2018 ASP Meeting

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A Retrospective Study on Human Parasitic and Mycotic Infections in the State of Mississippi, USA

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The Mississippi State Health Laboratory records covering the period from 1989 to 1997 were obtained and examined for human parasitic and mycotic infections. This study was carried out to know the pattern and distribution of these infections state-wide during the period surveyed. It was also to document it for posterity, the specimens for diagnosis were submitted by clinics and health centers from different counties in the State of Mississippi. They were diagnosed, collated, and tabulated. Of 26,219 parasitological specimens submitted, 7.6% were positive. With respect to fungal specimens submitted, 37.1% (2846) of 26,219 were positive. These included 5 different genera of protozoans, 6 Genera of helminthes and 4 Genera of mycotic flora. The overall occurrence of protozoans was 7.6%, helminthes, 2.0% and mycotic infection, 37.1%. For several years, the recording and reporting of state-wide prevalence of parasitic infections has dwindled and is now practically non-existent. It is recommended that this be resumed especially as people from different parts of the world, including Orientals and Mexicans are now establishing residence in the State where parasitic infections are endemic.

A comparison of the metazoan parasites of invasive Neopomacentrus cyanomos and the native damselfish Chromis multilineata in Cayo Arcas reef, Mexico

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Neopomacentrus cyanomos, a common planktivorous damselfish from Indo-west Pacific reefs, was first reported, in 2013, as an alien species in the southwest Gulf of Mexico. It is now widely distributed on both coral reefs and oil-platforms there. During a sampling program of invertebrate diversity at Cayo Arcas, N. cyanomos and the native planktivorous damselfish C. multilineata, which is abundant on that reef, were capture and examined for parasites. Since the arrival of N. cyanomos into the Gulf of Mexico apparently is a recent event, we expected significant differences in parasite species richness and composition between native and alien damselfishes. External and internal organs of 13 C. multilineata and 42 N. cyanomos were examined, and all metazoan parasites were counted and identified under the light microscope. A total of 13 morphospecies of parasites, all apparently native species, were identified from both host species. Nine species were digeneans (six in metacercariae stage, one juvenile and two adults), one monogenean, one cestode (larval), one didimozomid (larval) and one copepod. Eight morphospecies of parasites were found in N. cyanomos and 10 in C. multilineata. Of the 13 species of parasites, five were shared between both host species. N. cyanomos had significantly fewer parasite species per host than did C multilineata (0.4 ± 0.7 vs
3.1 ± 1.2), as well as substantially fewer individual parasites per host (1.2 ± 2.4 vs 115 ± 391). Our results support the idea of a recent arrival of *N. cyanomos* in the Gulf of Mexico as most individuals were infected with a single individual of a single species of parasite. In contrast, individual native damselfish typically were infected with 100+ individual parasites (maximum 600 in a single host) belonging to several species. Clearly, this alien species is acquiring the most common parasites that infect an ecologically similar native damselfish. What effect the addition of a common new host species has on fish-parasite populations in the southwest Gulf of Mexico remains to be seen.

A comparison of the parasites of yellow perch (*Perca flavescens*) between an oligotrophic and eutrophic lake in New York State, USA

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Over the past nine years the SUNY Oneonta fish parasitology lab has conducted a survey of the parasites found in yellow perch (*Perca flavescens*) in Otsego and Canadarago lakes in central east New York state. The sampling period included the summer and fall months, as well as winter. For every fish examined, all components of the digestive system were examined for parasites, and in many samples, full necropsies, i.e., examinations of most of the body organs, were performed. In total, 245 individual *P. flavescens* were examined, including 115 from Canadarago Lake, and 130 from Otsego Lake. Parasites that were collected were preserved and subsequently prepared as whole mount microscope slides using conventional parasitological techniques. In total in the survey, three species of acanthocephalans, two species of cestodes, two species of trematodes, and two species of nematodes occurred as adult worms in the digestive system of the fish. A larval cestode, a nematode, and at least two species of larval trematodes were encountered in other body organs. External parasites encountered included a species of leech, a monogenean, and four protists. Following identifications of parasites, comparisons were made between Canadarago Lake, a eutrophic lake, and Otsego Lake, a nearby oligotrophic lake. Although the parasite fauna of *P. flavescens* mostly overlapped in both lakes, there were several differences in occurrence, and in prevalence, of several species of parasitic worms. This poster presentation includes specific comparisons between the two lakes and comparisons with other studies of parasites of *P. flavescens*.

A comprehensive approach to assess monogeneans infection in aquaria settings

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Cichlidogyriasis is produced in the Nile tilapia Oreochromis niloticus by monogeneans of the genus Cichlidogyrus. During mild parasitic infections, growths of fishes are affected, but in severe cases fish mortality can occur. The main diagnostic test is based on parasitological diagnosis in fresh gills after necropsy of the tilapia. In the present study, we propose the implementation of non-lethal and/or non-invasive molecular diagnostic tools using samples from aquaria of *O. niloticus* infected with Cichlidogyrus spp. All the tests were based PCR: the first test was standardized with samples from the water column while the second test used samples from sediments or detritus. Both tests
were validated using a 2x2 contingency table against the gold standard (parasitology) using a Chi-square test with a confidence limit of 95%. Both were significant different (p<0.05). In a second approach, the same tests were compared from parasite’s genomic DNA in mucus samples from the fish gills. For this a Kruskall-Wallis test was employed and significant differences (p<0.05) were found by comparing the results of these three tests. Being the most efficient the test employing mucus swabs from the gills, followed by sediment or detritus. The PCR using samples from the water column was less efficient. Based on the results from this study, it was determined that the PCR test described herein under different landscapes, can be used as a non-invasive diagnostic tool for diagnosis of cichlidogyriasis in tilapia aquaculture.

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A morphological study of the acanthocephalan Neoechinorhynchus cylindratus (Van Cleave, 1913)

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Neoechinorhynchus cylindratus (Van Cleave, 1913) is a widespread fish acanthocephalan that has been reported from several species of fish hosts in North America, including economically important gamefish such as the centrarchids Micropterus salmoides (largemouth bass) and Micropterus dolomieu (smallmouth bass). In spite of the common occurrence of N. cylindratus, information about its morphology is relatively limited to studies that were done prior to the advent of modern tools such as scanning electron microscopy and DNA sequencing. As part of a long term fish-parasite survey at Otsego Lake in New York state, USA, adult specimens of N. cylindratus were encountered in five species of centrarchids: M. salmoides, M. dolomieu, Lepomis gibbosus, Lepomis auritus, and Ambloplites rupestris. The survey made possible the acquisition of a large number of specimens of N. cylindratus to subject to morphological analysis including traditional light microscopy and scanning electron microscopy, as well as DNA sequencing. This presentation summarizes additional information acquired on the morphology of newly collected specimens of N. cylindratus in comparison with the type specimens that were examined.

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A new species of cestode of genus Stillabothrium (Rhinebothriidea) from the Blackspotted Whipray, Maculabatis astra, from Australia

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Examination of the spiral intestines of two specimens of the Blackspotted Whipray, Maculabatis astra, from Australia led to the discovery of numerous (~100) specimens of a new species of the cestode genus Stillabothrium Healy and Reyda, 2016 within the order Rhinebothriidea. Species of Stillabothrium possess a scolex that consists of four bothridia with a combination of horizontally and vertically oriented muscular septa and loculi, structures that function in attachment to the intestinal wall of the stingray host. In this study, the new species of cestode is characterized using morphological measurements obtained with light microscopy of approximately 50 specimens, and with images obtained using scanning electron microscopy. The new species of Stillabothrium is distinguished from six of the seven other species in genus in its possession of a greater number of transverse septa.
A new species of cestode of genus Stillabothrium (Rhinebothriidea) from the leopard whipray, Himantura leoparda, from Australia

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Examination of the the spiral intestine of two specimens of Himantura leoparda, the leopard whipray, off the northern coast of Australia lead to the discovery of a new species of cestode. The use of light microscopy and images obtained with scanning electron microscopy were used to characterize the morphology and distinctive characteristics of this new cestode. The species we encountered is consistent with the genus Stillabothrium Healy and Reyda, 2016 within the order Rhinebothriidea, which possesses a scolex with four bothridia, each with horizontally and vertically oriented muscular septa and loculi. Unlike other species of Stillabothrium, this new species possesses seven horizontally oriented septa and loculi on the anterior portion of the bothridia, and those septa are not overlapped by the vertically oriented septa in the posterior portion of the bothridia. This feature alone is adequate to distinguish the new species from the seven previously described species in the genus. This is the third species of Stillabothrium from Australia. This project is part of a global collaboration of individuals working on describing new species of tapeworms from vertebrate animals.

A putative concomitant infection between Rickettsia like Organisms (RLOs) and PaV1 in spiny lobsters (Panulirus argus) from the Mexican Caribbean sea

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Rickettsia like organisms (RLOs), are Gram-negative bacteria that are considered opportunistic. They produce high mortalities and cause great economic losses in aquatic organisms. PaV1 primarily infects hemocytes that are the first line of defence of crustaceans, due the decrease in that number, the mechanism of recognition of pathogens is impaired and the host is susceptible to secondary bacterial infections such as RLOs. In this study we assessed whether there is a concomitant infection between PaV1 virus infection and RLOs. Thus, the present work was focused to first, establish whether there is a relationship between infection of PaV1 and RLOs and then determine if the RLOs are more frequent as it increases the severity of the infection with the virus, PaV1. Similarly, since the identification of RLOs is through histology, conditions were established to design a PCR test on the tissue through the amplification primers designed from a sequence of the 16S rRNA gene reported
for organisms of the genus Rickettsia. An analysis of 652 lobsters; 245 from Bahía de la Ascension, QRoo, and 407 from Puerto Morelos, QRoo were collected during four years. In the particular case of PaV1, juvenile lobsters are more susceptible to infection. None of the healthy lobsters presented RLOs. PCR and sequencing analysis showed similarities with sequences from bacteria of genera Rickettsia. Based on the results of this work documents for the first time a concomitant infection between the virus, PaV1, and RLOs in lobsters P. argus collected in the Mexican Caribbean as well as the first evidence of a molecular test for RLO’s in P. argus.

ANTISCHISTOSOMAL ACTIVITIES OF SYNTHETIC CURCUMIN ANALOGS IN TWO STRAINS OF SCHISTOSOMA MANSONI

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Praziquantel (PZQ) is the only drug now available for the mass treatment campaigns of schistosomiasis. However, PZQ has low efficacy against juvenile schistosoma worms and its extensive field use has raised concerns about development of PZQ-resistant strains. Our earlier studies with curcumin (CUR), the major curcuminoid in turmeric, popular yellow spice powder obtained from the rhizome of Curcuma longa L. have reported potent in vitro schistosomicidal activity against Schistosoma mansoni of Luis Evangelista (LE) strain. Although CUR showed low potency and poor absorption characteristics in animal studies, it remains an ideal lead compound for the design of more effective antiparasitic analogs. The aim of this study was to evaluate in vitro schistosomicidal activity of eighteen synthetic CUR analogs against LE and Porto Rican (PR) strains of S. mansoni parasites. The compounds were first screened against schistosomula of the S. mansoni PR strain in vitro followed by a re-evaluation of successful hits on adult worms. In parallel, the compounds were evaluated in vitro against adult worms of the S. mansoni LE strain. Morphological changes were determined against adult S. mansoni LE worms in vitro followed by cytotoxicity studies in mammalian cells. Seven of the eighteen compounds screened caused degeneration of 80-100% of the schistosomula and five of these caused high mortality against adult worms of S. mansoni PR strain at the concentration of 10 µM during 48-h incubation period. Active compounds were also tested on adult worms of S. mansoni LE strain and four CUR analogs revealed high activity (IC50<10.5 µM) after 48 h exposure. Further, these compounds induced tegumental damage to S. mansoni parasites. The results also showed that they were more toxic to adult S. mansoni worms than to mammalian cells under in vitro conditions. In conclusion, this study demonstrates that the new CUR analogs tested are promising candidates for further investigations for optimizing their antischistosomal activities against S. mansoni parasites of different strains. (FAPESP Grant number 2016/24456-1)

Age-related cytokine modulation in the experimental Trypanosoma cruzi infection.

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Introduction: Since intrinsic defects in aged immune response and altered release of immune cell-derived cytokines are classic hallmarks shared by aging and T. cruzi infection, we addressed whether the course of the acute T. cruzi infection is influenced by age-related changes. Methods: Male Wistar rats were infected with 1x10^5 blood trypomastigotes of the Y strain of T. cruzi and randomized into the groups: young control (YC), young infected (YI), aged control (AC) and aged infected (AI). For cytokine assays, serum obtained on 16th days post infection was used. Concentrations of IL-2, IL-12, IL-17, TGF-β and TNF-α were measured by specific two-site enzyme-linked immunosorbent assay (ELISA - Immuno Biological Laboratories-IBL America) with reference standard curves, using known amounts of the respective rat recombinant cytokines. Results: IL-12, IL-17 and TGF-β displayed a significant increase in its production (p < 0.05), on the 16th day of experiment, for aged and infected animals as compared to young and infected counterparts. On the other hand, a reduction in IL-2 and TNF-α levels (p < 0.05), was observed in aged infected animals as compared to young ones. Significant increased levels of TGF-β and TNF-α levels were observed for young infected animals. Conclusion: A better knowledge of the dynamic interplay of cytokines and age-related changes during the acute T. cruzi infection is necessary to improve the efficiency of immunotherapies against Chagas disease. Financial support: FAPESP.

Allometric patterns in parasite ecology: Are they repeatable across scales?

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Parasite populations are nested within their host, and the spatial scale of host-parasite interactions should reflect this aspect of the relationship. Several studies report that the population density of parasitic species scales with parasite body size, and the spatial scale of the interaction has been interchangeable and represented as within a host or ecosystem. Nonetheless, it remains unclear whether the patterns emerging from these analyses using different sampling units are repeatable, and it is also likely the processes generating the patterns are not comparable. The aim of this study was to directly compare the population density and body mass scaling across host-parasite pairs, while describing parasites nested within their host and ecosystem. We sampled three, 100-m transects along two riverine ecosystems, across 4 seasons to evaluate the effects of spatial scale of parasite population allometries in fish hosts. We used linear mixed effect models to assess the relationship between parasite density represented as n/m^2 stream sampled and n/gram host, and host and parasite body size. Host and parasite identity were used as random effects, to account for variation in sampling within species. In the fall, winter and spring we collected 793 fish individuals representing 23 fish species, and 26,771 parasite individuals representing 37 parasite taxa. Across both spatial scales of within the host and ecosystem, there was no significant scaling relationship between parasite density and parasite body size (ecosystem scale: slope = -0.05, CI (-0.16, 0.07); host scale: slope = 0.002, CI (-0.13, 0.14)). There was a positive scaling relationship between host body size and parasite density with an ecosystem (slope = 0.20, CI (0.04, 0.35)). However, we also report a strong negative relationship between parasite density and host body size at the level within a host (slope = -0.47, CI (-0.67, -0.27)). Our data reveals a lack of relationship between parasite body size and population density at both spatial scales, but shows scaling relationships with host body size that differ based on the definition of the boundary of a host-parasite interaction. Moving forward in these analyses, a more transparent use of parasite spatial scales is needed to evaluate how parasites fit into broad scale ecological theory.
An overview of the results of the Planetary Biodiversity Inventory Survey Project: Tapeworms from Vertebrate Bowels of the Earth

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Tapeworms from Vertebrate Bowels of the Earth was an 8-year NSF funded global collaborative effort to provide an updated appraisal of the cestodes that parasitize vertebrate animals across the planet, with focus on the discovery of novelty and assessment of interrelationships. While much progress was made in our understanding of the diversity, morphology, host associations, geographic distributions, and phylogenetic relationships across all orders, those orders parasitizing elasmobranchs arguably benefitted most from this concerted effort. Training was a major component of the project. A total of 5 post-doctoral fellows, 20 graduate students, and 40 undergraduate students were involved in all aspects of the elasmobranch cestode work. The results of this survey from more than 1,800 specimens of elasmobranchs of 215 species in over 18 countries revealed a global elasmobranch cestode fauna of over 1,000 species. In total, 149 new species were described and 30 new genera were erected. Sequence data were generated for 2 nuclear and 2 mitochondrial loci for ~360 specimens of 8 of the 9 elasmobranch-hosted cestode orders. A phylogenetic analysis including representation of the vast majority of elasmobranch-hosted genera confirmed that elasmobranch cestodes compose the backbone of the cestode phylogenetic tree overall. To resolve some of the paraphyly apparent in the classification based on these relationships, 2 new orders (the Phyllobothriidea and Onchoproteocephalidea) were erected. Molecular data were further used to assess phylogenetic relationships within 8 of the 9 elasmobranch-hosted cestode orders. The classification of several orders was revised to reflect these relationships: 2 new suborders of the Trypanorhyncha, and 4, 2, and 1 new families of the Lecanicephalidea, Rhinebothriidea, and “Tetraphyllidea” relics, respectively, were established. We learned that the discovery of additional novelty is best achieved by targeting unexplored host species rather than unexplored geographic regions. Moreover, sharks and stingrays continue to be the most productive hosts both in terms of prevalence and the number of species encountered within a single host species. That having been said, the Squaliformes (dogfishes, etc.) and “Scyliorhinidae” (cat sharks, etc.) are particularly poor hosts. In contrast, the Myliobatiformes (stingrays and eagle rays) and Rhinopristiformes (guitarfishes and their kin) are especially good hosts, and thus unexamined members of these groups make excellent candidates to target for future collecting work.

Analysis of complete mitochondrial genomes in the genus Monocercus (Cestoda, Dilepididae)

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The family Dilepididae is a diverse group of cyclophyllidean tapeworms including more than 750 species and about 90 genera. The majority of dilepidids parasitize birds, with only 8-10 genera infecting mammals. Numerous questions exist in the systematics of the Dilepididae including monophyly
of numerous genera and monophyly of the family, now based almost exclusively on morphology. DNA sequences, including whole mitochondrial genomes, provide new tools and additional sources of characters that are critical for resolving the systematic relationships among members of this large tapeworm group. We sequenced, assembled, and annotated the first mitochondrial genome of a dilepidid tapeworm, *Monocercus arionis* (type species), as well as the mitochondrial genomes of 9 additional taxa within the genus. Mitochondrial genomes were assembled from Illumina HiSeq whole genome sequence data. Bioinformatic programs NOVOPlasty and Geneious were used to isolate and assemble the mt genomes, which were then annotated using both Geneious (protein coding genes (12) and rRNAs (2)) and ARWEN (tRNAs (22)). The approximate average mt genome length was 13,500 bp with highly conserved gene locations for all worms sequenced. Divergent and ambiguously aligned regions of the protein-coding sequences were removed using the Gblocks server. Maximum likelihood and Bayesian inference methods for phylogenetic analysis were performed using MrBayes and RAxML, respectively, on the CIPRES Science Gateway. These mitochondrial genomes provide new resources for examining the evolutionary history of this diverse cestode family.

### Analysis of the communities of parasitic helminths of the French Grunt (*Haemulon flavolineatum*) in four location in the Yucatan Peninsula

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The French Grunt *Haemulon flavolineatum* (Haemulidae) is an abundant fish, ecologically important in reefs and a common source of food for other fish of commercial importance. Studies on parasites in *H. flavolineatum* have been mainly on the taxonomic topic, a total of 30 species have been reported, however, there are very few records for Mexico. Although this species represents a good model for the study of parasitic communities, due to their distribution, feeding, habitat and ecological importance in the reef communities, no analyzes of the parasitic communities have been carried out, nor the differences in the parasite-fauna present in its area of distribution. Therefore, the present study aims to analyze the communities of parasitic helminths of the French grunt (*H. flavolineatum*) in two localities of the Mexican Caribbean and two of the bank of Campeche. Samples of *H. flavolineatum* were collected from 5 July to August 10 2014, in four locations in the Yucatan Peninsula: Puerto Morelos and Banco Chinchorro (Mexican Caribbean) and Arrecife Alacranes and Cayo Arcas (Bank of Campeche). All specimens were captured, frozen and transported to the Marine Biology Laboratory of the UADY, in Mérida Yucatan for review. Once in the laboratory, the total length (cm) of each individual was obtained and an external and internal revision of the organisms was carried out. Parasites were identified at the lowest possible taxonomic level and the infection parameters (prevalence, mean abundance and mean intensity) were then calculated. We produced rarefaction and extrapolation sample-size-based and coverage-based curves to provide asymptotic diversity estimators based on Hill numbers to compare the communities. We calculated the first 3 Hill numbers, which are associated with estimators of species richness and species dominance. The first Hill number \(q = 0\) used in the analysis estimates the expected parasite species richness (the number of species) in each community. The second Hill number \(q = 1\) is the exponential of the Shannon entropy index and estimates parasite diversity with respect to equally common species and species richness. The third Hill number \(q = 2\) is the inverse Simpson concentration index and measures the dominance of parasite species (abundant and prevalent species). A total of 104 specimens of *H. flavolineatum* were examined. A total of 11 taxa (8 digenetic, 2 acanthocephalus and one nematode) were recorded, with the highest species richness observed at Banco Chinchorro and Arrecife Alacranes with 7 taxa of parasites each, followed by Cayo Arcas and Puerto Morelos with 6 each. However, when we extended all extrapolations up to double the reference sample size, there are no statistical differences between the helminth communities richness. There was not significative differences in Shannon diversity entropy index and Simpson concentration index between communities from Banco Chinchorro, Arrecife Alacranes and Cayo Arcas. However, Puerto Morelos showed lower values of those index. Those differences may be related with the fact that Puerto Morelos is a coastal locality, mean-
while the other three are insular. The digenetic *Postmonorchis orthopristis* presented more than 60% contribution of the parasites that constituted the localities of Puerto Morelos, Banco Chinchorro and Arrecife Alacranes, while *Dollfusitrema* sp. represented the highest percentage in Cayo Arcas. In addition, these parasites also represented the highest percentage of contribution to the differences between localities.

Anecdotes from the field and the lessons they teach us

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Decades of fieldwork in multiple countries across the globe, primarily in tropical and subtropical marine regions, have yielded some valuable lessons for the intrepid field Parasitologist. These include: identify in-country collaborators prior to your trip; there is no substitute for local “host” expertise; embrace local knowledge of potential hazards; aspire to make arrangements for the purchase of chemicals in advance as they are generally difficult to obtain in-country, especially on short notice; bring the correct power adaptor; patience is a virtue; humor is a must, as is a sense of adventure; there is no substitute for careful, detailed work even in the most extreme situations; obtain as much information on each host specimen as possible at the time of necropsy as host specimen identifications will be problematic; knives may be confiscated; it is, indeed, possible to dissect almost anywhere with the right equipment. In terms of staying healthy: filter your water or, if drinking bottled water, be absolutely certain the seal is intact; consume only recently cooked fresh foods; never underestimate the importance of non-perishable foods (i.e., dates, nuts, Nutella); the pros of probiotics; don’t count on life jackets. Effective fish market strategies (e.g., don’t pay too much for a host too soon, and when to establish a bounty for a host you really need/want, etc.), research vessel etiquette, and packing specimens for air travel (i.e., whirl-packs, sorbent pads, and dog food vaults) will also be addressed. In the end, at a minimum, you will be extremely well served by the following gear on any expedition to the tropics or subtropics: a light-weight rain jacket, light-weight down jacket, bathing suit, sarong, water shoes, hat, head lamp (with extra batteries), towel, “pod”, sleeping sheet, leather man, small UV water purifier, waterproof bag(s), cable ties, duct tape, first aid kit (including lomotil), sun screen, insect repellent, water bottle, copy of your passport (and green card if applicable), printed copies of Fish and Wildlife form 3-177. Finally, the following equipment and supplies will likely be difficult to obtain in-country and thus are best to bring with your from your home institution: archival pens, a field notebook that includes key information on the targeted regions, vials (glass and plastic with snap-cap), fine dissecting tools, glass pipettes and bulbs, squirt bottles, petri dishes, black cards, and, if at all possible, a dissecting microscope. Although not exhaustive, hopefully these tips will help facilitate the success of future parasitological fieldwork.

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**Apolipoprotein L-1 Builds Cation Selective Pores In African Trypanosomes: And Makes Them Pay For It**

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The human innate immunity factor Apolipoprotein L-1 (APOL1) protects against *Trypanosoma brucei brucei* infection. Endocytosis of APOL1 results in the necessary acidification within the endosome/lysosome that activates APOL1 and leads to eventual lysis of the parasite due to colloid-osmotic swelling. Previous studies have shown recombinant APOL1 (rAPOL1) inserts into planar lipid bilayers at an acidic pH 5.6 and forms a cation selective pore, which opens upon subsequent neutralization, pH 7.0. This corresponds with the pH changes APOL1 would encounter during endosome recycling, suggesting that APOL1 forms a pH-gated pore in the plasma membrane of the parasite. Structural characteristics of the APOL1 pore are poorly understood, but molecular structure prediction software indicates that the protein is primarily helical in nature and contains two putative transmembrane domains, along with a leucine-zipper motif in the C-terminal region. Utilizing *E. coli* derived rAPOL1, we are investigating the effects of single amino acid substitutions in trypanolysis assays and pore formation in planar lipid bilayers. We have identified key residues that govern APOL1 cation selectivity and control pH gating. Further amino acid analyses will yield more insight into the structural domains.

Arthrogryposis Multiplex Congenita due to Toxoplasma gondii infection in a newborn calf

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A rare case of Arthrogryposis Multiplex Congenita (AMC) due to *Toxoplasma gondii* infection in a newborn calf was examined. The affected calf, male of Brown Swiss breed from a farm in eastern Sicily (Italy), died shortly after birth and was submitted for necropsy examination. Flexure of the carpal and tarsal joints and flexure of the metacarpophalangeal and metatarsophalangeal joints combined with a moderate lateral rotation of the phalanges causing medial deviation of the fingertips were the only observed lesions. Laboratory examinations were performed to detect the main bacterial and viral infections. Histopathology samples were obtained from the central nervous systems, lung, liver, spleen, intestine, heart and kidney. Heart, spleen and brain samples were further examined for *T. gondii* by AF targeted real time PCR. Virological, bacteriological and parasitological tests were negative while the brain and spleen samples were PCR-positive for *T. gondii*. Furthermore the affected calf’s dam was seropositive for *T. gondii*. Congestion of meningeal vessels was the only lesion found in the central nervous system. Histopathologic features were characterized by microglial nodules scattered throughout the brain and other nonspecific and restricted occasional findings, such focal hemorrhages. A central nervous system involvement appears to be present in most cases of AMC and in this case the presence of *T. gondii* in calf’s brain was demonstrated through PCR examination. The parasite infection had resulted in a microglial cells proliferation associated with nodules scattered through the brain with no other evidence of acute inflammation. No vascular proliferation, endothelial hyperplasia, perivascular inflammatory infiltrate or necrosis was found. The brain lesion due to congenital *T. gondii* infection can therefore be considered the cause of the multiple joint contractures and subsequent AMC making this syndrome as a further outcome of toxoplasmosis among the well-known fetal abnormalities (hydrocephalus, cerebral calcifications, chorioretinitis).
**Leeches illuminate the evolution of bloodfeeding, habitat choice, and other traits**

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Leeches (Hirudinida) are an ancient and ecologically diverse group of annelids. They are crucial members of terrestrial and aquatic ecosystems, and the propensity for bloodfeeding in some taxa makes them integral to certain aspects of modern medicine. Despite their significance as parasites, predators, prey, and medical tools, the broadscale evolutionary relationships of Hirudinida remain ambiguous. Here, we present the first comprehensive, multilocus phylogenetic study of leeches encompassing all currently recognized families, and approximately half of the known species diversity. The resulting topology resolves several contentious relationships and overturns many long-standing groupings in leech systematics, most notably at the base of the tree. Harnessing this new understanding, we use various approaches, including ancestral state reconstruction, to investigate important evolutionary transitions within Hirudinida. Contrasting prior studies, our results support a marine origin of leeches, with subsequent shifts to terrestrial and freshwater habitats. Although the ancestral leech was almost certainly bloodfeeding, there have been repeated transitions to other feeding modes across the tree, with various taxa subsisting on haemolymph or ingesting large prey. Furthermore, it appears that the clades now known as “medicinal leeches” likely evolved from macrophagous ancestors. These dietary changes were accompanied — or, perhaps, facilitated — by modifications of the feeding apparatus in several groups. The earliest branching lineages of leeches possess proboscises, which they forcibly evert into the integument of their hosts, but more derived lineages have lost this structure, feeding instead by way of muscular jaws that are variously armed with teeth. Our results provide evidence for two distinct origins of teeth within the subclass encompassing leeches and their closest relatives. Moreover, we find evidence for repeated, independent reduction in the number of jaws, and convergent losses of teeth in macrophagous taxa.

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**Assessing a role for the gut microbiota in the murine response to infection with the rat tapeworm, Hymenolepis diminuta**

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While intuitive to accept an interaction between intestinal helminth parasites and the gut microbiota, it is only recently that data have been presented to support this position. Mice infected with *H. diminuta* expel the worm within 12-14 days of a primary infection and 2-3 days of a secondary infection. Given the role of the microbiota in the education of the immune system we asked if the gut microbiome affected the response to, and immunologically-mediated rejection of *H. diminuta*; and, reciprocally if infection with *H. diminuta* resulted in compositional changes in the colonic microbiota (*H. diminuta* is a parasite of the small intestine). Balb/c mice treated with broad-spectrum antibiotics showed no perturbation in their ability to expel *H. diminuta*, nor in their capacity to mobilize Th2 immunity (i.e. splenic interleukin-4, -5 and -10; blood eosinophilia; intestinal goblet cells) following infection with *H. diminuta*. The presence of the worm, or the murine hosts response to it, did lead to subtle shifts in the colonic mucosa-associated microbiota as assessed by Illumina sequencing of the...
V3-V4 region of the bacteria 16S gene; bacterial diversity, as gauged by alpha and beta indices, were increased in a time-dependent manner following infection with *H. diminuta*. Analysis of operational taxonomic units (OUT) indicated an increase in firmicutes (Lachnospiraceae) and this corresponded with increased levels of short-chain fatty acids in the feces of *H. diminuta*-infected mice. We have reported that infection with *H. diminuta* protects mice from colitis induced by intra-rectal delivery of the haptenizing agent, dinitrobenzene sulphonic acid (DBNS). Preliminary studies indicate that this anti-colitic effect of infection with *H. diminuta* is compromised in mice co-treated with antibiotics, implying a role for gram-positive bacteria. Thus, as we assess host-parasite interactions it is becoming clearer that these need to be viewed, at least in part, through a microbiology lens.

**BATHYCOTYLE SP.: A FISH TREMATODE LIVING OUTSIDE**

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As a result of a fishing competition we got several dolphinfish (*Coryphaena hippurus*) to perform parasitological analyses. The gills of these fish were not only infected by traditional ectoparasites but also by some trematodes, usually considered endoparasites. Their examination allowed us to distinguish some large individuals which seemed to use the gills as target site and thus, a more detailed analysis was performed. Thirty dolphinfish samples from Atlantic Spanish waters were collected during two years (2015 and 2016). Gills were examined for parasites and trematode specimens were isolated. Morphological analyses were conducted using specimens fixed in ethanol 70 and stained with ferric acetocarmine while specimens fixed in absolute ethanol were used for preliminary molecular studies. Histology was performed with formalin fixed specimens decalcified in 8% formic acid and embedded in paraffin. Sections of 5 μm were stained with haematoxylin-eosin and analyzed using a light microscope (40x–1000X). Total infection prevalence (P) was 23.3%, with P: 33.3%, mean abundance (MA): 0.86±1.59 and mean intensity (MI): 2.6±1.8 for 2015 and P: 13%; MA: 0.13±0.35 and MI: 1±0 for 2016. Specimens (N=5), 15.8±2.8 long and 2.2±0.3 wide, were mostly found free in the gill rinse although two of them were directly attached to the tip of the gill filaments with their ventral sucker. After morphological analyses, specimens were identified as adult *Bathycotyle* sp.. Comparison of molecular results with other sequences available in GenBank did not reveal significant coincidences but further studies are needed. The attachment of these trematodes modifies the gill filaments which become club-shaped and in histological sections appear to have a bended tip. Host tissue reaction was slight, affecting only one gill filament. This is in contrast with the severe reaction caused by other trematode species infecting gills: *Accacoelium contortum* in *Mola mola*.

**Bat Out of Helminth: Using Microbiomes to Understand Helminth Parasitism in Bats**

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Interactions among a community of organisms living in the host gut can exert strong pressures on co-evolutionary relationships between a host mammal and its endosymbionts. The gastrointestinal
tract of bats (Order: Chiroptera) harbors not only its resident microbiome, but also a complex assemblage of helminth parasites, including nematodes (Phylum: Nematoda), spiny-headed worms (Phylum Acanthocephala), and platyhelminths such as tapeworms (Class: Cestoda) and trematodes (Class: Trematoda). Because helminths share an environmental space with the host microbiota, it is possible that these two groups impact each other’s community structure and the host’s health. To date, there have been few studies addressing the impact of the host microbiota on helminth parasitism of the GI tract in mammals. Helminth colonization may result in alterations to the microbiota, but reciprocally, the microbiota may have an impact on the ability of helminth parasites to invade or persist in the host gut. We used 16S rRNA microbiome sequencing and collection of endoparasites from hosts to test for correlations between average microbiome diversity and parasite load in Neotropical bats. The results of this study will be incorporated in a neural network algorithm to predict endoparasitism in bats using host traits, host phylogeny, and microbiome attributes. This will lead to an increased understanding of the determinants of parasitism in the second most diverse clade of mammals.

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Beneficial impact of trematode infectious stages on zooplankton populations in the presence of predators

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Motile trematode cercariae represent a large amount of biomass in aquatic ecosystems, yet their interactions with other surrounding planktonic fauna are relatively obscure. Previous studies have shown that cercariae are consumed by many aquatic predators, including various larval insects, sometimes even preferentially so relative to other prey such as zooplankton. By serving as alternate prey, cercariae may thus decrease predation pressure on free-living animals with a similar trophic niche. The purpose of the current study was to examine the extent to which the presence of cercariae (Plagiorchis sp.) affected the population dynamics of common freshwater zooplankton (Daphnia sp.) in the presence of larval odonate predators known to consume both of these prey items. We used four treatments (12 replicates each) representing a factorial combination of infected or uninfected snails and the absence/presence of dragonfly larvae after seeding 48 mesocosms with starting populations of Daphnia. Weekly non-destructive samples of Daphnia were subsequently taken from each mesocosm over a four-week period to monitor their population size. Overall, there was trend for larger Daphnia populations in treatments containing Plagiorchis sp.-infected snails in comparison to those with uninfected snails. However, there was a highly significant interaction between the presence of infected snails and larval dragonfly predators for Daphnia population size. When faced with predation pressure, Daphnia reached higher numbers if accompanied by infected versus uninfected snails, suggesting a protective effect by cercariae that serve as alternate prey. Within natural settings, abundant snail populations harboring trematode infections may prove advantageous for zooplankton communities facing predation. Further studies will be needed to determine how this varies depending on the predator, trematode, and zooplankton taxa involved.

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Benznidazole combination therapy with cysteine protease inhibitor protects mice from acute experimental infection of Chagas Disease

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Currently, only two drugs are available for the treatment of the Chagas disease: nifurtimox and benznidazole (Bz). Thus, the only drug currently used for therapeutic purposes is Bz which is effective only during the acute phase of the infection but may present undesirable systemic toxicity. Therefore, the development of more efficacious and less toxic drugs, which can be used as alternatives for drug resistance is urgently needed. To identify new therapeutic drugs against *Trypanosoma cruzi*, we evaluated both in vitro and in vivo activity of the inhibitor of a cysteine-protease of T.cruzi named Neq674. The compound was tested, in vitro, against Tulahuen β–galactosidase strain combined or not with a suboptimal dose of Bz (1µM). The Neq674 successfully killed the intracellular amastigotes forms of the Tulahuen strain of T.cruzi (IC50(ama) = 4.8µM). This value was nearly than the Bz (IC50(ama) = 3.8µM) and showed moderate toxicity to healthy mammalian cells (EC50(LLCM-K2) = 33µM). The combination of Neq674+Bz was 26 times more effective against amastigotes forms of T.cruzi (IC50(ama) = 0.18µM). Due to encouraging in vitro results, the Neq674 was tested against acute stage of Chagas disease under the approval of ethics committee, n.100/2014. BalB/C female mice was infected with 10,000 forms of Y strain of T.cruzi. The treatment started at 5th post-infection (d.p.i) for 10 consecutive days. In 10mg/Kg day, Neq674 was not able to reduce the parasitaemia in the peak of infection. Although the combination of Neq674 and Bz, at the same concentration (10mg), showed 53.3% of reduction compared to saline treatment. This result was not fully credited to Bz’s action because 80% of Bz (10mg/Kg) treated-mice succumbed on first 30d.p.i. while that the combination protected 100% of mice. In addition, the combination showed a significant reduction of cardiac damage expressed by the decrease of the enzyme CK-MB on serum of mice after 15d.p.i. All together, these data indicate that the new combination therapy showed itself promising to new treatment against acute Chagas Disease.

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Blood transcriptomes illuminate the evolutionary history of the malaria parasites (Order Haemosporida)

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The malaria parasites (Order Haemosporida) are a widely studied group of apicomplexan blood parasites of mammals, birds, and squamates worldwide, though investigation of these parasites using genomic data has been limited to the few species that are model organisms and their close relatives. The small genome sizes and difficulty of separating malaria parasites from their host cells have made obtaining genomic data from most malaria parasite species unrealistic, rendering genome-scale studies of the malaria parasites incomplete. Here, we address this limitation by demonstrating that sequencing the blood transcriptomes of moderately to heavily infected hosts using minimal starting material can yield thousands of loci for phylogenomic and molecular evolutionary studies. Using less than 50 µl of blood from wild caught birds and lizards, we sequenced the blood transcriptomes of 25 samples infected with malaria parasites in the genera Plasmodium, Parahaemoproteus, and Leucocytozoon. We used a BLAST approach to filter out host transcripts, yielding 844 to 9,904 malaria transcripts per sample. We found a strong relationship between the number of transcripts recovered and the parasitemia of the parasite in the host blood, though even samples with infections that would typically be referred to as chronic (~0.5% parasitemia) yielded over 4,000 transcripts. This finding demonstrates the potential for low-intensity haemosporidian infections that are abundant in wild hosts to yield large quantities of genomic data. Using these data we construct the largest phylogenomic dataset for the malaria parasites assembled to date, providing novel insights into the evolutionary history of this globally important parasite lineage.
CARDIAC REGENERATION AFTER TGF-β INHIBITOR THERAPY IN AN EXPERIMENTAL MODEL OF CHRONIC CHAGAS DISEASE

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Studies published by our group demonstrated the involvement of TGF-β in Chagas cardiomyopathy development in Trypanosoma cruzi-infected animals during the acute phase of Chagas disease. TGF-β is the most important protein involved in fibrosis process. Activation of TGF-β signaling pathway was observed in the cardiac tissue of infected animals during the acute phase, favoring the increase of extracellular matrix components (ECM) expression. The metalloproteases degrade ECM and are directly involved in cardiac remodeling, and their activity is controlled by TIMPs. Concomitant data from the group indicates that T. cruzi infected animals during the experimental chronic phase of Chagas disease presented cardiac damage, increased expression and deposition of collagen in the heart and increased levels of circulating TGF-β. Treatment of the animals with the compound GW788388, inhibitor of TGF-β activity, 120 days post infection (dpi), was able to: improve cardiac electrical conduction; reverse expression and deposition of collagen in cardiac tissue; and decrease circulating levels of TGF-β. The aim of this study is investigate the mechanisms involved in the reversal of fibrosis after treatment with GW788388, and to evaluate possible candidates involved in cardiac regeneration during the chronic phase of experimental Chagas’ disease. To this end, animals C57Bl/6 were infected with T. cruzi colombian strain (10⁶) and treated orally with 3mg/kg GW788388 after 120 days post- infection (dpi) in two treatment schemes: once a week or three times a week during 30 days. The animals’ hearts were removed and total proteins and mRNA were extracted for investigation of the gene expression (RT-qPCR) and protein (Western blot) of MMP-2, MMP-9, as well as their proteolytic activities (Zymography). Also, the protein expression of TIMPs -1, -2 and -4 and troponin T was investigated; and the gene expression of cardiac regeneration markers: Gata-4, Gata-6, T-box5, Nkx2-5, troponin T, desmin and titin by RT-qPCR. Histological techniques were used to evaluate Cx43. In addition, the levels of circulating TIMP-1 were evaluated by ELISA. Our results demonstrate that the chronic infection induces to disorganization of Cx-43 and the treatment recovers the Cx-43 pattern. A reduction in MMP-2 and -9 activity was observed with the chronic infection.
and the treatment with GW788388 increased its activities in the animals’ hearts. Circulating TIMP-1 levels increase at 120 days post-infection and fall 150 days post-infection, treatment with GW788388 increases these levels. The expression of TIMPs -1, -2 e -4 increase at 150 dpi and the treatment with GW788388 reduces these expression. Chronic infection leads to decreased expression of all assessed cardiac regeneration markers, and increased expression of Gata-6 and T-box5 was observed in the treatment with GW788388 3x a week. Our data indicate that cardiac fibrosis reversion is mediated by the regulation of MMP -2 and -9 activity by TIMPs. In addition, the treatment with GW788388 appears to be able to induce cardiac regeneration in chronically infected animals and is promising as a new possibility of treating cardiac fibrosis observed in the chronic phase of Chagas disease.

Cell-free Expression, Purification and Immunoreactivity Assessment of Recombinant Fasciola hepatica Saposin-like Protein-2

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Cell free protein synthesis has emerged as a powerful technique for the high-throughput production of proteins that typically are difficult to express in living cells. The protein SAP2 of Fasciola hepatica (FhSAP2), has demonstrated to be both, a great vaccine candidate against experimental fascioliasis and a suitable antigen for serodiagnosis of human chronic fascioliasis. The production of this protein has been carried primarily by using an Escherichia coli expression system, often resulting in a laborious non-cost effective production protocol for this type of molecule. This is mainly due to its tendency to over-expressed in inclusion bodies. Moreover, FhSAP2 expressed in an E. coli -based expression system is poorly glycosylated, insoluble and often undergoes improper folding leading to reduced immunogenicity. In this work, we demonstrate that FhSAP2 can be expressed in vitro using the eukaryote cell free system, TNT T7 Quick coupled transcription / translation, that has been designed for the expression of PCR-generated DNA templates. FhSAP2 was expressed in 1.5 hours, then purified by an affinity chromatography method, which gave a protein yield of 500μg/ml as determined by BCA method. Additionally, Circular dichroism, Western blotting and ELISA analysis were used to confirm the secondary structure, purity and integrity of the resulting protein. This work describe for the first time the ability to express a Fasciola hepatica antigen that could be used for immunization experiments and immunodiagnostic techniques using a cell-free, endotoxin free, fast and highly efficient system.

Cercarial productivity in the North Branch of the Raritan River in New Jersey

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Cercarial productivity in the North Branch of the Raritan River in New Jersey.

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In this study we quantified the production of cercariae shed from the snail host Pleurocera virginica across seasons in the Raritan River. We sampled a 100-meter transect of the Raritan River using a surber sampler net to measure snail density and parasite infection. Collected snails were individually isolated for 24 hours in containers filled with river water to collect cercariae. After 24 hours, the water was stained with Blue Nile, filtered onto individual filters, and counted to estimate parasite daily production. All snail hosts were later dissected to confirm parasite infection and host shell length was measured. Pleurocera virginica population density varied across seasons, with mean densities of 68.89 and 61 n/m² in the Fall 2016 and 2017, 24.44 and 10.76 n/m² in winter 2017 and 2018, 27.98 n/m² in spring 2017. Parasite prevalence was seasonal, with a prevalence of 3.12 and 9.41% in Fall 2016 and 2017, 9.10 and 6.67% in winter 2017 and 2018, and 30.77% in spring 2017. Cercarial production was 98.39 and 211.44 n/m² in the fall, 94.82 and 69.35 n/m² in the winter 2017 and 2018, and 177.13 n/m² in spring 2017. Our study suggests parasites production is high and variable across seasons, and can function as an important component of a stream energy budget.

Characterization of the infection of Prochristianella sp. in Octopus vulgaris and Octopus maya from the Yucatan Peninsula, Mexico.

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The common octopus (Octopus vulgaris) and the red octopus (O. maya) are among the most important fisheries of the Yucatan Peninsula. Due to its economic importance, the research on their biology including the parasites affecting them in fishing areas are increasing. One of the most frequent parasites affecting octopuses are cestodes of the Prochristianella genus. Thus the objective of this study was to characterize the parasitic infection of the cestode Prochristianella sp. in the common octopus and the red octopus of the Yucatan Peninsula. We collected 85 O. maya octopuses in Puerto Progreso and 78 O. vulgaris octopuses in Río Lagartos. Using confocal microscopy and scanning electron, we compared the larvae of the cestode Prochristianella sp. (Cestoda: Tryanorhyncha) in the oral mass of the examined specimens from each octopus species. A prevalence of 98.9% was registered for the red octopus and 96.8% for the common octopus. The mean intensity (I) was higher in the common octopus than in the red octopus (I=295 ± 40 vs I=190 ± 19). For O. vulgaris, a positive correlation between octopus weight and parasite intensity was detected (r=0.4538, p=0.0001); we also observed that the mean intensity varied significantly between depths (Kruskal-Wallis H= 7.63 p= 0.0219), being the maximum (I=367) between 11 and 20 m of depth, and the minimum (I=97) between 21 and 30 m of depth. The results suggest that cephalopods are intermediate hosts of larval stages of the cestodes, playing a decisive role in the transmission of parasites to their definitive hosts, which are commonly the elasmobranchs.

Chasing parasites: permits, protocols, preparations, problems and parasites

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The pursuit of diminutive parasites in their natural environments (hosts) often involves herculean, detail oriented effort, to practically stepping out into your “backyard” or placing an order. Each of these efforts can produce interesting and compelling results, changed paradigms, and bring insight into the intimate and complex relationships of organisms that likely live in the most hostile environment, the host immune system. If you look, you will find parasites, but if you want a specific parasite(s), to look means you become your system, to be adaptable. But before you can chase after your parasite of choice, the 21st century has made a mountain of often opaque set of rules and regulations. And that is your first effort. But once you are past the permits and protocols, you can start to detail your trip, who, what when, where, risk, cost, comfort, and colleagues. Some of this effort will be determined by your permit and protocol permissions, among myriad other. Perhaps by the time you are ready to reconsider expeditions of any size, soon the joy of organizing the collections redirects you to focus on the acquisition of the anticipated hosts(s), and those beloved parasites (because that is why you are here!). Thus, what will be presented is an outline of how I go about field work, with an emphasis on international fieldwork, but certainly lots of ideas for work in the US. The outline will start with (topics not mutually exclusive!): a) where to go, what to collect, and collaborators, ideally; b) the legal permissions (and there are lots) to collect, and revisit collaborators, ideally; c) field site(s) background checks (weather, diseases, culture, politics, local events); d) the fun details of gathering what you need to be efficient, adaptable, the appropriate equipment and means of data recording; e) operating in the field; and finally f) all the stuff you didn’t plan for, but comes up. And in the end, there is the effort to make sure that all materials, including hosts when possible, have their ultimate resting home, vouchered in a museum collection, somewhere.

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Coevolutionary analysis of haemosporidian parasites and their avian hosts from across all eight Amazonian areas of endemism

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Host switching has been shown to be the main evolutionary force in shaping associations between avian hosts and their haemosporidian parasites as well as speciation of the latter. In highly diverse ecosystems, such as the Amazon basin, high host and vector diversity may support cospeciation between specific haemosporidian parasites and avian hosts. To understand how coevolutionary forces have influenced the diversity of haemosporidian parasites among Amazonian birds we analyzed 324 haemosporidian lineages (64 Parahaemoproteus, 260 Plasmodium) recovered from 4178 individual birds (434 host species) collected from all eight Amazonian areas of endemism. Separate tanglegrams of Parahaemoproteus and Plasmodium and their avian hosts were used to construct cost-event analyses to analyze the importance of four separate coevolutionary forces: cospeciation, duplication, host switching, and sorting (e.g., extinction). Data on both Parahaemoproteus and Plasmodium support a coevolutionary history dominated by host switching with occasional cospeciation and duplication. To understand where cospeciation may be important we focused on a single area of endemism, Belém, and conducted a global cospeciation analysis on 49 haemosporidian lineages (3 Haemoproteus, 6 Parahaemoproteus, 40 Plasmodium) recovered from 323 individual birds. Significant cospeciation links were identified between non-passerine hosts and their Haemoproteus and Parahaemoproteus parasites as well as between specific passerine species and their Plasmodium parasites. The hyper-diverse host and vector communities within Amazonian areas of endemism provide ample opportunities for host switching and subsequent speciation without significant selective pressure for cospeciation. However, some evidence of cospeciation does exist within Amazonia. The high host diversity may allow for cospeciation between specific haemosporidian lineages and their avian hosts. For these lineages, cospeciation could result from the movement of parasites between areas with their dispersing hosts, followed by geographic isolation of both.
Colonization of a Mayan freshwater environment by a complex of invasive alien species (Haplorchis pumilio, Melanoides tuberculata and Carassius auratus), due to aquarist activities from Mérida, Yucatán, México

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The invasive alien species (IAS) and adult parasite trematode of reptiles, birds and mammals (including humans), Haplorchis pumilio, is recorded for the first time from Yucatán state in México. Ornamental fish from pet stores from Mérida City, were necropsied searching for parasites. The trematode was identified based on molecular and morphological data of metacercariae collected from freshwater fish, Carassius auratus (“gold fish”), from 7 out of 10 aquarium stores sampled. 28S ribosomal gene region sequences and COI fragments corroborate the identifications based on morphological attributes. DNA sequences from GenBank, showed low genetic divergence in both genes from H. pumilio associated with three different host groups during larval and adult stages around the world, e.g., cercarie from Melanoides tuberculata, metacerarie from Trichogaster trichopterus and trematode adult from Homo sapiens. We infer that this trematode was likely introduced through pet store commerce together with the complex of the IASs, M. tuberculata and C. auratus. As Yucatán is the second major ornamental fish producer in México, accidental release of these IASs into the aquatic environmental could result in the introduction of H. pumilio into regional freshwater bodies. Its introduction in Yucatan is probably relatively recent and not spread yet, but its potential pathogenicity can cause an emergence of diseases not only in wildlife, but also in local human populations so, particular attention should be given to this finding. On the other hand, we do not know the possible impact of the population expansion of H. pumilio under the present scenario of the global climate change.

Comparative genomics and transcriptomics and the evolution of novel morphology in a bizarre elasmobranch tapeworm

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The saga surrounding the evolution of the bizarre morphology in Litobothrium aenigmaticum, relative to that of its congeners also parasitizing the pelagic thresher shark, continues. Our previous work demonstrated that not only does L. aenigmaticum lack all the characters of a typical litobothriidean tapeworm but it’s scolex contains an aggregate of 11 cell types that has not been observed in any other cestode. Our current work now focuses on elucidating the mechanisms that may have led to the evolution of this novel morphology. Specifically, this study employs comparative genomics and transcriptomics to investigate how changes in gene sequence, regulation, and/or expression have contributed to the evolution of L. aenigmaticum. To pursue this, genomes and transcriptomes have been generated from specimens that were collected in Taiwan in 2017. Scaffolded genomes for L. aenigmaticum, L. daileyi, and L. amplifica had repeat regions masked with RepeatMasker and are currently being annotated with two different pipelines, Braker and MAKER. The genomic data has shown that L. aenigmaticum has twice the number of unique repeat regions when compared to L. daileyi and L. amplifica (i.e., 746 vs 344 and 299, respectively). Yet, the total estimated repetitive content for all three species is similar at about 30%. Preliminary annotation results from Braker
have predicted approximately 15,744 genes and 17,140 protein sequences for *L. aenigmaticum* and 20,418 genes and 21,734 protein sequences for *L. daileyi*. De novo transcriptomes were generated for replicate specimens of *L. aenigmaticum*, *L. daileyi*, and *L. nickoli* from two different individuals of pelagic thresher sharks. These transcriptomes represent five individuals of *L. aenigmaticum*, three pools of individuals of *L. daileyi*, and six individuals of *L. nickoli*. These 14 transcriptomes were used to generate master transcriptomes for each of the 3 species of *Litobothrium*. The open reading frame was determined for each master transcriptome, trimmed transcripts were aligned to their species master transcriptome, and then these alignments were used to determine the transcript counts for evaluation of gene expression. Orthofinder was used to identify orthologous genes that are present in litobothriidean species. Functional annotation of genes in the master transcriptomes and representatives of the orthogroups is currently underway. The transcriptomic data has shown that the transcriptome for *L. aenigmaticum* is about twice the size of those of *L. daileyi* and *L. nickoli* (i.e., 38 Mb versus 17 and 19 Mb, respectively). This indicates that *L. aenigmaticum* either has greater allelic diversity or that there are many more alternatively spliced isoforms present compared to the other two species. The next steps in the project will allow for the comparison of syntenic regions, orthologous genes, gene family evolution, and differential gene expression. Furthermore, additional parsing of the Orthofinder results will be used to identify which genes are shared among species and which are unique to individual species.

**Complete mitochondrial genomes and phylogenetic relationships of multiple taxa in the genus Staphylocystoides**

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The Hymenolepididae is the largest family of tapeworms infecting mammals and birds. Even with about 90 genera and over 900 species, only 4 species have complete mitochondrial (mt) genomes sequenced, few species have more than a single gene sequenced, and most have no DNA sequences. Current phylogenetic framework of mammalian hymenolepidid tapeworms is based primarily on partial sequences of the 28S rDNA. *Staphylocystoides* is the largest genus within the Hymenolepididae infecting shrews (Eulipotypha), with 10 described and many undescribed species worldwide. Interspecific relationships within the genus remain unknown and the position of the genus within the family as a whole is unclear. The goal of this project was to assemble and annotate mt genomes of 16 *Staphylocystoides*. Protein coding amino acid sequences were used to generate a more comprehensive phylogeny of the genus. Mitochondrial genomes were assembled from Illumina HiSeq whole genome sequence data. Bioinformatic programs NOVOPlasty and Geneious were used to isolate and assemble the mt genomes, which were annotated using both Geneious (protein coding genes (12) and rRNAs (2)) and ARWEN (tRNAs (22)). The approximate average mt genome length was 13,500 bp with highly conserved gene locations for all worms sequenced. Divergent and ambiguously aligned regions of the protein-coding sequences were removed using the Gblocks server. Maximum likelihood and Bayesian inference methods for phylogenetic analyses were performed using MrBayes and RAxML respectively on the CIPRES Science Gateway. The 16 *Staphylocystoides* mt genomes will provide a foundation for greater understanding of the evolutionary history and of hymenolepidids and cestodes as a whole.
Could it be crypsis? Investigating the diversity of *Quinqueserisalis* trematodes in North America

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DNA sequencing has revealed that trematode species diversity is underestimated because of cryptic species. Poor taxonomic resolution may mask host-parasite interactions and make it difficult to test evolutionary hypotheses. There are questions about the taxonomy, life cycles, and host specificity of species within the trematode genus *Quinqueserisalis*. Using integrative taxonomy (morphology, DNA sequences and host use), we will determine if cryptic species are present and estimate the number of species in North America. Further, we will use field collections of intermediate and definitive hosts to better understand life cycles and host specificity. Intermediate snail and definitive mammal (voles and muskrats) hosts will be collected from four locations in the northern and southern extent of the parasite’s range in North America: Inuvik, Northwest Territories, Churchill, Manitoba, Winnipeg, Manitoba and Blacksburg, Virginia. Preliminary genetic and morphological analyses indicate at least two species. Adult parasites in voles from Churchill were genetically distinct from adult parasites in muskrats from Winnipeg (1000 bp of 28s rRNA, p-distance = 0.015). In contrast, adults from muskrats from Virginia and Winnipeg were genetically similar (p-distance = 0.004). Multivariate analyses with ten morphological features suggested no evidence for crypsis. Genetically dissimilar parasites were morphologically distinct with parasites from voles being generally larger than parasites from muskrats. Genetically similar muskrat parasites were morphologically similar. Further, DNA sequencing confirmed a novel intermediate host, *Promenetus* sp., for *Quinqueserisalis* sp. collected from Winnipeg. This study will determine whether *Quinqueserisalis* taxonomy has been affected by crypsis, and fill gaps in our knowledge about the fundamental biology of these parasites. By doing so, we can then use this trematode group to test how geography and host specificity have influenced trematode speciation.

Could ocean acidification and warming trigger spontaneous metamorphosis in trematode parasites?

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Ocean acidification and warming (OA&W) are altering many aspects of marine life, from the fitness of individual organisms to the structure of entire communities. Both acidification and warming are caused by increases in atmospheric carbon dioxide resulting primarily from fossil fuel combustion. Acidification affects the acid-base balance and calcification of marine organisms, while warming can alter many fundamental metabolic processes and cause substantial range shifts. The combined effects of these global-scale processes have received scant attention in the parasitological literature, despite the potential impact of either stressor on the important regulatory ecological role of marine parasites. As the ocean is being acidified and warmed simultaneously, it is vital to understand the combined effects of these novel stressors on parasites and host-parasite interactions to accurately predict future changes to the influence of parasites in marine ecosystems.

Trematodes may be particularly vulnerable to OA&W, as they possess complex life-histories that involve up to six physiologically distinct life-stages, each exhibiting differential tolerances to abiotic stressors, and which rely on physical and chemical cues to trigger transitions between life-stages. We investigated the prediction that OA&W could change the physical and chemical properties of seawater such that the stimuli for metacercarial formation are artificially triggered outside of the second intermediate host. To test our hypothesis we exposed echinostome cercariae to four temperature treatments (5, 15, 25, and 40°C), each nested within six pCO2 treatments (280, 317, 455, 587, 853, and 1000 ppm).
1600ppm), for a 28 hour period. Cyst formation was significantly affected by temperature (p<0.001) and the interaction of temperature and pCO2 (p<0.001), with the highest formation rates at 25°C and 853ppm. These results introduce an unanticipated consequence of OA&W which could have a significant impact on the regulatory role that parasites often play in marine ecosystems.

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DETERMINING THE EFFICACY OF RIBEIROIA ONDATRAE AS A NUTRIENT RICH FOOD SOURCE FOR HIGHER TROPHIC LEVEL CONSUMERS

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Increasing evidence suggests that free-living infectious stages of parasites contribute substantially to the overall biomass in aquatic ecosystems and where they may also fall prey to predators at higher trophic levels. The motile cercariae of trematode parasites are similar in size and abundance to zooplankton and are eaten by a range of predatory invertebrates and vertebrates. Zooplankton are a vital source of nutrients and energy, and are an especially important link for the movement of essential fatty acids across the plant-animal interface; a process which regulates aquatic ecosystem productivity through its effects on growth and reproduction. Although cercariae, by comparison, are, as yet, not fully defined as prey items, they may be contributing substantially to the flux of nutrients and energy in aquatic systems. We compared cercariae (Ribeiroia ondatrae) and zooplankton (Daphnia sp.) as prey items for dragonfly larvae (Leucorrhinia intacta); a top predator in freshwater systems. Forty similar-sized larval dragonflies were collected and individually maintained in the lab. Dragonfly larvae were fed (twice per week) one of two diet treatments consisting of wet mass equivalents of either Daphnia or R. ondatrae cercariae. Dragonfly larvae were fed for 5 weeks, after which they were re-measured and sacrificed for fatty acid analysis. We found no significant difference in growth or essential fatty acid (EFA) contents of dragonfly larvae fed either R. ondatrae or Daphnia. We suggest therefore that these trematode cercariae are an equally nutritional food source relative to Daphnia and that they can, through the trophic transfer of EFA, support dragonfly growth and fitness thereby contributing to overall aquatic ecosystem productivity. Furthermore, high connectivity exists between freshwater wetlands and terrestrial landscapes and in which insect emergence (and concomitant EFA subsidies) plays a key role. Cercariae have the potential to contribute to these subsidies, and which has important implications for the health and survival of terrestrial organisms.

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DEVELOPMENT OF COMPARATIVE CHEMICAL GENOMICS AS A TOOL TO IDENTIFY TARGETS OF SMALL MOLEClCLE INHIBITORS IN BABESIA PARASITES

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Babesia parasites are one of the most common blood parasites of vertebrates. Babesiosis is a significant cause of veterinary disease, and has been emerging as an important zoonotic infection in
humans driven by the emergence of Babesia microti in the United States. However, human Babesiosis is caused by several parasites including B. duncani and B. divergens, and their related parasites. Further, there are over 100 described species of Babesia parasites. Currently, there is no specific antibabesial therapy and current treatments are susceptible to de novo drug resistance. This vast diversity necessitates the identification of compounds with pan-babesiacidal activity. Our lab has previously identified several small candidates from the freely available Malaria Box (Medicines for Malaria Venture) that are effective against two distinct Babesia species as well as Plasmodium species. This shared chemosensitivity suggests that these compounds act on a conserved target, an ideal scenario for an antibabesial with broad activity. The 400 compounds in the Malaria Box are structurally diverse, and likely interact with a range of known and novel targets. The goal of this work is to identify the targets of several small molecular inhibitors in the Malaria Box using chemical genomics as a means of identifying both lead compounds for future development and discovery of novel druggable targets. To achieve this, compound resistant mutants are generated, sequenced and finally validated via reverse genetics as a means of target identification. However, traditional selections can be slow (longer than 400 days) and sometimes impossible. Consequently, we have optimized chemical mutagenesis with N-ethyl-N-nitrosourea to accelerate generation of resistant mutants. While not the focus of this study, the results of this work could be applicable in Plasmodium as well, and could serve to identify novel apicomplexan biology. To date, resistance has been achieved to several compounds, and target identification and validation is on-going. Further, characterization of the phenotype generated upon compound application has been performed as a method to inform the mechanism of action, which has revealed interesting morphological perturbations.

DIFFERENTIAL PERFORMANCE OF HELMINTH PARASITE GROUPS AS EFFECT BIOINDICATORS OF ENVIRONMENTAL IMPACT IN THE GULF OF MÉXICO

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Theory suggest that parasite communities are good effect bioindicators of environmental impact, because they respond to disturbances with changes in the frequency of appearance or number of individuals. However, we still do not know what group of parasites performs better as a good effect bioindicators of environmental impact. The objective of this work is to determine which taxonomic parasite group (Digenea, Cestoda, Nematoda or Acanthocephala) performs better as bioindicators of environmental impact in three sampling areas in the Gulf of México. Between 2015 and 2017, we collected 674 fish on the Yucatan platform: (260 fish in 18 stations), Campeche Sound (230 fish in 34 stations), and the Perdido region (184 fish in 11 stations). For each fish, we determined the number of individuals and species richness of each group of parasites, as well as the concentration of heavy metals and hydrocarbons in tissues (muscle and liver). Redundancy analysis (RDA) was carried out to determine the potential statistical association between the concentration of contaminants and the number of individuals and species richness of each group of parasites as well as the concentration of heavy metals and hydrocarbons in tissues (muscle and liver). Redundancy analysis (RDA) was carried out to determine the potential statistical association between the concentration of contaminants and the number of individuals and species richness of each group of parasites. The RDA analysis showed significant positive associations between the number of digeneans and the concentration of anthracene, fluoranthene and n-enicosane for the Yucatan platform (Overall Explained Variance (OEV): 78.8%; F = 4.66; p = 0.0002). For the Campeche Sound significant positive associations between cestodes, digeneans, and the concentration of 1-methylnaphthalene were found (OEV = 67.3%; F = 4.6; p = 0.002). In Perdido region, the significant positive associations found were between cestodes and the concentration of Vanadium, and between digeneans and the concentration of n-enicosano. The analysis explained 64.4% of the variance (F = 2.199, p = 0.003). Our results suggest that digeneans and cestodes when present, had significant statistical associations with heavy metals and hydrocarbons, supporting the view that they should be considered in environmental monitoring programs.
DOUBLE TROUBLE IN COWNOSE RAYS IN SENEGAL

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Two species of cownose rays, *Rhinoptera marginata* and *Rhinoptera peli*, have recently been determined to occur sympatrically off the west coast of Africa. Confirmation of the identities of cownose rays from this region has paved the way for work on cestodes from these hosts collected off Senegal over a decade ago during field trips in 2003 through 2005. Whole worms fixed in formalin were stained, mounted, and examined with light microscopy. Scolecites were also examined with scanning electron microscopy. Sequence data for the D1–D3 region of the 28S rDNA gene were generated for specimens preserved in ethanol. *Rhinoptera marginata* and *R. peli* were each found to host a distinct species of *Duplicibothrium*, both of which are generally consistent with the diagnosis of *Duplicibothrium*, and both of which appear to be new to science. The bothridia of the species discovered in *R. marginata* resemble those of *Duplicibothrium cairae*, however they lack the 2 longitudinal septa present in the latter species. The species of *Duplicibothrium* parasitizing *R. peli* most closely resembles *Duplicibothrium minatum* but is smaller and generally possesses fewer proglottids. *Rhinoptera peli* was also found to host a particularly interesting species that was initially difficult to assign to a genus. Although it bears proglottids that are anatomically consistent with those of typical *Duplicibothrium* species, its scolex differs conspicuously from that of other members of the genus. Rather than bearing bothridia that are longer than wide, and thus a scolex that is similarly proportioned, this species bears bothridia that are substantially wider than long. As a consequence the scolex is more than 3.4 times as wide as the strobila. In addition, each bothridium bears what appears to be an anterior sucker flanked by 4 small marginal loculi on either side, followed posteriorly by a single row of 5 large, rectangular facial loculi. In addition, at approximately 2 cm in total length, it is a giant among its congeners. Nonetheless, phylogenetic analyses of sequence data place this species robustly among members of *Duplicibothrium*. These findings suggest that scolex morphology is less conserved than proglottid anatomy. They also raise questions about the nature of the anterior loculus in other members of the genus, such as *D. paulum*. Not only is this the first record of *Duplicibothrium* from the eastern Atlantic Ocean, but description of these three species will double the number of valid species in the genus. Furthermore, this report increases the number of species of *Rhinoptera* known to host *Duplicibothrium* to four of the 10 species of *Rhinoptera* currently recognized.

Depletes serum concentration of IFN-γ in helminthes infection and after antihelminthic therapy in a rural population in Nigeria

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Interferon-gamma (IFN-γ) at a relatively high concentration in the serum exhibit pro-inflammatory properties but it has been confirmed to be anti-inflammatory at reduced seral value. The study was aimed at understanding the seral concentration of IFN-γ in helminths infections and the concentration after the expulsion of helminths in the gut. All volunteers were infected by intestinal helminths and confirmed free of infection of malaria parasites by thick film microscopy. Specific treatment was given; Albendazole 400mg STAT dose, Praziquantel (60mg/kg) in 5 hours apart and Artemether/Lumefantrine (20mg/120mg) to take care of larval and adult stage of Schistosoma infections. A repeat collection of blood and stool samples was done 2 weeks after treatment for post-treatment evaluation. The levels of immune molecule was quantified by enzyme-linked immunosorbent assays (ELISA) specific for IFN-γ manufactured by Peprotech LTD, US from sera obtained from
80 helminths infected people. The lower limit of detection (LOD) for analyte was 31.3 pg/ml. Samples which had OD values below or above the standard range were re-analyzed at appropriate dilutions. The median value of immune molecule was determined using ‘R’ Programming (2016 version). The ova of intestinal helminths infection found were scanty and they included: Schistosoma intercalatum, A. lumbricodes, S. mansoni, Trichuris trichiura, hook worm, Strogyloides and Tape worm. No parasites were detected at post-infection evaluation. The median level of IFN-γ in the serum of helminths infected participants was low (59.8 pg/ml) which was further depleted (36.2 pg/ml) after elimination of helminths from the gut. The low serum concentration of IFN-γ in helminths infections and after expulsion from the gut may have clinical relevance.

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Development of a Species-Specific Quantitative PCR Diagnostic Assay for the Sea Lion Lungworm Parafilaroides decorus

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In 2017 approximately 1,000 California sea lions, Zalophus californianus, were stranded in Southern California. Most are young California sea lions that are emaciated, dehydrated and very underweight. About 70% of these pups come into rehabilitation centers infected with some form of a parasitic infection. One species of lungworm that infects California sea lions, Parafilaroides decorus, causes symptoms such as anorexia, coughing, dyspnea, and blood in the stool and urine. These infections have a high mortality rate, and can be difficult to diagnose as there is very little known about Parafilaroides decorus. In order to assess the threat they pose to sea lions and other marine mammals, and to allow for the conservation of these species, rehabilitation facilities must be able to diagnose and apply targeted treatment protocols to living animals. We receive lungworm samples that have been contextually and morphologically identified by the The Marine Mammal Center in California. We perform barcoding PCR to molecularly identify these nematodes. These barcoding protocols rely on the information in the NCBI’s BLAST database that works by comparing the new sequences to a wide variety of genes and genomes already in the database. Unfortunately, there are limited sequences of few loci from Parafilaroides decorus in the NCBI database, and the similar lack of sequences for other Parafilaroides spp, limits our identification to the genus level. These barcoding protocols are also not sensitive enough to identify nematodes in samples such as feces and sputum, which does not allow for noninvasive monitoring of infections. One of our goals is to utilize whole-genome next generation sequencing of these nematodes to create a sensitive and species-specific repeat-based quantitative PCR assay to identify and diagnose Parafilaroides decorus infections from noninvasively collected samples.

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Differences in larval activation of a multi-drug resistant strain of Ancylostoma caninum

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Hookworm disease remains among the most important neglected tropical diseases, with an estimated cost of 2-4 million disability-adjusted life years. Treatment is limited to mass drug administration (MDA) programs, which require repeated administration of anthelmintic drugs due to high
re-infection rates in endemic areas. As such, emerging anthelmintic resistance in hookworm populations is a pressing concern, and the identification of resistance mechanisms and alleles is key to prolonging the efficacy of existing drugs. Previously, our lab isolated and characterized a multi-drug resistant strain of the canine hookworm *Ancylostoma caninum*, which we named KGR. This strain was isolated from a retired racing greyhound that had a history of monthly heartworm preventative, and was refractory to multiple courses of fenbendazole treatment. Resistance to thiabendazole, pyrantel, and ivermectin when compared to the wild type (WMD) strain was confirmed using larval development assays. During our investigation, we tested for differences between KGR and WMD strains in infective third stage larvae (iL3) in vitro activation. Infective iL3 from each strain were incubated in the presence of S-methylglutathione and <10kD filtrate of canine serum, which mimics the host signal and is known to stimulate feeding (a marker for activation) by the larvae. Culture medium only was used for negative controls. We observed a significant reduction in feeding in KGR iL3 when compared to WMD iL3. Previous work from this lab has shown that cGMP and insulin-like signaling pathways mediate iL3 activation in hookworms, and that when iL3 are incubated with the cell permeant analog of cGMP, 8-bromo-cyclic GMP (8-br-cGMP), feeding is initiated. When testing 8-br-cGMP activation on WMD and KGR iL3, we found no difference in feeding between strains. This suggests that the inhibition of feeding seen in KGR larvae occurs upstream of cGMP in the activation pathway. Additional inhibitors are currently under investigation to illuminate the cause of the decreased feeding in KGR worms.

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**Different Coasts and Different Hosts: Development of Quantitative Real-Time PCR Diagnostic Assays for Two Populations of the Phocid Seal Lungworm, Otostrongylus circumlitus**

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To the detriment of much marine life, global warming is predicted to increase the range, prevalence, and virulence of aquatic parasites (Harvell et al., 2002). Accurate identification of these parasites can help us better assess the threats to wild marine mammals and treat infections of those in rehabilitation facilities. Our goal is to create sensitive, species-specific assays for post-mortem identification as well as diagnostic use in rehabilitating wild animals. To diagnose a live animal, regular PCR is often not sufficiently sensitive to pick up trace amounts of DNA from parasite eggs or larvae in the feces or blood. By using a repeat-based quantitative PCR assay, we gain sensitivity. In nematodes these repeats evolve quickly enough that they are also species-specific (Pilotte et al., 2016). *Otostrongylus circumlitus*, known as harbor seal lungworm, is a metastrongyloid nematode that causes mild symptoms in its intended host, *Phoca vitulina*, such as bronchitis. However the parasite has also been found in the Northern elephant seal, *Mirounga angustirostris*, where it migrates to the heart, causing disseminated intravascular coagulation, a fatal condition (Elson-Riggin et al., 2001). We developed a qPCR assay to identify *O. circumlitus* infections based on a repeat found by high-throughput sequencing of a sample from the Atlantic. Curiously, nematodes morphologically identified as *O. circumlitus* from the Pacific appear negative on our qPCR test. While it is possible that the repeat is too specific, it is also possible that these two parasite populations are speciating, much like their harbor seal host became two different subspecies after their isolation ~2.5 million years ago (Stanley et al., 1996). Preliminary barcoding data suggests the Pacific population is distinct and derived from the Atlantic population. After next-generation sequencing of more samples from both the Atlantic and Pacific, we hope to further explore the divergence of these populations, as well as develop an assay that can identify Pacific *Otostrongylus* infections from fecal or sputum samples.
Different malaria vector species can differ in their susceptibilities to endectocides

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Insecticide-treated bed nets (ITN) and indoor residual spraying (IRS) of insecticides has dramatically reduced malaria by killing Anopheles vector species that feed indoors on humans. But some vector species feed outdoors (exophagy) and some are as likely to feed on livestock as they are on humans (=zoophagy). ITN and IRS will not work against these malaria vectors. One strategy being investigated against these types of malaria vectors is the treatment of livestock with endectocidal drugs such as ivermectin. In this study, we compared the relative toxicities of several endectocides currently registered for use in cattle (e.g., ivermectin, eprinomectin, doramectin, abamectin) when fed to two zoophagic malaria vectors – An. stephensi and An. albimanus. There were marked differences in drug toxicities between these two vector species. For example, ingested ivermectin was extremely toxic to An. stephensi (oral LC-50 = 7 ng/ml) but totally ineffective against An. albimanus (oral LC-50 = 1,468 ng/ml). Thus, ivermectin treatment of cattle would be a viable strategy to reduce zoophagic vector populations in India and Pakistan where An. stephensi is a major malaria vector, but it would be a rather poor strategy to use in Central America where An. albimanus is a major malaria vector. Endectocidal treatment of livestock holds great promise as part of an integrated approach to eradicate residual malaria transmission through the world. However, it is imperative that the efficacies of specific endectocides against major zoophagic malaria vector species are determined beforehand in the laboratory, prior to their deployment in the field.

Differential expression of immune genes in Aedes aegypti mosquitoes infected with ZIKV and reared under variable dietary conditions

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Arboviral diseases transmitted by mosquitoes are a major cause of mortality throughout the world. The transmission of such pathogens is dependent on competent mosquito vectors that have the capability of maintaining the pathogen and transmitting them to a subsequent vertebrate host. Studies of mosquito vector competence have suggested that the interaction of pathogens with the mosquito immune system plays an important role in determining competence. The capability of many viruses to manipulate the host antiviral immune response suggests that complex host-pathogen interactions could influence the efficiency of the mosquito immune response. Larval diet, including N input, has also been implicated in the development of the insect’s immune response. In this study, we analyzed relative expression levels of a suite of immune genes from Aedes aegypti that were ZIKV infected, vs. uninfected controls and in response to larval-rearing in a range of experimentally controlled settings. qRT-PCR using previously published primer sets was used to analyze relative expression. Differential expression was observed in mosquitoes from the infected group in all immune genes analyzed and the general trend was that these genes were down-regulated when compared to housekeeping genes following viral infection. Significant changes in expression were also observed in response to larval C:N ratio, although these data were more complex. These results represent a step towards illuminating the influence of larval diet and viral infection on the immune response in mosquitoes, which may in turn have important consequences vector competence. We will also discuss ongoing experiments using RNA-seq to gain a broader view of the effects of viral infection and larval diet on transcriptional regulation in this host-pathogen system.
Diseases of cultured sea cucumber Isostichopus badionotus, in Yucatán, México.

Karen Ascenet Arjona Cambranes; José Quinatzin García Maldonado; Miguel Ángel Olvera Novoa; Amelia Paredes Trujillo; Itzel Sánchez Tapia; Itzel González Mercado; Víctor Manuel Vidal Martínez

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The Sea cucumber is a highly sought-after organism in Asian markets for human consumption, but its high demand has led to the depletion of wild populations all over the world. In the continental shelf of the Yucatan Peninsula, overexploitation and illegal fishing have diminished the sea cucumber populations. An alternative method of growing their population led by Cinvestav has been to develop a sea cucumber aquaculture. However, the presence of diseases and the scarcity of information on the causal agents have limited the success of the aquaculture of this important resource. To date, infections caused by bacteria, fungal, protozoan, platyhelminthes and opportunistic copepods have been reported mainly from Asia, but in Mexico there lacks an updated list of parasites and diseases under aquaculture conditions. The aim of our work was to identify the infectious agents causing diseases in sea cucumber Isostichopus badionotus cultured in Cinvestav’s marine station at Telchac, Yucatán. We focused on sea cucumbers in the larval and juvenile stages with health problems using 18s rDNA as a molecular marker and morphological characteristics of the putative causal agents. The results of the identification of these microorganisms haven’t been conclusive, but at this point five benthic copepod morphotypes belonging to the order Harpacticoida have been recorded. While the most abundant of these morphotypes is of the Miracia genus, their identification at a species level is still in progress. Likewise, spherical amoebas of ~ 10 μ diameter have been found around the stomach of larvae, occasionally causing up to 100% mortality rates in those affected. Additionally, skin ulcers in juvenile sea cucumbers apparently associated with Vibrio spp. have been observed in the culture, and the specific identification of the causal agent is in process. This work is the first contribution to the unexplored realm of investigation of sea cucumber diseases cultured in Mexico.

Distribution and diversity of metazoan parasites infecting Texas freshwater turtles.

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With only two major surveys on the helminth parasites of freshwater turtles in Texas, both of which were confined to one or two locations, this state and these hosts have been greatly understudied. Many studies have cited parasites as key ecological indicators, revealing the effects of environmental change before their host species. With the rapid changes in human land use and the global climate, baseline knowledge of parasite diversity is vital to understanding the changes that are occurring locally and worldwide. For this project, red-eared sliders (Trachemys scripta elegans), common snapping turtles (Chelydra serpentina), and spiny softshell turtles (Apalone spinifera) were collected from locations across the state of Texas. The turtles were necropsied and examined for endo and ectoparasites. A total of five species of acanthocephalans, nine species of nematodes, twelve species of trematodes, five species of monogeneans, two species of cestodes, five species of leeches, a species of mite, and a species of pentastomid were recovered from 79 turtles sampled. Acanthocephalans of the genus Neoechinorhynchus were the most common parasite of T. s. elegans, while nematodes of the genus Falcaustra were most common in C. serpentina and nematodes of the genus Spiroxys were the most common in A. spinifera. While acanthocephalans and nematodes were typically more abundant, trematodes were more diverse. Most parasites examined appear to be site specific within
the host, but will venture into other locations with high intensity of infection. A total of 12 new host records and 9 new locality records are reported. Through this study, the known host associations and geographical locations of many parasite species have been expanded.

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Do schistosome parasites affect behavior via the microbiome?

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Schistosomiasis is a chronic disease of inflammation caused by flatworms that invade the vasculature of the intestine or urogenital organs. Chronic infection of school children in endemic countries has been shown to reduce cognitive ability and increase behaviors such as anxiety, hyperactivity, and “acting out”. We hypothesize that schistosomes can influence host behavior through the gut-brain axis indirectly by influencing the microbiome of the intestine. Schistosome eggs passing through the intestinal wall create local inflammation that is likely to influence the microbiome composition and function. In turn, these changes could alter host behavior as inflammation in other disease contexts drives microbiome associated changes in anxiety behaviors and memory in mice. This pilot study aimed to determine the effects of schistosome infection on the behavior of a mice as a model of human infection. Mice infected with schistosomes were subject to several behavioral tests to assay anxiety (step down and open field), obsessive compulsive behavior (marble burying), learning and memory (Morris Water Maze). We found that infection significantly increased obsessive-compulsive behaviors compared to controls and that infected mice were significantly impaired in in early development of spatial bias for long-term and short-term memory. In an open field test, infected mice showed more anxious behaviors that uninfected controls. These results support our hypothesis that schistosomes alter host behavior. Future analysis of gut microbiome changes of these mice will enable us to examine the role of the microbiome as well as other potential mechanisms of this behavior change.

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Do the allocreadiid trematode Creptotrematina aguirrepequenoii represent a single species along its distributional range? evidence from molecules and morphology

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Creptotrematina aguirrepequenoii is an allocreadiid trematode that belongs to the biogeographical core helminth fauna of characid freshwater fishes of the genus Astyanax. This trematode species was originally recorded as a parasite of the Mexican tetra, Astyanax mexicanus in Nuevo León state, northeastern Mexico; according with published records, the species is widely distributed; its distribution range extends from northeastern Mexico to as far south as Costa Rica, in Central America. Here, we investigated the morphological and genetic variation of this allocreadiid across its distributional range. Between 2014 and 2016 we sampled individuals of Astyanax spp. in locations across Middle America, including rivers in Costa Rica, Nicaragua, El Salvador, and 8 states of Mexico; 64 specimens of C. aguirrepequenoii were collected from the intestine of their hosts. Some specimens were
processed for morphological study, including SEM; DNA was extracted from some specimens and sequences of a nuclear gene (28S rRNA) and a mitochondrial gene (cox1) were obtained. Morphologically, specimens are very similar, although some differences in the development of the muscular lobes were observed through SEM microphotographs. Genetically, 28S rDNA sequences show very low divergence levels, varying from 0 to 0.3%, while divergence through cox1 was higher and varied from 0 to 7.4%. Phylogenetic analysis through Maximum Likelihood and Bayesian Inference recovered all samples of Caligus aguirrepequenoi as monophyletic assemblages, with high nodal support values. We conclude that this species of allocreadiid is the same species along its distributional range, but a geographic structure is recovered in the cox1 analysis.

Does scratching control avian ectoparasites?

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Preening is one of the most important ectoparasite defense behaviors of birds. There are, however, regions of the body that birds cannot reach with their bills such as the head and neck. Without alternative ectoparasite defenses, these regions may become overrun by parasites. Scratching with the foot may be an effective defense against parasites in these areas. We tested the effectiveness of scratching to remove lice on captive pigeons by experimentally infesting birds with identical numbers of lice and using hobbles to prevent some birds from scratching effectively. Microhabitat censuses were taken to determine where on the host lice were found. We discovered that birds that were unable to scratch normally had significantly more lice than those that could scratch normally, and that these lice were found in higher concentrations on the head and neck which are regions that pigeons cannot preen. These results suggest that scratching is an important defense against ectoparasites. This study was the first experimental test of the role of scratching in controlling avian ectoparasites.

Don’t panic: a parasitology guide to the field work galaxy

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Laboratory and theoretical approaches contribute greatly to our understanding of parasites and their influences at many biological levels, but all of our knowledge ultimately relates back to phenomena occurring in natural systems. Field work is often relatively complex and difficult; however, the experiences and information gained can be invaluable, as well as complement other empirical approaches. To effectively conduct field work, one must be prepared to communicate its importance both beforehand and afterwards. It is also essential to plan carefully in order to comply with regulations, minimize environmental impact, work safely, use optimal equipment and methodology, and maximize gains in the field by collecting the right data. Effective planning should additionally consider how to work with others, including the public, various levels of government, and non-government organizations. Despite the best laid plans, it is also important to expect the unexpected when conducting field work, and this can become an additional opportunity to refine approaches and gather value-added information. Done properly, field work can offer many intangible and tangible benefits that enrich research in parasitology and open up possibilities for questions encompassing multiple taxa as well as spatial and temporal scales.
ECHINOSTOME SPP. IN GIT OF BATS MICROCHIROPTERA (Pipistrellus pipistrellus / Pipistrellus pygaemus) IN MULTAN, PAKISTAN

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Bats play a vital role in the dissemination of various virus, bacteria and parasites. The parasitological survey of Gastrointestinal Tract was conducted on bats of microchiroptera (Pipistrellus pipistrellus/ Pipistrellus pygaemus) found cosmopolitan including Pakistan. Ten freshly naturally killed bats were investigated for the presence of GIT parasites. Upon investigation it revealed the presence of trematodes of Echinostome spp. dominating in the small intestine. The study revealed the presence of trematodes which might be indicator for the presence of intermediate/secondary host harboring the various stages of immature fluke(s). The excessive presence of flukes may indicate the abundance of food found for bats present in the area. Further molecular investigation may be conducted for the confirmation of trematode species and its zoonotic importance.

EVALUATION OF THE OCCURRENCE OF OOCYSTS OF CRYPTOSPORIDIUM SPP. IN THE DISTRIBUTION NETWORK OF THE WATER TREATMENT PLANT RUFINA ALFARO AND RAW WATER OF THE LA VILLA’S RIVER ASSOCIATED WITH PHYSICAL AND CHEMICAL PARAMETERS, IN DRY AND RAINY SEASON AND TOTAL COLIFORMS IN THE LOS SANTOS PROVINCE

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The Protozoan parasites such as Cryptosporidium and Giardia are one of the biggest problems in health public water supplies, since these parasites outweigh without difficulty (Castro-Hermida, 2011) systems. The main objective of this research was to evaluate the occurrence of oocysts of Cryptosporidium spp. in the distribution network of the water treatment plant Rufina Alfaro and the La Villa River raw water, associated with total coliforms and physico-chemical parameters, during dry and rainy; This study used a completely randomized, descriptive design, performing in two seasons of the year, the Seca who understood from February to March, 2013 and the Lluviosa, which comprised of August and September 2012, in Uptown and low pressure of the water distribution network, and at the entrance of raw water from the ground water treatment plant Rufina Alfaro, with a total 48 samples for two months, where we evaluated two biological parameters such as Cryptosporidium Oocyst count spp. and total coliform colonies, and four physical and chemical parameters such as pH, temperature, turbidity and chlorine. Found that differences in the occurrence of Cryptosporidium oocysts there spp., related to times, areas and weeks of sampling the results produced, increased occurrence of Cryptosporidium oocysts were spp., in the rainy season, as well as Total coliforms in drinking water distribution network in rainy season, as well as total coliforms in drinking water distribution network during the rainy season. There is no relationship between the occurrence of oocysts of Cryptosporidium spp., coliforms and the physical and chemical parameters in this study.
EXPLORING THE ROLE OF CHEMICAL COMMUNICATION IN PARASITE-MODIFIED HOST BEHAVIOUR

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Many parasites cause host behavioural changes which increase the likelihood of transmission to the next host. However, few studies elucidate the mechanisms underlying these changes. We performed a series of behavioural experiments with echinostome trematodes to determine if potential second host snails (*Helisoma trivolvis*) were more attracted to parasitized first host conspecific and heterospecific (*Lymnaea elodes*) snails. In a Y-maze tank a choosing snail (*H. trivolvis*) was placed in the base and a stimulus snail was restrained in either arm. Four treatments (30 replicates each) were each digitally filmed for 30 minutes: T1) control/no snail, T2) 1 non-parasitized stimulus, T3) 1 parasitized stimulus, T4) 1 parasitized and 1 non-parasitized stimulus. In the conspecific trials, there were no significant responses in any of the treatments. When the stimulus snail was heterospecific, snails spent more time with the stimulus and entered the snail arm more times than the non-snail arm in T2 and T3. Non-parasitized *L. elodes* in T2 were not chosen first, but parasitized *L. elodes* in T3 were. When both parasitized and non-parasitized stimuli were present (T4), responding snails spent significantly more time in proximity to parasitized heterospecific hosts. These results indicate that potential second host snails were more attracted to parasitized heterospecific first host snails than non-parasitized heterospecific and parasitized and non-parasitized conspecific snails. High-performance liquid chromatography was used to test whether parasites were affecting the chemical communication of the stimulus snail. We will discuss whether there were differences in the amount and type of signalling molecules such as eicosanoids in parasitized vs non-parasitized conspecific and heterospecific snail-conditioned water and report on the results of behavioural trials where only signalling molecules were used as stimuli. This study demonstrates that parasites alter the behaviour of second intermediate host snails and provides insight into the role of chemical communication in parasite-altered behaviour.

### Early-life disruption of gut microbiota increases later-life infection risk in frogs

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Host-associated microbiota can affect infectious disease risk, which might be inadvertently altered by pollutants. These interactions could be particularly important during the formative times of immune system development of the host. Here, I tested whether an early-life disruption of the microbiota of Cuban tree frogs (*Osteopilus septentrionalis*) affects later-life resistance to infections by manipulating the microbiota of tadpoles and challenging them with parasitic gut nematodes (*Aplectana hamatospicula*) as adults. Adult frogs with reduced bacterial diversity as tadpoles were more susceptible to infection compared to adults without their microbiota manipulated as tadpoles. Given these results, I then determined whether the microbiota of frogs mediates the effect of early-life exposure to pollutants on infection risk. Tadpoles were experimentally treated with an insecticide or solvent, crossed with either microbe-manipulated or non-microbe-manipulated water. After metamorphosis, adult frogs were exposed to *A. hamatospicula* nematodes. The insecticide-treated water, with and without microbes, decreased bacterial diversity in the guts of tadpoles, which was associated with increased infection risk in adults. These results support the idea that preventing an early-life disruption of host-associated microbiota, such as with pesticides, confers protection against infections later in life.
Ecological associations of acanthocephalans in freshwater snail hosts

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In many acanthocephalan life cycles, a vertebrate paratenic host is used to bridge an ecological gap between the intermediate and definitive hosts. However, there are a few reports of freshwater snails serving as paratenic hosts for acanthocephalans. To assess how commonly freshwater snails serve as hosts for acanthocephalans, 2 species of freshwater snails, Helisoma trivolvis and Physa acuta, were collected from 29 wetlands throughout Oklahoma. Additionally, snails were sampled monthly for a year from a single location to further investigate seasonal variation of infection. Snails were dissected for juvenile acanthocephalans by flattening snail tissue between slides. Based on complete ITS rDNA sequences, these juvenile acanthocephalans were identified as Neoechinorhynchus emydis, which infect turtle and ostracod hosts in nature. Lastly, we evaluated acanthocephalan recruitment and any growth that may occur in snail hosts by exposing laboratory-reared H. trivolvis snails to naturally infected ostracod hosts in field cage experiments. Each week, for 5 weeks, a sub-sample of snails was collected and necropsied from the cages. Among all sites sampled, 7 of 29 (24%) contained snails infected with acanthocephalans, with H. trivolvis being more commonly infected than P. acuta. Depending on the site, prevalence and mean intensity ranged from 4–79% and 1–3.6, respectively. Throughout the year, prevalence peaked at 73% during the summer and decreased to 0% during the winter. In field experiments, snail acanthocephalan infections were observed during weeks 2–5, with a prevalence of 15% and mean intensity of 1.3, with an average worm length of 1.2 mm (range 0.9–1.9 mm). However, there were no significant differences in average worm length between weeks (P > 0.05), strongly suggesting these acanthocephalans do not grow in their snail hosts. Our results suggest that 1) freshwater snails may be important hosts for the transmission of N. emydis to aquatic turtles that commonly feed on freshwater snails; 2) location and season have a strong effect on the variation of acanthocephalan infections in snails; and 3) acanthocephalans appear not to be growing within their snail hosts, which has important implications on transmission.

Ecology and Evolution of Parasitism

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It is well accepted that parasites evolved from free-living ancestors, but empirical evidence for how and why parasitism evolved is limited. A commonly proposed hypothesis posits that intermediate strategies, such as phoresy and facultative parasitism, serve as stepping-stones towards the evolution of obligate strategies. The fitness benefits gained from those intermediate associations then allowed some symbioses to evolve towards more obligate relationships by means of natural selection. We used a facultative parasite system to experimentally test assumptions and hypotheses surrounding the evolution of parasitism. First, we tested for the presence of additive genetic variation in infectious behaviour in the facultatively parasitic mite Macrocheles muscaedomesticae. Mites responded positively to selection for increased infectivity; realized heritability of infectious behaviour was significantly different from zero and estimated to be 16.6% (±4.4% SE). We then tested for the presence of evolutionary trade-offs potentially constraining the evolution of infectivity by comparing the longevity, fecundity and infection plasticity of selected and control mites. Our data did not support the presence of evolutionary trade-offs. Facultative parasitism in M. muscaedomesticae is potentially
maintained by the variable and unpredictable nature of their habitats or perhaps through the utilization of a bet-hedging strategy. Our study is the first to provide an estimate of additive genetic variation for infectious behaviour in a facultative parasite and provides insight into their life history, with important implications for the evolution of parasitism.

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Effects of Plastic Microbeads on Host Longevity and Cercariae Production in Trematode-Infected Snails

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There are enormous amounts of plastic in the environment that persist for long periods of time, with important possible consequences for aquatic fauna in particular; however, little is known regarding potential affects on host-parasite dynamics. The same is true for small plastics called microbeads that are primarily found in toiletries and other personal care products, which range between 0.1 μm and 5mm in size. Once these products get washed down drains, they are not captured by filters at wastewater treatment plants, and thus travel into waterbodies through effluent or in run-off from head-containing biosolids applied to agricultural fields. This becomes problematic, because microbeads are often ingested by various aquatic organisms due to their similar appearance to food such as phyto- or zooplankton, or by adhering to food sources. The purpose of the current study was to examine the effects of microbead ingestion on: 1) longevity of freshwater host snails (Stagnicola elodes) infected by the trematode Haematolechus sp.; and, 2) the emergence of Haematolechus sp. cercariae. Through this three weeks lab study, 135 individual host snails were fed food cubes containing either 0, 10 or 100 polyethylene microbeads sized 106-125μm. Another group of 45 infected snailswere fed the same microbead concentrations and thenumber of cercariae emerging were counted weekly for the same period. Snails exhibited a significant non-monotonic response to microbead diet in relation their average and total production of cercariae, with the lowest quantities seen in the 10 microbead treatment. However, the snails fed the highest amount of microbeads had the shortest longevity. These results indicate complex effects of microbead diet on potential transmission of cercariae to the next host in the life cycle. Future studies will be needed to explore the trend of greater snail growth in the highest microbead treatment, and other ways in which microbeads may affect host-parasite interactions.

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Effects of an Eye-Dwelling Parasite on the behavior of a New Zealand Fish

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The trematode family Diplostomidae contains many species that inhabit the eyes of fishes as metacercariae. Of these, members of the genus Diplostomum are often studied as potential manipulators that cause their fish host to be predated at a higher rate. Recently, the first diplostomid described in New Zealand, Tylodelphys spp., was found in the eyes of a native freshwater fish, the common bully Gobiomorphus cotidianus. Tylodelphys spp., as other congeners, resides in the humors of the
eyes of its fish host until it is eaten by the definitive host, a piscivorous bird. Common bullies are the most widespread of New Zealand’s native fish and are important prey items for predators (e.g. birds and eels). Our study investigates how infection by Tylodelphys spp. affects fish behaviors that rely on vision (e.g. predator avoidance, microhabitat selection, foraging). Fish were tested in aquarium conditions for their response to predators (using a computer animation simulating a shadow moving over the fish) and their preference for microhabitats with different lighting levels. Our results reveal the subtle ways in which parasite-induced vision impairment may influence fish behavior and possibly parasite transmission.

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Effects of habitat fragmentation on the microbiome of blood-feeding ectoparasites of bats

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As the body of research on host-microbiome interactions continues to grow, it is becoming more apparent that the host is not a perfect island that physically and biologically constrains the microbiome. The composition and diversity of the microbiome and abundance of its members are influenced by both its immediate environment (the host) and broader environment (where the host lives). Habitat fragment size and distance from a source are important variables influencing community composition of plants and animals, but their role in microbial community composition and turnover is unknown. It is hypothesized that host evolution and ecology influences the microbiome more than broader environmental factors, but this hypothesis has not been tested. To compare the relative influence of broader environment to that of host environment, we applied high-throughput sequencing of the V4 region of 16S rDNA from 226 obligate ectoparasitic bat flies (8 species) collected from 155 bats (5 species) from 10 habitat fragments in the Atlantic Forest of Brazil. By examining the microbiome of ectoparasitic bat flies, we are able to treat the bat fly as the host environment that is somewhat shaded from the effects of the broader environment, because parasites are dependent on their hosts as habitats. If the host bat fly controls composition and turnover of the microbiome more than the broader environment, then composition of the microbiome will vary according to bat fly phylogeny. If the broader environment influences the composition and turnover of the microbiome more than the host bat fly, than the microbiome will vary according to habitat fragment size and distance from the source. As habitat fragmentation continues to rapidly increase globally, understanding the influence of environment on organismal microbiomes is important for understanding the relationship between animal and plant development, physiology, and health.

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Efficacy of Cotrimoxazole on Malaria Parasitaemia, CD4+ count and Anaemia among HIV-infected pregnant Women in Jos, Nigeria**

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ABSTRACT
Although Cotrimoxazole is being used for antimalarial prophylaxis among HIV-infected individuals, its effectiveness against malaria parasitaemia, CD4+ count, PCV and placental malaria parasitaemia in HIV-infected pregnant women in Nigeria have not been sufficiently investigated. Two hundred and thirty-five (235) HIV-infected pregnant women were studied for the efficacy of prophylaxis with Cotrimoxazole (CTX) against malaria parasitaemia, CD4+ count, PCV and placental malaria parasitaemia. Study population was divided into 2 groups; Group A took CTX while group B took sulfadoxine-pyrimethamine (SP) for antimalarial prophylaxis in pregnancy. Peripheral blood samples were examined before and after prophylaxis, while placental blood was examined at delivery for malaria parasitaemia using the thick and thin blood smears. CD4+ count and PCV were determined using flow cytometry and capillary tube methods respectively. Cotrimoxazole reduced malaria parasitaemia by 89.4%, while SP gave 79.01% reduction. Mean CD4+ count (cells/µl) was significantly (P<0.05) increased from 223.55 to 300.54, and decreased from 570 to 534.4 with CTX and SP respectively. Mean PCV was significantly (P=0.015) increased from 33.09% to 33.20% after prophylaxis with CTX, but insignificantly (P=0.154) reduced from 33.11% to 32.90% with SP. No malaria parasites were detected in the cord blood of group A infants while 3.2% of group B infants were infected. Placental malaria was 5.9% among group A and 7.5% among group B. Mild anaemia was recorded in the placentas of both groups. Infants’ anaemia (PCV<42%), was statistically significantly (P<0.05) higher among study population who took Cotrimoxazole (CTX) for prophylaxis compared to those who took SP [(62%) and (42%) respectively]. Prophylaxis with Cotrimoxazole was associated with more reduction in malaria parasitaemia with reduced effect on both maternal and infant PCV.

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Efficacy of Sm-p80-based schistosomiasis vaccine in baboons: Mimicking natural conditions of chronic infection, treatment and reencounter with parasites

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Efficacy of Sm-p80-based schistosomiasis vaccine in baboons: Mimicking natural conditions of chronic infection, treatment and reencounter with parasites

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Schistosomiasis is a neglected tropical parasitic disease affecting over 200 million people with an additional 779 million people at risk of infection. Based on the hypothesis, that for every infected individual positive for schistosome egg there is an egg ‘negative’ infected counterpart, the current estimate for the number of infected people is between 400 and 600 million. Endemic in 79 countries, schistosomiasis accounts for 280,000 deaths annually with another 20 million individuals suffering from severe symptoms such as anemia, internal bleeding, chronic diarrhea, hepatosplenomegaly and organ damage. Vaccine development and implementation against Schistosoma spp. aims to decrease schistosomiasis disease burden among individuals and communities mostly at risk. In this study, the efficacy of the Sm-p80 based vaccine was evaluated against a Schistosoma mansoni infection in a baboon model designed to mimic vaccine intervention in endemic regions following establishment of a chronic infection and drug-treatment. This study utilized a new form of monitoring an infection by using a CAA assay, which correlates with the number of worms present in an infection. Through
this method, we saw a reduction of the CAA in serum by 28.2% and 64.5% in urine at time of necropsy. Immunization with Sm-p80 vaccine reduced worm and hepatic egg burdens by 13.9% and 38%, respectively. In addition, there was a significant reduction of 72.2% and 49.4% in egg load in the small and large intestines, respectively, of vaccinated baboons compared to the controls. More importantly, egg hatching rates were significantly reduced 60.4%, 48.6% and 82.3% in liver, small and large intestine respectively, by highlighting transmission-blocking capability of Sm-p80 vaccine. Confocal microscopy with immunofluorescence revealed the expression of Sm-p80 on the outside of both male and female worms as well as developed eggs, explaining why our vaccine efficiently decreased worm burden and affects the maturation and hatching of the eggs. Overall, data presented in this study on the anti-fecundity, anti-pathology and transmission-blocking efficacy of Sm-p80 supports further development of Sm-p80 as a vaccine against schistosomiasis.

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**Endoparasites from Bolivian Mammals: First record of hookworms (Ancylostoma) from Ctenomys**

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Endoparasites from Bolivian Mammals: First record of hookworms (Ancylostoma) from Ctenomys G. O. Drabik and S. L. Gardner, H. W. Manter Laboratory of Parasitology, University of Nebraska State Museum, University of Nebraska-Lincoln, Lincoln, Nebraska 68588-0514

In the last century, the American Museum of Natural History (AMNH), the Museum of Southwestern Biology (MSB), the Harold W. Manter Laboratory of Parasitology (HWML), and the Bolivian National Museum of Natural History in La Paz mounted joint collecting expeditions throughout Bolivia to survey and inventory Sylvatic mammals and their parasites. This work reports the discovery of hookworms of the genus Ancylostoma Dubini in subterranean rodents of the genus Ctenomys de Blainville collected in the lowlands of eastern Bolivia. From five individual Ctenomys boliviensis and three of Ctenomys steinbachii collected between August 1984 and June 1990 from near Santa Rosa de la Roca, Dept. of Santa Cruz, a total of 14 nematodes were recovered from the small intestines. From Ctenomys boliviensis collected from Santa Cruz Dept., near Santa Rosa de la Roca a 28.6% prevalence of Ancylostoma was observed, while Ctenomys sp. collected from Estacion El Pailon had a prevalence of 9.1%. Observations and measurements of standard morphological characters were made with a computerized Zeiss Axiophot microscope and these data were compared to published descriptions of species of Ancylostoma from the Neotropics. An previously unreported papilla (potential autapomorphy) on the copulatory bursa of our specimens was noted during our investigation. Currently we are calling these nematans A. buckleyi, originally described from Felis concolor L. necropsied in the London Zoo in 1957, the cat being wild-caught in Argentina. If this is not an undescribed species, this would represent the first record of A. buckleyi from an Hystrix-nathous rodent in the Neotropics. Funded by NSF grants: DBI1458139, BSR8612329, BSR9024816, DEB9496263, DEB9631295, BSR8408923, BSR8316740, and DBI0545051.

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**Endoparasites of public health importance in an invasive mammal species: the case of the American mink (Neovison vison) in Navarino Island, Region of Magallanes and Chilean Antarctica, Chile**

Francisco Ramirez Pizarro¹; Rene Ortega¹; Veronica Madrid¹; Italo Fernandez¹; Carlos Landaeta Aqueveque¹
The American mink (*Neovison vison*) is an invasive species in different countries of the world, including a country of South America, Chile. The mink presents a high impact in Chile because it depredates the wildlife and it can host endoparasites transmissible to other animals, including the human. The aim of this work was the search for *Cryptosporidium* and *Trichinella* in minks inhabiting Navarino Island, Region of Magallanes, and in Maullín and Ancud, Region of Los Lagos, Chile. 94 minks were examined and muscle samples were preserved in thimerosal 0.1%. Furthermore, 73 stool samples of minks were preserved in 70% ethanol. Muscle samples were subjected to artificial digestion to assess the presence of *Trichinella*. DNA was extracted from 0.2-0.4 grams of stool with two extraction kits: PowerSoil DNA Isolation Kit and QIAamp DNA Stool Mini Kit, following the manufacturer’s instructions. Molecular detection of *Cryptosporidium* was done by nested Polymerase Chain Reaction (PCR) on a SSU-rRNA gene fragment (~590 bp). Phylogenetic analysis was performed with software MEGA 7 to determine the species of *Cryptosporidium*. *Trichinella* larvae were not observed in muscle samples. DNA of *Cryptosporidium parvum* was detected in 3 minks (6.66%), all of them from Navarino Island. The prevalence of *Cryptosporidium* in the mink from Navarino Island is similar to other countries. For example, 5 of 81 minks (approximately 5%) from Ireland were positive for *Cryptosporidium* by PCR. Currently, the American mink has been reported as host of four species and two genotypes of *Cryptosporidium*: *C. andersoni*, *C. canis*, *C. meleagridis*, *C. parvum* “ferret” genotype and *C. “mink” genotype. These species and the “mink” genotype have the ability to colonize the gastrointestinal tract of the human, so they represent a risk to public health. Finally, the finding of *C. parvum* in the American mink from Navarino Island is the southernmost in the world and its presence suggests a role of the mink in the epidemiology of this parasite in southern Chile.

Evaluation of cyst fluid based enzyme electroimmune transfer blot for diagnosis of neurocysticercosis in a rural endemic population of North India

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Cysticercosis, caused by *Taenia solium* larva is a major public health problem in the developing world and neurocysticercosis (NCC) is considered to be the most common parasitic infestation of the central nervous system. Human is the only definitive host of *T. solium* harboring adult tapeworm in the intestine (taeniasis), while both human and pig can act as intermediate host and harbor the larvae in different internal organs (cysticercosis) including brain (NCC). NCC is identified as the single most common cause of community acquired active epilepsy; 26.3% to 53.8% active epilepsy cases in the developing world including India and Latin America are due to NCC. It is also becoming more common in the developed world because of increased migration of people with the disease or *Taenia solium* carriers and frequent travel to the endemic countries. Clinical symptoms associated with NCC are pleomorphic in nature. Radio-imaging tools are most reliable diagnostic tools. Hence, diagnosis of NCC at primary care facility is still challenging due to lack of reliable sero-diagnostic tools and absence of radio-imaging facilities. The existing sero-diagnostic tools are of limited use in highly endemic population like India, where patients have exposure to several other intestinal parasites. Enzyme electroimmune transfer blot (EITB) is considered an important serological test for diagnosis of neurocysticercosis (NCC). We evaluated *T. solium* cyst fluid based EITB and Lymphocyte transformation test (LTT) for the diagnosis of NCC in urban and in highly taeniasis endemic rural pig farming populations. Positive EITB (any band reactive <50kDa) was significantly associated with NCC in urban (p<0.001) but not in rural pig rearing populations (p=0.292). Similarly, the sensitivity and specificity of LTT for diagnosis of NCC was 93.8% and 96.2% respectively. However,
Evolution of precocious life cycles in the trematode genus Alloglossidium

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The addition or removal of a host from a parasite’s life cycle is not a trivial evolutionary event; life cycle transitions can have major consequences for parasite transmission, behavior, physiology, development, and mating systems. Hence, evolutionary change in life cycle complexity is of central importance in parasitology. Studying the causes and consequences of complex life changes hinges on knowing the evolutionary order of life cycle transitions, which in itself, requires data on phylogenetic relationships among species that differ in life cycle patterns. Unfortunately, such data remain scarce. We present a molecular phylogeny of the trematode genus Alloglossidium, which contains several species that display precocious (a.k.a., progenetic) life cycles (i.e., sexual maturation in what is typically regarded as an intermediate host). The genus was recognized early on as a model system to study parasite life cycle evolution and as such, has been the subject of morphological/life history-based phylogenetic hypotheses over the past 4 decades. The most recent of these hypotheses shows an ancestral 3-host pattern with a fish definitive host. There was then a single transition to a 2-host life cycle with maturation in a crustacean host, followed by a host jump into a leech definitive host. The molecular phylogeny also indicates an ancestral 3-host pattern using a fish definitive host, but similarities largely stop there. In contrast, the molecular data show an early split in the history of the genus. In one clade are all species with a 2-host life cycle and leech definitive host. The other clade has an ancestral 3-host life cycle using ictalurid catfishes as definitive hosts. Within the latter clade, there are at least 2 additional independent evolutionary events that lead to a precocious life cycle (loss of catfish host and maturation in a crustacean). We conclude by discussing how existing hypotheses on the evolution of precociousness, and parasite life cycle complexity in general, may or may not relate to the patterns observed in genus.

Evolution of the cationic ligand-gated ion channel subunit gene family in the nematodes

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Post-synaptic neurotransmitter receptors of the helminth neuromuscular junction (NMJ) are of particular interest as anthelmintic drug targets. These pentameric ligand-gated ion channels (pLGICs) are composed of subunits encoded by a diverse gene family that has expanded within the nematodes. The evolutionary mechanisms that gave rise to this diversity is largely unknown. Here we present a comprehensive annotation, phylogeny and bioinformatic analysis of the cationic pLGICs at the nematode NMJ with particular attention to subunit gene duplication and loss events. Ion channel subunit genes were manually curated from the Helminth Genome Project genomes. Multiple sequence alignments (MAFFT) and maximum likelihood gene trees (PhyML) were used to identify and
date gene duplication and loss events. Substitution rates were estimated along branches following gene duplication or gene loss events (PAML). Positively selected sites in subunits under elevated substitution rates (HyPhy) were mapped onto modelled structures (YASARA). A marked expansion of the pLGIC gene family occurred after divergence from the Clade I nematodes. Significant loss of subunit genes occurred within the filarial Clade III nematodes. Substitution rate analysis suggests subunits diverge in function following duplication. Of particular interest are the putative subunits of the levamisole receptor in filarial nematodes. All but one non-alpha type subunit has been lost while both alpha type subunits unc-38 and unc-63 show independent duplication, with one copy of each showing characteristics of the non-alpha type with increased substitution rates. The many species with genomes available provides an unparalleled opportunity to determine if prior loss of subunits predisposes persistence of other duplications or the reverse. Functional characterization of these subunits is ongoing to determine the mechanisms involved in their evolutionary change.

Excretory-secretory products from activated infective juveniles of the entomopathogenic nematode Heterorhabditis bacteriophora suppress the Drosophila antimicrobial peptide response.

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Due to its effectiveness as a biocontrol agent against insect pests and its relatively close common ancestry with important parasitic nematodes of humans, the entomopathogen Heterorhabditis bacteriophora represents an opportune model for the identification of generalized nematode virulence factors that target host immune systems. These immunomodulating factors are likely to be present in the excreted-secreted (ES) products of the nematode released early in infection in response to a host signal. To investigate the molecules, we incubated axenic H. bacteriophora strain TT01 infective juveniles (IJ) in hemolymph plasma of a natural lepidopteran host, Manduca sexta, followed by transfer to medium without hemolymph to collect the ES products. Following concentration, the ES products were injected into Drosophila melanogaster adult flies, and Drosophila RNA was collected for RT-qPCR-based analysis of immune gene expression at illustrative time points. The results of these assays demonstrated a specific and substantial suppression of diptericin (an antimicrobial peptide in Drosophila) expression, with upregulation dropping from approximately 600-fold in flies exposed to ES products from non-activated IJs to approximately 200-fold in flies injected with ES products from hemolymph-activated IJs. Broadly, this indicates Immune deficiency (Imd) pathway interference that could serve to diminish the host’s ability to respond to either the nematode’s pathogenic bacterial symbiont released during the infection, or to tissue damage inflicted by the nematode itself. In association with the concomitant inability of the ES products to elicit mortality in injected flies, this observed reduction in immune responsiveness demonstrates the presence of not directly toxic, but rather immunomodulatory virulence factors that could potentially be exploited to tune the nematode’s infective program with the aim of either enhancing the nematode-based biocontrol of agricultural pests, or diminishing the burden of infection on human hosts.

Experimental evaluation of behavioural changes in gilt-head seabream infected by brain-encysted Cardiocephaloides longicollis (Trematoda, Strigeidae) metacercariae

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Trophically transmitted parasites may increase their transmission efficiency by altering the behaviour of infected hosts, thus increasing their susceptibility to predation by next hosts. The strigeid trematode *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982, highly parasitizes, among other 30 fish species, the gilt-head seabream (*Sparus aurata* L.), one of the most important fish in Mediterranean aquaculture. The cercariae penetrate the skin and migrate into the brain, where they encyst as metacercariae. It is commonly believed that the cysts could cause significant alterations in fish behaviour increasing their transmission to the definitive host, as evidenced in other brain-infecting trematodes (e.g. *Fundulus parvipinnis*). However, this behavioral pathology suggested to be provoked by *C. longicollis* has never been experimentally studied. In this study, an experiment to detect differences in the behaviour of infected and uninfected fish was performed. First, 14 fish were experimentally infected with 180 *C. longicollis* cercariae, emerged from *Nassarius reticulatus*. Behaviour experiments were run 6 months post-infection to ensure that metacercariae were infective. Fish were placed in a plexiglass tube (200 cm height, 30 cm diameter) where an effective light-dark gradient was generated. The water column was vertically divided into 20 cm sections and the position of each fish at 1-min intervals for 30 minutes every 2 hours during 3 days was recorded in three assays, i.e. control, infected and mixed fish group. Preliminary results show significant differences in behaviour between control and infected fish. This may suggest that encysted metacercariae might provoke this behavioral alteration in infected fish within the tube associated to a neuronal disorder. Despite of the fact that most metacercariae infect the optical lobes, this behaviour might not be a consequence of a decrease of light perception, as infected fish occupied darker depths. However, other aspects of fish vision, such as visual acuity, could be affected, which has to be further studied.

**FLUCTUATING ASYMMETRY OF SCLEROTIZED STRUCTURES OF HALIOTREMA SPP. (MONOGENEA: DACTYLOGYRIDAE) AS A BIOINDICATOR OF HEAVY METAL CONTAMINATION IN THE COAST OF THE YUCATAN PENINSULA, MEXICO**

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The anchors are attachment structures that help monogeneans to resist physical dislodgement from the host. Natural random deviations in the shape or size of anchors can occur between the left and right sides, a pattern known as fluctuating asymmetry (FA). Few studies have successfully accounted for changes on patterns of symmetry in the anchors as bioindicators of environmental quality, but none has quantified the potential effect of heavy metal contamination on those patterns. First, we need to know the patterns of symmetry of the anchors on a region without heavy metal contamination as we assume it is the Yucatan Peninsula, and posteriorly compare similar patterns with monogeneans from oil extraction regions. Therefore, the aims of this study were to describe the intraspecific variability in the shape and size of the anchors of *Haliotrema* striatohamus and *Haliotrema* sp. from continental shelf of the Yucatan Peninsula. Data on grunts *Haemulon auriculatum* monogeneans and heavy metals were obtained from oceanographic cruises of the SENER Project granted to the CIGOM consortium. A Principal Components Analysis (PCA), was performed to explore potential group formation. Procrustes ANOVA was used to quantify the relative amounts of shape variation of asymmetry (left-right sides) and measurement error among anchors. A Generalized Additive Model (GAM) was applied to assess the potential effect of heavy metals on FA of the anchors of *Haliotrema* sp. PCA showed no clear group formation. We detected significant FA for anchor shape in *H. striatohamus* and *Haliotrema* sp., and for size in both anchors on...
the former. We found a significant positive relationship between Cd, Pb, Ni, V and FA in shape for Haliotrematoidei spp., and size with Ni, Cd and Pb for H. striatohamus. These significant relationships suggest that haptoral asymmetry of Haliotrema spp. may be useful bioindicator of heavy metal contamination.

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Finding (or not finding) old and new worms: Survey work of Neoechinorhynchus type localities

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Although freshwater fish parasitology has a long history in North America, there remain many opportunities for discovery. Many species discovered and described by researchers in the 20th century (e.g., Van Cleave and Mueller) have subsequently been neglected, so that there are many species that have not been analyzed with modern taxonomic tools like scanning electron microscopy and DNA sequencing. This is exemplified by species of the acanthocephalan genus Neoechinorhynchus Stiles and Hassall, 1905, which includes a diversity of species from North American freshwater fishes and turtles, and upon which we have chosen to focus our survey work. Not a single one of its species from the USA has previously been the focus of a modern integrative taxonomic study, so there is great room for taxonomic improvement. In our survey, we have placed emphasis on sampling of catostomid fishes since Neoechinorhynchus species are particularly diverse among members of this fish family. In order to effectively describe new or redescribe existing species, an important first step in this research program has been to revisit the type localities of Neoechinorhynchus to obtain newly collected specimens to subject to both morphological and molecular analyses. To date, we have conducted survey work at type localities for eight species in five states in the USA: New Hampshire, Minnesota, Oklahoma, Mississippi, and Tennessee. Although we successfully obtained catostomids at six of eight localities, we only encountered Neoechinorhynchus specimens in two. Many of the catostomids we examined were infected with other parasitic worms – including other acanthocephalans – but Neoechinorhynchus were absent. This is in stark contrast with our long-term survey work in central East New York – an area not previously reported for Neoechinorhynchus – where we have found three new species of Neoechinorhynchus from catostomids and see infection in other fishes year-round. This presentation summarizes the results of our survey work on Neoechinorhynchus in terms of discoveries, obstacles, and surprises.

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First Systematic Snapshot of Neoechinorhynchus from the United States and Canada

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Neoechinorhynchus Stiles and Hassall, 1905 is a highly diverse and widespread genus of acanthocephalan that parasitizes fishes and turtles on six continents. The literature base for the genus grew rapidly throughout the 20th century, mostly as a result of original descriptive work recognizing the diversity across the world. There have been, however, relatively few contributions regarding North American species of Neoechinorhynchus since the turn of the 21st century, and a particular lack of use of modern molecular tools. Several recent studies used DNA sequence data to understand species
relationships and uncover cryptic species of *Neoechinorhynchus* in Mexico, but there is still no molecular phylogenetic analysis focused on species of *Neoechinorhynchus* from the USA or Canada. Here we present the phylogenetic results of the first such study, based on nuclear DNA from the internal transcribed spacer (ITS) and large ribosomal unit (LSU) regions, including molecular data from multiple specimens of each of 17 total species (two from turtles and 15 from fishes) from the United States and Canada. We also present morphological evidence supporting the boundaries of several of these species. In addition to giving the first look at relationships between these species, this work has uncovered three previously uncharacterized species from white suckers of Central New York State. Plus, we have found that a species of another neoechinorhynchid genus, *Octospinifer*, nests among species of *Neoechinorhynchus*, lending further support to the recently recognized paraphyly of this large and somewhat enigmatic genus.

First record of lagochilascariosis in Quintana Roo

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Lagochilascaris minor is one of the five valid species within the genus and the only producing a rare and emerging infection called lagochilascariosis, which is a zoonotic disease caused in humans (also in cats and dogs) mostly reported in Central and South America. In Mexico, there are five human cases and this finding represents the sixth, but it is the fourth in the Yucatan Peninsula. The case report was a young man from the rural village Tres Garantías, southern Quintana Roo, Mexico, who harbored a subcutaneous abscess in the left back side of neck with purulent discharges containing nematodes in different developmental stages. Nematodes were small and were specifically identified based on the length of spicules and body. Patient was treated with ivermectin and abscess decreased in size, although he later underwent surgery. The clinical examination showed chronic erosion of brain tissue and some individual nematodes occurred within the mouth some days after surgery. The life cycle and transmission of this nematode are unknown, although man could become infected after eating the eggs from soil or infective larvae from an intermediate host (mice), although cats or other domestic hosts could act as potential disseminating agents or eventual infection agent for humans. Autoinfection is possible because the parasite is able to reproduce inside the lesion, thus resulting in the occurrence of different developmental stages.

From the rivers to the oceans or the opposite? Phylogeny of the leech family Ozobranchidae

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Ozobranchidae Pinto, 1921 is by far the least numerous leech family with only ten species arranged in three genera. Species of this group have numerous lateral digitiform branchiae and are mainly parasites of turtles. Species of *Ozobranchus* De Quatrefages, 1852 are distributed worldwide in the oceans whereas species of *Bogobdella* Richardson, 1969 and *Unoculubranchiobdella* Lobo Peralta, Rodrigues Matos & Serra-Freire, 1988 inhabit rivers in South America, Australia and India. Whether this group is marine with secondarily invasions to freshwater environments or the opposite has not
been investigated and so far no comprehensive phylogenetic studies of the group have been performed. Based on molecular markers, both nuclear and mitochondrial, we conducted a phylogenetic study of the group and mapped the habitat on the tree. Based on the current information, it is suggested that freshwater species invaded this habitat secondarily, from marine ancestors. Here, we present the most comprehensive phylogenetic analysis of the group and discuss about nomenclatural changes based on the tree topologies.

Functional genomic study in Strongyloides and related parasitic nematodes by transgenesis and CRISPR/Cas9 mutagenesis

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Animal parasitic nematodes make difficult subjects for functional genomic research, and this hampers the search for new interventions against these important pathogens. The genera Strongyloides and Parastrongyloides stand out as being more tractable in this regard, owing to their ability to undertake generations of free-living development. Transgenesis has been achieved in both these genera, opening the possibility of using transgene constructs encoding dominant mutations to interrogate function in genes of interest and lately, of using the CRISPR/Cas9 system to disrupt or edit such genes. We have now used both approaches to interrogate the function of the gene Ss-daf-16 in Strongyloides stercoralis. This gene encodes an insulin-regulated FOXO transcription factor homologous to the dauer regulatory factor DAF-16 that drives formation of dauer larvae in the free-living nematode Caenorhabditis elegans. Our published work has revealed that a transgene construct designed to suppress Ss-daf-16 brings about loss of intestinal and pharyngeal architecture and aberrant molting in S. stercoralis larvae developing towards the infective third-stage larval stage (iL3). More recently, we employed CRISPR/Cas9 to insert a 24 base pair oligonucleotide, containing stop codons in all reading frames, into exon-5 of Ss-daf-16, which is expressed in all message isoforms, presumably creating a null mutation. Having confirmed this insertional mutation by PCR, we screened F1 progeny of transduced free-living female worms for phenotypes. A mean of 57.3 +/- 8.5% of mutant progeny exhibited malformations of the pharynx and degradation of intestinal cells. Notably for both of the aforementioned studies, these anatomical features are remodeled substantially during formation of the iL3. Together, these studies, and those of our colleagues, underscore the potential of Strongyloides spp. as subjects of reverse genetic study through transgenesis and CRISPR/Cas9 mutagenesis. They represent substantial progress toward direct study of gene function in parasitic nematodes.

Gastrointestinal Helminth Prevalence in Lesser Scaup (Aythya affinis) Wintering on Lake Pontchartrain, Louisiana

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The continental population of Lesser Scaup (Aythya affinis; hereafter, “scaup”) has been in decline since the 1980s, and today exists at 51% of the original goal of 6.3 million set by the North American Waterfowl Management Plan. Endogenous nutrient stores accumulated by scaup females during the winter can influence initiation date, clutch size, and egg viability, and these factors are believed to
drive scaup populations. Gastrointestinal parasites accumulated on wintering grounds could negatively affect nutrient absorption, leading to a decline in body reserves and consequently lowering reproductive success.

We identified and enumerated helminth parasites in the lower gastrointestinal systems of 33 scaup collected from Lake Pontchartrain during the winter of 2016. We found a high prevalence of parasites, but at relatively low intensity. 75% of scaup were infected with a total of 465 parasites. The mean abundance was 14.09 parasites per scaup. Trematodes were the most common class of parasite (87%), while Cestodes (12%) and Nematodes (1%) were observed less frequently. Preliminary helminth counts suggest that the prevalence of gastrointestinal parasites borne by scaup wintering on Lake Pontchartrain is lower than those observed in scaup in the upper Midwest and elsewhere in Louisiana. Preliminary results indicate similar mean helminth abundance among sex and age classes of scaup, and we found no significant relationship between parasite intensity and body condition. In conclusion, we found a relatively low intensity of helminth parasites residing in the lower gastrointestinal systems of scaup, and no association between helminth infection and body condition. This suggests that, in Lake Pontchartrain, helminth infection is not currently contributing to the decline of Lesser Scaup.

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Gastrointestinal Parasites Among Pregnant Women Attending Anti-natal in Parts of Jos, Plateau State Nigeria

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Gastrointestinal parasites are endemic in Nigeria. Cases of asymptomatic infections also exist which maintains transmission of these parasites through ways which include vertical transmission. A total of five hundred and ten (510) stool samples were obtained from volunteer pregnant women attending anti-natal is some hospitals in Jos. they were analyzed using iodine and normal saline wet preparations as direct smears while saturated flotation and formol ether concentration methods were used. Pearson’s Chi-square test was used to compare infections in relation to age, trimesters, PCV and occupational groups. One hundred and sixty seven (32.7%) had single parasite infection, 32 (6.3%) were infected with two parasites while 3 (0.6%) had three parasites. The age groups >45 years had 3 (42.9%) infection, 16 - 25 years age groups had 35.8% and least infection was in the 26 - 35 years age group. infection across age groups showed no significant difference ($\chi^2 = 2.4078, df = 3, P > 0.05$). Those in the third trimesters had the highest infection (34.7%) and least in the first trimesters. Infection across trimesters showed no significant difference ($\chi^2 = 0.31645, df = 2, P > 0.05$). There was however significant difference in relation to PCV groups ($\chi^2 = 35.559, df = 2, P < 0.0001$) and occupational groups ($\chi^2 = 16.738, df = 2, P = 0.0001$). The highest infection was with Entamoeba histolytica, followed by Ascaris lumbricoides and least with Strongiloides spp. Of those infected, 18 (10.78%) were bloody, 14 (8.38%) were watery, 6 (3.59%) were mucoid and 2 (1.20%) had fatty stool. There was significant difference ($\chi^2 = 54.29, df = 6, P < 0.0001$) between parasites species pooled infection rates recorded in individuals. The result shows the need for routine screening of all pregnant women for gastrointestinal infection during anti-natal visits for treatment. Sensitization campaigns should be given to avoid habits that predispose to infection and effects.
Genetic differentiation of pinworm populations (Trypanoxyuris spp.) in howler and spider monkeys across their distributional range in Middle America

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Patterns of genetic variation among populations can reveal the evolutionary history of species. Since many of the genetic attributes observed in parasite populations are driven by their host own demographic and dispersal histories, host and parasite phylogeographic patterns are expected to be intertwined, especially in those highly host specific parasites. Here, we compare the genetic divergence and phylogenetic history of different pinworm populations (Trypanoxyuris spp) infecting howler monkeys, Alouatta palliata, and spider monkeys, Ateles geoffroyi, in locations of Mexico, Nicaragua and Costa Rica, to assess population structure and if the samples from Central America represent the same evolutionary significant unit as the one we previously sampled in Mexico. Pinworms were recovered from the feces of their hosts during 2017 and sequenced for cox1 and 28S rDNA genes. We observed genetic differentiation in cox1 between populations from Mexico and those from Central America in three of the four pinworm species studied from both primate species; the 28S gene showed no divergence among pinworms from different sampling locations. Pinworm populations infecting spider monkeys showed high levels of genetic divergence in cox1 (7.3 %) between Mexico and Central America; however no morphological differences were identified. The genetic variation observed among these pinworm populations could be a byproduct of the host biogeographic history and colonization process from South America through Central America up to Mexico, but also could be the result of a strong co-evolutionary association where different host subspecies harbor distinct genetic variants of pinworms.

Genetic diversity of Oncomegas wageneri (Cestoda: Trypanorhyncha) in the southern Gulf of Mexico

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Population genetics describes the genetic composition of the biological populations in a historical context. Helminth parasites (e.g., plathelminth cestodes) are ideal biological models to analyze the genetic diversity in a particular region (e.g., Gulf of Mexico [GoM]), because the parasitic lifestyle requires the presence of intermediate and definitive hosts (associated with the quality of the environment). To date, in the GoM 98 species of cestodes have been reported. However, no data on the genetic diversity of cestodes has been presented for the GoM. A biological model to obtain an overview of such genetic diversity in the GoM are the larval and adult stages of Oncomegas wageneri, a cestode using flatfish as second intermediate hosts, and stingrays as definitive hosts. In this work, we carried out phylogeographic and population genetic analyses of O. wageneri, using DNA sequences of the COI barcode region to delimit species and determine population structure. Furthermore, we analyzed partial sequences of 28S ribosomal DNA to test the phylogenetic position of O. wageneri within the Eutetrarhynchidae (Cestoda: Trypanorhyncha). Our phylogenetic analyses linked two life stages of O. wageneri: plerocercoid and adult. Based on haplotype network
analysis we detected high genetic diversity distributed along the southern GoM; e.g., 42 haplotypes in 9 oceanographic sampling sites. According to the population genetic and phylogeographic approaches used, the population expansion of O. wageneri dated around 150 thousand years ago, possibly related to glaciations/interglaciation events of the Pleistocene. The results suggest that certain platyhelminth parasites display long-distance dispersal capabilities by using different hosts (with distinct vagilities) during their life stages, showing that parasitic evolutionary ecology models can provide excellent resolution to describe genetic diversity in the GoM and a sensitive indicator that might be affected by catastrophic events such as large-scale oil spills.

Get more from publicly available data: ortholog development for target-capture phylogenomics in copepods

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Like many invertebrate taxa, copepod phylogenetics and classification have been hindered by limited molecular resources, including accurately identified reference sequence data. Planktonic and parasitic copepods have traditionally been preserved in formalin, reducing the amount of material suitable for molecular analysis. Fewer than 400 species, only 3% of described copepods, have been included in molecular phylogenetic studies. To build a better understanding of evolutionary relationships among the Copepoda, a taxon containing 12,000 described species, a highly scalable approach is needed to efficiently generate sequence data. Luckily, high-throughput NGS approaches enable the sequencing of hundreds of loci from hundreds of taxa in a single sequencing run. This study was undertaken with the goal of developing an ortholog set for an NGS target capture approach to copepod phylogenetics. By taking advantage of 2 publicly available copepod genome sequences, we used an ortholog identification pipeline to identify > 500 orthologous protein coding loci ranging from 200–1,000bp each. Recently, we assembled de novo transcriptomes for an additional 10 copepod species across 4 orders to validate our initial genomic orthologs and develop a more robust set of loci able to be captured efficiently across copepods and related crustacean taxa. The results of the ortholog identification pipeline are discussed, and we expand on methodology, pitfalls, and best practices for target capture marker development using copepods as a case study. This approach is replicable in most taxa with available transcriptomic and, ideally, genomic reference sequences.

Global patterns of emerging infectious diseases affecting wildlife

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Emerging infectious diseases (EIDs) are increasingly observed to impact human health, agriculture, and wildlife. Recent large-scale studies have demonstrated that for human EIDs, leading causes include pathogens evolving drug resistance and increased zoonotic disease spillover; for agriculture, monoculture cropping practices and globalization are leading causes. For wildlife, we lack an empirical understanding of the drivers that lead to EIDs in wildlife at a large scale. For this study, we used a literature survey to create a database of more than 700 EID records in wild vertebrates from around the globe over the last 150 years. EID events included records from all five vertebrate classes from 54 countries and infectious diseases included viruses, fungi, bacteria, helminths, protozoans, and ectoparasites. To evaluate factors associated with EID events, we summed events per country and
developed an information theoretic approach using general linear mixed models. Candidate models to predict wildlife EID events included each country’s gross domestic product as an apriori variable to control for bias in wildlife disease surveillance per country. Candidate models then included one or more of the following classes of variables measured per country: pathogen pollution, habitat change, total protected land area, biodiversity loss, and biogeographic climate metrics. Model averaged results demonstrated that invasive species were the strongest predictor of EID events across all vertebrates. When we investigated the dataset by vertebrate group, different subsets of factors emerged as predictors, particularly for mammals and amphibians. Overall, this study suggests that pathogen pollution (pathogen spillover resulting from human-mediated movement of animals outside their native range) is the largest driving factor for EID events affecting wildlife. These results underscore the importance of limiting invasive species and wildlife trade as a way to reduce the impact of infectious disease on animal diversity.

Global phylogenetic analyses of Paraorygmatobothrium

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The phyllobothriidean genus Paraorygmatobothrium was erected by Ruhnke (1994) for three cestode species parasitic in sharks. Since then, the genus has grown to include 26 species, with nearly all of them residing in galeomorph sharks. Early work on the genus included species described from only one host shark species, but the work of Jensen and Bullard (2009) and Cutmore et al. (2017) revealed a much more relaxed version of host specificity for some species. The present phylogenetic study is global in nature, contains 15/26 of the known species and includes samples of Paraorygmatobothrium species from Australia, Borneo, US Atlantic coastal waters, the Gulf of California, the Gulf of Mexico, Japan and Senegal. Samples of Paraorygmatobothrium have been sequenced from the following host genera (number of host species): Alopias (1), Carcharhinus (12), Galeocerdo (1), Hemigaleus (2), Hemipristis (1), Hemitriakis (1), Lamiopsis (1), Mustelus (2), Negaprion (1), Orectolobus (2), Prionace (1), Rhizoprionodon (5), Scoliodon (1) and Sphyra (3). Analyses of 28S, CO1 and ND1 included 34 species of Paraorygmatobothrium, at least 18 of which represent undescribed species. Inclusion of the related genera Doliobothrium, Nandocestus, Orectolobicestus, Ruhnkecestus, and Scyphophyllidium in the 28S analysis revealed that Paraorygmatobothrium is non-monophyletic with respect to those genera. Analyses of host distribution for species indicates that lack of host specificity may be the rule in this genus rather than the exception. Evidence exists for four widespread species of Paraorygmatobothrium.

Guts! Ingested DNA lends insights into the evolution and natural history of the North American medicinal leech

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Leeches represent an ancient, and highly successful, lineage of bloodfeeding annelids: they are found on all continents, in terrestrial, marine, and freshwater environments. Their propensity for sanguivory makes them indispensable to several aspects of contemporary medical practice, and compounds isolated from their salivary secretions remain the most powerful known anticoagulants on Earth. In
recent years, high-throughput sequencing has given us new insight into the molecular basis of blood-feeding in leeches, but attempts to study the evolution of this feeding mode in a comparative context have been stymied by the lack of reliable and comprehensive host records for nearly all species. This is especially troubling in light of preliminary findings, which suggest that many species are more general in their host preferences than previously assumed. Over the past decade, DNA barcoding of invertebrate bloodmeals has been applied to a variety of problems, including disentangling vector-borne transmission networks and conducting biodiversity surveys. Here, we use similar methods to gain a comprehensive picture of the feeding habits of Macrobdella decora, the North American medicinal leech. We sequenced two mitochondrial loci from over 100 samples of ingested DNA representing individuals collected from 16 localities across the species’ range, and documented several novel observations. These data deepen our understanding of the natural history of these organisms; help us discern how M. decora disperses across landscapes; and allows us to investigate correlations between their host preference and anticoagulant repertoire.

Helminth parasites of Lagodon rhomboides of coastal lagoon Celestun, Yucatan

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A total of 43 specimen of “Xlavita” Lagodon rhomboides were captured in Celestun coastal lagoon, at the Yucatan Peninsula, South Gulf of Mexico. Fishes were examined for the search of helminth parasites. A total of 22 helminth species were recovered (4 335 individuals), from them 17 helminths were platelmites including 15 digeneans (7 adults and 8 metacercariae), 1 monogenea, 1 cestode (larva), 4 were nematodes (adult and 3 larvae) and 1 acanthocefala (adult). From the 22-helminth species recovered, 14 represent new host records and 7 new geographical records. The helminth species composition was dominated by digeneans (15 of 22 parasite species). The number of larval species was slightly larger than the species in adult stage (12 vs 22). The metacercarie Stephanostomum sp.1 was the most dominant species (0.38% of all helminth individuals), the most prevalent (35% of infected hosts) and the most abundant (39 ± 365 individuals Stephanostomum sp.1 per host). The species accumulation curve confirmed that the sample size was good enough to collect most of the helminths parasites of this host at the studied location. Additionally, the helminth-fauna of L. rhomboides in Celestun, Yucatan was compared with recorded localities in northwestern Atlantic, north and south of the Gulf of Mexico.

Helminth parasites of deep sea fishes from Perdido fold belt, north-western Gulf of Mexico

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We present a taxonomic survey of the helminth parasites of deep sea fishes from the Perdido fold belt, northwestern Gulf of Mexico. The fish were obtained by trawling with the oceanographic vessel Justo Sierra (UNAM) at depths between 500 and 3500m, and examined on board. The taxonomic identification of both fish and parasites was undertaken by DNA barcoding and light microscopy. Fifty-nine individual fish were recovered and belonged to 5 families and 10 species: Gonostomatidae: Cyclothone accliniden, Cyclothone alba, Cyclothone braueri, Cyclothone pallida, Cyclothone pseudopallida and Cyclothone sp.; Myctophidae: Notolychnus valdiviae; Peristediidae: Perystedion barbiger; Sternoptychidae: Sternoptyx sp., and Stomiidae: Chauliodus sloani. The helminths collected were the nematodes Mooleptus rabuka from C. pallida (prevalence = 9%; intensity of infection = 1; n = 11 fish) and Anisakis sp. from C. sloani (25%; 1; n = 4), the digenean Lethadena profunda from Cyclothone sp. (5%; 1; n = 20), and two Tetraphillidean cestodes, one Scolex pleuronectis from C. pallida (9%; 1; n = 11) and another S. pleuronectis from C. pseudopallida (9%; 1; n = 11). The remaining fish species did not present with helminth infections. The prevalence and intensity of infection of the helminth parasites from the Perdido fold belt were lower than those of the Myctophid fish Myctophum punctatum (8-100% for the same helminths; 1-8; n = 89) and Notoscopelus kroyeria (8-100%; 1-6; n = 74) from surveys of deep sea fishes from the Mid-Atlantic Ridge (MAR), Central Atlantic. However, the helminth species richness and composition of the Perdido fold belt and MAR were remarkably similar, supporting the view that the helminth fauna in these deep sea regions is depauperate.

Helobdella stagnalis does not occur in North America

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Helobdella stagnalis was described by Linnaeus (presumably from Uppsala, Sweden) and has since been recorded from Europe, North America, South America, Africa and Asia. The species feeds primarily on snails, oligochaetes and insect larvae, but dubious accounts also exist for salamanders and frogs. The primary diagnostic character for Helobdella stagnalis is a dorsal nuchal scute, and this character is commonly used as the sole identifier for the species. However, other names have also been attributed to species possessing chitinoid scutes, such as H. modesta and H. adiastola in North and South America, respectively. Synonymization of these species with H. stagnalis was frequent among early taxonomists due to the lack of morphological data, brought on by the minute nature of the organisms. Recently, it has been proposed that North American and European specimens of H. stagnalis are phylogenetically distinct from each other, but the lack of multiple representatives from both continents, specimens from type localities and type specimens have, in general, obscured the taxonomic status of specimens possessing a chitinoid scute. Based on a phylogenetic analysis of cytochrome c oxidase subunit I (COI) from multiple representatives from Europe (including the presumed type locality) and North America, we propose a formal distinction between the North American and European specimens. Therefore, the name H. stagnalis is restricted to specimens from Europe (and Asia) and H. modesta Verril, 1872 should be resurrected for one clade of the North American representatives.
Hidden in plain sight! Overcoming Pleomorphic Trypanosoma Infections to Assess Amphibian Trypanosome Diversity

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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 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 morphologies. The inability to morphologically identify species is a major problem that greatly impedes studies on trypanosome biology and biodiversity. Additionally, using molecular techniques identify species is difficult because sequencing the 18s rRNA gene from blood containing multiple morphotypes yields mixed chromatograms with unusable sequence data. In order to circumvent the challenges presented by multiple morphotype infections, we used anion-exchange chromatography to separate trypanosomes from blood cells and isolate individual morphotypes of amphibian trypanosomes for DNA sequencing. DEAE cellulose is an anion-exchange chromatography resin that can separate trypanosomes from whole blood samples based on differences in their cell surface charge. Trypanosomes are more positively charged than host blood cells, and when blood is added to the chromatography column, trypanosomes pass through the column, while the negatively charged blood cells stick to the positively charged column. Once isolated, we sequenced the 18s rRNA and gGAPDH genes and we were able to definitively assign gene sequences to the individual *Trypanosoma* morphotypes from which they originated. As a first step, this study provides a novel technique to evaluate the diversity of trypanosome morphotypes in their amphibian hosts.

Hidden partners: microbes in host-parasite interaction

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Parasitic infections are complex, widespread, and produce a variety of phenotypic effects on the host organism. To understand inter-individual variations in the success of parasitic infection and associated symptoms, studies have traditionally focused on the host immune system, and parasite virulence factors. It is now becoming evident that these studies leave out crucial players: microbes. I will review evidence that viruses and bacteria associated with parasites can affect the outcome of the interaction, and that parasites can interact with host-associated microbes. I will then outline current research aiming at characterizing the level of specificity and evolutionary role of parasites interaction with microbes to determine to what extant microbes should be included in epidemiological models and could be used to develop new therapeutic strategies.

Highly diverse mitochondrial genome sequences found among the apicomplexan suborder Adeleorina.

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The suborder Adeleorina is composed of biologically diverse apicomplexan parasites with many still poorly understood. Nearly all of the Adeleorina utilize invertebrates as definitive hosts with the exception of Klossiella spp.; the latter monoxenous parasites infect mammals. The group includes haemogregarine parasites that infect blood-feeding invertebrate definitive hosts and a wide range of vertebrate intermediate hosts. Despite similar life history and morphology, the relationship of these parasites is often brought into question; particularly because their hosts are phylogenetically diverse. In a previous study the Adeleorina were determined to be monophyletic within the Apicomplexa based on molecular phylogenetic trees generated from nuclear 18S ribosomal DNA sequences. In the present study, the molecular relationship of the Adeleorina was evaluated based on whole mitochondrial (mt) genome sequences. Several Bartazoon species infecting Ontario ranid frogs and a Japanese Brown Spotted Pit Viper, Hepatozoon canis from an Israeli dog, Hepatozoon griseisciuri from Ontario Grey Squirrels, Klossiella equi from an Ontario horse and Haemogregarina balli from various species of Ontario turtles were sampled. Polymerase chain reaction (PCR) and Sanger sequencing completed mt genome sequences from each (~6kb) with the exception of H. canis. Shotgun NGS and assembly of NGS data from H. canis-infected blood revealed several contig sequences that mapped to apicomplexan mitochondrial genomes, including a cytochrome c oxidase I (COI) sequence, but a complete mt genome was not recovered. In most apicomplexan mt genomes, 3 protein coding regions, COI, COIII and cytochrome B as well as various fragmented rDNA sequences are found; unusually, organization of the CDS and rDNA fragments varied widely among adeleorinid taxa. With the exception of the piroplasms, most well-recognized groups of apicomplexan parasites, such as eimeriids or haemosporinids, have conserved mt genome organization among their members, even if the organization differs from those found in other major groups.

Histopathological Effects of Parasites on the Liver and Gills of Flatfish of Yucatán platform

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Histopathological examination provide insights into interactions between pathogens and their impacts on the host organism. Larval stages of parasites frequently produce histopathological effect due to their accumulation in the host. So, depending on the number the parasitic forms accumulated in a particular organ they can cause serious damage to the tissue and also alter the normal function of the organs. The histological study of liver and gills are a direct evidence of robust effect on the fish health and is helpful in providing the possible effect of parasitism on the functions of different body systems. Between 2010 and 2012 we collected 153 flatfishes of the genera Cyclopsetta and Syacium from the northeastern of the Yucatán shelf in order to identify histologically lesions associated with the presence of parasites in liver and gills. Microscopically examination of liver and gills tissue revealed different histopathological and cellular lesions in infected fishes. The pathological alterations in liver associated to the presence of parasites included: inflammation, accumulation of lipid droplets, and in severe cases extending melanomacrophages centers (MMC), granulomes, congestion blood of sinusoids and haemorrhage. Parasites in gills induced inflammation, hypertrophy, granulomes and blood congestion in the affected areas. We observed more damage in gills than liver. The prevalence of larval stages of parasites in the liver varied year to year being 21%, 25% and 17% during the 2010, 2011 and 2012 respectively, while in gills was 46%, 61% and 35% in the same periods. Larger values of prevalence can be due to the fact that the gill represent the first organ of entrance for parasites. Cestodes and Stephanostomum sp. were the principally larval forms that we found in liver and gills of flatfish. Tissue damage was associated with the quantity of parasites presents; a fish that is lightly
infected will show few lesions, while a heavily infected fish may present severe injuries extended histologically.

Host Specificity of Ectoparasitic Bat Flies (Diptera: Streblidae and Nycteribiidae) from Bats of Brazil

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Ecological studies on ectoparasites have the potential to clarify patterns of host specificity, coevolution, and population genetic structure of parasites and their hosts. Previous studies have shown that obligate ectoparasitic bat flies (Streblidae and Nycteribiidae) tend to be highly host specific, but these studies have been geographically limited in their sampling. Comparisons of host-parasite associations across broad geographic areas and ranges of host taxa may elucidate the degree of host specificity of bat flies at a broader scale. By studying community composition and host-specificity of bat flies, we can estimate potential for parasite host-switching in tropical communities. Toward this end, we collected bat flies from bats of the Atlantic Forest of Brazil (State of Rio de Janeiro). After identifying bats and bat flies using morphology and COI barcoding, we measured extent of host specificity of each bat fly species using the program ParaFit. To evaluate the factors that influence host-parasite associations (e.g., host bat phylogeny, host bat roosting ecology, environmental factors), we grouped host individuals based on their shared parasite species and used multiple regression on distance matrices to measure the influence of various host and parasite traits. This is the first time that the influences of various host and environmental factors on host specificity of bat flies has been examined in a broad geographical context.

How changes in host community diversity and composition affect parasite transmission

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Ongoing debate over the relationship between biodiversity and disease risk underscores the fundamental need to develop a more mechanistic understanding of how changes in host community composition influence parasite transmission, particularly in complex communities. While empirical investigations have emphasized correlations between host richness and various metrics of infection, experimental manipulations are essential to test underlying processes. A key challenge remains to determine how parasites select among potential hosts and whether this process shifts with community composition, host diversity, or both. Focusing on interactions between larval amphibians and trematode parasites, we first used choice trials to assess how the selectivity of cercariae for five host species varied with community composition. In subsequent trials, cercariae were allowed to contact and infect hosts, allowing comparison of host-parasite encounter rates (parasite choice) with...
infection outcomes (successful infections). While cercariae exhibited consistent preferences for specific host species, these preferences did not correlate with susceptibility. As a result, host species varied in the degree to which they functioned as dilution versus maintenance hosts. In the second dimension, we contrasted the effects of amphibian host richness (1, 2, or 4 species) and host composition (5 permutations per richness level) to understand whether diversity-mediated effects on transmission were additive (predictable from composition) or non-additive (an emergent property of richness). By combining experimental results with complementary field surveys on host community assembly from 375 ponds, our results indicated that diversity broadly inhibited infection success by all three trematode species tested; however, these effects were primarily additive, manifesting under ‘realistic’ rather than ‘randomized’ patterns of community assembly. These findings emphasize the potential for combining information on host traits and assembly patterns to forecast diversity-mediated changes in infection.

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**How does the order of infection by a virus, fungus, and parasite affect within-host community dynamics of amphibians?**

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Disease ecologists have historically focused on understanding how a single pathogen causes widespread mortality of multiple hosts. However, evidence suggests that hosts are regularly infected by multiple pathogens (i.e., coinfection), which have been implicated in drastic host declines across multiple ecosystems. The goal of our project was to understand the disease ecology of coinfections within hosts using an experimental set-up. Specifically, we asked if order of infection affects host feeding rates, growth rates, and immunological responses. We used 650 pacific tree frog, Pseudacris regilla, tadpole hosts, which were placed in either uninfected (control) or three infected (treatment) groups by exposing them to one of three infectious agents: ranavirus, Batrachochytrium dendrobatidis (Bd), or trematode cercariae. We then exposed tadpoles to one of the two remaining infectious agents. We expected the interactive effect (facilitative, antagonistic, or neutral) between the primary and secondary parasites to depend on if the primary parasite stimulates or inhibits host immune response. We euthanized, dissected, and sampled host tissues related to ranavirus, Bd, and trematode intensities from 25-40 individuals per day. We also examined blood samples for the abundance of lymphocytes, basophils, eosinophils, and neutrophils to measure immunological responses. Regarding host feeding rates, there was no significant difference in the quantity of food all infected individuals consumed when compared to uninfected individuals. However, there were significant differences between the growth rates of all infected individuals, whether individuals experienced single infections early in development, late in development, or were co-infected (p < 0.05). Tadpoles infected with trematode cercariae late in development experienced the largest negative growth rates (i.e. they grew smaller) compared to all other infection combinations. Individuals that were exposed to single infections early or late in development showed a decrease in lymphocyte production as well as a decrease in total immunological response (lymphocyte, basophil, eosinophil, and neutrophil counts combined) while co-infected individuals showed an increase in lymphocyte production and total immunological response. However, the differences between the immunological response variables were not significantly different between early single infections, late single infections, and coinfections. In summary, tadpoles grow less when infected during later developmental stages as well as when they are co-infected. While immunological responses did not differ significantly among single infections and coinfections, we observed a consistent pattern of individuals allocating energy away from growth to combat infections by ranavirus, Bd, or trematode cercariae.
Humancystic echinococcosisin Western Romania, 2007-2017

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Human cystic echinococcosis (CE), a serious life-threatening neglected zoonotic disease, is caused by tapeworms of the genus Echinococcus. Romania is recognized as a highly endemic area due to high rates of echinococcosis in humans and animals. The present study assesses the epidemiological aspects of CE in adult patients hospitalized in 6 surgical clinics in Timisoara, Western Romania, over a period of 10 years. In this retrospective study were included 228 consecutive patients diagnosed and hospitalized with CE, between January 1st 2007 and September 30th 2017. Clinical, laboratory and epidemiological data were collected from patients’ medical records. Patients were aged between 19-82 years (mean=46.2), 104 (45.6%) were males and 136 (59.6%) from rural area. The highest prevalence of CE was observed in patients aged 50-59 years (23.2%). The mean length of hospital stay was 13.4 days (range between 3 and 46 days) and was significantly higher in patients aged 60-69 years (16 days) compared to those aged 30-39 years (11 days), 40-49 years (7.5 days) and 50-59 years (11 days) (p<0.05). Liver (75.9%) and lungs (10.5%) were the most affected organs. Complications (anaphylactic shock, cardio-respiratory arrest, cystic rupture, biliary fistula, bacterial superinfection) were described in 51 (22.4%) patients. The mean length of hospital stay was significantly higher in patients with complications (15.7 days) compared to those without complications (12.7 days) (p=0.017) and in patients who underwent cystectomy (15.4 days) compared to those in whom percutaneous procedure (9 days) was performed (p<0.001). Seventy-two patients (31.6%) had two or more hospitalizations. CE remains a public health problem in Western Romania due to its severity and economic impact. Therefore, it is desirable to improve the CE surveillance system and control program, in order to reduce the human infection rates.

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Human infection by Gnathostoma spp in Ensenada, Baja California: A case report

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Human Gnathostomiasis—an infection acquired mainly by the consumption of freshwater products—has been reported in some parts of México. Here, we presented a case of a female patient, a resident of Ensenada, Baja California—a city close to the border with the United States—who says that she eats seafood frequently because her husband is a fisherman. She was 37 years old at the time of her first consultation. She suffered from a severe headache, predominantly a morning hematemesis followed by diarrheic evacuations during five days without blood or mucus, conjunctival jaundice, pains in the epigastrium and right hypochondrium without irradiation. No major alterations were detected in the laboratory studies and it was treated with an empirical antibiotic therapy. However, the patient returned two months later with vomiting, diarrhea, ocular problems and symptoms of anxiety. A cysticercosis test was performed, showing negative results. Therefore the patient was treated for Gnathostomiasis (Albendazole 10mg / kg for 21 days, and Ivermectin 0.2mg / kg for two days), which was finally confirmed by ELISA (IgG). After the treatment, the patient evolved satisfactorily. Gnathostomiasis is very common and its expansion is imminent, not only from freshwater fish, but also from seafood (Ceviche). In addition, this infection has a poor clinical diagnosis and inadequate treatments as it presents an atypical clinical picture and thus it can be confused with other pathologies.
IDENTIFICATION OF PATHOGENIC BACTERIA IN Sciaenops ocellatus CULTURE

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Losses caused by pathogenic bacteria in aquaculture can generate mortalities of up to 100%. In these culture systems high densities during fattening, harvesting and transportation favors factors that affect the quality and safety of the final product. The objective of this work was to identify pathogenic bacteria in the red drum S. ocellatus under culture conditions and in organisms surrounding the growing area. A total of 30 wild and 30 cultured organisms were collected in marine floating cages of Seybaplaya, Campeche. Isolation of the bacteria was performed by means of swab in four target organs: liver, kidney, spleen and gill, inoculated in BSA broth. Subsequently, the strains obtained were inoculated in BD BBLTM CrystalTM strips and were identified through readings in BIOMIC V3. To obtain an approximation of the bacteria present in five strains of interest from the samples under culture conditions, these were sequenced partially with the 16SrRNA gene. The results obtained from the readings in BIOMIC V3 showed that, of 15 isolated strains, five belong to the genera Vibrio and Aeromonas, which were identified in cultured organisms. The remaining 10 strains from the wild organisms belong to the genera Sphingomonas, Pseudomonas, Shewanella, Proteus and Halomonas. The strains with assignment of the genera Vibrio and Aeromonas were confirmed by specific growth in TCBS medium. The reference sequence 16SrRNA of the five strains sequenced showed the genera Bacillus, Micrococcus, Staphylococcus, and Vibrio, which are associated with skin lesions, lethargy and mass mortalities in intensive culture systems. These data suggest that the systems of culture of floating cage favor the growth of vibriosis that can generate gastrointestinal problems in humans. These results suggest the need for the development of agents for the control of pathogenic bacteria in aquaculture systems. The alginates from Sargassum horridum can be an option for the control of pathogenic bacteria in aquaculture due to the antibacterial activity related with these biomolecules.

INVESTIGATING THE TAPEWORM FAUNA OF THE CROCODILE SHARK

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The Crocodile shark, or thrako, Pseudocarcharias kamoharai, is a member of one of four monotypic families of lamniform sharks. Its tapeworm fauna has never been investigated. In May of 2014, we collected spiral intestines from eight individuals of this species at a fish market in Ecuador. Tapeworms from these specimens fixed in formalin were prepared as whole mounts for examination with light microscopy, as well as for examination with scanning electron microscopy. Sequence data for the D1-D3 region of the 28S rDNA gene were generated for a subset of specimens fixed in 95% ethanol. Bayesian and Maximum Likelihood phylogenetic analyses were conducted on these data along with comparable data from GenBank for potential congeners. Specimens of three new species, all belonging to the order Phyllobothriidea, were found. Two of these appear to represent new species of Clistobothrium. One of these resembles its congeners in both scolex morphology and proglottid anatomy. The other bears a scolex that diverges from the morphology typically seen in Clistobothrium in its possession of simple, circular bothridia; beyond a uterus that extends only to
the level of the cirrus sac, the anatomy of the proglottid also deviates substantially from that of the other members of the genus. The third new species appears to belong in Paraorygmatobothrium. It differs from 23 of its congeners in its possession of fewer than 60 testes. It is distinguished from its three remaining congeners, which also bear low testis counts, in terms of overall size and genital pore position. The results of the phylogenetic analyses confirm these generic placements for all three species. These results also indicate that the two Clistobothrium species are not each other’s closest relatives. Except that it is not a member of the well-supported Lamnidae, the affinities of Pseudocarcharias kamoharai among lamniform sharks are unclear. Its morphological resemblance to both mako sharks and thresher sharks is reflected in its common name, the thrako. It is thus interesting that this shark’s cestode fauna is a mixture of those seen in members of these two host groups. Clistobothrium species typically parasitize mako sharks and their relatives (i.e. Lamnidae). Although the majority of Paraorygmatobothrium species parasitize carcharhiniform sharks, several have been reported from thresher sharks (i.e. Alopiidae). It appears that the cestode fauna of the Crocodile shark is the result, at least in part, of one or more host switching events.

Identification and infection parameters of Larval tapeworms (Cestoda: Trypanorhyncha) from Flatfishes of the Southern Gulf of México

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In the southern Gulf of Mexico (SGOM), flatfishes sustain important fisheries, they are diverse in species number, with a geographical distribution restricted to the continental platform and have aquaculture potential. However, the benthic habits of flatfishes, make them susceptible for parasitic infections. Cestodes of the order Trypanorhyncha are the most frequent and abundant endoparasitic helminths groups that infect flatfish. In general, it is known that trypanorhynchids use benthic invertebrates as first intermediate hosts, flatfishes (among other fish species) as second intermediate host or as paratenic hosts), and elasmobranchs (sharks and rays) as definitive hosts. However, the life-cycles of many Trypanorhyncha larvae infecting flatfishes are poorly known because the identification is difficult given that their scoleces remain undifferentiated until they infect the definitive host. The objective of this research was to determine the lowest possible taxonomic level the larval trypanorhynchid cestodes infecting flatfishes, as well as their prevalence and mean abundance in the southern Gulf of Mexico. Based on samples of the flatfishes Syacium papillosum, S. gunteri, S. micrurum, Bothus robinsi and Cyclopsetta chittendeni, collected from oceanographic cruises in the southern of the Gulf of Mexico, a total of five cestode species in larval stage were found: five trypanorhynchids and one tetraphyllid. The trypanorhynchids were represented by plerocercoids of five species: Nybelinia sp., Kotorrella sp., Oncomegas wagneri, Lacistorhynchus sp., Trypanorhynchus gen. sp. 1. The present study will establish new host and locality records, and the most frequent and abundant larval cestode was Oncomegas wagneri infecting to C. chittendeni (prev = 73%, Ab+ -DS = 46.42±56.34), followed by Kotorrella sp. infecting to S. papillosum (prev = 64%, Ab+ -DS =13.88±11.33). Morphological and molecular identification for each larval species of cestodes recovered from flatfishes are provided for the SGOM. The overall pattern was that trypanorhynchids larval cestodes infecting flatfishes are very frequent and abundant in the SGOM.

Immune response of the bed bug, Cimex lectularius, to simulated traumatic insemination, starvation, and exposure to entomopathogenic bacteria.
The bed bug, *Cimex lectularius*, is an ancient and persistent human pest. Their bites can cause a medically significant reaction in some people and their numbers and range have been expanding recently due to increases in insecticide resistance and international travel. While bed bugs are thought to have evolved several immune-related adaptations to aid their unique lifestyle, the specific mechanisms involved in their innate immune system are poorly understood. Along with hemophagacy, bed bugs undergo major exposure to pathogens during the process of traumatic insemination (TI). During this process, male bed bugs pierce the female through their body wall and inseminate the body cavity directly. It has previously been shown that females have evolved physiological adaptations that abate potential fitness costs associated with TI. As the bed bug genome was completed in 2016, we now have the capability of measuring specific immune gene regulation in response to immune challenges. Here, we used simulated TI, and exposure to a panel of entomopathogenic bacteria followed by a qRT-PCR-based analyses to take a systemic approach to understanding the *Cimex* defensive response against hemocoelic inoculation with bacteria native to the *Cimex* cuticle. Our primary goal was to measure the transcriptomic response of a suite of conserved innate immunity-related genes: Prophenoloxidase, Nitric Oxide Synthase, C-type Lectin 8, Argonaute-2, and several antimicrobial peptides. Our results suggest that a subset of antimicrobial peptides and immune regulatory genes responded differentially to these immune challenges. Surprisingly, starvation seemed to have little effect on immune gene regulation. These results will be discussed as they relate to data from other hematophagous insects.

Immunoregulatory properties of melatonin and *T. cruzi* infection

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Introduction: In the absence of highly active chemotherapy against *Trypanosoma cruzi* and considering the lack of available data about the effects of aging during acute *T. cruzi* infection, the impact of melatonin treatment was evaluated. Methods: *T. cruzi*-infected Wistar rats were infected with 1x10^5 blood trypomastigotes of the Y strain of *T. cruzi* and the circulating levels of corticosterone, spleen thymocytes apoptosis and the percentages of CD4+ and CD8+ T cells were evaluated. Animals were randomized into the groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). Melatonin was administered through the gavage (05 mg/Kg) to young (5 weeks) and middle-aged (18 months) rats. All spleen samples were processed for flow cytometric analysis, using conjugated-specific monoclonal antibodies. Results: Melatonin affected the percentage of CD4+ and CD8+ T lymphocytes, since increased (*P<0.01 and *P<0.001*) percentages of these cells were found in melatonin treated middle-aged animals. Melatonin exerted an important anti-apoptotic agent by reducing the percentages of both, early and late apoptotic cells and by counteracting the exacerbated production of corticosterone no matter if young or middle aged animals. Conclusion: These findings undoubtedly open a new pathway for Chagas’ disease research, with melatonin emerging as a potentially important immunoregulator by counteracting the immunoendocrine imbalance during *T. cruzi* infection.

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Impact of multiple host invasions on native parasite assemblages: What happens when three non-native anurans invade a Florida frog pond?

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While much attention and resources have been given to the impact of free-living invasive species on native flora and fauna, few studies have focused on the impact invasive species have on native parasite assemblages. Further, many ecosystems are experiencing impacts from multiple non-native species, yet we do not understand how multiple introductions impact native hosts or parasites. Florida is well-known for its invasive species, including an increasing number of anurans (frogs and toads), many of which have already been surveyed for parasites. These surveys are useful to establish a baseline of presumably native parasites from anurans in the eastern United States. Our long-term survey of anurans from central Florida aims to compare a native parasite assemblage pre and post-invasion of 3 non-native anurans. We also aim to record any parasites introduced to the wetland from the non-native species. From September 2010 – April 2018, we sampled adult anurans from a permanent, shallow wetland adjacent to the Florida Southern College campus. Our samples were divided into wet (June-September) and dry (January-April) seasonal periods, and we attempted to collect at least 15 specimens of each available species in both seasons for parasite discovery. All anurans collected in 2010 – 2011 were species native to central Florida: Anaxyrus terrestris, Acris gryllus, Hyla cinerea, Rana grylio, and Rana utricularia, which hosted diverse parasite assemblages that included native parasites, such as Cosmocercella sp., Cosmocercoides sp., Rhabdias spp., Haematoloechus spp., Mesocoelium sp., and Lawrencarus sp. Invasive anurans first appeared in our wetland in spring 2012 with non-native Rana catesbiana, which was followed by exotic Rhinella marina (spring 2013) and Osteopilus septentrionalis (fall 2016). Besides the introduction of these non-native anurans, no obvious changes occurred to the wetland environment, yet we could not find A. gryllus after 2013 and once abundant R. grylio was rarely collected after 2014. The current anuran community consists of 4 native and 3 non-native species, which varies markedly from the original community of 5 native host species. We found many native parasite species were still present in the wetland’s anurans after the invasions, however some parasite species have apparently become locally extirpated. We also found that non-native hosts introduced new parasite species into the wetland, with some of these parasites likely being exotic to Florida. We will discuss differences between parasite parameters among host species and years. Overall, our study suggests that multiple introductions of non-native anurans and their parasites can impact native parasite assemblages.

In vitro inhibition of Trypanosoma cruzi by naphthalene-based compounds confirms in silico binding predictions for the REL-1 subunit of the editosome.

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Based upon in silico modeling, naphthalene-based compounds (NBCs) have previously been proposed as potential competitive inhibitors of the REL-1 subunit of the editosome RNA editing complex in Trypanosoma brucei. A similar modeling approach applied to REL-1 of T. cruzi found similar promising binding kinetics. Four naphthalene-based compounds (V1 - V4) were tested in vitro for efficacy against culture-derived BSF trypomastigotes, as well as in co-cultures of BSFs with DH-82 canine macrophages. Cultures containing 10, 50, or 100μm concentrations of V1 - V4 were sampled at 24, 48, and 72 hours for cBSF concentration and viability.
Results showed that BSFs were significantly reduced by the presence of any of the NBCs tested, with several effective at the 10µm concentration. The relative efficacy of the NBCs was consistent with the in silico predictions of their respective REL-1 binding affinities. When DH-82 cells were cultured in the presence of the NBCs they maintained the ability to replicate and adhere normally, even in the presence of 100µm concentrations of the NBCs. In a binding inhibition assay three of the four compounds demonstrated specificity, as well as a high affinity, for the ATP binding site of the T. cruzi REL-1 subunit. These compounds may provide a novel chemotherapeutic approach against T. cruzi, as well as potentially other infectious kinetoplasts.

In-vitro screening to identify potential candidates for treatment of Balamuthia Amoebic Encephalitis

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Balamuthia Amoebic Encephalitis (BAE) is a disease caused by the parasitic amoeba Balamuthia mandrillaris. Although considered a rare disease (<200 people since 1986), it has a 95-98% fatality rate worldwide. Not much is known about the amoeba’s lifecycle, genome, or chemical composition, bringing into question whether accidental diagnoses of Acanthamoeba has occurred instead of BAE. Amoebic cysts and trophozoite can change between stages depending on environmental factors and nutrient availability. Nicknamed the “Brain Eating Amoeba", patients with Balamuthia-Induced granulomatous amoebic encephalitis can experience skin lesions, paralysis, seizures, necrosis of brain tissue, brain stem interference, and death. Severe brain hemorrhages have been seen in patients who died from the disease. The focus of this study was to identify a compound or drug that could significantly reduce the population of Balamuthia trophozoite and cysts, in vitro, and lead to in vivo drug testing. Results of in vitro screening showed that Oligomycin A (an ATP-Synthase inhibitor), Sertraline (a selective serotonin-reuptake inhibitor), Colistin Sulfate (an antibiotic), and Pentamidine Isethionate (antiprotozoal drug) were effective against Balamuthia. The EC50s for the trophozoites were 1.5µm, 19.7µm, 10.1µm, and 2.3µm, respectively. EC50s against the cysts were 0.6µm, 16.6µm, 4.3µm, and 39.2µm, respectively. In this study, we identified four potential compounds that can be further tested in in vivo studies as possible candidates that may be used as life-saving treatments for patients with BAE.

Invasive species of freshwater fish helminths in Mexico: where are they now?

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In Mexico, around 40 exotic species of freshwater fish helminths have been recorded; however, only four of these species can be considered invasive because the number of parasitized hosts, their potential damage to native species as well as its wide distribution ranges: Centrocestus formosanus (Digena), Cichlidogyrus sclerosus (Monogenea), Schizocotyle aichleinogathii (Cestoda), and Pseudocapillaria tomentosa (Nematoda). The aim of this review is update the host spectrum and geographic range of the aforementioned species since their introduction in Mexico and briefly discuss the factors that have determined their invasive success. To accomplish this goal, we compiled data from the Colección Nacional de Helmintos, housed at IBUNAM and two electronic sites: ISI Web of Knowledge
and CABI, for the period 1965–2016. Since their first record in Mexico (1981), *S. acheilognathi* has been found in 11 fish families and 96 native species in 28 of the 32 Mexican states; *C. formosanus*, recorded for the first time in 1989, parasitize 77 native species belonging to 17 families in 19 states; *P. tomentosa* was recorded in 1986 and currently infects 11 native species of 3 families in 5 states and finally, *C. sclerosus* has been reported in only cichlid fishes (2 native species) in 10 states since 1985. The reduced host specificity of the 4 helminth species along with the uncontrolled introduction of carps and tilapias by anthropogenic activities are the mains factors that determined their invasive success in Mexico. However, the number of parasitized hosts by these species is probably underestimated, due to only 41% of the native freshwater fish fauna has been studied in some extent from a helminthological perspective. In addition, the environmental impact and economic and sanitary consequences of these biological invasions have not been evaluated yet.

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**Involvement of Nimrod family genes in the peristomial hemocyte immune response of a mosquito**

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In mosquitoes, the primary responders to infection are the hemocytes, which recognize and kill pathogens via phagocytosis, melanization, lysis, and other mechanisms. Some of the hemocytes associate with the ostia (valves) of the heart, where they mount immune responses in areas of high hemolymph flow. Hemocytes are always present at the ostia, at which point they are called peristomial hemocytes, but an infection results in the aggregation of additional hemocytes at these locations, thus amplifying their immune capacity. The molecular basis of peristomial hemocyte aggregation remains unknown, and thus, the focus of this study was to assess whether members of the Nimrod gene family are involved in the aggregation of hemocytes in the peristomial regions of the African malaria mosquito, *Anopheles gambiae*. Three members of this gene family - *draper, nimrod* and *eater* - were selected for investigation because they function in immune responses, and because some of their orthologs are involved in adhesion. In this talk we will present data from experiments involving RNA interference, organismal assays, fluorescence microscopy and infections with *E. coli*, that implicate *eater*, and possibly *draper*, in the process of peristomial hemocyte aggregation. Furthermore, we will present data showing the involvement of *eater* and *draper* in the phagocytosis of pathogens in the peristomial regions of the heart. This study begins to uncover the molecular basis of the genes involved in the physiological interaction between the mosquito circulatory and immune systems.

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**Isolation and proteomic analysis of Schistosoma mansoni extracellular vesicles released during in vitro miracidial transformation**

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Schistosome miracidia transform to primary sporocysts soon after entry into the snail intermediate host. This event is characterized by the shedding of ciliary epidermal plates concurrently with the formation of the sporocyst tegumental syncytium. During this transformation process a diverse array of larval proteins (larval transformation proteins or LTP) are released into surrounding snail
tissues, thereby representing a major source of host-interactive molecule. Previous proteomic analyses of whole LTP released in vitro by transforming Schistosoma mansoni miracidia included several marker proteins typically found in exosome-like extracellular vesicles (ELVs). Because ELVs have not been investigated in early developing schistosome larvae, we proceeded to isolate ELVs from LTP (24-hr cultures) and characterize their proteomic profile. ELVs isolated by sequential ultracentrifugations were subjected to nanoparticle tracking analysis (NanoSight LM10) to determine the ELV size distribution followed by nanoLC-MS/MS proteomic analysis. Nanosight results indicated the presence of ELV nanoparticles with a mean size of 130nm and mode of 66 nm. Protein comparisons between ELV-enriched samples and LTP-depleted or ELV-wash fractions revealed an enrichment of specific proteins, including known exosome-associated markers. Among the 25 most common proteins associated with exosomes (exoCarta), 10 were found enriched in ELV from 24h S. mansoni LTP, including tetraspanin/CD63 receptor, annexin, enolase, GAPDH and others. In addition, we observed an uptake of fluorescent ELVs by a subset of the snail hemocytes after in vitro exposure, suggesting a potential for ELV-hemocyte interaction during infection. Studies are currently in progress to further characterize ELVs released during early larval development and their potential roles in regulating snail/parasite compatibility.

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It’s All About Perspective: Using Integrative Taxonomy to Understand Echinostome Biodiversity

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Recognizing parasite diversity is important because treating several parasite species as one could be masking variation in infection dynamics, geographic distributions, and host use. DNA sequencing aids in parasite identification, but only using genetic sequences limits our ability to understand parasite taxonomy and evolutionary ecology. Using integrative taxonomy to link genetics, morphology and host use provides insight into levels of biodiversity (e.g. populations and species) that can inform our knowledge of life cycles and host specificity. Echinostomes are a species-rich group of trematodes that are found as adults in vertebrates such as birds and mammals worldwide. Within this group, DNA sequencing has suggested misidentifications and cryptic species occur, which implies that our knowledge of life cycles and host specificity may be inaccurate. We sequenced adult worm DNA from definitive hosts collected in Manitoba including 43 sequences from eight bird species and 102 sequences from 66 muskrats at partial ND1 and 28S rRNA genes. Phylogenetic and haplotype network analysis combining our data with GenBank sequences confirmed five known species/lineages: Echinostoma trivolvis lineage a and c, Echinostoma revolutum, Echinoparyphium lineage 3 and Hypoderaeum spp. Two new lineages of Echinostoma robustum and Echinoparyphium lineage 2 were discovered. The morphology of 56 sequenced individuals and 22 museum specimens was measured. Principal component analysis with 27 traits revealed that some lineages, like E. trivolvis a and c, were morphologically distinct and not cryptic species. In contrast, we also found morphological overlap among some species, which helps explain past misidentifications. Integrative taxonomy improved our understanding of what constitutes echinostome species and revealed more diversity in North America. Our data suggests that host specificity and geography have influenced speciation though more samples from a wider geographic sampling are required.

Ivermectin: from a Mouse to a House.

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There was no individual discoverer. There was collaboration and teamwork. There was a shared objective – the discovery of a novel antiparasitic agent. There was simplicity; for the efficacy of a new class of anthelmintic was first observed in a single mouse, without recourse to sophisticated instrumentation. There was complexity; for the process of turning an observation of efficacy into a useful medication is inherently complex. Participants had taken various scientific pathways to reach the project, sometimes with refreshing diversions along the way. And when ivermectin became a success, there was a celebration—and that is where a special house (or two) came into the picture.

Knocking-out TcNTPDase-1 Gene in Trypanosoma cruzi

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INTRODUCTION: The Ecto-Nucleoside Triphosphate Diphosphohydrolases are enzymes that hydrolyze tri- and/or di-phosphate nucleotides. Evidences pointed out to their participation in Trypanosoma cruzi virulence, infectivity and purine acquisition. OBJECTIVES: In this work, we develop and evaluated the viability of a recombinant parasite knockout for the NTPDase-1 gene, and confirm the gene deletion stability and survival of the parasite. MATERIAL/METHODS: For this purpose, intergenic regions upstream and downstream of the TcNTPDase-1 were amplified by PCR and these regions were inserted in vectors for knockout pNNeo2 and pHYG2 thus, epimastigote forms of the recombinant parasites Dm28c clone (Hygro and Neo #1 to #4) were cultivated at 28°C in 10% FCS supplemented LIT medium. RNA was extracted using TRizol®, followed by the quantification and treatment with DNase before the cDNA synthesis. The RT-qPCR assay were performed using TcGAPDH and TcCalmoduline as housekeeping genes. For assays of western blotting, we used anti-apyrase immune serum, and the protein of the clones was extracted by the freezing and thawing method.

RESULTS: We observed that the Hygro and Neo hemi-knockout showed a lower cell growth when compared to the wild type clone. In addition, we also verified that Hygro and Neo clones exhibited lower expression of TcNTPDase-1, when compared to wild type. These data observed in the mRNA levels corroborate with the data obtained in western blot experiments. Furthermore, we performed in vitro infection in VERO cells with the recombinant parasites, we observed a significant reduction in the adhesion and endocytic index. CONCLUSIONS: When T. cruzi was knocked-out in both alleles of the NTPDase-1 gene, it was not possible to observe the parasite survival. But, the hemi-knockout parasites were viable and presented an altered expression level of the NTPDase-1 gene. These results suggest the importance of this enzyme in the adhesion and internalization of the parasite in the host cell.
The capacity of a vector to spillover different pathogens to reservoirs is widely known. This has high importance because these pathogens are responsible for many of the health problems in human and animal populations. Nevertheless it is necessary to get more information about the species that we can find in a host. Rodents are considered an important reservoir for many pathogens because they can carry different vectors like lice, fleas and ticks. Nevertheless we do not know much about which species of ectoparasites they have. We made a list of the ectoparasites on rodents from the Scientific Research Station Las Joyas, in the core zone of the Biosphere Reserve Sierra de Manantlan. We trapped alive rodents with Sherman traps in human dwelling and secondary scrub vegetation. During a year round, ectoparasites were collected mainly by intense visual fur searching of hosts (February 2014 to January 2015). We registered 161 individuals of nine species (Heteromys irroratus, Neotoma mexicana, Oryzomys melanotis, Peromyscus hylopetes, P. maniculatus, P. spicilegus, Reithrodontomys sumichrasti, R. fulvescens, Sigmodon allenii) and 905 ectoparasites where collected. The highest amount of ectoparasites were collected in secondary scrub vegetation (846) followed by human dwelling (59). The Laelapidae family was the best represented (540 individuals), next was Ixodidae: I. tovari (206) and I. cookei (61) the last species is considered the main vector of Powassan virus (POW), Amblyomma sp. (8) and Trombiculidae (3). The class Insecta was represented by Polygenis (65) and Plusaetis (1); Cuterebra fly genera (16). The study of ectoparasites of wild fauna allows to know more about the ecological dynamics of vector-borne diseases, as well as to identify if there is any risk for human populations.

Larval Amphibian Disease Dynamics: Relationships Among Ribeiroia Trematode Infection, Chytrid Fungus (Batrachochytrium dendrobatidis), and Stress

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Vertebrates across the globe are experiencing alarming population declines. While this loss of biodiversity is a concern for all vertebrate taxa, it appears that amphibians are particularly vulnerable. These catastrophic declines can be attributed to multiple factors, most of which stem from anthropogenic disturbances. Amphibians, and vertebrates as a whole, have an endocrine response to these potential anthropogenic stressors that results in increased circulating corticosterone following hypothalamic-pituitary-interrenal axis activation. Corticosterone can, in part, act to mobilize energy reserves to potentially combat a stressor. To exacerbate the amphibian biodiversity crisis, stress-linked anthropogenic disturbances have been also been linked to a number of emerging infectious diseases within amphibian communities, including viral, fungal, and parasitic. Our study investigated dynamics surrounding a parasitic trematode, Ribeiroia, infection in two anuran species: California toads (Anaxyrus boreas halophilus) and Northern Pacific Treefrogs (Pseudacris regilla). We sought to determine the impact, if any, corticosterone and chytrid fungus (Batrachochytrium dendrobatidis) infection had on altering Ribeiroia infection in tadpoles. Exposure to B. dendrobatidis significantly decreased Ribeiroia infection in both anuran models. However, increased corticosterone did not impact Ribeiroia infection in our anuran models. Our results highlight the importance of understanding the complex dynamics associated with amphibian diseases in our attempts at continued conservation of such a keystone vertebrate taxa.

Latitudinal Gradients in Helminth Community Composition

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While the latitudinal diversity gradient is a well-documented pattern in biogeography, the influence of these patterns on parasite community composition has been less well studied. Parasite communities are influenced by multiple abiotic and biotic variables that often vary with latitude, including climate and species interactions, and might be expected to follow a latitudinal gradient in their species composition. To investigate possible latitudinal gradients in parasite community composition, I collected data on parasitic helminths of cricetid rodents from sites along a latitudinal gradient in North America in 2015 to 2017. At each site, I trapped and dissected rodents and collected their helminths; rodents and helminths were preserved according to standard techniques. In addition, abiotic and biotic data, like environmental and host characteristics, were collected to be used in further analyses to investigate factors affecting changes in community composition. Principal component analyses and generalized linear mixed models were used to investigate changes in helminth community composition and the abiotic and biotic variables that were significantly correlated with community composition. These analyses were conducted on two scales, both within and among rodent host species along a latitudinal gradient, with effects of host phylogeny controlled on the latter scale. Results of these analyses will be presented and discussed.

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 multidimensional 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.

Life history and multi-locus sequence based genotyping of the equine renal parasite Klossiella equi strongly support the phylogenetic placement of the genus Klossiella among the Adeleorina (Apicomplexa: Coccidia).
Species of the genus *Klossiella* are unique among the suborder Adeleorina because they are monoxenous in mammals exclusively while all other reported members of the Adeleorina utilize invertebrates as definitive hosts. Unlike other coccidia, all members of the Adeleorina undergo syzygy, the association of micro- and macrogamonts prior to maturation to gametes and syngamy. Despite being biologically similar to other members of the Adeleorina, the phylogenetic placement of *Klossiella* has been questioned based on its unique host affinity. In the present study, two cases of *Klossiella equi* were reported from the renal tissue of Ontario horses (*Equus ferus caballus*) examined in post mortem. The life history as well as mitochondrial (mt) and nuclear 18S ribosomal DNA (18s rDNA) sequences were analyzed to provide both morphological and molecular evidence for the phylogenetic placement of *Klossiella*. Initially, various stages of the life history were interpreted from histological slides prepared from the renal tissue. DNA was isolated from infected equine renal tissue. Polymerase chain reaction (PCR) and Sanger sequencing were used to generate a complete mt genome sequence (6,569 bp) and a partial 18S rDNA sequence (1,443 bp). The *K. equi* 18S rDNA sequence was aligned with various publicly available apicomplexan 18S rDNA sequences. This alignment was used to generate a phylogenetic tree based on Bayesian Inference. Multiple *K. equi* stages were identified including meronts, micro- and macrogamonts associating in syzygy as well as thin walled oocysts in various stages of sporagonic development. The mt genome of *K. equi* contained 3 coding sequences for cytochrome *c* oxidase I, cytochrome *c* oxidase III and cytochrome *b* as well as various fragmented ribosomal sequences. These components were arranged in a unique order that has not been observed in other apicomplexan mt genomes sequenced to date. The 18S rDNA sequence of *K. equi* positioned within the monophyletic Adeleorina clade, sharing a common most recent ancestor with heteroxenous adeleorinid parasites.

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**Light-response larval behavior in three capsalid monogeneans**

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Monogeneans cause serious problems in finfish aquaculture around the world. Oncomiracidia of some capsalid monogeneans possess phototaxis and this behavior can be useful for the development of a new control measure. We compared the larval phototactic behavior of three important capsalids, *Benedenia seriolae*, *Benedenia epinepheli* and *Neobenedenia girellae*. Behavioral response to repeated LED illuminations in freshly hatched (< 3 hr) oncomiracidia were video recorded and larval swimming speed and swimming patterns were analyzed on a PC. All three species showed strong attraction to light by moving straight toward a light source. The two *Benedenia* spp. show significant increase in the swimming speed during phototactic behavior. In contrast, swimming speed of larval *N. girellae* in response to light is relatively slow. When the light was turned off, larvae of all three species demonstrated photophobic responses with erratic swimming. These behaviors are likely adaptation for these ectoparasite to increase the chance of encounter and attachment to suitable fish hosts. The reasons for observed inter-specific difference in phototactic behavior, however, remain unclear.
Little Bo Peep, what’s causing diarrhea in your sheep? Identifying Eimeria species causing coccidiosis in sheep and goats using morphology and genotyping

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Protozoan parasites in the genus Eimeria are the pathogenic agents that cause coccidiosis. This diarrheal disease is an economical burden on the small ruminant industry due to its high morbidity and mortality rates. From this group of host specific species that infect sheep and goats only certain species are considered to be highly pathogenic. Less pathogenic species can be excreted at large numbers without causing any severe clinical effects and pathogenic species can cause so much intestinal damage that oocyst shedding is reduced rendering conventional enumeration methods unreliable indicators of the severity of infections. Therefore the identification of individual species, particularly those that are the most pathogenic, is crucial information for effective and targeted anticoxidial treatment. Currently identification of species infecting sheep and goats are done by the morphology of the exogenous oocysts but this method is unreliable due to overlapping morphological characteristics. Recently DNA barcoding techniques have been successfully used in identifying of Eimeria species infecting poultry. Differences in gene sequences at specific loci within the mitochondrial and nuclear genomes can be used to differentiate species. The goal of this project was to identify individual Eimeria species infecting sheep and goats using differences at specific genetic loci within the mitochondrial and nuclear genomes in comparison to conventional morphological techniques. Eimeria oocysts were isolated from sheep and goat fecal samples collected globally. Morphological data was collected from 50 oocysts per sample. Sequence data was collected from several genetic loci in the mitochondrial genome and 18S rRNA SSU gene using conventional PCR and amplicon sequencing. PCR amplicons containing mixes of species are to be sequenced using multiplexed next generation sequecing (NGS). Molecular phylogenetics was used to establish relationships among the identified species; morphometric data were then mapped onto the molecular tree to assist in selecting morphological characteristics that reflect the evolutionary relationships among these genotyped Eimeria species.

MECHANISMS FOR MATERNAL TRANSMISSION OF THE NEOGRE-GARINE, <i>Ophryocystis elektroscirrha</i> IN THE MONARCH BUTTERFLY, <i>Danaus plexippus</i>.

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The pathogenic neogregarine Ophryocystis elektroscirrha infects the hypodermal tissues of the monarch butterfly (Danaus plexippus). Currently, two major routes of transmission have been proposed for O. elektroscirrha and include horizontal transmission, when butterflies deposit protozoan oocysts onto milkweed leaves from scales on their abdomen, and more commonly through maternal transmission, when females deposit oocysts from scales on their abdomen onto eggs during oviposition. Caterpillars become infected when they ingest oocysts from milkweed leaves or egg cases after hatching. However, the mechanism for oocyst transfer from infected female butterflies to their eggs and the number of oocysts deposited on eggs in nature is unknown. Interestingly, recent studies indicate that over time infected female monarchs lose over 90% of the oocysts from their abdomens but the
average number of oocysts transferred to eggs remains constant. Additionally, laboratory studies indicate that a large percent of butterflies emerge deformed and die when caterpillars ingest more than 10 oocysts. To investigate these discrepancies, we first examined the abdomen and internal cuticular region of the ovipore of naturally infected female monarchs using SEM and histological techniques. Second, we evaluated the number of oocysts and scales deposited on eggs of experimentally infected monarchs at different time intervals during ovipositional events in cages. Finally, we evaluated deformities and survival in field collected, naturally infected 5th instar caterpillars that were allowed to pupate and emerge as butterflies in the laboratory. Our results indicated that all infected female monarchs contained oocysts of *O. elektroscirrha* among internal spines of the ovipore as well as on the scales of their abdomens. However, our laboratory oviposition studies indicated that only 20% of monarch eggs contained scales, which are deposited on eggs when females touch previously laid eggs during subsequent oviposition events. More importantly, eggs with scales contained significantly more oocysts (30 ± 39) than eggs without scales (2.2 ± 1.8; P < 0.0001). Additionally, the number of oocysts on eggs with scales decreased significantly with oviposition date (6.5 ± 6.7; P < 0.0001); whereas the number of oocysts on eggs without scales did not (1.4 ± 2.8; P = 0.08). Finally, none of the butterflies that eclosed in the laboratory from naturally infected 5th instar caterpillars were deformed or died. Our observations suggest that under natural conditions where female monarchs deposit one or two eggs per milkweed plant, a non-lethal dose of oocysts is transferred to their eggs from inside the ovipore of females and not from the scales of the abdomen. The implications of our findings are discussed in terms of conservation of migratory monarch butterfly populations.

**MONITORING THE HEALTH OF CANADA’S ARCTIC MUSKRATS: RELATIONSHIPS BETWEEN BODY CONDITION, PARASITES AND ENVIRONMENTAL CONTAMINANTS**

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Effective conservation requires baseline studies to validate whether populations have declined and if so, suggest what factors may have contributed. Indigenous trappers in the Mackenzie River Delta, Northwest Territories recently reported reduced muskrat populations. Scientists teamed up with local communities to test this hypothesis by assessing the density and health of the muskrats in this region. From trapper-donated carcasses, we measured body condition, parasitism, and environmental contaminants in 43 muskrats (27 males, 16 females) collected in 2016-2017. Preliminary regression analyses indicated a significantly positive relationship between log body mass and log body length of muskrats. An analysis of variance found an interaction between sex and parasite intensity that explained a significant amount of variation in muskrat body condition (partial residuals from regression of log mass and log length). Males had better body condition and were infected with significantly more parasites than females. Preliminary necropsies recovered at least four trematode genera including *Echinostoma, Notocotylus, Plagiorchis,* and *Quinqueserialis.* The latter parasite was the most prevalent (100%) and had the highest mean intensity (69-1,006 individuals). Our results suggest differences in parasite infection between the sexes. We will discuss how environmental contaminants such as organic and inorganic mercury and heavy metals relate to body condition and whether there is any interactive effect with parasites. By collaborating with communities in northern Canada, this baseline study will help distinguish potential drivers of muskrat population dynamics and health.

**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE CESTODA CLASS PARASITES IN Octopus maya (Voss y Solis, 1966), IN THE YUCATAN PENINSULA**
The cephalopod, Octopus maya is an endemic species of 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. Previous studies indicate the presence of an important amount of larval cestodes, in both, number of species and in individuals. Therefore, in the present study we aim to identify parasites of the Cestoda class and subsequently characterize them using molecular tools and microscopy techniques. From September to December 2017, 15 octopus were collected in each one of the port localities of Sisal, Progreso, Dzilam de Bravo and Ría Lagartos in the Yucatan Peninsula. The organisms were obtained through commercial fishing. All specimens were measured, weighed and dissected searching for cestodes in all internal organs. Cestodes were identified in fresh and counted, subsequently fixed for both, microscopic identification and molecular techniques. Prevalence, mean intensity and mean abundance for each of the taxa identified in fresh were calculated. Each parasitc taxon was described based on Light and Scanning Electron Microscopy (SEM) images and molecular techniques. Six species of cestodes were identified: Prochristianella hispida, Eutetrarhynchus spp., Phyllobothrium spp., Prosobothrium spp., Echeneibothrium spp., and Kotorella sp. The most frequent species was P. hispida, collected in the bucal mass, with 100% prevalence in all localities and a mean abundance of 362.60 (±804.60); the least frequent was Echeneibothrium spp., collected in the ink sac, with a prevalence of 10%. It is presumed that the high abundances of some species of cestodes are a product of the constant infection (through feeding on intermediate hosts) from the juvenile stages of O. maya, since the larvae of this class of parasites accumulate over time.

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Mapping and filling gaps: Revision of proteocephalid tapeworms (Cestoda), neglected parasites of freshwater fishes in North America

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The freshwaters of North America harbor an extraordinarily rich fauna of bony fishes, ranging from archaic groups such as sturgeons (Acipenseridae), bowfin (Amia calva Linnaeus) and gars (Lepisosteidae) to several more recently evolved perciform families (e.g., Centrarchidae, Percidae). These fishes are hosts of a great variety of helminth parasites including tapeworms (Cestoda). Some of the cestode groups occurring in North America were revised at the beginning of the 20th Century, such as members of the family Proteocephalidae La Rue, 1911. However, attention to fish cestodes (and parasites of freshwater fishes in general) has dramatically decreased since the second half of the last century and the current knowledge of their diversity, life cycles, host associations, distribution and phylogenetic relationships is insufficient. Collections of proteocephalideans over the last two decades in Canada and USA, in our possession, enabled us to begin a critical examination of the validity of many North American proteocephalids. As many as 36 species of Proteocephalus Weinland, 1858 have been described from a broad spectrum of fishes in North America since the end of the 19th Century. A forthcoming series of taxonomic papers focused on tapeworms is being prepared with the goal of providing a robust baseline for future studies on one of the dominant groups of intestinal helminths parasitizing freshwater fishes in North America. Revision of species of the Proteocephalus-aggregate from perciform fishes of the families Centrarchidae (bass) and Percidae
(perch, pikeperch) has revealed that the actual number of valid species is considerably lower than that of nominal taxa described from North America. In contrast, a preliminary evaluation of species from sticklebacks (Gasterosteiformes) indicates the existence of a new species from Culaea inconstans. Targeted collection of fresh specimens across North America are planned so to overcome the absence of properly fixed material suitable for morphological and molecular evaluation.

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Mate Preference of the Horsehair Worm, Paragordius varius (Nematomorpha)

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Knowing how parasites mate can help us understand the evolutionary relationships among closely related parasites and could lead to new medical treatments. Horsehair worms infect various terrestrial arthropods, which they manipulate into entering water where the adults emerge, mate and lay eggs. Previous studies suggested that adult horsehair worms (Paragordius varius) encounter each other non-randomly in the laboratory. We tested the mate preference of a focal worm in the presence of two stimulus worms in a 10-gallon tank filled to 5 cm with RO water. Worms were reared in Acheta domesticus crickets (Millbrook) that were infected with field-collected snails (Physa spp) known to contain P. varius cysts. Worms were isolated immediately after exiting the cricket to prevent mating. The two stimulus worms, a male and a female, were placed at opposite ends of the tank and held in nylon bags. The focal worm (either a male or a female) was placed in the center of the tank and its movements were video taped for 15 minutes. The worm’s position was also recorded at one-minute intervals using a scale: from -2 (far left side) to zero (center) to 2 (far right side). Analysis of the scale data from the last five minutes of 26 trials suggests that the focal worm exhibits a statistically insignificant (t-test, p = 0.06) preference for the side of the tank containing the stimulus worm of the opposite sex. However, the 12 male focal worms significantly preferred the side containing the female stimulus worm (p = 0.029) whereas the 14 female focal worms did not show a preference (p = 0.69). Additional analysis of the video data will focus on the time spent on each side of the tank, time spent in contact with each stimulus worm and the number of times the focal worm crossed the center of the tank. Mass spectrometry will analyze the pheromones that adult horsehair worms use to locate each other. Results could lead to the development of drugs that interrupt the mating behavior of closely related human nematode parasites.

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Measurement of the entomopathogenic capacity of Heterorhabditis sp. native of Yucatan in the wax moth Galleria mellonella

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Chemical insecticides of third generation such as pyrethroids and organochlorines have been used for the control of the kissing bug, mosquitoes and other arthropods which act as vectors of important sicknesses for humans such as Malaria, Dengue fever or Chagas disease. According to The World Health Organization (WHO) in the case of Chagas disease there are already 15 000 000 of people infected worldwide. However, one of the major problems with insecticides is that the arthropods eventually develop resistance to these chemicals. As an alternative, the use of entomopathogenic
nematodes for biological control has been proposed but in Mexico, there are very few records of the distribution and effectiveness of these nematodes. While using a survey looking for entomopathogenic nematodes in several localities of Yucatán, we found a new undescribed species: *Heterorhabditis sp.* The objective of the present study was to test the entomopathogenic capacity of *Heterorhabditis sp.* on the larvae of the moth *Galleria mellonella* to determine whether this nematode has a future as a biological control agent. We exposed one hundred and five individuals of *G. mellonella* to infected soil with *Heterorhabditis sp.* and after seventy-two to ninety-six hours, the moths became infected and died. The mean number of infective juveniles of *Heterorhabditis sp.* per *G. mellonella* individual was 20166 ± 2000. Our results suggest that *Heterorhabditis sp.* has an interesting entomopathogenic capacity, and that further experimental infection of larvae of arthropod vectors such as kissing bugs or mosquitoes should be undertaken.

**Melatonin protects against thymic oxidative damage during Trypanosoma cruzi infection**

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Melatonin protects against thymic oxidative damage during Trypanosoma cruzi infection  
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Introduction: It is well established that oxidative stress generated during acute T. cruzi infection as well as the inefficient scavenging of reactive oxygen species (ROS) seem to worsen tissue damage. Methods: Melatonin was administered by gavage (05 mg/kg) to young (5 weeks old) and middle-aged (18 months old) male Wistar rats infected with 1x10⁵ blood trypomastigotes of the Y strain of T. cruzi and randomized into groups: young control (YC), young melatonin control (YMC), young infected (YI), young melatonin infected (YMI), middle-aged control (MC), middle-aged melatonin control (MMC), middle-aged infected (MI) and middle-aged melatonin infected (MMI). Oxidative stress was evaluated through lipid peroxidation analysis of thiobarbituric acid-reactive substances (TBARS), superoxide anion (O₂⁻) generation, superoxide dismutase (SOD) activity and expression of SOD1 and SOD2. Thymus samples were processed for flow cytometric analysis, using conjugated-specific monoclonal antibodies. Results: Infected and melatonin treated animals displayed reduced levels of O₂⁻ and TBARS. Increased SOD activity as well as SOD1 and SOD2 expression were observed for all middle-aged melatonin treated animals, followed by an increase of thymus weight and total number of thymocytes. Conclusion: The modulation of oxidative damage and thymic weight loss by the antioxidant ability of melatonin was revealed by our results. For this reason we propose the use of melatonin as a co-adjuvant compound for the treatment of Chagas’ disease. Financial support: FAPESP.

**Metazoan parasites on the lionfish Pterois volitans (Pisces: Scorpaenidae) from the Yucatán Peninsula**

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Abstract
The lionfish Pterois volitans is an invasive and predatory species into the Gulf of Mexico and the Caribbean Sea. Due to its position in the food webs (top predator) it represents a threat to the biodiversity of marine ecosystems, overall to coral reef areas. At the same time its presence could be adversely affecting the transmission of parasites in invading areas. For the Yucatán Peninsula there is little knowledge about the helminth fauna of lionfish and its interaction in parasite transmission. Therefore, this work is the first step to bring light on the fish-parasite interactions that are affected by the lionfish presence. A total of 76 lionfish from 6 localities (Cozumel, Arrecife Alacranes, Cabo Catoche, Cayo Arcas, Cayo Arenas and the eastern tip of the Yucatán Platform) were collected between 2011 and 2017 searching for parasites. A total of 27 helminth species belonging to 18 genera were recovered including: 12 Digeneans, 5 Cestodes, 7 Nematodes and 3 Acanthocephala most of them as larval stages. The Eastern zone of the Yucatán Peninsula presented the highest number of parasite species (18), while in the Alacranes Reef only 4 species were recovered. Siphoderina sp., Stephanostomum sp. and Diphtherostomum sp. were collected in 4 out of 6 locations, while Lecithochirium sp. appeared in all of them. Our results suggest that helminth communities of the lionfish P. volitans are richer in number of species compared with those reported for this fish species in the Bahamas, Puerto Rico, the Canary Islands and Coral Reef System of Veracruz from the Gulf of Mexico. Nevertheless, only 4 species were found in adult stage, therefore, the lionfish is a deadly end to most of the helminth species found.

Microparasite dispersal in metapopulations: a boon or bane for the host population?

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Although connectivity can promote species persistence over a larger metapopulation, dispersal may also act as an agent for disease transmission by bringing hosts and parasites into contact. This problem is further complicated by the fact that parasite distribution can impact dynamics. We investigated the effects of connectivity and initial distribution of parasites throughout the metapopulation on microparasite-host dynamics, using guppies and the monogenean ectoparasite Gyrodactylus turnbulli. We created metapopulations of four tanks of eight fish and introduced either two parasites to all tanks or eight to one tank. Every 10 days, one randomly selected fish from each tank was moved to the next tank in the metapopulation. Additionally, we introduced either two or eight parasites to isolated tanks of eight fish that allowed no dispersal of fish or parasites. Parasite counts were performed on each marked fish every other day until no parasites were found in the system for two consecutive counting days or for 120 days. We analyzed the effects of connectivity and of initial conditions on parasite persistence, host mortality, peak parasite load and mean abundance. Parasites persisted longer in metapopulations than isolated tanks. Mortality was lowest in isolated tanks where two parasites were introduced and did not differ among connected tanks. Peak parasite load
and mean abundance were influenced by the interaction of connectivity and parasite introduction levels. When parasites were introduced at low levels, being in a metapopulation helped the parasite persist longer but had little effect on host parasite burden or death. However, when parasites were introduced at high levels, being in a metapopulation also benefited the hosts, as parasite burdens were lower. These findings have important implications for disease management and species conservation.

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**Microparasites with high potential impact in endemic poeciliid fishes on the periphery of a Mexican reserve of the biosphere**

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The development of a study to reproduce in captivity to vulnerable endemic poeciliids of Lake Catemaco in Veracruz State, showed that microparasites (ciliated protozoa, myxozoan and gyro-dactylid monogeneans) could seriously curb the possibility of attaining it. This study shows data about episodes of mortality in the lab of: 1) wild fry of *Poecilia catemaconis* (N=141) (collected in May 2016), 2) wild fry of *Pseudoxiphophorus tuxtlensis* (N=75) collected in October 2016, 3) sentinel naïve fries of 4 species of poeciliids exposed 96 hrs. in cages on Lake Catemaco in September 2016, and 4) the temporal presence of such parasites in 4 species of poeciliids: *Poecilia catemaconis*, *Pseudoxiphophorus tuxtlensis*, *Xiphophorus kallmani* and *X. milleri* on 6 surveys (N = 15-30) in dry, windy and rainy seasons from 2015-2016. The length of fish were recorded, and parasitological examinations included the search for ecto and endo microparasites; the Lake’s water temperature was recorded 2 times per week throughout the study. The mortality of *P. catemaconis* fries was 85% with two peaks at 28 and 40 days post sampling, and was associated with myxozoans in body muscle. The mortality of *P. tuxtlensis* was 55% with two peaks at 5 and 14 days post sampling, and was associated with *Gyrodactylus* sp. (30-50 worms/parasitized fry), *Trichodina* sp., *Ambiphrya* sp. and *Ichthyophthirius multifiliis* ("Ich"). The 4 species of sentinel poeciliids presented "Ich" (70-80% prevalence). Respect to seasonal data, *I. multifiliis* presented the highest prevalences (74-87%) in fries of *Xiphophorus kallmani*, *X. milleri* and *Pseudoxiphophorus tuxtlensis* in February (19.5±1.5 °C), but peaks of prevalence were also observed (55 to 66%) in juvenile fishes in June (temperature 31±1.0°C) in *X. kallmani* and *X. milleri*, respectively. Finally, *Gyrodactylus* sp. showed the highest prevalence (43%) in rainy season (N=30) and the lowest in dry season (6%), 0-10 monogeneans/analyzed fish (N=16).

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**Migratory and resident haemosporidian assemblages remain dissimilar despite host range overlap during wintering migration**

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Up to 50% of individuals of migratory birds are infected with haemosporidian parasites (order Haemosporida) prior to travelling to wintering areas. Although 1,600 bird species undertake seasonal migrations and migratory birds are declining both regionally and globally, little is known about how migration contributes to dispersal of avian vector-transmitted parasites. We asked whether, during wintering, migratory birds retain haemosporidian lineages acquired in the breeding grounds, or gain lineages endemic in the resident host assemblage of wintering areas. We surveyed infections in migratory and resident birds sampled during January in the Dominican Republic. We surveyed 1,128 samples from 25 resident species, and 299 samples from 11 migratory avian species. We sequenced 299 infections of 19 haemosporidian lineages; 281 of which were found in year-round residents and only 8 were detected in migrants. Using a linear mixed model controlling for sampling year and host species identity, we determined that the odds of resident birds being infected with haemosporidian parasites is 1.2 (OR, 95%CI=1.1–2.4) higher compared to infections in migratory birds. Seven out of the 8 infections detected in migrants were lineages that commonly occur in North American breeding grounds, and only one migrant host carried a lineage endemic of the Caribbean. Resident birds were only infected with West Indies endemic haemosporidian lineages. Our results support that wintering migratory lose or suppress most infections transmitted in the breeding grounds, and rarely carry parasite lineages endemic to Tropical wintering territories. We found no evidence of established host switching of parasite lineages from North America to resident hosts of Hispaniola, and vice-versa. Haemosporidian assemblages remain mostly dissimilar despite range overlap of migratory and resident species in wintering areas. We hypothesize migratory hosts suppress within host parasite populations during migration and wintering, thereby limiting parasite transmission in wintering grounds.

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Missing the target? Exploiting suitable genetic loci for PCR- and sequence-based molecular typing of coccidia.

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PCR-based assays have revolutionized the rapid identification of coccidian parasites, but the multitude of *Eimeria* species that can contribute to coccidiosis infections in domestic poultry poses a challenge for such assays. A PCR-based assay becomes complex because of varying numbers of closely related species that can all be present simultaneously. There is a variety of genetic loci that have been targeted in attempts to identify coccidia but serious limitations exist for some of these loci.

The nuclear ribosomal RNA genes (18S rDNA) and neighboring ITS regions have been the mainstay of *Eimeria* species identification of these and other parasites; however the sequence divergence among closely related coccidia can limit PCR primer possibilities. Genetic loci in the mitochondrion (especially cytochrome c oxidase subunit I, mtCOI) have been exploited more recently because interspecific sequence divergence is usually 1% or greater, whereas intraspecific variation is usually one-tenth as great. The mtCOI locus has become used widely for sequence-based genotyping (i.e. "DNA barcoding") and species differentiation of eukaryotic taxa including coccidia; this same locus is a useful target for designing species-specific PCR primers.

This presentation will review existing molecular targets that have been used for diagnostics of *Eimeria* species in poultry and summarize PCR optimization protocols required for diagnostic assays. A previously unused locus, the mitochondrial cytochrome c oxidase subunit 3 (mtCOIII) CDS, has features that make it a highly suitable locus for species-level molecular differentiation. The higher sequence divergence of the mtCOIII locus compared with mtCOI provides better differentiation of closely related species. Side by side PCR assay results will be compared for the commonly used loci and diagnostic applicability will be discussed. Preliminary PCR reactions show promise for the mtCOIII to reliably identify *Eimeria* species infecting turkeys and chickens.
Mitochondrial tRNA duplication in Placobdella spp. How widespread is it?

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The genus Placobdella (Clitellata: Glossiphoniidae) is integrated by hematophagous ectoparasitic leeches that feed mainly on freshwater turtles. So far, 24 Placobdella species are recognized, but there is great potential for new species discovery. Most species are distributed in North America, with Placobdella costata Müller 1864 as the only European species described (Sawyer, 1986; Oceguera-Figueroa & Siddall, 2008). Oceguera-Figueroa et al. (2016) analyzed the mitochondrial genomes of three hematophagous leeches species, including Placobdella lamothei and Placobdella parasitica. Both species have a duplication of the trnD gene (aspartic acid) located between genes cox2 and atp8, which has not been observed in any other annelid, so it may be restricted to this genus. Both copies of the gene are transcribed into tRNAs with a regular and apparently functional secondary structure. Nevertheless, while in P. parasitica there is no overlapping or spacing between the copies, in P. lamothei there are 128 bp of intergenic space. Furthermore, in P. parasitica both copies hold a GUC anticodon, while in P. lamothei one of them has been modified and possesses an AUC anticodon. The region between cox2 and atp8 exhibits great gene variability within the phylum Annelida (for which the ancestral condition is the possession of a single trnD copy), as well as in hymenopteran insects and has been called a hot spot for gene rearrangements (Dowton et al., 1999). We designed primers specific for the amplification of this genomic region for Placobdella species and obtained new sequences that fairly represent the phylogeny of the genus, which were later analyzed using MITOS Web Server to check for genes present and obtain tRNA secondary structures. We found different arrangements of genes at this position including not only duplications, but apparent translocations of tRNA genes (trnl, for example). These rearrangements appear to be a phenomenon exclusive to this genus and potentially a synapomorphy of the group.

Molecular and Morphological Identification of Echinostomes in the Mud Snail, Ilyanassa obsoleta, the Ribbed Mussel, Geukensia demissa, and the Herring Gull, Larus argentatus, in a Delaware Salt Marsh

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The echinostome, Himasthla quissetensis (Miller and Northrup, 1926) (Hq hereafter) has been reported for > 40 years from the marine gastropod, Ilyanassa obsoleta, along the U.S. east coast. Many reports are from Rehoboth Bay, DE, near Savages Ditch with >85% prevalence in I. obsoleta. Gulls (Larus spp.) serve as the definitive hosts. We hypothesized that the second intermediate host in the Hq life cycle at Savages Ditch is the ribbed mussel, Geukensia demissa, based the presence of echinostome metacercariae and the proximity of G. demissa to I. obsoleta at this locality. Our objective is to elucidate the life cycle of Hq using morphological and molecular data. To confirm the presence of Hq in this locality, we examined I. obsoleta (n=1,534) and G. demissa (n=98) for rediae and metacercariae, and Larus argentatus (n=9) for adult echinostomes. We identified the echinostomes using morphological evidence and tested our identifications with DNA barcoding. In 2016-2017, the prevalence
of echinostome rediae in *I. obsoleta* was 4.3-6.4% and metacercariae in *G. demissa* was 65-75%. Echinostomes were photographed and sequenced for partial mitochondrial CO1 and NAD1 and partial nuclear 28S and ITS1 rDNA loci. A BLAST search in GenBank revealed that the sequences belong to the Echinostomatoidea. Phylogenetic analysis of the results grouped all adults, 14 metacercariae, and 5 rediae into one clade (Clade Hq, confirmed by adult morphology), 15 rediae and 9 metacercariae into a second clade (Clade E), and 1 metacercaria did not fall within either clade. Pairwise distances were calculated within and between the clades for CO1 and NAD1. For CO1 there was a 0.07% difference between clades Hq and E and a 0.005-0.01% difference within each of these clades. For NAD1 there was a 0.07% difference between clades Hq and E and a 0.008% within each of these clades. The metacercaria that did not place within clades Hq or E was 0.14-0.15% different for both genes. These results indicate that the echinostome specimens sequenced represent 3 echinostome species, including Hq, at Savages Ditch.

### Molecular and functional dissection of the insect immune response against nematode-bacteria complexes

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Despite important advances in the field of insect innate immunity, our understanding of host immune responses to entomopathogenic nematode infections lags behind. Nematodes in the genus *Heterorhabditis* and *Steinernema* maintain mutualistic relationship with the bacteria *Photorhabdus* and *Xenorhabdus*, respectively. Unlike other animals associated with mutualistic bacteria, *Heterorhabditis* and *Steinernema* nematodes are viable in the absence of their associated bacteria. Consequently, each partner of this mutualistic/pathogenic relationship can be separated and studied in isolation and in combination, thus enabling the insect host immune reactions against each player of the interaction to be studied separately or together. Recent work to analyze the interactions between insects and entomopathogenic nematode-bacteria complexes has begun to take advantage of the powerful genetic and genomic tools of the fruit fly *Drosophila*. We have used this tripartite system to understand the molecular and mechanistic basis of insect immune defenses against these nematode parasites and their bacteria. We have generated fundamental information on the immune detection, the transcriptional regulation of signaling pathways in the fly, as well as the number and nature of the effector immune process that *Drosophila* activates in response to entomopathogenic nematode-bacteria complexes. Our findings provide novel insights on insect anti-nematode immune activities that could be considered when devising alternative strategies for the control of noxious insects of agricultural or medical importance.

### Molecular evidence linking the larval and adult stages of *Mexiconema cichlasomae* (Dracunculoidea: Daniconematidae): from Celestun coastal lagoon, Yucatán, México, with notes on its phylogenetic position in the Dracunculoidea

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The study of the life cycles of nematodes of aquatic organisms in tropical America is scarce, and restricted to *Anisakidae* (5 life cycles) and to *Camallanidae* (4 life cycle) families. In Mexico, the only partially described nematode life cycle is that of the dracunculid *Mexiconema cichlasomae*. This
nematode uses the crustacean ectoparasite *Argulus yucatanus* (Crustacea: Branchiura) as first intermediate host and the adult stages are found in the Mayan cichlid *Cichlasoma urophthalmus* (Percomormes: Cichlidae) both from Celestun coastal lagoon, Yucatán, Mexico. However, the lack of distinguishing characteristics in the larval stages cast doubts about whether they truly belong to *M. cichlasomae*. Therefore, our aims were two-fold: testing the possible conspecificity between larval stages in *A. yucatanus* and *M. cichlasomae* adults in *C. urophthalmus* using morphological features and SSU molecular marker, and re-evaluating the phylogenetic position of *M. cichlasomae* into the Daniconematidae family by molecular analysis. We obtained sequences from the SSU rDNA marker from larval stages of *M. cichlasomae* in *A. yucatanus* and adult nematodes in *C. urophthalmus*. Our morphological and molecular results support conspecificity between *M. cichlasomae* larvae in *A. yucatanus* and the adult stages in *C. urophthalmus*. Furthermore, our phylogenetic analyses provide evidence of, the monophyly of *M. cichlasomae* among the Daniconematidae associated with as branchiurid intermediate hosts. This is the first complete life cycle of Daniconematidae nematode of fishes for tropical America.

**Molecular phylogenetic analyses uncover a species complex in the nematode Rhabdochona mexicana, a parasite of Astyanax spp.**

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The genus *Rhabdochona* represents one of the most speciose groups of freshwater fish nematodes with ca. 100 described species around the world. Most species in the genus are host specific towards their fish hosts, especially at family level. Species diversity in the genus has been based on morphology, and few attempts have been made to analyze such diversity using molecular data. Here, we conducted a molecular prospecting study on *Rhabdochona mexicana* to detect the potential existence of cryptic species. This nematode is host specific to characids, a Neotropical fish group; it was described from the intestine of *Astyanax mexicanus* in central Mexico, and latter found in other localities of central and southern Mexico. Between 2014 and 2016, we conducted samplings in 15 localities between northeastern Mexico and northern Guatemala, including the known distribution range of *R. mexicana*. DNA sequences of three molecular markers were obtained (18S, 28S, and cox1), and phylogenetic analyses were performed through Maximum Likelihood and Bayesian Inference. Our results show that *R. mexicana* represents a species complex, where two additional evolutionary significant units (candidate species) were recognized. We based our conclusions on evidence from reciprocal monophyly, genetic divergence, geographical distribution, and host association. Further morphological analysis of one of the uncovered genetic lineages for which we sampled a large number of individuals, resulted in finding some traits that allowed us to distinguish this as a separate new species. Molecular tools are then required to fully estimate species diversity within Rhabdochona. This kind of studies are required particularly for other congeneric species with a wide geographic range and/or wide host spectrum such as *R. kidderi*, *R. acuminata* and *R. canadensis*.

**Molecular phylogenetic analysis of the Cyathocotylidae**

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The Cyathocotylidae Mühling, 1898 is a small, but globally distributed family of digeneans parasitic as adults in the intestine of reptiles, birds and, rarely, mammals. The remarkable combination of worldwide distribution, parasitism in a broad variety of hosts and adaptation to both freshwater and saltwater environments poses a number of intriguing questions regarding the evolution, hosts associations, historical biogeography and systematics of the family. The monophyly of the family, its sub-families and larger genera are among these questions along with the need to understand the occurrence and nature of evolutionary host switching events in shaping up current host associations of cyathocotylids. An answer to these and a number of other questions requires knowledge of phylogenetic relationships among cyathocotylid taxa. The compact size of the family makes it possible to answer the majority of questions posed above with relatively few taxa. Members of only 4 out of 16 currently recognized genera have been previously included in molecular phylogenetic analyses. In this study, we used partial sequences of the nuclear ribosomal 28S gene to examine phylogenetic interrelationships of several cyathocotylid species from reptiles, birds, and mammals from Africa, Australia, Europe, and North America. Our analysis has demonstrated host switching events within some cyathocotylid clades including those encompassing species parasitic in reptiles and birds. Combination of morphological and molecular analyses revealed mistakes in morphological descriptions of some known taxa and allowed for reassessment of systematic value of some morphological characteristics traditionally used in systematics of this group. Several revisions in the system of this family are proposed. This study was funded in part by the National Science Foundation project number DEB 1120734 and the University of North Dakota Academic Programs and Student Awards Committee Research Stipend.

Molecular survey of helminths infecting ground dwelling birds in the grouse subfamily Tetraoninae

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Alaskan grouse and ptarmigan (Galliformes) are important avian game species in Alaska. Interestingly, gallids harbor a fairly diverse helminth fauna, particularly cestodes, which may make birds with high parasite loads more susceptible to predation. Unfortunately, there is limited information available on the helminth fauna of Alaskan gallids, and no molecular surveys. The present study aims to develop baseline data on diversity of intestinal and subcutaneous (filariid) helminth infections in galliforms using morphological and molecular (DNA) approaches. These data can then be used to better understand changes in helminth community structure given current environmental volatility. The intestines, cloaca, liver and kidneys of 72 Alaskan gallids (ptarmigan: Lagopus lagopus, L. muta, L. leucura and grouse: Falcipennis canadensis, Bonasa umbellus, Tympanuchus phasianellus, and Dragapus fuliginosus) and blood/tissues of 628 birds were examined for the presence of helminths (nematodes, cestodes, digeneans). Ten helminth species, including 3 digeneans, 4 cestodes, and 3 nematodes, were found infecting 72 dissected birds. Prevalence of infection was 84% across all species. Falcipennis canadensis, L. lagopus, and L. muta harbored the highest species richness. PCR and sequencing of 28S rDNA and COI mtDNA verified morphological species designations from DNA extracted from the adult helminths. Real Time PCR using TaqMan probes targeting the 18S rDNA gene was used to test for the presence of filarial nematodes in 628 blood/tissue DNA samples [L. lagopus (n = 242), L. muta (n = 161), L. leucura (n = 23), F. canadensis (n = 156), B. umbellus (n =
23), *T. phasianellus* (n = 15), and *D. fuliginosus* (n = 8)]. This is the first molecular survey of Alaskan grouse and ptarmigan parasites.

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Monogenean community traits affected by species abundance

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Monogeneans are direct life cycle parasites found throughout freshwater and marine ecosystems. In order to determine the ecological factors that shape communities of gill monogeneans, we sampled three New Jersey lakes for Centrarchid fish, recovered parasites by dissection, and measured individual worms. We also investigated the effects of host density using mesocosm tanks to identify parasite population responses to host-specific stimuli. Overall, the data shows that monogenean abundance increased significantly in high host density tanks relative to wild parasite populations with both *Onchocleidus* sp. and *Actinocleidus* sp. growing larger populations. Abundance of *Urocleidus dispar*, common in natural lakes, showed no changes in abundance as host density increased, but this parasite was less prevalent (53%) in mesocosms when compared to natural populations (100%). There was a significant relationship between parasite size and parasite abundance; *Onchocleidus* sp. size increased with species abundance. These results suggest that transmission may be improved with higher host densities for some species more than others and that transmission could be a more important limiting factor for those species than others in nature.

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Mutualisms Between Bacterial Endosymbionts and Blood-Feeding Leeches

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Hematophagous leeches of the genus Placobdella (family Glossiphoniidae) possess a unique bacteriome organ that is attached to their esophagus. This structure, sometimes erroneously referred to as mycetome, has no other obvious function than housing intracellular bacteria. The function of these bacteria remains elusive though precursory evidence suggests a mutualism between the leech and bacteria. The bacteriomes and their microbes are absent in non-hematophagous genera of the family (e.g., *Hellobdella, Glossiphonia*) implying an endosymbiotic association relevant to blood-feeding diets. Preliminary phylogenetic analyses revealed that Placobdella endosymbionts are members of α-proteobacteria (αPB). This class of bacteria includes several invertebrate endosymbionts, such as Buchnera in aphids and Wigglesworthia in tsetse flies, that function to supplement proteins or vitamins missing in their respective host’s diets. Endosymbionts residing in Placobdella’s sister genus *Haementeria* have been identified as γ-proteobacteria. Sequences of the Haementeria-associated microbes have coded protein domains involved in synthesizing vitamin B, suggesting the supplementation of this essential vitamin absent in the leech diet. Here, I present genome-level sequence data, acquired through several lanes of Illumina sequencing of enriched bacterial DNA, for several Placobdella-bacterial endosymbionts. I provide evidence for their function within their leech hosts, discuss their genomic makeup and sizes, as well as their interesting phylogenetic position among plant-related, nodule-forming Rhizobiaceae.
Nematode-Specific Amplicon Sequencing in Studying Scat Samples from South African Mammals

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Gastrointestinal parasitic nematode infections are prevalent among many African mammals. The diversity and prevalence of parasitic nematode infections found within African mammals is influenced by numerous factors including habitat, feeding patterns and predation. This area of research is vastly understudied due to the often obscure and indirect effects parasites have on host survival. Gaining knowledge in regards to host-parasite relationships, infection rates, and nematode diversity found within hosts is of great importance to conservation efforts. Major obstacles for conservationists are being able to 1) detect parasitic nematodes and 2) properly identify different nematode species in fecal samples. It is often difficult to distinguish nematodes using solely morphological keys, and thus there is a need for developing a molecular approach to properly classify gastrointestinal nematodes found in scat samples. Next-generation amplicon sequencing is used to identify nematode parasites in scat samples from a variety of African mammals including cheetahs, zebras and rhinoceroses. Identified nematode parasites are then subjected to our next-generation sequencing and Galaxy-based RepeatExplorer pipeline (Pilotte et al, 2016) to identify repetitive DNA targets that are used as targets for the development of novel, PCR-based diagnostic assays. The aim of these studies is to develop PCR-based assays to screen for nematodes present in the fecal samples of various African mammals in order to help study parasite prevalence to assist in future wildlife conservation efforts.

Neospora caninum infection in Backyard Chickens from Southern Punjab, Pakistan

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Abstract:

Neospora caninum infection is widespread in wild and domestic animals including birds and backyard chicken that can act as sentinels of their infection. The current study was planned to investigate the prevalence of IgG antibodies to N. caninum in backyard chicken from Southern Punjab, Pakistan. Sera from 341 birds were evaluated to determine the prevalence of anti-Neospora antibodies by using a commercial accessible cELISA (VMRD, Inc., Pullman, USA). Antibodies (IgG) to N. caninum were detected in 89 (26%) of 341 birds. The positivity rate to N. caninum by breeds of chicken was 41.5% (35/89), 26.9% (24/89), 19.1% (17/89) and 14.6% (13/89) for non-descriptive, Mini red, Fumi and Aseel breed chicken. Statistically, a significant (P<0.05) difference was recorded among pure breeds and non-descriptive chickens. However, no significant association (P<0.05) was found within the pure breeds of chicken. The prevalence was higher in birds raised in condition having access to ruminants and pets (68.5%) that of birds raised without contact with ruminants and pets (31.4%), indicating a significant (P<0.05) association among two categories. Our findings suggest that presence of antibodies to N. caninum in backyard chicken is a good indicator of soil and environmental contamination with oocysts as chickens fed from the ground.

Keywords: Neospora caninum, chicken, Aseel, Mini Red, Fumi, backyard
New species of avian schistosomes from Argentina: from Gondwanaland to gulls

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In the blood fluke family Schistosomatidae, the diversity and natural history of the marine species are less well-known than the fresh water species. Most of the diversity of marine schistosomes has been reported from gastropod intermediate hosts, the focus on this host in part because of their role in transmitting species that cause cercarial dermatitis (swimmer’s itch). As part of a larger effort to discover and describe the schistosome diversity in Argentina, avian hosts spend some time in marine habitats were examined. Two species gulls were examined, the kelp gull (Larus dominicanus) and the brown-hooded gull (Chroicocephalus maculipennis). The former species is found throughout the southern hemisphere, populations largely sedentary in fresh and marine waters, and the latter species is a South American endemic, populations spending most time in freshwater habitats and intertidal marshes, but also in marine coast. Two different species of schistosomes were recovered, one from each of the two species of gull hosts. These schistosomes were different both genetically and morphologically. Schistosomes from L. dominicanus from Patagonia marine coast has neither oral nor ventral suckers and the male has a short gynaecophoric canal without transversal bands. Conversely, schistosomes from C. maculipennis from Buenos Aires province, a northern inland site, also has neither oral nor ventral suckers, but the gynaecophoric canal of the male is longer and begins further down from the anterior end than that of the former species. The molecular phylogenetic results showed two distinct clades. The first clade is a species that has been found previous in Argentina from both Larus and the marine gastropod, Siphonaria lessonii, as well as is aligned with a specimen from penguins from South Africa. The second clade is unique and did not group with any defined species or clade, other than members of the large freshwater clade of long, thin adult worms that use a diversity of freshwater, and some marine, gastropods. Not surprisingly, the diversity and distribution of these schistosomes is correlated with the vagility and distribution of their bird hosts. Such that worms from hosts distributed in the southern hemisphere are more closely related to each other than species from northern hemisphere. However, how these current distributions might relate to the gastropod evolutionary history and presence in the supercontinent, Gondwanaland, most importantly, yet awaits more sampling. These two species add to the list of about 10 avian schistosome species from Argentina alone, with 5 avian schistosome species put within a molecular phylogenetic context.

ORAL ADMINISTRATION OF GW788388 REVERSES CARDIAC DAMAGE DURING CHRONIC PHASE OF CHAGAS DISEASE

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Studies published by our group demonstrated the involvement of TGF-β in Chagas cardiomyopathy development in *Trypanosoma cruzi*-infected animals during the acute phase of Chagas disease. Activation of TGF-β signaling pathway was observed in the cardiac tissue of infected animals during the acute phase, favoring the increase of extracellular matrix proteins expression. TGF-β is the most important protein involved in fibrosis process. The aim of this study is investigate the effect of GW788388 treatment in TGF-β signaling pathway during the chronic experimental model of Chagas disease. To this end, animals C57Bl/6 were infected with *T. cruzi* colombian strain (10²) and treated orally with 3mg/kg GW788388 after 120 days post– infection (dpi) in two treatment schemes: once a week or three times a week during 30 days. Electrocardiograms were performed after 120 and 150 dpi, before and after the treatment. The hearts of infected animals treated or not with GW788388 were collected and total proteins were extracted for the investigation of fibronectin and type I collagen expression by Western blot methods. In addition, collagen deposition was measured in the cardiac tissue of animals by histological methods. Also, circulating levels of TGF-β were evaluated by ELISA. Our data suggested that the chronic model presents 100% of cardiac damage after 120 dpi. GW788388 treatment improved the electrocardiographic state of infected animals: reduced the bradycardia, the PR interval and P wave duration. GW788388 treatment also improved heart remodeling since restored the decreased LV ejection fraction. Furthermore, GW788388 treatment, three times a week, was able to reverse collagen expression in the heart of infected animals. We also observed that TGF-β circulating levels were increased due to *T. cruzi* infection and GW788388 treatment reverse these levels significantly. To date, the results are promisor and suggested a new possibility of fibrosis treatment in the chronic phase of Chagas disease.

**Occurrence of divergent Cryptosporidium parvum and Cryptosporidium hominis subtypes in Macaca fascicularis**

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Nonhuman primates are often infected with *Cryptosporidium hominis* subtypes that are circulating in humans in the same area. They can also be infected with a host-adapted *C. hominis* subtype IiA17, which is occasionally found in humans. The occurrence of *C. parvum* and divergent *C. hominis* subtypes in these animals is not well studied. In this study, to understand *Cryptosporidium* transmission in laboratory monkeys, 1,221 fecal specimens were collected from carb-eating macaques (*Macaca fascicularis*) on one farm in Hainan, China during April 2016-October 2017. *Cryptosporidium* spp. were
detected and genotyped by using PCR and sequence analysis of the small subunit rRNA gene, with the \textit{C. parvum} and \textit{C. hominis} identified being subtyped by PCR and sequence analysis of the 60 kDa glycoprotein gene. Divergent \textit{C. parvum} and \textit{C. hominis} subtypes identified were also sequenced for whole genomes. Altogether, \textit{Cryptosporidium} spp. were detected in 57 (4.7\%) of sampled animals. The infection rate was significantly higher in females (8.4\%, 42/498) than in males (1.6\%, 9/576), in monkeys with diarrhea (7.7\%, 26/339) than those without -diarrhea (3.5\%, 31/882), and in monkeys aged 1–2 (6.5\%, 36/532) and 2–3 (4.3\%, 18/421) years than in monkeys aged over 3 years (1.1\%, 3/278). Two \textit{Cryptosporidium} species were identified, including \textit{C. hominis} in 42 animals and \textit{C. parvum} in 15 animals. The \textit{C. parvum} identified belonged to subtypes in three known subtype families, including IIoA14G1 (in 12 animals), IIdA19G1 (in 2 animals) and IIaA17G2R1 (in 1 animal). In contrast, the \textit{C. hominis} identified mostly belonged to two new subtype families Im and In, which are genetically related to the well-known subtype families Ia and Id, respectively. The \textit{C. hominis} subtypes identified included ImA18R3 (in 25 animals), InA14 (in 3 animals), InA17 (in one animal), InA26 (in 2 animals), and IiA17 (in 1 animal). Whole genome sequencing of 9 isolates from the study supported the genetically divergent nature of \textit{C. parvum} IIo and \textit{C. hominis} Im and In subtype families. Crab-eating macaques on the study farm were apparently infected with diverse \textit{C. parvum} and \textit{C. hominis} subtypes, including several genetically divergent ones.

\textit{emphasized text}

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One or multiple leech species of Limnobdella from Mexico

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\textit{Limnobdella mexicana} Blanchard, 1893 was described, surprisingly enough, based on specimens collected in Central Mexico and Baja California. The original description was brief so latter taxonomists got confused and named \textit{L. mexicana} to almost every leech with five pairs of eyespots arranged in parabolic arch and five annuli between gonopores. In particular, Caballero (1932) collected some specimens in Mexico City and used them to describe the internal morphology of what he thought was \textit{L. mexicana}. Latter studies by Richardson, 1971 demonstrated that the specimens studied by Caballero actually correspond to a different species that he described as \textit{Percymoorensis caballeroi} Richardson, 1971, eventually transferred to the genus \textit{Haemopis}. Oddly enough, when Caballero studied leeches that actually belong to \textit{Limnobdella}, he erected a new name to accommodate this apparently new taxon: \textit{Potamobdella}. Latter on, Caballero recognized his own mistake and transferred his own species to \textit{Limnobdella} and then described at least two more species under this genus. Preliminary analyses of recently generated molecular data (sequences of the cytochrome c oxidase subunit I) from specimens of \textit{Limnobdella} from Veracruz, Jalisco, Nuevo León and Oaxaca, suggest that only two species of this group exist in Mexico: \textit{Limnobdella mexicana} and \textit{Pintobdella cajli}. The geographic distribution of \textit{L. mexicana} is unusually widespread and includes localities from the Neotropical and Nearctic regions, maybe as a result of their preference for blood of large mammals. Further studies, including samples from more localities as well as the generation of new molecular markers and morphological studies will clarify this taxonomic problem.

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PARASITE FAUNA OF THE INVASIVE LIONFISH PTEROIS VOLITANS (PISCES: SCORPAENIDAE) IN THE MEXICAN CARIBBEAN
The Mesoamerican Reef System is the second largest barrier reef in the world and has great ecological and economic importance. In this system, fish play a very important ecological role, and have received special attention from the scientific, commercial and tourist areas. In this region of the Atlantic, it has recently been reported the invasive lionfish Pterois volitans (family Scorpaenidae), widely known for its use as ornamental fish. The main impacts reported by the introduction of this species (native from the Indo-Pacific) in wild environments include negative impacts on biodiversity and economic losses (fisheries). However, little is known whether this invasive species could also be carrier of parasites dangerous to native species or even to humans if consumption is promoted as a strategy control. The objectives of the present study were: to identify the parasite fauna in P. volitans in different reef localities of the Mexican Caribbean and to determine if these species could be pathogenic for humans. Lionfish specimens were collected by snorkeling and scuba diving in 37 reef locations, between February 2011 and February 2016. In a total of 630 P. volitans individuals (total length between 380 and 455 mm) six main groups of parasites were recorded (Digenea: Stephanostomum sp., Siphoderina sp., Lecithochirus sp. L. musculus, L. microstomum, L. floridense, Brachyphallus parvus; Nematoda: Ascarophis mexicana, Spirocamallanus sp., Capillaria sp., Histerothylacium sp.; Acanthocephala: Gorgorhynchus sp., Serrasentis sp.; Cestoda: Prochristianella sp.; Isopoda: Cymothoa excisa; Copepoda: Caligus wilsoni y Caligus xystercus). In both copepod species P. volitans is a new host record and extend their known geographic range. This species richness of parasites was relatively low when compared to studies conducted on this invasive species in other locations in the Caribbean Sea. The species of parasites recorded here do not represent a risk to human health.

PARASITES FROM THE GRASSLANDS OF EAST AFRICA, THE SKY ISLANDS OF NORTH AMERICA, AND THE BIDDING WARS ON EBAY: USING LIFE CYCLES TO DISCOVER THE HIDDEN BIODIVERSITY OF PARASITES!

Matthew Bolek

Parasitism is the most common way of life. However, one of the hallmarks of parasitic organisms is that most species have incredible life cycle strategies used to infect their hosts. Over the last decade our laboratory and collaborators have utilized those life cycle stages to discover and understand the hidden diversity of various parasitic groups. In this presentation I will highlight some of the tricks of the trade to discover new species of parasites using these novel techniques. In addition I will outline the benefits and challenges we have faced over the years of dealing with collecting various hosts and parasitic life cycle stages in the field and bringing those live parasites back to our laboratories for host infections.
PROTEASOME-ASSOCIATED DEUBIQUITINASES INHIBITION EFFECTS ON WORM VIABILITY AND TEGUMENTAL CHANGES IN SCHISTOSOMA MANSONI

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The 26S proteasome is a barrel shaped structure with a 20S catalytic core that is flanked by 19S caps on either side. Our group has been showed that 26S proteasomes are critical for Schistosoma mansoni development and survival, being that more than 95% of worms pairs treated with the proteasome inhibitor MG132 showed alteration on egg laying and viability. The 19S cap is the regulatory complex and functions in unfolding and deubiquitinating the proteins before their entry into the 20S complex using constitutive deubiquitinating enzymes (DUBS). Recently, it has been demonstrated that inhibition of the DUBS, UCHL5 and USP14, which are reversibly bound to the 19S particle of the 26S complex, called b-AP15, results in cell death because it leads to endoplasmic reticulum stress in a variety of mammalian cells. This study is the first to document the potential role of b-AP15 as a schistosomicide and the ultrastructural changes induced by b-AP15 in couple worms of S. mansoni.

The following methods were used to analyse alterations: Transmission Electron Microscopy (MET), Colorimetric quantitative assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), activity of the proteasome through the substrate Suc-Leu-Le-Al-Tyr-AMC specific for chymotrypsin activity and western blotting. They were tested various concentration of the drug (0.2; 0.4; 0.8; 1.6; 3.2 up to 50 µM) and from 1.6µM b-AP15 inhibited the egg production of adult worm pairs in vitro with 24 h of treatment and did not change the viability with 1.6 µM, but showed changes from the dosage of 3.2µM. From 1.6 µM cellular and tegumentary alterations occurred and 50 µM adult worms were dead. The Western blotting showed accumulation of high molecular weight polyubiquitin proteins in the presence of 3.2µM without changes in the 20S proteasome activity. Tank together the results showed that b-AP15 alters the oviposition, viability and leads to death of couples of parasites, reinforcing the hypothesis that ubiquitin proteasome system are essencial to S. mansoni biology.

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Parasite communities of Fundulus diaphanus in River and Lake Ecosystems in New Jersey

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The assembly of parasite communities is dependent on a suite of local environmental conditions, and in this study we describe differences in parasite community composition across lotic and lentic ecosystems using the host, Fundulus diaphanus. We sampled F. diaphanus individuals from 4 populations, 2 lotic (Raritan and Passaic River) and 2 lentic (Assunpink and Mercer Lake) in fall, spring and summer to evaluate differences in parasite communities. From the fall data we report a total of 12 and 8 parasite species from Raritan and Passaic River respectively, with 7 species overlapping in the Rivers. The two lake systems harbored 4 parasite species, and all species were shared between lakes. Despite being a host to the same parasite species among lakes, infection levels differed. For example, mean abundance of Eustrongylides sp. was 1.053 in Mercer and 0.077 in Assunpink, while
Posthodiplostomum minimum mean abundances were 0.917 and 10.128 respectively. Parasite mean species richness differed across sites after correcting for differences in host body size (ANCOVA: F4,173 = 17.27, p <0.0001). Our data suggests the identity of the parasites within the community depends on whether the hosts sampled came from a lotic or lentic system, but community composition differs substantially across systems.

Parasite mediation of community dynamics in freshwater crustaceans

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Parasites can influence important interactions among hosts such as competition and predation. These impacts can affect local biodiversity, distribution and relative abundance of host and non-host species, as well as community and ecosystem structuring. Yet, despite the significance of parasites and extensive theoretical modeling of their critical role in shaping ecosystems, little has been empirically measured to examine parasites’ far-reaching impacts; it has proven particularly difficult to modulate parasite abundance in naturalistic experiments. Here, to quantitatively examine the role of parasites in structuring communities, we set up multigenerational mesocosms with different levels of parasite exposure. We used four invertebrate species from the same community that share up to five species of parasites. We found that the trematode Maritrema poulini influenced population dynamics of these hosts in species-specific ways. For instance, the survival and recruitment of the amphipod Paracalliope fluviatilis were greatly reduced, indicating that parasites may impact their long-term persistence in the community. The relative abundance of different host species was also influenced by parasite exposure levels, thus demonstrating the parasites role in host community structuring. Asymmetrical effects of parasites on sympatric host species are likely widespread structuring forces in communities as parasites are ubiquitous components of ecosystems.

Parasite-oriented strategy of anti-bacterial treatment to kill adult filarial nematodes.

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Over 120 million people in the world are at high risk of suffering the debilitating effects of the parasitic filarial nematodes Wuchereria bancrofti, Brugia malayi, and B. timori that live in the lymphatic system and cause human lymphatic filariasis. Onchocerca volvulus is the causative agent of onchocerciasis and is responsible for ocular or dermal pathologies, including blindness. Unfortunately, the treatments currently used for mass drug administration initiatives, such as ivermectin, produce only microfilaricidal effects – they block transmission but have no effect on the adult parasites. These microfilaricidal drugs require long-term, yearly administration and are contraindicated in areas co-endemic with Loa loa. Therefore, macrofilaricidal drugs that kill adult worms are required to overcome these limitations. Recent data demonstrate the unique potential of using Wolbachia, the mutualistic bacteria of filarial nematodes, as a novel chemotherapeutic target against human
Filarial infections. Antibiotic treatment (doxycycline, in particular) kills the endosymbionts and has macrofilaricidal effects. To explore this potential, we introduce a novel approach: a host (parasite)-oriented treatment that induces a host innate defense and forces filarial nematodes to eliminate their endosymbiont. In this work we focus on autophagy, the primary intracellular defense mechanism by which eukaryotic cells can maintain a healthy intracellular environment and protect cells against intracellular invaders (e.g., bacteria, viruses). We proved the concept that induction of autophagy in filarial nematodes can eliminate Wolbachia, induce apoptosis in developing embryos of females, and consequently kill adult worms. We showed that Wolbachia can be recognized by autophagic proteins and then lysosomes that can fuse to bacteria and digest it in B. malayi parasites. We tested a small library of autophagy inducers (including FDA-approved drugs and natural products) and found 24 compounds that reduce Wolbachia loads in B. malayi microfilariae in amounts equal to or greater than doxycycline after a 6-day in vitro treatment. This new strategy of anti-bacterial treatment will yield a new class of active compounds and allow for the repurposing of existing drugs, for use as alternatives to or in combination with current anti-filarial treatments.

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Parasites of Freshwater Crabs of Sri Lanka with a revision of crab hosts and evidence that Paragonimus compactus and Paragonimus siamensis are the same species

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Fresh water crabs (FWC) of Sri Lanka (SL) harbor 16 metacercariae including 3 Paragonimus (P) species. FWC taxonomy has changed since original descriptions. All FWC were considered Paratelphusa, whereas present taxonomy includes 51 species, belonging to 5 genera, none of them Paratelphusa. According to the new taxonomy FWC hosts belong to Genera Ozietelphusa, Ceylonthelphusa and Perbrinckia. Miyazaki and Wykoff (1965) reported P siamensis as a new species in Thailand. P siamensis was morphologically identical to P compactus. However, using the analogy of P ohirai and P iloktsuenensis where adults are morphologically similar but metacercariae differ, metacercaria of P compactus being unknown they reported them as separate species. The first report of P compactus was from an Indian mongoose that died in London Zoological Gardens. The author found Paragonimus sp in a mongoose in Sri Lanka, which was reported P siamensis. FWC metacercaria in the area, when fed to experimental animals revealed, adults identical to both P siamensis and P compactus. During joint studies with the author Miyazaki could not distinguish between the adults. Hence the author believes they are the same species. P compactus was known to exist in SL prior to these studies. FWC in SL also found to harbor 3 nematode larvae and a leech. Field studies and results of animal experiments will be presented.

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Parasites of the Burbot, Lota lota from Green Bay of Lake Michigan with an evaluation of Eubothrium rugosum

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This report is part of an ongoing study on the helminth parasite fauna of the Burbot, *Lota lota*, from Green Bay of Lake Michigan. In the summer (May – August) of 2017, 19 adult Burbot (475 – 815 mm TL) were examined for helminth parasites. Six species of helminth parasites were found: 2 cestodes (juvenile and adult *Eubothrium rugosum*, and immature *Proteocephalus* sp.), 1 necrosed and unidentifiable nematode embedded in the intestine, and three species of acanthocephalans, *Neoechinorhynchus tumidus*, *Neoechinorhynchus rutili* and *Echinorhynchus salmonis*. This appears to be the first report of *N. tumidus* from Burbot. No gravid females of these acanthocephalans were found and *N. rutili* and *N. tumidus* occurred as immature males only. These acanthocephalan infections are likely incidental because burbot overlap, ecologically, with their true definitive hosts, such as salmonids. This study also allowed an evaluation of a North American population of *Eubothrium rugosum* and a comparison with its European counterpart. Partial sequences of the 18S (1933 bp) and 28S (716 bp) rDNA from *E. rugosum* in this study showed 5 bp and 1 bp differences, respectively, with corresponding sequences of *E. rugosum* from *L. lota* in Europe. ITS-2 sequences (716 bp) from 8 individual *E. rugosum* were identical to one another and showed a 3 bp difference with ITS-2 sequences from European *E. rugosum*. These molecular data can be added to the morphological differences noted by Wardle and McLeod between North American and Eurasian *E. rugosum*, one of which (a greatly distended apical region of the scolex in N. American *E. rugosum*) was also observed in our study. Furthermore, the scoleces of all *E. rugosum* were deeply embedded in a capsule-like formation in the submucosa of the ceca.

Parasitic infections in goldfish *Carassius auratus* from Pet-shops of Mérida, Yucatán, México

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The goals of this paper were to taxonomically identify, and estimate the prevalence and mean intensity, of parasites in goldfish (*Carassius auratus*) from pet-shops of Mérida Yucatán, México. Between October 2016 and April 2017, a total of 210 fish were examined for parasites from 10 of the 25 active pet shops in Mérida. During the parasitological review, 8 species of parasites were recovered, including 6 ectoparasites: *Ichthyophthirius multifiliis*, *Trichodina* sp., *Gyrodactylus* sp., *Dactylogyrus* sp, *Argulus* sp. and *Lernea cyprinacea*, and 2 endoparasites: *Haplochiris pumilio* and *Centrocestus formosanus*. It was found that *Dactylogyrus* sp. was the most frequent and abundant species (prevalence of 67% and 11 ± 20 individuals/host), followed by *Gyrodactylus* sp., (65%, 10 ± 56). The digeneans *C. formosanus* (50%; 25 ± 56) and *H. pumilio* (15%; 2.25 ± 2.37) were in the metacercarial stage in *C. auratus*. *Trichodina* sp. and *C. formosanus* presented the highest parasite loads with 3453 and 2656 individuals respectively. We consider that the strict enforcement of sanitary measures such as quarantines and use of non-destructive diagnostic methods would be of great practical value for containment of parasites of exotic ornamental fish like *C. auratus*. 

Parasitic mites have non-consumptive effects on the fitness and energy budgets of flies

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The intersection of parasitology and community ecology offers connections between parasite-host systems and predator-prey systems. Predators negatively impact prey fitness even when those prey are not consumed, and it is hypothesized that parasites can have similar non-consumptive effects on potential hosts. Exposure to parasites alone, even in the absence of direct contact, can alter the behavior and metabolic rate of potential hosts. We aim to test if proximity to an ectoparasitic mite (Macrocheles subbadius), separate from contact or infection, reduces the fitness of Drosophila nigrospiracula. Female flies were exposed to mites from the time of eclosion to death. We measured the longevity and lifetime fecundity of flies chronically exposed to mites relative to control, unexposed flies. The fly and mites were separated by a mesh to prevent contact or infection from occurring, ensuring we only observed the non-consumptive effects of the mite on the fly. Proximity to mites reduced both the longevity and lifetime fecundity of female flies: D. nigrospiracula that were housed with mites lived on average 38% shorter lives and produced 13% fewer offspring relative to flies housed without mites. We also tested possible underlying mechanisms of these fitness costs, including energetic costs of grooming. Using flow-through respirometry, we found that flies subjected to an irritant produced 43% more carbon dioxide than control flies (indicative of activity and/or stress). Flies induced to groom for half an hour with an irritant were less able to resist subsequent infection by mites. These results suggest that energetically demanding host defenses such as grooming divert resources away from somatic maintenance and reproduction, and make hosts more vulnerable to future parasite threats. Persisting in environments with parasites can negatively impact host fitness, and impose trade-offs between survival, reproduction, and resistance.

Parasitic nematodes in lutjanids (Perciformes: Lutjanidae) of the Mexican South Atlantic

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Lutjanids (snappers) are one of the main fishing resources in the tropical and subtropical zones, due to its excellent food quality and high value in market and also represent a very important fishery in the Mexican South Atlantic (Southern Gulf of Mexico and Caribbean). Because of its commercial and ecological importance, it is imperative to know diverse aspects of their biology, as the parasite fauna. Therefore, the objective of this work was to determine the nematode fauna and its infection parameters in lutjanids of the Mexican South Atlantic. From February 2016 to November 2017, 428 individuals of 10 lutjanid species were collected in seven localities of the Mexican South Atlantic. Only seven of the 10 host species were parasitized by 2,081 individuals belonging to 12 nematode species. *Pseudoterranova* sp. was registered in the seven species of lutjanids and also showed the highest prevalence (21%), while the remaining had lower values (< 10%). *Lutjanus griseus* harboured the highest species richness (9), follow by *L. apodus* (7). Most localities were similar in terms of the number of nematodes species, but differed in the specific composition; Xcalak had the highest diversity. Eight new records of host are included, thus increasing to 20 species of nematodes in lutjanids in the region. There exists a potential risk for the public health due to the presence of nematode with zoonotic potential and the custom of eating raw fish (ceviche).

Partial purification and characterization of Superoxide dismutase from the somatic tissue of Gastrothylax crumenifer (Trematoda: Digenea)
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Abstract
Superoxide dismutase (SODs) enzymes play a crucial role in the neutralization of oxygen free radicals (O2−) and seize the progressive oxidizing chain reactions that cause extensive cellular damage. Reactive oxygen species (ROS) are generated by incomplete reduction of oxygen during respiration in mitochondria and as side products of a variety of normal metabolic reactions in cytoplasm, endoplasmic reticulum, plasma membrane and peroxisomes. In helminths, three types of SODs have been reported; the cytosolic copper/zinc (Cu/Zn)-SOD, mitochondrial manganese (Mn)-SOD and extracellular Cu/Zn-SOD. SOD enzymes have been studied in a variety of helminth parasite including Fasciola hepatica, Schistosoma mansoni, Ascaris suum, Paragonimus westermanii, Trichinella spiralis, Taenia solium.

Gastrothylax crumenifer is an amphistome parasite which parasitizes the foregut of Indian water buffalo, Bubalus bubalis causing the disease amphistomosis. Such diseases are common in tropical and subtropical regions across the world. Adult worms inhabiting rumen have low pathogenicity, while the migrating immature stages produce severe pathological disturbances causing high morbidity and mortality resulting in considerable economic losses. Pathological disorders in amphistomosis might be attributed to more than twenty species of ruminal worms including G. crumenifer. Adult amphistomes cause acute catarrhal and haemorrhagic inflammation in the abomasum, duodenum and jejunum with associated anaemia and hypoproteinaemia whereas the migrating immature amphistomes cause severe pathological disorders including acute catarrhal and haemorrhagic inflammation associated with anaemia, hypoproteinaemia and oedema, often leading to general debility, enteritis, development of foetid diarrhoea and death. Despite the fact that amphistome infection in domesticated livestock lead to severe economic loss, little attention has been paid to design and develop sustainable amphistome control strategies.

In the present study, SOD enzyme was partially purified from the somatic tissue of G. crumenifer and the basic biochemical characters were analysed. In brief, SOD enzyme in the somatic tissue of G. crumenifer was precipitated at 60-80% ammonium sulphate salting out concentration. The precipitated SOD was partially purified by gel filtration chromatography and its biochemical characterization was carried out. We observed that the Sephacryl S-100 HR column eluted a total of sixteen fractions out of which the ninth fraction contained maximum SOD enzyme activity. The purified SOD protein resolved as a single band corresponding to the enzyme subunit in SDS-PAGE with an apparent molecular weight of 16 kDa. The thermal stability of the purified SOD were tested in a temperature range of 4ºC to 80ºC and the enzyme was found to be fairly stable up to 37ºC, beyond which the enzyme activity was steadily diminished. The pH tolerance of the obtained SOD molecule showed the pH optima of 8.5 and the enzyme activity decreased abruptly at both the extremes of acidic and basic pH range.

To the best of our knowledge, this is the first study related to purification and biochemical properties of SOD enzymes in the amphistome parasite, G. crumenifer. It was envisaged that a thorough and selective study on SODs from the rumen infecting amphistomes will shed some light towards better understanding of SODs of helminth origin. This will be useful in generating a base line data so that the salient enzymatic properties can be compared with other known SOD from different parasitic fauna.

Pattern of diversity in larval Posthodiplostomum

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Research on the diversity and relatedness of parasitic species is important for understanding patterns of infection, host specificity, distribution and transmission. Cryptic diversity in trematodes is being discovered at an extremely high rate. For example, it had been assumed that a single species of Posthodiplostomum exists throughout much of North America in a variety of second intermediate fish hosts. Recent studies have documented cryptic diversity in this genus, however most of the specimens for those studies were obtained from the northern United States and Canada. The goal of this study was to determine the genetic diversity among Posthodiplostomum metacercariae from various species of fish hosts collected in Texas. Thus far, the internal transcribed spacer (ITS) gene has been sequenced for each specimen for analysis of evolutionary relationships. The phylogenetic results will be discussed as to whether our specimens represent a single species or a complex of cryptic species.

Phylogenetic analysis of species of Foleyellides (Nematoda: Onchocercidae) from southern Mexico, based on mitochondrial DNA sequence.

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Phylogenetic position of five genera of onchocercid nematodes from North and Central America

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Filarial nematodes are well distributed around the world. Onchocercidae is the family that includes the filarial worms of main medical and veterinarian importance. The phylogenetic relationship between the members of this family has been investigated including only specimens from Europe, Asia, Africa and South America, without any representative from North and Central America. Accordingly, several authors suggested that some of the questions about their classification, origin and evolution remain unknown. The inclusion of New World filarial nematodes to the phylogeny turns out to be important to better understand the relationships between them, being that the main purpose of the present work. Hosts (lizards, bats and toads) were collected in six localities from Mexico and were dissected in order to recover the nematodes. Additionally, we also studied specimens deposited in the Colección Nacional de helmintos at UNAM. Specimens were cleared with a glycerin-alcohol mixture and observed under a microscope for identification (Ochoterenella, Foleyellides, Piratuba, Litomosoides and Pelecitus). Total DNA was extracted using the QIAGEN kit, and PCR were performed to generate molecular markers: two nuclear fragments 18S and 28S, and two mitochondrial: 12S and COI. Sequences were aligned using MAFFT and phylogenetic inferences were performed under Maximum likelihood and Parsimony methods. The resulting trees were congruent between them and with the previous phylogenetic hypotheses of the group, recovering the five original clades; the inclusion of Ochoterenella Foleyellides and Piratuba from Mexico and Costa Rica, supports the monophyly of ONC 1 (Oswaldfiliarinae + Waltonellinae); three species of Pelecitus (two from the New World and one European) do not constitute a monophyletic group. Finally, the two Mexican species of Litomosoides were found nested within the South American representatives of the genus, supporting their monophyly.

Polycyclic aromatic hydrocarbon (PAHs) in Oncomegas wageneri (Cestoda: Trypanorhyncha) intestinal parasite of Mexican flounder Cyclopetta chittendeni in the southern Gulf of Mexico

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The concentrations of some polycyclic aromatic hydrocarbons (PAHs) and their bioconcentration factors (BCFs) were determined in larval stages of Oncomegas wageneri recovered from the intestine of the Mexican flounder (Cyclopetta chittendeni), in the southern Gulf of Mexico. The bioaccumulation of PAH in parasites was measured using spectrofluorometry and compared with PAHs concentration in bile host and sediments. The parasites acquired PAHs in markedly higher concentrations than host tissues or sediments. The highest BCF values were obtained for hydroxypyrene (OHP) and Benzo-a-Pyrene (BaP), which is result of parasites PAH concentrations 4 orders of magnitude above the host PAHs concentrations. A significant negative relationship was found between parasite PAHs concentrations (as dependent variable) and the number of parasites, and proximity oil wells (as independent variables) using a general linear model (GLM). Low BFC and host PAHs concentration values showed a positive effect on parasite PAHs concentration, while high values of BFC and host PAHs concentration had a negative effect on the parasite PAHs concentration. The present study is the first evidence of PAHs accumulation capacity in intestinal parasites, highlighting role of fish parasites as indicators of bioaccumulation, even above their hosts and habitats.
Coccidiosis, caused by *Eimeria* spp., is a disease of major impact to the commercial poultry industry. Oocysts are shed in feces of infected birds, and sporulated in the environment to become infective. Infection is self-limiting and highly immunogenic, with immunity conferred by one *Eimeria* species being specific to that species; these factors make live vaccination an effective means of coccidiosis control. High prevalence of drug resistance and increasing consumer demand for “Raised Without Antibiotics” (RWA) products suggest an increasing role for vaccination in the control of coccidiosis going forward. Vaccination requires establishment of infection with sufficiently numerous viable oocysts to stimulate development of protective immunity, but few enough to avoid disease. Knowledge of actual viability of oocysts is therefore paramount to the determination of effective vaccine dosage. No rapid and accurate method for determination of oocyst viability presently exists; time-consuming and expensive live-infection trials are the current gold standard. Difficulty in assessing oocyst viability ultimately impedes successful implementation of vaccination programs. We have proposed an *in vitro* assay for the determination of *Eimeria* spp. oocyst viability, using transcript abundance as a proxy for viability. Specific assay targets have been investigated using standard RT-qPCR; early data show a strong correlation between target abundance and oocyst viability. Ongoing work aims to identify optimal targets through transcriptomic analysis of fully sporulated oocysts. An optimal target will allow for determination of individual species’ contribution to viability in mixed samples. Assay optimization of transcript quantification protocols, assessing one- and two-step RT-qPCR strategies and the use of digital droplet PCR (ddPCR) aim to increase assay sensitivity and decrease impacts of endogenous inhibitors. Other potential assay applications include as an epidemiological tracking tool, and in validation of strategies for environmental control of *Eimeria* and related parasites.

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**Population dynamics of Trypanosoma cruzi in vectors and reservoir hosts in the Southeastern United States.**

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Although Trypanosoma cruzi is endemic to the southern tier of the United States, little is known of the lifecycle, distribution, and infection prevalence in the regional vector and reservoir populations. Vector and reservoir specimens were collected from two sites, one in the Appalachian plateau and another from a coastal barrier island and adjoining mainland. Specimens were analyzed by PCR for the presence of *T. cruzi* specific DNA sequences. From the Appalachian plateau site, of the 102 Sigmodon hispidus trapped, 75 (73.50%) were PCR positive, while 28 of 64 (43.80%) *Peromyscus gossypinus* tested positive, as well as 2 of 9 (22.20%) Short-tailed shrews. In this same site, of 23 raccoons (*Procyon lotor*) trapped, 15 (65%) tested positive. Mammals collected as part of a separate survey on coastal Georgia (Camden County) found that of 17 mammalian species tested, 14 were positive, including 5 of 6 species of bats tested. Of 412 Triatoma sanguisuga collected from throughout the southeast, 365 have been tested, with 78 (21%) being PCR positive for *T. cruzi*. A monitored population of *Triatoma sanguisuga* showed that this vector species hatched primarily in
early to mid-summer months, then wintered over mostly as 4th and 5th stage nymphs, molting into the adult stage in the spring, with males appearing earlier than females. Although the frequency of autochthonous infections remains unknown, this confirms a wide-spread and active endemic cycle for T. cruzi in the Southeastern United States.

Population genetic analysis of Guinea worm (Dracunculus medinensis) and the peculiar case of dog infections in Chad

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Since being formally targeted for eradication, efforts to eliminate Guinea worm (Dracunculus medinensis) have been remarkably successful. Of the 21 countries with endemic transmission at the eradication campaign’s inception, 18 have interrupted transmission and 16 have been certified free of the disease by WHO. Four countries remain classified as having endemic transmission, though Mali has reported no human cases for 2 years and South Sudan interrupted transmission as of 2017. However, over the past 7 years an unprecedented number of Guinea worm infections in dogs has generated concern, particularly in Chad where the number of infected dogs vastly outnumbers human cases. In response, a comprehensive research effort is under way to investigate the biological and epidemiological factors underlying this ostensible host shift in Chad and examine avenues to improve ongoing interventions. Here we present results from ongoing genetic studies aimed at resolving questions of the parasite’s taxonomy among African host species and elucidating patterns of parasite transmission within Chad. Using sequence data from 4 mitochondrial genes and microsatellite allelic variation from 23 loci, we find no evidence that parasites from human and non-human hosts are separate species or maintain isolated transmission cycles. Geographic origin, both within and among countries, appears to have more influence on parasite population subdivision than definitive host species. Additionally, the Chadian Guinea worm population has maintained considerable genetic diversity, even at the scale of the individual village, leading us to conclude that Chadian dog infections are not the product of incidental spillover infections from human cases or a recent population expansion. Ultimately, the preponderance of data currently suggests that Guinea worm transmission in Chad is being maintained by dogs. And while D. medinensis infection of non-human hosts is not unique to Chad, the extent of the Chadian situation does currently appear to be novel.

Potential effect of water temperature in intensity of infection and biology of Argulus yucatanus (Crustacea: Branchiura) infecting Cichlasoma urophthalmus (Pisces: Cichlidae)

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Global warming is expected to affect transmission patterns of aquatic parasites. However, the biological outcome of an increase in temperature is unknown, for example maturation, egg production or infection intensity in the final hosts. In temperate latitudes, theory suggests that with increase in temperature, the abundance of parasites will also increase up to a certain level. However, for tropical coastal lagoons this kind of information is almost inexistent. Using the life cycle of fish lice Argulus yucatanus in the definitive host Cichlasoma urophthalmus as a model, we experimentally studied the effect of water temperature increase in maturation, egg production and infection intensity of this ectoparasite. The experiment consisted of three treatments (28º, 32º and 36ºC) and one control (24ºC), each one with 3 replicates. In each treatment, a C. urophthalmus was placed with 14 A. yucatanus, which were previously sexed and classified according to their degree of maturity. At 32 ºC and 36 ºC, the infection intensity of A. yucatanus was significantly higher, and maturation and egg production shortest in fish lice females compared with control (24 ºC). However, at 32 ºC and 36 ºC the number of viable eggs was significantly smaller than that of the 28ºC treatment. In fact, at 28ºC, both the egg production and hatching of the nauplii of A. yucatanus were significantly higher. At temperatures > 32 ºC, A. yucatanus hatching was inhibited. Our results suggest that the increase in water temperature in the tropics due to global warming would increase the infection intensity of fish lice. However, the same increase in temperature will negatively affect critical reproductive variables such as maturation time and egg viability of A. yucatanus, especially if this increase goes beyond the temperature range that the ectoparasite is able to bear.

Pre-Adult Morphological and Developmental Patterns in Neotropical and Nearctic Mesocestoides (Cestoda: Cyclophyllidea)

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Mesocestoides is a common and cosmopolitan cestode genus. The adults use numerous mammals (including humans) and birds as definitive hosts from which their oncosphere larvae are carried within a distinctive paruterine organ within intact gravid proglottids. The metacestode juveniles, tetrathyridia, infect numerous amphibian, reptile, avian, and mammalian intermediate hosts, and when eaten are infective to definitive hosts. Despite decades of work by many researchers, infection of an intermediate host by hexacanths has never been observed. As part of ongoing collaborations with many labs, we collected Mesocestoides pre-adult stages from diverse hosts in North and South America and examined them by light microscopy of whole mounts and histological sections. We compared ours with specimens provided by other researchers and museums. Herein we present new data on post-hexacanth pre-adult stages examined from North America and South America, and integrate our novel findings with the body of data available from the extensive literature. We propose four morphological categories of tetrathyridia taken from field-collected hosts, listed here in descending order of prevalence: 1) normal non-proliferative from many host species globally; 2) aberrant proliferative with budding from body undergoing neoplastic malignant transformation, with or without a scolex, from many host species globally; 3) aberrant proliferative with scolex dividing medially at the apical massif, only from lizards and rodents in western North America; 4) normal non-proliferative with an apical (rostellar) sucker, from a lizard in North America. Based on our data and the literature, we propose that Category 4 is a pre-tetrathyridium transitional metamorphosis stage. Our new data constitute critical new information on postlarval metamorphosis, but no definitive insights into the host sequence. Nevertheless, our new findings might suggest a life cycle with only one intermediate host, in contrast to two successive intermediate hosts frequently proposed in the literature.

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The importance of vegetables to human health and wellbeing cannot be over emphasized as they form a major component of a healthy diet. This study is aimed at determining the prevalence of human parasites associated with vegetables in Maikatako Market. A total of two hundred (200) vegetable samples were collected in Maikatako market of Bokkos Local Government Area between the months of October through December, 2016 for analysis. The vegetables sampled include cabbage, carrot, garden egg, lettuce, spinach, spring onions, tomatoes, cucumber, bitter garden egg and green pepper. The vegetables were screened for the presence of parasites using normal saline sedimentation technique. Of the 200 samples collected and analyzed, 69 (34.5%) were positive for the presence of intestinal parasites. Tomatoe had the highest contamination rate of 14 (7.0%) followed by spinach 10 (5.0%), while garden egg and green pepper had contamination rate of 3 (1.5%) each. Among the parasites encountered, Entamoeba histolytica had the highest prevalence rate of 27 (13.5%) followed by hookworm with a prevalence rate of 16 (6.0%) while Gardia lamblia and Ascaris lumbricoides each had the least rate of prevalence of 6 (4.0%). Statistically, there is a significant difference between the parasites isolated and the vegetables studied (P<0.05). The result of this research is an indication that human parasites can be acquired through the consumption of these vegetables, especially when not properly and hygienically prepared before taken to market.

Problematic Identification of Azygia Species in New York Freshwater Fishes

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This study focuses on species of Azygia that were encountered during a fish parasite survey and two lakes and their tributaries in central east New York that took place over the course of nine years. During the nine years of survey work 34 species of fish were examined for parasitic worms. Fish were collected via seining, electrofishing, angling or gill netting. Fish were anesthetized and dissected, and the entire digestive system was examined for parasites with the aid of a dissecting microscope. Multiple species of nematodes, acanthocephalans, cestodes and trematodes were encountered during the study. This study, however, focuses on the species of trematode genus Azygia that were found in the stomachs of eight different species of fish: Esoc niger (Esocidae), Perca flavescens (Percidae), and six species of centrarchids. Based on observations of the morphology using light microscopy, it seems that there are three species of Azygia present, two of which were previously reported elsewhere, and one that is potentially new. Azygia longa occurs in E. niger and in P. flavescens, but gravid specimens of this taxon can vary drastically in overall body size, and in whether their digestive cecae are straight or convoluted. Azygia angusticauda occurs in P. flavescens and less frequently in several other fish species that were examined. Among the three species of Azygia encountered, A. angusticauda was the most readily distinguished from the other two based on its smooth tegument and distinctive body shape. Specimens representing a potentially new species of Azygia were found in
the stomach of *Ambloplites rupestris* and, less commonly, in the other species of centrarchids. These specimens were notably smaller in length than any of the *A. longa* that were encountered, and were gravid. Efforts to identify distinguishing features of this putative new species raised questions about the consistency of the morphology of *A. longa* with which it seems to overlap. Overall survey data and specific morphological features of each of these three species of *Azygia* are presented in this poster.

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**RELATIONSHIP OF FEEDING, GROWTH, AND PARASITISM IN ACHETA DOMESTICUS INFECTED WITH PARAGORDIUS VARIUS (PHYLUM: NEMATOMORPHA).**

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The presence of parasites and pathogens in a host can induce changes that can be detrimental to host life history traits and behavior. Because body size is often a predictor of fecundity in insects, changes in body size due to resource allocation in parasitized insects could affect reproductive output in female insects. Dietary intake can also affect resource allocation to physiological processes such as reproduction and is often altered in response to parasite infection. Hairworms are macroparasites of crickets and other arthropods with a complex life cycle utilizing aquatic paratenic hosts and terrestrial final arthropod hosts. The purpose of this study was to examine the feeding behavior of *A. domesticus* infected with *P. varius* and examine the relationship of growth and feeding when provided two complementary diets of proteins and carbohydrates. Compared to uninfected controls, infected crickets consumed the same proportions of protein to carbohydrates but consumed 14% less food than the uninfected controls, despite parasites increasing their body size more than 5000x in infected crickets. On average, infected crickets had less mass and shorter body lengths and therefore should require less food than control crickets. Therefore, we predicted that infected and control crickets should gain mass proportionally to the amount of food they eat. After accounting for body water content, uninfected control crickets consumed 23.15 mg of food to gain 1 mg of lipids. In comparison, infected crickets had to consume 74.21 mg of food to gain 1 mg of lipid. Taken together, the increase of food required to produce 1 mg of lipid and the smaller body size of infected crickets suggests that the developing hairworm sequesters most of the energy ingested by crickets that cannot be initially observed by cricket body size alone.

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**Relationships of endoparasite diversity and feeding ecology in the seabird complex of south Florida**

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Endoparasite community structure likely varies as a function of trophic guild, preferred feeding sites, and migratory habits, but these relationships have been poorly studied in migratory birds, particularly among the seabirds of south Florida. We examined parasite communities in seven south Florida seabird species: brown pelican *Pelecanus occidentalis* (n=33), northern gannet *Morus bassanus* (n=31), double-crested cormorant *Phalacrocorax auritus* (n=33), laughing gull *Leucophaeus atricilla* (n=40), herring gull *Larus argentatus* (n=12), royal tern *Thalasseus maximus* (n=30), and osprey *Pandion haliaetus* (n=26). We identified 23 parasite taxa: 3 nematodes, 2 cestodes, 2 acanthocephalans, and 15 digeneans (tracheal mites were found but excluded from the analyses). ANOSIM of Bray-Curtis community similarity (square root transformed data; R=0.463, p=0.001) found significant differences among species. Subsequent pairwise tests and similarity profile analysis identified three distinct clusters with similar parasite community structures: (1) pelicans and gannets; (2) cormorants and ospreys; and (3) terns and both gull species. Mean infracommunity observed species richness differed among species (ANOVA; R²=0.401, DF=6, F=22.01, p<0.001) was highest in pelicans (3.3±0.3) and gannets (3.9±0.3), and lowest in herring (0.9±0.4) and laughing (0.4±0.2) gulls; a similar pattern was observed for infection intensity (ANOVA; R²=0.370, DF=6, F=19.34, p<0.001). Thus, species that forage further offshore (pelicans and gannets) host more diverse parasite communities and experience higher infection intensities than those that forage inshore (gulls and cormorants). Our results suggest that host feeding ecology (e.g., preferred foraging habitat and range, dietary niche breadth, preferred prey and prey size) likely plays a major role in structuring parasite community species composition, as well as species richness and infection intensity.

Resident and invasive trypanosome parasites have differential geographic histories in endemic rodents of Sulawesi

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The Indonesian island of Sulawesi is a globally significant biodiversity hotspot with substantial undescribed biodiversity, particularly wildlife pathogens such as blood-borne parasites. Sulawesi’s parasite diversity is likely to affect the health and conservation of Sulawesi’s unique wildlife as well as being of relevance to the biogeography of pathogens across the Indo-Australian region. Our study systematically sampled rodents and their trypanosome parasites from two isolated forested mountains on Sulawesi separated by 218 kms of lowland forest and agricultural land. In 2016, we collected blood smears and liver samples from 641 specimens representing 21 species of rodents. We screened liver samples from 501 specimens (21 species) for trypanosomes using PCR targeting the 18S rRNA gene. We detected trypanosome infections in 151 specimens (10 species) with a mean prevalence of 30.1% (95% CI: 26.1 – 34.2%). We sequenced all positive samples and identified 21 unique Trypanosoma spp. haplotypes. Based on phylogenetic comparison with existing sequences on GenBank, we recovered four haplotypes infecting 34 specimens (6 species) that were nested within the *T. lewisi* clade. We also recovered 17 haplotypes infecting 117 specimens (8 species) that were nested within the *T. theileri* clade. Haplotypes from the *T. lewisi* clade were reciprocally monophyletic between mountains, had limited genetic diversity, and we recovered identical sequences in two samples from the invasive rodent, *Rattus exulans*. Together these results suggest that the occurrence of *T. lewisi*-like trypanosomes on these two mountains on Sulawesi resulted from two separate and historical invasions. In contrast, haplotypes from the *T. theileri* clade were not reciprocally monophyletic between mountains, displayed substantial phylogenetic structure and diversity, and were not recovered...
from invasive species. Together these results suggest that *T. theileri* represent a native trypanosome that has long-infected Sulawesi’s endemic rodents.

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**Revealing biotic diversity: how do complex environments influence human schistosomiasis transmission?**

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The growing discipline of disease ecology emphasizes understanding the biotic context in which disease transmission occurs. Such a perspective is important for understanding transmission of human schistosomiasis in complex tropical aquatic habitats. In western Kenya, *Schistosoma mansoni* is transmitted by *Biomphalaria pfeifferi* in streams and we have undertaken bi-monthly surveys from Jan 2014-Jan 2018 at six different sites along Asao Stream where we collect data on abiotic factors, snail densities, and prevalence of larval trematode infections. Our study has revealed that stream transmission sites harbor dynamic populations of 8 different snail species, some of which may compete with *B. pfeifferi* or serve as dead-end hosts for *S. mansoni*. We have also established a dominance hierarchy among the trematode species infecting *B. pfeifferi* that includes consideration of both predatory interactions and facilitation among the species involved. In addition to *S. mansoni*, molecular analysis has shown *B. pfeifferi* to harbor 14 additional trematode species. Cattle-transmitted amphistomes and *S. mansoni* are positively correlated with one another (R² = 0.683) and local prevalence, particularly because of the abundance of amphistomes, can rise to 25%, with overall *S. mansoni* prevalence of 4.9%. We found priority effects among amphistomes and *S. mansoni*, where *B. pfeifferi* infected with amphistomes rely on a secondary exposure of *S. mansoni* to continue their development. We combined our observational and experimental data to create a mathematical model to understand the extent to which *S. mansoni* transmission may be dampened by the complex webs of competing trematodes present in typical transmission sites to better understand the implications of biodiversity on human schistosomiasis transmission.

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The family Hystrignathidae Travassos, 1920 (Nematoda: Oxyuridomorpha: Thelastomatoidea) includes about 100 species of parasitic nematodes specific of the gut caeca of passalid beetles. The genus Longior Travassos & Kloss, 1958 (Hystrignathidae), includes six nominal species of monoxenous parasites from passalid beetles (Coleoptera: Passalidae). All of these species have been described from the Neotropical region: Brazil, Panama and the West Indies (Saint Lucia and Cuba). This genus was revised, including by first time SEM and molecular techniques. SEM allowed the study of the cephalic structures of both sexes, as well as the pattern of copulatory pattern of the
males. An amended generic diagnosis was given. L. alius García & Coy, 1994 was synonymized with L. longior Morffe & García, 2011 (from Cuba). The species L. longicollis Travassos & Kloss, 1958 (type species of the genus, from Brazil), L. semialata Hunt, 1991 (from Saint Lucia), L. panamensis Morffe & García, 2012 (from Panama), L. similis Morffe, García & Ventosa, 2009 and L. elieri García, Ventosa & Morffe, 2009 (both from Cuba) keep their taxonomical status. Three new species from Mexico and Dominican Republic were described for science. DNA studies were performed using 28S LSU rDNA and 18S SSU rDNA markers. All the species of Longior form a monophyletic clade. Such clade is included in another monophyletic clade containing other available sequences of hystrignathids, with Travassosinema and Cameronia as sister-group.

Richard Pearson Strong, Harvard University Museum of Comparative Zoology, and the Emergence of the American Society of Parasitologists

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The American Society of Parasitologists (ASP) was founded in 1924, contributing to one of the greatest revolutions in the medical and life sciences as our understanding of infectious diseases was exploding with transformational research. The founding scientists of ASP were just revealing that many parasites are carried by intermediate hosts and vectors, which are thus potential keys to controlling disease. Among these leaders was Prof. Richard Pearson Strong, a U.S. Army medical officer whose research on pneumonic plague in the Philippines during and after the Spanish-American War led to his appointment as Harvard University’s first Professor of Tropical Medicine. Shortly afterward, his globally celebrated success in controlling epidemic typhus in Serbia during World War 1 was a key element in the establishment of health as a major priority in U.S. foreign policy. His work at the Harvard University Medical School led to his research role in Harvard’s Museum of Comparative Zoology (MCZ), where he deposited many of his specimens of parasites from his work in South America, Central America, and Africa. Recognizing the role of wild and domestic vertebrates in the dynamics of zoonotic parasites, he studied animal as well as human parasites, collaborating with many parasitologists, including Henry Baldwin Ward, who contributed to the MCZ. In 1927, Strong followed Ward and C.W. Stiles to become the third president of the ASP, bringing his substantial international notoriety to the fledgling society. Strong worked with many parasite taxa ranging from helminths to protists to arthropods across several continents. Strong’s monumental work with onchocerciasis in Guatemala stands as perhaps his greatest contribution to the rising stature of American parasitology. In 1942, at the age of 70, Strong again joined the Army, providing expertise on parasitology and tropical medicine to support the World War 2 effort. He served through the war’s end until 1946, and died in 1948, leaving a lifetime legacy of serving humanity through parasitology.

Salivary gland transcriptome of Cimex lectularius and functional assessment of select salivary components

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The resurgence of the bed bug, *Cimex lectularius*, in the United States and Europe exemplifies its role as a tenacious parasite. Their ability to infest homes and the cutaneous and systemic reactions resulting from their bites warrants further investigation into the mechanisms underlying this component of their lifecycle. Through Illumina-based RNA-seq analyses using the recently published reference genome for the Harlan strain of *Cimex*, we analyzed the salivary transcriptome from adult females of *C. lectularius*. Through relative expression and comparative analyses we have highlighted genes suspected to play important roles in their ability to serve as a persistent nuisance species. This includes several novel genes not identified in previous EST-based studies. This project is augmented by a parallel project working to silence or knock-out a major component of the bed bug sialome, Nitrophorin, and to use human-based bioassays to test for this protein’s involvement in inflammation. Collectively, this data offer new insights into the evolutionary history and functionality of bed bug salivary proteins.

Schistosome and Diplostome infections in Gulls (Larus spp.) in Maryland and Delaware (U.S.A.)

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During an investigation of echinostome parasites of three species of gulls (*Larus* spp.) in coastal areas of Maryland and Delaware, specimens in the families Schistomatidae and Diplostomidae were also collected from intestine veins and intestine lumen, respectively, and became the focus of two undergraduate research projects. Avian schistosomes are of interest due to their potential for causing cercarial dermatitis in humans and possibly other hosts. Furthermore, little is known of schistosomes in marine habitats. Diplostomids are parasitic in birds and mammals and are known to cause diplostomiasis (eyefluke disease) when metacercariae infect fish eyes, thus impairing vision and their ability to forage, avoid predators, and likewise, survive. Thus far, we have examined 3 herring gulls, *L. argentatus*, 2 great black-backed gulls, *L. marinus*, and 2 ring-billed gulls, *L. delawarensis* for schistosomes and diplostomes. Schistosomes were present in *L. argentatus* and *L. marinus*, but not in *L. delawarensis*. The schistosomes we collected are in the genus *Austrobilharzia*, and we tentatively identify the species to *A. lari* (MacLeod, 1937) Penner 1953. Trematodes in the genus *Diplostomum* were obtained from all three gull species. We hypothesize that the specimens we collected thus far are *Diplostomum spatheceum* (Rudolphi 1819) and *D. pseudospathaceum* Niewiadomska, 1984. Future observations of morphometric and meristic data will finalize our identifications for specimens in both trematode families. We are also planning additional collections of gulls to shed light on the biodiversity of these parasites along the mid-Atlantic coast of the U.S.A.

Schistosome sulfotransferases: Mode of action, expression and localization

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Human schistosomiasis is a disease caused by species of the genus Schistosoma, which globally affects over 260 million people. The major species affecting humans are S. mansoni, S. haematobium, and S. japonicum. There is currently only one method of treatment (monotherapy), the drug Praziquantel. Constant selection pressure through mass chemotherapy has yielded evidence of resistance to PZQ both in the laboratory and the field. The goal of this research is to develop a second drug for use in conjunction with PZQ. Previous treatment of S. mansoni included, among others, the use of oxamniquine (OXA), a prodrug that is enzymatically activated in S. mansoni but is ineffective against S. haematobium and S. japonicum. The OXA activating enzyme was identified, described, and crystallized by our laboratories as being a sulfotransferase (SmSULT). The focus of this research is to reengineer OXA to be effective against S. haematobium and S. japonicum. In this regard we isolated the S. haematobium (ShSULT) and S. japonicum (SjSULT) sulfotransferases. Of the 230 derivatives that we have tested in an in vitro killing assay, one stands out CIDD-0066790 as it will kill all three major human schistosome species. One question is whether the mode of action of killing is the same as for OXA-the parent drug. RNAi experiments demonstrate that knockdown of the SmSULT, ShSULT and SjSULT results in resistance to CIDD-0066790. Therefore they have the same mode of action. A second question is the expression of the respective schistosome SULTs throughout development and compared to each species. Male worms express more SULT than female worms. SmSULT has a higher expression than ShSULT than SjSULT. A third question is the localization of SmSULT in S. mansoni. We used in situ hybridization and uptake of radiolabeled OXA to determine what cell types in the adult schistosome worm express SULT? Our studies demonstrate that OXA derivatives do kill all three major human schistosome species and thus will be an useful complement to praziquantel.

Secondary extinctions of parasites

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Parasites probably go extinct as much as or more than free-living species. Theoretical models suggest parasites should adapt well to specialize on dependable hosts, but this might lead to mass parasite extinctions when conditions change. The fossil record documents a few parasite extinctions, such as the loss of several heterakid nematodes specific to extinct moa in New Zealand. And we can see modern parasite extirpations following host extirpations. Two examples are the local extinction of a specialist stomach nematode (Mastophorus muris) following rat eradication at Palmyra Atoll. Less expected was the local extinction of a generalist mosquito (Aedes albopictus), which was not able to persist on the small human population. Humans sometimes try to drive parasites extinct - Guinea worm, being a hopeful example, and the condor louse being a regretful one. Whether we wish to preserve parasite diversity remains an open question for society.

Selective Dendritic Cell Apoptosis Driven by a Fatty Acid Binding Protein of Fasciola hepatica (Fh12)

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The parasitic helminth *Fasciola hepatica* secretes different proteins that modulate the host’s immune response toward an anti-inflammatory environment. Fatty acid binding protein (Fh12) is one of these molecules. Fh12 targets the LPS-binding pocket on the CD14 co-receptor, blocking the LPS-CD14 binding and stopping the entire TLR4 activation cascade. Concurrently, Fh12 also suppresses the phosphorylation of various kinases (p38, JNK and ERK) downstream the TLR4 signaling cascade, induces activation of macrophages (MOs) by an alternative pathway and inhibits the capacity of MOs to phagocyte microbes. Dendritic cells (DCs) are antigen-presenting cells that play a key role at early phases of innate immunity and that are essential in the development of adaptive immune response. The main objective of the current study was to investigate the effect of Fh12 on the activation and function of these cells. DCs isolated from bone marrow of naïve C57BL/6 mice were stimulated in vitro with Fh12 1h prior to LPS stimulation for 18 hrs. ELISA was used to quantify the amount of secreted cytokines in supernatant. FACS was used to determine the activation of MHCII, co-stimulatory molecules and to determine cell viability. In addition, allogeneic co-culture of DCs from BALB/c mice and splenocytes from C57BL/6 mice were incubated and cytokines measured by ELISA. Results demonstrated that Fh12 significantly inhibits the production of IL-12p70 (p<0.03) and IL-6 (p<0.0454), reduced the expression of MHCII and co-stimulatory molecules CD80. Fh12 also prevented the priming of naïve T-cells and consequently suppressed the expression of IFN-γ. In contrast to the effect observed in macrophages, Fh12 induced early and late apoptosis of DCs and this effect was found to be dose-dependent. Further studies are in progress to elucidate the mechanisms through which Fh12 induces such selective apoptotic effect in DCs. The strong suppressive effect that Fh12 exerts on DC-activation could have relevant implications in the subsequent development of adaptive immune responses to microbial pathogens.

Sensitivity of four molecular methods for the diagnosis of the virus Cyprinid herpesvirus 3 (CyHV-3) in goldfish (Carassius auratus)

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Cyprinid herpesvirus 3 (CyHV-3) is the causal agent of an important notifiable disease, known as Koi herpesvirus (KHV). This virus has caused high mortality rates around the world in common and koi carp, and has been reported in more than 30 countries. However, there are no official reports confirming the presence of CyHV-3 virus in Mexico. The detection of CyHV-3 in asymptomatic carriers dispersing this disease is one of the main problems in carp aquaculture and the ornamental fish industry nowadays. Therefore, it is important to develop fast and efficient yet sensitive and non-destructive diagnostic methods to detect the virus. The aim of this study is to evaluate the sensitivity of four molecular methods: polymerase chain reaction (PCR), nested polymerase chain reaction (nPCR), quantitative polymerase chain reaction (qPCR) and isothermal mediated amplification loops (LAMP) for the diagnosis of KHV in goldfish (Carassius auratus). Eighty-four goldfish acquired from commercial pet shops in Mérida, Yucatán, Mexico were sampled. Preliminary results showed a 16% prevalence using PCR with specific primers for the thymidine kinase gene. However, using a second pair of internal primers (nPCR) the prevalence increased to 69%, with this technique being the most sensitive so far. It is still necessary to test the sensitivity of two techniques (qPCR and LAMP) to obtain definitive results.
Sequences of RNA-binding proteins (RBPs) in amitochondriates parasites: E. histolytica and G. duodenalis

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Entamoeba histolytica and Giardia duodenalis, are two protozoa that cause intestinal diseases worldwide. E. histolytica is the causative agent of amebiasis, while G. duodenalis causes giardiasis. Both parasites need iron for their survival and virulence; however, iron regulation mechanisms still in study. In mammals, iron homeostasis is regulated at the post-transcriptional level by the IRE/IRP system (Iron Response Elements/Iron Regulatory Proteins). The cytoplasmic proteins IRP-1 and IRP-2 interact with IREs, which are conserved stem-loops structures found in the untranslated regions (UTR) of the mRNAs that encode proteins related to iron homeostasis. This mechanism was previously described in T. vaginalis, for Tvcp4 and Tvcp12 proteins which are regulated at the post-transcriptional level. Due to their evolutionary closeness we hypothesized the existence of the IRE/IRP system in E. histolytica and G. duodenalis. Previously, our research group found IRE-likes structures in the mRNA of E. histolytica by in silico analysis. In addition, proteins that interact with human IRPs antibodies are found in the cytoplasmic extracts of both parasites. Therefore, our objective was to search in silico RNA binding proteins that would participate in the post-transcriptional regulation of iron in the amitochondriate parasites E. histolytica and G. duodenalis, in order to determine the presence of this IRE/IRP-like mechanism. Thus, we perform an in silico analysis, in which the amino acid sequences of the human IRP1 and 2 proteins were searched. In addition, T. vaginalis IRP-likes (α-TvACT3, HSP70-4 and Actin) were used as probes in both in E. histolytica (AmoebaDB) as in G. duodenalis (GiardiaDB) genomes; subsequently, possible orthologous were analyzed for RNA-binding motifs in the SMART® program. The analysis revealed 106 possible orthologous for E. histolytica, of which only 11 showed RNA-binding domains. On the other hand, G. duodenalis genome showed 22 possible orthologous however none of them showed RNA-binding sites; possible the binds would occur once the protein acquires the tertiary structure. These results suggest the presence of an iron post-transcriptional mechanism IRE/IRP-like in E. histolytica and G. duodenalis. Likewise, studies are in progress to identify and characterize IRP-like proteins. The knowledge derived from this work can help in the future for the development of new diagnostic methods.

Significance of Fasciola hepatica Glutathione S-transferase glycans and conformation in the suppression of toll-like receptors stimulation

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Fascioliasis is a parasitic disease caused by one of the most prevalent parasitic helminths called Fasciola hepatica. This parasite is well known as a master of immunomodulation because of the ability to suppress the Th1 immune response and elicit a strong Th2/T-reg immune response by releasing excretory-secretory products (ESPs), which comprise myriad of glycoproteins, which it believe play an important role in immune recognition and response. Parasite glutathione S-Transferase (GST) is one of the ESP components recognized for its anti-oxidant properties and capacity to provide part of parasite defense by detoxifying the secondary products of lipid peroxidation produced via immune initiated free-radical attack on host or parasite membranes. Our preliminary studies demonstrate that FhGST also exerts a strong suppressive effect on the activation TLR4 of macrophages, which is evidenced by significant suppression of NF-kB activation and down-regulation of TNF and IL1B in response to LPS-stimuli. We have been speculated that such suppressive effect could be due in part to the glycans that decorate the structural moiety of FhGST. To test our hypothesis, we used a cell line of human monocytes (THP1-CD14) that express most TLRs, as well as an NF-κB-inducible, secreted embryonic alkaline phosphatase reporter gene. Cells were stimulated with a whole extract of 1 x10⁸ cells Klebsiella pneumonia (Kp) in the presence or absence of native FhGST or denatured by heating, which was demonstrated by CD analysis or treated with PNGase F, an amidase that removes almost all N-linked oligosaccharides. Our results demonstrate that native FhGST suppressed the NF-kB in 86.3%, whereas the heat denatured protein suppressed the NF-kB activation in an 84%. After PNGase F treatment FhGST suppressed the NF-kB activation induced by Kp in 84%. Based on these results we concluded that in contrast to expected, the integrity of secondary structure of FhGST as well as the presence of glycans are not essential to determine the capacity of FhGST to suppress the TLR4 activation induced by K. pneumonia.

**Specific interaction of two IRE-like from Entamoeba histolytica to human iron regulatory proteins**

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**Entamoeba histolytica** infect human causing amebiasis, that is cataloged as the third most frequent parasitic cause of death worldwide. In *E. histolytica* has been reported that iron has the ability to modulate the expression of genes related to the virulence of the parasite. However, the mechanism by which this expression is regulated has not been well described. In mammals, the posttranscriptional regulation of iron by the IRE/IRP system (Iron Responsive Element/Iron Regulator Protein) is the most studied. The iron regulatory cytoplasmic proteins, IRP-1 and IRP-2, interact with Iron Responsive Elements (IREs), which are conserved stem-loop structures located in the untranslated regions (UTR) of the mRNAs that encode proteins related to iron homeostasis. Recently, our research group has reported the existence of a posttranscriptional iron regulation system parallel to the IRE/IRP system in the protozoan parasite *Trichomonas vaginalis*, this mechanism has been described for some proteins of *T. vaginalis* virulence. In addition, our group has found stems-loops structures in *E. histolytica* mRNAs coding EhCP5, Ehmbp26, Actin, EhPRL21 and EhFeSOD proteins, in some of which it has been shown that are modulated by iron. However, the functionality...
of the IRE-like structures has not been demonstrated. Therefore, the aim of this work was to determine the specific interaction of two IREs of the parasite (Ehhmbp26 and Actin) to human cytoplasmic proteins. Gel retardation assays were carried out using these IREs of the parasite with cytoplasmic extracts of HeLa cells. Our results showed specific interaction of IRE-IRP type. In addition, by UV-crosslinking assays we observed proteins bands of 95 and 110 kDa. These data suggest the presence of an IRE/IRP system in *E. histolytica*.

### Studies on Parasitic Contamination of Soil and Local Drinking Water Source in Doma Local Government Area of Nasarawa State, Nigeria

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Water is a natural resource that is essential to all living things on earth, but if contaminated can pose a lot of risk to human health when consumed. This study evaluates the parasitic contamination of drinking water sources and surrounding soil in Doma Local Government Area of Nasarawa State, Nigeria. A total of 48 water and soil samples around water sources were collected from different selected sources (wells, streams and boreholes) between the months of March to July 2017. The water samples were analyzed using the Calcium carbonate (CaCO3) floatation method while a modified Baermann technique was used to examine the soil samples microscopically for the presence of parasites. A total of 32 water samples were found with parasites. These include 2 species belonging to the protozoan group (Entamoeba histolytica, and Giardia lamblia) 3 from the nematode group (Trichuris trichiuria, Ascaris lumbricoides and Hookworm), and 1 trematode group (Fasciola hepatica). The nematode group had the highest contamination rate 19 (59.38%) followed by the protozoan group 12 (37.50%) while the trematode was least with 1 (3.13%). The most contamination was in stream water sources for late dry and early wet season with 69.23% and 94.74% respectively. The wells had 30.77% and 5.26% in late dry and early wet season respectively. The boreholes had zero contamination for both seasons. Prevalence of parasites in relation to sources of water showed a high significant differences ($\chi^2 = 49.741$, df = 2, $P = 0.0000001$), while there was no significant difference in relation to late dry and early rainy seasons ($\chi^2 = 2.3438$, df = 1, $P = 0.1258$). Geohelminths was highest around the borehole area 15 (35.71%) followed by the well area 14 (33.33%) and was least encountered in the stream area 13 (30.95%). However, there was no significant difference ($\chi^2 = 0.94915$, df = 2, $P = 0.6221$) in the prevalence of geohelminths in relation to sources of water area. Result indicated high rate of parasitic contamination of soil and drinking water sources in the study area. There is need for advocacy and enlightenment on the importance of proper drinking water treatment.

### Surveillance of Blood Parasites and Ticks in the Maryland White-Tailed Deer Population

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White-tailed deer (Odocoileus virginianus, WTD) are found throughout Maryland. Apicomplexan parasites such as Plasmodium, Babesia and Theileria spp. and obligate intracellular bacteria such as Ehrlichia and Anaplasma spp. are transmitted by mosquitoes and ticks and infect WTD. The aim of this study was to ascertain the prevalence of these parasites in the Maryland WTD population by sampling deer harvested during management programs. Blood samples and ticks were collected from Maryland WTD harvested during two separate managed hunt seasons from 2017-2018. Locations were Fair Hill NRMA State Park (FH, Harford County), Gunpowder Falls State Park-Sweet Air (GPF, Baltimore County), Catoctin Mountain National Park (CMP, Frederick County), Seneca Creek State Park (SC, Montgomery County), and Cabin John-Rock Creek (CJ-RC, Montgomery County). Samples ranged from n=5-27 from the various parks. Giemsa-stained blood smears were analyzed microscopically to quantify parasitemia. Genomic DNA was extracted from selected samples and used for molecular detection of parasite species. The 16S and 18S ribosomal RNA (rRNA) genes were PCR amplified and gene sequences were compared to NCBI databases. Microscopy found intracellular piroplasms and bacteria in the blood of all deer from the five parks. The overall average parasitemia detected at each park was: CMP (0.84±0.34%, n=12), GPF (0.54±0.20%, n=31), FH (1.34±0.8%, n=47), SC (1.36±0.67%, n=26), and CJ-RC (1.62±0.33%, n=14). PCR analysis of 15 selected samples from the different parks showed T. cervi (n=9) and B. odocoilei (n=6) were present. In three parks (CMP, FH, and CJ-RC), both species were present. Only B. odocoilei was found at GPF and only T. cervi was found at SC. A nested 16S rRNA PCR using Anaplasma-specific primers amplified a fragment in a FH sample, but the species could not be determined. This work demonstrates the spread of piroplasms in WTD throughout Maryland. Further studies will focus on analysis of ticks to determine their role in parasite transmission in the various parks.

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TAXONOMIC DESCRIPTION OF HELMINTH PARASITES FOUND IN THREE BIRD SPECIES (Phalacrocorax olivaceus, Egretta thula and E. tricolor) FROM THE RÍA CELESTÚN BIOSPHERE RESERVE, YUCATAN, MEXICO

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Aquatic birds are frequently the definite hosts of a wide variety of helminth parasites that also parasitize fish and invertebrate species. Extensive study has been done of the helminth parasites of fish and invertebrates from Celestún lagoon, in the state of Yucatán, México. In contrast, records of and research on the parasites of birds in the area are rare. To extend the available data on the helminth parasites of birds in this region, five birds from each of three species (Phalacrocorax olivaceus, Egretta thula and E. tricolor) were collected from Celestún lagoon. Morphometric measurements were taken of the birds and necropsies done. Each organ and all tissues were examined under a stereoscopic microscope in search of helminth parasites. Platyhelminthes were dyed using the Mayer’s paracarmine technique, cleared with clove oil and finally mounted in Canada balsam. Morphometric characterization was done of each identified species. A total of fifteen helminth species were collected: three from P. olivaceus; seven from E. thula; and five from E. tricolor. Of these fifteen species, ten were digeneans, one was a nematode, one a cestode and one an acanthocephalan. Taxonomic descriptions are currently available for six of the species: Renicola sp., Renicola hydranassae, Renicola lari, Phagicola diminuta, Carneophallu trilobatus, Contracaecum sp. and Southwellina hispida. In Celestün lagoon, cercaria of Renicola sp. have been reported in the snail Cerithidea pliculosa and its metacercaria in snails of the genus Melampus. The fluke P. diminuta has been recorded in Poeciliidae fish as secondary intermediate hosts. The larval stages of Contracaecum sp. and S. hispida have only been reported in fish.
TGF-β POLYMORPHISMS ARE A RISK FACTOR FOR CHAGAS DISEASE

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Transforming growth factor β1 (TGF-β1) is an important mediator in Chagas disease physiopathology. It was observed that patients with cardiac damage show elevated plasmatic levels of TGF-β compared to asymptomatic patients and those patients presented a worse clinical outcome after 10 years of follow up. The polymorphism at codon 10 in the TGF-β1 gene has been described to influence the susceptibility to Trypanosoma cruzi infection in patients from Colombia and Peru. The present study assessed polymorphisms of the TGF-β1 gene in patients with chronic Chagas disease, correlating the presence of polymorphisms on TGF-β1 gene with the susceptibility and severity in Brazilian population. 152 patients with Chagas disease (53 with the indeterminate form and 99 with the cardiac form) and 37 non-infected controls were included. We investigated five SNPs (-800G>A, -509C>T +10T>C +25G>C and +263C>T) by sequencing of PCR products. In addition, serum levels of TGFβ1 were measured by ELISA. We observed a significant difference in the distribution of the -509C/T and +10 T/C between the controls and patients groups, suggesting a genetic influence of these TGFβ1 variants on T. cruzi infection susceptibility. Genotype C/T at position +10 of the TGF-β1 gene was also more frequent in Chagas disease patients than in non-infected controls. Genotypes C/T and T/T at position -509 of the TGF-β1 gene were more frequent in Chagas disease patients than in non-infected controls. However, we could not find any significant differences in the distribution of any of the studied TGF-β1 polymorphisms between patients with the indeterminate form or different stages of the cardiac form of Chagas disease. Furthermore, TT genotype at position -509 was associated with higher serum levels of TGF-β1. Taken together these results show that -509 C/T and +10 T/C TGF-β1 polymorphisms are associated with Chagas disease susceptibility in a Brazilian population.

THE HYDROPHOBIC NATURE OF <i>Ophryocystis elektroscirrhosa</i> (Apicomplexa: Neogregarinorida) Oocysts, A PARASITE OF MONARCH BUTTERFLIES, <i>Danaus plexippus</i>, REVEAL BIOLOGICALLY RELEVANT MECHANISMS FOR PARASITE TRANSMISSION

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The pathogenic neogregarine *Ophryocystis elektroscirrha* infects the hypodermal tissues of monarch (*Danaus plexippus*) and queen (*D. gilippus*) butterflies. Currently, three routes of transmission have been proposed for *O. elektroscirrha* and include vertical, horizontal, and sexual transmission. Vertical transmission is the most common route and happens when females scatter protozoan oocysts directly on eggs during oviposition. Horizontal transmission occurs when butterflies scatter oocysts on milkweed leaves. Additionally, sexual transmission occurs when infected butterflies contaminate the scales of uninfected butterflies with oocysts during copulation, and contaminated females subsequently scatter oocysts onto eggs and/or milkweed leaves. Caterpillars become infected when they ingest oocysts from egg cases or milkweed leaves after hatching. However, currently it is unclear if or how oocysts of *O. elektroscirrha* adhere to milkweed leaves after they are scattered from butterfly scales. In this study, we evaluated the adherent nature of scattered oocysts on milkweed leaves using SEM and light microscopy before and after milkweed plants were exposed to artificial and natural conditions. Our results indicated that oocysts of *O. elektroscirrha* were extremely hydrophobic and difficult to concentrate in water using centrifugation. Our SEM images of oocysts on milkweed leaves indicated that their hydrophobic nature allowed them to bond with the waxy cuticle of milkweed leaves, which prevented them from being dislodged when submerged for long periods of time under running water or during intense rain, hail and wind events. Our study is the first to evaluate the adherent nature of *O. elektroscirrha* oocysts and our findings are discussed in terms of transmission of *O. elektroscirrha* to monarch butterfly caterpillars.

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**Take a Breather: Parasitic Effects on Host Respiration**

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Parasites by their nature cause damage to their hosts, which can perturb physiological functions such as metabolism and respiration. Host metabolism can vary greatly depending upon a large number of factors including the intensity of parasite infection. While a large body of literature has examined respiration in insects, few studies have examined the impact of parasites on host respiration and metabolism. Herein we hypothesized that the metabolic rate of the fruit fly, *Drosophila nigrospiracula* is dependent upon infection by *Macrocheles subbadius*, a facultative parasitic mesostigmatid mite. We predicted that host metabolic rate would increase with the number of mites. Flies employ behavioural defences to fend of mites, which is also energetically costly. Hence we examined the interaction between infection and movement on a host’s respiratory rate. Our prediction is that fly metabolic rate would increase with the level of parasite infection and host movement: restricted uninfected flies < free uninfected flies < restrained flies infected by mites < free flies infected by mites. We found that higher infection intensities induced higher levels of host carbon dioxide production. Together these data indicate that *M. subbadius* parasitism induces an increase in its insect host’s respiration, and this may be mediated by changes in host physiology and/or activity level.

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**Targeting Wolbachia as an approach to combat Filarial Disease**

Laura Chappell

Lymphatic filariasis and Onchocerciasis are human diseases caused by filarial nematodes, small hair-like parasitic worms of the Filarioidea family. These diseases affect millions of people across the globe, and there are currently no drugs that effectively kill the adult stage of the parasite. These worms share a symbiotic relationship with an intracellular bacterium called Wolbachia, and they require this bacteria for reproduction and survival. Taking advantage of this crucial symbiosis, we
aim to find drugs that can kill the adult parasite by targeting Wolbachia. A high-throughput primary drug screen has tested hundreds of thousands of compounds in Wolbachia-infected cells to find hits that reduce levels of the bacteria. A secondary screen has tested these hits for Wolbachia-reducing activity in a species of filarial nematode, Brugia pahangi. So far, we have identified 13 promising compounds.

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Taxonomy and ecology of Clausidium vancouverense (Haddon, 1912) associated with two callianassid ghost shrimp taxa from the eastern Pacific.

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Clausidium vancouverense (Haddon, 1912) is redescribed based on specimens collected from two callianassid ghost shrimp (giant ghost shrimp, Neotrypaea gigas (Dana, 1852) and bay ghost shrimp, Neotrypaea californiensis (Dana, 1854)) from British Columbia (Canada), Washington, Oregon, California (United States), and Baja California (Mexico). Comparisons with copepod specimens identified by Wilson (1935) as Clausidium californiensis Wilson, 1935 from N. californiensis (as Callianassa californiensis) from Newport Beach, California also revealed that they are conspecific with C. vancouverense. Interspecific variations of the labrum, postmaxillipedal sternite, anal somite, mandible, and leg 5 are discussed. Additional ghost shrimp specimens were collected from two southern California mudflats (Cabrillo Salt Marsh, San Pedro and Santa Ana River, Huntington Beach) to obtain C. vancouverense prevalence and intensity data. In total, 182 N. gigas and 67 N. californiensis were collected and 423 and 225 C. vancouverense were removed, respectively. The prevalence of C. vancouverense at Cabrillo Salt Marsh (59.6% for N. gigas and 54.8% for N. californiensis) was similar to that at Santa Ana River (52.3% for N. gigas and 52.8% for N. californiensis). By contrast, the mean intensity of C. vancouverense at Cabrillo Salt Marsh (3.38 for N. gigas and 8.59 for N. californiensis) was higher than that at Santa Ana River (2.65 for N. gigas and 4.16 for N. californiensis). A significant positive correlation was found between host carapace length and C. vancouverense intensity (p = 0.0239 for N. californiensis; p < 0.0001 for N. gigas).

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Temporal and Spatial Prevalence of Giardia lamblia in bivalves Collected from Orchard Beach and Soundview Park, NY from 2014 to 2017

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Giardia lamblia, a flagellated protozoan parasite, if ingested, infects the lumen of the small intestines in humans. This parasite is found in various animals, such as dogs, cats, and birds. G. lamblia is most commonly transmitted to humans via ingestion of contaminated food and water. Its presence in aquatic environment is relatively unorthodox and its presence in public waterways is a public
The goal of this study is to determine the prevalence of *G. lamblia* in Atlantic oysters (*Crassostrea virginica*) and ribbed mussels (*Geukensia demissa*) collected from Orchard Beach and Soundview Park, Bronx, NY. The mussels were collected on September 15, 2016 at low tide. Tissues were dissected followed by DNA extraction and PCR analysis. Thus far, we found a prevalence of 77% (21/28) and a prevalence of 65% (11/17) of *G. lamblia* in the oysters collected in Soundview Park and Orchard beach, respectively. In contrast, the prevalence of *G. lamblia* was 29% (11/39) in the ribbed mussels collected at Orchard Beach, and 19% (7/37) in those collected from Soundview. This is interesting since those two beaches are only 6 miles away from each other. In conclusion, Atlantic oysters and ribbed mussels can be used as a biological sentinels to detect *G. lamblia* in public waterways and reservoirs.

### Temporal dynamics of parasites of Mexican and vulnerable poeciliid fishes

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The ichthyofauna of Lake Catemaco represents an important group of fishery resources in the region of “Los Tuxtlas” (Veracruz State). Among the different fish families, Poeciliidae includes 5 endemic species, *Xiphophorus milleri*, *Xiphophorus kallmani*, *Poecilia catemaconis*, *Poeciliopsis catemaco* and *Pseudoxiphophorus tuxtlaensis*, which form an important component of trophic chains, being main food of some aquatic vertebrates as birds (migratory and resident) or commercial fish in the region. Currently the American Fisheries Society (AFS) has listed 4 of the 5 poeciliids as vulnerable species. Despite the importance of fish, little is known about the parasitic fluctuations over time. Two samples were taken per season (dry, rainy and windy), fish were captured alive and were reviewed in search of external and internal parasites (protozoa, myxozoan, helminths and arthropoda), which were usually identified at the level of genus or species. The water temperature and precipitation during the samplings were recorded periodically. A total of 249 fish were examined, finding myxozoan (10 infected sites), all of them were considered as one species, protozoa (5), flukes (1 adult and 8 larvae), monogeneans (2), cestodes (5 larvae), nematodes (2 adults and 6 larvae), acanthocephala (1 adult and 1 larva) and 1 crustacean. The windy season presented the largest number of parasitic species (29) compared with rain (24) and dry (26), as well as the largest number of larvae of cestodes (5), as opposed to dry and rainy season (2). Protozoa presented the highest prevalence in the windy season (93.3%) compared with the lowest in dry season (66.7%). The larvae of flukes were present in all samples with high prevalences, but with higher average intensity during the dry season, particularly *Ascocotyle felippei* (183.3±29) in the heart of *P. tuxtlaensis*. The higher frequency and abundance of nematodes and cestodes were recorded in the windy season.

### Temporal variation of metazoan parasites of Dorosoma petenense, native fish of fishing importance of Catemaco Lake, Veracruz, Mexico

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Dorosoma petenense is a fish highly prized for its taste in the region of Los Tuxtlas, Veracruz. Despite its fisheries and ecological importance as a fish filter and member of trophic chains, few is known about their parasitic and temporary fluctuation of their populations. Therefore, the temporal variation of the parameters of their metazoan parasites infection were analysed. Total reviewed 90 fish, 30 by climate (2016-2017): northwind (December-February), dry (March to may) and rainy season (July to September). In total there were six species of parasites: four larvae of trematodes: *Echinochasmus macrocaudatus*, *Phagicola nana*, *Timonella loossi*, *Strigeidae* sp., as well as the larva of a nematode *Procamallanus* sp. and a copepod *Ergasilus* sp. *E. macrocaudatus* was the numerically most important species where 99% of the total number of registered individuals (83,982), belonged to the species. It was during rainy season where arose the greater intensity of parasites (F2, 90=14.5165, P<0.001): *E. macrocaudatus* 1,899 ± 2,020 and greater species richness (5) where the temperature reaches its annual maximum values (31 ± 0.7) and rainfall is 8.45 ± 0.7 in comparison with northwind arose where the lower average intensity of *E. macrocaudatus* 323 ± 249 and the lowest richness of species (3). We register for the first time in Catemaco Lake the presence of *E. macrocaudatus*, as well as the seasonal variation in their frequency and abundance.

The IMD pathway controls antimicrobial-peptides expression in Rhodnius prolixus fat body

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*Rhodnius prolixus* is a major vector of *Trypanosoma cruzi*, the etiological agent of Chagas disease, which infects over 8 million people worldwide. *Rhodnius prolixus* is also a hemathophagous hemipteran that has served for decades as a model organism in insect physiology. The publication of the genome of this insect revealed that the immune-related IMD pathway was incomplete, an observation shared with the Fea aphid (*Acyrthosiphon pisum*) and the bedbug (*Cimex lectularius*). Despite the incomplete and possibly non-functional pathway the data suggest that the IMD pathway does control the expression of Defensin A in the midgut. We used RNAi strategies to determine if the IMD pathway also regulates the expression of antimicrobial peptides (AMPs) in the fat body of *Rhodnius prolixus*. We knocked down different genes of the IMD pathway and then injected those insects with Gram-positive or Gram-negative bacteria. We then used real time PCR to measure gene expression of AMPs regulated by the IMD and toll pathways in the Rhodnius prolixus fat body.

The Importance of Microbiome Assessment in Parasitology

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Complex organisms evolved in the presence of microbes, forming symbiotic relationships. Historically, the majority of investigations of this system have focused on individual parasitic microbe/host relations. While these studies have yielded vital knowledge for disease and health research, there is a pressing need to incorporate microbial community assessment. Recent sequencing advances have stimulated an entire field dedicated to understanding the complex relationship between communities of symbiotic microbes (also known as the microbiome) and their hosts. As parasitic microbes are only one component of a microbiome, it is important to consider the community dynamics of the entire system. Due to the high variability of microbiomes in wild host species, study-specific analyses are imperative. In this study, I lay down the framework for incorporating microbiome community investigation into field-based parasitology research.

The advantage of using mucus for the detection of ectoparasites in Nile tilapia by endpoint PCR and multiplex PCR

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The ectoparasites cause damage to many animals including fishes. They can be specific or infect a great range of hosts. Likewise, some monogeneans can be vectors of other pathogens like virus or bacteria. Transportation and introduction of animals from different places promote the translocation of pathogens into new areas. Infection of the ectoparasites like Ichthyophthirus multifiliis, Trichodina spp., Cichlidogyrus spp., and Gyrodactylus spp. are associated to damage, morbidity and mortality in hatcheries of Nile tilapia Oreochromis niloticus. Currently, diagnosis of these ectoparasites is based on necropsy of fish and subsequent morphological identification. Our aims were developed simplex and multiplex PCR tests to detect the presence of monogeneans Cichlidogyrus spp. and Gyrodactylus spp., as well as protozoans I. multifiliis and Trichodina spp. DNA was isolated from each ectoparasite and from the mucus of tilapia. The primers Ekgyro1, Ekich5, Ektri3 and Cicly4 designed for each ectoparasite were selected sequences from the GenBank® database. The sensitivity, specificity, PVP and PVP were of 100% for simplex PCR using DNA from parasites and from mucus. Six individual sequences to Cichlidogyrus spp., 16 sequences to Gyrodactylus spp., four sequences to I. multifiliis and one sequence to Trichodina spp. were identified, and these had high homology with the sequences of the same genus. The multiplex PCR validation in samples of mucus provide a non-invasive test for simultaneous detection. In conclusion, we obtained four non-invasive techniques by simplex PCR to diagnose ectoparasites on Tilapia O. niloticus and developed the conditions of multiplex PCR for simultaneous detection of the same ectoparasites.

The effects of maternal and grandmaternal flea infestation on offspring quality and quantity in Meriones crassus (Mammalia: Gerbillinae)

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Parental effects may alter offspring phenotype in a beneficial manner if the parental environment sufficiently predicts the offspring environment. Parasitism is a common stressor across generations;
therefore, parental infestation could reliably predict the likelihood of infestation for offspring. Few experiments have investigated the relationship between parasitism and maternal effects on offspring phenotype and none have investigated this relationship across multiple generations. We investigated how maternal and grandmaternal infestation with fleas (Xenopsylla ramesis) affected offspring quality and quantity in a desert rodent (Meriones crassus). We used a fully-crossed design with control and infested treatments to examine litter size, pup body mass at birth, and pup mass gain before weaning for combinations of maternal and grandmaternal infestation status. No effect of treatment on litter size or pup body mass at birth was found. However, maternal and grandmaternal infestation status significantly affected pre-weaning body mass gain, a proxy for rate of maturation, in male pups. Furthermore, pups gained significantly more weight before weaning if maternal and grandmaternal infestation status matched, regardless of the treatment. Thus, pups whose mothers and grandmothers experienced similar risk of parasitism, either both control or both infested, would reach sexual maturity more quickly than those pups whose mother’s infestation status did not match that of their grandmothers. These results support the contention that parents can receive external cues, such as risk of parasitism, that prompt them to alter offspring provisioning. In turn, this provisioning is beneficial if the parental environment matches that of the offspring. Therefore, parasites could be a mediator of environmentally-induced maternal effects and could affect host reproductive fitness across multiple generations.

The future of cestode diversity, phylogenetic relationships, and classification: Looking beyond the Planetary Biodiversity Inventory (PBI) Project

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While many major elements of the phylogenetic relationships among cestodes were resolved as a result of work on the PBI project, many others remain uncertain. For example, erection of the new order Onchoproteocephalidea remedied the non-monophyly of the Proteocephalidea relative to a suite of elasmobranch-hosted genera, but the interrelationships among the latter genera are still unclear and a robust morphological synapomorphy for this new order has yet to be identified. Establishment of the new order Phyllobothriidea emphasized morphological similarities across a wide array of genera that parasitize sharks, but the interrelationships among these genera are highly uncertain. By far the most problematic are the taxa that remain in what is now referred to as the "tetraphyllidean" relics, which is clearly a polyphyletic assemblage of taxa. These deficiencies have major implications for the classification of cestodes. But, it is now clear that resolution of these issues will require development of genetic markers beyond the handful typically used. In an attempt to expand that repertoire, we have begun an effort, informed by the de novo assembly of genomes for representatives of 8 of the 9 orders of cestodes that parasitize elasmobranchs, to identify a large number of orthologous protein coding genes for use in phylogenetic reconstruction using a targeted gene capture approach. We anticipate recognition of as many as 10 additional independent groups of elasmobranch-hosted cestodes, each of which share similarities in morphology and/or host associations. In terms of global cestode diversity, elasmobranchs were determined to be key to the evolution of most other orders of tapeworms. Not only do they span the full breadth of the cestode phylogeny, but they also host an unexpectedly high proportion of the overall diversity of genera, families, and orders relative to other vertebrate groups given they constitute only about 2% of the planet’s 68,000 vertebrate species. Our best estimate based on a combination of positive and negative infection data, as well as host taxa that have not yet been examined for cestodes, is that a total of 20,000 species of cestodes will ultimately be found parasitizing the planet’s vertebrate species.
The influence of Euhaplorchis californiensis on California killifish (Fundulus parvipinna) behavior across contexts and ontogeny

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Research examining parasite manipulation of host behavior usually suffers from a variety of methodological limitations. For example, some studies examine behavior of hosts from infected and uninfected populations, but these populations may differ in behavior for a variety of reasons other than infection status (e.g. differences in habitat quality or predator abundance). Even when experimental infections are employed, hosts usually originate from populations that do not share a recent coevolutionary history with the parasite. Also, infection timing and dose may not reflect natural infection regimes, and infections often fail to reach ecologically relevant intensities. Finally, most behavior-modification research does not use a positive control, which allows researchers to begin to tease out the general impacts of infection from adaptive manipulation of behavior. All of these limitations preclude robust understanding of the strength and development of parasite manipulation of host behavior in nature. Here, we address these limitations in a now classic behavioral manipulation system. Euhaplorchis californiensis is a brain-infecting trematode whose intensity is correlated with the frequency of "conspicuous behaviors" in its second intermediate host, California killifish (Fundulus parvipinna). Infected killifish are more likely to be consumed by final-host predators. We collected gametes from a killifish population that co-occurs with E. californiensis, and hatched uninfected killifish in the lab. We divided killifish into one of four treatment groups: high doses of E. californiensis, low doses of E. californiensis, negative control (seawater), and positive control (a low dose of trophically transmitted cyathocotylid trematode). We then mimicked natural infection timing and doses by repeatedly infecting killifish with cercariae twice a week for 9 months. We also collected videos at multiple times throughout the day (day, night, dusk, dawn, routine behavior, foraging, and infections), and throughout the lives of the killifish, to examine how killifish microhabitat use (time spent near the water surface) and the frequency of conspicuous behaviors change across contexts and ontogeny. Hence, this study provides a more ecologically grounded examination of the impacts of manipulation over a host’s lifetime.

The role of ontogenetic habitat shift on parasite communities in five south Florida fishes

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Many reef fishes recruit into mangroves, and migrate to reef habitats as they grow and mature. Each ontogenetic habitat shift exposes migrants to previously unencountered parasite taxa, potentially increasing parasite species richness and driving changes in parasite community structure. However, studies on this topic rarely attempt to distinguish between the effects of habitat shifts (i.e. location) vs simple increase in size (i.e. size class). Therefore we contrasted parasite community richness and structure in Great Barracuda Sphyraena barracuda (N=84), Atlantic Needlefish Strongylura marina (N=49), Crevalle Jack Caranx hippos (N=59), White Mullet Mugil curema (N=90), and Yellow-fin...
Mojarra Gerres cinnerus (N=60) from mangrove, inshore seagrass beds, and offshore reef habitats. Mullet harbored the highest species richness (S=26, mean infracommunity S=2.4±1.6) and Needlefish the lowest (S=8, mean infracommunity S=0.5±0.8). A global model including species, location, and size class was significant (R²=0.654, DF 17, F=35.91, p<0.001), with location (LogWorth 6.0) and size class (LogWorth 4.9) having the strongest effect; furthermore there was a significant species by location interaction (p<0.001, LogWorth 14.6). Regarding parasite community structure, PERMANOVA on Bray-Curtis similarities found that both location and size significantly structured parasite communities for all species, with habitat shift (pseudo-F 3.3) having a larger effect than size (pseudo-F 1.8). As with species richness there was a significant location by species interaction (pseudo-F 4.6). Ordination analyses suggested that parasite community structure was similar among species during their juvenile mangrove stage, but changed significantly as they initiated their shift to seagrass beds; community structural changes associated with the final shift to reef habitats were less pronounced in all taxa except mullet. Our results suggest that ontogenetic habitat shifts and (to a lesser extent) host size class were important drivers of parasite community composition and structure in these fishes.

The underestimated biodiversity of Onchocerca nematodes associated with native North American ungulates

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Until recently, a single species of the genus Onchocerca (Nematoda; Filarioidea: Onchocercidae) was known to be associated with native North American ungulates. Onchocerca cervipedis Wehr & Dikmans, 1935 has been reported from five cervid hosts: white-tailed deer (WTD, Odocoileus virginianus), mule deer (Odocoileus hemionus), moose (Alces americanus), caribou (Rangifer tarandus), and the wapiti (Cervus canadensis), as well as the pronghorn (Antilocapra americana). Records range from Costa Rica to sub-Arctic Western Canada and Alaska. Molecular characterization of adult nematodes from moose, and white-tailed deer tissue samples containing skin-dwelling microfilariae proved the existence of an undescribed, cryptic species in the Northeastern US (Onchocerca sp. 1). Additional sampling, and the molecular characterization of adult nematodes in WTD from West Virginia (Onchocerca sp. 2), showed that this species is phylogenetically closer related to O. cervipedis sensu Verocai, from Western North America, than these from WTD from upstate New York. Therefore, WTD is host for at least two Onchocerca species. Moreover, another study screening host-seeking, female blackflies (Simulium tescorum, and Simulium vittatum s.l.) from Los Angeles County, southern California, found DNA belonging to Onchocerca sp. 1. Interestingly, only mule deer is endemic to this area, suggesting that this cryptic species has a vast geographic distribution, and at least two cervid hosts. Phylogenetic analysis support at least two separate events of invasion of North America from Eurasia, and likely colonization of other ungulates with or without speciation. Onchocerca cervipedis is a species complex of at least three species associated North American ungulates. Together, these findings highlight the need for a reassessment of the Onchocerca biodiversity in the continent, and for elucidating its complex biogeography by means of geographically extensive sampling of nematode specimens, host tissues, and vectors; and integrated classical and molecular approaches.

The use of morphometrics and genotyping to describe Eimeria species in Alectoris chukar

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Coccidian parasites of the genus *Eimeria* are commercially important pathogens in the poultry industry that can result in substantial losses for a producer if not controlled. While commonly researched in chickens and turkeys, information regarding coccidiosis in commercially raised gamebirds such as partridge is limited. Modern descriptions of *Eimeria* spp. require identifying key morphological characteristics of the oocysts and endogenous stages as well as the genotype of the parasite. Inoculating naïve birds with oocysts is useful in determining the pathogenesis and life history of the parasite. Total collection of fecal material and determination of oocysts per gram (OPG) is useful for estimating the parasite burden within the birds. Documenting the morphometrics of the oocysts and sporocysts is supplemented with observations on the number, morphological features and location(s) of endogenous stages within the infected host to provide a comprehensive description for an *Eimeria* species. Genotyping provides a useful adjunct to the morphological features included in a species description that can help to define the morphological variation exhibited by a single parasite species. The mitochondrial genome of *Eimeria* species has been found to be an ideal genetic target for species-level sequence-based genotyping.

Recurrent coccidiosis at a commercial chukar partridge farm in southern Ontario was investigated. Fecal samples were collected from the facility and oocysts were isolated from feces for morphometric characterization. DNA isolated from the same isolate of oocysts was used for sequencing of the complete mitochondrial genome and 18S nuclear ribosomal RNA gene (rDNA). Only single sequences were found at each genetic locus suggesting the presence of only one genotype; the uniformity of oocyst morphometrics supported that only a single *Eimeria* species was present.

To further characterize the *Eimeria* species responsible for the coccidiosis in this commercial flock, twenty-one day old birds were inoculated in increasing doses every day for the period of a week to document the endogenous development of this parasite. To determine the pre-patent and patent periods of the causative *Eimeria* species, feces were collected every eight hours for total oocyst output. In addition to quantifying the oocysts shed, the morphometrics of the shed exogenous stages were determined. Birds were necropsied every 12 hours over the duration of the endogenous development to provide distribution and morphometrics of endogenous stages in the birds. Generation of such baseline biological and genetic data for pathogenic *Eimeria* species may help partridge producers maximize animal health through the control of coccidiosis.

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**Ticks and Tick-Borne Infections in Some Livestock Slaughtered at Gwagwalada Abattoir, in The Federal Capital Territory, Abuja, Nigeria.**

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Generally, the parasites transmitted by ticks constitute a major health problem in livestock and the livestock industry. The lean information on the ticks and their borne infection in Abuja necessitated the survey to provide a checklist of ticks found in the study area. A survey of ticks and tick-borne infections was carried out between June and November 2010. Of the two hundred (200) animals were sampled. Only the cattle were infected with Babesia marginale (4.00%), Anaplasma bigemina (2.00%) and Anaplasma central (2.00%) but none of the in sheep and goats were infected with any of the parasite. Among the ticks collected were Amblyomma variegatum (38:45.78%) with higher occurrence, while others include; Hyalomma truncatum (20:24.10%), Boophilus decoloratus (16:19.28%) Repiephallus spp (8:9.64%) and Ornithodorus spp (1:1.26%) being the least. The relationship between infection rate and sexes of cattle showed a significant difference (P<0.05) while there was no significant difference (P>0.05) in relation to age of the cattle using Chi-Square. The current study was able to provide a baseline information on prevalence of ticks and their borne infections. This therefore poses great consequences to livestock in Gwagwalada, FCT Abuja, Nigeria.

Keywords: Ticks, Tick-borne Infections, Livestock, Slaughter, Gwagwalada-Abattoir.
Trophic ecology and parasitism of a deep-pelagic fish assemblage

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Mesopelagic (open ocean, 200-1000 m daytime depth) fishes are important consumers of zooplankton and are prey of oceanic predators. Some mesopelagic fishes (e.g. myctophids and stomiids) undertake a diel vertical migration where they ascend to the near-surface waters during the night to feed and descend into the depths during the day to avoid predators. Other mesopelagic fishes (e.g. Sternoptyx spp.) remain at depth throughout the day. While in the epipelagic zone (surface ~ 200 m depth), vertically-migrating fishes become prey to upper-trophic level predators, such as tunas and billfishes. Benthic fishes (e.g. macrourids) often vertically-migrate as well, ascending into the pelagic zone to feed on holoplanktonic organisms. Although fishes of different depths experience different food webs, vertical migration patterns likely lead to overlaps, linking surface, deep-pelagic, and benthic food webs. The study of gut contents and parasites can yield insights into ecological processes occurring within these assemblages. We examined the gut contents and parasite communities of 26 mesopelagic fish species in the Gulf of Mexico. Based on gut content analyses, we identified six different feeding guilds within this assemblage: copepodivores, copepods and other zooplankton, copepods and euphausiids, gelatinivores, generalists, shrimpivores, and upper-trophic level predators. Although parasitism rates were generally low, deep-pelagic fishes hosted a diverse assemblage of parasites, including larval and adult digeneans, larval cestodes, and nematodes. Parasite community differed significantly among host feeding guilds. For example, generalists feeding on more diverse prey items (e.g. Sternoptyx spp.) had more diverse parasite communities, whereas larger upper-level predators feeding on squid and fish (e.g. Nealtotus tripe) hosted abundant infections of single taxa, typically tetraphyllidean larvae. These data are being used to develop and refine models aimed at understanding ecosystem structure and resilience to large-scale disturbances.

Trypanorhynchs from batoids of Mozambique.

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Trypanorhynchus is the most speciose of the 9 elasmobranch-hosted cestode orders comprising 315 valid species. Formidable hooked tentacles and notoriously low host-specificity set species in this order apart from other elasmobranch-hosted metazoa. While reports of trypanorhynchines from both bony fishes (as larvae) and elasmbranchs (as adults) are commonplace, parts of the world remain from which trypanorhynchines—particularly as adults—are understudied. Mozambique is one such place. Larval trypanorhynchines have been reported from species of bony fish and penaeid shrimp from Mozambique, but only 3 reports of adults exist, each from a single species of wedgefish, skate, and gulper shark. For this study, specimens of 6 species of batoids (4 species of stingrays, and a species each of wedgefish and cownose ray) collected from Mozambique in 2016 were examined; 5 of these batoid species have not previously been reported as hosts for trypanorhynchines. Trypanorhynchines were examined morphologically using light and scanning electron microscopy. Species representing 7 trypanorhynch genera in 4 families were identified, including 1 of the 3 species previously reported from elasmbranchs from Mozambique. Morphological data suggest that most of these species are described species previously reported from other host species and other localities, but representing new host and locality records. At least one species from a cownose ray, however, appears to be novel. This species was identified as a member of the parapyletic “Rhinopericonidae” Carvajal & Campbell, 1975 based on its possession of four bothria, solid hooks, and heteroacanthous typical armature, and its lack of gland cells within the bulbs. It is most similar to Rhinopericonidae
megacantha Carvajal & Campbell, 1975 (the only member of its genus) but differs notably from that species in, for example, proglottid number and hook shape and size. Molecular sequence data would provide valuable insight to help inform the placement of this potentially novel taxon, as well as to confirm identifications of previously described species.

Turkey coccidiosis in the Great White North: Diverse Eimeria spp. lacking immunological cross-protection found on Canadian turkey farms.

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Despite its importance to commercial production, the diversity of Eimeria spp. infecting Canadian turkeys hasn’t been studied for decades. To begin addressing this paucity of data, fecal samples from commercial farms were screened for coccidia using standard fecal flotations; parasite diversity was determined using a nested-PCR assay targeting mtCOI that reliably differentiates species (i.e. determining national coccidial biodiversity in the field samples). Forty samples from 27 farms were obtained (20 farms from Ontario, 2 from Saskatchewan, 3 from British Columbia, one from Alberta and one from Nova Scotia). Thirty-four of the fecal samples were coccidia-positive and 6 Eimeria spp. were found in various combinations in the samples (>1 species in most samples). To evaluate the potential immunological cross-protection of the 6 Eimeria spp. of turkeys, an in vivo cross-immunity trial was performed. High dose (virulent) challenge infections were used to assess protection, if any, against macroscopic lesion development and reduced body weight gain (BWG). Using a lower challenge dose (100 oocysts/bird), total oocyst output over a 7-day shedding period was used to assess immunological impact on parasite fecundity. High and low dose homologous challenge infections demonstrated a high degree of protection with respect to lesion scores and BWG (not different from sham-challenged poult), and parasite fecundity (95.4-100% reduction in oocyst output). In contrast, results from the heterologous challenge infections provide no support for immunological cross-protection among the 6 Eimeria spp. with regards to lesion development and parasite fecundity. Eimeria spp. were found to be diverse and plentiful in commercial turkey flocks and no practical immunological cross-protection detected among these parasites. This work has highlighted that coccidia pose a continued threat to the Canadian turkey industry; the generated data herein may be important for both producers and live coccidiosis vaccine manufacturers to optimize management of coccidiosis in commercial turkey operations.

Using an iterative process in the development of novel therapeutics to treat human schistosomiasis

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Human schistosomiasis is a debilitating, life-threatening disease affecting more than 250 million people in as many as 78 countries. Currently, schistosomiasis is labeled a neglected tropical disease and is second only to malaria as "the most devastating parasitic disease". There is only one drug of choice effective against all three major species of *Schistosoma*, Praziquantel. However, as with many monotherapies, resistance is emerging in the field and can be selected for in the laboratory. Previously used therapies include Hycanthone and Oxamnique, however shortcomings such as carcinogenicity and affordability, respectively, resulted in their discontinuation. Our collaborations with medicinal chemists and structural biologists have enabled us to develop and test novel drug derivatives to treat this disease. Using an iterative process for drug development, we have successfully identified one derivative that is effective against all three species of the parasite at varying levels and shows promising preliminary *in vivo* efficacy, CIDD-0066790. As CIDD-0066790 is a racemic mixture, we have isolated the R and S enantiomers of this derivative and identified the more effective form. Furthermore, we have identified three more successful derivatives that are highly effective against *S. mansoni*. Our goal is to generate a secondary therapeutic that can be used in conjunction with Praziquantel to overcome the ever-growing threat of resistance. The ability and need to design, screen, and develop future, affordable therapeutics to treat human schistosomiasis imperative, and can greatly benefit the lives of those in disease ridden areas.

Using metabolic networks to probe the symbiosis between *Wolbachia* and filarial nematodes, and identify novel drug targets

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Filarial nematodes represent one of the leading causes of disability in the developing world. Many filarial worm species, including *Brugia malayi*, one of the causative agents of lymphatic filariasis, have an obligate endosymbiotic relationship with the alpha-proteobacteria *Wolbachia*. To better understand the molecular interplay between these two organisms, we profiled the transcriptomes of *B. malayi* and *Wolbachia* across the life cycle of the parasite using dual RNA-seq. With these data, we built a co-expression network for the two organisms using weighted gene correlation network analysis. This allowed us to pinpoint functional pathways involved in this essential symbiotic relationship provided by the co-expression of nematode and bacterial genes. For example, during female worm development we find that *Wolbachia* upregulate genes involved in ATP production and purine biosynthesis, as well as genes involved in the oxidative stress response. We have also identified co-expressed pathways required for molting of the worm from L3 to L4, the molt which marks the establishment of infection in the human host. In parallel efforts, we are using these data to characterize the endosymbiotic relationship at the metabolic level using Flux Balance Analysis, identifying choke points that could be exploited for therapy. By creating a draft metabolic network for *B. malayi* and *Wolbachia*, and using *in silico* knockouts, we have determined the necessary pathways for growth and virulence and examined how these pathways are influenced by the presence of *Wolbachia*. We are now testing these predictions using RNAi and small molecule inhibitors in order to validate novel drug targets.

White blood cell differentials of amphibians naturally infected with multiple trypanosome morphotypes
Disease is one of the leading factors contributing to the global decline of amphibians. While diseases such as chytrid fungus and ranavirus have been implicated in amphibian decline, considerably less is known about how blood protozoa, such as trypanosomes, affect amphibian populations. Despite the common occurrence of trypanosome infection in amphibian populations, we still know relatively little about how amphibians respond to trypanosome infection. In this study, we characterize differential white blood cell counts—the relative proportion of five different immune cell types in the blood—in two anuran species (*Rana catesbeiana*, *R. sphenocephala*) with and without trypanosomes. Frogs were wild caught and naturally infected with one to four distinct trypanosome morphotypes, differing in size, presence of a free flagellum, and cell motility. Trypanosome species can have multiple morphological stages in their life cycles, however the species associations of amphibian trypanosome morphotypes are not known. Coinfection with different trypanosome species or different morphotypes within the same species could have differential effects on the relative abundance of white blood cells. We hypothesize that amphibians infected with trypanosomes will have altered white blood cell counts when compared with uninfected individuals and that white blood cell profiles will differ depending on the trypanosome morphotypes present. This study will help characterize one of the immunological responses of anuran amphibians to trypanosome infection and provide insight into how blood protozoa interact with their amphibian hosts at an immunological level.

Worth their weight in gold: estimators of biomass for small parasites

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Parasite biomass is considered as a determinant trait enhancing changes in wild communities. It can be measured directly, mainly by weighing individuals, but most often indirect estimators are required. We developed two indirect non-destructive methods to estimate parasite biomass in small parasite specimens; one based on clay modelling and another based on image analysis, implemented with open-source software. Furthermore, we tested the accuracy of the two new methods and the widely used geometric approximation method by using direct measurement as benchmark. We applied these three different methods (the modelling method, the image analysis method and the geometric approximation method) to three morphologically disparate parasite model species: a flatworm, an acanthocephalan and a crustacean, whose size allowed direct estimation of their biomass. To validate our indirect estimations and to test their accuracy, we weighed specimens of the three species and calculated their tissue densities. Finally, we propose an easy technique to estimate thickness of individuals under a microscope, a required measured for two of the three indirect methods tested. The indirect methods proposed in this paper provided the best approximation to direct measurements. Despite its wide use, the geometric approximation method showed the lowest accuracy. Our indirect methods to estimate biomass from images (i) provide much more accurate results than approximating body morphology to regular geometric forms; (ii) can be applied to both fresh and permanently mounted material; (iii) are non-destructive; (iv) are time- and cost-effective. This approach is timely due to the recently increasing number of projects requiring reliable parasite biomass estimates to explain overlooked processes in ecosystems.
¿CAN PARASITES ALTER TROPHIC INTERACTIONS OF SHARKS? EVALUATING THE HOST-PARASITE SYSTEM OF Rhizoprionodon terraenovae USING δ13C AND δ15N.

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The decrease in the abundances of a species has great repercussions on the stability of an ecosystem. Due to the constant decrease of elasmobranch populations, the potential ecological impact will be alerted, since some species are classified as health indicators of an ecosystem. In the coast of Campeche, Rhizoprionodon terraenovae is considered as a key species in the balance within the community, however, like other shark species there has been a notable decline in its abundance and it is not yet clear whether this downward trend is due to fishing or ecological changes. Some ecological causes that have been documented in the decline of a species, is the increase in the diversity of parasites. It has been documented that parasitism has a great influence on the structure and function of an ecosystem and its increase is related to habitat alteration because of anthropic effects (e.g., water pollution). Therefore, 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 host-parasite system and the alteration of trophic interactions of a species is with the isotopic composition (specifically δ15N and δ13C) of tissues with different turnover rates (e.g., muscle, blood). Analyzing δ15N and δ13C of muscle and blood enables one to elucidate trophic structures and infer energy and matter flows in food webs. δ15N is used to estimate resource use, while δ13C reflects the relative contribution of different food sources or habitats to the diet. Despite the relevance, few studies have been conducted on the parasitic fauna of elasmobranchs, and none have been conducted on the host-parasite system in R. terraenovae on the Campeche coasts. In this context, the aim of this study was to assess the relation of the host-parasite system in R. terraenovae based on the isotopic composition (δ13C and δ15N) of two tissues (muscle and blood, reflecting about months and days of information, respectively). The identification of possible changes in resource and habitat use (δ13C and δ15N) by both sexes during ontogenetic development related with parasitism may lead to the generation of baselines to understand the energy flow between a predator of higher trophic levels with respect to its endoparasites fauna, aspect named as a key by several authors worldwide and step to follow in the field of research of trophic networks and interactions, for the assessment 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 blood. Specimens of R. terraenovae were collected from 2015 to 2017. Of the 146 sharks sampled, there were 91 males and 55 females. The recovered parasites were preserved for further staining (Carmin Meyer’s-Schuberg’s) or rinsed (Glycerin at different concentrations) (depending on the class) to be identified at the lowest possible taxon. The intervals of infection (prevalence and mean intensity) were calculated. For the analysis of stable isotopes, muscles were treated with deionized water to eliminate the urea and a mathematical correction was applied for the values of the blood, to eliminate the influence of the lipids, then, the isotopic composition of the tissues (muscle and blood, which was divided into serum and plasma) and parasites, collected from R. terraenovae, was analyzed by means of an Isotope-ratio mass spectrometry. To know if there is a correlation between the possible changes in niche width and trophic overlap, the SIBER method was used in the SIAR package of the R program. Of the total sample (n = 146), 56 individuals were parasitized (13 for CDC and 43 for SFC); with the presence of nematodes (Anisakis spp.), acanthocephala (Gorgorhynchus spp.) and cestodes (Dasyrhyynchus spp. and Phoreiotobothrium spp.). The highest prevalence and mean intensity were for cestodes in SFC (52.9% and 23.6, respectively). The presence of acanthocephala was only recorded...
in SFC. The isotopic composition of the parasites (δ15N 10.43 and δ13C -18.54) was similar with the serum (δ15N 11.31 and δ13C -18.82). This shows us that the parasites are feeding on the host’s blood serum and this would be giving us results of a spare hour rate, and it reflects a slight isotopic enrichment, but it is only visible on small time scales, however, no differences were found in the isotopic comparison between sexes and regions (SFC and CDC). This results are relevant since it is the first records of parasites in this species of elasmobranch in this region, moreover, show us how parasites feed into the organism (mainly serum), which doesn’t affect in the trophic interactions of R. terraenovae, thus, the decline in the abundance of this shark species could be related with others ecological aspects but no with parasitism.

“A native entomopathogenic nematode from Yucatan, Mexico as an alternative for biological control of mosquito Aedes aegypti larvae”

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Mosquito-borne diseases such as dengue, chikungunya and zika are of enormous public health concern worldwide. Dengue is the most geographically wide-spread disease in the world, affecting from 50 to 100 million people annually. Its principal vector is Aedes aegypti, a mosquito distributed in tropical and subtropical regions. Over the last century, A. aegypti has expanded its geographical distribution to regions where it was not present before, becoming also insecticide-resistant. One alternative for combating vectors such as A. aegypti is biological control with entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae and their endosymbiotic bacteria of the genera Xenorhabdus and Photorhabdus. These nematodes parasitize mosquito larvae reaching the hemocele, where their bacteria release toxins causing septicemia in 24 to 48 hours. The objectives of this paper was to determine the infectivity of a native nematode of the genus Heterorhabditis recovered from citrus orchard in Yucatan, to A. aegypti larvae. The infectivity of the nematodes was tested exposing mosquitoes larvae to nematodes in 1-L plastic jars: 20 jars for the experimental treatment and 20 for the controls without nematodes. Four concentrations of nematodes were used: 1,260:1 nematodes / mosquito larvae (n/lv); 2,520:1 n/lv; 3,780:1 n/lv and 5,040:1 n/lv. Water environmental variables (temperature, pH, oxygen concentration and conductivity) were not controlled during the experiment. A multivariate analysis of variance (MANOVA) was carried out to determine possible significant differences between treatments and controls, considering the interaction with environmental variables. All treatments were significantly different from control (P < 0.05). The best treatment reached 60 % cumulative mortality at 1,260 n/lv, suggesting that Heterorhabditis sp. and its endosymbiotic bacterium could be used as a bioinsecticide alternative against A. aegypti larvae. However, further tests should be undertaken to determine the identity and infectivity of the endosymbiotic bacterium.