of nematode proteins. Whereas the proteins and metabolites produced by the bacteria have been studied in earnest, the details of the nematode contribution are underexplored, leading to a widespread assumption that the nematode serves primarily as a vector for the bacteria, which is the pathogenic partner. However, it has been shown that EPNs in the genus Steinernema contribute to pathogenesis through their secreted/excreted products. We have shown that the mixture of proteins released by activated IJs is toxic at low doses to a variety of insect hosts. Both the nematode and the bacteria contribute to host death. In addition, we have identified several fatty acid- and retinol-binding proteins (FARs) released by activated IJs. We demonstrate that these FAR proteins are potent modulators of insect immunity, decreasing their resistance to bacterial infection. Together, our data suggest that activated steinernematid IJs release a complex mixture of proteins into their insect host, and that these proteins modulate host immunity and contribute to host death, even in the absence of the nematodes’ bacterial symbiont.

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**Evaluation of antimalarial and antioxidant activities of ethanol leaf extract of Vernonia amygdalina Delile on mice infected with Plasmodium berghei.**

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Ethanolic extract of Vernonia amygdalina obtained by 72 hours maceration with constant shaking using GLF shaker was evaluated for antimalarial, antioxidant activities, acute toxicity (LD50) and phytochemical constituents. Mice (20 -32g) infected with 1 × 10⁷ P.berghei were used to test the suppressive and curative antimalarial activities through oral administration. The antioxidant activity of the extract was evaluated in vitro using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical and hydrogen peroxide scavenging ability assays. DPPH effect of the extract showed concentration dependent increase in its percentage scavenging activity. At the highest concentration (500µg/ml), the percentage inhibition of V. amygdalina was 68% which was not comparable to 92% of ascorbic acid (standard). Also, the extract at highest concentration scavenged 49.6% and ascorbic acid scavenged 91% of H2O2 at the same concentration. Percentage suppression of parasites at 150mg/kg was 63.7% and 77.0% at 300mg/kg while chloroquine at 10mg/kg gave 81.7% suppression of parasitaemia in early malaria infection. V. amygdalina extract at the same doses of 150mg/kg and 300mg/kg exhibited significant (P<0.0001) and dose related increase in parasitaemia suppression (66.2% and 75.8% respectively) in the curative model which was very close to 78.4% suppression caused by chloroquine (10mg/kg). Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, saponins, tannin, sterol, phlobatanin, C. glycosides and phenol. No death was recorded in the LD50 test even with the highest concentration of the extract. Oxidative stress of the mice was accessed using catalase, glutathione reductase and malondialdehyde (MDA). The result showed significant increase (P<0.0001) in level of glutathione reductase but significant (P<0.0001) decrease in catalase activity and malondialdehyde level of all the extract and chloroquine treated groups when compared to the negative control group. However, ethanolic leaf extract of Vernonia amygdalina has demonstrated a potent antimalarial and antioxidant activities and is safe up to a dose of 3200mg/kg.

### Evaluation of biochemical assays for the in vitro testing of drug response in the canine heartworm Dirofilaria immitis microfilaria

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Heart worm disease caused by Dirofilaria immitis is a source of great suffering in dogs and cats. Prophylactic use of monthly macrocyclic lactone preventatives is the single most effective way to control the disease. Resistance development in isolates of Dirofilaria immitis to the approved macrocyclic lactones (MLs) is emerging in the United States and is a cause for great concern for veterinarians and pet owners. Current tests to diagnose resistance involve infecting experimental dogs and demonstrating treatment failure in them. Genetic markers of resistance have been recently described. But these methods are time-consuming and/or require special training, and cannot be easily conducted in diagnostic lab settings. There are no reliable in vitro biochemical tests that can reliably demonstrate response of the microfilarial stage to drugs. We report the evaluation of biochemical assays to test responses to drugs in validated resistant and susceptible isolates of heartworm microfilariae.

### Evidence for incipient co-speciation: Protopolystoma xenopodis (Monogenea: Polystomatidae) is tracking the divergence of its host, Xenopus laevis (Anura: Pipidae)

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The globally invasive amphibian *Xenopus laevis* exhibits marked phylogeographic structuring across southern Africa, its native range, resulting in high genetic variability in some invasive populations. However, geographic and genetic barriers between host lineages in the native range remain unresolved. In such cases, host-specific parasites can provide increased phylogeographic resolution to elucidate the more recent evolutionary history of the host. The monogenean flatworm *Protopolystoma xenopodis* emerges as an ideal magnifying glass to study the process of incipient speciation in *X. laevis*.

In this study, co-phylogenetic methods, both event-based and global fit methods, were employed to evaluate phylogeographic congruence between *X. laevis* and *P. xenopodis*, ultimately shedding light on the processes involved in the divergence within this host-parasite association. The mitochondrial 12S rDNA and COI genes, amounting to more than 900 base pairs, of 30 hosts and their corresponding parasites, were utilised to provide a robust phylogeny that formed the basis of the co-phylogenetic analyses. Both invasive and native sites were sampled, representing all seven known phylogeographic lineages of the host in southern Africa, France and Portugal.

Substantial divergence was seen in both the host and parasite, as evidenced by an overall mean model-uncorrected distance of 4% for the host and 6% for the parasite. Results show significant overall congruence in phylogeny between the interacting host and parasite, with evidence for co-diversification of the two main phylogeographic lineages. This suggests that this process, in allopatry, can also play a role during the first tentative steps of speciation in host-parasite associations.

### Expanding species diversity in the genus Paraorygmatobothrium

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The phyllobothriidean genus *Paraorygmatobothrium* was erected by Ruhnke (1994) for three species and has since expanded to 26 species. The genus is the most speciose in the order, but published work and examination of collected material suggests *Paraorygmatobothrium* may be substantially even more diverse in terms of species number than is currently documented. Understanding the species diversity of the genus is complicated by a relaxed pattern of host specificity and difficulties in morphological discrimination between species that are easily distinguished via DNA sequence comparison, as discussed by Cutmore et al. (2017). Thus far, 27 species of *Paraorygmatobothrium* have been included in analyses of CO1 and ND1 mitochondrial DNA and over 50% of these (14) are putative new species. Study series have been prepared for five of these new species. Discrimination of these new species from existing ones is difficult, and examination of sequence alignments are ongoing to identify unique sequence differences between species of the genus.

### Extension and validation of a real-time PCR assay for detection of Cyclospora cayetanensis on pre-cut romaine lettuce salad

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The phyllobothriidean genus *Paraorygmatobothrium* was erected by Ruhnke (1994) for three species and has since expanded to 26 species. The genus is the most speciose in the order, but published work and examination of collected material suggests *Paraorygmatobothrium* may be substantially even more diverse in terms of species number than is currently documented. Understanding the species diversity of the genus is complicated by a relaxed pattern of host specificity and difficulties in morphological discrimination between species that are easily distinguished via DNA sequence comparison, as discussed by Cutmore et al. (2017). Thus far, 27 species of *Paraorygmatobothrium* have been included in analyses of CO1 and ND1 mitochondrial DNA and over 50% of these (14) are putative new species. Study series have been prepared for five of these new species. Discrimination of these new species from existing ones is difficult, and examination of sequence alignments are ongoing to identify unique sequence differences between species of the genus.
Cyclospora cayetanensis is a protozoan parasite causing an intestinal illness in humans, cyclosporiasis, associated with the consumption of contaminated fresh produce or water. Extensive foodborne outbreaks affecting hundreds of people have occurred in the U.S. since the mid-1990s. In the most recent outbreak in 2018, 511 laboratory-confirmed cases of C. cayetanensis spanning 16 states were reported in an outbreak associated with people who had consumed a variety of salads containing romaine lettuce from McDonald’s restaurants in the Midwest. During the investigation, two samples of domestically grown romaine lettuce tested positive for C. cayetanensis. The present study extended the validated the FDA Bacteriological Analytical Manual (BAM) Chapter 19B to include romaine lettuce, a type of produce that had not been previously validated by this method. The FDA method entails produce washing, DNA extraction, and a TaqMan real-time PCR assay targeting the 18S rDNA gene of C. cayetanensis. The matrix extension was performed by examining 25 g samples of ready to eat bagged pre-cut romaine salad, either un-spiked (n=8) or spiked with 5 (n=10) or 200 (n=8) C. cayetanensis oocysts. The BAM Chapter 19B sample preparation and detection methods were used with no modifications to wash produce, extract DNA, and perform molecular detection using a qPCR analysis specific for C. cayetanensis. The method was robust and reproducible in romaine salad, detecting as few as 5 oocysts in 25 g samples. The detection rate for the bagged pre-cut romaine salad samples seeded with 5 oocysts was 80.0%. All bagged pre-cut romaine salad samples seeded with 200 oocysts were positive; all unseeded bagged pre-cut romaine salad samples were negative. No inhibited qPCR reactions were identified based on the performance of the internal amplification control (IAC). This study validates the use of BAM Chapter 19B method in romaine salad in advance of potential future outbreak investigations.

Fish parasites as indicators of host biology in a changing water body in New York

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This presentation provides specific examples of cases in which parasites can be used to infer information about the fish hosts in a changing water body. The examples are from a long-term fish parasite survey in various water bodies in Otsego County in central east New York that took place 2008–2018. In total, 1,637 individual fish representing 44 species were examined for intestinal helminths. Parasites encountered in the digestive system of fishes included a diversity of digeneans, cestodes, nematodes and acanthocephalans. Representatives of these groups were also encountered in other body organs of the fish. The parasites that were encountered in Walleye (Sander vitreus), Lake whitefish (Coregonus clupeaformis), Largemouth bass (Micropterus salmoides), Chain pickerel (Esox lucius), and Golden shiners (Notemigonus crysoleucas) made it possible to infer additional information about specific aspects of host biology. Otsego Lake Walleye lack host-specific parasites that are present in adjacent water bodies, reflecting their recent reintroduction to that water body via stocking. The parasites that were encountered in Chain pickerel, including a difficult-to-identify species of trematode and various other post-cyclically transmitted helminths, reflect the highly piscivorous diet of the host. The parasites that were present, or absent, in Largemouth bass among the different water bodies examined provide information about the differences in mollusk and other invertebrate populations among the water bodies. The presence of the Asian fish tapeworm (Schyzocotyle acheilognathi) in Golden shiners and in other cyprinids in Otsego County water bodies raises the possibility of bait bucket introductions of cyprinid species in different areas. That introduction was followed by the spread of that generalist tapeworm into fish species that are generally free of intestinal helminths. These examples illustrate both the value of parasitological information in helping understand fisheries and the importance of continued fish parasite survey work in changing water bodies.
Fitness effects of helminth parasites in a matrotrophic fish

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Gambusia affinis (western mosquitofish) serves as a host for a variety of larval and adult parasites and is an incipient matrotroph, exhibiting adjustments in post-fertilization provisioning to some offspring within a brood using recently acquired resources. Nutrient transfer to the embryos is expected to result in larger offspring that are more likely to survive. Therefore, maternal contributions should increase fitness. The presence of parasites reduces the available resources for developing offspring, and may lower host fitness. Our objective was to investigate the effects of parasitism on host fitness in an incipient matrotroph. Gambusia affinis were collected from 3 sites monthly from June 2015 through August 2016. All helminth parasites were collected and identified. Brood size and embryo developmental stage were recorded for each female fish. For each fish collected from May through August 2016, 10 ova/embryos of each stage were haphazardly selected and individually weighed. The effect of parasites on brood size was assessed with a generalized linear model and on embryo weight with a generalized linear mixed-effects model. Brood size was not only influenced by collection site, the number of days between collection and dissection, maternal body condition, the number of daylight hours and water temperature, but also by two parasites. Neoechinorhynchus cylindratus cystacanth intensity had a negative effect on brood size, however intensity of both plerocercoid and adult Schyzocotyle acheilognathi had a positive effect. Embryo weight increased with presence of either Camallanidae or Contracaecum multipapulatum, as well as with embryo developmental stage and relative host density. The results indicate that some parasitic helminth species negatively affect the fitness of G. affinis, whereas others may have a positive effect on reproductive output. Further, that effect can vary with intensity. This increase in reproduction associated with infections of some parasites may be a temporary investment reflecting a tradeoff with future reproduction.

Genetically investigating the trypanosomes of amphibians with gene sequencing of single cell isolates.

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The genus Trypanosoma consists of flagellated protozoans that infect the circulatory system of all classes of vertebrates. Trypanosomes display a high level of morphological diversity, both between species and within the life cycle stages of a single trypanosome species. As a consequence, a single host individual is often infected with multiple trypanosome morphotypes, and it is unclear whether these morphotypes represent distinct species or a single species with multiple morphological life stages. The inability to identify species using morphological characters is a major problem that greatly impedes studies on trypanosome diversity and specific host parasite associations. Amphibians are commonly infected with 1-4 trypanosome morphotypes simultaneously and therefore represent an excellent host group for studies differentiating Trypanosoma species. In the following study, we aimed to investigate trypanosome morphological diversity by isolating single trypanosome cells from naturally infected amphibians, recording their morphology, and sequencing the 18s rRNA and gGAPDH genes from the isolated cells. Thus far, we have found that trypanosome morphotypes that commonly infect the same individual frog are genetically distinct, suggesting that these cells are not life stages of the same species or lineage. Additionally, we compared morphotype morphometrics (such as cell length, cell width, and organelle positions, etc.) of morphotypes across their host range. Morphological differences exist between morphotypes in bullfrogs (Rana catesbeiana) when compared to the same morphotypes infecting green frogs (Rana clamitans). These differences suggest...
different host-parasite interactions occurring in different hosts, strongly suggesting that molecular data is critical for their identification. This study provides a novel methodology to investigate the species/lineage associations of trypanosomes.

Genomic Typing of *Cyclospora cayetanensis* via Targeted Next Generation Sequencing of Mitochondria Genomes

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*Cyclospora cayetanensis* is a coccidian apicomplexan responsible for large foodborne outbreaks globally, including the most recent event affecting 2299 individuals in 2018 in the US. Only one-third of the cases could be linked to specific food exposures through the epidemiologic investigations due to the lack of molecular epidemiology tools. Hence, the development of genomic typing tools to track the source of *C. cayetanensis* contamination in foods is essential for the prevention and management of outbreaks. Our previous work, based on geographical metadata, showed that SNP profiles of multi-copy mitochondria genomes of *C. cayetanensis* exhibit significant discriminatory power. In this study we developed a very sensitive genomics workflow to obtain complete mitochondrial genome sequences for typing of *C. cayetanensis* isolates from foods, and water contaminated with oocysts and from clinical stool samples. The 6274 bp *C. cayetanensis* mitochondrial genome was amplified using Illumina Ampliseq Targeted Next Generation Sequencing technology. Genomic DNA was extracted directly from various food matrices such as fresh produce and prepared dishes spiked with known numbers of *C. cayetanensis* oocysts. Targeted sequencing libraries of the samples were prepared using the Illumina Custom Targeted Panel and sequenced using MiSeq. Sequence reads were mapped to a reference *C. cayetanensis* mitochondrial genome, and analyzed using the Geneious program. This targeted sequencing approach allowed us to obtain near-complete mitochondrial genomes directly from food samples seeded with as low as five *C. cayetanensis* oocysts, a level of contamination usually detected in the contaminated food samples during outbreak events. This level of sensitivity to collect high resolution genome data with discriminatory power is a critical milestone in genomic typing of *Cyclospora*. Altogether, this new tool will facilitate epidemiologic investigations by helping to link *C. cayetanensis* identified in clinical and food samples during outbreaks.

Go your own way: the role of geography and host specificity in the speciation of *Quinqueserialis* (Notocotylidae)

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Species-level taxonomic resolution is essential for understanding the ecological role and evolutionary history of parasites. However, few studies identify parasites to species, leading to poor taxonomic resolution, knowledge gaps, and biased conclusions. Thus, to reveal parasite species diversity, an integrative approach must be implemented. Here, we use geographic distribution, host use, morphology, and gene sequences to investigate the species diversity of the trematode group *Quinqueserialis* (Notocotylidae). Questions remain as to the number of valid species, as well as incomplete
life cycle information in this genus. Intermediate snail and definitive mammal (voles and muskrats) hosts were field-collected from six locations in the northern and southern extent of the parasite’s range in North America. Museum-deposited adult specimens were also morphologically analyzed to increase the geographic range represented. Preliminary genetic analysis suggests at least two species that differ by 1.6% and 10% (p-distance at 28S and CO1 genes, respectively). Multivariate analyses with 13 adult features show that these genetic groups form distinct morphological clusters. Further, there was a third cluster consisting only of museum specimens (no sequencing possible). The three species appear to have different geographic ranges with two being more restricted and a third being widespread. Interestingly, two species co-occurred only at one site and were both found in the same host species (voles). Preliminary results from larval sequencing indicate a broader host range for one species, *Q. quinqueserialis*, that includes snails *Promenetus exacous* and *Gyraulus crista*. Genetic and morphological analyses indicate at least three species occur in North America, and host-induced phenotypic plasticity occurs in *Q. quinqueserialis*. Through integrative taxonomy and broad geographic sampling, this research revealed a potential hotspot of parasite co-speciation and suggests that factors influencing speciation vary between species.

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**Helminths of bats in the State of Pará, Brazil**

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Bats (Chiroptera) is the second largest order of mammals reaching the greatest diversity in tropics. In Brazil bats are represented by 9 families, 64 genera and 178 species. Bats have been studied in only a quarter of the territory of the Amazonian biome and yet, 146 species of bats have been reported. Very few Amazonian bats have been examined for parasites so far. In order to close this gap in knowledge we studied the helminths fauna of bats occurring from several localities and different habitats in the State of Pará. The bats were collected by mist netting, euthanized according to the approved protocols and examined for helminth. Upon removal from the host, helminths were placed in saline solution and fixed in hot 70% alcohol. Nematodes were studied on temporary mounts cleared with lactophenol. Flatworms were stained with alum carmine and mounted permanently on slides. Sixty out of 237 bats (25.3%) collected and examined during 2017 had helminth parasites. Representatives of the digenean genera *Anenterotrema, Metadelphis, Paralecithodendrium, Ochoterenatrema, Limatulum, Urotrema*, the cestode genus *Vampirolepis*, the nematode genera *Histiostrongylus, Cheiropteronema* and an unidentified Capillariidae, were found. Three new digenean species and two new cestode species were discovered and are being described using combined morphological and molecular approaches. New geographical and host records are also reported. The low overall prevalence of helminth infections is explained by the high proportion of fruit bats in our samples. Our study demonstrates that South American bats and particularly those found in Amazonian ecosystems, likely harbor significant undescribed diversity of parasitic worms still awaiting their discovery and formal description. The collecting was carried out under the permit nº56638-1 from ICMBio and the study was done according to the protocol nº 6319260717 approved by the Committee on Ethics in Research with Animals/UFPA.

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**High prevalence of divergent Cryptosporidium species and Cryptosporidium parvum subtypes in farmed Chinese bamboo rats (Rhizomys sinensis)**
Bamboo rats are widely farmed in southern China for meat, but their potential in transmitting pathogens to humans and other farm animals remains un-elucidated. In this study, to understand the transmission of Cryptosporidium spp. in these animals, 709 fecal specimens were collected from Chinese bamboo rats (Rhizomys sinensis) on nine farms in Jiangxi, Guangxi, and Hainan provinces, China. They were analyzed for Cryptosporidium spp. using PCR analysis of the small subunit rRNA gene. The Cryptosporidium species or genotypes present were identified by sequence analysis of the PCR products, and further subtyped by sequence analysis of the 60 kDa glycoprotein gene. Altogether, Cryptosporidium spp. were detected in 209 (29.5%) of sampled animals. The infection rate was significantly higher in animals under two months of age (107/142 or 75.4%) than adult animals (46/410 or 11.2%). Four Cryptosporidium species/genotypes were identified, including C. parvum (in 74 animals), C. occultus (in one animal; a parasite of rats), a new genotype that is genetically related to C. ubiquitum (in 83 animals), and another new genotype that is genetically related to C. parvum (in 36 animals). Among them, C. parvum (27,610±71,911 oocysts/gram of feces) and the C. parvum-like new genotype (38,679±82,811 oocysts/gram of feces) had higher oocyst shedding intensity than the C. ubiquitum-like genotype (2,470±7,017 oocysts/gram of feces). The C. parvum identified belonged to two rare subtype families, including subtypes IIpA9 (in 45 animals), IIpA6 (in 6 animals), and IloA15G1 (in 3 animals). Therefore, bamboo rats on the study farms were apparently infected with diverse Cryptosporidium species and divergent C. parvum subtypes, indicative of their likely origin of native habitats. However, similar C. parvum subtypes have been recently detected in farmed macaques and humans. As rodents are suspected to be the origin of the dominant C. parvum subtypes in livestock and humans in China, attentions should be paid to the potential role of these new farm animals in the transmission of zoonotic pathogens.

Host Hybridization: Is there support for vigor or susceptibility?

The effects of host hybridization on parasite infection are inconsistent. When two species of hosts hybridize, the hybrid may experience a greater or lesser parasite load. If the hybrid has fewer parasites compared to the parental species, then the hybrid may confer a selective advantage for parasitic resistance, contributing to hybrid vigor. Alternatively, the hybrid could display hybrid susceptibility, in which the hybrid harbors a greater parasitic burden than the parental species. The conflicting results in this field highlights a need to understand how hybridization contributes to host-parasite interactions. Here, we performed a literature review and meta-analysis to examine patterns of helminth infections in animal versus their parental species. Following the PRISMA guidelines, a literature search was conducted using the keywords Hybrid AND Host AND Parasit within the Web of Science Core Collection database. A total of 1,147 articles were assessed for infection measurements from at least one parental species and hybrid hosts. In addition to infection parameters, we compiled other variables including: host taxon, parasite taxon, endo- versus ectoparasite, parasite transmission mode, and whether either of the two parental species are an introduced species. The results pertaining to the role hybridization plays in host-parasite interactions will be discussed.
Host mating status affects vulnerability to infection but not parasite preference in a Drosophila-Macrocheles system

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Heterogeneity in infection is observed in many macroparasite systems and can be driven by preferences for certain hosts by the parasite as well as differential host susceptibility and/or exposure. Trade-off theory describes how investment in one beneficial trait can require reductions in other fitness-improving traits. We hypothesize that mating and reproduction will be energetically costly to a host, and in return reduce resources available to resist parasite infection. Additionally, physiological and behavioural changes during and after mating could affect the metabolic rates of female insects, and by extension the amount of energy available to parasites; this hypothesis predicts a preference in mites to infect mated flies. We predicted that 1) mated female flies will accrue more infections than virgin females due to reduced resistance capabilities and 2) mites will preferentially infect mated females in a metabolism-mediated manner. To test the first prediction we housed female flies with male flies at a 2:1 ratio for 48 hours, then exposed individual mated or virgin flies to 5 infectious-stage mites for 1 hour. We found that mated flies had significantly higher infection intensities (1.38±0.27, mean±1 SE) than virgin flies (0.71±0.18). We also tested if male harassment contributed to increased susceptibility to infection among mated females by giving female flies 24 hours of rest following mating. However, females given time to recover still showed higher infection intensities (1.17±0.23 mites) than virgin females (0.5±0.20 mites). To test the second prediction, we used respirometry to test if mated flies have higher metabolic rates than virgins, but found no significant difference in respiration rate between mated and virgin flies (0.063±0.0072 μL/min and 0.058±0.0043 μL/min respectively). In pair-wise choice tests, we found no preference for mated flies over virgin flies (52% mated infected, 48% virgin). Understanding trade-offs between key life history traits and parasite resistance may help predict which potential hosts are at greatest risk for infection.

How does ROP23 Contribute to Toxoplasma pathogenesis?

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Toxoplasma gondii is an intracellular Apicomplexan responsible for the zoonotic disease toxoplasmosis. T. gondii utilizes the contents of specialized secretory organelles at its apical end to exert its virulence. Specifically, T. gondii injects proteins (ROPs and RONs) from the rhoptry organelles directly into its host during invasion. While many rhoptry proteins have been implicated in the parasite’s pathogenic processes, only a handful have been fully characterized to date. Our project focuses on the putative rhoptry protein, ROP23. Though expressed in all phases of infection, rop23 transcripts are significantly increased during the chronic stage of toxoplasmosis. Given the functions of known rhoptry proteins to T. gondii virulence, we postulate that ROP23 is directly secreted into the host cell from the rhoptries where it modulates host processes. To address this hypothesis, we generated an insertional mutant parasite line at the rop23 locus (Δrop23), which had no significant on growth in vitro. However, preliminary data in a murine model of toxoplasmosis indicate that ablation of rop23 renders the parasite avirulent in CBA/J mice. To determine how ROP23 contribute to virulence, we generated an HA-tagged version of the protein for localization studies. Immunofluorescence assays, however, showed no indication of ROP23-HA in tachyzoites. We are currently assessing the expression of ROP23-HA in bradyzoites.
INTESTINAL PARASITE IN RELATION TO HAEMOGLOBIN LEVEL AND NUTRITIONAL STATUS AMONG PRIMARY SCHOOL CHILDREN IN EGBUOMA IMO STATE, NIGERIA.

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The prevalence of intestinal parasite in relation to haemoglobin level and nutritional status among two primary schools in Egbuoma, Imo-State Nigeria was conducted. Faecal and blood samples were collected from the respondents and taking to laboratory for analysis using direct wet mount for stool and blood dilution techniques respectively. Anthropometric studies were also conducted to determine their height and weight, while structured questionnaire was used to determine the socioeconomic factors enhancing the transmission of the parasite. Of the 100 samples screened, 47% were infected with different types of parasites. Taenia spp had highest prevalence of 19%, followed by hookworm 13%, Ascaris lumbricoides 8% and E.histolytica recorded the least prevalence with 7% respectively. Fifty-six percent (56%) were well nourished while 44% were malnourished. The malnourished children were more infected than the nourished with 70.5% and 28.6% respectively. The age group between 7-10 years had the highest infection with 56.7%. Both male and female has the same chances of contaminating the disease. The overall prevalence of anaemia was 24%. The prevalence of anaemia among infected children (38.6%) was higher than non-infected children (12.5%). Infection was higher among pupils who used bush pathway for defaecation than those of latrine and water closet. The rate of infection was higher among those whose parents were farmers and traders than those of civil servants. Sanitation should be encouraged in order to control the disease since high prevalence was found to be associated with unsanitary habits. There is also need for Iron supplementation programme in the study area.

Identification of TGF-β superfamily homologues in mouse whipworm, Trichuris muris

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TGF-β is an immunoregulatory growth factor known to modulate the mammalian immune system promoting homeostasis and repair. TGF-β plays a vital role in regulating inflammation during chronic infection with gut-dwelling helminths, a process known to enhance parasite survival in the host. Given the current evidence that helminths can encode TGF-β-like ligands to modulate the immune response, we wanted to better understand the immunomodulatory potential of Trichuris spp by identifying homologous TGF-β superfamily members within the model whipworm Trichuris muris. Using a phylogenetic and functional approach, we show for the first time that Trichuris muris shares a common eukaryotic TGF-β superfamily member with mammals. This ortholog shares C-terminal cysteine residues and the furin cleavage site characteristic of the TGF-β superfamily. The ortholog was confirmed in Trichuris muris by conventional PCR and sequencing and is expressed both in larval and adult stages of the parasite (RT-PCR and sequencing). Significantly, antibodies to mammalian TGF-β successfully immunoprecipitated the protein from adult worm homogenate. Our findings suggest that the conservation of this TGF-β family member across many gut dwelling helminth species provides mutualistic benefit, utilising the mammalian host to enhance immune regulation during gut infection.
If not cophylogeny, then what?

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Previous work on elasmobranch tapeworms has revealed that host specificity is not necessarily indicative of cophylogenetic signal. There are currently no methods for objectively assessing other factors that could account for host/parasite associations including host diet, geographic distribution, geological age, etc. This has led to a collaborative effort to generate such a method. Robust empirical host/parasite datasets of a variety of groups are required to inform the development of this method. The Rhinoptera/Duplicibothrium system is one of the datasets being assembled for this project. The genus Rhinoptera, commonly known as the cownose rays, is the sole genus of the batoid family Rhinopteridae. In addition to the 8 described species, previous collections from southeast Asia revealed a ninth, potential novel species in this host genus. As a result of our global fieldwork, tissues are available from all 9 host species for the generation of data for multiple genes and thus also a robust molecular phylogeny. The distributions of these rays are complementary and thus provide replicated, geographically distinct sets of distributions, with 2 species found in the western Atlantic Ocean, 2 in the eastern Atlantic Ocean, 2 in southeast Asia, 1 off northern Australia, and 1 in the eastern Pacific Ocean. Although available diet data are rather coarse, in general, they feed primarily on molluscs, crustaceans, and other small benthic organisms. The fossil record shows that Rhinoptera is a relatively recently diverged group of batoids, with the earliest known fossils being from the late Paleocene period, approximately 56 million years ago. Whole mounts were prepared for material of cestodes of the genus Duplicibothrium that parasitize these hosts. A molecular phylogeny was generated from sequence data from multiple genes from material of these species preserved for molecular work. The cestode genus Duplicibothrium is known to parasitize species of Rhinoptera. It currently houses 3 described species. However preliminary work has led to the discovery of as many as 10 potential undescribed species across the 9 species of Rhinoptera. Each cestode species appears to exhibit tight specificity for individual species of Rhinoptera. The availability of comprehensive host and parasite phylogenies, tight host specificity, host geographic distribution, diet, and age, make this an ideal host/parasite system to help inform development of the new software for exploring the lack of cophylogenetic signal.

Illuminating Schistosome Diversity: Targeted sequence capture of ultra-conserved elements within Schistosomatidae

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Schistosomatidae is a medically important and biologically fascinating group of trematodes. They use mammals and birds, a diversity of snail host families, inhabit 3 different intra-host habitats, are sexually dimorphic as well as morphologically diverse and vary in life history strategies. Since the first molecular phylogeny of Schistosomatidae in 2000, there remain persistent questions on their evolution and biogeography as the phylogeny is unresolved at deep branches. This research utilized a targeted sequence capture approach to recover novel loci for phylogenetic reconstruction and testing evolutionary hypotheses. A novel probe kit was designed to target approximately 2,000
ultra-conserved elements (UCE) within the schistosome genome, and libraries were sequenced via an Illumina platform. Using a modified sample purification and library preparation protocol for low (<0.1 µg) and degraded template DNA, we recovered an average of 1,500 loci per sample, at a sequencing depth of approximately 350X. In total 72 individual schistosomes were sequenced. Additionally, published trematode genomes (n=12) were mined for shared UCE loci. From our data we generated 15 alignments of variable completeness (80%-100%, 49-62 taxa). Alignments ranged from 244 - 539,670 nucleotides and 1-1,699 loci. Maximum likelihood and coalescent-based methods were used and both provided clarity to familial relationships and stabilizing some genera which have historically lacked phylogenetic placement. Reconstructed phylogenies were used to estimate character evolution within the family, specifically: intra-host habitat, host use, and body size. Overall this effort represents the largest and most comprehensive dataset to address phylogenetic relationships within a group of trematodes.

Impact of Selective Predation on Host Susceptibility

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Predation is a fundamental ecological process with the potential to shape community structure. Because predator-prey interactions are embedded within complex systems, the direct effects of predators on their prey can produce cascading effects on other processes, such as host-pathogen interactions. While the impact of predators on disease dynamics has received considerable attention, research has focused on selective predation on infected prey. There is, however, substantial evidence that some predators avoid infected prey, preferentially attacking uninfected individuals. Such different strategies of prey selectivity by predators modulate host-parasite interactions changing the fitness payoffs both for hosts and their parasites. Do predators’ feeding preferences affect the co-evolutionary dynamics of their prey and prey’s parasites? Here we take a first step towards answering this question by investigating the effects of selective predation on the evolution of host susceptibility. In particular, we use a host (Daphnia dentifera) - parasite (Metschnikowia bicuspidata) system to artificially manipulate predation to represent selective removal of infected or uninfected prey over multiple generations. We collected data on population patterns of D. dentifera, to examine differences in population densities and infection rates. Subsequently, we calculated population susceptibility to determine if predation treatment affects host susceptibility. After ten weeks of selective predation pressure on the D. dentifera populations, preliminary data suggest no differences in overall population densities across predation treatments. In contrast, there were trends suggesting differences in infection rates based on predation strategy. Populations in which uninfected individuals were removed to simulate selective predation had higher infection rates compared to populations where either infected or randomly selected individuals were removed. Despite strong predation pressure, there were no obvious changes in host susceptibility in response to selection over multiple generations. These preliminary patterns suggest that the benefits of low susceptibility in these predation treatments may be outweighed by associated tradeoffs (e.g., reduced fecundity). Alternatively, it may be that limited heritability precludes an evolutionary response in host susceptibility. Overall, these results suggest that while selective predation can alter infection rates, these effects do not always cascade across multiple generations through changes in genotype frequencies.

Impacts of a presumed manipulative parasite on the responses and susceptibility of fish to simulated predation

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Parasites manipulating their host to facilitate trophic transmission is a widespread and diverse phenomenon. Trematode eye-flukes in the family Diplostomidae infect a variety of fish species as metacercaiae, many residing in the eyes. A recently described diplostomid, *Tylodelphys darbyi* from the South Island of New Zealand has been found to infect the freshwater fish *Gobiomorphus cotidianus*. Within the fish, the metacercariae move about freely in the liquid parts of the eye and are quite large. We hypothesized that increasing intensity of *T. darbyi* infection will result in increasing visual impairment, thus reducing the ability of *G. cotidianus* to identify and react to a predatory threat. To test this hypothesis, we performed experiments to 1) examine the fish’s reaction to a purely visual predator cue, and 2) test their ability to avoid simulated predation under natural levels of infection. Among the 64 fish used in our experiments, *T. darbyi* had a prevalence of 98.7% with an average of 17.6 worms per fish. However, there was no relationship between *T. darbyi* intensity and either the fish’s reaction to a visual predator stimulus or their ability to escape a simulated predator. Our findings indicate that despite being present in large numbers in the eyes of its fish host, the parasite appears incapable of improving its chances of trophic transmission to its avian definitive host. The results also suggest that the fish *G. cotidianus* could be using other senses (e.g. olfaction, lateral line, etc.) to compensate for visual impairment, and detect and respond to predators.

**Imperfect Timing: Influence of Infection Maturity on Co-Infection Success with a Dominant Competitor**

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The distribution and prevalence of parasites is dependent on the ability of the parasite to locate a host, exploit host resources, and compete with other parasites. Coinfection with trematodes in an intermediate host is rarely observed in the wild, and it has been demonstrated that competitive ability differs among taxa. However, there has been little research into the priority effects on trematode competition. Using a freshwater snail host (*Biomphalaria glabrata*), we studied the ability of a highly competitive trematode, *Echinostoma caproni*, to establish and reproduce in a host previously infected with a less competitive trematode species, *Schistosoma mansoni*. Snails were exposed to *S. mansoni* and co-exposed to *E. caproni* either simultaneously or after 1 week, 4 weeks, or 6 weeks. Control treatments were established as unexposed, exposed to *E. caproni* only, or exposed to *S. mansoni* only. Co-exposure decreased the infection prevalence of *S. mansoni* for all treatment groups relative to the *S. mansoni* exposure control. Infection prevalence of *E. caproni* was lower for the 4 week exposure group than for all other treatment groups and the *E. caproni* control. *E. caproni* infections 4 and 6 weeks after *S. mansoni* exposure took longer to reach patency than infections in *E. caproni* control snails. Survivorship of co-exposed snails did not significantly differ from survivorship of *E. caproni* controls but was significantly lower than survival of *S. mansoni* controls. These results indicate that the timing of infection is important for parasite competition, affecting successful parasite establishment and developmental time.
Ecotones can increase free-living species richness, but little is known about parasites at ecotones. Here, we described gastrointestinal parasite communities in raccoons (*Procyon lotor*) in relation to two ecotones. To help characterize the parasite community, we used published parasite lists to classify parasites as core or satellite species. We then surveyed raccoons in coastal California to describe how proximity to two ecotones altered the parasite community. Raccoons near ecotones had more satellite and fewer core parasite species. Near freshwater ecotones, a moderate decrease in core species was almost offset by an increase in satellite species, leading to no significant change in total richness. Near marine ecotones, a weak decrease in core species was overwhelmed by a strong increase in satellite species that used marine intermediate hosts. We hypothesize that increased parasite richness at the marine ecotone resulted from increased diet diversity, but that raccoons were sinks for some marine parasites. Thus, the marine ecotone increased parasite diversity by adding maladapted satellite species to a persistent core community, whereas the freshwater ecotone shifted the parasite community from core to satellite species without a net change in overall parasite richness.

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**Invasive copepod infections of introduced salmonids in Lake Ontario**

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Salmincola californiensis (Subclass Copeoda: Family Lernaeopodidae) parasitizes the gills of salmonids of genus Oncorhynchus. Three species of Oncorhynchus salmon native to the Pacific Northwest, Oncorhynchus mykiss (rainbow trout), Oncorhynchus tshawytscha (chinook salmon), and Oncorhynchus kisutch (coho salmon) have been reported as hosts for *S. californiensis* since 1852. These three salmonids have been introduced to the Great Lakes intermittently since the mid 1800's. The introduction of these salmonids to the Great Lakes was followed, at some point, by the introduction of their parasitic gill copepod, *S. californiensis*. Given anecdotal accounts of *S. californiensis* in introduced salmonids in Lake Ontario since 2012, we chose to conduct a survey to formally document the occurrence of this invasive species. Our survey took place in 2018 during the spring, summer and fall at the south-eastern side of Lake Ontario. The Salmon River Fish Hatchery in Altmar, New York provided fish during the spring and fall spawning runs of 2018 while lake fishing charters provided fish during the summer of 2018. Examination of fish occurred post-mortem in all cases and each gill arch was examined by eye. Prevalence and intensity of infection was recorded and parasites were removed and preserved for analysis. Our survey results indicate the prevalence of *S. californiensis* to be 69% with a mean intensity of 2.71 in the 61 rainbow trout examined, and a prevalence of 39%
with an intensity of 1.56 in the 223 chinook salmon examined. *S. californiensis* was not found in the 100 coho salmon examined. The prevalence of 69% in rainbow trout is of great concern considering that it is nearly double that compared to the prevalence of *S. californiensis* in rainbow trout in its native range, (35%). This is the first formal documentation of *S. californiensis* in Lake Ontario. These data, in combination with future work investigating potential relationships of infection with fish age, sex and length, will be of potential use in fisheries management decisions in Lake Ontario and its tributaries.

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**Is allopreening a stimulus-driven defense against ectoparasites?**

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Allopreening occurs when one bird preens another bird. The behavior is normally directed at the head and neck of the recipient, i.e. regions that the bird cannot self-preen. Studies of penguins, pigeons, and other groups suggest that allopreening plays a role in the control of ectoparasites, such as ticks and feather lice. However, it is not known whether allopreening increases in response to increases in parasite load, or whether it is a programmed response that occurs at a regular interval independent of parasite load. We conducted a simple experiment using wild-caught Feral pigeons (*Columba livia*) to test the relationship between ectoparasite load and allopreening rate. We added feather lice (*Columbicola columbae*) to captive pigeons and tested for changes in their allopreening rates, compared to control birds with no lice added. Allopreening rates were negatively correlated with self-preening rates, but were not affected by the addition of lice.

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**Lazy hosts: evaluating methods of including uninfected hosts in the analysis of patterns of infracommunity similarity.**

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Dimensionality reduction allows for the evaluation of multivariate patterns by reducing variables to a coordinate system representing the pattern using a smaller number of variables. Nonmetric multidiimensional scaling (NMDS), in conjunction with the Bray-Curtis index of dissimilarity, has been shown to be among the most robust ordination analyses for the analysis of patterns of community similarity. As with most similarity indices, a distance measure for sites with no individuals present cannot be determined, i.e., the metric is undefined. Thus, for infracommunity data, uninfected hosts must be left out of the analysis, eliminating potentially important data related to transmission probabilities. One solution to this problem has been the assignment of a dummy variable that has a positive value for uninfected hosts, and is null for infected hosts. The utility of the use of dummy variables as a means of including uninfected hosts in ordination analyses was examined by computer simulation of patterns of infracommunity similarity. Infracommunities were generated in 2-dimensional space defined by 2 orthogonal gradients that influence the mean abundances of a subset of the parasite species present. Three categories of dummy variables were generated as follows: a single variable distinguishing infected from uninfected hosts, a dummy variable for each parasite species distinguishing infected from uninfected, and a dummy variable for each parasite species that produced
larger distances between uninfected and heavily infected hosts than between uninfected hosts and those with low abundances. Ordinations with the dummy variables, and excluding uninfected hosts were compared to the coordinates from which the infracommunities were generated by Procrustes analysis to determine which method produced the best representation of the underlying patterns of similarity.

Linking larvae of cestodes of Octopus maya and their role as intermediary host

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Octopus maya is an endemic species of cephalopod from the Yucatan Peninsula with a great economic importance, locally and nationally. Due to the interest for this species for aquaculture, in the last decade there is a growing interest to identify the parasites and symbionts that infect this resource. As well know, cestodes are more closely linked to the biology of their hosts than most other groups of parasites. The life cycles of some Trypanorhyncha, and some Tetraphyllidea, seem to involve two intermediate hosts, in addition to the definitive host, and may occur the phenomenon of paratenesis. The aims of the study are to enhance the 28S and 18S rDNA datasets for cestodes by adding of new sequences of species of the order Trypanorhyncha and Tetraphyllidea, that are parasites of Octopus maya, and link the larval stages of the cestodes, to learn more about the role of the octopus as an intermediate host for the closure of life cycles. We conducted the review of 60 specimens of Octopus maya, from which we obtained several cestode larvae that were fixed in absolute ethanol and in 70% ethanol for later processing for phylogenetic and morphological analyses. The morphological analysis did not allow us to assign all the larvae up to species level. However, we were able to identify the larvae to the following four genera: Kotorella and Prochristianella (Trypanorhyncha), plus Acanthobothrium and Phoreibothrium (Tetraphyllidea). On the other hand, the results of the phylogenetic analysis allowed us to associate the larvae found in the octopus with other cestodes found in marine fish of the Gulf of Mexico. In the case of the trypanorhynchids, these were linked with plerocercoids found in Diplectrum formosum and in Opsanus beta, while tetraphyllidids were linked with adult cestodes parasitizing Sphyra tiburo and Dasyatis say. Our results allow us to know that in the octopus the same larval stage develops that can occur in other fish that act as intermediary guests, as well as to know which are the elasmobranchs that consume the octopus.

MERGENCE OF ALTERNATIVE MALE MORPHOTYPES IN BLUEGILL SUNFISH (LEPOMIS MACROCHIRUS) MASKS DIFFERENCES IN PARASITISM

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Bluegill sunfish (Lepomis macrochirus) are sexually dimorphic North American sport fish possessing multiple male morphotypes that differ in territoriality, diet, and behavior. The larger α-males invest
heavily in courtship and parental care, while the subordinate β-males partake in mating strategies involving sneaking or mimicking behaviors. Previous studies regarding parasitism in *L. macrochirus* have not accounted for these distinct morphotypes, but this study investigated whether these behavioral differences influenced parasitism and if combining these morphotypes, as examined in previous studies, actually masks potential differences between sexes of *L. macrochirus* hosts. Over 1,500 *L. macrochirus* were collected from 14 lakes and ponds in northwestern Virginia and assessed for parasites infecting the different morphotypes. Significant differences in parasite abundance between male morphotypes were observed in 13 parasite species, while differences between at least one male morphotype and females were found in 16 parasite species. Conversely, when the male morphotypes were combined, only 3 parasite species differed between males and females, indicating that combining male morphotypes in analyses of parasitism in *L. macrochirus* actually masks the differences, not only between the male morphotypes, but between sexes. Future studies regarding parasitism in *L. macrochirus* must take the male morphotypes into account because combining them may result in missing key explanatory variables.

**Malaria eradication in Central America: potential role of drug-treated cattle.**

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Malaria is a tropical disease caused by the protozoan *Plasmodium* and transmitted to humans by *Anopheles* mosquitoes. In Central America, there are hopes that malaria eradication can be achieved in our lifetime. Eradication will require strategies to control *Anopheles* species that feed both on humans and livestock. One strategy is to treat livestock with drugs and render cattle “poisonous” to mosquitoes that feed on them. My research tested the effectiveness of two drugs used routinely in cattle to control ticks (i.e., ivermectin and fipronil) against *Anopheles albimanus*, a Central American malaria vector. A small pilot trial involving six heifers was conducted in Belize, using wild-caught local mosquitoes. The fipronil-treated heifers killed mosquitoes up to two weeks. An ivermectin-treated heifer killed mosquitoes up to one week. Ovarian development was hindered in the surviving treated mosquitoes. This project demonstrates that treating livestock with commercially-available drugs could assist malaria eradication efforts in Central America.

**Metazoan parasite infracommunities of the dusky flounder (*Syacium papillosum*) as bioindicators of environmental conditions in the continental shelf of the Yucatan Peninsula, Mexico**

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We assessed metrics of the metazoan parasite infracommunities of the dusky flounder (*Syacium papillosum*) as indicators of aquatic environmental health of the Yucatan Shelf (YS) during the 2015 north wind season (November–April). Our aims were: 1) to determine whether the parasite infracommunity metrics of *S. papillosum* exhibit significant differences among YS sub-regions, 2) to determine
whether the number of its parasite species and individuals were affected by physicochemical variables at the seascape level, and 3) to determine whether there were statistical differences between the parasite infracommunity metrics of *S. papillosum* from YS and those of *Syacium gunteri* from the Campeche Sound. Multivariate statistics and generalised additive models (GAMs) were used to examine the potential statistical associations between physicochemical variables and parasite community metrics, and the maximum entropy algorithm (MaxEnt) was used to characterise the habitat’s suitability for the parasites. We recovered 48 parasite species from 126 *S. papillosum*, with larval cestodes and digeneans being the most numerically-dominant. Multivariate analyses showed significant differences among Western YS, Mid YS and Caribbean sub-regions, with the latter being the richest in species but not in individuals. The GAM and MaxEnt results indicated a negative effect of top predators (e.g. sharks and rays) removal on parasite metrics. The parasite infracommunities of *S. papillosum* were twice as rich in the number of species and individuals as those reported for *S. gunteri*. The significant differences among sub-regions in parasite metrics were apparently due to the interruption of the Yucatan current during the north wind season. The fishing of top predators and low concentration of hydrocarbons coincides with an increase in larval cestodes and digeneans in *S. papillosum*. The dusky flounder inhabits a region (YS) with a larger number of parasite species compared with those available for *S. gunteri* in the Campeche Sound, suggesting better environmental conditions for transmission in the YS.

Micro-CT imaging of the accessory glands of macrobdellid leeches

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Micro computed tomography (Micro-CT) is a powerful tool for visualizing internal structures of objects and preserved specimens. The resulting scans are combined into high resolution 3D reconstructions of the specimen for study. This is a handy tool for soft-bodied invertebrates, such as leeches, because the specimen remains intact, unlike with serial sectioning, and can be scanned or examined at a later date. We used micro-CT scanning to compare the internal reproductive morphology of members of two closely related species of Macrobdellidae, the North American medicinal leeches. The accessory organ (or gland) and pores are unique to *Macrobdella* and *Philobdella*, both members of Macrobdellidae. The accessory organ is a glandular structure that is thought to exude an adhesive substance through the pores during copulation. The number and arrangement of the accessory pores have been reliable, easily recognizable external morphological characters that are diagnostic of Macrobdella species. The exception is *Macrobdella mimicus* that has 4 accessory pores arranged in 2 columns in 2 rows, the same configuration as *Macrobdella decora*, the type species of the genus. Two specimens each of *M. mimicus* and *M. decora* were stained with 0.3% phosphotungstic acid and 3% DMSO in 70% ethanol for 2 weeks and scanned using a GE Phoenix v|tome|x micro-CT scanner with the 180 kV Nanofocus tube configuration. The best scan of each species was segmented for 3D reconstruction using the software package Amira version 6.7. Scans revealed a network of tubing inside the accessory organ leading to the individual pores as well as the first pair of testisacs embedded within the organ of each scanned leech. The internal structure of the vagina and male atrium was further defined for each species. This is a promising technology for documenting fine scale internal morphology of soft-bodied invertebrates, such as leeches, without altering external morphology through dissection.

Microbiota biofilm dysbiosis induced by enteropathogens or in IBD: The path towards new therapies.

Andre G. Buret¹
Abnormalities in the commensal gut microbiome (dysbiosis) contribute to the pathogenesis and Inflammatory Bowel Disease (IBD) and a variety of other disorders. Phenotypic and functional abnormalities of the dysbiotic microbiota remain incompletely understood. Gut microbiota live on the intestinal mucus as poly-microbial communities called "biofilms". Beyond abnormalities in their taxonomic representations, a better understanding of how disruptions in these commensal mucosal biofilm communities are regulated will pave the way towards new therapies.

Dietary iron supplementation leads to disease exacerbation and a higher risk of infection in IBD, via unclear mechanisms. In this study we have identified a novel hydrogen sulfide (H2S)-releasing drug (ATB-429 – which we found had anti-inflammatory properties) that could correct microbiota dysbiosis.

Multispecies microbiota biofilms obtained from human colonic biopsy tissues were studied ex vivo under anaerobic conditions. These biofilms were exposed to ATB-429 at 0.05-40 μM. The ability of ATB-429 to chelate, restore microbiome biofilm and mucus homeostasis, and reduce inflammation, all critical hallmarks of IBD.

Funding for these studies was provided by the Natural Sciences and Engineering Research Council of Canada, The Canadian Institutes of Health Research, and Crohn's and Colitis Canada.

References:
- Reti et al, Infect. Immun. 2015;83(12):4571-4581

Micronutrient Deficiencies among Plasmodium falciparum infected Pregnant Women in a Tertiary Health Institution in Nigeria

Frederick Akinbo¹; Omobolanle Alabi¹; Sophia Akinbo²; Joseph Aiyeyemi³

¹ University of Benin
² Inter Mountain Hospital
³ Federal Medical Center, Owo, Nigeria

Abstract

The two important barriers to a successful pregnancy outcome are maternal under nutrition which contributes an estimated 800,000 neonatal mortality annually, and malaria, estimated to cause about 900,000 low birth weight deliveries and over 100,000 infant deaths yearly. This study was conducted to determine the micronutrient deficiencies among P. falciparum infected pregnant women in a tertiary health institution in Nigeria. Two hundred and fifty four participants age 18-42 years consisting of 154 pregnant women attending antenatal clinics at the Federal Medical Center, Owo and 100 apparently healthy non-pregnant women as controls were enrolled in this study using random sampling technique. Blood specimen was collected and analyzed for the detection of P. falciparum using 10% Giemsa staining technique while micronutrients (calcium, copper, iron and zinc) were analyzed using Atomic Absorption Spectrophotometer (AAS). An overall prevalence of 27.9% and 3% of P. falciparum infection among pregnant and non-pregnant women was observed. Pregnancy status was a significant risk factor for micronutrients deficiency (P<0.0001). Pregnancy status reduced significantly the investigated micronutrients (P<0.0001) values with more reduction in iron levels (59.1%). There were cases of multiple micronutrient deficiencies only among pregnant women with more reduction of the combination of iron and calcium levels (50%) observed. Age and gestational age significantly affected the prevalence of P. falciparum infection among pregnant and non-pregnant women was observed. Pregnancy status was a significant risk factor for micronutrients deficiency (P<0.0001). Pregnancy status reduced significantly the investigated micronutrients (P<0.0001) values with more reduction in iron levels (59.1%). There were cases of multiple micronutrient deficiencies only among pregnant women with more reduction of the combination of iron and calcium levels (50%) observed. Age and gestational age significantly affected the prevalence of P. falciparum infection among pregnant women with micronutrient deficiencies (P=0.0109; P=0.0234). Being primiparous pregnant women with micronutrient deficiencies significantly affected the prevalence of P. falciparum infection (P=0.0303). Seasonal variation and iron deficiency strongly affected P. falciparum infected pregnant women

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Abstract

The two important barriers to a successful pregnancy outcome are maternal under nutrition which contributes an estimated 800,000 neonatal mortality annually, and malaria, estimated to cause about 900,000 low birth weight deliveries and over 100,000 infant deaths yearly. This study was conducted to determine the micronutrient deficiencies among P. falciparum infected pregnant women in a tertiary health institution in Nigeria. Two hundred and fifty four participants age 18-42 years consisting of 154 pregnant women attending antenatal clinics at the Federal Medical Center, Owo and 100 apparently healthy non-pregnant women as controls were enrolled in this study using random sampling technique. Blood specimen was collected and analyzed for the detection of P. falciparum using 10% Giemsa staining technique while micronutrients (calcium, copper, iron and zinc) were analyzed using Atomic Absorption Spectrophotometer (AAS). An overall prevalence of 27.9% and 3% of P. falciparum infection among pregnant and non-pregnant women was observed. Pregnancy status was a significant risk factor for micronutrients deficiency (P<0.0001). Pregnancy status reduced significantly the investigated micronutrients (P<0.0001) values with more reduction in iron levels (59.1%). There were cases of multiple micronutrient deficiencies only among pregnant women with more reduction of the combination of iron and calcium levels (50%) observed. Age and gestational age significantly affected the prevalence of P. falciparum infection among pregnant women with micronutrient deficiencies (P=0.0109; P=0.0234). Being primiparous pregnant women with micronutrient deficiencies significantly affected the prevalence of P. falciparum infection (P=0.0303). Seasonal variation and iron deficiency strongly affected P. falciparum infected pregnant women

References:
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with micronutrient deficiencies (P<0.0173; P=0.0013 respectively). Emphasis on health education, adequate intake of a well-balanced diet consisting of the appropriate micronutrients in the right proportion and intake of nutritional supplements are advocated.

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Molecular characterization of parasites of the silver therapon, Leiopotherapon plumbeus, a unique fish species endemic to the Philippines.

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The silver therapon or ‘ayungin’, Leiopotherapon plumbeus (Terapontidae), is a freshwater fish species endemic to the Philippines. A study of its parasites from Laguna de Bay (Laguna Lake) in 2016 and 2018 yielded 4 species of endohelminths, including Opegaster minima (Trematoda: Opecoeliidae) and two acanthocephalans, Pallisentis sp. (Quadrigyridae), and a single Neoechinorhynchus sp. (Neoechinorhynchidae). This study focuses on O. minima, using molecular (DNA sequence) data. DNA sequence data from the rRNA gene array (18S and 28S rDNA) of O. minima from silver therapon were identical to those of O. minima from the type host, the tank goby Glossogobius giuris (Gobiidae) also from the Philippines. Phylogenetic analyses did not place O. minima with the only other Opegaster species for which molecular data are available. Further, O. minima does not conform to the current morphological diagnosis of Opegaster. This study also provides molecular data from a Neoechinorhynchus species from the Philippines. Finally, we provide the first report of Pallisentis sp. from the silver therapon, likely caused by the established presence of the snakeheads, Channa spp. (Channidae) in Laguna Lake, in which this acanthocephalan is common.

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Molecular phylogenetic analysis of the Uvulifer Yamaguti, 1934

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Uvulifer Yamaguti, 1934 (Diplostomidae: Crassiphialinae) is a globally distributed genus of digeneans parasitic as adults in the intestine of kingfishers (Aves: Alcedinidae). This genus is one of the causative agents of the infamous black spot disease in fish including many species of game fishes. Uvulifer includes arguably between 16 and 19 species worldwide, however, only 6 species are found in the New World. Remarkably, only 1 named species of Uvulifer has been included in previous molecular phylogenetic analyses; however, sequences from multiple unidentified species from North and Central America have been previously published. In this study, we used partial sequences of the nuclear ribosomal 28S gene and the mitochondrial CO1 gene to examine phylogenetic interrelationships of several Uvulifer species from North, Central and South America. The combination of morphological and molecular analyses revealed the presence of 2 new species of Uvulifer. The Uvulifer species used in our study did not form clear patterns or clades associated with either geographic regions or traditionally used differentiating characters of adults. Our analyses support very close relationships among Uvulifer species throughout the New World. This study was funded in
Monogenean body size increases with infrapopulation abundance in three species

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Among populations, there is a trend of decreasing mean body size with increasing population abundance, primarily due to limited availability of resources. This typically leads to a lowered birth rate and higher death rate as the population reaches a carrying capacity (K) and fluctuates around K. Parasites on a host (an infrapopulation) will never encounter conspecifics outside on other hosts, and competition, if it exists, occurs at this infrapopulation level. We report that, unlike most free-living organisms, the mean infrapopulation body size of three monogenean species in Bluegill sunfish show a positive relationship with infrapopulation abundance of conspecifics. Moreover, there was no significant relationship between body size and abundance on single gills, so the relationship is unlikely to be the result of interactions with other monogenean worms. These data indicate a lack of intraspecific competition and suggests that a whole-host reaction to parasite infection induces this phenotypic response. Body size and abundance have important implications for reproductive output in monogeneans, both result in higher egg production per worm, and should not be considered separately. A larger body size provides a mechanism for increased egg production via a higher capacity for vitelline storage. Thus, we postulate that increased abundance results in increased body size that increases reproductive output. These findings have implications for understanding monogenean population growth and their effects on hosts.

More Than Meets the Eye: Genetic Analysis of Myxosporean Parasites of Amphibians Reveals Cryptic Diversity

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Once classified as protozoans, myxozoans are now recognized as highly specialized multicellular parasitic cnidarians. They are most commonly found infecting fish, but infections have been reported in other vertebrate hosts (amphibians, reptiles, birds, and mammals). The known myxozoan diversity in these other hosts is dwarfed by what is known in fish. A constant challenge in describing this biodiversity is the fact that morphological features of myxozoans are few, thus many reports are often attributed to the same parasite. Advances in DNA-based methods have revealed cryptic diversity in myxozoans of fish, and in this study, the diversity in amphibians in the Southeastern and Southcentral United States was investigated. Frogs and salamanders were collected from counties in Oklahoma and Arkansas. Both Cystodiscus and Chloromyxum species were regularly encountered infecting the gall bladder of several host species. Partial DNA sequences of the small and large subunit ribosomal RNA gene were obtained for each specimen. Samples categorized to a particular genus appear morphologically similar to one another, but genetic analysis revealed 4 distinct DNA
sequence types for *Cystodiscus* (syn. *Myxidium*), and 5 for *Chloromyxum*. The *Chloromyxum* species samples were all collected from salamanders (*Eurycea* spp.) and 4 of the genetic types likely represent variants of *Chloromyxum salamandrae*. The *Cystodiscus* species infect a wide range of hosts, but some differences were observed for specific genetic types. For example, *Cystodiscus* type B was only found in salamanders in Arkansas. Type A and type C were only found in frogs but were widespread. Type D likely represents *Cystodiscus melleni*, and was found infecting frogs and salamanders. These data reveal a diverse complex of myxozoan species or subspecies that differ in their host specificity and geographic range. As further studies investigate the potential impacts of these parasites on their amphibian hosts, this diversity must be considered because different species may have dramatically different effects on their hosts.

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**Morphological and molecular characterization of a new species and genus of turtle blood fluke (Platyhelminthes: Digenea: Schistosomatoidea) from the six-tubercled Amazon River turtle, *Podocnemis sextuberculata* (Pleurodira: Podocnemididae) in South America (Peru, Amazon River Basin).**

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Turtle blood flukes comprise 103 named species assigned to 22 accepted genera. Most of the named turtle blood flukes infect hidden necked turtles (Testudines: Cryptodira; 263 spp. distributed in the northern and southern hemisphere) but the side necked turtles (Pleurodira; 93 spp.; southern hemisphere only) remain vastly underexplored for infections. During a research expedition to the Amazon River (Peru) in 2016, we discovered infections in the heart of six-tubercled Amazon River turtles (*Podocnemis sextuberculata* [Pleurodira: Podocnemididae]) that represented a new species and a new genus. This record, combined with two other new species from another turtle in the region, collectively represent the first records of blood fluke infections in South American freshwater turtles. The new species resembles the other new sympatric species by having a dorsoventrally flattened, ovoid body, an oral sucker with anteroventral spines, two inter-cecal testes arranged in a column, inter-gonadal terminal genitalia, an inter-cecal post-ovarian Laurer’s canal pore, and Y-shaped excretory bladder. The new species has an anterior to posterior sequence of ventral sucker, anterior testis, cirrus sac, ovary, and posterior testis. It differs from all other nominal TBFs by having the combination of an aspinose body that lacks mammillae, ventral sucker, slightly M-shaped or U-shaped ceca, a deeply-lobed (dendritic) transverse ovary, and a transverse uterus. As predicted by morphology, a phylogenetic analysis of the nuclear large subunit ribosomal DNA (28S) revealed that South American freshwater turtle blood flukes are monophyletic and sister to several marine turtle blood flukes. The present study comprises the 8th species of turtle blood fluke reported from a hidden-necked turtle, the 3rd freshwater TBF species from South America, and the 1st blood fluke infection recorded from a member of Podocnemididae.

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**Morphological and molecular characterization of an unnamed Klossia species (Apicomplexa; Adeleidae) infecting pulmonate land snails, *Triodopsis hopetonensis* (Polygyridae), in Arkansas USA.**

Elizabeth G. Zeldenrust; Alexandre N. Leveille; John R. Barta
Klossia species (Apicomplexa; Eucoccidiorida; Adeleidae) are monoxenous apicomplexan parasites that have been described infecting the kidneys of molluscs. The type species, Klossia helicina Schneider 1875, was described from the White-lipped Snail Cepaea hortensis (Helicidae). It is the only Klossia species for which any sequence data was publicly available and these were nuclear (nu) 18S rDNA sequences; sequence data from the mitochondrial (mt) or plastid genome are not available. Klossia species have not been described in North American pulmonate land snails. Polygyrid land snails (Polygyridae) tentatively identified as Magnolia Three-Tooth snails, Triodopsis hopetoniensis, were collected in Arkansas USA. The kidneys of these snails were removed and examined microscopically for oocysts typical of Klossia spp. Two kidneys contained polysporocystic oocysts and sporocysts consistent with the genus Klossia. Oocysts measured ~85 um and contained dozens of sporocysts (~13 um in diameter) that contained 4 (rarely 8) sporozoites and a small residual body. Host and parasite DNA extracted from the tissues was combined with PCR primers specific for adeleorinid nu 18S rDNA to amplify portions of this nu gene; to avoid amplification of host DNA, one adeleorinid specific PCR primer was used in each of two PCR reactions to provide overlapping amplicons for sequencing. The nu 18S rDNA from this unnamed Klossia sp. from Arkansas (1800 bp; 42.9% GC) had <96% pairwise sequence identity with the existing K. helicina 18S rDNA sequences. In addition, the mt genome was sequenced and compared to the mt genomes of other adeleorinid coccidia; this is the first mitochondrial sequence data from any monoxenous adeleorinid parasite infecting invertebrates. The taxonomic (different families) and geographic isolation (different continents) of their snail hosts combined with pairwise identities of <96% of the nu 18S rDNA locus between K. helicina and the Klossia sp. observed in the Arkansas snails supports the conclusion that this is an undescribed species of Klossia.

NOVEL 4-AMINO-7-CHLOROQUINOLINE APPENDED [1,2,3]-TRIAZOLES CAN SIMULTANEOUSLY TARGET ASEXUAL STAGES AND GAMETOCYTES OF P. FALCIPARUM

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Drug resistance in malaria parasites and absence of safe and effective transmission-blocking drugs are the two major roadblocks which have severely halted the progress of malaria elimination campaigns. One way to combat these problems is to develop novel antimalarials which are safe and effective. The advantage of hybrid antimalarial molecules is their ability to surpass the threat of drug resistance due to the presence of multiple pharmacophores and hence multiple mechanism of action. In the present study, 4-amino-7-chloroquine appended [1,2,3]-triazoles hybrids are designed, synthesized and screened for their ability to simultaneously target asexual stages and sexual stages (gametocytes) of P. falciparum.

The study begins with development of a continuous in vitro culture of P. falciparum along with identification of gametocyte producing Indian field isolates for drug screening experiments. Further, a very simple and cost effective gametocytocidal-drug screening assay, also applicable to less affluent laboratory setups, was developed. Then, pharmacokinetic properties of the synthesized group of quinoline-triazole hybrids were predicted and their cytotoxicity evaluated. Further, their antiplasmodial potential was investigated against asexual and sexual stages of chloroquine sensitive and resistant P. falciparum. After optimizing the asexual stage culture and gametocyte production, two field isolates RKL-9 and JDP-8 were identified as high gametocyte producers and were deemed suitable to screen gametocytocidal drugs. Addition of [1,2,3]-triazoles to a 7-chloroquine nucleus resulted in compounds which demonstrated potency in nanomolar range against chloroquine sensitive P. falciparum with
three compounds appreciably more potent than chloroquine against chloroquine resistant field isolate (RKL-9) showing IC50 of $<100 \text{ nM}$. Further, the lead compounds proved to be gametocytocidal and were observed to be causing morphological deformations in stage V gametocytes which lead to their conversion into abnormal pyknotic forms. All compounds displayed attractive pharmacokinetic profile whilst majority demonstrated little or no cytotoxicity. This is the first study which presented two gametocyte producing lines of Indian origin which can be further used in anti-gametocyte drug discovery. Further, the most active quinoline-triazole hybrids represent promising candidates for further evaluation of their schizontocidal and gametocytocidal potential and may have the potential to be used as prototypes for the development of effective multi-stage antimalarials against P. falciparum.

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NTD Diagnosis: Importance of Isothermal Techniques

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World Health Organization (WHO) enlisted 17 Neglected Tropical Diseases (NTDs) mostly parasitic diseases account for almost one third of the world population and thousands every year for deaths and disability-adjusted life years (DALY). Of this list schistosomiasis is being considered as the deadliest NTD accounted for more than 200 million people and 90% of the cases occurring in sub-Saharan Africa. Also, Strongyloidiasis (>100 million people), hookworm infection (>1 billion people) and co-infection with malaria posing a serious threat to the most vulnerable population in the world. Global health challenges related with NTDs are becoming complex due to range of reasons – weak healthcare delivery systems, large population stuck in war, migration, increasing populations in developing countries with poor performing health systems, changing epidemiology just to name a few. The changing disease pattern and reduced infection prevalence due to mass drug administration (MDA) call for changes in strategies particularly in detecting low-level infection with isothermal approach, which can be adapted as a point-of-care (POC) test for resource-limited and improvised areas. This approach will address the issues, such as sensitivity, specificity, ease of use, and cost-effectiveness. Isothermal DNA amplification has been used for several major infectious diseases in recent years, such as Malaria, Tuberculosis, Ebola, Visceral Leishmaniasis, Schistosomiasis, Trypanosomiasis and Chagas. In addition, the scope of multiplexing and detecting multiple parasites from single sample such as, non-invasive urine sample via isothermal techniques makes them an ideal candidate for POC. Effective and appropriate diagnosis will correctly identify people those need treatment. Moreover it will reduce over and under treatment, which can ultimately reduce the drug inefficiencies and drug resistance in future.

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New records of Capsaloides cornutus (Verrill, 1875) Price, 1938 (Monogenoidea: Capsalidae) in gills of Istiophorid fishes from the Western Atlantic Ocean

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The Family Capsalidae (Platyhelminthes; Monogenoidea) includes about 200 species in 9 subfamilies that inhabit skin, gill, mouth, and olfactory chambers of marine, pelagic fishes. Capsalid hosts include advanced teleosts (e.g., scombrids and istiophorids), primitive teleosts (sturgeons) and elasmobranches (sharks and batoids). Capsaloides cornutus (Verrill, 1875) Price, 1938 is 1 of 36 species
in the Subfamily Capsalinae Baird, 1853, and 1 of 7 species in the genus Capsaloides Price, 1938. Capsaloides spp. infect gills of billfishes (Suborder Xiphiodei) in its 2 families: Istiophoridae (marlins, spearfishes and sailfish) and Xiphiidae (1 species, the swordfish). Known host records for Capsaloides spp. are: black marlin (Istiompax indica), Pacific sailfish (Istiophorus platypterus), striped marlin (Kajikia audax), Pacific blue marlin (Makaira nigricans), shortbill spearfish (Tetrapturus angustirostris), Mediterranean spearfish (T. belone), longbill spearfish (T. pfluegeri) and swordfish (Xiphias gladius). Records of C. cornutus are scarce. The 1875 species description (Tristoma cornutum Verrill, sp. nov.) named the type host “bill-fish (Tetrapturus albidus)” and did not provide a drawing. Verrill (1885) provided a nebulous drawing. Fifty-four years later, Price examined Verrill’s type specimen, and other specimens deposited in the USNPC and obtained from K. albida, and produced a species redescription and drawing for C. cornutus, still lacking in detail. We examined gills of white marlin (Kajikia albida), Atlantic blue marlin (M. nigricans), and roundscale spearfish (T. georgii) caught in the Atlantic Ocean and landed in Ocean City, Maryland for capsalines and all except blue marlin were infected with C. cornutus. Specimens were heat-killed, stained in hematoxylin, cleared in clove oil and mounted in Canada balsam, and detailed drawings and photomicrographs were produced. Tetrapturus georgii represents a new host record, and K albida represents a new geographic record. Our drawings and photomicrographs reveal new anatomical details as well as egg morphology.

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Occurrence of turtle Neoechinorhynchus species (phylum: Acanthocephala) in ostracod intermediate and snail paratenic hosts

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Although little information exists on the role of invertebrate intermediate and paratenic hosts in the life cycles of acanthocephalans, the North American turtle acanthocephalan, Neoechinorhynchus emydis, has been reported from freshwater ostracod intermediate and snail paratenic hosts. However, few studies have examined ostracods and snails for acanthocephalan infections. In this study, two species of freshwater snails, Helisoma trivolvis and Physa acuta, were examined for acanthocephalan infections from 23 wetlands and streams throughout northcentral Oklahoma. Additionally, freshwater ostracod intermediate hosts were examined for acanthocephalans at 2 locations. The complete Internal Transcribed Spacer (ITS) region of nuclear rDNA was then sequenced from juvenile acanthocephalans from ostracods and snails and adults of 5 species of Neoechinorhynchus from turtle definitive hosts. Of the 23 locations sampled, 7 (30%) had at least 1 snail infected with juvenile acanthocephalans, with prevalence ranging from 5 – 70% and intensity as high as 56 worms per snail. In contrast, prevalence in ostracod intermediate hosts ranged from 0.05 – 0.2% with a mean intensity of 1 among the 2 locations. The ITS sequences of juvenile acanthocephalans recovered from snail hosts were identical to ITS sequences generated from adult N. emydis from turtle hosts. In contrast, the ITS sequences of 2 juvenile acanthocephalans from ostracods were identical to N. emydis, as well as N. pseudemydis from turtles. These results suggest that snails play an important role in the transmission of N. emydis to turtle definitive hosts, whereas N. pseudemydis appears not to utilize snail paratenic hosts. Additionally, this study provides baseline ITS rDNA sequence data to serve as a genetic barcode for acanthocephalans from ostracod, snail, and turtle hosts, which can help with the identification of potentially new intermediate and paratenic hosts in the life cycles of acanthocephalans.

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Opportunities in Clinical Parasitology

Blaine Mathison¹
There are several interesting opportunities in the field of Clinical Parasitology for individuals with a Bachelor’s degree in Microbiology and/or Medical Laboratory Science. Mr. Mathison will discuss the different pathways to becoming a specialist in Parasitology in a clinical microbiology laboratory. Mr. Mathison will also discuss ways microbiologists and medical technologists can expand and improve their knowledge in Parasitology and contribute to the field. Mr. Mathison has been working in Parasitology for about 22 years, including managing the CDC’s DPDx website for 9 years. He has published extensively on the subject and has taught all education levels from MLS students to practicing technologists, and from fellows and residents to practicing physicians. He will talk about the typical day at the bench in clinical parasitology and the opportunities for contributing to education, research and patient care.

Ova and Parasite Exam Positivity at a Large Reference Laboratory

Despite the increasing use of molecular test methods in the clinical parasitology laboratory, microscopic examination of stool specimens using the ova and parasite (O&P) exam remains the gold standard for detection of most intestinal parasites. The O&P exam utilizes microscopic examination of both concentrated and unconcentrated stool specimens to detect protozoa, helminth eggs, and nematode larvae. The aim of this retrospective study was to determine the type and number of parasites detected by stool O&P exam during January-December 2018 at Mayo Clinic Laboratories (MCL), a large national reference laboratory. We also sought to determine if seasonal trends were present for specific parasites. A total of 33,841 specimens were tested during the study period, of which 1756 (5.2%) were positive. The majority of parasites were protozoa, with only 76 specimens (0.2%) containing helminth eggs or larvae. The most common intestinal parasites detected were Blastocystis hominis (586), Endolimax nana (261), Giardia duodenalis (178) and Entamoeba coli (140). Several parasites had notable seasonal peaks; Giardia duodenalis was detected throughout the year (mean 14 cases/month) but experienced a significant peak (up to 26 cases/month) in the summer and fall, while Cryptosporidium cases increased from 4 cases/month, on average, to 17 cases in July. These parasites are endemic to the United States and are commonly acquired through ingestion of contaminated food and water. Detection of the non-endemic protozoan parasite, Cyclospora cayetanensis, increased dramatically in June and July (34 and 36 cases/month, respectively) compared with other months (≤2 cases/month), which corresponded with two nation-wide outbreaks associated with imported produce. Lastly, Entamoeba histolytica/dispar (average of 5 cases/month) rose to 19 cases in August, indicating a possible localized outbreak. These findings correlate with available recent national data and provide important information regarding the expected range of parasites identified through the year.
Vector control is an integral part of the global strategy for management of mosquito-borne diseases and insecticide application is the most crucial part of this strategy, the development and spread of insecticides resistance is a major concern in mosquito control. The effect of piperonyl-butoxide (PBO) synergist on DDT and pyrethriods resistant Anopheles gambiae s.l., Culex quinquefasciatus and Aedes aegypti in Lekki, Lagos State was evaluated. Larval of Anopheles, Aedes and Culex mosquitoes collected from different breeding sites in Lekki were reared and 2-3 days old adults were subjected to susceptibility assays using WHO kits, test papers and procedure. Batches of 20 adult mosquitoes were exposed simultaneously to DDT (4%) and permethrin (0.75%) alone and then pre-exposed to PBO (4%) for 1 hour before exposing to permethrin and DDT, identification was done by morphological differentiation only. The knock down rate after 60min and mortality at 24hr were recorded. Resistance to DDT was recorded with percentage mortalities of 55%, 60% and 87.5% for Anopheles gambiae s.l., Culex quinquefasciatus and Aedes aegypti species. Pyrethriods resistance was also recorded across the three mosquito species with an average of 74% in all the three species. Pre-exposure of mosquitoes to PBO significantly (P<0.05) suppressed resistance to both DDT and permethrin at an average of 96% and 88% respectively in the mosquito species. The results indicate that metabolic enzymes are highly involved in the resistant mechanism observed in this area. Therefore PBO should be incorporated in insecticide resistance management strategies in the area and for the effective control of mosquito populations.

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PLANNING FOR YOUR FUTURE: THINGS YOU CAN DO AS A STUDENT TO PREPARE FOR YOUR CAREER IN PARASITOLOGY

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Do you clam up when people ask you what you are going to do with that career in parasitology? Did you think parasites were super cool, but you never gave a thought as to how you could use them in your future? I want to give you some tips on how you can prepare for your future career in parasitology and in the process make yourself a more competitive applicant. I will discuss ways in which you can build your curriculum vitae during your time as a student using a scaffolding strategy. These strategies will be a preface to the myriad of parasitology careers you will hear about during our student symposium.

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Parasite Persistence: Horsehair Worm [Nematomorpha] Prevalence and Intensity in Eastern Nebraska Twenty Years Later

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Parasites with complicated life cycles may not persist in rapidly changing environments. Horsehair worms [Nematomorpha] have complicated life cycles that consist of two arthropod hosts, one aquatic and one terrestrial, as well as a short aquatic free-living adult period. They also encyst in aquatic snails, which infect easily because they lack internal defenses. Thus, we can survey horsehair worm prevalence at various aquatic sites by counting cysts found in aquatic snails. Because of their complicated life cycles, we predict that the presence of horsehair worm cysts will serve as an indicator of water quality. To test this, we expand upon a 2001 study to determine the prevalence and intensity of horsehair worm cysts in 20 snails collected from 50 sites, varying in water quality. Each snail is examined for cysts following the original study’s methods. A YSI probe collects data on the temperature, pH, dissolved oxygen, nitrate, and salinity of each site. Current data is compared to 2001 data and correlated with water quality data. Preliminary data from one site (Site 28) yielded a prevalence of 0.9 and mean intensity of 39.95, which compares to the 2001 study that yielded a prevalence of 1.0 and mean intensity of 115.2. Based on this preliminary data, we predict a general decrease in horsehair worm prevalence and intensity at sites with poor water quality. Our results will help assess the feasibility of using horsehair worms as indicators of water quality. It will also help assess the resilience of this host-parasite system to survive environmental change.

Parasites of Cockroaches in Two Selected Hospitals and Hostels in Lafia and Doma Local Government Areas of Nasarawa State, Nigeria.

Parasite communities in Gambusia affinis and Cyprinella lutrensis

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Parasites with complex life cycles depend on different host species and often trophic transmission to complete their life cycles. Therefore, parasite communities can vary between similar hosts depending on host ecology and habitat use. Gambusia affinis (western mosquitofish) are commonly found in shallow waters on the banks of rivers and streams. Although Cyprinella lutrensis (red shiner) are habitat generalists, they are usually found in shallow pools located in the middle of streams and rivers. Differences in habitats can affect interactions with other community species, which are potentially hosts of different parasites. The objective of this study was to examine the parasite communities of G. affinis and C. lutrensis in the Colorado River at Timberlake Biological Field Station. Due to the variation in ecology and habitat, we predicted G. affinis and C. lutrensis to support different parasite communities. Twenty G. affinis and seventeen C. lutrensis were collected using seining and dip netting in the Colorado River at Timberlake Biological Field Station. Fish were necropsied and all tissues were searched for parasites. Prevalence and abundance were calculated, and component community composition was analyzed. Community structure had significant overlap with Posthodiplostomum spp., Rhabdachona canadensis, and Schyzocotyle acheilognathi found in both species of fish. Differences in community structure are likely driven by greater prevalence of R. canadensis in C. lutrensis than G. affinis. Further, the anchor worm, Lernaea cyprinacea, and an unknown nematode were only found in C. lutrensis. For all other parasite species, there was no difference in prevalence between host species. Further, there was no difference between hosts in abundance of any parasite species. Although these two fish hosts shared a few parasite taxa, the differences found may be due to host ecology and/or host specificity.
Cockroaches are one of the ubiquitous insects around human habitations. Their filthy habits make them potential carriers of pathogens. Cockroaches from two selected hospitals and hostels were handpicked between 7pm – 11 pm and parasites from the external were obtained by washing each insect in normal saline, centrifuged and the sediments stained with Lugol’s iodine. Each insect was then dissected and intestinal parasites sought for. A total of 213 cockroaches were collected of which 174 (81.69%) were infected and infection rate was significant (χ²=85.5634, df=1, P<0.0001). Also 132 were picked from hospital wards with 87.87% of them infected while 81 were picked from hostels out of which 71.60% were infected. There was no significant difference in the rate of infection from the two areas (χ² =7.511, df=3, P=0.05728). Cockroaches were most abundant in toilets 40.85%, then female wards 25.35%, male wards 23.0% and 10.80% in children’s wards with no significant difference, (χ²= 18.2926, df=3, P=0.003828). Infection rate was highest in cockroaches from toilets 87.88%, and least from male hostels 71.60% of the infected with significant difference in parasite infestation (χ²= 38.92.49, df =3, P<0.0001). In single infection, *Ascaris lumbricoides* was most prevalent at 53.33%, *An- cylostoma duodenale* and *Enterobius vermicularis* were least with 6.67% each. Of the single infection 88.24% were nematodes and 11.76% cestodes. Protozoa were found alongside other parasites only in multiple infections. Other frequently encountered co-parasitizing organisms included *Hammer-schmidtiella diesingi, Strongyloides stercoralis, Taenia species* and *Trichuris trichiura*. These infections were mostly internal and were significant. Therefore harboring internal and external parasites by cockroaches is a risk and of public health importance to humans who are exposed to their presence. Control measures are therefore needed to be promoted to curb the presence and contact to cockroaches in places of human activities to minimize infection which may not be documented.
mississippiensis, wild-caught spotted gar *Lepisosteus oculatus* collected in Louisiana were subjected to parasitological examination. Gar were dispatched at Nicholls State University and transported back to MSU CVM for full parasitological examination, yielding nymphs and metacercariae of alligator parasites as well as adult parasites of spotted gar. Comparison of mitochondrial cox1 sequence data indicate that these are the nymphs and metacercariae of *S. mississippiensis* and *A. coranarium*, respectively providing a link between the predator (*A. mississippiensis*) and their prey (*L. oculatus*). Data generated from these studies will contributed to our understanding of the systematics and life cycles of these understudied parasite groups from alligators and spotted gar and trophic linkages within the aquatic ecosystems they occupy.

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**Parasites of the Burbot, Lota lota, from Green Bay of Lake Michigan, with a description of Eubothrium sp. (Cestoda: Amphicotylidae) from this fish host**

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In the summer (May – August) of 2017 and 2018, 28 adult Burbot, *Lota lota*, (TL 393 – 815 mm) from Green Bay of Lake Michigan were examined for parasites. Six species of helminth parasites were identified: 2 cestodes (juvenile and adult *Eubothrium* sp., and immature *Proteocephalus* sp.), 1 nematode (1 encapsulated *Contracaecum* sp.), and 3 species of acanthocephalans, *Acanthocephalus dirus*, *Neoechinorhynchus saginatus* and *Neoechinorhynchus tumidus*. Partial 28S rDNA sequences of the acanthocephalans were obtained from hologenophores, and confirmed the identifications of and *A. dirus* and *N. saginatus*. This appears to be the first report of *N. tumidus* from Burbot. A study of the *Eubothrium* species in Burbot using stained whole-mounted specimens, Scanning Electron Microscopy (SEM), histology, and molecular data from the 18S and ITS-2 regions of the rRNA gene array, and cox-1 gene, suggests that it is a ‘new’ species different from *E. rugosum* of Eurasian Burbot. Museum specimens of "*Eubothrium rugosum*" from Burbot in North America are also of this new species.

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**Parasites of the peamouth, Mylocheilus caurinus (Cyprinidae) and the Olympic mudminnow Novumbra hubbsi (Umbridae) from Oregon and Washington**

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We report on the parasites of two species of freshwater fishes, the peamouth *Mylocheilus caurinus* (Cyprinidae) and Olympic mudminnow *Novumbra hubbsi* (Umbridae) from the western coastal drainages of North America (Oregon and Washington). A sample of 8 adult peamouth, collected from near the confluence of the Willamette and Columbia Rivers, yielded 9 species of parasites. These included the monogeneans *Octomacrum* sp. and *Dactylogyrus* sp. from the gills, the caryophyllidean cestode *Edlintonia* (cf *pychocheila*) and the trematode *Plagioporus* sp. (Opecoelidae) from the intestines, metacercariae of *Diplostomum* sp. from the eyes, *Posthodiplostomum* sp. from the viscera.
and of unidentified heterophyids (Heterophyidae) from the gills, and two species of Myxobolus (Myxozoa) from the gills and musculature, respectively. Morphological and molecular data (28S rDNA sequences) from Plagioporus sp. indicate that it is a species new to science. Two species of parasites were found in 12 Olympic mudminnow from Olympic National Park; an unidentified monogenean on the gills and the hemiuroid trematode Deropegus sp. (Derogenidae) in the stomach. Deropegus sp. was compared, using morphology and molecular data (28S rDNA sequences), with Deropegus aspina collected from cutthroat trout, Oncorhynchus clarkii (Salmonidae), and found to be the same species. However, the seminal vesicle and pars prostatica of Deropegus aspina are enclosed in a common sac, which contradicts a key feature of its generic diagnosis.

Parasitism influences producers and nutrients in mesocosm ecosystems

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Parasitism is increasingly recognized as an important component in ecosystem function, such as nutrient cycling. Despite a number of theoretical reviews on the subject, empirical studies supporting a role for parasites at the ecosystem scale are sparse. Yet, the indirect influence of parasites on host behavior, physiology, and mortality has been known for some time. We explored host-parasite-nutrient interactions using mesocosm "ecosystems". We seeded 150 liter mesocosms with local sediment, algae, zooplankton and snail communities. After establishment, uninfected and echinostome-infected Helisoma spp. snails were used to create a gradient in parasitism (0%, 40%, and 100% infected Helisoma) across mesocosms. Increasing infection prevalence was positively correlated with the biomass of Wolffiella gladiata, a regionally rare floating aquatic plant. Additionally, periphyton ash-free dry mass (a measure of periphyton nutrient content) was significantly higher at 40% Helisoma infection prevalence than in 0% control treatments. Interestingly, Helisoma mortality was significantly lower in 40% treatments compared to 0% control treatments. We suggest that greater ash-free dry mass associated with parasitism may increase forage quality, prolonging the host lifespan, and generating a positive feedback on infection. These results suggest that parasitism likely has an underappreciated role in ecosystem functioning and warrants further exploration.

Plumage coloration predicts haemosporidian infection incidence in birds

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Plumage coloration in birds can be determined by exogenously sourced pigments such as carotenoids. Because carotenoids come from the diet, carotenoid-based plumage is an honest signal of an individual's ability to forage and compete for carotenoid-containing resources. Carotenoids also play a role in mounting immune response and neutralizing the by-products of cytotoxic activity. We determined whether colorimetric descriptors of carotenoid-based plumage predict infection incidence with malarial parasites. We captured birds in an area of sensu strictu Cerrado, in southeastern Brazil, and used PCR to molecularly detect haemosporidian infections. We collected five feathers from the region below the furcula of each individual to retrieve plumage coloration descriptors (saturation, carotenoid chroma, hue and the maximum reflectance at the ultraviolet spectrum). Assuming that colorimetric variables can predict infection status, we tested the hypothesis that decreased foraging capacity measured by an individual's body condition would be predicted by plumage color, and would be associated to higher likelihood of infection incidence. We used the scaled mass index as a proxy of body condition. We analyzed feathers of 67 individuals from three bird species: lesser elaenias, flavescent warblers and red-pileated finches. Color saturation and carotenoid chroma predicted the individual incidence of infection with malarial parasites. Body condition was not related to any of the colorimetric variables. We suggest that infected individuals may trade carotenoid investment between the immune system and plumage coloration, as carotenoid chroma was negatively associated with parasite incidence. We hypothesize that infected individuals have diminished foraging capacity than uninfected individuals, since we found that less color saturated individuals were more likely to be infected with haemosporidian parasites. Our results show that haemosporidian parasites are important in shaping physiological trade-offs in natural bird populations, with potential consequences for sexual selection and reproductive fitness.

Polystome (Polystomatidae: Monogenea) diversity in American freshwater turtles

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With less than 200 species reported, the Polystomatidae includes 26 genera that parasitize semi-aquatic vertebrates, such as the Australian lungfish, amphibians, freshwater turtles and the common hippopotamus. Polystomes are globally distributed within frogs and chelonians. Whereas they are recovered mostly from the bladder of amphibian hosts, they can be found in the bladder, pharyngeal cavity or conjunctival sacs of chelonian hosts. Because of limited morphological variation, numerous polystome species in the past, particularly within amphibians, were described solely on host identity. Some species were regarded as host-generalists, particularly within American chelonians. Together, this raised the question as to the validity of several polystome species, whether in amphibians or turtles.

Because of the great diversity of polystomes in the USA and the lack of taxonomic studies, our first objective was to revise the systematics of American chelonian polystomes. We used COI and 28S as DNA barcodes, which provide an invaluable tool in comparison to morphological characters that are usually not conclusive for species delimitations. The second objective of our study was to study the role of the red-eared slider (Trachemys scripta elegans) in polystome dissemination across American wetlands. This species, which is regarded as one of the worst global invasive species, was shown to act as a vector of parasites for freshwater turtles across European aquatic ecosystems.

With increasing difficulty obtaining permits to collect and dissect freshwater turtles and ethical issues regarding the methods used for euthanasia of turtles, it is becoming increasingly difficult to obtain parasite specimens. In an attempt to reduce the number of turtles euthanized, DNA was
extracted from parasite eggs harvested from live turtles, but also from parasites recovered from road-killed turtles (eye, bladder and/or pharyngeal cavity) or from live turtles using cotton swabs (pharyngeal cavity).

Fieldwork was conducted in July 2015 in New York state, Connecticut, and North Carolina and in July 2018 in North Carolina and Florida. Based on molecular markers, at least twelve polystomes were identified from distinct host species/subspecies, namely Apalone ferox, Chelydra serpentina, Chrysemys picta marginata, Kinosternon baurii, Pseudemys concinna, P. floridana, P. nelsoni, P. peninsularis, P. rubriventris, T. s. elegans and T. s. scripta. Among these parasites, five polystome species are likely new to science. Finally, T. s. elegans was the single host species to harbour more than three non-specific species of polystomes suggesting Trachemys may act as a reservoir for numerous American polystomes. We therefore discuss the need to revise polystome classification and the risks of parasite dissemination posed by translocation of T. s. elegans in the US and potentially around the world.

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Progenetic larval forms parasitic in fresh water crabs with a simple hypothesis to explain their existence

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Trematode metacercariae usually possess well developed gastrointestinal tracts, nervous and excretory systems but no reproductive primordia. The development of precocious life cycles in the trematode genus Alloglossidium, is described as a non-trivial evolutionary event. Alloglossidium in fresh water fishes, cray fish, shrimp and leeches is known for precocious development. Fully developed nematode, Rhabdochona cascadilla (spiruroidae) has been reported in nymphs of the mayfly Hexagenia. The author found two progenetic lecithodendrid metacercariae and a nematode (Rhabdochona praecox) in fresh water crabs (FWC). R praecox has mature eggs while in FWC. Of the two metacercariae, Pleurogenoides sitapurii has eggs close to maturity while in the fresh water crab. Excysted metacercariae developed mature eggs in saline at room temperature in 48 hours. The other progenetic metacercaria was not fully identified. Frogs (Rana hexadactyla) are the final hosts of P. sitapurii. The final hosts of Rhabdochona spp. are small freshwater fish. R praecox was found in small streams and collections of water with no room for larger fish. Both hosts are incapable of consuming a fully grown FWC with a hardened chitinous shell. If the crabs are consumed while they are small, the larval forms could mature in the final host. Otherwise, the larvae remain in FWC during their entire life span, resulting in maturation in the intermediate host. Other metacercariae found in FWC, Paragonimus westermani, P. siamensis and P. macrorchis produced eggs in 6 weeks in experimental hosts. However, most trematode metacercariae mature in a few days. The life span of FWC in aquariums is approximately 2 years. The persistence of larval forms in the intermediate host for a prolonged period may result in further maturation of larvae while in FWC. This hypothesis may not explain the situation in Alloglossidium or R. cascadilla, but provides an explanation for the precocious development of R. praecox and P. sitapurii.

Progression of intestinal permeability during Eimeria maxima infection

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Poultry coccidiosis, caused by protozoa of the genus Eimeria, is an important disease affecting productivity. The parasite invades the intestinal mucosa, induces villus damage, crypt dilation and disruption of the intestinal barrier, resulting in watery diarrhea, impaired nutrient utilization, weight loss, poor feed conversion and mortality. However, the dynamics of disruption of gut epithelial during Eimeria infection requires detailed investigation. The objective of this study is to elucidate the dynamics of the intestinal barrier and parasite development of a pure single oocyst isolate of E. maxima in commercial broilers. One hundred and ten 14-days old Ross708 broiler chickens were infected via gavage with 2x10^5 Eimeria (E.) maxima sporulated oocysts suspended in water. Another 110 chickens were mock infected with water (control). Five birds each from the infected and control groups were sampled daily for 10 days. On each day, the birds were gavaged with 2.2 mg/mL of fluorescein isothiocyanate dextran (FITC-d) for the assessment of intestinal permeability. Intestinal tissue samples, fecal oocyst shedding, lesion scores and weight gain data were collected. Data were analyzed using two-way-ANOVA at the 5% significance. Multiple comparisons were corrected using Bonferroni’s method. Intestinal integrity was severely reduced at 5 and 6-days post infection (dpi) as compared to the control groups (P<0.0001), while oocyst shedding assessed by oocyst per gram of feces were significantly increased at 6 (15753 ± 9083) and 7 dpi (64068 ± 38635) when compared to the controls (0; P ≤ 0.0006). Macroscopic intestinal lesions were significantly higher from 4 to 7 dpi (P ≤ 0.016), while microscopical lesions were significantly higher from 2 to 7 dpi (P ≤ 0.016) as well as 10 dpi (P ≤ 0.016). The data indicate that damage to the intestinal barrier, starts at 5 dpi, prior to oocyst shedding and at 7 dpi the gut show signs of repair. Management of chickens infected with E. maxima is critical at 5-7 dpi to limit other opportunistic infections leading to necrotic enteritis.

Rapid experimental evolution of reproductive parasite isolation from a single parasite population

Sarah Bush None; Sarah Villa None; Dale Clayton None

Ecological speciation occurs when local adaptation generates reproductive isolation as a by-product of natural selection. Although ecological speciation is a fundamental source of diversification, the mechanistic link between natural selection and reproductive isolation remains poorly understood, especially in natural populations. Here we show that experimental evolution of parasite body size over four years (ca. 60 generations) leads to rapid reproductive isolation in natural populations of feather lice on birds. When lice are transferred to pigeons of different sizes they rapidly evolve differences in body size that are correlated with host size. These size differences trigger mechanical mating isolation between lice that are locally adapted to the different sized hosts. Size differences among lice also influence the outcome of competition between males for access to females. Thus, body size directly mediates reproductive isolation through its influence on both inter-sexual compatibility and intra-sexual competition. Our results confirm that divergent natural selection acting on a single phenotypic trait can cause reproductive isolation to emerge from a single natural population in real time.

Arthropods and their Pathogens Symposium / 286

Re-visiting the potential for transmission of human pathogens by bed bugs

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Bed bugs are resurging pests and human ectoparasites that are now common in diverse environments
across the globe. While the clinical consequences of bed bug bites are well-documented, their ability to vector human pathogens is still not fully clear. To date, no case of transmission from bed bug to human in a natural setting has been conclusively documented, and it appears unlikely that these insects are major vectors of any known infectious agents. No less, a number of laboratory studies demonstrate that bed bugs are biologically-competent vectors of several important vector-borne pathogens. Further, several instances of transmission in natural settings have been speculated based on pathogen detection studies. Given the ongoing spread of bed bugs, stochastic nature of pathogen emergence, and increasing globalization of human society, understanding what pathogens bed bugs may be able to transmit, and the contexts in which they may do so, may be critical to proactively preventing bed bug-borne infections. Here, historical evidence and recent studies on the role of bed bugs as pathogen vectors will be highlighted. In addition, results from our own ongoing pathogen screens and vector competence tests in these insects will be discussed.

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Regulation of Glycogen Content in the Parasitic Protist Trichomonas vaginalis

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Trichomonas vaginalis is the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection (STI) in the world. Trichomoniasis is easily cured with oral nitroimidazole antibiotic therapy, though resistant strains of T. vaginalis are emerging. Additionally, treatment is not always tolerated well by some patients. Like all parasitic organisms, Trichomonas is dependent on the host for many of its nutritional needs. While the microenvironment of the vaginal epithelium is rich in carbohydrates, the availability of nutrients in the male urethra is not well understood. Previous studies have demonstrated that in culture, T. vaginalis accumulates large amounts of glycogen. It is thought that these glycogen stores may support parasite survival during periods of nutrient limitation. Here we demonstrate this glycogen content varies significantly in response to changes in carbohydrate availability. Such changes in carbohydrate availability are likely to be experienced by the parasite during transmission between hosts. Using a carbohydrate starvation and replenishment protocol to simulate changes in nutrient availability during parasite transmission, we are evaluating changes in glycogen content and the activity of enzymes involved in glycogen synthesis and degradation. Currently our laboratory has measured the activity of two different enzymes involved in the synthesis of glycogen, glycogen synthase and UDP-glucose pyrophosphorylase (UDPGase). We have demonstrated significant changes in glycogen content in response to carbohydrate availability but have so far not detected changes in glycogen synthase or UDPGase activity. Future studies will investigate additional enzymes and substrates involved in glycogen synthesis or degradation within T. vaginalis. Our hope is that these studies may provide additional insight into the metabolism of T. vaginalis, and lead to the development of additional treatment options for this common STI.

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Repeated reduction in parasite diversity in invasive populations of Xenopus laevis: a global experiment in enemy release

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The introduction of species to multiple continents creates natural experiments suited to the evaluation of ecological hypotheses. For the *Enemy Release Hypothesis* (ERH), which postulates that the success of invasive populations hinges upon release from the effects of their natural enemies, assessments of parasite loss during invasion across independent geographical replicates are scarce. This study is the first to assess the ERH for a globally invasive amphibian, *Xenopus laevis*, a successful invader on four continents with a well-described parasite fauna.

In this study, the metazoan parasite communities of *X. laevis* from 20 invasive and 27 native sites in five regions (southern Africa, California, Chile, France and Portugal) and three continents were compared. An overall pattern of reduced parasite diversity in invasive *X. laevis* was not yet countered by acquisition of novel parasites. Invasive *X. laevis* harboured impoverished parasite communities that were distinct from those of native *X. laevis* from undisturbed habitats. Conversely, parasite communities from native *X. laevis* from disturbed habitats were similar to those from the invasive range. Accompanying parasites were common in the native range, present in native regions historically linked to export and included both generalists with indirect and specialists with direct life cycles.

Our findings emphasise that parasite loss is characteristic of the invasion process of *X. laevis* and possibly contributes to its success as a global invader. The ERH is supported in terms of metazoan parasites as natural enemies, irrespective of the geographical origin, climatic conditions and invasion history of the host populations. This study also draws attention to parasites that co-invade with their hosts as invaders in their own right.

Ultimately, the results raise questions about the factors contributing to parasite loss in the native range where the host is a domestic exotic and pioneer species, as well as whether parasite loss truly plays a role in invasive potential.
Schistosoma mansoni antigen Sm-p80: prophylactic efficacy using TLR4 agonist vaccine adjuvant glucopyranosyl lipid A-Alum in murine and non-human primate models

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Sm-p80, the large subunit of Schistosoma mansoni calpain, is a leading candidate for a schistosomiasis vaccine. The prophylactic and antifecundity efficacy of Sm-p80 has been tested in three animal models (mouse, hamster and baboon) using a multitude of vaccine formulations and approaches. In our continual effort to enhance the vaccine efficacy, in this study, we have utilized the adjuvant, synthetic hexa-acylated lipid A derivative, glucopyranosyl lipid A (GLA) formulated in aluminum (GLA-Alum) with recombinant Sm-p80. The rSm-p80+GLA-Alum immunization regimen provided 33.33%–53.13% reduction in worm burden in the mouse model and 38% worm burden reduction in vaccinated baboons. Robust Sm-p80-specific immunoglobulin (Ig)G, IgG1, IgG2a and IgM responses were observed in all immunized animals. The rSm-p80+GLA-Alum coadministration induced a mix of T-helper (Th) cells (Th1, Th2 and Th17) responses as determined via the release of interleukin (IL)-2, IL-4, IL-18, IL-21, IL-22 and interferon-γ.

Seasonal occurrence of Neoechinorhynchus emydis (phylum: Acanthocephala) in the freshwater snail, Helisoma trivolvis

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Neoechinorhynchus emydis infects freshwater turtle definitive and ostracod intermediate hosts, and is the only acanthocephalan species reported to infect snails as potential paratenic hosts. Currently, no information is available on the seasonality of N. emydis in snail hosts. To address this, the seasonal distribution of acanthocephalans in the freshwater snail, Helisoma trivolvis, was examined from a single location in northcentral Oklahoma. Seasonally, prevalence of N. emydis in snails was highest (50%) during the summer and lowest (0%) during the winter. Snail shell diameter was smallest in the winter, suggesting that larger and/or older snails were dying during the winter. However, it was unclear whether the seasonal variation of acanthocephalan infections was a result of snail mortality due to snail age, additional parasite infections, or a combination of these factors. To control for some of these factors, additional field and laboratory experiments were implemented. First, laboratory-reared H. trivolvis snails were exposed to naturally infected ostracods in field cages. Second, a laboratory survival experiment was conducted by testing the life span of field-collected snails naturally infected with acanthocephalans and/or trematodes. Data from snail cage infections were consistent with the seasonal field survey, with N. emydis infections being highest in the summer (20%) and lowest (0%) in the winter, suggesting that snails were not ingesting infected ostracods during the winter. However, the fewest snails survived in field cages during the winter, suggesting that snails may die more often during the harsh winter conditions. Finally, the snail survival experiment indicated that snails co-infected with trematodes and acanthocephalans died at a faster rate than snails only infected with acanthocephalans. Taken together, these results suggest that the occurrence of acanthocephalans in snails throughout the year may be partially influenced by the abundance of infected ostracods, co-infections with trematodes, and snail population fluctuations during the year.
Sex Ratios of Aulonocephalus pennula in Northern Bobwhites from Southern Texas

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Literature on nematode reproduction strategies suggests that sex ratios at the component population level should be female-biased at low prevalence and mean intensity and approach 1:1 as prevalence and mean intensity increase. However, reproduction occurs at the infrapopulation level. Sex ratios of a commonly occurring cecal nematode, \textit{Aulonocephalus pennula}, in northern bobwhites (\textit{Colinus virginianus}) were examined to learn more about sex ratios at the infrapopulation and component population level. We used a database containing 174 bobwhites (\textit{A. pennula} prevalence 85\%, mean intensity 73, range 1–745 individuals) collected during the 2016–2017 hunting season and 106 bobwhites (\textit{A. pennula} prevalence 81\%, mean intensity 127, range 1–603 individuals) from the 2017–2018 hunting season in southern Texas. \textit{Aulonocephalus pennula} individuals were partitioned into 5 groups: 5–30, 50–80, 100–150, 200–300, and 300+ worms, with five replicates each. Infrapopulation and component population sex ratios of \textit{A. pennula} were determined for each group. We report our results and discuss our findings on productivity and persistence of \textit{A. pennula}.

Something to ruminate about: Identifying genotypes and morphotypes of Eimeria species infecting sheep and goats

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Coccidiosis in small ruminants is caused by \textit{Eimeria} spp. (Apicomplexa, Eimeriidae) and is characterized by bloody diarrhea and, in severe cases, death. Individual \textit{Eimeria} spp. (~12 spp. infecting sheep; ~9 spp. infecting goats) vary in their ability to cause clinical disease; therefore, it is important to identify individual species. Traditionally \textit{Eimeria} spp. were identified using morphometrics of fecal oocysts but overlapping features and measurements make identification unreliable. Sequence-based genotyping using loci from the mitochondrial (mt) and nuclear (nu) genomes have been successful at distinguishing \textit{Eimeria} spp. infecting poultry and should be applicable to \textit{Eimeria} spp. of small ruminants. \textit{Eimeria} positive fecal samples from small ruminants were collected from various geographic locations. Morphometric data were recorded from ~50 oocysts per sample using light microscopy. Initial genotype data were collected by sequencing loci within the mt (i.e. COIII, COI) and nu genomes (i.e. 18S rDNA, ITS-1) using Sanger sequencing of PCR amplicons to identify samples containing a single \textit{Eimeria} spp. predominantly. For those "single-species" samples, whole mt genome and nu 18S rDNA sequences were generated. For mixed-species samples, next generation sequencing (NGS) of amplicons was used to estimate species diversity following PCR of mt or nu loci. We were able to link morphometric features used to describe these \textit{Eimeria} spp. with whole mt genome and nu 18S rDNA sequences to provide the data necessary to unequivocally identify individual \textit{Eimeria} spp. infecting small ruminants. With these morphotypes linked to specific genotypes, the multi-locus sequence data could then be used to estimate the phylogenetic relationships among these \textit{Eimeria} spp. using Bayesian inference (MrBayes). Morphometric data could then be mapped onto the resulting phylogenetic tree. Sequences from phylogenetically informative loci will support the creation of molecular assays to identify individual \textit{Eimeria} spp. for accurate diagnostics and application of selective anticoccidial treatment.
Spatio-Temporal variation of fish parasite communities from Perdido Region, Gulf of Mexico

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In view of the current intensity at which marine resources are being exploited in the Gulf of Mexico (GoM) (e.g. oil extraction, fisheries, etc.), there is an urgent need of bioindicators of environmental impact. In the Campeche Sound (southern GoM), the metazoan parasite communities of flatfishes have been used as bioindicators of environmental impact associated to oil extraction activities. However, the main limitation of these studies has been the lack of data on the temporal variation of the metrics of these parasite communities. As part of a larger program to determine the environmental health of the Mexican Gulf of Mexico, we performed 4 cruises in the Perdido region (P1-P4) (Northwest GoM) during which fish were collected by trawling on board of an oceanographic vessel at 25 and 500 m depth during 2016-2017 dry and rainy seasons. Our aim was to determine the spatio-temporal variation of the metrics of the parasite communities of benthic fishes from Perdido. All cruises visited the same 23 sampling sites at depths between 25 and 500 m. A total of 24,229 individuals belonging to 56 parasite species were collected from 22 fish species through P1-P4. Species richness and the mean number of parasite individuals per fish did not show significant differences through P1-P4. However, the identity of the numerically dominant species changed, since in P1 and P2 was the nematode Hysterotylacium reliquens, in P3 was the cestode Oncomelas wageneri and in P4 the cestode Tetraphyllidea gen. sp. The rare species composition of these parasite communities also changed substantially through cruises. The most relevant spatial pattern through P1-P4 was that of a higher number of species and individuals in sampling sites near to the coast. Concluding, our results reflect the relatively “natural” temporal fluctuation of the metrics of the parasite communities of benthic fish from Perdido, not exactly a pristine region as it is under chronic coastal environmental disturbance due to the Rio Grande discharge.

Species specific IgSF HMM profiles provide accurate predictions of anti-trematode FREPs in Biomphalaria glabrata

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Fibrinogen-related proteins (FREPs), composed of one or two N-terminal immunoglobulin superfamily (IgSF) domains and one C-terminal fibrinogen-related domain (FBG), have been found in hemocytes and plasma of the freshwater snail Biomphalaria glabrata. FREPs can both bind to trematode larvae and precipitate soluble trematode antigens. The automated identification of FREP sequences is a challenge because the successful identification rate of IgSF domains in invertebrate proteins is low with conventional protein domain prediction software. We propose a new way of identifying IgSFs in FREPs using the classic hidden Markov models (HMM) profile building process but starting with species-specific protein sequence alignments. The IgSF domain sequences were first extracted
from proteins sequences previously identified as *B. glabrata* FREPs, then clustered based on 30% identity. For each cluster, sequences were aligned to build an HMM profile. The new IgSF HMM profiles were combined with an FBG HMM profile to predict FREPs from annotated proteins. The traditional protein domain prediction programs showed poor performance recovering FREPs. Among all 55 manually confirmed FREP proteins, Gene3D, Superfamily, and SMART were able to recover 29, 3, and 2 FREPs, while the new method identified all of them. The number of IgSF domains of each FREP were predicted accurately when compared to a manual check. Two different IgSF HMM profiles (newIg1 and newIg2) were built from the new method. The order of the two IgSF in proteins is consistent with previous findings in that: 1) the newIg2 is at the N-terminus in dual-IG FREPs, and 2) all single-IG FREPs contain just the newIg1 domain. The new method offers an accurate way to identify IgSF domains in snail proteins, therefore the automated FREP's identification process became possible in large scale genome and transcriptome searches. The new model with further modification could be applied to search IgSF/FREPs among Mollusca or invertebrates more generally. Supported by NH grant R01 AI 101438 and P30GM110907.

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**Stratification Effect on Larvicidal Potential of Skimmia laureola Collected from Swat, Khyber Pakhtunkhwa, Pakistan**

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**ABSTRACT**

The present study is based upon the characterization and finding larvicidal potential of *Skimmia laureola* ethanolic extract collected from different altitudes i.e. 1200 and 800 ft at Swat named as SLA and SLB respectively. The plant extract was obtained through maceration technique in ethanol and stock solution was prepared in distilled water. Characterization was carried out through phytochemical screening, FTIR, UV-VS and GCMS analysis. The LC50 and LC90 values were calculated using probit analysis for different dilutions of plant extract, prepared from stock solution against *Culex quinquefasciatus*. Larvae of *C. quinquefasciatus* were collected from stagnant water situated in the stream side of Quaid-i-Azam University, separated the 1st and 3rd instar for experimental purpose kept in plastic containers with net covering. The bioassay was designed using concentrations 100 to 500 ppm, with a difference of 100 taking 25 larvae per container per concentration of both SLA and SLB. The observations were taken after every 12 hours for three days. Three replicates of each set were run parallel with control. The results revealed that SLA plant showed the high percentage mortality of 89.2% and 99.4% at 400 and 500 ppm respectively as compared to the percentage mortality of SLB 84.8% and 92.8% with same concentrations whereas no mortality was observed in control in the experimental days. The comparatively more SLA larvicidal efficacy may be due to the chemical difference in both plants extracts as shown by FTIR. The identified functional groups were hydroxy alcohol, alkanes, carbonyl group, nitro compounds, alcohols, carbonyl & ether and aliphatic fluoro-compounds under 19 peaks of different wavelengths in both SLA and SLB, while SLB showed 16 peaks of different wavelengths for functional groups. The phytochemical screening showed the presence of phenols, tannins, flavonoids, steroids, terpenoids, glycosides, carbohydrates and saponins in SLB, while SLA having all the phytocompounds excluding saponins and carbohydrates. The UV-VS (the strong peaks with maximum absorbance at 662.5nm with absorbance of 0.27 in SLA while SLB showed a strong peak at 664.65nm with absorbance of 0.8. The GCMS analysis of *S. laureola* revealed the identification of certain phytocompounds which were matched with the NIST library (National Institute of Standards and Technology). The GC-MS analysis showed the presence of 15 compounds in SLA while 17 in SLB. In GCMS analysis, some of the phytoconstituents screened were, Alpha-D-Glucopyranoside, Dictamine, Phytol, Skimmianin and Octadecanoic acid. The retention time and peak area percentage in mass spectra were interpreted and identified with the help of literature. It is concluded that *S. laureola* plants have larvicidal potential against *C. quinquefasciatus* whereas with the difference in altitudes the larvicidal potential varies. In the present study the difference of 400 ft in altitude, SLA collected from 1200 ft showed relatively more larvicidal potential as compared to SLB at 800 ft of altitude. With the increase in elevation of 400 ft, the chemical
composition of the plant *S. laureola* altered as detected by phytochemical screening, FTIR, UV-VS and GCMS analysis.

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**THE MAKING OF A SOLDIER: WHAT CAN NEOGREGARINES IN THE GENUS OPHRYOCYSTIS TELL US ABOUT THE EVOLUTIONARY HISTORY OF THEIR DANAID BUTTERFLY HOSTS?**

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The pathogenic neogregarine *Ophryocystis elektroscirrha* infects the hypodermal tissues of monarchs (*Danaus plexippus*) and queen butterflies (*D. gilippus*). Three transmission routes have been proposed for *O. elektroscirrha* and include horizontal transmission, maternal transmission, and sexual transmission. Caterpillars become infected when they ingest oocysts from milkweed leaves or egg cases after hatching. However, the diversity of these parasites in other butterfly species is not known. To evaluate this, we examined 40 species of milkweed butterflies from 9 genera collected on 5 continents; including all 11 *Danaus* species (monarchs, tigers, and queens) for *Ophryocystis* infections. Based on oocyst morphology and complete ITS rDNA sequences, only 4 species of milkweed butterflies in the genus *Danaus* and from 2 subgenera were infected with 3 lineages of *Ophryocystis*. Importantly, oocyst morphology and host pathology, was conserved within host clades but distinct among host clades. More interestingly, for maternally and sexually transmitted parasites, the distribution of *Ophryocystis* lineages on the *Danaus* phylogeny was peculiar. For example, *Ophryocystis* occurred in 3 of 4 species of queens (subgenus *Anosia*), 0 of 4 species of tigers (subgenus *Salatura*; the sister clade to queens) and 1 of 3 species of monarchs (subgenus *Danaus*). Because recent genomics studies suggest postspeciation gene flow between queens (*D. gilippus*) and monarchs (*D. plexippus*), and queens and their sister species the soldier (*D. eresimus*), we conducted crosses between monarchs and queens, and evaluated a mitochondrial gene (partial COI) for various subspecies of monarchs, queens and soldiers. Our results suggest that mitochondrial introgression between monarchs, queens and their hybrids resulted in multiple lineages of soldiers, some of which are more closely related to monarchs while others are more closely related to queens. The implications of our findings are discussed in terms of host switching events for sexually transmitted parasites and conservation efforts for monarch butterflies.

**THE OTHER OKLAHOMA LAND RUSH: FIELD AND EXPERIMENTAL OBSERVATIONS ON A NEW GORDIUS SP. (NEMATOMORPHA: GORDIIDAE) WITH THE FIRST DOCUMENTED TERRRESTRIAL LIFE CYCLE FOR THE PHYLUM**

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All gordiids have complex life cycles that use a terrestrial arthropod host but are considered aquatic in their free-living adult phase. However, we discovered a new Gordius sp. in Oklahoma which occurs in terrestrial habitats. To describe this new species, investigate their behavior in terrestrial systems, and understand their transmission strategies a total of 2202 male and 539 female adult free-living worms, and 996 earthworm paratenic hosts were collected from 20 sites in Payne Co., OK during 2014-2019. Field observations indicated that adult free-living worms emerged after heavy rains, and began mating on lawns, open fields, and road gutters. Additionally, large numbers of free-living worms were located entangled in grass roots, where some females were depositing egg strings. Finally, earthworms from locations where adult free-living worms were observed, indicated they were commonly infected with Gordius type cysts suggesting gordiid larvae were present in the soil. To test our field observations, we performed comparative laboratory assays on egg laying behavior of the new Gordius sp., Gordius difficilis, a burrowing aquatic gordiid, and the aquatic non-burrowing gordiid, Paragordius varius. When worms of all 3 species were placed on soil, all individuals of the aquatic P. varius died and dried up, 100% of G. difficilis females burrowed and 80% of the Gordius sp. females collected from terrestrial habitats burrowed within minutes into the soil. More importantly, some female Gordius sp. began depositing egg strings within days of burrowing under the soil. Examination of the eggs of the new Gordius sp. indicate they are unlike the eggs of any other hairworm species and contain double membranes suggesting these eggs may be resistant to desiccation. Finally, in the laboratory, when larvae of the new Gordius sp. were inoculated into soil, earthworms became infected with Gordius type cysts. Taken together, our observations and laboratory experiments strongly suggest that this species represents the first documented hairworm species with a terrestrial life cycle.

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**Taxonomy and host associations of lice (Phthiraptera) parasitizing lemurs**

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In recent years, we have analyzed and described several new species of sucking lice collected from lemurs in Madagascar. While the single known species of chewing louse known from lemurs, *Tri-chophilopterus babakotophilus*, shows little host specificity and parasitizes at least 6 species of larger lemurs, all sucking lice known to date from lemurs are highly host specific. Three described and 1 undescribed species in the sucking louse genus *Phthirpediculus* are each known to parasitize a single species of target lemur, and 5 described and 1 undescribed species of *Lemurpediculus* are each known to parasitize a single species of smaller lemur. Tracking individually tagged lemur lice through a wild lemur population has allowed host home ranges and contacts between host individuals to be inferred. Lemur lice are not known to transmit pathogens or parasites to their hosts. Because their hosts are endangered, these lice, and other indigenous lemur parasites, should warrant co-conservation status.

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**Testing parasites as potential biological indicators of wetland health through food web models**

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Wetland management and restoration efforts require tools for monitoring ecosystem conditions. The use of biological indicators has the potential to provide specific and reliable information of environmental stressors. Recently parasites have been proposed as potential biological indicators of community abundance, diversity and trophic (feeding) links. Our research objective was to use a combination of field surveys and food web analysis to contribute to the development of parasites as potential biological indicators of wetland health. For three summers (2014-2016), we measured the abundance, distribution, and diversity of aquatic and semi-aquatic organisms in two cattail marshes to create food webs of sites differing in anthropogenic impact. One site in northeastern Illinois is part of a nature preserve surrounded by restored upland habitat, while the second site in southeastern Wisconsin is in a mix of agricultural and suburban habitat. Importantly, our research included surveys of trematode and cestode parasites that are transmitted by consumption to their hosts. Therefore, we gain information about predator-prey interactions that may be difficult to directly observe or quantify from gut contents. The wetland in the more intact ecosystem had a total of 36 free-living and four parasite taxa connected by 615 interactions, while the wetland in the more disturbed habitat had 31 free-living and four parasite taxa connected by 389 interactions. The species interactions at the sites also differed in terms of how species connect to one another and the relative importance of species in interactions based on several standard metrics: graph density (0.78 vs. 0.65), alpha centrality (0.31 vs. -2.26), and betweenness centrality (16.12 vs. 15.22). Management implications include understanding the presence and the interactions of species contributing to stability and resilience to disturbance such as land use change and offering managers a novel tool for ecosystem assessment.

The Arms Race Between Host and Parasite: Primate Specific Serum Resistance in Zoonotic Trypanosomes and the fitness cost

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Innate resistance to African trypanosomes is due to specific complexes of proteins and lipids, trypanosome lytic factors (TLFs), which circulate in the blood and tissues spaces of Old World Monkeys and Great Apes. TLFs are high density lipoproteins (HDLs, the good cholesterol) that carry two primate specific proteins, haptoglobin related protein (receptor ligand) and apolipoprotein L1 (APOL1).

Upon endocytosis of TLFs by the African trypanosomes, APOL1 is released from TLF complexes within the acidic endosome/lysosome of the parasite. The APOL1 dimerizes and inserts into the membrane of the endo/lysosome, forming an ion channel. Depending on the primate source of the ion channel protein it will open (baboon) or remain closed (human and gorilla) within the acidic environment of the endo/lysosome. Recycling of the ion channel to the plasma membrane of the trypanosome and exposure to neutral pH of the extracellular milieu drives complete opening of the ion channel, allowing the selective flux of cations (K+, Na+, Ca2+) down their concentration gradients. Therefore, APOL1 is a pH gated cation selective channel, carried on HDLs synthesized by the liver in an inactive form in blood.

Baboon APOL1 kills all African trypanosomes, human APOL1 cannot. However, human variants of APOL1, termed G1 and G2 that can kill human infective trypanosomes, were selected in Africa 4000-10,000 years ago. Heterozygous or homozygous carriers of these variants are protected from human infective trypanosomes, however homozygous carriers have a 15-50% increased risk of kidney disease. We show that akin to the trypanocidal mechanism of action of APOL1, endogenous G1 or G2 APOL1 made by cells within the kidney, forms ion channels in the plasma membrane, leading to ion imbalance and cell death. In contrast, Baboon APOL1, in baboons or transgenic mice does not
cause kidney damage and kills all African trypanosomes, and thus forms the basis of our transgenic livestock project in Africa.

Keywords: trypanosome lytic factors, APOLIPOPROTEIN L1, ion channel, primates, transgenic, kidney

The Missing Host: Confirming Heptageniidae as the Aquatic Insect Host of Chordodes morgani [Nematomorpha]

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Horsehair worms [Nematomorpha] have complicated life cycles that require a terrestrial arthropod and an aquatic arthropod host. The life cycles for most hairworms remain unknown. In July of 2018 we failed to find cysts of Chordodes morgani in 10 different families of insects from sites known to harbor this horsehair worm. But we found 108 C. morgani cysts in three specimens of the flatheaded mayfly larvae (Heptageniidae), suggesting it serves as an aquatic host of C. morgani, which lays its eggs on sticks. To confirm this, we will collect sticks and their accompanying invertebrates from three sites near Lincoln, NE known to harbor C. morgani. We will note the presence of C. morgani eggs on each stick and place them in labeled zip lock bags. We will also measure (twice a week) the temperature, pH, dissolved oxygen, nitrate, and salinity of each site with a YSI probe. Atrazine strips will measure relative atrazine levels. The zip lock bags will be frozen at -80 C until the invertebrates can be identified in the lab. The invertebrates will then be crushed and examined microscopically for the presence of hairworm cysts. We predict that we will find the highest number of cysts in invertebrates that are mobile scrapers collected from sticks with eggs. To confirm that flatheaded mayfly larvae serve as the aquatic host, we will feed specimens containing cysts to lab-reared wood roaches. If the worms successfully develop in the wood roaches, then we have strong evidence that those species serve as the aquatic host in nature. We will also correlate the water chemistry data with the presence and absence of C. morgani at the three sites. Understanding the life cycle of this horsehair worm will allow researchers to rear the species in the lab, which could become a model for parasite research. Also, this hairworm could be used as an indicator for the quality of water. If the aquatic insect host(s) lives in clean water, then the presence of C. morgani would indicate high water quality.

The Role of the Laboratory Director in Clinical Parasitology

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The field of Clinical Parasitology offers exciting opportunities for individuals with a doctoral degree who are interested in contributing directly to patient care. Dr. Pritt will discuss the different pathways to becoming a laboratory director of a clinical microbiology laboratory and her view on the positive and negative aspects of this career path. Dr. Pritt is the laboratory director of the Clinical Parasitology laboratory at Mayo Clinic and is the director of the Mayo Clinical Microbiology Fellowship Program. She is also the medical director of the Mayo Clinic Medical Laboratory Science program and has directly mentored over 100 students in these fields. She will talk about the ‘typical’
day in clinical parasitology and the opportunities she has for contributing to education, research and patient care.

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The Unusual Suspects: Challenging Cases and the Role of the Clinical Parasitologist

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The broad range of parasites capable of infecting humans encompasses single-celled protozoa, multicellular helminths, and arthropod ectoparasites. While commonly-encountered parasites such as *Giardia duodenalis*, *Cryptosporidium species*, *Enterobius vermicularis*, *Pediculus humanus capitis*, and *Sarcoptes scabei* are covered in most medical laboratory science, medical school, infectious diseases fellowship and pathology residency curricula, less common but clinical relevant parasites and parasite mimics receive little to no coverage. The gap between the parasitology training provided to medical/laboratory professionals and the spectrum of clinically-relevant parasites that may be seen in clinical practice highlights the need for parasitology specialists in the clinical laboratory. Mayo Clinic Laboratories (MCL) is a high volume international reference laboratory that performs over 3 million microbiology tests each year, including >300,000 tests for human parasites. In addition to the 22 medical laboratory scientists trained in clinical parasitology, the parasitology laboratory has a dedicated technical specialist, education specialist, three clinical microbiology fellows and medical laboratory director, who are tasked with evaluating and identifying unusual parasites submitted from patients to MCL. A retrospective review of uncommon parasites identified at MCL during 2008 – 2018 identified five primary categories of unusual parasites which required specialist review: tissue parasites, zoonotic parasites, arthropods, pseudoparasites, and parasites found in histopathology preparations. Examples of each category and algorithms for identification are presented, along with relevant biology, epidemiology, and clinical significance.

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The effect of hybridization on parasite communities infecting sunfish (*Lepomis* spp.)

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Hybridization among members of the animal kingdom not only results in genetic recombination of 2 parental haplotypes, but also creates a new environment for parasites. Investigation into the effects of hybridization on parasite communities is largely unexplored, particularly regarding endohelminth infections. Sunfish (*Lepomis* spp.) are a common inhabitant of freshwater lakes and ponds in North America, which breed around the same time, creating hybrid zones, where genetically distinct populations produce sexually viable hybrids. The hybrid offspring have a new genetic combination of both parents, which may impact several aspects of fish health, including parasitism. This study investigated parasitism differences between hybrid sunfish and their parental species by sampling and necropsying *Lepomis* spp. from 2 ponds in northwestern Virginia. North Pond had populations of Bluegill sunfish (*L. macrochirus*), Green sunfish (*L. cyanellus*), and their hybrids, while Blackbird Pond contained *L. macrochirus*, Redear sunfish (*L. microlophus*), and their hybrids. The hybrid sunfish were infected by mostly generalist parasites that infect all *Lepomis* spp.
only harbored parasite species that were found in at least one of the parental hosts. In addition, hybrids typically had intermediate parasite abundance compared to their parental species, which may be beneficial to the hybrid sunfish by decreasing the disease burden compared to their more heavily infected parental species. *Lepomis macrochirus* and *L. cyanellus* had distinctly different parasite communities, resulting in greater parasite diversity in their hybrid offspring, while parasite assemblages did not differ between *L. macrochirus* and *L. microlophus*, leading to decreased parasite diversity in their hybrid progeny. These results suggest that the parasite assemblage and diversity in the hybrids is dependent on the similarity of the parasite communities between the parental species.

### The fruit fly as model host to explore the insect immune response to entomopathogenic nematodes

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Although significant progress has been made in the broad field of innate immunity, our understanding of the molecules and signaling pathways that are involved in the insect immune response to entomopathogenic (EPN) nematode infections remains incomplete. The fruit fly Drosophila melanogaster is a widely appreciated model organism for studying fundamental biological processes including innate immune signaling and function. We have established an infection model of Heterorhabditis bacteriophora in *D. melanogaster* to uncover the molecular determinants that direct host defense against EPN. We have performed RNA-sequencing transcriptomic analyses to identify the number and nature of genes in *D. melanogaster* that are differentially regulated in response to *H. bacteriophora* and its mutualistic bacteria Photorhabdus luminescens, separately or together. We have identified certain components in the TGF-beta signaling pathway that participate in the regulation of resistance towards the nematodes and their associated bacteria as well as the persistence of the parasites during infection. In addition, we have used gene expression and functional assays to elucidate the role of thioester-containing protein (Tep) genes Tep2 and Tep4 in the humoral and cellular response of *D. melanogaster* adult flies to *P. luminescens* challenge. Using this unique infection model and experimental approach we anticipate to expose the molecular players that dictate insect host response to EPN infection, which will potentially reveal orthologous mechanisms in mammals, perhaps including humans, leading to novel parasitic nematode control strategies.

### The life cycle of a new species of fish blood fluke (Digenea: Aporocotylidae) infecting variable coquina clams (Donax variabilis) and lesser electric rays (Narcine bancroftii) in the Gulf of Mexico.

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Fish blood flukes (Digenea: Aporocotylidae; FBFs) number 161 species that collectively infect freshwater, marine, and estuarine fishes, are occasional pathogens of cultured fishes, and are the ancestor to the human-pathogenic blood flukes (schistosomes) that debilitate >218 million people annually. The life cycles of FBFs include an invertebrate intermediate host (mollusk or polychaete) and a fish definitive host. A total of 18 life cycles for FBFs have been elucidated but none of those elucidated include a chondrichthyan definitive host. Our objective was to morphologically characterize a new species of FBF infecting a ray, search for larval infections among sympatric mollusks, histologically characterize infections in those mollusks, and use genetic sequence data to infer the life cycle of and reconstruct a phylogeny. From 2012–2017, the heart of 14 of 54 (26%) lesser electric rays, *Narcine bancroftii* (Narcinidae) from coastal Alabama were infected with adults of a new FBF species.
Adults of the new species resemble those of *Ogawaia glaucostegi*, which infect the giant shovelnose ray, *Glaucostegus typus* (Glaucostegidae) in the southwestern Pacific Ocean off Australia by having inverse U-shaped ceca, a looping testis, a post-cecal ovary, and ascending and descending portions of the uterus. It differs by having a vermiform body (vs. lancelet), a testis with 34 curves (vs. 52), a seminal vesicle that is 1/2 body width (vs. 1/4), and a uterus posterior to the testis and ovary. From 2017−2018, six of 1,174 (0.5%) variable coquina clams, *Donax variabilis* (Donacidae) were infected by a FBF comprising 4−6 cercariae within each sporocyst. This cercaria resembles *Cercaria asymmetrica*, which infects variable coquina clams from coastal Florida by having concentric rows of spines about the anterior sucker, tegumental spines, a dorsal finfold, and asymmetrical furcae without finfolds. Sequences of the large subunit rDNA (28S) from these adult and larval FBFs were identical, indicating conspecificity. This is the first documented life cycle for a FBF infecting a chondrichthyan.

The lionfish *Pterois volitans*, the invader invaded by local parasites: the case of *Lecithochirium*

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The lionfish *Pterois volitans* was brought from Asia and accidentally introduced into the American Atlantic coral reefs. This fish represents an important ecological risk because it preys on young native coral reef fish, but at the same time acquires native parasites. Among all the species of helminths reported for the lionfish from Mexican coral reefs, the generalist adult digenean *Lecithochirium floridense* has always been the most prevalent. In this study we investigate the genetic variability of *L. floridense* infecting the lionfish from Cozumel, in the Mexican Caribbean in relation with those collected from native fishes from other localities in the Yucatan Peninsula. From 21 *P. volitans* dissected, all the *L. floridense* obtained were fixed in 70% and in absolute ethanol for morphological and molecular identification respectively. We generated 28S rDNA sequences for phylogenetic analyses. For comparison in the phylogenetic analysis, specimens of *Lecithochirium* from other fish species of the Yucatan Peninsula were obtained. The molecular identification showed that the *Lecithochirium* specimens belong to at least 3 different species (*Lecithochirium floridense*, *Lecithochirium* sp. 1 and *Lecithochirium* sp. 2). This result suggests that, mixed infections can be found in the same lionfish. We conclude that due to its voracity, the lionfish is intruding into the food webs from which other native fish species are acquiring their food items. This is the case of *Achirus lineatus*, *Rhomboplites aurorubens*, *Syacium papillosum* and *Syacium guttatum* that are the natural hosts in which we have found *L. floridense*, *Lecithochirium* sp. 1 and *Lecithochirium* sp. 2 in the Yucatan Peninsula. It remains to be seen what the future will hold for these native fish species over the next few years in view of the voracity of this successful intruder.

The road not taken: Host infection status influences parasite host choice

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The manipulation of host organisms by their parasites has captured the attention of behaviorists, parasitologists, and the public. However, our knowledge of parasite behavior independent of a host
Parasite behaviors can help explain trematode community structure, the aggregation of parasites within host populations and can potentially be harnessed in biocontrol measures. In this study, we used a simple choice chamber design to examine whether trematode parasites can detect the infection status of a potential host and avoid hosts infected with a competitively dominant species. Our results show that *Schistosoma mansoni*, a competitively subordinate species, can detect and avoid hosts infected with a competitively dominant parasite, *Echinostoma caproni*. However, *Echinostoma caproni*, despite showing a significant preference for snails infected with *S. mansoni* over uninfected snails, showed little ability to detect the infection status of the host or even the hosts’ presence. We propose subordinate species may be under stronger selection to avoid dominant competitors, whereas dominant competitors may be more strongly selected to find any suitable host regardless of infection status. Previous research has focused on parasites distinguishing between 'host' and 'non-host', which has variable evolutionary costs and benefits. However, the ability of parasites to determine the infection status of a host presents a consistent evolutionary advantage.

The scaling of the energetic cost of parasitism from hosts to ecosystems

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Parasites are thought to play a significant role in ecosystem energy flow, but we currently lack a mechanistic framework to integrate parasite energetics at broad scales. Here, we develop a metabolic based framework to evaluate the energetic cost of parasitism across spatial scales from hosts to ecosystems. First, we develop and test theory that link host metabolism (R) to the energy flux of parasitic communities (F) spanning 28 host taxa. Specifically, we test whether the fraction of a host’s energy budget that is allocated to parasitism is invariant with respect to host body size, meaning parasites use a constant proportion of a host’s metabolism across host taxa. Our data affirms an allometric relationship between host metabolic rate and parasite community flux (p < 0.001), although the slope of the relationship was shallower than the expected isometric relationship based on theory (observed slope: 0.76; 95% CI: 0.56, 0.95). This relationship suggests the fraction of energy taken by parasites declines with host metabolic rate. The empirical equation $F = 0.002 * R^{0.76}$ implies that the fraction of energy a parasite community uses, $\delta$, varies with host metabolic rate as: $\delta = F/R = 0.002 * R^{(-0.24)}$. Finally, we extend this framework to explain the scaling of host and parasite community energetics at the ecosystem scale. Across three riverine ecosystems, we found a strong relationship between the energy flux of parasites infecting fish communities and the energy flux of those fish communities ($R^2 = 0.93$, p < 0.0001). Specifically, a common scaling exponent describes host and parasite energy flux with all three ecosystems (slope = 0.41; 95% CI: 0.35, 0.48), but intercept values varied based on ecosystem identity. Overall, energetic-based models outperformed those using biomass when describing parasite community energetics within hosts and ecosystems.

The schistosome tegumental ectoenzyme SmNPP5 hydrolyzes host purinergic signaling nucleotides to potentially exert both anti-inflammatory and anti-thrombotic effects.

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Schistosomiasis is a debilitating disease that affects > 200 million people worldwide. This disease is caused by intravascular platyhelminths of the genus Schistosoma that can survive in the host for decades. We hypothesize that host interactive proteins on the worm’s surface are key in preventing worm elimination. We have functionally expressed the tegumental ectonucleotide pyrophosphatase/phosphodiesterase SmNPP5 which can cleave ADP and prevent platelet aggregation. SmNPP5 additionally cleaves nicotinamide adenine dinucleotide (NAD) and can block NAD-induced T cell death in vitro. We hypothesize that SmNPP5 degradation of NAD in vivo can act to prevent the apoptosis of regulatory T cells (Tregs) and maintain a more schistosome-friendly, immunotolerant environment within the host. Furthermore, SmNPP5 (or living adult worms) can hydrolyze the proinflammatory DAMP (damage associated molecular pattern) ATP and can block ATP-induced T cell death in vitro. Finally, SmNPP5 cleavage of NAD and ATP both yield AMP and this can be further broken down by schistosome tegumental alkaline phosphatase to generate adenosine—a potently immunosuppressive metabolite. Thus, SmNPP5 is a key schistosome tegumental ectoenzyme that, by cleaving host purinergic signaling nucleotides, could exert both anti-inflammatory and anti-thrombotic effects.

The “Rhinoptericolidae” revisited: Less host specific, more diverse, and more broadly distributed than previously thought

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For decades, the elasmobranch tapeworm order Trypanorhyncha has been plagued by taxonomic and systematic instability. Some groups of trypanorhynchs, however, have been more affected by this instability than others. One of the most apparent examples of a group whose diagnosis, membership, and phylogenetic associations have changed markedly through time is the family “Rhinoptericolidae”. Since its erection in 1975 to house the monotypic and atypically host-specific Rhinoptericola, the family has been synonymized, resurrected, moved between three superfamilies, and has variously included members of several unusual trypanorhynch genera. Today, the “Rhinoptericolidae” is placed in the superfamily Eutetrarhynchoidea and houses the two monotypic genera Rhinoptericola and Nataliella. Based on the most recent phylogenetic hypothesis for interrelationships in the order, the family is paraphyletic with respect to the superfamily Tentacularioidea. Findings from recent global elasmobranch collections have called into question the identity of this family once more. Data collected using light and scanning electron microscopy reveal an alternative, simpler interpretation of hook arrangement in the type genus Rhinoptericola, and suggest Shirleyrhynchus (a former “rhinoptericolid”) to be a junior synonym of Rhinoptericola. Molecular sequence data (partial 28S rDNA) support this synonymy, and in addition, reveal two novel species of Rhinoptericola morphologically consistent with the revised hook pattern. Furthermore, sequence data show that Rhinoptericola megacantha—previously assumed to parasitize the Atlantic cownose ray in the Chesapeake Bay only—parasitizes several additional species of cownose rays and a species of whipray, and enjoys a circumglobal distribution. Phylogenetic analysis including additional partial 28S rDNA sequence data, which significantly expands upon previous species-level sampling within the Trypanorhynch, supports monophyly of the family, but suggests the Eutetrarhynchoidea and Tentacularioidea to comprise a single superfamily.

Thioester-containing proteins (TEPs) in the snail Biomphalaria glabrata

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None
Schistosoma mansoni is a trematode parasite and the cause of the human disease schistosomiasis, affecting over 200 million people in tropical and subtropical countries. *S. mansoni* parasites require the infection of an intermediate host, the freshwater snail Biomphalaria glabrata. Interestingly, there are resistant and susceptible snail strains that have genetically based susceptibility to schistosome infections. Although the molecular mechanisms that lead to protection in *B. glabrata* are not fully understood, the host’s humoral immune components are known to play an important role. One such group of molecules are the thioester-containing proteins (TEPs), which have been reported in both vertebrates and invertebrates. TEPs are traditionally classified into three subfamilies: 1) alpha-2-macroglobulins (A2Ms); 2) components of the complement system, and 3) thioester proteins (TEP/CD109). TEPs have shown to be involved in immunological functions against pathogens including blocking proteolytic attacks, opsonization, and cell lysis. Using the recently published snail genome, 12 members of the TEP superfamily have been identified in strains of *B. glabrata*, these include three members from the A2M group, three complement C3-like molecules, and six classical TEP-related proteins. The present study reports the characterization of *B. glabrata* TEP transcripts, their phylogeny and gene expression after exposure to microbial challenge, including *S. mansoni*. Results show high sequence identity between snail strains and the presence of conserved protein domains, such as the characteristic GCGEQ thioester motif. Phylogenetic analysis clustered these TEPs into the three main groups alongside homologs from other species. Finally, preliminary results showed the various TEPs respond differentially depending on the type of challenge, and that constitutive TEP expression differed between strains. This study offers new information regarding the evolution of TEP molecules, their expression, and possibly immune function during early response to infection.

**Tick Pathogen Survey on Pine Ridge Reservation, SD**

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Ticks are one of the most common ectoparasites that infect humans in North America and are one of the most common vectors of zoonotic diseases such as Lyme disease and Rocky Mountain Spotted fever. Changes to the environment have altered transmission patterns of arthropod-borne diseases. Additionally, few tick surveys have been conducted on Pine Ridge Reservation which suffers from poverty, high rates of diabetes, alcoholism, unemployment, and suicide. Thus, it may be more heavily impacted by the spread of diseases such as those vectored by ticks. The goal of this study is to obtain information about the identify and distribution of ticks and their pathogens to produce an epidemiological map. To accomplish this goal, we will sample eleven previously identified sites in Pine Ridge as well as any new sites that represent good habitats for ticks. Sampling technique will involve sweeping a white flannel across the vegetation and collecting any ticks that grab on as well as any that we may find attached to ourselves. Additionally, we will sample ticks collected by the local Oglala Sioux Parks & Recreation and Oglala Lakota College from roadkill and trapped rodents. They will record collection information with regards to time and place when possible. After ticks are collected PCR and gel electrophoresis will confirm the identification of the ticks and their pathogens. A preliminary collection in 2018 yielded 196 ticks (103 males and 93 females) identified as the American dog tick (*Dermacentor variabilis*). These ticks tested positive for the Spotted Fever Group, which includes *Rickettsia* and *Francisella tularensis*. Some also tested positive for *Ehrlichia* spp. We expect similar findings in this more extensive survey. Our results will assist health care assist public health officials in Pine Ridge Reservation and prompt other state-wide surveys by universities, health centers and state agencies.
USE OF SNAILS AS INDICATORS OF ECOSYSTEM HEALTH

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Many studies associate healthy freshwater ecosystems with aquatic insect diversity and, more recently, with trematode diversity. However, few studies have asked if the relationship between aquatic freshwater snail diversity correlates with a healthy ecosystem. To address this question, we gathered snails from three freshwater sources in Pine Ridge, South Dakota and one reference site at Lacreek National Wildlife Refuge. At each site, three researchers spent 15 minutes inside a 3 x 10 meter transect sampling for snails. The next 15 minutes were dedicated to collecting aquatic insects. Snails were identified before being dissected for trematodes, which were also identified. We calculated the Simpson diversity indices for snails, insects and trematodes. Three of the four sites had either zero or one trematode species. The reference site (Lacreek) had a Simpson index of 0.55 for trematode diversity. Insect diversity (at the family level) at all four sites had Simpson indices that ranged from 0.57 (at the reference site) to 0.51 at the most disturbed site. Snail diversity was highest at a rural park in Pine Ridge (0.68) and lowest at a rural fishing site in Pine Ridge (0.08). Our results suggest that insect diversity may be a more reliable measure of ecosystem health than snail or trematode diversity. However, further analysis of the insect data (at the genus level) may change this conclusion.

Understanding the Ecology of the Hairworm [Nematomorpha], Chordodes morgani in Creeks Near Lincoln, NE.

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Hairworms [Nematomorpha] have a complicated life cycle requiring multiple hosts. Free-swimming adults mate and lay eggs, which hatch into larvae that encyst in aquatic insects. When these aquatic insect hosts metamorphosize into flying adults, they are preyed upon or scavenged by a terrestrial insect host. The hairworm matures in this terrestrial host and then manipulates the insect into entering water where it can complete its life cycle as an adult hairworm. Previous studies found adults of the hairworm Chordodes morgani in Elk Creek (a first order stream) near Lincoln, NE in July, but not in nearby creeks. To understand the ecology of Chordodes morgani we sampled Elk Creek and nearby creeks in the month of July. Using a novel hairworm trap, we found adults of Chordodes morgani in Elk Creek July of 2017, indicating the presence of a stable parasite population. We also found adults at two additional sites: Upper Elk Creek (four miles north of the Elk Creek site) and the first order stream, Maple Creek (two miles west of Elk creek). We did not find Chordodes morgani in West Oak Creek, which is only two miles east of Elk creek. Chemical properties (temperature, DO, conductivity, salinity, pH and nitrate), physical properties (average depth, average width and stream flow) and vegetation mass at each site did not consistently correlate with the presence of Chordodes morgani. We found evidence that the wood cockroach (Parcoblatta pennsylvanica) serves as the terrestrial host. Pitfall traps showed that the wood cockroach is present at all four sites, suggesting that the terrestrial insect host does not limit parasite distribution.
Unraveling the complex interactions between members of the Schistosoma haematobium group and Bulinus snails in and around Lake Victoria in West Kenya.

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Schistosoma haematobium, the causative agent of urogenital schistosomiasis, has an obligate dependence on intermediate hosts of the genus Bulinus. Interactions between S. haematobium and related schistosomes like Schistosoma bovis, and their bulinid hosts are complex. One complexity is that Bulinus species differ in their ability to harbor S. haematobium. To assess this in West Kenya, we examined natural populations of Bulinus ugandae, Bulinus globosus, Bulinus nasutus, Bulinus truncatus trigonis, and Bulinus forskali for infections, and established laboratory colonies of each species. Of particular interest are B. ugandae, which lives on the lakeshore, and B. t. trigonis, from deeper waters. This is because Lake Victoria is a hyperendemic area for S. mansoni transmission, yet S. haematobium transmission seems rare or lacking in the lake itself. Experimental infections of each snail species using S. haematobium miracidia are underway. We seek to learn if lake-dwelling bulinids are refractory/resistant to schistosome infection. Additionally, our ongoing studies suggest that snail-schistosome interactions are more complicated when other trematode larvae co-infect the snails. For example, Bulinus tropicus only supports development of S. bovis if first exposed to Calicophoron microbothrium (Southgate et al. 1989). A similar interaction seems to be occurring in West Kenya. A recent survey in Tiengre, Kenya found both B. nasutus and B. forskali in the same habitats. Amphistomes were commonly found in B. forskali and the only schistosome found, S. bovis, occurred in a co-infection with amphistomes. Conversely, for B. nasutus, no amphistomes were found yet snails shedding S. bovis were present. One interpretation of this pattern is that amphistome infection may facilitate S. bovis development in B. forskali. Because differences in compatibility among snails influence schistosome transmission, potentially they can be manipulated to limit transmission. This study was supported by NIH grants P20GM103452 and R37AI101438.

Unveiling the hidden fauna in North America’s freshwater biodiversity hotspot: the importance of freshwater snail species, river basin, and habitat on the distribution of Trematoda: Digenea in Alabama, USA.

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Alabama is a global hotspot for biodiversity including the highest freshwater snail diversity in North America with 204 of 716 species occurring here. Many species are state endemics due in large part to the varied geology, unparalleled river drainage densities, and a large availability of freshwater habitats. Although many freshwater snail species are known to co-evolve with digeneans, much of the diversity and distribution of these worms in Alabama is unknown. Yet, the high degree of endemism of snail fauna by river basin and habitat type suggests that parasite diversity may also be explained by these factors. We addressed the significance of snail host specificity, river basin, and habitat on the distribution of digeneans based on the collection of aquatic snail species across four major river basins in both pond and riverine habitats in Summer 2015/2016. Trematodes were detected from
snail tissue smears viewed under a compound microscope and morphotyped based on gross morphology. Genetic sequencing of 18S rDNA from 110 samples were used to generate phylogenetic analyses and obtain putative worm identifications based on comparisons to published sequences available on NCBI Genbank. Fifteen families from both Plagiorchiida and Diplostomida lineages were recovered in the tree although several additional lineages were recovered that are poorly represented on Genbank. At least six families of digeneans were found in snails not previously reported as hosts in the literature. In general, specificity of digenean taxa for their snail hosts was supported for worms that infected either Coenogastropoda (operculate, gill breathing snails) or Bassomotophora (lung-breathing snails) albeit specificity at finer scale taxonomic levels was low. Also, most taxa were found in at least two major river basins in both lentic and lotic habitats. The large range sizes of many trematode taxa recovered in our study may be due, in part, to high vagility of secondary intermediate and definitive hosts that allow connectivity across habitats and river basins.

Use of δ13C and δ15N to evaluate the host-parasite system in Rhizoprionodon terraenovae in the Campeche coasts.

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The decline in the abundances of a species has been shown to have great repercussions on the stability of an ecosystem. The constant decrease of elasmobranch populations is a warning about the possible ecological impact that this will have, since the functional trophic role played by some species such as Rhizoprionodon terraenovae, which consumes fish with lower trophic levels, is considered key in the balance of a The community, acting as a dense regulator, similarly serves as health indicators of an ecosystem. Among the multiple causes related to the decline of a species, the increase in parasite diversity has been documented. It has been shown that parasitism exerts a great influence on the structure and function of an ecosystem and its increase is related to habitat alteration as a result of anthropic effects (e.g., water pollution). On the whole, an increase in parasitic diversity with a habitat alteration can cause changes in trophic interactions between species, specific case, elasmobranchs vs prey. One way to evaluate the level of alteration of trophic interactions between a predator and its prey as a result of the parasitic diversity that it presents, is from the stable isotope analysis, (δ13C and δ15N) in different tissues (eg blood and muscle), since it allows the assessment of the structure and dynamics of ecological communities. Despite the relevance, few studies have been carried out on the parasitic fauna of elasmobranchs, and no studies on the host-parasite system in R. terraenovae on the coasts of Campeche. The present study determines the quantitative relationship of the host-parasite system in R. terraenovae, which constitutes the baseline for understanding the energy flow (stable isotopes) between a predator of higher trophic levels with respect to its endoparasitic fauna. This aspect has been considered as key by several authors worldwide and steps to continue in the field of research of trophic networks and interactions, for the evaluation of the ecological stability of an ecosystem before natural and anthropic impacts, which has been underestimated to date and in some cases with null research records. In the present study, monthly samplings were made in the Campeche coasts (Ciudad del Carmen (CDC) and San Francisco de Campeche (SFC)) for the collection of spiral valves, dorsal muscle and R. terraenovae blood. 146 spiral valves were collected and reviewed, of which 91 correspond to males and the remaining, 55 valves to females. Recovered endoparasites were preserved for staining (Carmin de Meyer’s) or rinsed (glycerin at different concentrations) (depending on the class) and identified at the lowest possible taxon. Infection parameters (prevalence, abundance and mean intensity) were calculated. For the analysis of stable isotopes, by isotopic ratios mass spectrometry (IRMS), the isotopic composition of the tissues (muscle and blood, that was divided into serum and plasma) and the parasites...
collected from R. terraenovae were analyzed. To find out if there is a correlation between the possible changes in amplitude, overlap and trophic position, the SIBER method was used in the SIAR package of the R program. As a result, a total of 56 individuals were parasitized (13 for CDC and 43 for SFC); the presence of nematodes is confirmed (genus Anisakis, with a prevalence of 36%, mean intensity of 1.22 and an abundance of 0.44 for CFS, for CDC 15.21%, 3.14 and 0.47, respectively), acanthocephala (genus Gorgorhynchus, with a prevalence of 4 % and mean intensity of 1.25 and an abundance of 0.05 for CFC) and cestodes (genera Dasyrhynchus, Callitetrarhynchus, Nybelinia and Phoreiobothrium, with a prevalence of 58% for CFC and 26.09% for CDC and a mean intensity of 16.31 for CFC and 11.83 for CDC ), which is relevant since it is the first records of parasites in this elasmobranch species for the Campeche region. Regarding the stable isotopes, washes were made with deionized water to the muscles to eliminate the urea, a mathematical correction was applied to the values of the blood, to eliminate the influence of the lipids. It was observed that the value of the parasites (average δ15N 10.43 and δ13C -18.54) slightly below the muscle values (average δ15N 11.93 and δ13C -16.52) and blood (average δ15N 12.36 and δ13C -17.12), but not so with serum (average δ15N 11.31 and δ13C -18.82). MixSIAR indicated that blood serum contributes 54% to parasites, however, according to SIBER, no significant differences were found for δ13C and δ15N vs. parasitic diversity, so it is concluded that the current host-parasite relationship is not altering the trophic interactions of R. terraenovae present in the Campeche coasts.

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Using ecological niche modeling to predict the suitable habitat for Trichinella species in cougars (Puma concolor) from Colorado

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Little information exists on the occurrence of Trichinella species in cougars throughout North and South America. However, species distribution models can be useful to predict the suitable habitat for elusive species with limited occurrence data. Here, we used the occurrence data from a recent study that found larvae of three Trichinella species in 44% (17/39) of cougars from five counties in Colorado. Environmental layers were constructed in ArcMap and included elevation, land cover, precipitation, and temperature. Habitat suitability models were created using MaxEnt, and models were projected to the extent of Colorado. The resulting models for infected (AUC=0.67) and uninfected (AUC=0.79) cougars were then combined to refine the final model, yielding distinct areas of presences and absences for Trichinella spp. The final model shows areas directly surrounding mountains to be the most suitable for Trichinella spp. Future work would benefit from sampling in predicted suitable areas to confirm species presences or absences. To date, this is the first ecological niche model of Trichinella spp. in cougars from Colorado.

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Using parasites to investigate habitat quality for striped bass (Morone saxatilis) across the Chesapeake Bay

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Ecosystems with high free-living biodiversity tend to have equally high levels of parasite diversity, which is evident in the diet and subsequent body condition of predators in ecosystems. For this study,
we are examining the richness and abundance of parasites associated with the diet and infecting striped bass (M. saxatilis), an anadromous fish and dominant predator in estuaries and coastal waters along the Atlantic coast of the USA. To do this, we have collected striped bass across seasons (spring, summer, fall) and ages (young of year to spawning age) from several tributaries in the upper and lower portions of the Chesapeake Bay. The assessment of parasites is twofold including 1) dissection and microscopic examination of multiple organs (gills, intestines, muscle) for the presence, diversity, and abundance of parasites and 2) the use of amplicon-based high throughput sequencing (SSU and COI genes) to assess parasites present in the gut contents. Parasites found in dissections include multiple species of nematodes, cestodes, monogenean trematodes, acanthocephalans, and copepods (Ergasilidae). Nematodes and copepods were the most abundant parasites found across tributaries and seasons. A few individuals had heavy infections (>100 individuals) of one or two parasites, but most individuals had a minimal abundance of multiple parasites across the tissues examined. We also found variation in parasite diversity and abundance across age groups, with young of year fish having more trematode infections than adults. Gut content analysis is currently ongoing. Thus, our preliminary results show that striped bass, regardless of age, tributary sampled, or season, are host to a variety of helminth and crustacean parasites. The infection intensity and diversity of parasites varies across these factors, which we hypothesize may be indicative of the different prey items and habitats utilized by different age groups across the tributaries.

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Virus-like Particle Display of the α-Gal Carbohydrate for Vaccination against Leishmania Infection

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Secreted and surface-displayed carbohydrates are essential for virulence and viability of many parasites, including for immune system evasation. We have identified the α-Gal trisaccharide epitope on the surface of the protozoan parasites Leishmania infantum and Leishmania amazonensis, the etiological agents of visceral and cutaneous leishmaniasis respectively, with the latter bearing larger amounts of α-Gal than the former. A polyvalent α-Gal conjugate on the immunogenic Qβ virus-like particle was tested as a vaccine against Leishmania infection in a C57/BL6 α-galactosyltransferase knockout mouse model, which mimics human hosts in producing high titers of anti-α-Gal antibodies. As expected, α-Gal-T knockout mice infected with promastigotes of both Leishmania species showed significantly lower parasite load in the liver and slightly decreased levels in the spleen, compared with wild-type mice. Vaccination with Qβ-α-Gal nanoparticles protected the knockout mice against Leishmania challenge, eliminating the infection and proliferation of parasites in the liver and spleen as probed by qPCR. The α-Gal epitope may therefore be considered as a vaccine candidate to block human cutaneous and visceral leishmaniasis.

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Waiter, There’s a Fly in my Eye, or...Myiasis: Maggots Muchin’ on Man!

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Myiasis is contamination with fly larvae (maggots). A large number of fly larvae are capable of causing myiasis in humans, with the primary clinical categories being obligatory, facultative, and
incidental myiasis. Myiasis is rarely seen in the United States, with most infections acquired in travelers to Africa and South America. Five cases of human myiasis are presented covering the spectrum of clinical manifestations, including cases of *Dermatobia hominis*, *Cordylobia anthropophaga*, *Oestrus ovis*, *Lucilia* sp. and *Cuterebra* sp. The biology, epidemiology, and clinical presentation will be covered for each case. Means of laboratory identification of genera and species of flies whose larvae cause myiasis in the human host will be discussed, as well as differentiation of pseudoparasite mimics commonly submitted to clinical, diagnostic, and reference laboratories.