The 88th Annual Meeting of the American Society of Parasitologists and the 13th Annual Québec Molecular Parasitology Meeting

Loews Le Concorde Hôtel
Québec City, Canada, June 26-29, 2013

Program & Abstracts

Thanks to Everyone Who Helped Make this Meeting Possible …

The American Society of Parasitologists gratefully acknowledges the following for their support, sponsorship, and hard work in putting together this year's annual meeting.

Local Arrangements Committee

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The American Society of Parasitologists
ESTABLISHED 1924

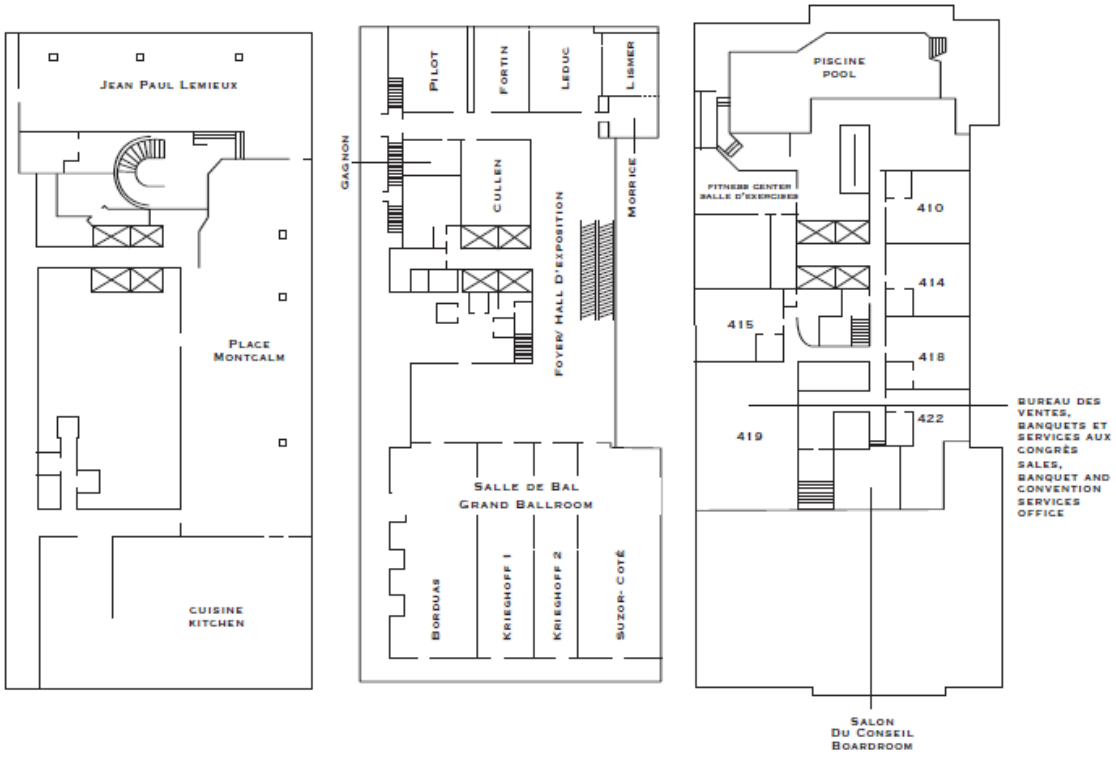
Welcome

We would like to welcome you to the 88th annual meeting of the American Society of Parasitologists (ASP).

The ASP is a diverse group of over 1500 scientists from industry, government, and academia who are interested in the study and teaching of parasitology. Founded in 1924, ASP members have contributed not only to the development of parasitology as a discipline, but also to primary research in systematics, medicine, molecular biology, immunology, physiology, ecology, biochemistry, behavior, and more.

Herman Eure and Kelli Sapp, Scientific Program Officers

Floor Plan, Loews Le Concorde Hôtel



ÉTAGE INFÉRIEUR
LOWER LEVEL

TROISIÈME ÉTAGE
THIRD FLOOR

QUATRIÈME ÉTAGE
FOURTH FLOOR

Day/Times	Activity/Function	Room/Space
June 26 (Wednesday)		
8:00 a.m.-Noon	ASP Council	Pilot
1:00-5:00 p.m.	Ecology I	Suzor Coté
1:00-4:30 p.m.	Genetics, Molecular, Vector Biology	Krieghoff
1:30-4:30 p.m.	Host Parasite Interactions I	Borduas
2:45-3:15 p.m.	Coffee Break	Foyer
7:00-10:00 p.m.	Welcome Reception	Suzor Coté/Krieghoff
June 27 (Thursday)		
7:00-8:30 a.m.	Editorial Board Breakfast	Pilot
8:30-10:30 a.m.	ASP President's Symposium	Borduas/Krieghoff I
10:30-11:00 a.m.	Coffee Break	Foyer
11:00-Noon	Stoll Stunkard Lecture	Borduas/Krieghoff I
1:00-3:00 p.m.	Ecology II	Suzor Coté
1:00-3:00 p.m.	Taxonomy, Systematics, Phylogeny I	Leduc/Fortin
1:00-2:30 p.m.	Biochemistry/Physiology, Chemotherapy and Drug Resistance, Immunology	Borduas
1:00-3:00 p.m.	Life Cycles & Epidemiology I	Krieghoff
3:00-3:30 p.m.	Coffee Break	Foyer
3:30-5:30 p.m.	ASP Students' Symposium	Leduc/Fortin
5:30-6:30 p.m.	ASP Student Social	Pilot
3:00-6:00 p.m.	Auction Set Up	Place Montcalm
6:00-7:00 p.m.	Auction Preview	Place Montcalm
7:00-9:00 p.m.	23rd Annual ASP Student Auction	Place Montcalm
June 28 (Friday)		
8:00-10:00 a.m.	Associate Editors Symposium	Suzor Coté
9:00 -11:45 a.m.	45th Coccidiosis Conference	Leduc/Fortin
9:00-11:30 a.m.	Life Cycles & Epidemiology II	Krieghoff
9:00-10:00 a.m.	QMP Keynote Speaker	Borduas
10:30-12:30 p.m.	QMP:ASP Host Parasite Interactions II	Borduas
10:00-10:30 a.m.	Coffee Break	Foyer
1:00-2:00 p.m.	ASP President's Address	Suzor Coté
2:15-5:45 p.m.	Ecology III	Suzor Coté
2:15-5:45 p.m.	Taxonomy, Systematics, Phylogeny II	Leduc/Fortin
2:15-5:00 p.m.	Host Parasite Interactions III	Krieghoff
1:45-3:00 p.m.	QMP: Host Parasite Interactions and Immunology	Borduas
3:30-5:30 p.m.	QMP:ASP Biochemistry, Molecular Biology and Genetics I	Borduas
3:00-4:00 p.m.	Coffee Break	Foyer
4:00-5:30 p.m.	Authors may set up posters	Place Montcalm
7:00-10:30 p.m.	Hall of the Musée de la Civilisation de Québec	

June 29 (Saturday)

8:00-10:45 a.m.	Ecology IV	Suzor Coté
8:00-10:45 a.m.	Taxonomy, Systematics, Phylogeny III	Leduc/Fortin
8:00-9:00 a.m.	QMP Keynote Speaker	Krieghoff
9:00-10:15 a.m.	QMP:ASP Biochemistry, Molecular Biology, and Genetics II	Krieghoff
9:15-9:30 a.m.	ASP Coffee Break	Foyer
10:30-11:00 a.m.	QMP Coffee Break	Foyer
8:30-10:30 a.m.	Authors complete poster set up	Place Montcalm
11:00-1:00 p.m.	Poster Session, coffee, snacks	Place Montcalm
1:00-2:00 p.m.	R. Barclay McGhee Lecture	Suzor Coté
2:00-3:00 p.m.	H.B. Ward Lecture	Suzor Coté
2:00-3:45 p.m.	QMP: Chemotherapy, Drug Resistance and Drug Targets	Krieghoff
3:30-4:30 p.m.	ASP Awards and Business Meeting	Suzor Coté
3:45-4:30 p.m.	QMP Closing Remarks and Awards	Krieghoff

Wednesday Morning, 2013-06-26

08:00 am – Noon *ASP Council Meeting, Pilot*

Presiding: E.S. Loker, University of New Mexico

Wednesday Afternoon, 2013-06-26

1:00-5:00 pm Ecology I

Location: Suzor Coté

Presiding: J. Camp, Purdue University
N. Smith, Eckerd College

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (1)† **E.M. Warburton** and M. Vonhof. RELATIVE ROLES OF HOST EXPOSURE AND PARASITE ESTABLISHMENT IN DETERMINING HELMINTH BURDENS OF *EPTESICUS FUSCUS* (MAMMALIA: CHIROPTERA).

1:15 (2)† **M.R. Zimmermann**, K.E. Luth and G.W. Esch. DIFFERENTIAL INFECTION PATTERNS OF *ECHINOSTOMA* SPP. LARVAL STAGES IN SNAILS FROM THREE DIFFERENT FAMILIES.

1:30 (3) **E.S. Durkin.** EFFECTS OF SEX AND AGE ON A COMPONENT COMMUNITY OF CHEWING LICE (PHTHIRAPTERA) OF BROWN-HEADED COWBIRDS (*MOLOTHRUS ATER*).

1:45 (4)† **E.F. Bauer** and C.M. Whipps. CASCADE OF ENEMY RELEASE: IMPACTS OF AN INVASIVE SPECIES (*NEOGOBIUS MELANOSTOMUS*) ON THE PARASITE COMMUNITY OF A NATIVE PREDATOR (*MICROPTERUS DOLOMIEU*).

2:00 (5) **A. Sellers**, M. Torchin, G. Ruiz and B. Leung. LATITUDINAL GRADIENTS IN PARASITISM IN THE INVASIVE LIONFISH.

2:15 (6) **J. Wojdak**, R. Edman, J. Wyderko, S. Zemmer and L. Belden. EXPLORING THE DILUTION EFFECT: HOST DENSITY MEDIATES HOST DIVERSITY EFFECTS ON COMMUNITY-WIDE PARASITE INFECTION.

2:30 (7)† **E.C. Ogburn**, K.E. Limburg and C.M. Whipps. FISH PARASITES IN THE HUDSON RIVER ESTUARY'S LITTORAL HABITATS: A PRELUDE TO RESTORATION.

2:45-3:15 pm **COFFEE BREAK**

3:15 (8)† **N. Ortega**. THE EFFECT OF PRESCRIBED BURNS ON PARASITE FAUNA IN CUBAN TREEFROGS.

3:30 (9)† **J.S. Hernandez-Orts**, F.J. Aznar, I. Blasco-Costa, G. Alama-Bermejo, E.A. Crespo, J.A. Raga and F.E. Montero. DESCRIPTION, MICROHABITAT SELECTION AND INFECTION PATTERNS OF SEALWORM LARVAE (*PSEUDOTERRANOVA DECIPIENS* SPECIES COMPLEX, NEMATODA: ASCARIDOIDEA) IN FISH FROM PATAGONIA, ARGENTINA.

3:45 (10) **R. Bernot**, M. Bernot, L. Caffo, C. Crismore, D. Elias, P. Flores, J. Justice, J.H. Lee, H. Madinger, and R. Osborne. PARASITES AS INDICATORS OF DRUG CONTAMINATION: RELATIONSHIPS AMONG PARASITES, GASTROPODS, AND PHARMACEUTICALS IN MIDWESTERN STREAMS.

4:00 (11)† **S. Staicer**, T. Cook, and A. Smith-Herron. SPATIAL AND TEMPORAL PATTERNS OF GREGARINE INFECTIONS IN DAMSELFLIES IN FOUR TEXAS ECOREGIONS.

4:15 (12)† **K. Luth**, M.R. Zimmermann and G.W. Esch. A SUCCESSFUL WORM INDEED: INFECTION DYNAMICS OF A UBIQUITOUSLY DISTRIBUTED TAPEWORM, *PROTEOCEPHALUS AMBLOPLITIS* (EUCESTODA: PROTEOCEPHALIDEA).

4:30 (13) **R.B. Gagne** and M.J. Blum. DECREASED PRECIPITATION ALONG A NATURAL RAINFALL GRADIENT IS ASSOCIATED WITH INCREASED ABUNDANCE OF AN INTRODUCED NEMATODE PARASITE INFECTING ENDEMIC HAWAIIAN STREAM FISHES.

4:45 (14) **D. Pech**, V. Vidal-Martínez and L. Aguirre-Macedo. LONG TERM SPECIES CO-INFECTION PATTERNS OF HELMINTHS INFECTING A FRESHWATER TROPICAL FISH.

1:00-4:30 pm Genetics, Molecular Biology, Vector Biology

Location: Krieghoff

Presiding: T. Geary, McGill University
J. Porter-Kelley, Winston-Salem State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (15) **J. Humphries**, B. Harter, H. Jost, E. Ohlrogge and E. Weinlander. GENE REGULATION OF IMMUNE RESPONSES IN *BIOMPHALARIA GLABRATA*.

1:15 (16) **J.F. Hillyer**, T.Y. Estevez-Lao, S.N. Gomez and L.T. Sigle. EFFECT OF INFECTION ON MOSQUITO HEART PHYSIOLOGY.

1:30 (17) **F.O. Akinbo**, C. Okaka, R. Omoregie, H. Adamu and L. Xiao. UNUSUAL *ENTEROCYTOZOON BIENEUSI* GENOTYPES AND *CRYPTOSPORIDIUM HOMINIS* SUBTYPES IN HIV-INFECTED PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY.

1:45 (18) **J.D. Stoltzfus**, S.M. Bart and J.B. Lok. *STRONGYLOIDES STERCORALIS* INSULIN-LIKE PEPTIDES REGULATE INFECTIVE THIRD-STAGE LARVAE.

2:00 (19) **M. Koinari**, S. Karl, J. Ng, A.J. Lymbery and U. Ryan. NOVEL AND ZOONOTIC *CRYPTOSPORIDIUM* SPECIES/GENOTYPES IDENTIFIED IN FISH AND GOATS PAPUA NEW GUINEA.

2:15 (20)† **M. Klodnicki**. *HISTOMONAS MELEAGRIDIS*: RNASEQ ANALYSES AND TARGETED DRUG SENSITIVITY.

2:30 (21) **D. Bellamy**, K. Abernathy, D. Nichols, O. Seshie and J.M. Porter-Kelley. CHARACTERIZATION OF THE PUTATIVE ENDOCHITINASE IN *LEISHMANIA BRAZILIENSIS*.

2:45-3:15 pm **COFFEE BREAK**

3:15 (22) **V. Barrere**, K. Keller, G. von Samson-Himmelstjerna and R. Prichard. EFFICIENCY OF A GENETIC TEST TO DETECT BENZIMIDAZOLE RESISTANT *HAEMONCHUS CONTORTUS* NEMATODES IN SHEEP FARMS IN QUEBEC.

3:30 (24) **G.T. Souza**, **R**.J. da Graça, L.S. Gasques, S.M. Prioli, A.J. Prioli and R.M. Takemoto. SEQUENCING OF METACERCARIAE OF *CLINOSTOMUM* SP. (DIGENEA: PLATYHELMINTHES) IN A NEOTROPICAL FLOODPLAIN IN BRAZIL: ITS AND COI REGIONS.

3:45 (25) **L.E. Camp**. POPULATION GENETIC ANALYSIS OF *BAYLISASCARIS PROCYONIS* USING MICROSATELLITE MARKERS.

4:00 (26)† **M.E. Ogedengbe**, A. Leveille, M.A. Hafeez and J.R. Barta. SMALL IS NOT BORING: THE TINY, BUT VARIABLE, MITOCHONDRIAL GENOMES OF COCCIDIA, PIROPLASMS, HAEMOGREGARINES AND HAEMOSPORINIDS (APICOMPLEXA).

4:15 (27) **K.M. Pagenkopp Lohan**, R.C. Fleischer, K. Holzer, K.J. Carney and G.M. Ruiz. PROTISTAN DIVERSITY IN BALLAST WATER REVEALED BY AMPLICON-BASED 454 PYROSEQUENCING.

1:30-4:30 pm Host Parasite Interactions I

Location: Borduas

Presiding: C. Bayne, Oregon State University
S. Hallett, Oregon State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:30 (28) **E.B. Holt**, J.R. Palmieri and S.F. Elswaifi. UNUSUAL PRESENTATION OF CHRONIC GIARDIASIS REFRACTORY TO INITIAL TREATMENT.

1:45 (29) **J.J. Daly**. A COMPARISON OF POPULATION PARAMETERS OF *CLINOSTOMUM MARGINATUM* FROM SMALLMOUTH BASS TAKEN FROM DIFFERENT UPLAND STREAMS IN ARKANSAS.

2:00 (30) **A. Rodríguez González**, J.A. Balbuena Díaz-Pinés and C. Llopis Belenguer. PHENOTYPIC PLASTICITY IN HAPTORAL STRUCTURES OF *LIGOPHORUS CEPHALI* (MONOGENEA: DACTYLOGYRIDAE) ON THE GILLS OF *MUGIL CEPHALUS* (TELEOSTEI: MUGILIDAE): A GEOMETRIC MORPHOMETRIC APPROACH.

2:15 (31)† **S.K. Buddenborg**, M. Misra, M.A. Gordy, I.E. Lindquist, E.L. Agola, G.M. Mkoji and E.S. Loker. 454 SEQUENCING STUDIES OF FIELD-DERIVED UNINFECTED AND *SCHISTOSOMA MANSONI*-INFECTED *BIOMPHALARIA PFEIFFERI* FROM ASAO STREAM, WEST KENYA.

2:30 (32)† **E. Walther**. DISEASE DYNAMICS OF AVIAN HAEMOSPORIDIA IN A CALIFORNIA SONGBIRD COMMUNITY.

2:45-3:15 pm **COFFEE BREAK**

3:15 (33) **A. Ardila-Garcia** and N. Fast. MICROSPORIDIAN INFECTION DURING THE DEVELOPMENT OF A FREE-LIVING MARINE NEMATODE.

3:30 (34)† **K.L. Sheehan**, R.J. Johnson and G.K. Yarrow. PARASITE ASSEMBLAGES AS USEFUL INDICATORS OF HOST MOVEMENT.

3:45 (35)† **A.E. Garcia-Vedrenne**, A.M. Kuris and R.F. Hechinger. IN VITRO SYSTEMS FOR INTRAMOLLUSCAN STAGES OF TREMATODES- WILL THE TRICK USED FOR FRESHWATER SYSTEMS ALSO WORK FOR MARINE ONES?

4:00 (36)† **D. Keeney**, S. Lindley and P. Yurco. GENETIC STRUCTURE OF TWO INTERMEDIATE SNAIL HOSTS THROUGHOUT NEW YORK WITH COMMENTS ON THEIR SYMBIONTS.

4:15 (37) **F.D. Chibwana** and G. Nkwengulila. THE NERVOUS SYSTEM OF DIPLOSTOMID METACERCARIAE (DIGENEA) INFECTING THE CATFISH *CLARIAS GARIEPINUS* (CLARIIDAE) IN FRESHWATERS OF TANZANIA.

Wednesday Evening, 2013-06-26

07:00 - 10:00 PM WELCOME RECEPTION – Suzor Coté/Krieghoff

Thursday Morning, 2013-06-27

7:00 – 8:30 am Editorial Board Breakfast

Location: Pilot

8:30-10:30 am ASP President's Symposium

Location: Borduas/Krieghoff I

Presiding: D.S. Lindsay, Virginia Tech

Theme: "The Biology of Parasite Control"

8:30 INTRODUCTION.

8:40 (38) **D.G. Colley**. SCHISTOSOMIASIS: WHAT INNING ARE WE IN - AND WHAT'S THE SCORE?

9:10 (39) **J.P. Webster**. THE BIOLOGICAL IMPACTS OF CONTROL PROGRAMS: DO SCHISTOSOMES ADAPT AND EVOLVE TO A CHANGING WORLD?

9:40 (40) **J.M. Hawdon**. CONTROLLING SOIL TRANSMITTED HELMINTHS: TIME TO THINK INSIDE THE BOX?

10:10-10:30 Questions, Closing Remarks.

10:30-11:00 am **COFFEE BREAK**

11:00 am-NOON Stoll Stunkard Lecture

Location: Borduas/Krieghoff I

Carl Nathan
Stoll Stunkard Lecture
2013

Presiding: M. Siddall, American Museum of Natural History

11:00 Introduction of 2013 Stoll Stunkard Lecturer
C.F. Nathan, Weill Cornell Medical College

11:10 (41) **C.F. Nathan**, "Drug Discovery for Neglected Diseases: Challenges for Science and Society."

Thursday Afternoon, 2013-06-27

1:00-3:00 pm Ecology II

Location: Suzor Coté

Presiding: R. Bernot, Ball State University
R. Sehgal, San Francisco State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (42) **B. Belgrad** and N.F. Smith. EFFECTS OF PREDATION AND PARASITISM ON CLIMBING BEHAVIOR ON THE MARINE SNAIL, *CERITHIDEA SCALARIFORMIS*.

1:15 (43)† **J.A. Mischler**, A. Townsend, P. Walker, P. Johnson and V. McKenzie. EFFECTS OF METACERCARIAL INFECTION ON BIOGEOCHEMICAL CYCLING: *POSTHODIPLOSTIMUM MINIMUM* AND *COTYLURUS FLABELLIFORMIS*.

1:30 (44) **C. Blanar** and D.J. Marcogliese. PLAYING WITH PRINKLES: FACTORS SHAPING PARASITE COMMUNITY STRUCTURE IN THREESPINE STICKLEBACKS *(GASTEROSTEUS ACULEATUS*).

1:45 (45)† **H.A. Stigge** and M.G. Bolek. EXPERIMENTAL EVIDENCE FOR ACQUIRED IMMUNITY TO *HALIPEGUS* SPECIES IN TWO SPECIES OF FRESHWATER SNAILS.

2:00 (46)† **W.D**. **Helenbrook**, W.M. Shields and C.M. Whipps. INFLUENCE OF FOREST STRUCTURE AND HUMAN ENCROACHMENT ON PARASITE COMMUNITIES OF MANTLED HOWLER MONKEYS, *ALOUATTA PALLIATA*.

2:15 (47)† **J.A. Kolman**, R.E. Clopton and D.T. Clopton. OOCYST PRODUCTION AND SPOROZOITE VIABILITY ARE INVERSELY RELATED TO ENVIRONMENTAL TEMPERATURE OF DEVELOPING GAMETOCYSTS.

2:30 (48)† **S.M. Steele**, R.E. Clopton and D.T. Clopton. VIABILITY AND ENVIRONMENTAL LONGEVITY OF GREGARINE OOCYSTS.

2:45 (49)† **S. Weinstein**. TRANSMISSION OF RACCOON ROUNDWORM, *BAYLISASCARIS PROCYONIS*, IN SOUTHERN CALIFORNIA.

3:00-3:30 pm **COFFEE BREAK**

1:00-3:00 pm Taxonomy, Systematics, Phylogeny I

Location: Leduc/Fortin

Presiding: F. Reyda, State University of New York
S. Rios, University of North Dakota

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (50)† **S.R. Catalano**. THE SECRET LIVES OF DICYEMID PARASITES: AN INSIGHT INTO CEPHALOPOD HOST POPULATIONS VIA ANALYSES OF THEIR DICYEMID PARASITE FAUNA.

1:15 (51)† **A.R. Lapierre**, S.A. Locke and D.J. Marcogliese and D. McLaughlin. MOLECULAR PHYLOGENY OF THE DIPLOSTOMIDAE AND STRIGEIDAE (DIGENEA).

1:30 (52)† **E.L. Kasl**, T.J. Fayton, W.F. Font and C.D. Criscione. DISCOVERIES REVEAL EVOLUTIONARY PATTERNS OF LIFE CYCLE COMPLEXITY AND THE NEED FOR GENETIC DATA TO SUPPORT MORPHOLOGICAL DESCRIPTIONS IN THE GENUS *ALLOGLOSSIDIUM*.

1:45 (53)† **D.F. Rosim**, G.A. Boxshall and P.S. Ceccarelli. A NEW COPEPOD (ERGASILIDAE) FROM THE GILLS OF A FRESHWATER FISH FROM BRAZIL.

2:00 (54)† **T.J. Fayton** and A. Choudhury. PHYLOGENETIC AFFINITIES OF *PLAGIOPORUS* STAFFORD, 1904 (DIGENEA: OPECOELIDAE) AND CLOSELY ALLIED PLAGIOPORINE GENERA OF THE HOLARCTIC.

2:15 (55)† **J.R. Fauver**, R.E. Clopton and D.T. Clopton. THE PROMISE AND PREDICAMENT OF MORPHOMETRIC SPECIES RECOGNITION IN GREGARINES.

2:30 (56) **K. Jensen**, A. Waeschenbach, J.J. Cielocha, J.N. Caira and D.L. Littlewood. INTERRELATIONSHIPS AMONG THE LECANICEPHALIDEAN TAPEWORMS BASED ON MOLECULAR SEQUENCE DATA: A PROPOSED FAMILY-LEVEL CLASSIFICATION.

2:45 (57) **J.J. Cielocha** and K. Jensen. *CEPHALOBOTHRIUM* SHIPLEY & HORNELL, 1906 REVISITED: RESURRECTION OF THE CEPHALOBOTHRIIDAE PINTNER, 1928 (PLATYHELMINTHES: EUCESTODA: LECANICEPHALIDEA).

3:00-3:30 pm **COFFEE BREAK**

1:00-2:30 pm Biochemistry/Physiology, Chemotherapy & Drug Resistance, Immunology

Location: Borduas

Presiding: R. Kuhn, Wake Forest University

Time (Abstract No.)

1:00 (58) **V. Dufour**, C.R. Caffrey, R.N. Beech, P. Ribeiro, J.A. Dent and T.G. Geary. MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL GLUTAMATE-GATED CHLORIDE CHANNEL SUBUNITS FROM *SCHISTOSOMA MANSONI*.

1:15 (60) **V. Samoil**, M. Zamanian, R. Murali, M. Stevenson, A. Jardim and P. Ribeiro. COMPARATIVE ANALYSIS OF EXOSOMES RELEASED BY *SCHISTOSOMA MANSONI* AND THE FREE-LIVING FLATWORM, *DUGESIA TIGRINA*.

1:30 (61) **J.F. Hillyer** and J.G. King. MAPPING THE MOSQUITO CELLULAR IMMUNE RESPONSE: HEMOCYTE DISTRIBUTION THROUGHOUT THE HEMOCOEL.

1:45 (62) **S E. Ruiz Lancheros** and T.G. Geary. STRATEGIES TO DEORPHANIZE AND CHARACTERIZE G PROTEIN-COUPLED RECEPTORS FOR NEUROPEPTIDES IN THE MODEL NEMATODE, *CAENORHABDITIS ELEGANS*.

2:00 (64) **C. Bulman**, C. Bidlow, S. Lustigman, F. Cho-Ngwa, M. Samje, A. Rascón, K. Lim, B. Suzuki, L. Rojo-Arreola, G. Knudsen, S. Gunatilleke, A. Barrios, J. McKerrow, A. Debnath and J. Sakanari. REDISCOVERING GOLD: AURANOFIN AS A MACROFILARICIDAL DRUG CANDIDATE.

2:15 (65) **J.M. Porter-Kelley**, K. Brown, L. Dixon, S. Peoples, C. Adams, S. Battle, S. Lea, R. Robinson, E. Masatanna, R. Wilson, F. Akinbo and G. Mayer. PREVALENCE OF *PLASMODIUM* IN HIV-INFECTED PATIENTS IN BENIN CITY, EDO, NIGERIA.

3:00-3:30 pm **COFFEE BREAK**

1:00-3:00 pm Life Cycles & Epidemiology I

Location: Krieghoff

Presiding: D. Kyle, University of South Florida

U. Ngenegbo, Nnamdi Azikiwe University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (66) **S. Georgieva**, A. Faltynkova, A. Kostadinova, C. Selbach, M. Soldanova and B. Sures, K. Skirnisson. CRYPTIC DIVERSITY WITHIN THE *ECHINOSTOMA 'REVOLUTUM'* SPECIES COMPLEX (DIGENEA: ECHINOSTOMATIDAE).

1:15 (67) **D.E. Kyle**, S.V. Siegel, B.L. Colon, G.P. Noblet and I. de Buron. TEREBELLID POLYCHAETES IDENTIFIED AS INTERMEDIATE HOSTS FOR *CARDICOLA LARUEI* (DIGENEA: APOROCOTYLIDAE) IN SPOTTED SEA TROUT (*CYNOSCION NEBULOSUS*).

1:30 (68) **P.F. Armstrong**, S. Fly, J. Payne and J. Gunderson. METACESTODES FROM THE SQUID *LOLLIGUNCULA BREVIS.*

1:45 (69)† **S.A. Orlofske**, S.M. Flaxman, B.A. Melbourne and P.T. Johnson. BEYOND FREQUENCY AND DENSITY-DEPENDENCE: AN EXPERIMENTAL DEMONSTRATION OF THE IMPORTANCE OF NON-LINEAR TRANSMISSION DYNAMICS IN A HOST-MACROPARASITE SYSTEM.

2:00 (70) **G. Alama-Bermejo**, J.A. Raga and A.S. Holzer. SEASONAL DYNAMICS AND TRANSMISSION OF THE MARINE MYXOZOAN *CERATOMYXA PUNTAZZI*.

2:15 (71) **K.R. Price**, J. Bulfon and J.R. Barta. PRACTICAL APPLICATION OF THE *EIMERIA* LIFE CYCLE WITH LIVE VACCINATION, REPLACEMENT LAYER PULLET PRODUCTION AND THE CAGED ENVIRONMENT.

2:30 (72)† **M.J. Andres** and R.M. Overstreet. CETACEAN AND FISH ANISAKID NEMATODES SUGGEST BENTHIC-PELAGIC COUPLING BY SOME MESOPELAGIC FISHES IN THE GULF OF MEXICO.

2:45 (73)† **E.A. Zieman**, J. Reeve and A. Jimenez. THE LIFE CYCLE, PATHOGENICITY AND GENETIC STRUCTURE OF *DELADENUS PROXIMUS*, NEOTYLENCHID PARASITE OF THE WOODWASP *SIREX NIGRICORNIS* (HYMENOPTERA).

3:00-3:30 pm **COFFEE BREAK**

3:30-5:30 pm ASP Students' Symposium

Location: Leduc/Fortin

Presiding: **L.E. Camp**, University of California – Davis

Theme: "Emerging Infectious Diseases"

Time (Abstract No.)

3:30 pm **L.E. Camp**, Introduction.

3:40 (74) **G. Conboy**. METASTRONGYLOIDS OF RED FOX (*VULPES VULPES*): EMERGING THREATS TO DOGS.

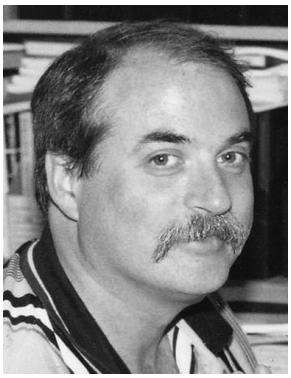
4:10 (75) **M. Ndao**. LOSING THE T OF THE NTD (NEGLECTED TROPICAL DISEASES).

4:40 (76) **M.J. Yabsley**. CHAGAS DISEASE IN THE UNITED STATES – UNDERSTANDING THE SYLVATIC CYCLE.

5:10 Questions, Closing Remarks.

5:30-6:30 pm *The Steve Upton Party for ASP Students*

Location: Pilot



Steve J. Upton

Dr. Steve J. Upton (6/14/1953-7/29/2010) was an active member of the ASP. His research focused on numerous coccidia and *Cryptosporidium* species. He taught a variety of courses including human parasitology and medical parasitology during his tenure at Kansas State University as a Professor of Biology. Dr. Upton published 225 original research papers, 11 book chapters and 3 books. In 1996, he was the recipient of the H.B. Ward Medal.

Thursday Evening, 2013-06-27

6:00-7:00 pm **Auction Preview**

7:00-9:00 pm **23rd ANNUAL ASP STUDENT AUCTION**

Location: Place Montcalm

Friday Morning, 2013-06-28

8:30-10:00 Associate Editors Symposium

Location: Suzor Coté

Presiding: G.W. Esch, Wake Forest University
M. Sukhdeo, Rutgers University

Time (Abstract No.)

8:00 (77) **G.W. Esch** and M.V. Sukhdeo**.** Introduction, ASSOCIATE EDITORS SYMPOSIUM – 2013.

8:20 (78) **C. Goater**. ECOLOGICAL EPIDEMIOLOGY OF AN UNLIKELY INVADER: THE LANCET LIVER FLUKE, *DICROCOELIUM DENDRITICUM*, IN ALBERTA, CANADA.

8:40 (79) **D. Zelmer**. PARASITES OF *LEPOMIS AURITIS* IN THE EDISTO RIVER: THE RELATIONSHIP BETWEEN HOST AND PARASITE COMMUNITY STRUCTURE.

9:00 (80) **S.L. Perkins**. MALARIA AND ITS MANY MATES: REVISING HAEMOSPORIDIA.

9:20 (81) **I. de Buron**. TO BE BAD OR TO BE GOOD? THAT IS THE QUESTION….

9:40 G.W. Esch and **M.V. Sukhdeo**, Questions, Closing Remarks**.**

10:00-10:30 am **COFFEE BREAK**

9:00-11:45 pm 45th Coccidiosis Conference

Location: Leduc/Fortin

Presiding: **J.R. Barta**, University of Guelph

Time (Abstract No.)

9:00 am Introduction.

9:15 (82) **H.D. Chapman**. COCCIDIOSIS VACCINATION: PROBLEMS AND SOLUTIONS.

9:35 (83) **K.R. Price** and J.R. Barta. PARASITES, PLACES AND POULTRY: ENVIRONMENTAL INFLUENCE ON LIVE COCCIDIOSIS VACCINE SUCCESS IN COMMERCIAL POULTRY REARING.

10:00-10:30 am **COFFEE BREAK**

10:30 (84) **M.C. Jenkins**. OPPORTUNITIES AND CHALLENGES FOR ACHIEVING UNIFORM PROTECTION OF CHICKENS AGAINST COCCIDIOSIS WITH LIVE EIMERIA OOCYSTS VACCINES.

10:50 (85) **J.R. Barta**, M.A. Hafeez and M.E. Ogedengbe. DNA BARCODING OF COCCIDIA – PROMISE AND PITFALLS.

11:10 (86) N. Sundar, M.A. Miller, J.M. Wendte, K. Haman, E.R. James, P. Keeling, P.A. Conrad and **M.E. Griggs**. VIRULENCE SHIFT IN A SEXUAL CLADE OF *TOXOPLASMA GONDII* INFECTING WILDLIFE IN NORTH AMERICA.

11:30 Questions, Closing Remarks.

9:00-11:30 pm Life Cycles and Epidemiology II

Location: Krieghoff

Presiding: L. Camp, University of California-Davis
K. Sapp, High Point University

Time (Abstract No.)

9:00 (87) **K.A. Davila**, C.E. Jones, N.J. McLean and C.M. Adema. CANID OCULAR FILARIASIS IN NM.

9:15 (88) **R. Krause**, N. Sandoval, K. Koski and M.E. Scott. CONTAMINATION OF SOIL WITH HELMINTH PARASITES AROUND HOMES IN RURAL PANAMA.

9:30 (89) **A.I. Paredes Trujillo**. SPATIAL DISTRIBUTION OF HELMINTH PARASITES IN TILAPIA FARMS IN THE STATE OF YUCATáN, MéXICO.

9:45 (90) **J. Ye**, L. Xiao, J. Li, W. Huang, S. Amer, Y. Guo and Y. Feng. MOLECULAR IDENTIFICATION OF ENTERIC PARASITES IN LABORATORY *MACACA FACICULARIS* IN GUANGXI, CHINA.

10:00-10:30 am COFFEE BREAK

10:30 (91) **D.S. Lindsay** and A.E. Houk. *CYSTOISOSPORA CANIS* (APICOMPLEXA: SARCOCYSTADIIAE): DEVELOPMENT OF MONOZOIC TISSUE CYSTS IN HUMAN CELL LINES.

10:45 (92) **S. Amer**, S. Zidan, H. Adamu, J. Ye, D. Roellig, Y. Feng and L. Xiao. PREVALENCE AND CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPP. IN DAIRY CATTLE IN NILE RIVER DELTA PROVINCES, EGYPT.

11:00 (93) **A. Gilabert**, S. Harvey and J. Wasmuth. EVOLUTION OF THE ARRESTED LARVAL STAGE DEVELOPMENT PATHWAY IN NEMATODE SPECIES.

11:15 (94) **M.S. Tucker**, L.B. Karunaratne, R.C. Peoples and F.A. Lewis. IMPROVEMENT OF *S. JAPONICUM* INFECTION IN MICE USING FETAL BOVINE SERUM AND A TAIL IMMERSION METHOD.

9:00-12:30 pm QMP-ASP Host Parasite Interactions II

Location: Borduas

Presiding: B. Papadopoulou, Chairman of LOC, Laval University

9:00 **B. Papadopoulou**, Opening Remarks.

9:10 Keynote Speaker: **N. Fasel**
Leishmania RNA virus-a backseat driver to metastatic leishmaniasis.

10:00-10:30 am **COFFEE BREAK**

Presiding: D. Richard, Laval University
M. Olivier, McGill University

Time (Abstract No.)

10:30 (95) **L.E. Luque de Johnson**. CYTOSKELETAL REARRANGEMENT IN HUMAN RED BLOOD CELLS INDUCED BY THE *PLASMODIUM FALCIPARUM* PROTEIN EBA-175.

10:45 (96) **L.M. Starr**, K.G. Koski and M.E. Scott. DIFFERENTIAL IMPACTS OF MATERNAL NEMATODE INFECTION AND PROTEIN DEFICIENCY ON HORMONE, CYTOKINE AND ANTIBODY COMMUNICATION AT THE MATERNAL-FETAL INTERFACE IN MICE.

11:00 (97) **F.A. Kassa**, E. Boilard and M. Olivier. ABSENCE OF APOLIPOPROTEIN E PROTECTS MICE FROM CEREBRAL MALARIA.

11:15 (98) **Q.D. Miao**, B. Ward, C. Santamaria, D. Bailey and M. Ndao. *TRYPANOSOMA CRUZI* INFECTION CAUSES THE TRUNCATION OF APOLIPOPROTEIN A1 IN HOST HIGH DENSITY LIPOPROTEINS (HDL).

11:30 (99) **V. Diniz Atayde**, K. Hassani, H. Aslan Suau, M.A. Gomez, N. Saravia, S. Kamhawi and M. Olivier. *LEISHMANIA* EXOSOMES AND THEIR INFLUENCE IN THE DEVELOPMENT OF LEISHMANIASIS.

11:45 (100) **N. Moradin**, D. Matheoud, W.J. Hong, M. Desjardins and A. Descoteaux. *LEISHMANIA* INHIBITS THE PHAGOSOMAL RECRUITMENT OF SEC22B.

12:00-12:30 **M. Marti**. MECHANISMS OF GAMETOCYTE FORMATION AND SEQUESTRATION IN THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*.

Friday Afternoon, 2013-06-28

1:00-2:00 *ASP President's Address*

Location: Suzor Coté

Presiding: B. Christensen, University of Wisconsin - Madison

1:00 Introduction of Dr. Eric S. Loker.
University of New Mexico

1:10 (101) **E.S. Loker**. "This de-Wormed World?"

Eric S. Loker
ASP President

2:15-5:45 pm Ecology III

Location: Suzor Coté

Presiding: R. Blaylock, University of Southern Mississippi
H. Eure, Wake Forest University

Time (Abstract No.)

2:15 (102) **B. Quiroz-Martínez** and G. Salgado-Maldonado. TAXONOMIC COMPOSITION, ENDEMISM AND PATTERNS OF DISTRIBUTION OF THE HELMINTH PARASITES OF FRESHWATER FISHES OF MEXICO.

2:30 (103) **J M. Soldánová**, C. Selbach and B. Sures. CERCARIAL EMERGENCE OF *TRICHOBILHARZIA* SPP. (DIGENEA: SCHISTOSOMATIDAE) FROM TWO LYMNAEID SNAIL HOSTS UNDER DIFFERENT LABORATORY CONDITIONS.

2:45 (104) **J.J. Forest**, D.J. Marcogliese and J.D. McLaughlin. HELMINTH INFECTIONS IN JUVENILE ROUND GOBY *NEOGOBIUS MELANOSTOMUS* IN THE ST. LAWRENCE RIVER, CANADA.

3:00 (105) **A. Olsen**, A. Bruno, A. Fedynich and D. Rollins. *OXYSPIRURA PETROWI* IN NORTHERN BOBWHITES FROM SOUTH TEXAS AND THE ROLLING PLAINS.

3:15 (106) **A. Bruno** and A.M. Fedynich, D. Rollins. OPERATION IDIOPATHIC DECLINE: SURVEY AND ASSESMENT OF PARASITIC INFECTIONS OF NORTHERN BOBWHITES IN THE ROLLING PLAINS ECOREGION.

3:30 (107) **J. Koprivnikar**, J. Huver, P.T. Johnson and S. Whyard. ENVIRONMENTAL DNA: AN EFFECTIVE METHOD TO DETECT PARASITE PRESENCE IN WATER BODIES.

3:45 – 4:00 pm **COFFEE BREAK**

4:00 (108) **J F. Maure**. HOST BEHAVIOURAL MANIPULATION OF THE SPOTTED LADY BEETLE BY A PARASITIC WASP.

4:15 (109) **S.A. Locke**, G. Bulté, D.J. Marcogliese and M.R. Forbes. REDUCED PARASITISM AND DIET BREADTH IN A NATIVE PREDATOR (*LEPOMIS GIBBOSUS*) FEEDING ON INTRODUCED PREY (*DREISSENA POLYMORPHA*).

4:30 (110) **D.J. Marcogliese**, A.D. Gendron, J.J. Forest and J.D. McLaughlin. EXPANSION OF THE INVASIVE ASIAN FISH TAPEWORM IN THE LOWER GREAT LAKES AND ST. LAWRENCE RIVER.

4:45 (111) **C.D. Criscione**. ESTIMATION OF THE EFFECTIVE POPULATION SIZE (NE) AS A GENETIC EPIDEMIOLOGICAL TOOL TO MONITOR METAZOAN PARASITE POPULATION AND TRANSMISSION DYNAMICS.

5:00 (112) **C.P. Tadiri**, **F**. Dargent and M.E. Scott. RELATIVE BODY CONDITION OF THE GUPPY, *POECILIA RETICULATA*, AND FOOD AVAILABILITY INFLUENCE *GYRODACTYLUS TURNBULLI* (MONOGENEA) EPIDEMIC DYNAMICS.

5:15 (113) **R.L. Grunberg** and M.V. Sukhdeo. SEASONALITY OF GREGARINE (EUGREGARIONORIDA) INFECTIONS OF AMPHIPODS (*GAMMARUS*) IN THE NORTH BRANCH OF THE RARITAN RIVER.

5:30 (114) **A. May-Tec**. TEMPORAL VARIATION OF *ARGULUS YUCATANUS* IN THE MAYAN CICHLID *CICHLASOMA UROPTHALMUS* IN CELESTUN, YUCATAN, MEXICO.

2:15-5:45 pm Taxonomy, Systematics, Phylogeny II

Location: Leduc/Fortin

Presiding: A. Jiménez, Southern Illinois University
S. Perkins, American Museum of Natural History

Time (Abstract No.)

2:15 (115) **L.E. Reid**, T.J. Cook and M.L. Thies. HELMINTHS OF *MICAELAMYS NAMAQUENSIS* AND *AETHOMYS CHRYSOPHILUS* (RODENTIA: MURIDAE) FROM NORTHWESTERN BOTSWANA.

2:30 (116) **N.A. Al-Zanbagi** and A. Hassan. A MULTIVARIATE ANALYSIS OF SOME DIGENEAN SPECIES COLLECTED FROM SEVERAL RED SEA FISHES IN SAUDI ARABIA.

2:45 (117) **H.H. Mejía-Madrid**. HISTORICAL BIOGEOGRAPHY OF *RHABDOCHONA* SPECIES.

3:00 (118) **J. Kvicerova**, P. Siroky and N. Dvorakova and V. Hypsa. PHYLOGENETIC RELATIONSHIPS AND GENEALOGY OF A BLOOD PARASITE *HEMOLIVIA MAURITANICA*.

3:15 (119) **A. Smythe**, K. Forgrave, A. Patti, R. Hochberg and M. Litvaitis. ECOLOGY, TAXONOMY, AND EVOLUTION OF THE *CHAETOGASTER LIMNAEI* (OLIGOCHAETA, ANNELIDA) SPECIES COMPLEX.

3:30 (120) **R. Míguez-Lozano**, J.A. Balbuena, V. Sarabeev and I. Blasco-Costa. MOLECULAR PHYLOGENY OF SPECIES OF *LIGOPHORUS* (MONOGENEA: DACTYLOGYRIDAE) AND THEIR AFFINITIES WITHIN THE DACTYLOGYRIDAE.

3:45 – 4:00 pm COFFEE BREAK

4:00 (121) **D. Willsey**, K. Herzog and F. Reyda. RELAXED HOST SPECIFICITY IN A NEW CESTODE GENUS FROM *DASYATIS* AND *HIMANTURA*.

4:15 (122) **J R. Devkota**, S.V. Brant and E.S. Loker. SCHISTOSOMIASIS IN NEPAL.

4:30 (123) **S.V. Brant** and E.S. Loker. ARE SCHISTOSOMES A SPECIAL CASE OR TYPICAL STORY OF PARASITE DIVERSIFICATION?

4:45 (124) **J.N. Caira**, F.P. Marques, K. Jensen, R. Kuchta and V. Ivanov. PHYLOGENETIC ANALYSIS AND RECONFIGURATION OF THE GENERA IN THE CESTODE ORDER DIPHYLLIDEA.

5:00 (125) **A.J. Phillips**, B.B. Georgiev, A. Waeshenbach and J. Mariaux. NOVELTY AND HOST SPECIFICITY OF PARUTERINID CESTODES FROM SOUTH AMERICAN BIRDS.

5:15 (126) **N.M. Luchetti** and F.P. Marques. LINEAGE DIVERSITY, MORPHOLOGICAL VARIABILITY AND HOST SPECIFICITY WITHIN *POTAMOTRYGONOCESTUS* PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS.

5:30 (127) **C.T. Olivares** and F.P. Marques. MOLECULAR DIVERSITY AMONG LINEAGES OF *POTAMOTRYGONOCESTUS* PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS: TESTING MORPHOLOGY-BASED SPECIES BOUNDARIES.

2:15-5:00 pm Host Parasite Interactions III

Location: Krieghoff

Presiding: I. de-Buron-Connors, College of Charleston
J. Hillyer, Vanderbilt University

Time (Abstract No.)

2:15 (128) V.E. Lemmons and **A.W. Shostak**. PARASITISM BY *HYMENOLEPIS DIMINUTA* INCREASES EGG CANNIBALISM BY *TRIBOLIUM CONFUSUM*.

2:30 (129) **M.C. Curran**, B.A. Brinton and J. LaBarre. THE EFFECT OF THE BOPYRID ISOPOD PARASITE *PROBOPYRUS PANDALICOLA* ON THE BEHAVIOR OF *PALAEMONETES PUGIO* AND THE PREDATION PREFERENCES OF *FUNDULUS HETEROCLITUS*.

2:45 (130) **S. Edaye** and E. Georges. *PLASMODIUM FALCIPARUM*: CHARACTERIZATION AND CELLULAR LOCALIZATION OF ABCG PROTEIN.

3:00 (131) **V.M. Frankel** and M.E. Torchin. INFECTION PREFERENCE OF AN INVASIVE TREMATODE, *CENTROCESTUS FORMOSANUS*, TO AN INVASIVE CICHLID, *CICHLA OCELLARIS*, IN THE PANAMA CANAL.

3:15 (132) **M.R. Laidemitt**. PATTERNS OF HOST USE OF PARAMPHISTOMOID FLUKES FROM KENYA, WITH IMPLICATIONS FOR *SCHISTOSOMA MANSONI* TRANSMISSION.

3:30 (133) **M. Bolek** and R. Shannon. GORDIID (PHYLUM NEMATOMORPHA) CYST DEVELOPMENT AND SURVIVAL TO DRYING IN PARATENIC HOSTS.

3:45 – 4:00 pm **COFFEE BREAK**

4:00 (134) **C.C. Reed**. ADVANCES IN APPLIED MARINE PARASITOLOGY STUDIES IN SOUTH AFRICA.

4:15 (135) **J.G. King**, OVERCOMING OBSTACLES TOWARDS THE IN VITRO ANALYSES OF HEPATOCYTE INVASION AND EXO-ERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM*.

4:30 (136) **M.A. Hudgell**, M.A. Gordy and E.S. Loker. EXPOSURE OF *BIOMPHALARIA HAVANENSIS* TO SCHISTOSOMA MANSONI: EXPLORATION OF THE BASIS OF INCOMPATIBILITY IN THIS HOST-PARASITE SYSTEM.

4:45 (137) **P.M. Estrella** and B. Hanelt. GOING IT ALONE? CHARACTERIZING THE RESPONSE OF A PARASITE UPON IMMUNE CHALLENGE OF THE HOST-PARASITE UNIT.

1:45-3:00 pm QMP Host Parasite Interactions and Immunology

Location: Borduas

Presiding: A. Descoteaux, Institut Armand Frappier
S. Stager, Institut Armand Frappier

Time (Abstract No.)

1:45 (138) **M. Olivier**, F. Kassa and M. Shio. ROLE OF INNATE IMMUNITY IN THE DEVELOPMENT OF MALARIA RELATED PATHOLOGIES.

2:10 (139) **J. Éstaquier**, V. Rodrigues, M. Laforge, A. Ouaissi, A. Cordeiro-da-Silva and R. Silvestre. ABORTIVE FOLLICULAR HELPER DIFFERENTIATION IS ASSOCIATED WITH DEFECTIVE HUMORAL RESPONSE IN *LEISHMANIA INFANTUM*-INFECTED RHESUS MACAQUES.

2:30 (140) **S.J. Reiling**, G. Tadeus and P. Rohrbach. CHLOROQUINE'S MODE OF ACTION IS NOT RESTRICTED TO THE *PLASMODIUM FALCIPARUM* DIGESTIVE VACUOLE.

2:45 (141) **K. Sonzogni-Desautels** and T.G. Geary. CRYPTOSPORIDIUM PARVUM SURFACE PROTEINS AS CANDIDATES FOR A CRYPTOSPORIDIOSIS VACCINE TO PROTECT NEWBORN CALVES.

3:00 – 3:30 pm **COFFEE BREAK**

3:30-5:30 pm QMP-ASP Biochemistry, Molecular Biology and Genetics I

Location: Borduas

Presiding: B. Papadopoulou, Laval University
A. Jardim, McGill University

Time (Abstract No.)

3:30 (142) **L.I. McCall**, W.W. Zhang , S. Ranasinghe and G. Matlashewski. LIVE IMMUNIZATION AGAINST VISCERAL LEISHMANIASIS USING A NATURALLY ATTENUATED CUTANEOUS *LEISHMANIA DONOVANI* ISOLATE FROM SRI LANKA.

3:45 (63) **E. Gazanion**, C. Joly-Beauparlant, A. Droit, B. Papadopoulou, J. Corbeil and M. Ouellette. GENOME-WIDE MAPPING OF HISTONE H3K4 TRIMETHYLATION AND GENE EXPRESSION REGULATION IN *LEISHMANIA.*

4:00 (144) **T. Duguet**. FUNCTIONAL DIVERSIFICATION OF LEVAMISOLE RECEPTORS IN THE TRICHOSTRONGYLID NEMATODE *HAEMONCHUS CONTORTUS.*

4:15 (145) **A.E. Davidsen** and A. Jardim. INSERTION OF THE *LEISHMANIA DONOVANI* PEROXIN 5 INTO GLYCOSOMAL MEMBRANES.

4:30 (23) **H. Azizi**, T. Patrícia Romão Pompílio de Melo, C. Dumas and B. Papadopoulou. MECHANISTIC INSIGHTS OF SIDER2 RETROPOSON-MEDIATED MRNA DECAY IN *LEISHMANIA.*

4:45 (147) **Z. Lu**, H.S. Najafabadi, V. Mehta, V.H. Gazestani, V. Adoue and R. Salavati. REGULATION OF RNA METABOLISM BY ARE-BINDING PROTEINS IN *TRYPANOSOMA BRUCEI.*

5:00 (148) **A. Dupé**, C. Dumas and B. Papadopoulou. ALBA DOMAIN PROTEINS IN *LEISHMANIA*: DIFFERENTIAL PROTEIN LOCALIZATION DURING AMASTIGOTE DIFFERENTIATION AND THEIR ROLE IN AMASTIN MRNA DEVELOPMENTAL REGULATION.

5:15 (149) **K. Ordonez**, M. Woodard, A. Wake and M. Porter-Kelley. IDENTIFICATION AND CHARACTERIZATION OF POST-TRANSCRIPTIONAL REGULATORS IN *LEISHMANIA BRAZILIENSIS*.

4:00-5:30 pm Poster Display Boards delivered

Location: Place Montcalm

Authors may set up posters during this time.

Friday Evening, 2013-06-28

7:00 – 10:30 pm Evening at the Hall of the Musée de la Civilisation de Québec

Saturday Morning, 2013-06-29

8:00-10:30 am Authors complete set up for poster session

Location: Place Montcalm

8:00-10:45 am Ecology IV

Location: Suzor Coté

Presiding: K. Luth, Wake Forest University
K. Sheehan, Clemson University

Time (Abstract No.)

8:00 (150) **R.N. Sehgal**. HABITAT ELEMENTS IMPACT THE PREVALENCE AND HOST SPECIFICITY OF AVIAN HAEMOSPORIDIAN PARASITES IN TROPICAL ECOSYSTEMS.

8:15 (151) **B. Hanelt**, M.G. Bolek and A. Schmidt-Rhaesa. SKY ISLAND HAIRWORMS: BIODIVERSITY OF FRESHWATER NEMATOMORPHS FROM ISOLATED MOUNTAIN TOPS IN SOUTHEASTERN ARIZONA.

8:30 (152) **A. Per-Arne**, K. Lafferty, R. Knudsen, R. Primicerio, R. Kristoffersen, A. Klemetsen and A. Kuris. NEW PARASITES AND PREDATORS FOLLOW THE INTRODUCTION OF TWO FISH SPECIES TO A SUBARCTIC LAKE: IMPLICATIONS FOR FOOD-WEB STRUCTURE AND FUNCTIONING.

8:45 (153) **E.T. Gendron**, E.S. Loker, V.V. Tkach and S.V. Brant. VARIATION IN HOST ECOLOGY CAN LEAD TO DISCORDANT POPULATION DEMOGRAPHICS AMONG PARASITES WITHIN A *TRICHOBILHARZIA* SPECIES COMPLEX.

9:00 (154) **J.P. McLaughlin**, A.M. Kuris and K.D. Lafferty. ADDING PARASITES TO FOOD WEBS.

9:15-9:30 am COFFEE BREAK

9:30 (155) **M.L. Aguirre-Macedo**, A.L. May-Tec, C.M. Vivas-Rodríguez, F. Puc-Itza, D. Pech and V.M. Vidal-Martínez. LONG-TERM DYNAMICS OF THE METAZOAN PARASITE SPECIES RICHNESS AND DIVERSITY OF *CICHLASOMA UROPHTHALMUS* FROM CELESTúN, YUCATáN, MEXICO.

9:45 (156) **F. Reyda**. THE FOOD WEB OF OTSEGO LAKE, NEW YORK FROM A PARASITOLOGICAL PERSPECTIVE.

10:00 (157) **V.M. Vidal-Martínez**, D. Romero and M.L. Aguirre-Macedo. THE EFFECT OF PH AND OTHER ENVIRONMENTAL VARIABLES ON THE SPATIAL DISTRIBUTION OF FLATFISHES AND THEIR HELMINTH PARASITES IN THE GULF OF MEXICO.

10:15 (158) **J.V. Hopper**, C. White, J. Lorda, S.E. Koch, A.M. Kuris and R.F. Hechinger. REDUCED PARASITISM OF A MARINE WHELK, *KELLETIA KELLETII*, IN ITS EXPANDED GEOGRAPHIC RANGE.

10:30 (159) **S.L. Hallett**, G.R. Buckles, R.A. Ray and J.L. Bartholomew. LONG-TERM SURVEILLANCE OF A SALMONID PARASITE BY RIVER WATER SAMPLING AND QPCR.

8:00-10:45 am Taxonomy, Systematics, Phylogeny III

Location: Leduc/Fortin

Presiding: S. Seville, University of Wyoming
M. Zimmermann, Wake Forest University

Time (Abstract No.)

8:00 (160) **F.P. Marques**. THE ROLE OF MORPHOMETRIC DISCONTINUITIES IN TAXONOMIC PRACTICE: ARE WE MEASURING ENOUGH WORMS?

8:15 (161) **D.J. Machado**, F.P. Marques and F.B. Reyda. THE PHYLOGENETIC POSITION OF UNARMED CESTODES PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS.

8:30 (162) **L.M. Abbott** and J.N. Caira. A NOVEL GENUS AND TWO NEW SPECIES OF DIPHYLLIDEAN CESTODES FROM THE YELLOWSPOTTED SKATE, *LEUCORAJA WALLACEI*, FROM SOUTH AFRICA.

8:45 (163) **V.M. Bueno** and J.N. Caira. THE OTHER SIDE OF THE WORLD: ASSESSMENT OF THE DIVERSITY OF ECHENEIBOTHRIINAE.

9:00 (164) **J. Bernot**. SITE SPECIFICITY OF TAPEWORMS OF THE GENUS *CALLIOBOTHRIUM* IN THE SPIRAL INTESTINE OF SMOOTHHOUND SHARKS (CARCHARHINIFORMES: TRIAKIDAE).

9:15-9:30 am COFFEE BREAK

9:30 (165) **T.J. Katz**. A REVISED LOOK AT THE GENUS *CAULOBOTHRIUM* (CESTODA: TETRAPHYLLIDEA): NOVEL SPECIES AND THEIR INTRIGUING HOST ASSOCIATIONS.

9:45 (166) **M. Pickering** and J.N. Caira. CESTODES FROM DEEP-WATER SQUALIFORM SHARKS SURVEYED OFF AZOREAN SEAMOUNT REVEAL ADDITIONAL DIVERSITY OF ADULT AND LARVAL FORMS.

10:00 (167) **F.A. Jimenez**, R.P. Scheibel, B.M. Byles and S.L. Gardner. THE METAZOAN PARASITES OF OPOSSUMS IN BOLIVIA: AN INVENTORY OF 25% OF MARSUPIAL DIVERSITY.

10:15 (168) **M.A. Hafeez**, I. Stasiak, P. Delnatte, D.A. Smith and J.R. Barta. MOLECULAR EVIDENCE SUGGESTS THAT TWO *ISOSPORA* SPECIES CAN CAUSE VISCERAL ISOSPOROSIS ('ATOXOPLASMOSIS') CONCURRENTLY IN CAPTIVE-BRED SUPERB GLOSSY STARLINGS (*LAMPROTORNIS SUPERBUS*).

10:30 (169) **S. El-Sherry**, M.A. Hafeez and M.E. Ogedengbe, H.D. Chapman and J.R. Barta. *EIMERIA* SPECIES OF TURKEYS: LINKING MOLECULES AND MORPHOMETRICS.

8:00-10:15 am QMP-ASP Biochemistry, Molecular Biology and Genetics II

Location: Krieghoff

Presiding: J. Éstaquier, Laval University
S. Sato, Laval University

8:00 Keynote Speaker, **J. Weinstock**
Helminths and Autoimmunity.

Time (Abstract No.)

9:00 (170) **M. Mitreva**. USING EXISTING DRUGS AS LEADS TO COMBAT NEGLECTED DISEASES.

9:15 (171) **F. Dargent**, M.E. Scott, A.P. Hendry and G.F. Fussmann. RELEASE FROM A KEY PARASITE (*GYRODACTYLUS* SPP) IN THE WILD LEADS TO REPEATABLE SEXUALLY ASYMMETRIC EVOLUTION OF RESISTANCE.

9:30 (143) **G. Mayer**. CHARACTERIZATION OF A NOVEL *PLASMODIUM FALCIPARUM* CALCIUM-BINDING EXPORTED PROTEIN.

9:45 (173) **M. Rashid**, M. Kimber, T. Day and P. Ribeiro. PUTATIVE CATION-SELECTIVE NICOTINIC ACETYLCHOLINE RECEPTORS OF *SCHISTOSOMA MANSONI*.

10:00 (174) **C. Valentim**, D. Cioli, L. Picca-Mottaccia and A. Guidi, F. Chevalier, T. Anderson and P.T. LoVerde. ANTI-SCHISTOSOMAL MODE OF ACTION OF OXAMNIQUINE.

10:30-11:00 am **COFFEE BREAK**

Saturday, 2013-06-29

11:00-1:00 pm QMP-ASP Poster Session, beverages and snacks

Location: Place Montcalm

All authors must stand with your posters from 11:00-1:00.

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175 **J. Brown**, K. Campanaro, M.S. Mesbahuddin, M. Teghtmeyer, E. Walden, J. Yee and S.P. Rafferty. HEME PROTEINS OF *GIARDIA LAMBLIA.*

176 **R.T. Mathew**, K. Thivierge, and J.P. Dalton. THE M1 ALANYL AMINOPEPTIDASES OF *PLASMODIUM FALCIPARUM* (PFM1AAP) MALARIA: BIOCHEMICAL AND MUTATIONAL ANALYSIS.

177 **A.H. Kottarampatel**, N. Cyr, R. Strasser and A. Jardim. INTERACTION OF *LEISHMANIA DONOVANI* PEX14 WITH GLYCOSOMAL MEMBRANE.

178 **V.N. Mehta**, H. Moshiri and R. Salavati. IN VITRO SCREENING OF COMPOUNDS IDENTIFIES RNA EDITING INHIBITORS.

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179 **S. Hallée** and D. Richard. CHARACTERIZATION OF AN NEW PUTATIVE RHOPTRY PROTEIN IN *PLASMODIUM FALCIPARUM*.

180 **X. Wu**, C. Jackson, M. Truscott, R. Ocadiz-Ruiz, R. Geske, I.W. Chalmers, K.F. Hoffmann and T.P. Yoshino. VENOM ALLERGEN-LIKE (VAL) PROTEINS IN EARLY INTRAMOLLUSCAN LARVAL STAGES OF *SCHISTOSOMA MANSONI*.

CHEMOTHERAPY AND DRUG RESISTANCE

181 **M. O'Neill**, E. Burkman, A.R. Moorhead, C.D. Mackenzie and T.G. Geary. MORPHOLOGICAL ALTERATIONS OF FEMALE BRUGIA MALAYI FOLLOWING EXPOSURE TO FLUBENDAZOLE: EFFECTS OF VARIATION IN THE EXPOSURE PROFILE.

182 **J. Wunderlich** and P. Rohrbach. THE EFFECT OF QUINOLINE DRUGS ON INTRACELLULAR PH IN *PLASMODIUM FALCIPARUM*.

183 **R.L. Monte Neto** and M. Ouellette. DNA AMPLIFICATION, LOCUS DELETION AND A POINT MUTATION CONTRIBUTE TO ANTIMONY RESISTANCE IN *LEISHMANIA (VIANNIA) GUYANENSIS*.

184 **A. Renteria**. NOVEL COMPOUND AGAINST *CRYPTOSPORIDIUM PARVUM* INFECTIONS IN BOTH IN VITRO AND IN VIVO.

185 **J. Porter-Kelley**, K. Brown, L. Dixson, S. Peoples, C. Adam, S. Battle, D. Bellamy, L. Shyneque, R. Robinson, M. Eraifej, R. Wilson, F.O. Akinbo and G. Mayer. PREVALENCE OF *PLASMODIUM VIVAX* AND MALARIAE IN HIV-INFECTED PATIENTS IN BENIN CITY, EDO, NIGERIA.

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186 **J.R. Palmieri**, S. King and A. Santo. ZOONOTIC AND HUMAN RELATIONSHIPS OF LYME DISEASE IN VIRGINIA.

187 **M. Horther** and T. Sparkes. EGG SHAPE DIVERSITY IN ACANTHOCEPHALANS: EFFECTS OF HABITAT TYPE.

188 **C. Selbach**, M. Soldánová and B. Sures. TREMATODE COMMUNITIES IN FRESHWATER SNAILS FROM THE RUHR AREA IN GERMANY WITH A FOCUS ON BIRD SCHISTOSOMES.

189 **J.A. Mischler**, S.V. Brant, E.S. Loker and A. Townsend. CHANGES IN A HIGH ELEVATION MOLLUSCAN-TREMATODE COMMUNITY OVER THE LAST 50 YEARS IN CRESTED BUTTE, CO.

190 **V.V. Tkach**, S. Rios and S.V. Brant. FIRST REPORT ON AVIAN SCHISTOSOMATIDS FROM BIRDS AND MOLLUSKS IN NORTH DAKOTA.

GENETICS AND MOLECULAR BIOLOGY

191 **K. Horlock-Roberts**, C. Reaume, G. Daye and J. Yee. THE *GIARDIA INTESTINALIS* CELL CYCLE.

192 **M. Koinari**, S. Karl, A. Elliot, U. Ryan and A. Lymbery. IDENTIFICATION OF ANISAKIS SPECIES (NEMATODA: ANISAKIDAE) IN MARINE FISH HOSTS FROM PAPUA NEW GUINEA.

193 **O. Zghidi-Abouzid**, P. Padmanabhan, M. Samant, C. Dumas and B. Papadopoulou. THE ROLE OF AN ARGONAUTE-LIKE PIWI PROTEIN HOMOLOG IN *LEISHMANIA*.

194 **B. Li**, A.H. Rush and G.J. Weil. IN SITU EXPRESSION PATTERNS OF *BRUGIA MALAYI* CYS-LOOP LIGAND-GATED ION CHANNEL GENES CONFIRM A ROLE IN REPRODUCTION.

195 **S. Kuk**, U. Cetinkaya, M. Yuruk and S. Yazar. MOLECULAR DISCRIMINATION OF *ECHINOCOCCUS GRANULOSUS* AND *ECHINOCOCCUS MULTILOCULARIS* BY SEQUENCING AND A NEW PCR - RFLP METHOD WITH THE POTENTIAL USE FOR OTHER *ECHINOCOCCUS* SPECIES.

196 **S. Yazar**, S. Kuk, U. Cetinkaya and M. Yuruk. DEVELOPING OF ELISA BASED ON RECOMBINANT CATHEPSIN L1 PROTEIN FOR DIAGNOSIS OF *FASCIOLA HEPATICA*.

HOST-PARASITE INTERACTIONS

197 **J.R. Palmieri**, D. North and A. Santo. FURUNCULAR MYIASIS OF THE FOOT CAUSED BY THE TUMBY FLY, *CORDYLOBIA ANTHROPOPHAGA*.

198 **M. Sampson**, D. Brunet, S.F. Elswaifi, J.E. Powers, F. Rawlins II and J.R. Palmieri. DETERMINING THE BURDEN OF INTESTINAL PARASITES IN PATIENTS WITH GASTROINTESTINAL SYMPTOMS IN VERóN, THE DOMINICAN REPUBLIC.

199 **J.R. Palmieri**, C.F. Skinner and S.F. Elswaifi. GIARDIASIS REVISITED: GLOBAL SOCIOECONOMIC IMPACT AS A REEMERGING ZOONOTIC DISEASE.

200 **H.J. Peng**, J.R. Palmieri, Z.Z. Wang Y. Lan, F.S. Niu, Q.B. Ge, X.B. Wu, W.W. Ouyang and X.N. Lu. ORAL MYIASIS ASSOCIATED WITH TERTIARY SYPHILIS-INDUCED BRAIN STEM HEMORRHAGE.

201 **G. St-Pierre**, P. Bhaumik, V. Milot, C. St-Pierre and S. Sato. GALECTIN-3 FACILITATES NEUTROPHIL RECRUITMENT AS AN INNATE IMMUNE RESPONSE TO A PARASITIC PROTOZOA *LEISHMANIA MAJOR* CUTANEOUS INFECTION.

202 **A. Sassi**, J. Geary, A. Moorhead, D. Whitten, C. Mackenzie and T. Geary. IDENTIFICATION OF EXCRETED-SECRETED PROTEINS OF FILARIAL NEMATODES AS NEW DIAGNOSTIC REAGENTS.

203 **J. Christian**, S. Arulthas, P.A. Tessier and M. Olivier. IMPACT OF *LEISHMANIA*-NEUTROPHIL-INTERACTIONS ON MACROPHAGE SIGNALING.

204 **A. Ricciardi**, J.P. Dalton and M. Ndao. DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST *SCHISTOSOMA MANSONI* INFECTION USING CATHEPSIN B AND PEROXIREDOXIN 1 ANTIGENS.

205 **J.F. Chehayeb**, R. Martin, A.P. Robertson and T.G. Geary. ANALYSIS OF *ASCARIS SUUM* FLUID COMPARTMENTS USING A PROTEOMICS AND BIOINFORMATICS APPROACH.

206 **V. Gazestani** and R. Salavati. COMPUTATIONAL RECOGNITION OF CIS-REGULATORY ELEMENTS IN TRYPANOSOMATIDS.

207 **N. Nikpour**, I. Mak, H. Shateri Najafabadi, V. Hajihosseini and R. Salavati. INSIGHTS INTO THE ARCHITECTURE AND PROTEIN INTERACTION NETWORK OF RNA EDITING ASSOCIATED COMPLEXES IN *TRYPANOSOMA BRUCEI*.

208 **M.I. Kardoush**, B.J. Ward, P. Ribeiro and M. Ndao. IDENTIFICATION OF CANDIDATE SERUM BIOMARKERS FOR *S. MANSONI* INFECTED MICE USING MULTIPLE PROTEOMIC PLATFORMS.

209 **C.E. Montelongo**, B.B. Herrera and M.G. Castillo. IDENTIFICATION AND INITIAL EXPRESSION STUDIES OF A MANNOSE BINDING LECTIN (MBL)-LIKE PROTEIN IN SUSCEPTIBLE AND RESISTANT STRAINS OF *BIOMPHALARIA GLABRATA* UPON CHALLENGE WITH *SCHISTOSOMA MANSONI*.

210 **K. Van Den Ham**, M.T. Shio and M. Olivier. ATTENUATION OF CEREBRAL MALARIA PATHOLOGY BY PARENTERAL IRON OVERLOAD.

211 **J.P. Ek-Huchim**. MOLECULAR VARIABILITY OF *PERKINSUS MARINUS* IN OYSTERS *CRASSOSTREA VIRGINICA* FROM GULF OF MEXICO.

212 **G. Arango Duque**, M. Fukuda and A. Descoteaux. LEISHMANOLYSIN-MEDIATED DEGRADATION OF SYNAPTOTAGMIN XI LEADS TO DEREGULATED CYTOKINE SECRETION.

213 **A. George**, E. McElroy and I. de Buron. THE EFFECTS OF TWO PARASITES ON SWIMMING PERFORMANCE IN THE SPOTTED SEATROUT (*CYNOSCION NEBULOSUS*).

214 **N. Sharma**, N. Patocka and P. Ribeiro. RNA INTERFERENCE (RNAI) SCREEN OF PUTATIVE NEUROMUSCULAR RECEPTORS OF *SCHISTOSOMA MANSONI*.

215 **R.C. Peoples**, L.B. Karunaratne and M.S. Tucker. ANALYSIS OF *NAVICULA PELLICULOSA* GROWTH AND *NAVICULA PELLICULOSA*-FED *ONCOMELANIA HUPENSIS* SSP. SNAILS UNDER EXPERIMENTAL CONDITIONS.

216 **A. Hamami** and S. Stager. IL-10 LIMITS CD8+ T CELL DURING EXPERIMENTAL *LEISMANIA DONOVANI* INFECTION.

217 **M. William** and M. Jaramillo. MACROPHAGE TRANSLATIONAL CONTROL BY *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE.

218 **V.H. Gazestani** and R. Salavati. COMPUTATIONAL RECOGNITION OF CIS-REGULATORY ELEMENTS IN TRYPANOSOMATIDS.

IMMUNOLOGY

219 **K. Thivierge**, S. Cotton, D.A. Schaefer, M.W. Riggs, J. To, M.E. Lund, M.W. Robinson, J.P. Dalton and S. Donnelly. CATHELICIDIN-LIKE HELMINTH DEFENSE MOLECULES (HDMS): ABSENCE OF CYTOTOXIC, ANTI-MICROBIAL AND ANTI-PROTOZOAN ACTIVITIES IMPLY A SPECIFIC ADAPTATION TO IMMUNE MODULATION.

220 **M.T. Shio**, J.G. Christian, Y. Jung, K.P. Chang and M. Olivier. IMPACT OF *LEISHMANIA* METALLOPROTEASE GP63 ON NLRP3 INFLAMMASOME NETWORK.

LIFE CYCLES AND EPIDEMIOLOGY

221 **S.T. Britt**, D. Byler and J. Gunderson. A *POLYPOCEPHALUS* METACESTODE FROM BAY SCALLOPS LIVES AS AN ADULT IN COWNOSE RAYS.

222 **M. Taliercio**, T. Darden, W.A. Roumillat and I. de Buron. STRIPED BASS, *MORONE SAXATILIS*: A NEW HOST FOR A PARASITE OF PUBLIC HEALTH CONCERN IN SOUTH CAROLINA.

223 **K.F. Sonderman**, T.M. Norton, T.D. Tuberville, R.M. Lock and M.J. Yabsley. HAEMOGREGARINE PARASITE OF GOPHER TORTOISES (*GOPHERUS POLYPHEMUS*): SEARCH FOR A VECTOR.

TAXONOMY, SYSTEMATICS AND PHYLOGENY

224 **N. Dvorakova**, J. Kvicerova, V. Hypsa and P. Siroky. LOW HOST SPECIFICITY IN HAEMOGREGARINES FROM AFRICAN TERRAPINS OF THE FAMILY PELOMEDUSIDAE.

225 **E. Darpino**, R. Russell and F. Reyda. DIGENETIC TREMATODES OF THE FISHES OF OTSEGO LAKE, NEW YORK.

226 **J. Westenberger**, A. Borden and F. Reyda. NEMATODES OF THE FISHES OF OTSEGO LAKE, NEW YORK.

227 **I. Dykova**, A. Kodadkova, I. de Buron, I. Fiala and W.A. Roumillat. *SINUOLINEA* INFECTIONS IN THE URINARY SYSTEM OF *CYNOSCION* SPECIES (SCIAENIDAE) AND PHYLOGENETIC POSITION OF THE TYPE SPECIES OF *SINUOLINEA* DAVIS, 1917 (MYXOZOA: MYXOSPOREA).

228 **J.A. Balbuena**, R. Míguez-Lozano and I. Blasco-Costa. PACO: A NOVEL PROCRUSTES APPLICATION TO COPHYLOGENETIC ANALYSIS OF HOSTS AND PARASITES.

229 **A. Sendkewitz** and F. Reyda. CESTODES OF THE FISHES OF OTSEGO LAKE, NEW YORK.

230 **K. Herzog**, D. Willsey and F. Reyda. MORPHOLOGICAL DIVERSITY OF RHINEBOTHRIINAE NEW GENUS 3 (CESTODA: RHINEBOTHRIIDEA).

231 **W.M. Kistler**, M.J. Yabsley and S.E. Gibbs. MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF HEMATOZOAN PARASITES OF AMERICAN BLACK DUCKS (*ANAS RUBRIPES*) IN THE EASTERN UNITED STATES.

VECTOR BIOLOGY

232 **B.C. Shock**, S. Cohen, P. Williamson, A.C. Moncayo and M.J. Yabsley. UTILITY OF TESTING BLOOD-FED AND QUESTING TICKS FOR PIROPLASMS FOR IDENTIFICATION OF NOVEL VERTEBRATE HOSTS OR VECTORS.

1:00 – 2:00 pm R. Barclay McGhee Lecture

Location: Suzor Coté

Presiding: J.N. Caira, University of Connecticut
M. Siddall, American Museum of Natural History

1:00 Introduction of 2013 R. Barclay McGhee Recipient.
D. Duszynski, University of New Mexico

1:10 (233) **D. Duszynski**, "Historical perspectives and Another new coccidium, so what!"

Don Duszynski
R. Barclay McGhee

2:00 – 3:00 pm H.B. Ward Medal Lecture

Location: Suzor Coté

Presiding: R. Carreno, Ohio Wesleyan University

2:00 Introduction of 2013 H. B. Ward Medal Recipient.
S.A. Nadler, University of California - Davis

2:10 (234) **S.A. Nadler**, "Standing on the shoulders of benevolent giants."

Steve Nadler
H. B. Ward Medal

2:00-4:30 pm QMP Chemotherapy, Drug Resistance and Drug Targets

Location: Krieghoff

Presiding: R.K. Pritchard, McGill University
M. Ouellette, Laval University

Time (Abstract No.)

2:00 (235) **M. Ouellette**. GENOMICS OF DRUG RESISTANCE IN *LEISHMANIA*.

2:30 (236) **T. Mani**, C. Bourguinat and K. Prichard. ROLE OF P-GLYCOPROTEIN IN THE MACROCYCLIC LACTONE RESISTANCE MECHANISM IN *DIROFILARIA IMMITIS*.

2:45 (237) **F. Baakdah** and E. Georges. *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER (PFCRT) INTERACTING PROTEINS.

3:00 (238) **I.M. Vincent**, S. Weidt, L. Rivas, K. Burgess and M. Ouellette. NEW PROGRESS ON THE MODE OF ACTION OF MILTEFOSINE IN *L. INFANTUM* USING METABOLOMICS.

3:15 (239) **S. Ashraf** and R. Prichard. *HAEMONCHUS CONTORTUS*: TUBULINS AND IVERMECTIN SELECTION.

3:30 (240) **M.C. Laffitte**, A. Mukherjee, D. Légaré and M. Ouellette. MRE11 INVOLVEMENT IN DNA REPAIR AND DRUG RESISTANCE IN *LEISHMANIA.*

3:45 – 4:30 Closing Remarks and Awards.

3:15 PM - 4:15 PM ASP Awards and Business Meeting

Location: Suzor Coté

Jonas King
Ashton Cuckler Award
2013

ASP AWARDS

ASHTON CUCKLER NEW INVESTIGATOR AWARD

Presiding: C. Adema, University of New Mexico

The recipient of the 2013 New Investigator Award is **Dr. Jonas King**, W. Harry Feinstone Department of Molecular Microbiology & Immunology and the Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health.

WILLIS A. REID JR., STUDENT RESEARCH GRANT AWARDS

Presiding: M. Bolek, Oklahoma State University

BEST STUDENT PRESENTATIONS AND MARC DRESDEN TRAVEL GRANT AWARDS

Presiding: J. Koprivnikar, Brandon University

ASP BUSINESS MEETING

Presiding: E.S. Loker, University of New Mexico

Thank you for attending this year's ASP meeting and have a safe trip home.
*See you **July 24-27, 2014** at our next meeting in **New Orleans, Louisiana**!*

Abstract Listings

(1)

RELATIVE ROLES OF HOST EXPOSURE AND PARASITE ESTABLISHMENT IN DETERMINING HELMINTH BURDENS OF *EPTESICUS FUSCUS* (MAMMALIA: CHIROPTERA)

E.M. Warburton and **M. Vonhof**, Western Michigan University

In most host-parasite systems, variation in parasite burden among hosts is a major driver of transmission dynamics. Heavily infected individuals introduce disproportionate numbers of infective stages into host populations or the surrounding environment, this, in turn, may cause sharp increases in frequency of infection. Parasite aggregation within the host population may result from both heterogeneous exposure to infective propagules and heterogeneous establishment of parasites in the host. We sought to quantify the relative roles of exposure and establishment in producing variation in parasite burdens in order to predict which hosts are more likely to bear heavy burdens using big brown bats (*Eptesicus fuscus*) and its helminths as a model system. We captured bats from seven colonies in Michigan and Indiana, assessed their helminth burdens, and collected data on variables related to both exposure (capture location, capture date, water contact) and establishment (host sex, age, body condition, immune function, genetic heterozygosity). Digenetic trematodes had the highest prevalence (64%) and mean intensity (34 worms) while cestodes and nematodes had much lower prevalence (9% and 10%, respectively) and mean intensity (8 and 2 worms per host, respectively). Structural equation modeling revealed the best-fitting *a priori* model (AIC=11.704) for both all taxa and trematodes alone included host genetic diversity and distance of colony to nearest body of water. The best-fitting model for cestodes and nematodes (AIC=10.64) included month of capture and host genetic diversity. Thus, differential host exposure and differential parasite establishment both appear to play significant roles in creating heterogeneous helminth burdens. However, variables that impact trematode burdens differ from those that impact cestode and nematode burdens. Thus, transmission dynamics are not a one-size-fits-all affair and we must carefully consider the biology of both host and worm when attempting to predict helminth burdens.

(2)

DIFFERENTIAL INFECTION PATTERNS OF *ECHINOSTOMA* SPP. LARVAL STAGES IN SNAILS FROM THREE DIFFERENT FAMILIES

M.R. Zimmermann, **K.E. Luth** and **G.W. Esch**, Wake Forest University

More than 4,500 pulmonate snails were collected from 11 states in the mid-Atlantic and Midwestern United States in the summer of 2012. These snails were necropsied and echinostome metecercariae were commonly observed infecting the snails as 2nd intermediate hosts (20.0%). The snails comprised species of 3 genera (*Physa*, *Lymnaea*, and *Helisoma*) with distinct differences in the infection patterns of the *Echinostoma* spp. metacercariae among them. *Physa* spp. snails exhibited a significantly higher prevalence of infection (23.5%) than both *Lymnaea* sp. (11.6%) and *Helisoma* spp. (14.2%) ($P < 0.05$), with no difference in prevalence observed between *Lymnaea* sp. and *Helisoma* spp. ($P > 0.05$). The intensity of metacercariae within the snail hosts was significantly different between the 3 genera ($P < 0.05$), with *Lymnaea* sp. having the highest intensity (24.3 ± 5.6), followed by *Physa* spp. (15.2 ± 1.5) and *Helisoma* spp. (5.0 ± 0.9). Differences in prevalence and intensity were also observed when the different snail families co-habited the same body of water. The disparities in infection patterns are likely due to distinct differences in the behavioral and feeding ecology of the snail hosts.

(3)

EFFECTS OF SEX AND AGE ON A COMPONENT COMMUNITY OF CHEWING LICE (PHTHIRAPTERA) OF BROWN-HEADED COWBIRDS (*MOLOTHRUS ATER*)

E.S. Durkin, Northern Michigan University

Chewing lice (Phthiraptera) are permanent residents of their hosts and are transferred nearly exclusively through direct forms of body contact such as copulation and care of young. For most bird species, direct contact generally occurs with those of the same species. Brown-headed cowbirds (*Molothrus ater*) are brood parasitic and thus have the opportunity to come in contact with different bird species and their lice. Brown-headed cowbird offspring are cared for by a host parent and adult female cowbirds come in contact with the host nest while egg-laying. Both activities have the potential to facilitate the transfer of chewing lice. I investigated the effects of sex and age on chewing louse prevalence, intensity and diversity of brown-headed cowbirds. Four hundred and one brown-headed cowbirds were collected from Kirtland's warbler breeding habitat in Michigan and examined for lice. Lice were collected by blowing compressed air on euthanized brown-headed cowbirds in an enclosed chamber. Lice that had fallen from the brown-headed cowbirds within the chamber were counted and identified. Sixty percent of the brown-headed cowbirds in this population were infected (242/401) with at least one genus of chewing louse. Five louse genera (*Brueelia*, *Philopterus*, *Menacanthus*, *Myrsidea* and *Machaerilaemus*) were identified. Significantly more males were infected (189/293) with chewing lice than females (53/108). Chewing louse intensity and diversity were similar between the sexes. Significantly more second-year (younger) males were infected (72/100) with chewing lice than after second-year (older) males (117/193). Older males had greater infection intensity (*Mdn*=9 Range= 1-242) than younger males (*Mdn*=5.5 Range=1-96). Louse diversity was the same between younger and older males.

(4)

CASCADE OF ENEMY RELEASE: IMPACTS OF AN INVASIVE SPECIES (*NEOGOBIUS MELANOSTOMUS*) ON THE PARASITE COMMUNITY OF A NATIVE PREDATOR (*MICROPTERUS DOLOMIEU*)

E.F. Bauer and **C.M. Whipps**
SUNY-ESF, State University of New York, College of Environmental Science and Forestry

Enemy release has been proposed to explain the lack of parasites in species translocated from their native habitats. Round goby (*Neogobius melanostomus*) have been used as an example of an enemy released invasive in the Great Lakes, having become ubiquitous and abundant in many waters of New York State. In invaded ecosystems round gobies can be a significant dietary component of native piscivores, and have been especially exploited by smallmouth bass (*Micropterus dolomieu*). Round gobies in the Great Lakes are known to harbor fewer parasites than in their native range, and as smallmouth bass consume more gobies they may be less likely to be exposed to trophically transmitted parasites that occur in native prey. Furthermore, largemouth bass (*Micropterus salmoides*) tend to have less habitat overlap with gobies than smallmouth bass and served as a control species for comparison. To investigate if consumption or presence of an invasive prey species may lead to depauperate parasite diversity and abundance in bass, 464 total largemouth and smallmouth bass were examined from multiple sites where gobies were either present of absent. Gastrointestinal contents were examined to estimate frequency of round goby consumption. Among sites, goby frequency ranged from 0% to 75% and mean parasite species richness from 1.3 to 6.7 per fish. Correlations of mean parasite diversity by site indicate that in smallmouth bass, goby frequency (R^2= .66) was a better predictor of mean parasite richness than fish length (R^2= .34). Diversity of parasites that are unlikely to be influenced by goby presence were better explained by fish length (R^2= .68) than by goby frequency (R^2= .35). These correlations suggest bass parasite richness may

be influenced by presence of invasive round gobies. Analyses are ongoing to account for confounding factors and to determine individual parasites that may be impacted by gobies. Survey results may aid in predicting the impacts of imminent goby invasion of Oneida Lake, which has a multimillion dollar bass fishery.

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(5)

LATITUDINAL GRADIENTS IN PARASITISM IN THE INVASIVE LIONFISH

A. Sellers, McGill University
M. Torchin and **G. Ruiz**, Smithsonian Tropical Research Institute
B. Leung, McGill University

Biotic resistance to biological invasions is hypothesized to be strongest at low latitudes due to higher native diversity and stronger biotic interactions. We examined this hypothesis by comparing the abundance, species richness, and effect of metazoan parasites infecting the invasive lionfish (*Pterois volitans*) across 13 sites in the western Atlantic encompassing 17 degrees of latitude. We predicted that diversity and abundance of parasites infecting *P. volitans* would be highest at low latitudes, and that parasite abundance would have a negative effect on host condition. Overall, *P. volitans* were infected by few parasites. At any given site no more than 4 parasite taxa were recovered from the sample of hosts. Parasite prevalence pooled across all parasite taxa and sites was 63.5%. Though parasitism was low, the trematode *Lecithochirium floridense* was relatively abundant, particularly in Belize (prevalence=92.5%, max intensity=62). Furthermore, this was the only parasite species found at all 17 sites. Species richness and abundance of ectoparasites was significantly higher at low latitudes, however no such pattern was observed for endoparasites. We did not find an association between parasite abundance (within or across parasite groups) and host condition at any site. Our results suggest that even though lionfish seem to be more parasitized at low latitudes, the invaders are experiencing little resistance from native parasites across their introduced range. Furthermore, results from our study are comparable to results from past studies on parasite biogeography. Our ongoing work aims to examine how latitudinal variations in the diversity of prey consumed by invasive lionfish affect the diversity of the trophically transmitted parasites that infect the species in the Western Atlantic.

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(6)

EXPLORING THE DILUTION EFFECT: HOST DENSITY MEDIATES HOST DIVERSITY EFFECTS ON COMMUNITY-WIDE PARASITE INFECTION

J. Wojdak, Department of Biology, Radford University
R. Edman, **J. Wyderko**, **S. Zemmer** and **L. Belden**, Department of Biological Sciences, Virginia Tech

Host species diversity can dramatically alter disease transmission in natural communities. Whether diverse hosts dilute or amplify disease depends critically on species traits, particularly on how hosts affect each other's densities. Here we studied a community of potential hosts and decoys (i.e. non-competent hosts) for two trematode species, *Echinostoma trivolvis* and *Ribeiroia ondatrae*, which commonly infect wildlife across North America. We manipulated the density of a focal host (green frog tadpoles, *Rana clamitans*), in concert with manipulating the diversity of alternative hosts, to simulate communities where alternative species replace the focal species (substitution) or add to total density (additivity). We found that parasite transmission remained roughly equal (or perhaps decreased) when alternative hosts replaced focal hosts, but parasite transmission was higher when alternative hosts were added to a community without replacing a focal host. How host communities assemble should help determine species diversity effects on wildlife disease transmission.

(7)

FISH PARASITES IN THE HUDSON RIVER ESTUARY'S LITTORAL HABITATS: A PRELUDE TO RESTORATION

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Banded killifish (*Fundulus diaphanus*) parasite communities were examined from three different littoral habitat types (main channel fringe, secondary channel, and contiguous backwater) in New York's upper Hudson River Estuary with the goal of understanding ecosystem dynamics that could inform prospective restoration efforts. Killifish were collected at 6 sites in July and August 2012 for a spatio-temporal comparison of parasite component communities; in all 285 killifish were dissected. Twenty eight parasite species were found and the component communities from each site differed significantly. Abundance of *Posthodiplostomum minimum,* proteocephalid metacestodes, and *Contracaecum* sp. cysts explained most of these differences. Mean July abundance of *P. minimum* was highest in the secondary channel habitat (41.76) and lowest in the main channel fringe habitat (10.39). The opposite was true of the metacestodes: lowest mean abundance (13.29) occurred in the secondary channel and highest (31.45) in the main channel fringe. For *Contracaecum* sp. cysts, abundances ranked as secondary channel > contiguous backwater > main channel fringe. All sites showed high prevalence of the myxozoan gill parasite *Myxobolus funduli*. The severity of this infection implies detrimental health effects from probable impaired respiration. Sites shared some species in common as indicated by high Sørensen's similarity coefficients (0.743-0.865). Parasite diversity, as expressed using the Shannon-Wiener Index, was high at all sites and highest in the two secondary channel sites. The secondary channel had the highest parasite species abundance and diversity, suggesting that host species abundance and diversity was also high in the secondary channel habitat.

(8)

THE EFFECT OF PRESCRIBED BURNS ON PARASITE FAUNA IN CUBAN TREEFROGS

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In forming management plans for the monitoring or controlling infectious wildlife diseases, the natural ecology behind the mechanistic forces that lend to fluctuating or emerging diseases must be investigated. One such naturally occurring process that possesses the ability to alter the dynamics of natural ecosystems is fire. Fire and disease occur naturally but are also highly manipulated by humans. For the last 30 years, the Southwest Florida Water Management District has conducted periodic compartmental burns at Flatwoods Park in Hillsborough County, Tampa, FL; due to location, size, and accessibility, this site is ideal for a continued effort in understanding the relationship between fire and pathogens. My project aims to gather information on the impact fire could have on host-pathogen interactions as a result of the fires killing and thereby reducing soil parasites. The objective is to determine if prescribed burns alter soil-dwelling parasitic nematode abundance and composition in soil, and thus, influence host-parasite interactions with Cuban treefrogs, *Osteopilus septentrionalis*. Our preliminary results indicate that Cuban treefrogs collected from wetlands with a 1-2 year post-burn history have significantly fewer helminths than those collected 5-6yrs post-burn. The results of changes in soil parasites due to fire, coupled with known beneficial ecological alterations of habitat and biodiversity during prescribed burns, could better address necessary measures for managing wildlife diseases.

(9)

DESCRIPTION, MICROHABITAT SELECTION AND INFECTION PATTERNS OF SEALWORM LARVAE (*PSEUDOTERRANOVA DECIPIENS* SPECIES COMPLEX, NEMATODA: ASCARIDOIDEA) IN FISH FROM PATAGONIA, ARGENTINA

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Third stage larvae of the *Pseudoterranova decipiens* species complex (also known as sealworms) have been reported in at least 40 marine fish species belonging to 21 families and 10 orders along the South American coast. Sealworms are a cause for concern because they can infect humans who consume raw or undercooked fish (e.g. ceviche, a popular seafood dish from South America). However, despite their economic and zoonotic importance, morphological and molecular characterization of species of *Pseudoterranova* in South America is still underway. In this study we provide, molecular, morphological, and ecological data on the sealworm larvae in 542 individuals of 20 fish species from the neritic zone of the Patagonian Sea (Southwestern Atlantic). A total of 635 sealworm larvae were collected from 12 fish species. The most infected fish species was *Prionotus nudigula* (n = 32; prevalence [95% C.I.]: 100% [89.5-100]; mean intensity [95% C.I.]: 16.2 [12.5-20.9]), followed by *Paralichthys isosceles* (15; 26.7% [9.7-53.4]; 2.8 [1.0-5.5]), and *Pseudopercis semifasciata* (31; 25.8% [12.6-43.4]; 1.4 [1.0-1.6]). Most of the sealworms were collected from the muscles (mainly in the epaxial musculature), and to a lesser degree, from the mesenteries and liver. Sequences obtained for the mitochondrial cytochrome c oxidase subunit 1 gene (cox 1) of sealworms from *P. nudigula* formed a reciprocally monophyletic lineage with published sequences of *P. cattani* from definitive hosts. Morphology of sealworms from all the fish did no differ, but a discriminant analysis suggests that specimens from *P. nudigula* were significantly larger than those from other fish. Our results suggest that the most economically important fishes from this locality, i.e. *Merluccius hubbsi*, *Genypterus blacodes* and *Seriolella porosa* have low sealworm infections. Interestingly, despite the high density of pinnipeds (definitive hosts) inhabiting the Patagonian coast, levels of sealworm infection in large demersal fish from this area seem to be much lower than infections reported in demersal fish from the Northern Hemisphere.

(10)

PARASITES AS INDICATORS OF DRUG CONTAMINATION: RELATIONSHIPS AMONG PARASITES, GASTROPODS, AND PHARMACEUTICALS IN MIDWESTERN STREAMS

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Parasite diversity within an ecosystem depends on multiple biotic and abiotic factors and is increasingly being used as an indicator of ecosystem health. The growing presence of contaminants such as pharmaceuticals in the environment is an emerging concern due to their unknown effects on ecosystems and potential persistence in human drinking water sources. We sampled pharmaceuticals, physiochemical characteristics, gastropods, and parasites in 19 third order streams in central Indiana, USA, and used structural equation modeling to explore potential causal relationships among parasites, gastropods, pharmaceuticals, and stream variables. Caffeine (24-135 ng/L), carbamazepine (1-88 ng/L), cotinine (4-

34 ng/L), naproxen (5-15 ng/L), and paraxanthine (49-62 ng/L) concentrations varied among sites. Trematode prevalence in gastropods varied among sites and was positively related to gastropod host diversity, pharmaceutical richness, and phosphorus concentration. Trematode infection prevalence in gastropods was lower in sites with higher total dissolved solids. Relationships between pharmaceutical concentrations and trematodes depended on gastropod host and pharmaceutical identity. Trematode prevalence in *Physa acuta* was negatively related to carbamazepine concentration as well as *Chaetogaster limnaei* presence and abundance within hosts. Metacercarial cysts in *P. acuta* were more prevalent and abundant in sites with lower pharmaceutical concentration. These data establish broad links between parasites and contaminants of emerging concern and provide a baseline for using parasites as ecosystem indicators.

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(11)

SPATIAL AND TEMPORAL PATTERNS OF GREGARINE INFECTIONS IN DAMSELFLIES IN FOUR TEXAS ECOREGIONS

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Gregarines (Apicomplexa: Eugregarinida) are a diverse assemblage of parasites that utilize invertebrates as hosts and have historically been thought to exhibit strict host specificity. This view of strict host specificity has been supported by laboratory cross-infections of hosts and is thought to be a product of simultaneous cospeciation of hosts and parasites. However, some laboratory cross-infection experiments and extensive field surveys are challenging the notion of strict host specificity. Field surveys are the only way to elucidate host use when hosts are not able to be reared in the lab, as in damselflies (Odonata: Zygoptera). Extensive field surveys of hosts can reveal spatial and temporal patterns of host use. This study surveyed gregarine assemblages of Texas damselflies across four ecoregions throughout the 2012 flight season. Over 1,400 damselflies were collected representing 2 families, 7 genera, and 16 species. Damselflies were dissected and examined for gregarine infections, resulting in the collection of 256 gametocysts with an overall prevalence of 641 infected individuals (45.69%). Tentative identifications demonstrate that several gregarine species are able to parasitize a variety of hosts: *Nubenocephalus nebraskensis* infected *Enallagma basidens* and *Argia apicalis; Nubenocephalus sedundus* infected *A. apicalis* and *Argia sedula; Domadracunculus janovyi* infected *Ischnura ramburii* and *E. basidens*; and *Calyxocephalus karyopera* infected *Enallagma civile* and *I. ramburii*. Tentative new species of *Hoplorhynchus, Prismatospora, Steganorhynchus, Nubenocephalus,* and *Calyxocephalus* were collected from *I. ramburii, Argia moesta, Argia fumipennis, Argia translata, and E. civile* respectively. However, morphometric analysis is still ongoing to determine if these tentative new species are new or known taxa. Soil sample analyses (pH, percent organic matter, and particle size) were also performed to determine if soil characteristics influences gregarine species communities in differing ecoregions.

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(12)

A SUCCESSFUL WORM INDEED: INFECTION DYNAMICS OF A UBIQUITOUSLY DISTRIBUTED TAPEWORM, *PROTEOCEPHALUS AMBLOPLITIS* (EUCESTODA: PROTEOCEPHALIDEA)

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The cestode, *Proteocephalus ambloplitis*, has a complex life cycle that includes 3 hosts, i.e., a cyclopoid microcrustacean, a centrarchid or percid fish, and a small- or largemouth bass. Juveniles are found parenterally in first and second intermediate hosts, with adults being found exclusively in the gut of bass definitive hosts. Previously published results, coupled with the results of the current study, suggest that *P. ambloplitis* has a cosmopolitan distribution in the US. In the present study (conducted between 05/2011 and 10/2012), 140 lakes and ponds were sampled across 14 states (IL, IN, KS, KY, MD, MI, NC, NE, OH,

PA, TN, VA, WI, WV); *P. ambloplitis* was recovered from all 14 of these states (7 of which are first reports). Fish hosts, mostly belonging to the family, Centrarchidae, e.g., species of *Lepomis*, *Micropterus*, and *Pomoxis*, were obtained primarily via angling, eviscerated in the field, and the viscera were stored in 95% ethanol for subsequent necropsy and worm recovery. In all, 1,393 fish were captured and necropsied and >3,000 plerocercoids were collected. Infection dynamics, e.g., prevalence, intensity, and life stages present, of *P. ambloplitis* in the fish hosts were determined for every lake sampled, and the influences of environment, host, and parasite community on these dynamics were explored. Furthermore, a sample of all parasites (other than *P. ambloplitis*) was recovered from every host necropsied providing a large-scale survey of centrarchid fish parasite communities. Finally, in addition to providing the large-geographic-scale ecological data set, the tapeworms recovered in the current study will make a lake-to-lake study of the genetic structure of *P. ambloplitis* populations possible.

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(13)

DECREASED PRECIPITATION ALONG A NATURAL RAINFALL GRADIENT IS ASSOCIATED WITH INCREASED ABUNDANCE OF AN INTRODUCED NEMATODE PARASITE INFECTING ENDEMIC HAWAIIAN STREAM FISHES

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Parasite and pathogen infections of wildlife have increased as a result of anthropogenic movement of animals and plants across the planet. For example, the introduction of several species of poeciliid fishes into the Hawaiian Islands has resulted in the co-introduction of parasites that have subsequently infected native fishes. Climate warming is expected to further alter parasite densities and distributions through increased temperatures and related environmental changes. In Hawaii, reduced net precipitation resulting from climate warming is predicted to reduce stream flow, which may influence the abundance, intensity and prevalence of non-native parasites infecting native stream fishes. In this study, we utilized a natural precipitation gradient across the Hamakua coast on the island of Hawai`i to assess the effects of reduced precipitation on infection of *Awaous guamensis*, a native amphidromous goby, by an introduced nematode *Camallanus cotti*. We found that a decrease in precipitation was related to an increase in parasite abundance in *A. guamensis*. These results suggest that abundance of introduced parasites will increase in native Hawaiian fishes as a result of climate driven reductions in precipitation or through water extraction for agricultural and urban uses.

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(14)

LONG TERM SPECIES CO-INFECTION PATTERNS OF HELMINTHS INFECTING A FRESHWATER TROPICAL FISH

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The infracommunity of the tropical fish *Cichlasoma urophtalma* inhabiting the Celestun coastal lagoon was surveyed along four years in a monthly basis in order to understand the way in which co-infecting parasites interact among them within the host. To accomplish this task we started identifying the temporal distribution patterns of each one of the helminth parasites species by using time series models based in Fourier series, and then we looked for temporal association among parasites and environmental factors and host characteristics using canonical correspondence analysis. A total of 7 “core” species were observed, two external: the crustaceans *Argulus* sp. and *Ergasilus* sp., and five internal parasites: the nematods *Mexiconema cichlasomae*, *Contracaecum* sp., and the digeneans *Astrophaecum astorquii*, *Olygogonotilus manteri* and *Phagicola nana* were found. Additionally 26 satellite species were identified,

occurring at least once along the four years of survey. Fourier analysis shows that the core species shows maximum peaks of prevalence once in a year suggesting a periodicity in the host infection. Canonical correspondence analysis shows that the infection by digeneans mostly occurs during the dry season characterized with high environmental temperatures (28-34° C) and the nematodes and crustacean mostly infect the host during the rainy season. We also suggest that satellite species occurs as random infections along the year. We also suggest that metazoan parasites co exists in C. *urophtalma* by exploiting the host at different time during a year cycle and forming strategic aggregations based in taxonomic guilds

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(15)

GENE REGULATION OF IMMUNE RESPONSES IN *BIOMPHALARIA GLABRATA*

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Biomphalaria glabrata snails differ in their susceptibility to infection with the trematode parasite, *Schistosoma mansoni*. Following *S. mansoni* penetration of resistant *B. glabrata,* parasites are sealed off in a cellular encapsulation in which they are killed most likely due to host-derived reactive oxygen and/or nitrogen intermediates. In contrast however, susceptible snails are compatible hosts and can harbor a chronic schistosome infection. A number of differences between these strains have previously been identified including contrasting gene expression profiles. Therefore it is possible that differences in the regulation of gene transcription are partially responsible for the disparity between resistant and susceptible strains. To date however, only a few transcription factors have been described in *B. glabrata* and an understanding of gene regulation in this species is lacking. The NFkB family of transcription factors has been shown to regulate gene expression in a wide variety of biological processes, including immune and inflammatory responses. As a result, the NFkB pathway is a prospective candidate for the regulation of immune-related genes in *B. glabrata*. The aims of this study were: 1) to identify transcription factor binding sites, in particular kappa-binding sites, upstream of immune-related genes, including p38 MAPK, superoxide dismutase (SOD) and inhibitor of NFkB (IkB), 2) to compare the regulatory regions of these genes between resistant and susceptible *B. glabrata* strains. Regulatory regions upstream of genes of interest were identified using preliminary data from the *B. glabrata* genome project and amplified via PCR. Following, transcription factor binding sites were identified using TFSEARCH. Consequently several signature transcription factor binding sites, including putative kappa-binding sites, have been identified in the 5' regulatory region of *B. glabrata* immune-related genes. Furthermore, resistant and susceptible strains display differences in the regulatory regions upstream of the previously mentioned genes.

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(16)

EFFECT OF INFECTION ON MOSQUITO HEART PHYSIOLOGY

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Pathogens that enter the hemocoel (body cavity) of a mosquito are subject to swift hemolymph (blood) currents. These currents are modulated by the contractile action of the dorsal vessel, which is a muscular tube that spans the length of the insect and is divided into a thoracic aorta and an abdominal heart. Hemolymph enters the heart through paired valves, called ostia, which are present in each abdominal segment, and once inside the heart hemolymph is propelled in both anterograde (toward the head) and retrograde (toward the posterior abdomen) directions. Recently, we showed that when a mosquito acquires an infection, immune cells called hemocytes migrate to the heart and bind the regions surrounding the ostia. At these locations, periostial hemocytes phagocytose bacteria and malaria parasites,

and likely release soluble antimicrobial factors. In the present study we asked whether infection induces changes in circulatory physiology in the mosquito, *Anopheles gambiae*. By means of intravital video imaging, we found that infection induces a decrease in heart contraction rates, but that control of the infection results in a return to basal cardiac levels. In follow-up experiments, we are assaying the molecular mechanisms driving the infection-induced reduction in heart contraction rates, and have identified a hemocyte-produced factor that influences circulatory physiology. Together with our earlier data on hemocyte biology, these data show that multiple organ systems work in concert to combat systemic infections.

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(17)

UNUSUAL *ENTEROCYTOZOON BIENEUSI* GENOTYPES AND *CRYPTOSPORIDIUM HOMINIS* SUBTYPES IN HIV-INFECTED PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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Human immunodeficiency virus (HIV) infected persons are commonly infected with *Cryptosporidium* species and *Enterocytozoon bieneusi* in both developed and developing countries, particularly in patients with CD4+ cell counts below 200 cells/μL. Two hundred and eighty five HIV-infected patients on HAART were enrolled in this study and both stool and blood specimens were collected from participants. The stool specimens were analyzed and typed for *E. bieneusi* and *Cryptosporidium* spp. by PCR and DNA sequencing. CD4 count was analyzed using flow cytometry. *E. bieneusi* and *Cryptosporidium* were detected in 18 (6.3%) and 4 (1.4%) patients, respectively. The *E. bieneusi* detected mostly belonged to a new genotype group that thus far has only been found in a few humans: genotype Nig4 in two patients and two new genotypes related to Nig4 in 12 patients. The *Cryptosporidium* detected included *C. hominis* (2), *C. parvum* (1), and *C. felis* (1), with the two *C. hominis* infections belonging to an unusual subtype family. Further studies are required to determine whether some *E. bieneusi* genotypes and *C. hominis* subtypes are more prevalent in HIV patients on HAART.

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(18)

STRONGYLOIDES STERCORALIS INSULIN-LIKE PEPTIDES REGULATE INFECTIVE THIRD-STAGE LARVAE

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The infectious form of the parasitic nematode *Strongyloides stercoralis*, like that of filarial worm and hookworm species, is a developmentally arrested third-stage larva (L3i). *S. stercoralis* L3i are morphologically similar to the developmentally arrested dauer larva in the free-living nematode *Caenorhabditis elegans*. We hypothesize that insulin/IGF-I-like signaling (IIS), which is critical in regulating *C. elegans* dauer development, plays an important role in controlling both *S. stercoralis* L3i arrest and activation. In previous studies, we have demonstrated that the forkhead transcription factor *Ss*-DAF-16, the terminal signaling molecule in the *C. elegans* IIS pathway, regulates *S. stercoralis* L3i arrest. In a recent RNAseq study, we have identified all of the major components of an *S. stercoralis* IIS pathway and have shown that the transcript abundance of several insulin-like peptides (ILPs) is dramatically altered during L3i development. Specifically, *Ss-ilp-1* transcripts decrease over the course of post-free-living development, while *Ss-ilp-7* transcripts increase from post-free-living L1s to a maximum in both L3i

and *in vivo* activated L3 (L3+); *Ss-ilp-6* transcripts increase nearly 10-fold from L3i to L3+. In recent experiments, we have used promoter::*egfp* reporter constructs expressed in the *S. stercoralis* post-free-living generation to identify the tissues in which these three ILP promoters are active. While both *Ss-ilp-1* and *Ss-ilp-6* promoters are active in the hypodermis and neurons, the *Ss-ilp-7* promoter is active in the intestine and a pair of head neurons. Mis-regulation of the *Ss-ilp-6* promoter appears to alter morphogenesis of transgenic *S. stercoralis* larvae, a phenomenon not observed with the other constructs. Furthermore, we have observed that application of the membrane permeable cyclic guanosine monophosphate (cGMP) analogue 8-bromo-cGMP results in potent activation of *S. stercoralis* L3i, as measured by resumption of feeding, with 85.1±2.2% of L3i resuming feeding in 200 μM 8-bromo-cGMP in comparison to 0.6±0.3% in the buffer diluent. Application of 200 μM 8-bromo-cGMP results in a significant increase in *Ss-ilp-1* transcripts (46.76±9.84-fold increase in comparison to a 2.5±0.72-fold increase for the buffer diluent, both normalized to an unstimulated control), as determined by RT-qPCR. Together, these data provide additional evidence that an IIS pathway in *S. stercoralis* regulates L3i development. Understanding the mechanisms controlling L3i development in parasitic nematodes may lead to new treatments for infections caused by these organisms as well as environmental control strategies.

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(19)

NOVEL AND ZOONOTIC *CRYPTOSPORIDIUM* SPECIES/GENOTYPES IDENTIFIED IN FISH AND GOATS PAPUA NEW GUINEA

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Very little is known about the prevalence of the enteric parasite *Cryptosporidium* in humans, domesticated animals or wildlife in Papua New Guinea (PNG). In order to better understand the epidemiology of *Cryptosporidium* in PNG, a total of 504 faecal samples were randomly collected from goats (*n*=193) and sheep (*n*=311) in villages and institutional farms and a total of 614 fish were collected from freshwater and saltwater systems in PNG. All samples were screened for the presence of *Cryptosporidium* using PCR at the 18S locus and the positive isolates were sequenced and phylogenetic analyses were conducted. The overall prevalences were 5.2% (10/193) for goats, 1.9% for sheep (6/311) and 1.14% (7/614) for fish. The species/genotypes identified were *C. hominis* (*n*=6, only in goats), *C. parvum* (*n*=11, sheep, goats and fish), *C. andersoni* (*n*=1, in sheep), *C. bovis* (*n*=1, in a goat), and *C. scrofarum* (*n*=1, in a sheep), rat genotype II (*n*=1, in a goat) and a novel piscine genotype (*n*=2, in fish). This is the first report of *Cryptosporidium* spp. identified in sheep, goats and fish in PNG. The detection of *C. hominis* in goats suggests these animals may serve as reservoir for human infection.

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(20)

HISTOMONAS MELEAGRIDIS: RNASEQ ANALYSES AND TARGETED DRUG SENSITIVITY

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Histomonas meleagridis, a flagellated protozoan of the Order Trichomonadida, is the causative agent of blackhead disease in gallinaceous birds. Few genes have been identified in this organism; thus, little is known regarding the molecular basis for its metabolism, virulence, and antigenicity. To identify new genes, a cDNA library derived from a lab strain of H. meleagridis was sequenced and annotated. Data obtained from these experiments identified 3,425 *H. meleagridis* genes. Analysis of the data allowed the identification of 81 genes coding for putative hydrogenosomal proteins, and was used to determine the

codon usage frequency. Sequence information also identified bacteria that are cultured with *H. meleagridis*. *In vitro* assays of cultured parasites derived from a strain originating in Buford, Georgia, have illuminated future therapeutic strategies. Solutions of dilute cupric sulfate and zinc sulfate significantly inhibit *in vitro* growth at concentrations within recommended feed limits for turkeys and chickens. Parasite cultures at differing stages of growth were probed for sensitivity to each solution. Initial i*n vivo* translational studies using supplemental zinc in feed and water proved ineffective for experimentally-infected turkeys. Additional studies targeting the therapeutic delivery of supplemental copper to the turkey's ceca are currently underway. Future analysis of these transcriptomic data should provide valuable molecular insights into *H. meleagridis* and provide the platform for molecular studies aimed at understanding the pathogenesis of blackhead disease, while future biochemical studies are needed to determine the mechanism by which the metals kill the parasites.

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(21)

CHARACTERIZATION OF THE PUTATIVE ENDOCHITINASE IN *LEISHMANIA BRAZILIENSIS*

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Leishmania braziliensis causes cutaneous leishmainasis, a new world disease, prevalent throughout America, Mexico, and Argentina. During its life cycle, *Leishmania* transitions between two morphological forms. Through the bite of the sandfly (*Lutzomyia* or *Psychodopygus* (*sp*)), *Leishmania* promastigotes are transmitted to it mammalian host where they transform into the amastigote form. Changes in the morphology of this parasite are a result of gene expression regulated by cues it receives from the host environment. The goal of our lab is to determine if microRNA play a role in the gene expression in this protozoan parasite. Based on miRNA complement genome search endochitinase appear to be the target of a newly discovered miRNA, Lb-miR-1. To verify this experimentally, we must understand the gene expression and biology of the putative endochitinase. Here we show our work on the characterization of the putative endochitinase in *L. braziliensis*.

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(22)

EFFICIENCY OF A GENETIC TEST TO DETECT BENZIMIDAZOLE RESISTANT *HAEMONCHUS CONTORTUS* NEMATODES IN SHEEP FARMS IN QUEBEC

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Haemonchus contortus is a haemophilic nematode which infects sheep and causes anaemia and death of lambs. Benzimidazole drugs are used to remove these parasites, but the phenomenon of resistance has arisen worldwide. Fecal Egg Count Reduction Test (FECRT) which consists in counting parasite eggs in feces before and after treatment and comparing these results is used to detect anthelmintic resistance but is applicable only after treatment like all the current parasitological tests we have. A test to detect resistance before treatment would be a useful tool to enable farmers to anticipate the efficiency of the drug before drenching the flock. Benzimidazole resistance in *H. contortus* has been related to three mutations on the β-tubulin isotype 1 gene at codons 167, 198 and 200. These three mutations can be used as markers to detect benzimidazole resistance. In this study we compared a test to detect benzimidazole resistance based on detection of genetic markers in *H. contortus* before treatment with the FECRT. Eleven (11) farms from different regions of Quebec (Canada) participated in the study. Fecal samples were collected from 20 sheep before treatment then 10 of them were treated with a single dose of fenbendazole

and formed the treated group; the other 10 sheep remained untreated and formed the control group. Three samples of fresh fecal samples were randomly collected from the pasture in each farm. Nine (9) days after the first visit, fecal samples were collected on the 20 animals of treated and control groups. *H. contortus* eggs were counted specifically in pre and post treatment samples from control and treated groups. To differentiate between nematode species, a protocol based on peanut agglutinin conjugated to fluorescein isothiocyanate which specifically binds to *Haemonchus* eggs and not to those of other trichostrongyle species was applied; the *Haemonchus* eggs were visualized under ultraviolet illumination. With these data, we calculated the efficacy of the drug on *H. contortus*. Ten (10) pools of *H. contortus* eggs were prepared for each farm and the level of resistance was estimated based on resistant allele frequency at the three markers of resistance on β-tubulin isotype 1 gene. We observed a low occurrence of the nematode (Mean epg before treatment = 541 epg) but a high level of resistance to benzimidazole (mean efficacy of the drug = 29%). Comparing the genetic test with samples from individual sheep or off pasture, with the FECRT, we conclude that the genetic test can be applied to field samples to estimate benzimidazole resistance levels before treatment for *H. contortus* infections. This genetic test could be developed as a routine test in laboratories. Detection of resistance before treatment saves a considerable amount of time and money on farms where large numbers of sheep need to be treated.

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MECHANISTIC INSIGHTS OF SIDER2 RETROPOSON-MEDIATED MRNA DECAY IN *LEISHMANIA*

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We have shown previously that truncated versions of formerly active retroposons, SIDER2 (Short Interspersed DEgenerate Retroposons), located mainly in 3'UTRs of Leishmania transcripts, promote mRNA decay by a novel mechanism involving endonucleolytic cleavage without prior deadenylation. Endonucleolytic cleavage occurs at the beginning of the second conserved 79-nt SIDER2 signature sequence. Mutagenesis studies to alter sequence or structure within the putative cleavage region were carried out and confirmed the importance of secondary structure for endonucleolytic cleavage and mRNA decay. Integration of a reporter gene into genomic loci regulated or not through the SIDER2 pathway revealed that only transcripts harboring an active SIDER2 element were subjected to a rapid decay in a species and a stage-specific manner. We further showed that introducing a hairpin structure upstream of a reporter gene regulated by SIDER2 blocked translation and increased reporter transcript accumulation, suggesting that SIDER2-mediated decay requires ongoing translation. We are currently searching for protein factors involved in SIDER2-mediated mRNA decay using a tethering approach based on the co-transfection of the bacteriophage MS2 coat protein with its cognate RNA hairpin located upstream of a SIDER2-harboring 3'UTR as part of a reporter gene construct. Also, in situ hybridization studies are under way to assess if SIDER2-mediated mRNA decay takes place in a specific cytoplasmic location as opposed to non-SIDER2 stable transcripts. Collectively, these studies provide new insights into the SIDER2-mediated decay mechanism of unstable transcripts in Leishmania.

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(24)

SEQUENCING OF METACERCARIAE OF *CLINOSTOMUM* SP. (DIGENEA: PLATYHELMINTHES) IN A NEOTROPICAL FLOODPLAIN IN BRAZIL: ITS AND COI REGIONS

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Clinostomum species had been focus of a great number of taxonomic reviews especially because of their high interspecific morphological similarity. On the metacercarial phase, these parasites cause the "yellow spot disease", and are usually found in fishes from the upper Paraná River floodplain, Brazil. Although the specimens collected in this region had been previously identified by morphological characters as *Clinostomum marginatum* (Rudolphi, 1819), there are some uncertainty about specific classification, if these parasites are really of the same specie or just congeners. The aim of this study was to use molecular tools (ITS and COI sequences) to confirm the taxonomic classification of these metacercariae collected on the upper Paraná River floodplain. Metacercariae were recovered from *Loricariichthys platymetopon* (Siluriformes: Loricariidae) collected between June/2011 and September/2012. It was used the anterior third of parasite specimens body to the DNA extraction. The posterior regions of the body were preserved to subsequent morphological identification. The sequences were obtained by using 6 specimens in ITS and 5 in COI. When ITS sequences of sampled metacercariae were compared to those present on GenBank and Bold System, the p distance estimated were 0.01-0.02 (1-2%) between them and *C. marginatum*, with an intraspecific variation of 0.00-0.01 (0-1%). However, when COI sequences were performed, it was observed a great divergence between *C. marginatum* and the sampled specimens the p distance was 0.08-0.17 (8-17%), what showed that they weren't the same species. The same occurred when these metacercariae were compare with *C. complanatum* (p distance= 0.19). The intraspecific variation in COI analysis (0-2%) of Brazilian specimens showed that all they could be included in the same species. These results suggest that ITS sequences are not good molecular markers for these species, but the COI sequences were able to separate them satisfactory. Moreover, the Brazilian Clinostomidae species could be another one, different of those found in North America and in Europe.

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POPULATION GENETIC ANALYSIS OF *BAYLISASCARIS PROCYONIS* USING MICROSATELLITE MARKERS

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Raccoons are widespread and abundant throughout the United States. The ability of raccoons to utilize anthropogenic resources often puts them into conflict with humans, and in addition to property damage, contact with raccoons puts humans at risk for zoonoses such as *Baylisascaris procyonis*, or raccoon roundworm. Although human infections are rare, the consequences of larva migrans (LM) caused by *B. procyonis* are serious and life threatening. Many aspects of *B. procyonis* have been described because of its zoonotic potential, but population genetic investigations have been limited. Understanding the patterns of genetic structure in natural populations of *B. procyonis* will provide information on parasite gene flow throughout host populations, and may in turn inform patterns of population structure in raccoon hosts. Preliminary work based on cox-1 mtDNA haplotypes suggests high levels of gene flow in *B. procyonis* populations from the Northeast and Midwest, whereas populations in the western U.S. appear to have more restricted gene flow. This study presents the first application of microsatellite markers to assess the genetic diversity of *Baylisascaris procyonis* collected from raccoon populations across the U.S.A. These nuclear markers provide an independent assessment of genetic structure from that based on mtDNA haplotypes. Specimens were obtained from 12 U.S. states, and screened with 10 polymorphic microsatellites developed for *B. procyonis*.

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SMALL IS NOT BORING: THE TINY, BUT VARIABLE, MITOCHONDRIAL GENOMES OF COCCIDIA, PIROPLASMS, HAEMOGREGARINES AND HAEMOSPORINIDS (APICOMPLEXA)

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Mitochondrial genome sequencing has been limited for parasites in the Apicomplexa due to lack of suitable primers and presence of nuclear-encoded mitochondrial sequences (numt's) that hamper PCR-based methods of obtaining MT genome sequences. Although mitochondrial genomes of Apicomplexa are relatively conserved with respect to their small sizes (6-7kb in most cases) and with each encoding three critical enzyme genes (cytochrome *c* oxidase subunit I [COI], cytochrome *c* oxidase subunit III [COIII] and cytochrome B [CytB]) and various fragmented rRNA's, their structures and gene arrangements are highly variable. PCR-based amplification, sequencing and genome cloning approaches generated mitochondrial (Mt) sequences from a number of apicomplexan parasites. Three complete Mt genomes were obtained from a single-oocyst-derived clonal line of *Eimeria mitis* (USDA 50). Novel start codon positions are suggested for both COI and COIII that appear conserved among all *Eimeria* genomes available. Interestingly, sequence variation was found among the concatenated copies of the Mt genome of *E. mitis*. The first complete Mt sequence from an adeleid parasite, *Hepatozoon clamatae*, was obtained that had a unique Mt genome organization within the Apicomplexa. Despite considerable effort, only partial Mt sequences have been obtained from the Toxoplasmatinae (*Cystoisospora suis, Cystoisospora felis, Hammondia heydorni* and *Toxoplasma gondii*) likely due to numerous numt's in these parasites. Across the phylum Apicomplexa, genome structure [order and direction of the CytB, COI, and COIII genes] was conserved among closely related parasites (e.g. eimeriid coccidia) but highly divergent between groups of parasites such as coccidia, piroplasms, haemogregarines and haemosporinids. Molecular phylogenies based on Mt and nuclear (NU) sequences are in reasonable agreement suggesting that the combined data may be capable of producing a 'stable' evolutionary history for apicomplexan parasites.

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PROTISTAN DIVERSITY IN BALLAST WATER REVEALED BY AMPLICON-BASED 454 PYROSEQUENCING

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Over the last few decades, the shipping industry has experienced an increase in vessel traffic worldwide resulting from an increased demand in international trade markets. The steady increase in shipping volume has the potential to have major impacts on the marine realm due to the transfer of both macro and microorganisms in ballast tanks and on the underwater surfaces of vessels. Previous studies of ballast water communities have focused on only characterizing the presence of one to a few target microbial species (e.g. *Vibrio* spp.) at a time, thus not characterizing the total diversity present. Past studies have reported toxic dinoflagellates transported in ballast water, and many additional parasitic and toxic taxa, in addition to free-living microorganisms, are likely to be transported in ballast water. In this study, we utilized amplicon-based 454 pyrosequencing using two universal primer sets that amplify either the V4 or the V9 domain of the small subunit of the ribosomal gene complex in order to examine protistan diversity associated with ballast water from bulk carriers entering the Chesapeake Bay from foreign ports. Sequences were subjected to quality filtering and analysis using QIIME, Megan, and the phyloseq package for the R software. Sequences were obtained from a wide range of groups that contain free-living unicellular marine organisms including Choanoflagellida, Centroheliozoa, Cryptophyta, Rhodophyta, Strameophiles, and Rhizaria. However, the majority of protistan sequences obtained belonged to members of the Alveolata. Within the Alveolata, sequences were recovered from a number of parasitic groups including Coccidia, Blastodiniales, Syndiniales, and Perkinsida. In addition to the Alveolata, sequences were also recovered from other potentially parasitic groups including Amoeboza, Euglenozoa, Fungi, and Ichthyosporea. Sequences from parasitic groups were recovered from ballast water taken at the surface as well as water from the bottom of the tank. This study provides the first documentation for a wide range of protistan taxa, including parasitic taxa, in the ballast water of ships, suggesting such transport should be considered in the management of ballast water and also the biogeography of microorganisms.

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UNUSUAL PRESENTATION OF CHRONIC GIARDIASIS REFRACTORY TO INITIAL TREATMENT

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Chronic giardiasis can present with mild symptoms months to years after the initial infection and may often result in a missed diagnosis. Internationally, chronic giardiasis can result after multiple treatment regimens due to newly emerging drug resistant strains. In this case report, the medical record of a 29-year-old female patient with chronic giardiasis is reviewed. The patient remained infected and undiagnosed for seven months until a routine laboratory fecal examination found active trophozoite stage *Giardia intestinalis* (*G. duodenalis*) in her stool sample. The patient had a 7-year lag time between an episode of acute diarrheal illness in Mexico and the onset of persistent, mild-to-moderate gastrointestinal symptoms. Perhaps this unusual clinical picture contributed to her physicians' low index of suspicion for giardiasis. The patient required multiple treatments with metronidazole and albendazole separately and later taken together, before a clinical response was observed. Chronic giardiasis should be considered in any patient with persistent nonspecific gastrointestinal symptoms, especially if travel to a developing country has ever occurred in the patient's history. Infection should be diagnosed using accepted laboratory standards. The failure of treatment of a presumed infection does not rule out giardiasis because chronic cases can be refractory to single drug regimens or the *Giardia intestinalis* may be resistant to the prescribed medication. Cases are most likely undiagnosed or misdiagnosed because of a lack of access to proper healthcare in rural or underserved areas or due to inadequately trained healthcare providers. The epidemiology of *Giardia* has changed dramatically over the past decade due to the presence of the different *Giardia* species, strains, and genotypes and their wide and variable host ranges. Only molecular testing (e.g. polymerase chain reaction) can be used to accurately identify the subtypes of *Giardia,* although there are currently many new methods being developed. Our understanding of giardiasis transmission can be improved through the systematic use of molecular diagnostic tools in well-designed epidemiological studies conducted in various socioeconomic and geographic settings.

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A COMPARISON OF POPULATION PARAMETERS OF *CLINOSTOMUM MARGINATUM* FROM SMALLMOUTH BASS TAKEN FROM DIFFERENT UPLAND STREAMS IN ARKANSAS

J.J. Daly

Smallmouth bass (*Micropterus dolomeiu*) were taken from 16 different locales on three Ozark and Ouachita upland streams in Arkansas and examined for *Clinostomum marginatum* offering the opportunity to see what similarities or dissimilarities existed between the spatially different populations of the parasite. Population parameters examined by regression analysis were prevalence, maximum abundance, mean abundance, mean standard deviation, mean intensity, mean intensity standard deviation, dispersion, skewness and kurtosis. Examination of parasite population curves showed similar geometries and all but two adhered to a negative binomial distribution reinforced by standard deviations being larger than the means. Maximum abundance, mean abundance, standard deviation, mean intensity, and mean intensity standard deviation showed very high correlations (< 0.001 p) and predictive values. Prevalence showed little significance with any of the other parameters. This is partially due to when high levels of prevalence (close to or at 100%) are reached that the infection may still have increasing intensities. Dispersion was significantly correlated with maximum abundance and standard deviations. Skewness and kurtosis showed little significant relationships with any of the other population parameters. Skewness and kurtosis showed close affinity as expected since they are descriptors of the shapes of the population curves. The data shows that despite disparate distances of locales and different intensities of infection that the biological and environmental conditions at the different locales produce similar parameter-related populations of *C. marginatum*.

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PHENOTYPIC PLASTICITY IN HAPTORAL STRUCTURES OF *LIGOPHORUS CEPHALI* (MONOGENEA: DACTYLOGYRIDAE) ON THE GILLS OF *MUGIL CEPHALUS* (TELEOSTEI: MUGILIDAE): A GEOMETRIC MORPHOMETRIC APPROACH

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A geometric morphometric analysis was carried out to assess the morphological variation of shape and size of sclerotized haptoral structures of *Ligophorus cephali*. The aims of this study were (i) to investigate the phenotypic plasticity of the ventral and dorsal anchors according the site selection in the gills, and (ii) examine to what extent the size and shape of these structures covary. We examined 213 *L. cephali* from *Mugil cephalus*, collected in the Albufera Lake, Valencia, Spain. Variation in shape was examined using geometric morphometrics, which is a statistical analysis of shape based on Cartesian coordinates, after separating shape from the overall size, position and orientation of the landmark configurations. The significance of interspecific variation in the shape of haptoral structures was analyzed by a permutational analysis of variance (PERMANOVA) using a crossed design on the matrix of variable shape, where gill arch, gill section, and gill area were considered as fixed factors, host as a random factor and centroid size as a covariate. A PCA analysis revealed important shape changes in the haptoral structures, with the increase in size and linear change in shape of the selected landmarks. A Canonical Variate Analysis revealed significant morphological differences within *L. cephali* in relation with gill arch and section. The PERMANOVA showed significant associations between dorsal anchor morphology and gill-arch-section and with respect to ventral anchor the arch-section-area designs. Thus, our results indicate that morphological variation in *L. cephalus* is due to differences in shape in the specific site selection and likely are influenced by gill morphology at the site of attachment. Therefore *L. cephali* offers an interesting model to investigate to what extent the phenotypic variability of the attachment organ within species is related to environmental variables or to host specificity.

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454 SEQUENCING STUDIES OF FIELD-DERIVED UNINFECTED AND *SCHISTOSOMA MANSONI*-INFECTED *BIOMPHALARIA PFEIFFERI* FROM ASAO STREAM, WEST KENYA

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E.S. Loker, University of New Mexico

The majority of the world's cases of *Schistosoma mansoni* occur in sub-Saharan Africa. Although the Neotropical snail *Biomphalaria glabrata* has been and will remain an important model host for *S. mansoni*, it is also important we understand how this parasite interacts with its widespread African snail hosts, the hosts in which this parasite first evolved. One such snail is *Biomphalaria pfeifferi*. Also of interest is a need to explore host-parasite relationships focusing on naturally infected snails from the field. With these goals in mind, we have undertaken 454 sequencing of field-derived *Biomphalaria pfeifferi* to define the transcriptional repertoire of an uninfected snail, and a snail with a natural, patent *S. mansoni* infection (both taken from Asao stream, west Kenya, and processed directly for sequencing). Over one million reads from the uninfected snail and >1.2 million reads from the infected snail were returned. A hybrid analytical approach using both reference-assisted and *de novo* methods of assembly and assessment was used. Three databases have been compiled and used as a reference: 1) *B. glabrata* genome, 2) *S. mansoni* genome, and 3) a compilation of Archaea 16S, completed bacterial genomes, completed viral genomes, bacteria 16S, fungi 18S, and fungi 28S. Total reads were compared against each

database individually, as well as in parallel to obtain the best match for each read. Homologs of ten genes found in previous studies with *B. glabrata* to be responsive to *S. mansoni* infection have been found in *B. pfeifferi*. These are FREP3, FREP4, Dermatopontin, HSP70, Peroxiredoxin, SOD Cu-Zn, FREP2, Galectin-4, MIF and Matrilin-A. The 454 transcriptomics data acquired from uninfected *B. pfeifferi* indicates a uniformly high level of homology with the corresponding *B. glabrata* genes, ranging from 78-98% nucleotide identity. This is not surprising given the close phylogenetic relationship between the two snail species. Further analyses are underway with respect to identifying transcripts derived from *S. mansoni* intramolluscan stages, and possibly from third party symbionts (viruses, bacteria, fungi among others) that may also be commonly expressed in snails from the field. This study was supported by NIH grants P20RR18754 and R01 AI101438.

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DISEASE DYNAMICS OF AVIAN HAEMOSPORIDIA IN A CALIFORNIA SONGBIRD COMMUNITY

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Plasmodium and the malaria-like genera *Haemoproteus* and *Leucocytozoon* - collectively known as haemosporidia - are relatively common in California birds, which have coevolved with the parasites; however, although avian malaria has been shown to affect the health and fitness of birds worldwide, very little information is available on the prevalence and distribution of these blood parasites in California. While many studies have addressed prevalence (% of population infected) and parasitemia (parasite load in individuals) for avian haemosporidia, we know little about seasonal variation in diversity and prevalence within populations. In addition, in many studies, birds are sampled only once during a study, or are sampled multiple times within a short time, e.g., the period during which an adult is caring for nestlings. This provides an indication of infection status and prevalence within a population at a single point in time, but does not increase understanding of the temporal dynamics of chronic infections or the conditions under which recrudescence occurs. The research presented here identifies some of the key players and seasonal dynamics of avian haemosproidia transmission and persistence by resampling avian hosts in a California riparian songbird community, over multiple seasons for two years. Our results confirm a high diversity of haemosporidia in the avian community being studied, including 24 distinct lineages in 112 of 400 (28% overall prevalence) bird blood samples analyzed to date. Our research shows strong patterns of seasonal parasite diversity and a dominant parasite infecting a broad range of hosts. In addition, a range of migrant and resident avian host species of varying ages and breeding status have been sampled, allowing for insights into host-specificity at the parasite species, lineage, or, even, genotype level, as well as correlations between infection status and life history traits. Also, twenty of the individuals captured to date have been recaptured at least once, and the infection status of some of these birds has changed from “present” to “absent”. Blood smears are used to confirm infections in these birds where PCR may have failed to do so, and to identify new morphospecies and investigate coinfections. The spread of avian malaria continues to pose a serious threat to the conservation of naïve endemic island birds throughout the world, especially in light of the potential effects of global warming on hosts, vectors and parasites. Although birds that have co-evolved with the parasites appear to be more resistant to haemosporidia, it can cause varying degrees of pathology and mortality, especially in naïve bird species. Recent studies have shown that, in non-naïve hosts, infected parental birds experience fitness costs; specifically, a decrease in the number and quality of offspring they can raise. Although the project sampling is based on a California songbird community, the results obtained in this avian model system have the potential to inform other biological systems, with implications in conservation biology, human epidemiology and vector control.

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MICROSPORIDIAN INFECTION DURING THE DEVELOPMENT OF A FREE-LIVING MARINE NEMATODE

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Microsporidia are unicellular fungal parasites of animals. Their life cycle may be divided into two stages: a sporal extracellular stage and an obligate intracellular stage (meront). Recently, we discovered a novel microsporidian species, *Sporanauta perivermis*, infecting the free-living marine nematode *Odontophora rectangula*. *S. perivermis* infection was chronic, mild, and highly prevalent (over 80%). Infection was restricted to the hypodermal and muscle tissues of adult nematodes. In addition, we showed that eggs were infected in adult females suggesting that *S. perivermis* may be transmitted vertically. Most recently, we have examined *S. perivermis* infection in juveniles of *O. rectangula* nematodes by fluorescent *in situ* hybridization (FISH) and transmission electron microscopy (TEM). *S. perivermis* appeared to be at the meront stage during most of the development of infected juveniles. It also appeared that *S. perivermis* meronts differentiate into spores (sporogony) during the last juvenile stage before nematodes molt into adults. Overall, our results suggest that *S. perivermis* life cycle is linked to the developmental changes of its host.

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(34)

PARASITE ASSEMBLAGES AS USEFUL INDICATORS OF HOST MOVEMENT

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The distribution of trophically transmitted parasites is defined largely by the geographical ranges and movements of their hosts. Changes in host distribution have the potential to alter the ranges of parasites. The Double-crested Cormorant (*Phalacrocorax auritus*, Pelicaniformes) has undergone both range contraction and expansion over the last century, potentially altering the occurrence of parasite infections in intermediate hosts of digenetic trematodes, such as recreationally and commercially important fishes. Interestingly, the recent population bottleneck of the Double-crested Cormorant has muddied the delineations of subspecies and populations. Here, we show how parasite assemblages can be used to assess colony cohesion and determine the likelihood of cross-seasonal movement and interactions among *P. auritus* in the United States. For this assessment, we used a suite of the 16 parasites recovered from 218 *P. auritus* collected from 11 total locations across Mississippi, Alabama, Minnesota, and Vermont. Most of the parasites were digenetic trematodes including *Histeromorpha tribloba*, *Austrodiplostomum ostroweskiae*, and *Drepanocephalus spathans*, although Cestodes (Dilipidae) and Nematodes (Capillaridae and Anisakidae) were also included. The suites of parasites used in our assessments were chosen based on ordination of presence/absence data using a Principle Coordinates Analysis. Parasite presence appears to be a metric as powerful as infection intensity in assessing dissimilarity of parasite assemblages among hosts, although prevalence may be more useful when assessing similarities of parasite communities of different host populations or colonies. Here we show that similarity of parasite assemblages is not geographically explicit and we further demonstrate the degree to which resident avian colonies differ in their parasite infections when compared to migratory birds of the same species.

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(35)

IN VITRO SYSTEMS FOR INTRAMOLLUSCAN STAGES OF TREMATODES- WILL THE TRICK USED FOR FRESHWATER SYSTEMS ALSO WORK FOR MARINE ONES?

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In vitro systems for trematode parthenitae provide a valuable tool for parasitology research, allowing us to tackle questions concerning trematode sociality, interspecific interactions, and development. However, development of appropriate media has been particularly challenging. Following years of effort, a highly successful co-cultivation method was developed for freshwater systems by using the *Biomphalaria glabrata* embryonic (*Bge*) cell line. However, much less is known about *in vitro* systems for trematodes infecting marine snails. Marine *in vitro* systems reported so far are axenic and use the commercially available Leibovitz's L-15 medium, adjusting osmolarity as needed for marine invertebrates. These techniques have had only partial, short-term success. For instance, trematode rediae that infect the marine snail *Cerithidea californica* remain viable for only 23 days on average (60 days max). The rediae merely survive during that time period, with no further growth or development. Hence, we lack a successful, long-term medium for *in vitro* culture of marine trematodes. Is it possible to follow the success of freshwater *in vitro* methods and use snail cell lines for marine trematode culture? The freshwater *Bge* cell line will likely die in the highly saline conditions of marine media. Unfortunately, it is currently impossible to use a marine snail cell line, as no such lines have been successfully established. However, two findings from freshwater systems using the *Bge* cell line suggest a possible approach. First, co-cultivation with *Bge* cells is not necessary for culture success; media that has been only preconditioned with *Bge* cells has permitted parthenitae growth and development. Second, the *Bge* cell line works even for trematodes that use freshwater snails other than *B. glabrata* as first intermediate host. Hence, we asked whether the *Bge* cell line releases factors that can promote the *in vitro* development of marine trematode parthenitae. We conducted a series of *in vitro* tests using different kinds of media: i) The previously described, marine L-15 medium, ii) *Bge*-conditioned marine medium and iii) Co-cultivation with *Bge* cells. We present results from these studies, and compare them to performance observed in previous studies.

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GENETIC STRUCTURE OF TWO INTERMEDIATE SNAIL HOSTS THROUGHOUT NEW YORK WITH COMMENTS ON THEIR SYMBIONTS

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The geographic distribution of intermediate host genetic variation has important implications for the evolution and adaptation of parasites and other symbionts. While freshwater snails often appear to have limited innate dispersal capabilities, many species have broad distributions, encompassing waterways connected by various degrees. For many species, the historical and contemporary factors influencing the genetic connectivity of populations are unknown. The goals of the present study were to examine and compare the geographic distribution of genetic variation within the pulmonate snail *Promenetus exacuous* and prosobranch snail *Valvata tricarinata* throughout New York, and collect preliminary information on all associated symbionts for future comparative work. Initial analyses utilizing mitochondrial DNA sequences revealed a lack of geographic isolation of major genetic lineages, with several lineages dispersed among major watersheds throughout the state, especially for *P. exacuous*. The commensal oligochaete *Chaetogaster limnaei limnaei* was the most common symbiont encountered and was associated with 3% of *V. tricarinata*, representing two out of 11 collections sites, and 51% of *P. exacuous*, representing 11 out of 12 sites. Trematodes were recovered from 1% of *V. tricarinata*, representing two sites, and 10% of *P. exacuous*, representing six sites.

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THE NERVOUS SYSTEM OF DIPLOSTOMID METACERCARIAE (DIGENEA) INFECTING THE CATFISH *CLARIAS GARIEPINUS* (CLARIIDAE) IN FRESHWATERS OF TANZANIA

F.D. Chibwana and **G. Nkwengulila**, University of Dar es Salaam

The nervous systems of three diplostomid metacercariae (two *Tylodelphys* species and *Diplostomum mashonense*) occurring in the cranial cavity of the catfish, *Clarias gariepinus,* were examined by the activity of acetylthiocholine iodide (AcThI) in order to better understand the biology of these parasites and thus provide information for taxonomic purposes. Enzyme cytochemistry demonstrated a comparable orthogonal arrangement in the three metacercariae like in other digenean trematodes; the central nervous system (CNS) consisting of a pair of cerebral ganglia, from which anterior and posterior neuronal pathways arise and inter-link by cross-connectives and commissures. However the number of transverse nerves was significantly different in the three diplostomid metacercariae; *Tylodelphys* sp. 1 (30), *Tylodelphys* sp. 2 (21) and *D. mashonense* (15). The observed differences in the nervous system of the three metacercariae reflect three systems derived from three different cercariae yet to be described in freshwaters of Tanzania.

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SCHISTOSOMIASIS: WHAT INNING ARE WE IN - AND WHAT'S THE SCORE?

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The SCORE, in this case, is the Schistosomiasis Consortium for Operational Research and Evaluation. And to run the baseball analogy into the ground – based on two World Health Assembly (WHA) resolutions (WHA 54.19 & WHA 65.21) on schistosomiasis we soon need to start hitting more "singles, double, triples and homeruns," and be getting ready to bring in our "closer." However, while it might be time for that in some ballparks, in a lot of others we are still in the early innings – but at least the game has started! Founded on a lot of research over many years and based upon having a therapeutic drug (praziquantel) that is now donated and associated with only minor side effects, which allows for mass drug administration (or Preventive Chemotherapy) over the last 12 or so years the world of schistosomiasis control and elimination has changed rapidly. SCORE seeks to play a large role in providing data resulting from operational research that will help develop sound policy and guidelines to assist countries in gaining and sustaining morbidity control of schistosomiasis – and where feasible to move on to eliminate transmission of this disease of more than 240 million people. The SCORE Secretariat is based at the University of Georgia, but the consortium currently consists of more than 50 investigators, in 24 institutions, in 17 countries. SCORE members are asking research questions that we hope will provide data upon which to make decisions about how to most effectively deliver praziquantel in different transmission settings and the optimum combination of interventions to move to the elimination of transmission. In addition SCORE investigators are optimizing and evaluating different diagnostic tools to assist in gaining and sustaining schistosomiasis control and moving on to the elimination of schistosomiasis. For example, based on its evaluation in 5 African countries, SCORE has recommended the use of a commercially available point-of-contact, cassette-based, urine-based antigen detection tool for the mapping needed to allow better integration and design of control programs. This presentation will outline what SCORE is and what it does, and will demonstrate the critical links between basic and operational research and the informational and research feedback loops that will be essential if we are to drive in the winning run in the 9th inning.

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THE BIOLOGICAL IMPACTS OF CONTROL PROGRAMS: DO SCHISTOSOMES ADAPT AND EVOLVE TO A CHANGING WORLD?

J.P. Webster, Imperial College London, Faculty of Medicine

Parasite evolution is increasingly being recognized as one of the most important challenges in applied evolutionary biology. Environmental change, through natural and/or anthropogenic factors, can modulate infection prevalence, intensity and disease severity. Understanding how parasites maximize fitness whilst

facing such diverse challenges and selective pressures is central to successful disease control and offers a novel testing ground for evolutionary theory. For schistosomes, the causative agents of the water-borne disease schistosomiasis, recent changes in selective pressures following, for instance, increased mass drug administration programmes, combined with new dam constructions/irrigation systems and/or altered agricultural practices, may all impact the availability of suitable definitive and intermediate hosts for such parasites, and hence potential for both intra- and inter-specific interactions within such hosts. Furthermore, when humans and their livestock come into closer water contact, novel zoonotic hybrid schistosome species may be predicted to evolve and establish as a consequence, with subsequent change in parasite life history traits, transmission potential and virulence. However, we often lack empirical studies of such potential evolutionary changes under natural conditions, which are imperative if we are to fully understand the patterns of schistosome distribution and transmission across changing environment with the ultimate aim of controlling this disease of profound medical and veterinary importance. Here, using data gathered from a range of experimental and epidemiological studies encompassing both the laboratory and field, I demonstrate how schistosomes maximize their fitness under: a) carefully controlled differential selective pressures in the laboratory; b) long term disease control programme selective pressures across Asia; and c) recent disease control programme selective pressures across sub-Saharan Africa. The results presented are discussed in terms of their theoretical and applied implications.

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CONTROLLING SOIL TRANSMITTED HELMINTHS: TIME TO THINK INSIDE THE BOX?

J.M. Hawdon, The George Washington University Medical Center

Soil transmitted helminths (STH) continue to plague poor people in developing countries. Over 1 billion people are infected with one or more of the STHs, *Ascaris lumbricoides, Trichuris trichiura,* and the hookworms *Ancylostoma duodenale and Necator americanus*. While rarely fatal, infections cause anemia, cognitive and physical stunting, and malnutrition. The World Bank estimates that more than 20% of the disability-adjusted life years (DALYs) lost from communicable diseases among school-age children living in endemic areas are a direct result of intestinal nematodes. The socioeconomic costs act as a brake on development, and perpetuate cycles of poverty. Currently, there are two control strategies that are actively being pursued. Anthelmintic treatment can help reduce morbidity by removing a proportion of an individual's worm burden. Several mass drug administration (MDA) programs are currently underway, usually as an add-on to control programs for other parasites. The second control strategy is development of a hookworm vaccine. The Human Hookworm Vaccine Initiative (HHVI) is pursuing a recombinant vaccine against *N. americanus*, and has brought 2 antigens to Phase 1 clinical trials. In this presentation, I will discuss these strategies in detail, including why neither strategy is a viable long-term solution for STH control. A new, innovative multi-faceted approach towards STH control in China will be discussed.

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DRUG DISCOVERY FOR NEGLECTED DISEASES: CHALLENGES FOR SCIENCE AND SOCIETY

STOLL STUNKARD LECTURE

C.F. Nathan, Weill Cornell Medical College

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EFFECTS OF PREDATION AND PARASITISM ON CLIMBING BEHAVIOR ON THE MARINE SNAIL, *CERITHIDEA SCALARIFORMIS*

B. Belgrad and **N.F. Smith**, Eckerd College

In mangrove marshes in southern Florida, the marine snail, *Cerithidea scalariformis* exhibits a tidally-induced climbing behavior in which snails forage on the muddy substrate during low tides, then climb the aerial roots of mangrove trees on incoming and high tides. To test the conventional explanation that snails vertically migrate to avoid predators, we conducted field and laboratory studies to determine the role of predation and parasitism (by larval trematodes) in explaining this behavior. In field experiments with tethered snails, individuals experienced significant rates of predation between 0 – 8 cm above the substratum, which declined to zero at tethered heights at or above 28 cm. Behavioral data show a significant and positive association between snail climbing distance and tidal height regardless of infection status, and there was no significant effect of trematode infection on climbing distance. We conducted a laboratory experiment to determine if climbing behavior is an avoidance mechanism from predatory blue crabs, *Callinectes sapidus*, and if the behavior is influenced by parasitic infection. Results show that in the presence of blue crabs, uninfected snails climbed more than 53% higher than parasitized snails, and 37% higher than they would without the presence of a predator. In tanks without blue crabs, parasitized snails migrated significantly less than uninfected snails. Our data suggest that predation and parasitism are significant driving forces behind the climbing behavior of *C. scalariformis* whereby parasitic infection may affect their interactions with other salt marsh species. Reduced climbing distance may be an adaptive benefit that keeps snails submerged or closer to the substratum, providing favorable conditions for free-swimming cercariae to find a second intermediate host such as crab or fish.

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EFFECTS OF METACERCARIAL INFECTION ON BIOGEOCHEMICAL CYCLING: *POSTHODIPLOSTIMUM MINIMUM* AND *COTYLURUS FLABELLIFORMIS*

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Parasitic infection alters the metabolic demands of the host organism leading to changes in nutrient cycling at the organismal level. The additional and distinct metabolic needs of parasitic infection can shift host stoichiometric needs and lead to differences in assimilation, feeding, and excretion rates for different nutrients. These changes within the host are significant at the ecosystem level if both host and parasite biomass are sufficiently large as to exert overall control over ecosystem biogeochemical cycling as has been shown for both aquatic snails and fish. Here, we explored this potential at two different locations: (1) a Colorado state fishery were infection with the metacercarial stage of the trematode parasite *Posthodiplostimum minimum* is known to be widespread in bluegill, and (2) a series of 20 ponds in northeastern Colorado in which prevalence of the metacercarial stage of the trematode parasite *Cotylurus flabelliformis* varies. All 20 ponds were surveyed for water chemistry and *Cotylurus flabelliformis* infection in August 2010 and 19 of these 20 ponds were resurveyed during the summer of 2011 for water chemistry, infection, and periphyton nutrients. In August 2012, 115 snails were collected from a single pond and sealed individually into centrifuge tubes for a 3 hour excretion experiment. Following the experiment, water and snail feces were collected from the centrifuge tubes for nutrient analyses. Snails were dissected to determine infection intensity of *Cotylurus flabelliformis* and snail feet were collected for nutrient analyses. On September 20, 2012, 40 second-year bluegill were obtained from the Colorado Division of Wildlife's Wray Fish Hatchery in Wray, CO. All fish were from the same cohort and reared in the same pond. Fish were placed individually into buckets for a 1 hour excretion experiment. Following the experiment, water was collected for nutrient analyses and fish were taken to the lab to determine infection intensity with *Posthodiplostimum minimum*. *Posthodiplostimum minimum* tends to

concentrate within the liver with other infection sites being the mesentaries, kidneys, and pericardium. Bluegill livers were removed and squash slides were used to count the number of *Posthodiplostimum minimum* metacercariae per liver. Fish and liver tissues were collected for nutrient analyses. While no significant effects on phosphorus cycling were found for either experiment, nitrogen excretion was positively correlated with infection for both the snail – *Cotylurus flabelliformis* ($p<0.05$) and the bluegill – *Posthodiplostimum minimum* ($p<0.05$) systems, probably as a result of the use of protein catabolism to support infection. Highly infected snails had significantly less body nitrogen ($p<0.05$). These parasite-mediated increases in nitrogen excretion can have significant consequences for ecosystem-wide biogeochemical cycling. Fish rearing operations are already concerned about ammonia toxicity and nitrogen leakage to surrounding environments. It could be that parasitic infection could exacerbate these already serious problems. In the pond complex in NE Colorado, *Cotylurus flabelliformis*-mediated excretion rates led to decreases in the TDOC:TDN ratio ($p<0.05$, $R^2=0.43$) and the accumulation of TDN ($p<0.05$, $R^2=0.17$) in the water column of these strongly nitrogen-limited ponds. Parasite-driven trends in biogeochemical cycling must be taken into account as they can be ecologically significant.

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(44)

PLAYING WITH PRINKLES: FACTORS SHAPING PARASITE COMMUNITY STRUCTURE IN THREESPINE STICKLEBACKS *(GASTEROSTEUS ACULEATUS)*

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D.J. Marcogliese, Environment Canada

The factors that structure parasite communities likely differ depending on the scale of observation: at larger scales, community structure may be determined by biogeography and evolutionary relationships between host and parasite; at smaller scales, communities may be shaped by environmental filters, hydrology, habitat quality, and the distribution or abundance of hosts. We examined the relative importance of selected ecological processes operating at different scales (local, regional, continental) in shaping parasite community structure and composition in Atlantic threespine sticklebacks (*Gasterosteus aculeatus*). Sticklebacks from 18 sites from across eastern Canada (n=553) were collected and examined for macroparasites. We identified 28 taxa, the most abundant being *Gyrodactylus* sp., *Brachyphallus crenatus*, *Podocotyle atomon*, *Contracaecum* sp., *Proteocephalus* sp., and *Schistocephalus* sp. Seven taxa were observed for the first time in threespine sticklebacks (*Ascocotyle* sp., *Lepocreadium setiferoides*, *Uvulifer* sp., *Cosmocephalus obvelatus*, *Cystidicola farionis*, *Paracuaria adunca*, and *Rhabdochona* sp.). We also compiled previously published data on the abundance of 16 parasite taxa from 22 European Atlantic coastal populations of sticklebacks (n=2337). We combined the two datasets and used GLM and multivariate approaches to compare the significance of ecological processes operating at different scales. At smaller scales, the only factors that significantly affected parasite community structure were host size distribution and salinity. At larger scales, parasite communities were largely structured by distance among sites, and to a lesser extent climate and latitude. Interestingly, examination of MDS and hierarchical clustering revealed that European and North American communities were significantly different, and North American sites were divided into two distinct clusters: one comprising all Southern Gulf of Saint Lawrence sites, and the other including all sites located north of the Laurentian channel, i.e. Newfoundland and Quebec. The implications of this result for the biogeography of North American stickleback populations include the possibility of multiple postglacial refugia for this species. Overall our data suggest that continental- and regional- scale factors play significant roles in structuring parasite communities in Atlantic threespine sticklebacks.

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(45)

EXPERIMENTAL EVIDENCE FOR ACQUIRED IMMUNITY TO *HALIPEGUS* SPECIES IN TWO SPECIES OF FRESHWATER SNAILS

H.A. Stigge and **M.G. Bolek**, Oklahoma State Univeristy

It is well known that larval trematodes can cause extensive pathology in snails, including complete castration. Previous studies show that some snails are able to clear infections of sporocysts and reverse castration; whereas castration caused by rediae is likely permanent. However, recent field work suggests that wild snails can lose infections with rediae of *Halipegus occidualis*. Therefore, *Halipegus* species may be good model systems to examine if snails infected with rediae are capable of self-curing and reversing castration. The goals of this study were to 1.) Determine if *Helisoma trivolvis* and *Physa gyrina* are able to clear infections of closely related *Halipegus* species, *H. occidualis* and *H. eccentricus*; 2.) Document the rates of recovery and castration reversal; 3.) Investigate the susceptibility of snails to reinfection. A total of 500 lab reared *H. trivolvis* and *P. gyrina* were isolated, starved, and fed eggs of a *Halipegus* species. Then, snails were isolated and observed for cercariae. Dead snails were dissected and examined for rediae and gonads. Snails that stopped releasing cercariae were challenged with a second infection by re-exposing them to eggs 21 days later. After an additional 90 days, they were dissected. A total of 274 *H. trivolvis* were infected. Impressively, 91 of them cleared the infection. None became reinfected and 25 snails laid eggs. In contrast, 379 *P. gyrina* were infected, but only 18 of the snails stopped releasing cercariae. Most of the *P. gyrina* died during prepatent or patent periods. These results indicate that *H. eccentricus* may be more pathogenic to *P. gyrina* than *H. occidualis* is to *H. trivolvis*. Given the life spans of these two hosts, it seems likely that the recovery of *H. trivovlis* could be an adaptation to increase fitness. *Helisoma trivolvis* is long-lived (2-3 years) so living through the infection and reversing castration can greatly increase its fitness over its lifespan; whereas, *P. gyrina* is comparatively short-lived (3 months), therefore it may be more beneficial to invest in reproduction during prepatent periods.

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(46)

INFLUENCE OF FOREST STRUCTURE AND HUMAN ENCROACHMENT ON PARASITE COMMUNITIES OF MANTLED HOWLER MONKEYS, *ALOUATTA PALLIATA*

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Most newly emerging diseases in humans are zoonotic, with 40% of those found in the tropics originating in wild primates. Several lines of evidence suggest that anthropogenic disturbances such as human encroachment upon tropical forests, agriculture, deforestation, and climate change all play an important role in transmission between people and wildlife. However, the pathway by which ecological change leads to pathogen emergence is still not fully understood. In order to identify the role that humans play in emerging diseases and to understand the causal chain leading to pathogen transmission, this study measured indicators of forest degradation and analyzed gastrointestinal parasites collected from human and Ecuadorian mantled howler monkey (*Alouatta palliata*) fecal samples using morphological and genetic methods. Across 96 howler monkey samples, eight genera of protozoa including two apicomplexans were detected (*Balantidium* sp., *Blastocystis* sp., *Chilomastix* sp., *Dientamoeba* sp. *Entamoeba* spp., *Iodamoeba* sp., *Cyclospora* sp. and *Isospora* sp.), along with four nematodes (*Enterobius* sp., *Capillaria* sp., *Strongyloides* sp., *Trypanoxyuris* sp.), and one platyhelminth (*Controrchis* sp.). Humans had lower parasite species richness and abundance when compared to monkeys, harboring *Entamoeba* spp., *Blastocystis* sp., and *Capillaria* sp. A multinomial logistic regression analysis was used to compare parasite species richness among howler groups with group size, forest structure, and distance to humans. Grouping of specific parasite species was analyzed across sampled howlers using repeated measures ANOVA (Tukey) and parasite burden was described using cluster analysis. Taken together, we present the intricate relationship that exists between anthropogenic disturbance of rainforest, human encroachment of wildlife habitats, and parasite communities of primate populations.

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OOCYST PRODUCTION AND SPOROZOITE VIABILITY ARE INVERSELY RELATED TO ENVIRONMENTAL TEMPERATURE OF DEVELOPING GAMETOCYSTS

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Generational persistence of a parasite in an ecosystem depends upon the production and maintenance of viable infective stages to ensure successful transmission. Gregarines undergo gametogenesis, fertilization, and sporogony in environmental gametocysts to produce infective oocysts, but these developmental phases are subject to the vagaries of environmental conditions. Prior studies have demonstrated that environmental temperature and humidity significantly impact oocyst production, but no prior study has isolated the effects of temperature on both oocyst production and oocyst viability. In this study, gametocysts of *Blabericola migrator* were collected the tiger striped hissing cockroach, *Princisia vanwaerebeki*, surface sterilized, and incubated at 10, 18, 27, 35, and 40 °C in a saturated water vapor environment. Gametocysts were observed daily over 8 days to assess both the rate and percentage of oocyst production (dehiscence) at each temperature. After dehiscence, an excystation bioassay was used to assess percent viability of oocysts produced at each temperature. No gametocyst produced an oocyst and thus no oocyst viability was observed at 10 or 40 °C. Within these extremes, the rate of dehiscence increased with temperature from 72% at 18 °C to 95% at 35 °C; however, oocyst viability dropped over the same temperature range from 57% at 18 °C to 2% at 35 °C. Thus the relationship between temperature and oocyst production is not a simple matter of temperature mediated growth rate, suggesting that oocyst production and sporozoite viability are linked but environmentally independent phenomena. Over a range of viable temperatures, the mechanisms underlying oocyst production and dehiscence respond directly to increasing temperature while those underlying sporozoite viability respond inversely to increasing temperature. Whether this pattern is specific to *B. migrator* or is a more generalized gregarine developmental pattern will be clarified by extending the experiment to other gregarine species.

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VIABILITY AND ENVIRONMENTAL LONGEVITY OF GREGARINE OOCYSTS

S.M. Steele, **R.E. Clopton** and **D.T. Clopton**, Peru State College

Persistence of a parasite in an ecosystem depends upon an encounter rate between viable infective stages and new hosts sufficient to maintain viable reproductive parasite populations. Infective stage quiescence is an obvious evolutionary strategy increasing persistence by increasing amount of time individual propagules remain viable in the environment. Gregarines transmit using environmental oocysts, but the longevity of oocysts in the environment is unknown. Prior anecdotal evidence has suggested that gregarine oocysts are long-lived but there are no formal studies that quantify viability or differences in viable longevity among species. In this study, gametocysts of *Blabericola migrator* were collected from the tiger striped hissing cockroach, *Princisia vanwaerebeki*, surface sterilized, and incubated at 22.2 °C in a saturated water vapor environment. Gametocysts were collected in cohorts over a 12-hour period and incubated for 3 days. Gametocysts dehiscing to produce oocysts were maintained in incubation and their percent viability was tested weekly over time using an excystation bioassay. On dehiscence, 78% of oocysts tested were viable. Percent viability declined over the first 7 days (68%) and was reduced by 40% 14 days after dehiscence (46% viability). Twenty-one days post-dehiscence viability was reduced to less than 1%. Environmental persistence of infective stages is predicted for a parasitic species with direct transmission, but this does not appear to be the case for the oocysts of *B. migrator*. Whether this pattern is specific to *B. migrator* or is a more generalized gregarine developmental pattern will be clarified by extending the experiment to other gregarine species.

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TRANSMISSION OF RACCOON ROUNDWORM, *BAYLISASCARIS PROCYONIS*, IN SOUTHERN CALIFORNIA

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Larval migration caused by the raccoon roundworm, *Baylisascaris procyonis,* can cause severe disease in both humans and wildlife. We are using a combination of field studies, lab work, and models to study the transmission of this parasite in raccoon populations in southern California. We have found *B. procyonis* in 80% of raccoons necropsied with higher prevalence and intensity observed in younger hosts. In infected raccoons, total fecal egg output increases with infection intensity and some of the variability in egg production may be attributed to both worm size and sex ratio. Pathology in raccoons is minimal; however, ingestion of eggs and the resulting tissue migrations of larval worms can cause severe disability and death in other animals. Rodents and birds that forage at raccoon latrines may increase their contact rate with infectious eggs. We monitored latrine sites and identified species with increased exposure risk. The pathology caused by larval *B. procyonis* infection in rodents and birds may increase parasite transmission to raccoons. Wildlife cameras were used to estimate how often raccoons consume infected hosts and to identify other predators and scavengers that may be exposed to larval *B. procyonis*.

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THE SECRET LIVES OF DICYEMID PARASITES: AN INSIGHT INTO CEPHALOPOD HOST POPULATIONS VIA ANALYSES OF THEIR DICYEMID PARASITE FAUNA

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Dicyemid mesozoan parasites are microscopic organisms found with high intensities in the kidneys of benthic cephalopods. They have uncertain affinities to the Protozoa or Metazoa, hence their intermediate 'Mesozoa' classification. Whilst these parasites body organisation is simple, with individuals comprised of only 8 to 40 cells, their life cycle is complex, with two stages of development and two modes of reproduction. Although 114 species of dicyemids have been described worldwide, information on the dicyemid parasite fauna of cephalopod species in Australian waters is scant, and nothing is known on dicyemid genetic diversity, not only in Australia, but worldwide. This gap in knowledge has lead onto my research investigating the dicyemid parasite fauna of southern Australian cephalopods. In particular, 10 cephalopod species from southern Australian waters were collected and analysed for dicyemid parasites using a combined morphological and molecular approach. For morphological analyses, stained kidney smears were examined using light microscopy to initially establish if hosts were infected, and then dicyemid characters were measured and drawn for new species descriptions. Seven out of 10 cephalopod species were found to be infected by dicyemid parasites, with 11 new dicyemid parasite species documented. For molecular analyses, the complete dicyemid COI gene plus non-coding region (~1,560bp, both found on a minicircle) was sequenced for each new dicyemid species and phylogenetic trees (Maximum Likelihood – PhyML and Bayesian Inference – MrBayes) were generated. The population structure of three endemic cephalopod species, including the iconic, economically-important, yet threatened giant Australian cuttlefish (*Sepia apama*), was then investigated using morphological and molecular analyses of their respective dicyemid parasite fauna. Note that previous studies have used parasite genotypes to assign hosts to their population of origin with higher probability than using the host's own genotype, due to the greater subdivision of parasites among their hosts. Currently all three cephalopod species are treated as single populations in the two gulfs, Spencer Gulf (SG) and Gulf St Vincent (GSV), in South Australian waters, Australia, as inferred from host morphology and host molecular analyses. However, based upon respective differences in the dicyemid parasite fauna of each of

the three cephalopod species, distinct host population subsets in SG compared to GSV were found. Such a finding has implications for the management and conservation of cephalopod stocks, especially *S. apama* whose unique mass breeding aggregation population in SG is currently under threat and in decline. If the SG population is a distinct species, as may be inferred from my result, recruitment from the neighboring GSV population will not sustain this unique breeding population. Furthermore, if appropriate management plans are not put in place, an iconic and economically-important species may be lost from the system in the future. In conclusion, morphological and molecular analyses of dicyemid parasite faunas could be used as a tool in other systems to assess the population structure and potential species status of cryptic cephalopod species, with greater insight compared to host morphological and molecular analyses.

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MOLECULAR PHYLOGENY OF THE DIPLOSTOMIDAE AND STRIGEIDAE (DIGENEA)

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Evolutionary relationships within the Strigeidae and Diplostomidae (Digenea: Diplostomoidea), which are cosmopolitan parasites of vertebrates, are poorly understood. In this study, the phylogenetic relationships of selected genera within these families were studied using full small (SSU), partial large (LSU), and full internal transcribed spacer regions 1 and 2 (ITS) sequences of ribosomal DNA and partial sequences of cytochrome oxidase I (COI) from mitochondrial DNA. Sequences from 10 diplostomid genera (19 species) and 6 strigeid genera (9 species) were analyzed using maximum parsimony and maximum likelihood methods. Markers were analyzed independently and in total evidence combinations. All molecular topologies indicate a monophyletic clade but paraphyletic relationships among the families. Total evidence maximum parsimony analyses of concatenated sequences of SSU-ITS-COI and SSU-LSU-ITS-COI produced identical trees concordant with the fewest evolutionary changes based on a matrix of 32 morphological and life-history characters. The basal taxa, in ascending order, included the diplostomids *Hysteromorpha, Diplostomum*, *Tylodelphys*, *Alaria* and *Neodiplostomum*. The remaining taxa formed a paraphyletic clade in which the strigeids *Apharyngostrigea*, *Parastrigea*, *Apatemon* and *Cotylurus,* were separated from *Ichthyocotylurus* and *Cardiocephaloides* by the diplostomids *Posthodiplostomum, Mesoophorodiplostomum, Ornithodiplostomum*, *Uvulifer* and *Bolbophorus*. The morphological characters supporting the division of the basal branches from the remaining taxa are metacercarial features associated with encystment and limebody morphology. The basal group of diplostomids *Hysteromorpha, Diplostomum*, *Tylodelphys* and *Alaria* all have a diplostomulum type metacercaria that is unencysted and has enclosed limebodies. *Neodiplostomum* has an encysted metacercaria but enclosed limebodies. The remaining taxa in the paraphyletic clade have encysted metacercariae and free limebodies. None of the other morphological or life-history characters analyzed within this study differed between the two strigeid groups. These results provide further evidence that the classification of these groups needs to be reassessed.

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DISCOVERIES REVEAL EVOLUTIONARY PATTERNS OF LIFE CYCLE COMPLEXITY AND THE NEED FOR GENETIC DATA TO SUPPORT MORPHOLOGICAL DESCRIPTIONS IN THE GENUS *ALLOGLOSSIDIUM*

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Within the digenean genus *Alloglossidium*, there is interspecific variation in the number of hosts needed to complete the life cycle and the types of final host species used (catfish, crustacean, or leech). Like many digeneans, species of *Alloglossidium* with a 3-host life cycle sexually reproduce in a vertebrate final host; however, 70% of the species exhibit a 2-host pattern where precocious development leads to sexual maturation in what is typically considered the second intermediate host. Prior to 2011, there were 14 species of which only 2 non-precocious species were reported from catfish hosts (*A. corti* and *A. geminum*). As both of these species had broad host and geographic ranges and because subtle morphological differences separated them, the potential for cryptic species seemed high. Indeed, recent sequence data supported the resurrection of *A. kenti* and description of *A. fonti* (Tkach & Mills 2011). We present a new species found in madtoms from Florida and discuss potential traits for delimiting the species that infect catfishes. Results indicate there is no single trait simultaneously discriminating the 5 species, and traits often overlap. Thus, previous descriptions (and subsequent morphology-based phylogenies) that are not supported with genetic data are difficult to interpret due to possible past non-recognition of distinct species. Sequence (mtDNA and nuclear DNA) data allowed us to revisit the relationships within the genus. Prior morphology-based phylogenies hypothesized a single transition from a 3 to 2-host pattern with a later host switch from crustaceans to leeches. In contrast, our molecular-based phylogeny shows the 2-host life cycle was derived independently in leeches and crustaceans. Also, while the species from leeches form a clade, those from crustaceans show 3 independent losses of the vertebrate host. Future work will assess the correspondence between morphological and genetic species delimitation within the genus and examine correlations between life cycle patterns and morphological traits.

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A NEW COPEPOD (ERGASILIDAE) FROM THE GILLS OF A FRESHWATER FISH FROM BRAZIL

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A new species of the monospecific genus *Vaigamus* Thatcher & Robertson is reported from the gills of *Hoplias malabaricus* (Bloch), a Neotropical freshwater fish from the family Erythrinidae, collected from the Cristalino River, a tributary of the Tocantins-Araguaia River Basin, in the state of Mato Grosso, Brazil. The copepod is fully described using light and scanning electron microscopy. It can be distinguished from young ovigerous female of *V. retrobarbatus* Thatcher & Robertson found in plankton from lakes in the Amazonas River Basin, Brazil: by morphological differences observed in antennulary setation patterns and in the surface ornamentation of spinules on the limbs and anal somite; in the distribution of pigment on the prosome; in the size of rostral spine; in the number of eggs in the egg sac; and by having a 2-segmented exopod on the leg 4 as the majority of ergasilids. Additionally, the new *Vaigamus* possesses a segmented antenna comprising a robust coxobasis armed with a naked seta distally, a 3-segmented endopod and the curved terminal claw, with the fourth segment (third endopodal segment) reduced to an incomplete hoop of sclerotized cuticle. The family Vaigamidae was previously proposed to accommodate highly modified fish parasitic copepods found in the nasal fossae of fish that are characterized by bearing movable posterolateral stylets (retrostylets) on the cephalic shield, and a rostral spine. However they are currently recognized as a specialized lineage within the family Ergasilidae. The distribution of the genera previously placed in the Vaigamidae is summarized and discussed.

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PHYLOGENETIC AFFINITIES OF *PLAGIOPORUS* STAFFORD, 1904 (DIGENEA: OPECOELIDAE) AND CLOSELY ALLIED PLAGIOPORINE GENERA OF THE HOLARCTIC

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The systematics of *Plagioporus* Stafford, 1904 and closely allied genera have long been a subject of tedium and confusion; there are many morphologically similar taxa of opecoelids with few subtle differences that tend to grade into one another. Preliminary molecular phylogenetic analysis of 28S rRNA gene fragments of plagioporines, including those collected from freshwater environments of the Nearctic and Palearctic, indicate that not only do plagioporines from freshwater hosts form a monophyletic group, but also that *Plagioporus* is clearly paraphyletic. We discuss the taxonomic implications of these sequence data and note that the position of the uterus relative to the testis is a poor generic character for plagioporines from freshwater hosts.

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THE PROMISE AND PREDICAMENT OF MORPHOMETRIC SPECIES RECOGNITION IN GREGARINES

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By all estimates, most of the species diversity of septate gregarines remains undescribed. Morphological studies within the group have been very successful in recognizing generic and higher level differences among taxa, but the results are less conclusive at the species level, primarily because few unique species-level characters have been discovered. As a result, few intensive taxonomic studies of congeneric species have been conducted and the relative value of metric and shape characters at the species level remains enigmatic. The genus *Protomagalhaensia* includes 4 described gregarine species parasitizing cockroaches: *Protomagalhaensia granulosae* from *Blaberus discoidalis, Protomagalhaensia wolfi* from *Nauphoeta cinerea, Protomagalhaensia blaberae* from *Blaberus boliviensis*, and *Protomagalhaensia cerastes* from *Phoetalia pallida*. None of these species possesses a recognizable autapomorphy, but they are readily distinguished phonetically using morphometric characters (size) and morphometric ratios (shape). In any phenetic analysis separation of taxa is reflected by discontinuities in population variance among taxa, but how do we recognize these discontinuities as phenetic space becomes more crowded? This talk reviews the nature and variation of available morphological characters and uses data from undescribed species of *Protomagalhaensia* to evaluate the relative value of existing gregarine size and shape characters and to demonstrate their utility in the recognition of gregarine species. Within *Protomagalhaensia*, many traditional size and shape characters are of limited utility while others are of significant discriminatory power although they are rarely used. Traditional gregarine taxonomy has assumed that a limited number of common morphological and morphometric characters are sufficient to delineate species within genera as well as across families. We suggest that the relative utility of morphological and morphometric characters in gregarine taxonomy is much more restricted.

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INTERRELATIONSHIPS AMONG THE LECANICEPHALIDEAN TAPEWORMS BASED ON MOLECULAR SEQUENCE DATA: A PROPOSED FAMILY-LEVEL CLASSIFICATION

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Euzet (1994) considered five genera to be valid in his comprehensive treatment of the Lecanicephalidea almost 20 years ago. He placed these genera in four families: Anteroporidae, Lecanicephalidae, Polypocephalidae and Tetragonocephalidae. Since that time, ten new genera have been erected and an additional three previously described genera have been resurrected. However, familial placements of these genera have been severely hampered for two reasons: (1) most new genera represent scolex morphologies that differ dramatically from existing forms and thus are not easily reconcilable with existing generic diagnoses; and (2) a robust phylogenetic hypothesis of the interrelationships among lecanicephalidean genera upon which familial constituency can be based is lacking. However, concerted collection efforts globally over the past 20 years have resulted in the availability of material representing most major groups of lecanicephalideans that is suitable for generating molecular sequence data. Two mitochondrial and two ribosomal genes were sequenced for representatives of 15 of the 18 valid lecanicephalidean genera, three genera currently considered to be *genera inquirendae*, and five genera new to science. Phylogenetic analyses of these data suggested the presence of up to eight independent lineages; however, relationships among these lineages were not fully resolved. In general, genera lacking apical structures were found to be basal in position. Interestingly, genera within each of the eight lineages were found to exhibit similar proglottid anatomies, but apical structure morphologies that varied substantially. Conversely, similar scolex morphologies were not necessarily found to be indicative of common ancestry, as they were often associated with considerable differences in proglottid anatomy. Familial assignments of genera are made, but remain preliminary in the absence of a more resolved phylogeny.

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CEPHALOBOTHRIUM SHIPLEY & HORNELL, 1906 REVISITED: RESURRECTION OF THE CEPHALOBOTHRIIDAE PINTNER, 1928 (PLATYHELMINTHES: EUCESTODA: LECANICEPHALIDEA)

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For over a century *Cephalobothrium* Shipley & Hornell, 1906 has served as a repository for species with "sucker-like" apical organs. This is largely due to the lack of important morphological detail in the original description of the type species, *Cephalobothrium aetobatidis* Shipley & Hornell, 1906. This limited information led to its status as a *genus inquirendum*, most recently recognized in the Lecanicephalidae. As part of a revision of the genus, cestodes consistent with the original description collected from the type host *Aetobatus ocellatus* off northern Australia allowed for the redescription of *C. aetobatidis* and therefore an updated generic diagnosis. The 17 nominal species currently in the genus form an eclectic assemblage of apparently unrelated taxa, many of which are now inconsistent with the concept of the genus. For example, *C. abruptum* and *C. pteroplateai* were found to actually be members of *Hexacanalis* while *C. variabile* and *C. rhinobatidis* were found to represent a new genus. Moreover, morphological data suggest that *Hexacanalis* and the new genus share features with *Stoibocephalum*, not

Cephalobothrium. Towards determining the closest relatives of *Cephalobothrium* and confirming familial belonging, sequence data of 2 mitochondrial and 2 ribosomal genes were generated for 23 specimens representing 13 lecanicephalidean genera. With respect to the genera in question, parsimony and likelihood analyses strongly support a clade of *Cephalobothrium* and *Adelobothrium* and a separate clade of *Hexacanalis*, *Stoibocephalum*, the new genus, *Tylocephalum,* and *Lecanicephalum*. This suggests that *Cephalobothrium* is not a member of the Lecanicephalidae. Resurrection of the Cephalobothriidae is suggested for *Cephalobothrium* and *Adelobothrium* and supported by morphological data (presence of radiate ovaries and lack of post-ovarian vitellaria). These results are preliminary until a comprehensive phylogenentic analysis of the Lecanicephalidea is completed.

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MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL GLUTAMATE-GATED CHLORIDE CHANNEL SUBUNITS FROM *SCHISTOSOMA MANSONI*

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Cys-loop ligand-gated ion channels (LGIC) mediate fast ionotropic neutransmission. They are proven drug targets in nematodes and arthropods, but are poorly characterized in flatworms. This study characterized the inhibitory, non-acetylcholine Cys-loop LGICs from *Schistosoma mansoni*. We have previously cloned 3 glutamate-gated chloride channel (GluCl) subunits from *S. mansoni* (Sm), and characterized them by two-electrode voltage clamp (TEVC) in *Xenopus* oocytes. Concentration-response relationships revealed that the SmGluCl receptors affinity for glutamate is among the highest reported for GluCl to date, with EC_{50} values of 6.87- 26.28 μM. In addition, TEVC showed that SmGluCl receptors are insensitive to ivermectin (IVM), indicating that they do not belong to the highly IVM-sensitive GluClα subtype group. Phylogenetic analyses suggested that SmGluCl subunits belong to a novel clade of flatworm GluCls, which also includes putative genes from other trematodes and cestodes. This flatworm GluCl clade is evolutionarily distinct from the nematode-arthropod and mollusc GluCl clades, and from all GABA receptors. Using confocal microscopy, we showed that SmGluCls are distributed throughout the central and peripheral nervous systems of *S. mansoni*. Further work is in progress to provide a detailed description of SmGluCl distribution in males, females, cercaria and somules. Finally, we have initiated RNAi-based functional studies to assess the roles played by SmGluCls in schistosomes. Altogether, these results provide the first molecular evidence showing the contribution of GluCl receptors to L-glutamate signaling in *S. mansoni*, an unprecedented finding in flatworms. This project has uncovered a completely new aspect of neuronal modulation in flatworms, and brings attention to very appealing new anthelmintic targets which could be used to address the urgent need for new chemotherapeutic options for schistosomiasis.

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RECEPTOR-CARGO COMPLEXES FORM IN THE CYTOSOL OF *LEISHMANIA DONOVANI* FOR TRAFFICKING TO THE GLYCOSOME

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Leishmania donovani has a unique microbody organelle called the glycosome that compartmentalizes a variety of metabolic pathways essential for parasite survival. Trafficking and import of newly synthesized proteins depends on cytosolic receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LPEX7), which bind cargo proteins containing a PTS1 (peroxisomal targeting signal) or PTS2 motif, respectively. These receptor-cargo complexes bind to peroxin 14 (LdPEX14) docking complex on the cytosolic face of the glycosomal membrane. This network of protein-protein interactions is required for proper protein targeting. Biochemical analysis of *L. donovani* cytosolic fractions revealed that LdPEX5 and LPEX7 form heteromeric complexes loaded with PTS1 and PTS2 cargo proteins. To further dissect the molecular process associated with the trafficking and translocation of proteins across the glycosomal membrane we have initiated *in vitro* studies using the recombinant receptor proteins LdPEX5 and LPEX7 and several model PTS1 and PTS2 proteins. To facilitate these studies we have developed strategies to produce large amounts of the highly hydrophobic LPEX7 in an *E. coli* expression system. This recombinant receptor protein has been shown to self-oligomerize, and can exist as a monomer, dimer, and tetramer in solution; however, the monomer is sufficient for binding the PTS2 cargos. LPEX7 also forms complexes with LdPEX5, in the presence and absence of PTS1 cargos, which bind the native, glycosomal LdPEX14. This association with LdPEX14 causes the docking protein to undergo a conformational change that drastically increases resistance to protease digestion, most probably via membrane insertion and recruitment of other membrane proteins. The availability of these building blocks will permit studies to re-constitute and characterize the complexes involved in glycosomal cargo protein import.

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COMPARATIVE ANALYSIS OF EXOSOMES RELEASED BY *SCHISTOSOMA MANSONI* AND THE FREE-LIVING FLATWORM, *DUGESIA TIGRINA*

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Vesicles of endocytic origin, called exosomes, are released by many cell types and organisms, both *in vitro* and *in vivo*. Interest in exosomes has intensified after it was observed that they can participate in intercellular communication in a variety of physiological and pathological conditions, notably in the communication between immune cells, the response to pathogens and tumor progression. Recent evidence has shown that exosomes released by protozoan parasites are an important mechanism of protein export, which is used to modulate the host's immune response. By comparing parasitic and free-living worms of the same phylum we hoped to identify exosomal proteins or other molecules that are unique to the schistosome and potentially significant for infection. In this study we tested whether exosomes are also released from a multicellular parasite, the human bloodfluke *Schistosoma mansoni*. Adult worms were cultivated in vitro for up to 48 hr either in serum-free medium or medium containing exosome-depleted serum and vesicles were isolated from the culture media by ultracentrifugation at 105,000g, according to standard methods. Similar experiments were performed with a related free-living flatworm, the planarian *Dugesia tigrina*, which was cultured in water for the same period of time. Crude vesicular preparations from both species were floated on sucrose gradients. The results showed a

significant proportion of vesicles in densities typical of exosomes (around 1.15 g/ml) but also in other fractions (densities > 1.25 g/ml,) indicating the presence of heterogeneous vesicle populations. Electron microscopy analysis of the ≈ 1.15 g/ml fraction confirmed the presence of exosome-like vesicles of the correct size and shape in samples from both worm species. Further SDS-PAGE analysis of the purified exosome-like fraction revealed large numbers of proteins, including several exosomal markers, as determined by Western blot analysis. Purified vesicles were digested with trypsin and the resulting peptides were subjected to LC-MS/MS analysis. Mass-spectra were analyzed by Mascot and MassMatrix search engines. The comparative analysis identified numerous proteins that are common to all exosomes and are present in both worm species, including heat shock proteins, endosomal sorting complex (ESCRT) proteins, tetraspanins, signaling, vesicular trafficking and cytoskeletal proteins among others. The analysis also revealed several hypothetical proteins, some which are present in both species and some that are unique to *S. mansoni*. Studies are under way to determine if exosome release is biologically relevant in these worms, if it occurs in vivo within the infected host, and whether it contributes to immunomodulation associated with schistosome infection.

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MAPPING THE MOSQUITO CELLULAR IMMUNE RESPONSE: HEMOCYTE DISTRIBUTION THROUGHOUT THE HEMOCOEL

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Mosquitoes respond to infection by mounting powerful immune responses. The primary regulators of these immune responses are cells called hemocytes, which kill pathogens via phagocytosis and produce soluble antimicrobial factors. Mosquito hemocytes are circulated throughout the hemocoel (body cavity) by the swift flow of hemolymph (blood), and recent data show that some hemocytes also exist as sessile cells that are attached to tissues. The purpose of this study was to create a physical map of hemocyte distribution in the mosquito, *Anopheles gambiae*. Using correlative imaging methods we found that the number of hemocytes in a mosquito decreases with age, but that regardless of age, approximately 75% of the hemocytes occur in circulation and 25% occur as sessile cells. Injury does not alter hemocyte numbers, but infection induces an increase in the number of hemocytes, with this increase being primarily due to hemocyte mitosis in circulation. Within the sessile hemocyte population, the majority of hemocytes are present on the abdominal wall, although significant numbers of hemocytes are also present in the thorax, head, and several of the appendages. Within the abdominal wall, the areas of highest hemocyte density are the regions surrounding the ostia, or valves of the heart. These periosital hemocytes are rapid responders to infection and sequester pathogens that circulate with the hemolymph. Together, these data describe the spatial and temporal distribution of mosquito hemocytes, and map the cellular response to infection throughout hemocoel.

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STRATEGIES TO DEORPHANIZE AND CHARACTERIZE G PROTEIN-COUPLED RECEPTORS FOR NEUROPEPTIDES IN THE MODEL NEMATODE, *CAENORHABDITIS ELEGANS*

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The neuropeptidergic system in free living and parasitic nematodes plays critical roles in locomotion, feeding and reproduction behaviors. Finding neuropeptide endogenous receptors and characterizing ligand-receptor interactions are crucial steps to understand the neuropeptidergic signalling and search for new targets for new anthelmintics. To deorphanize (match) *Caenorhabditis elegans* G-protein-coupled receptors (GPCRs) with potent neuropeptides from the FMRFamide-like peptide (FLP) family, we

designed an *in situ* low throughput screening assay for matching GPCRs. The *in situ* assay consisted in identifying locomotion phenotypes in bisected wild-type (WT) worms upon exposure to individual FLPs. Those phenotypes were used as a read-out to screen 28 *C. elegans* strains with loss-of-function mutations in individual GPCR genes. Using this approach, we associated 7 FLPs, including AF2, AF1, AF8 and FLP18, with their cognate GPCR as their respective knockout strain did not retain the FLP-phenotype observed in WT worms. We also expressed matched GPCRs in modified *Saccharomyces cerevisiae* strains for *in vitro* receptor activation assays. The heterologous expressed receptors T19F4.2 (AF2R) and Y58G8A.4 (FLP18R) respond to their respective peptides in a concentration-dependent manner, confirming the *in situ* associations. Finally, we used both *in situ* and *in vitro* assays to study FLP18R interactions with truncated analogues of FLP18 (DVPGVLRFa) and an alanine scan series (sequential replacement of each amino acid by alanine). The **VLRFa** fragment is necessary for FLP18R activation *in vitro* as well as conservation of FLP18 phenotype *in situ*, suggesting that these 4 amino acids determine the ligand-receptor interaction. We also observed that the Ala^1 analog, **A**VPGVLRFa, is as potent as FLP18 and a better agonist; while the Ala^2, Ala^3 and Ala^4 modifications affect FLP18R sensitivity producing a shift to right in the EC_{50}. Our results suggest that the strategies used are suitable to deorphanize neuropeptide GPCRs and study their pharmacology. In addition, these tools can be used to find non-peptide agonist for nematode GPCRs as candidate anthelmintics.

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GENOME-WIDE MAPPING OF HISTONE H3K4 TRIMETHYLATION AND GENE EXPRESSION REGULATION IN *LEISHMANIA*

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Drug resistance in *Leishmania* induces major structural rearrangements of the genome such as gene copy number variation and changes in ploidy. Previous genomic and transcriptomic analyses highlighted a strong correlation between RNA abundance and gene copy number in *L. infantum* antimony- and methotrexate-resistant parasites. However, some discrepancies were observed for genes distributed on the same chromosome and these discrepancies might be due to gene expression regulation at the chromosomal level. The mechanism of transcription initiation in *Leishmania* is poorly understood but recent studies pinpointed the importance of histone and DNA modifications in the regulation of gene expression. Using LC-MS/MS analysis, we identified histone modifications in *Leishmania* and found several methylated and acetylated residues in the core domain of histones as well as in their N-termini and C-termini. We also investigated the genome-wide regulation of transcription in response to the parasite's differentiation and drug resistance development using chromatin immunoprecipitation followed by deep-sequencing of enriched DNA fragments (ChIP-Seq), which maps the precise location of the trimethylation state of lysine 4 of histone H3 (3meH3K4). This epigenetic mark is well-defined in mammals and is associated with active transcription, while in *Trypanosoma brucei* this modification is localized at the start site of divergent polycistronic transcription units. ChIP-Seq experiments were carried out with the two parasite life stages and with resistant parasites. Our preliminary analysis indicates that 3meH3K4 is enriched in the divergent polycistronic transcription units in the *Leishmania* genome and that differences exist between the strains studied. A complete analysis of our epigenetic study will be presented.

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REDISCOVERING GOLD: AURANOFIN AS A MACROFILARICIDAL DRUG CANDIDATE

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Auranofin is a gold-containing drug that was approved by the FDA over 25 years ago for the long-term treatment of rheumatoid arthritis. Studies have shown that auranofin is also an effective compound against a number of parasites including *Schistosoma mansoni*, *Echinococcus granulosus*, *Trypanosoma brucei*, *Leishmania* spp., *Entamoeba histolytica* and *Plasmodium falciparum*. We tested auranofin on adult female *Brugia malayi*, *Onchocerca volvulus* L3, female and male adult *O. ochengi*, and microfilariae *of O. ochengi Loa loa in vitro* and found that this compound is effective in inhibiting these worms at IC50s of 400 nM, 340 nM, 270 nM, 380 nM, 3 μM and 12.8 μM, respectively. Importantly, the drug is less effective on *L. loa* than on *O. ochengi* microfilariae, a desired drug property for treatment of *O. volvulus* in *L. loa* co-endemic regions. Auranofin also inhibited the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) by the *Brugia malayi* recombinant thioredoxin reductase, the presumed target of auranofin. To validate this drug as a candidate for the treatment of lymphatic filariasis and onchocerciasis, gerbils infected with *B. pahangi* were treated with auranofin for 4 weeks, which resulted in a significant loss of adult female worms. This work was funded by the Bill & Melinda Gates Foundation.

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PREVALENCE OF *PLASMODIUM* IN HIV-INFECTED PATIENTS IN BENIN CITY, EDO, NIGERIA

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Malaria is a parasitic disease transmitted by the bite of an *Anopheles* mosquito. The parasite of the genus *Plasmodium* includes five species that infect human: *P. falciparum, P. vivax, P. malariae*, *P. knowlesi*, and *P. ovale.* Each species of *Plasmodium* has a distinct life cycle and various severities of infections. Most malarial infections are caused by *P. vivax*, which is prevalent in tropical and temperate regions, while *P. falciparum* causes the greatest morbidity and mortality among those infected. Our collaborators at the University of Benin Hospital observed that HIV-infected patients are typically treated with Highly Active Antiretroviral Therapy (HAART) and, as a prophylactic, the anti-malarial Artemisinin Combinational Therapy (ACT). Yet they still have malaria parasites in their blood. These observations prompted the questions: Are these parasites resistant to ACT? Does HAART inactivate ACT? Are there other strains of malaria present? To begin to answer these questions, we isolated DNA from blood

samples and used polymerase chain reaction (PCR) to determine the prevalence of *Plasmodium* infections using primers against four human species of *Plasmodium*. Thus far, we have determined a prevalence of 28.7 % for *P. falciparum* infections.

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CRYPTIC DIVERSITY WITHIN THE *ECHINOSTOMA 'REVOLUTUM'* SPECIES COMPLEX (DIGENEA: ECHINOSTOMATIDAE)

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The *Echinostoma* '*revolutum*' group of species, with 37 collar spines qualifies as cryptic due to the interspecific homogeneity of characters. In the most recent revision of this group only five species were considered valid: the Eurasian *Echinostoma revolutum* (Frölich, 1802), *E. echinatum* (Zeder, 1803) and *E. jurini* (Skvortsov, 1924), the North American *E. trivolvis* (Cort, 1914) and the African *E. caproni* Richard, 1964). However, recent molecular studies have demonstrated higher diversity within the group and extended the knowledge on species geographical ranges. Here, we present results of an extensive study of the *Echinostoma* spp. parasitising lymnaeid snails in the central and northern Europe. A total of 3,537 snails (*Radix auricularia*; *R. peregra* and *Stagnicola palustris*) were examined in lakes in Germany and Iceland. Detailed morphological examination of the cercariae allowed us to differentiate two *Echinostoma* spp. Molecular phylogenetic analyses were performed using partial sequences of the mitochondrial *nad*1 gene, combined with previously published sequences of *Echinostoma* spp. retrieved from GenBank. Both, Neighbour-joining (NJ) and Bayesian inference (BI) analyses revealed strongly supported reciprocally monophyletic lineages for the European isolates.

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TEREBELLID POLYCHAETES IDENTIFIED AS INTERMEDIATE HOSTS FOR *CARDICOLA LARUEI* (DIGENEA: APOROCOTYLIDAE) IN SPOTTED SEA TROUT (*CYNOSCION NEBULOSUS)*

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Aporocotylidae comprises a diverse family of fish blood flukes, with adults found in the blood or body cavity of marine, brackish, or freshwater fish. Aporocotylids are unique among Digenea with development of larval forms in polychaetes (Annelida) or bivalves. Few cercariae of the family Aporocotylidae have been described previously and the complete life cycle has been elucidated for only two species that develop in marine polychaetes. Examination of *Enoplobranchus sanguineus* and *Amphitrite ornata*, both terebellid polychaetes collected from the South Carolina (SC) coast revealed infections with sporocysts and cercariae not previously described. The cercariae observed from *E. sanguineus* and *A. ornata* most closely resemble the cercariae of the family Aporocotylidae. The morphological characteristics of the cercariae include being apharyngeate, brevifurcate, and possessing an anterior organ instead of an oral sucker. Analysis of ITS-2 sequences from sporocysts and cercariae revealed 100% identity of sporocysts dissected from the coelom of both polychaetes. Whereas lsrDNA and ITS-2 sequences revealed close identity with the *Cardicola* clade of the Aporocotylidae, these were not identical to any sequence available from GenBank. Conversely, ITS-2 sequences were 100% identical to those of adult *Cardicola laruei* collected from local spotted seatrout *Cynoscion nebulosus*. This parasite is highly prevalent in SC and our

results confirmed conspecific infections in spotted seatrout and the terebellid polychaetes. This is the first report of larval aporocotylids in *E. sanguineus* and *A. ornata* and the first life cycle of aporocotylid discovered in the western Atlantic Ocean.

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METACESTODES FROM THE SQUID *LOLLIGUNCULA BREVIS*

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Specimens of *Lolliguncula brevis* were collected by trawl near the coast of Mississippi and examined for metacestodes. DNA was extracted from the cestodes after they were photographed. Small subunit (SSU), large subunit (LSU), and internal transcribed spacer (ITS) regions of the ribosomal RNA genes were amplified and sequenced. Fifty one parasites belonging to five species were collected. Two *Anthobothrium* species were found, one of which had sequences identical to those of an *Anthobothrium* collected by us from the blacktip shark, *Carcharhinus limbatus*. A second *Anthobothrium* sequence obtained was very similar to a group of sequences present in GenBank and identified there as coming from *Anthobothrium* spp. found in the sharks *Carcharhinus isodon*, *C. limbatus*, and *Rhizoprionodon terranovae*. A *Heteronybelinia* species present in *L. brevis* had rRNA sequences identical to those of *Heteronybelinia* previously collected by us from *C. limbatus*, and nearly identical to that of an *H. estigmena* specimen collected from *C. limbatus* caught in Malaysia (GenBank accession number DQ642789.1; 3 base differences in 1180 positions of overlapping LSU sequence). A second tentaculariid species having an rRNA sequence not matching any in GenBank was also present in *L. brevis*. Finally, metacestodes were found with rRNA sequences matching those of adult *Kotorella pronosoma* collected from the ray *Dasyatis say* in the northern Gulf of Mexico.

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BEYOND FREQUENCY AND DENSITY-DEPENDENCE: AN EXPERIMENTAL DEMONSTRATION OF THE IMPORTANCE OF NON-LINEAR TRANSMISSION DYNAMICS IN A HOST-MACROPARASITE SYSTEM

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Understanding the form of pathogen transmission is important for modeling disease impacts on host population dynamics, forecasting disease persistence in host populations and establishment in new populations, and understanding the evolution of virulence. An embedded assumption in contemporary disease ecology is that the form of transmission is either density or frequency dependent. However, there are surprisingly few attempts to empirically test transmission forms, particularly for competing models beyond frequency and density dependent transmission. Here, we used a novel experimental approach in which we vary four different factors (duration of exposure, numbers of parasites, numbers of hosts, and parasite density) influencing transmission. We investigated transmission using the trematode *Ribeiroia ondatrae* and larval amphibian hosts. This macroparasite system offers several advantages as a model system (e.g., ease of manipulating infective stages and lack of intrahost replication). Furthermore, by manipulating host behavior we were able to isolate the influence of parasite behavior on transmission dynamics. We evaluated seven candidate transmission functions using maximum likelihood methods to identify the best fitting model to the experimental data according to Akaike's information criterion. Our results indicated that, among the candidate models considered, non-linear forms of transmission involving either a power law or negative binomial function were the best fitting models and consistently outperformed classical density and frequency dependent functions. The power law function was the best fitting model for experiments varying the duration of exposure and host number, while transmission

dynamics in experiments varying parasite number independently of parasite density was best represented by the negative binomial function. These functional forms are consistent with saturating infection with high parasite exposures. The negative binomial function remained the best fitting model when parasite behavior was isolated from host behavior using anesthetized hosts. Upon re-analysis of previous empirical data from other macroparasite systems, we found that non-linear functions were a superior fit to the data relative to density or frequency dependence, suggesting that non-linear transmission dynamics are general across multiple host-parasite systems. These functions highlight important parallels with models of other species interactions, including predator-prey and host-parasitoid dynamics. Suggested mechanisms for non-linear transmission include heterogeneity in susceptibility or distribution or density-dependence in the parasite population. Our results have implications for disease management and provide a basis for conceptually integrating models for pathogen and consumer resource systems.

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SEASONAL DYNAMICS AND TRANSMISSION OF THE MARINE MYXOZOAN *CERATOMYXA PUNTAZZI*

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Myxozoans are metazoan microparasites, mainly known for the diseases they provoke in fisheries and aquaculture. The bile-inhabiting myxozoan *Ceratomyxa puntazzi* affects the sharpnout seabream *Diplodus puntazzo*, a recent aquaculture species in the Mediterranean. Our knowledge about the transmission and seasonality of marine myxozoans is limited, however it is of considerable importance for the design of management strategies for aquaculture. In order to better understand the seasonality and dynamics of *C. puntazzi* transmission and to determine if parasite stages can be transmitted from fish to fish we conducted: 1. *In vitro* transmission trials using different parasite developmental stages and 2. Monthly sentinel fish exposures in a *C. puntazzi* enzootic-environment in combination with monthly qPCR quantifications of infective spore stages in seawater, throughout a whole year. Our results demonstrate that *C. puntazzi* presents a marked parasite density-related seasonality, with a double-peaked prevalence of infection in sentinel fish in spring and late summer/autumn. Blood invasion occurred all year round, with covert infection during the winter months. The sentinel fish exposure along with the quantification of infective stages in the water allowed us to attribute this pattern in *C. puntazzi* densities to higher numbers of actinospores, while myxospores are predominant in summer and winter. We suggest that actinospore production in the invertebrate host is triggered by temperature increase and that the life cycle dynamics of the invertebrate host explain the double-peaked infection in fish. Experimental transmission of different *C. puntazzi* developmental stages was unsuccessful which indicates that fish-to-fish transmission is unlikely to occur in aquaculture facilites. Our data recommend initial exposure of fish/transfer to sea cages to be done in late autumn/early winter, resulting in higher resistance to re-infection with high numbers of parasites in the following spring. This is the first model studying seasonality and infection dynamics of a marine myxozoan.

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PRACTICAL APPLICATION OF THE *EIMERIA* LIFE CYCLE WITH LIVE VACCINATION, REPLACEMENT LAYER PULLET PRODUCTION AND THE CAGED ENVIRONMENT

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Live *Eimeria* vaccination stimulates immunity using a small dose of vaccine oocysts enhanced through fecaloral cycling. Successful live vaccination is most likely with uniform vaccine administration and environmental control in the barn. Day old chicks are frequently vaccinated in coarse spray cabinets that may lead to non-uniform ingestion of vaccinal oocysts. Without adequate cycling to complement vaccination, limited immunity puts birds at risk of coccidiosis when challenged. Studies were designed to assess variation in vaccine uptake and the level of protection against challenge depending on caged-environment modifications. The first experiment confirmed variation in spray vaccine uptake at 6 days post inoculation via counting oocysts shed. A second trial indicated that low relative humidity (<25%) during the initial vaccination period had a negative impact on vaccine success. For the third experiment, birds were separated into 4 vaccine uptake groups and reared on 3 different cage floor modifications to examine protection against challenge infection. Vaccine uptake groups were: oral-gavage (*E. acervulina*, *E. brunetti* and *E. tenella*); fecal-oral transmission, "contact-vaccinated"; positive and negative controls. Cage floor modifications were: 0% cage floor coverage (CFC); 40% CFC with thick paper; and 40% CFC with fibre trays. In general, vaccinated and "contact-vaccinated" birds reared on 40% CFC with paper or trays during the treatment phase had significantly higher body weight gains than positive controls during challenge infection with *E. acervulina* at 3 weeks of age. However, no significant difference was noted for birds reared on 0% CFC. Birds challenged with homologous *E. brunetti* or *E. tenella* did not follow this trend, which may have been due to a decrease in relative humidity during the initial oocyst sporulation stage in the barn. Understanding the parasite life cycle and its practical application can improve live vaccination success in replacement layer pullet production.

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CETACEAN AND FISH ANISAKID NEMATODES SUGGEST BENTHIC-PELAGIC COUPLING BY SOME MESOPELAGIC FISHES IN THE GULF OF MEXICO

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Mesopelagic fishes of the families Myctophidae and Sternoptychidae undergo diel vertical migrations and may represent an important link in the mesopelagic foodweb as shown by some anisakid nematodes. Previous authors have reported a truncation of this migration in instances of proximity to land, abrupt changes in bathymetry (seamount), and ontogenetic shift of some mesopelagic fish species. These truncations were thought to be brief and insignificant but are beginning to be thought of as important trophic couplings of the benthic and pelagic environment. To investigate such coupling in the Gulf of Mexico (GOM), we dissected potential paratenic hosts for anisakid nematodes. We examined 15 species of 3 mesopelagic families (Myctophidae, Sternoptychidae, and Stomiidae), 4 species of 1 epipelagic family (Clupeidae), and 5 species of 2 demersal/benthopelagic families (Macrouridae and Phosichthyidae) from 2010 through 2012. Following morphological examination for juvenile anisakids and subsequent sequencing of their ribosomal DNA internal transcribed spacer regions (ITS-1, 5.8S, and ITS-2), we found 9 species, but will focus on 3. Two mature in cetaceans, *Aniskais typica* (from offshore delphinids) and *Anisakis brevispiculata* (from kogiids), and 1 matures in fish, *Raphidascaris camura* (from the bluefish, *Pomatomus saltatrix,* and pelagic carangids). The examined epipelagic fishes (n=38) were infected with *R. camura* only and had a prevalence of 80%. The prevalence of *A. typica* in examined

demersal/bathypelagic fishes (n=26) was 45%, and prevalence values for all 3 parasites in examined mesopelagic fishes (n=307) were 5% (*A. typica*), 8% (*A. brevispiculata*), and 25% (*R. camura*). *Raphidascaris camura* infected both myctophids and sternoptychids with about 28% prevalence but did not infect the stomiid. *Anisakis brevispiculata* was about equally prevalent in sternoptychids and in the stomiid, but more prevalent in strenoptychids than in myctophids. Myctophids were the only mesopelagic fishes that harbored *A. typica*, but only individuals >75 mm were infected. Our results suggest that the life cycle for *A. typica* in the GOM is associated with benthic first intermediate or paratenic hosts, while the life cycles of *A. brevispiculata* and *R. camura* may include pelagic first intermediate or paratenic hosts. Furthermore, our results suggest that an important cause of truncation concerns ontogenetic shifts in mesopelagic fishes. The myctophids, more so than sternoptychids, feed more near the benthos and are therefore more associated with benthic-pelagic coupling of intermediate and final hosts in the GOM. Funded by NSF No. 0529684l; NOAA, OHHI award NA08NOS4730322; and USWFP CIAP, award M10AF20151 MS.R.798.

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THE LIFE CYCLE, PATHOGENICITY AND GENETIC STRUCTURE OF *DELADENUS PROXIMUS*, NEOTYLENCHID PARASITE OF THE WOODWASP *SIREX NIGRICORNIS* (HYMENOPTERA)

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Deladenus proximus (Neotylenchidae) is a nematode associated to pine trees and to the woodwasps, *Sirex nigricornis* (Hymenoptera), previous to this study little was known on the geographic distribution and variability of *D. proximus*. Herein we present information relative to their life cycle, pathogenicity, and variability. The life cycle is similar to that of other species of *Deladenus* in that it includes phloemophagous and entomopathogenous stages. Fertilized female nematodes penetrate siricid larvae and grow in the body cavity releasing thousands of larvae. These larvae invade the gonads, mycangia (sac containing symbiotic fungus) and eggs upon metamorphosis of the host. Females posit infected eggs and spores of fungus (*Amylostereum chailletii*) into stressed trees, nematodes mature and feed on the phloem, completing the life cycle of the nematode. Our study indicates variable prevalence across localities but in every infected wasps all eggs contained nematodes, thus were sterilized. Between 2009 to 2012 a total of 1,635 woodwasps were collected from Arkansas, Illinois, Louisiana, and South Carolina. Woodwasps were dissected and live nematodes were reared on cultures of *A. chailletii* and examined upon maturation. Reared nematodes were compared against type specimens of *D. ipini* and published descriptions of *D. proximus*. In addition we compared diagnostic characteristics of adult nematodes from each locality and found no significant difference in their size and structures depending on location. Reared specimens possess a conspicuous post uterine sac, which is the proposed diagnostic character of *D. ipini*. This and other morphometric differences including a,b,c, and V suggest that *D. ipini* is a junior synonym of *D. proximus*. DNA was isolated and amplified from individual nematodes including 18S, ITS, and CO1. Nuclear DNA was invariable from all 4 locations and had 99% identity to the invasive species *Deladenus siricidicola*. Analysis of mitochondrial DNA showed more variability and was used to evaluate the distinction of populations across these localities. The analysis of a portion of CO1 suggests the presence of 8 haplotypes and the absence of any geographic clusters or subpopulations. The lack of geographic structure may be due to the fact that each female wasp is infected with only one adult female nematode and therefore larvae within a wasp are siblings. With a generation time of 2 weeks these nematodes can have 20 generations without immigration or emigration, suggesting these nematodes are inbred. The pattern of transmission of this nematode and pathogenicity is similar to that of *Deladenus siricidicola*, which is used as a biocontrol against the invasive species *Sirex noctilio*. Experimental infections of *Deladenus proximus* in *Sirex noctilio* are recommended to test their viability as a biocontrol agent.

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METASTRONGYLOIDS OF RED FOX (*VULPES VULPES*): EMERGING THREATS TO DOGS

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Angiostrongylus vasorum (French heartworm) and *Crenosoma vulpis* (fox lungworm) are metastrongyloids infecting the cardiopulmonary system of various wild and domestic canids. Both nematodes share the same life cycle involving red fox (*Vulpes vulpes*) as a natural definitive host and terrestrial gastropods as intermediate hosts. In addition, frogs can serve as either an intermediate or paratenic host for *A. vasorum*. The geographic distribution of *A. vasorum* includes various countries in Europe, Africa, South America and a single endemic focus in North America in Newfoundland-Labrador, Canada. The distribution for *C. vulpis* includes parts of the temperate regions of Europe and North America. Dogs are susceptible to infection with both parasites and the frequency of diagnosis has increased with both parasites over the last few decades making them emerging diseases in dogs. Subclinical to fatal cardiopulmonary disease occurs with *A. vasorum* infection in dogs; *C. vulpis* infections are nonfatal but can result in a chronic cough condition. Diagnosis in dogs for both parasites occurs by the detection of first-stage larvae (L1) shed in the feces of infected animals by Baermann fecal examination (BFE). The potential for under-diagnosis for both parasites occurs due to the infrequent use of BFE for diagnostic surveillance in current clinical veterinary practice and the difficulties associated with larval identification. The reason(s) for the apparent rise in infections in dogs has not been determined. Speculation has included the geographic spread to nonendemic areas due to the ease and frequency of travel coupled with a relative lack of restrictions to animal movement and the environmental effects of global warming favoring parasite transmission. Additionally, the rise in diagnoses could reflect heightened awareness in clinical veterinary practice of the importance of these parasites as causative agents of canine cardiopulmonary disease resulting in an increased use of the appropriate diagnostic methodology. The latter appears to be most likely in the case of *C. vulpis* infection in dogs. With *A. vasorum*, there has been an alarming trend towards an increase in infection risk to dogs within established endemic areas and an expansion into new geographic regions. Whatever factors are involved in the apparent increase in infection risk to dogs has affected widely separated endemic regions located on 3 different continents.

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LOSING THE T OF THE NTD (NEGLECTED TROPICAL DISEASES)

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It is estimated that approximately half the world's population is affected by foodborne, waterborne, and bloodborne infections to which parasitic zoonoses significantly contribute. Food- and water-borne diseases are a major cause of mortality in peoples of Northern Canada. More than 70% of adults in northern communities harvest natural resources for subsistence through hunting and fishing and are thus exposed to unusual zoonotic diseases such as echinococcosis. In North America, cystic hydatid disease is the most common, and is caused by the “cervid” bioform of the canine cestode *E granulosis*. It inhabits the small intestine of dogs, wolves and possibly foxes infecting intermediate hosts such as caribou and moose. Approximately ~50% of moose in Ontario, Quebec and British Columbia are infected with this parasite. Based on information from the United Nations World Tourism Organization each year, more than 1 billion persons cross international borders. It is estimated that approximately 3% of the world’s population live permanently outside their country of birth and that there are approximately 81 million migrant workers. Our previous studies demonstrated that several immigrants/refugees from malaria endemic countries are asymptomatic. As a result, an increase of local transmission of autochthonous malaria cases have been noted in developed countries. Therefore some bloodborne parasitic diseases are no longer an exotic disease.

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CHAGAS DISEASE IN THE UNITED STATES – UNDERSTANDING THE SYLVATIC CYCLE

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a significant health concern throughout many parts of Latin America. In the past several decades, vector control efforts in southern Latin America has decreased or interrupted transmission in many countries or regions; however, because of migration of people, cases continue to be diagnosed in control regions, in city centers where cases were once rare, and in numerous countries where *T. cruzi* is not endemic (e.g., Spain, Japan, etc.). In the United States, human cases are uncommon, but the parasite is common in many species of wildlife and triatomine bugs. Also, in many regions of the US, Chagas disease in domestic dogs is a veterinary concern. I first became interested in *T. cruzi* as an undergraduate student and subsequently conducted a MS project on *T. cruzi* and wildlife reservoirs. My interest continued through my PhD project, which focused on tick-borne pathogens, but then upon obtaining a faculty position, I came back to my interest in this parasite. Since then, a long list of students and I, have continued studies to increase our understanding of *T. cruzi* in the United States with an emphasis on factors that allow this parasite to be maintained in a complex cycle involving hundreds of species of wildlife, vectors, and domestic animals. These studies have involved field-based studies to understand the host range, prevalence, and range; genetic typing studies to understand the role of parasite genetics on host range and infection dynamics; experimental trials to investigate parasite-host relationships and immune responses to US strains; and lab-based experiments to biologically characterize US isolates. During my presentation I will present an overview of the work my students and I have conducted on *T. cruzi* during our efforts to improve our understanding of the ecology of this parasite in the US.

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ASSOCIATE EDITORS SYMPOSIUM

G.W. Esch, Department of Biology, Wake Forest University
M.V. Sukhdeo, Rutgers University

The Associate Editors Symposium speakers will include Drs. Isaure de Buron, Cameron Goater, Susan Perkins, and Derek Zelmer, who are currently serving, or who have served, as Associate Editors for the Journal of Parasitology. Each presenter will be giving a talk that will feature their own research, or provide an update on a topic of their choice. Dr. Michael Sukhdeo, current Editor of the Journal, will chair the Symposium. Dr. Gerald Esch, immediate past Editor of the Journal, will provide an introduction of the Symposium speakers and their topics. We look forward to seeing you.

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ECOLOGICAL EPIDEMIOLOGY OF AN UNLIKELY INVADER: THE LANCET LIVER FLUKE, *DICROCOELIUM DENDRITICUM*, IN ALBERTA, CANADA

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The invasion of parasites into naïve host populations is a widespread phenomenon. But our understanding of how parasites enter new host populations and how they subsequently spread is poor. This is particularly so for generalist parasites that invade multi-host communities. The lancet fluke was introduced into Cypress Hills Park in southeastern Alberta in the mid 1990's. The pathway of invasion from European source populations is unknown. Prevalence in samples of cow, elk, mule deer, and white-tailed deer in the Park is usually 100%, with intensities ranging from 1 to over 5,000 worms per host. We use experimental exposures into sheep and cattle, molecular fingerprinting of European and North American worms, GIS technologies, and field monitoring of definitive and intermediate hosts to understand the ecological epidemiology of this invasive fluke at a range of scales. The sub-population of yearling cattle contribute over 70% of the millions of eggs shed onto pasture each year by the overall population of definitive hosts. Adult cows develop a strong immunity, indicating that the annual addition of susceptible yearlings each spring is probably required for $Ro > 1$. Cattle and other co-grazers are most at risk of ingesting ants when they graze in mid- to late summer within habitats that are dominated by aspen trees. The occurrence of aspen is not only a key predictor for metacercariae-to-ungulate transmission, but the peak rate of aspen encroachment into the park (due to fire suppression) coincides with the emergence of lancet fluke in the region. In some ants, at least 50% of cysts contain dead metacercariae. This unexpected result best explains the consistently low recruitment (< 20%) of metacercariae into sheep and cattle. Further, metacercariae-induced alteration in ant behaviour is temperature-dependent, restricting transmission into ungulates to a narrow temporal window in mid- to late summer. Our results show that the emergence and subsequent spread of this invasive parasite is influenced by broad-scale landscape and host characteristics that determine overall rates of parasite transmission, and fine-scale processes occurring within intermediate and definitive hosts that determine worm survival.

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PARASITES OF *LEPOMIS AURITIS* IN THE EDISTO RIVER: THE RELATIONSHIP BETWEEN HOST AND PARASITE COMMUNITY STRUCTURE

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It has long been hypothesized that helminths should be utilized as multi-level monitors of ecosystem changes. To evaluate the chances that these were the circumstances, we sampled the expanses of a Carolina drainage. Redbreast sunfish were collected (widely spread, but small home ranges) to see the spatial changes. Structure of the community of parasites appeared to be associated with that of the fish present at each site. Structure of the landscape did not affect the NMDS plot, and benthic inverts at a spot, showed a pattern much less tight. Dividing parasites by life-cycles shed some extra light; it seems Wisniewski was right. Spatial patterns may not translate temporally and the reponse rate is a factor that is critical for measurements of change, so a single site was surveyed, to see if host changes relayed to the worms, and was it delayed throughout an annual range. The monthly data showed how the community was arranged, but would the parasites change? Again, parasite assemblage bore a very strong resemblage to fish ordinations, when a one month lag was introduced. NMDS ordination, showed a much less strong relation, of invert participation, but a longer lag produced, a pattern more similar to the one spatially deduced; worthwhile variance reduced? Given that we see congruence, between time and spatial

influence, it appears that fish assemblage does play a consistent role. Thus it is reasonable to infer, that fish host changes can confer, a shift in parasite structure, but it's clearly not the sole effect so we must establish how inverts exert control, in order to meet our goal.

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MALARIA AND ITS MANY MATES: REVISING HAEMOSPORIDIA

S.L. Perkins, American Museum of Natural History

The five species of *Plasmodium* that cause the disease malaria in humans are part of the family Haemosporidia, which shows a large breadth of vertebrate hosts and insect vectors as well as morphologies and life histories. The classification of the parasites into genera and higher-level clades has had a tumultuous history with scores of reorganizations, splittings, and lumpings over the decades. Greater taxonomic sampling from diverse sources combined with multilocus molecular phylogenetic analyses using maximum likelihood and Bayesian algorithms have begun to shed light on the stability of various genera and their relationships to one another. I will present the latest results that include both new and re-discovered species including several recently found in turtle and bat hosts. These large analyses have greatly improved the stability of the phylogeny of this family. However, if we follow proper taxonomic principles, several long-standing and widely studied groups may need to be changed.

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TO BE BAD OR TO BE GOOD? THAT IS THE QUESTION….

I. de Buron, College of Charleston

The spotted seatrout, *Cynoscion nebulosus* (Perciforme: Sciaenidae) is an important fish, both economically and ecologically, in the southeastern USA. In South Carolina (SC) estuaries, this fish is found to be infected with numerous parasites, of which four new species have been described in the recent years. Two species, *Kudoa inornata* (Myxozoa: Multivalvulida) and *Cardicola laruei* (Digenea: Aporocotylidae) are highly prevalent in SC and will be the particular focus of this talk. *Kudoa inornata* infects the skeletal muscle and *C. laruei* the heart of the fish. Whereas both parasites induce morphological modifications of their habitats and appear to negatively impact the overall health of their hosts, the swimming performance of infected fish is enhanced as parasite densities increase. Although the mechanism of enhanced performance is not yet understood, this paradoxal consequence of host infection by these two parasite species raises questions about their adaptive interactions with the host fish.

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COCCIDIOSIS VACCINATION: PROBLEMS AND SOLUTIONS

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It is sixty years since the first vaccine was introduced for the control of coccidiosis in broiler chickens. Today a variety of vaccines comprising live oocysts are available which differ in a variety of characteristics such as the species of *Eimeria* included, the number of oocysts present, whether or not they are attenuated, and the method of administration. Immunological control is recognized as the only major practical alternative to chemotherapy for the control of coccidiosis and yet surprisingly little research has been published on fundamental and practical aspects of vaccine use such as the nature of the protective immune response, the extent of protection induced, and the important role that the environment plays in

vaccine efficacy. Here some desirable vaccine characteristics are described and some of the unresolved questions and gaps in our knowledge considered. The role of live vaccines in sustainable coccidiosis control programs is discussed.

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PARASITES, PLACES AND POULTRY: ENVIRONMENTAL INFLUENCE ON LIVE COCCIDIOSIS VACCINE SUCCESS IN COMMERCIAL POULTRY REARING

K.R. Price and **J.R. Barta**, Department of Pathobiology, Ontario Veterinary College, University of Guelph

The life cycles of *Eimeria* species consist of endogenous and exogenous phases that can be exploited by live vaccination and proactive environmental management, respectively. Although acceptance has been far from uniform, live vaccination for coccidiosis control is being adopted increasingly in the commercial poultry industries in a variety of poultry production systems and countries. Despite coccidiosis vaccines being present since the 1950s, their recent use coupled with dramatic changes in housing systems has altered the ways in which methods of environmental management can be used to influence live coccidiosis vaccine success in poultry rearing. A live coccidiosis vaccine administered to the flock without additional management may provide a "protective base" to initiate control of the in host parasite development and generate partial immunity. However, for the live vaccine to reach its full potential for mixed *Eimeria* species infection, management techniques must allow for prolonged oocyst cycling with the minimum numbers of infective oocysts required to elicit this immunity. Since live coccidiosis vaccines use live parasites, the environmental factors that would impact oocyst infectivity (such as temperature, relative humidity as well as oxygen levels) and transmission dynamics during an infection apply during vaccination. Accordingly, flock and housing environment management can directly impact oocyst cycling (exogenous development and transmission) and, indirectly, endogenous development of the parasite. As a result, managing the parasite during vaccination is dependent on both the vaccine administration method and the control of the parasite in the barn environment.

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OPPORTUNITIES AND CHALLENGES FOR ACHIEVING UNIFORM PROTECTION OF CHICKENS AGAINST COCCIDIOSIS WITH LIVE EIMERIA OOCYSTS VACCINES

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A hallmark of Eimeria infection in avians is the establishment of immunity against clinical signs of coccidiosis. The rapid development of immunity after a primary infection is the basis of coccidiosis vaccines used in the poultry industry, which is seeking alternatives to medication of feed with anticoccidial drugs. Our research has shown that current methods of vaccine administration are neither uniform nor efficient, and may result in placement of fully susceptible chicks on litter containing high numbers of Eimeria oocysts. As a means of improving vaccine delivery, gel beads containing a mixture of *E. acervulina*, *E. maxima*, and *E. tenella* oocysts were tested for stimulation of protective immunity against coccidiosis in day-old broiler chicks raised in battery cages. This research showed that uniformity of vaccine uptake by gel bead application was greatly improved over spray vaccination, and was equivalent to oral gavage. Chickens ingesting Eimeria oocysts gel beads were protected against high dose Eimeria challenge 4 weeks after immunization. This technology was tested in quasi-poultry house conditions by allowing chicks to ingest Eimeria gel beads, and then raising the chicks in contact with litter (thus allowing "oocysts cycling" and boosting of immunity). Complete protection against Eimeria oocysts-challenge infection was observed in chicks that had been immunized by ingesting Eimeria-containing gel beads. These studies indicate that gel bead delivery of Eimeria oocysts is a viable alternative to current

methods of vaccine administration. Studies are underway to extend moisture retention of gel beads, and to apply this vaccination method to commercial poultry houses.

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DNA BARCODING OF COCCIDIA – PROMISE AND PITFALLS

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Coccidia represent a cosmopolitan group of apicomplexan parasites that are found infecting the digestive tracts and tissues of a tremendously wide variety of hosts. Thousands of species have been described but the majority of these species descriptions are based on the morphology and morphometrics of a single life cycle stage of these organisms – the sporulated (or not) oocyst. The molecular characterization or identification of coccidia has been proposed as an adjunct to biological, morphological and morphometric details of these parasites. Use of a standardized, sequenced genetic locus for species identification has been termed 'DNA Barcoding'. For coccidia, nuclear 18S rDNA sequences have been used in species identifications and phylogenetic analyses for many years; although not termed 'DNA Barcoding' at the time, use of 18SrDNA sequences to assist identifications was functional DNA barcoding. The limitations of the nuclear rRNA genes for molecular identifications have become increasingly apparent. Recognition of paralogous, comparatively divergent rDNA copies within a single coccidian species, as well as insufficient genetic distance between closely related coccidia based on rDNA sequences, has made this genetic target less than optimal for species identification and characterization. Sequence variation that includes single/multiple base indels makes sequence alignment and assignments of positional homology in alignments problematic. Sequences from a mitochondrial gene such as cytochrome c oxidase subunit I (COI) are comparatively free of issues related to sequence alignment because these represent protein-coding regions that are free of introns; translations can be used to assist in establishing positional homologies. For the coccidia at least, mitochondrial genes do suffer from the presence of numt DNA (mitochondrial DNA translocated into the nuclear genome) and relatively limited comparative datasets. Even with these limitations, COI-based sequence analysis shows great promise for species identifications, linking lifecycle stages within different tissues and/or hosts, and uncovering previously unrecognized coccidia.

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VIRULENCE SHIFT IN A SEXUAL CLADE OF *TOXOPLASMA GONDII* INFECTING WILDLIFE IN NORTH AMERICA

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Waterborne outbreaks of coccidian parasites such as *Toxoplasma gondii* are increasingly causing fatal disease in marine wildlife. Using population genetic and molecular methods to study the evolution, emergence and transmission of pathogenic strains of *Toxoplasma,* our work has identified a marine invasion of new genetic variants produced when two parasites mate inside their definitive felid hosts. Population expansions of felids near marine estuarine environments have led to increased deposition of highly infectious oocysts and widespread exposure of marine wildlife to these pathogenic pollutagens after storm events. We have obtained *Toxoplasma* isolates from >150 cetaceans, pinnipeds and mustelids since

1998. Based on genome-wide PCR-DNA sequencing of plastid, mitocondrial, intron and antigen-coding markers across all 14 chromosomes, two distinct lineages were identified: Type II and a new clade of strains, called Type X (also referred to as haplogroup 12), that possess distinct alleles from archetypal strains at the majority of loci sequenced. Over 72% of marine mammal *Toxoplasma* infections were Type X. Type X strains have also been identified infecting a wide-variety of terrestrial animals and birds in the US, including humans, and its definitive felid hosts (bobcats and mountain lions). Whole genome shotgun sequencing has identified Type X as an admixture of strains from a cross between a Type II and sylvatic line we now refer to as gamma (g).Support for the Type X admixture model is based on STRUCTURE, Network, eBURST, TCS statistical parsimony analyses and phylogenetic incongruence among locus-specific trees. When assayed through mice, a distinct subset of Type X strains were highly virulent (LD_{100}= 1 parasite). The genetic basis for the altered virulence patterns among Type X strains is currently being assessed and will be presented.

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CANID OCULAR FILARIASIS IN NM

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Increasingly, veterinarians are diagnosing a new kind of parasite infection in pet dogs ranging from northwest to central New Mexico. Parasitic worms are present in the conjunctival tissues surrounding the eyes of dogs affected by this emerging infectious disease. Infection associates with eye damage and blindness, such that owners may choose to have the dogs euthanized. Necropsy of an individual dog yielded worm samples that were identified as the threadworm *Onchocerca lupi* (Filaria, Nematoda), based on morphology and ND5 and CO1 sequences. This parasite is endemic to the Mediterranean region but recently it is also encountered in dogs from several regions in the Southwest of the United States. The ND5 and CO1 sequences from the NM sample show high nucleotide identity (100-98%) to independently characterized *O. lupi* isolates from California, Utah and Europe. Furthermore, detection by nested PCR of specific 16S sequence indicates that *O. lupi* from NM also harbors *Wolbachia*, an obligate bacterial endosymbiont that is present in several other species of filariae. This may add antibiotics to options for management of *O. lupi* infections. *Onchocerca lupi* produces numerous larvae (microfilaria) that migrate throughout the host tissues and that can be detected from small skin samples (so-called skin snips). The *O. lupi* ND5-specific PCR protocol was adapted to facilitate (early) diagnosis from total DNA extracted from ethanol-fixed skin snips from dogs. This method is applied to study prevalence of *O. lupi* using skin-snip samples routinely collected from dogs that enter animal shelters in endemic regions of NM.

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CONTAMINATION OF SOIL WITH HELMINTH PARASITES AROUND HOMES IN RURAL PANAMA

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Contamination of soil with helminth parasite eggs and larvae may pose an important human health risk, particularly in poor, rural areas of developing countries where potable water supply and adequate

sanitation are minimal. The objective of this study was to evaluate the level of parasite contamination of soil around homes with preschool children in rural Panama. The study was conducted at 172 homes in 15 communities in the province of Veraguas in Panama. We collected a 100g (10cm wide x 10cm long x 1cm deep) soil sample from the area where preschool children in the household were reported to play, and also from the area adjacent to the faucet of the 94 houses that had a water faucet in the yard. The child play area was typically within a few metres of the house, whereas faucets were located at a distance of 0 to 30m from the house. In total we collected 258 samples. After collection, soil samples were refrigerated for up to four weeks before they were analyzed for free-living helminth and protozoan parasite stages using the formal-ether flotation technique. The parasites found included eggs and larvae of the hookworm and strongyloides groups, and eggs of *Ascaris* spp., *Taenia* spp., *Toxocara* spp., other Ascaroidea, and cysts of coccidia. Of the samples collected, 39.9% were contaminated with at least one parasite. Significantly more of the samples collected from the area near the tap were contaminated (52.1%) than from the area where the children played (32.9%) (p=0.0137). We examined the data for relationships between soil contamination and presence of infections in preschool children in the household, open defecation behaviours of the children, presence of a latrine at the home, domestic animals present at the home (dogs, chickens, pigs), and yard characteristics such as vegetation cover and shade cover. This study is part of a larger examination of environment and intestinal parasite infections within the context of communities practicing subsistence agriculture and participating in a government-run food security intervention program.

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SPATIAL DISTRIBUTION OF HELMINTH PARASITES IN TILAPIA FARMS IN THE STATE OF YUCATáN, MéXICO

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Infectious diseases are a major source of economic losses in aquaculture worldwide, due to mortality, treatment cost and production decline. In Yucatán (a tropical region), helminth parasites and specially monogeneans produce economic losses for tilapia farms. Their presence and persistence is due to the high fish densities, poor water quality, and fish stocking without adequate sanitary measures. However, the presence and distribution of these parasites has not been adequately recorded for Yucatán. A useful tool to prevent the translocation of these parasites from farm to farm is to use Geographical Information Systems (GIS) to map their distribution. Therefore, the main goal of this study was to determine the spatial distribution of helminth parasites in tilapia farms in Yucatán. A database of 29 extant farms of tilapia (*Oreochromis niloticus*) were used provided by the Yucatán Aquatic Animal Health Committee. 386 tilapias were analized and 14 parasite species were colleted: 10 species of monogeneans belonging to the genus (*Cichlidogyrus*, *Gyrodactilus* and *Enterogyrus*) two protozoan (*Trichodina* and *Vorticella*) one copepod (*Ergasilus*) and one nematode (*Phylomrtridae*). Geographic Information Systems (GIS) were used to generate maps and detect most prevalent farms. The results of the spatial distribution show high prevalence of introduced Africans monogeneans. Prevalence values ranged between 1 and 80%, where *Cichlidogyrus sclerosus* (75%), *C. tilapiae* sp. 1 (79 %) and *Gyrodactilus* sp. (40%) were the most prevalent. Mean abundance values were 1 to 65 per examined host. The affected farms were Boquinete and Acuacultores Mulchechen, located in Dzilam Bravo and Kanasin. The high prevalence can be attributed to lack of proper sanitation (water without the use of filters, excess organic matter, excess fish ponds). This study demonstrates that introduced parasites are a potential health risk to cultured fish populations, causing severe diseases in larval and juvenile fish, besides affecting the fertility of native species and also influence the population dynamics of their hosts.

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MOLECULAR IDENTIFICATION OF ENTERIC PARASITES IN LABORATORY *MACACA FACICULARIS* IN GUANGXI, CHINA

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Nonhuman primates have been identified as common hosts of *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium hominis*, and *Cyclospora* spp,. and are potential reservoirs of some enteric parasites in humans. However, few studies have examined the effect of animal management on the occurrence zoonotic pathogens in captive nonhuman primates. In the present work, 205 fecal specimens were collected from three groups of captive *Macaca facicularis* kept in different densities in a laboratory animal facility in Guangxi, China, and examined by PCR for *E. bieneusi*, *G. duodenalis*, *Cryptosporidium* spp., and *Cyclospora* spp. The infection rates of *E. bieneusi* and *G. duodenalis* were 72.2% and 11.1% in group 1 (35 juvenile monkeys/cage; n=18), 31.6% and 5.3% in group 2 (~25 adult monkeys/cage; n=19), and 11.3% and 1.2% in group 3 (one monkey/cage; n=168), respectively. Sequence analysis of PCR products showed the presence of five *E. bieneusi* genotypes, with genotype D (in 16/36 genotyped specimens) and a new genotype (in 15/36 genotyped specimens) as the dominant genotypes. All five *E. bieneusi* genotypes belonged to the zoonotic group (Group1). The *G. duodenalis* genotypes (assemblages AII and B) in five specimens and *C. hominis* subtype (IdA14) in one specimen were also known human-pathogens, although the *Cyclospora* sp. seen in one animal appeared to be new species unique to *Macaca* spp. The higher infection rate in juvenile animals reared in groups and common occurrence of zoonotic genotypes indicated that human-pathogenic *E. bieneusi* could be transmitted efficiently in captive nonhuman primates. Thus, crowding could be a risk factor for transmission of zoonotic pathogens in nonhuman primates in research facilities. (Supported by the National Natural Science Foundation of China Project No. 31110103901 and 31229005)

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CYSTOISOSPORA CANIS (APICOMPLEXA: SARCOCYSTADIIAE): DEVELOPMENT OF MONOZOIC TISSUE CYSTS IN HUMAN CELL LINES

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Development of most *Cystoisospora* species in cell culture is usually limited to repeated divisions by endodyogeny. Development of intestinal coccidial is normally superior in host cells from the appropriate definitive host. Sporozoites of *Cystoisospora canis* penetrated and developed to monozoic tissue cysts in CV-1 cells (monkey kidney) and BT cells (bovine turbinate) cells in a study by Mitchell et al. (2009). The present study was done to determine if development of *C. canis* occurred in 4 different human cell lines and 2 canine cell lines. Excysted *C. canis* sporozoites penetrated and developed to monozoic tissue cysts in all cell lines. No asexual division was observed although multiple infection of a single cell was seen. Each *C. canis* monozoic tissue cyst consisted of a single centrally located zoite surrounded by a granular tissue cyst wall. Zoites contained the cellular organelles characteristic of infective asexual stages of coccidial parasites. The nucleus was centrally located and a large posterior crystalloid body about 5 microns thick was present. The tissue cyst wall was about 1 micron thick and located adjacent to the parasitophorous vacuole membrane. Monozoic tissue cysts were observed to survive for up to 121 days in human pigmented retinal epithelial cells. The tissue cyst wall surrounding zoites stained with *Dolichos*

biflorus lectin indicating a similar molecular make up to the tissue cyst wall of *Toxoplasma gondii*. We believe that *C. canis* can be used as a model system to study extra-intestinal monozoic tissue cysts stages of *C. belli* of humans and latent tissue cysts of *T. gondii*.

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PREVALENCE AND CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPP. IN DAIRY CATTLE IN NILE RIVER DELTA PROVINCES, EGYPT

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Cryptosporidium infections in calves are associated with the occurrence of diarrhea, retarded growth, and mortality, and are of public health concerns. In this study, we examined the prevalence and molecular characteristics of *Cryptosporidium* in dairy cattle in four Nile River delta provinces, Egypt. Modified Ziehl-Neelsen acid-fast microscopy was used to screen *Cryptosporidium* oocysts in 1,974 fecal specimens from animals of different ages on 13 farms. All studied farms were positives, with an overall prevalence of 13.6%. The prevalence rates were 12.5% in pre-weaned calves, 10.4% in post-weaned calves, 22.1% in heifers, and 10.7% in adults. PCR-RFLP analyses of microscopy-positive fecal specimens revealed the presence of the four major *Cryptosporidium* species, including *C. parvum*, *C. ryanae*, *C. bovis*, and *C. andersoni*. In pre-weaned calves, *C. parvum* was the dominant species (~43.5%), but *C. ryanae* (18.8%), *C. bovis* (~10.1%), and *C. andersoni* (~10.1) were also present at modest frequencies. Mixed infections of *C. parvum*, *C. ryanae,* and *C. bovis* were common. In contrast, *C. andersoni* was the dominant species in post-weaned calves and older animals. Subtyping of *C. parvum* based on sequence analysis of the 60 kDa glycoprotein gene showed the presence of subtypes IIdA20G1 in eight specimens and IIaA15G1R1 in 28 specimens. The common occurrence of the two dominant *C. parvum* subtypes recently found in humans in Egypt suggests calves can be potential reservoirs of zoonotic cryptosporidiosis. (Supported by the Arab Fund for Economic & Social Development “Zamalat Program” and National Natural Science Foundation of China Project No. 31229005 and 31110103901)

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EVOLUTION OF THE ARRESTED LARVAL STAGE DEVELOPMENT PATHWAY IN NEMATODE SPECIES

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The transition from a free-living to parasitic life-history has arisen at least 20 times in the Nematoda. For many species from these incongruous groups, the third larval stage is considered the infective stage (L3i), where the worm displays arrested development. The free-living nematode, *Caenorhabditis elegans*, has a similarly arrested stage, known as the dauer, which has several behavioural and morphological characteristics consistent with survival and dispersal under severe environmental conditions. These observations have led many to suggest that the dauer and the infective stages are similar and that the

dauer larva may have been important step in the evolution towards parasitism. If dauer and infective stages are actually analogous stages, then the pathways controlling dauer formation in *C. elegans* should be conserved in the free-living and parasitic species. Four signalling pathways are responsible for dauer initiating development and regulation in *C. elegans*: the guanylyl cyclase pathway, the TGF-β and the Insulin/IGF-1 signaling pathways which act in parallel, and the steroid hormone signaling pathway which functions downstream. Here, we have investigated the evolution of the genes involved in these four pathways in nine free-living and seven parasitic species of nematodes, including *Trichinella*, *Brugia* and *Haemonchus*. Anticipating potential high sequence diversity, we used profile Markov models to scan the genomes with high sensitivity. Manual curation was required for many gene models. Gene expression data (ESTs and RNA-seq) from different life-cycle stages were used to predict the role of the likely dauer gene homologues. The general architecture of the dauer pathways are conserved across the phylum, though the details present a more complex scenario. We found lineage-specific duplications of genes that exist as single-copy in *C. elegans*. Key components of pathways were also absent in some species. Further, the expression studies show patterns of regulation of parasitic genes that were both consistent with or contrary to *C. elegans*, depending on the species. This suggests that the functions of these genes might not have been conserved during the evolution. The results suggest an ancient origin for these pathways, though a conserved role in regulating dauer-like developmental arrest across the phylum is unlikely.

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IMPROVEMENT OF *S. JAPONICUM* INFECTION IN MICE USING FETAL BOVINE SERUM AND A TAIL IMMERSION METHOD

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Schistosoma spp. are normally cycled through a small animal model to propagate their life cycles in the laboratory. We commonly utilize mice as the definitive host for *S. mansoni* and expose them to cercariae via the tail. This method gives highly reproducible results and enables quantification of penetration rates, a critical parameter to track in the laboratory. Although *S. japonicum* can be passed through mice, traditional methods utilize abdominal exposure, which requires anesthesia before cercariae can be applied to the abdomen. Exposure of mice this way is preferred because cercariae of *S. japonicum* possess a unique stickiness. This characteristic makes cercarial exposure via the tail inefficient because of the manipulations involved and ability of cercariae to stick to plastic, glass, etc. Although effective, abdominal exposure is laborious in that it involves a hair loop or similar device that allows accurate counting and efficient transfer of cercariae. Determination of cercarial penetration rates cannot be determined. Due to these factors, we investigated a method that would help optimize infection of mice with *S. japonicum*. Previous studies found that protein in medium could be used to negate stickiness of cercariae and help in laboratory manipulations. We examined if fetal bovine serum (FBS) could be used to facilitate tail infection with *S. japonicum*. Preliminary studies in our lab showed that a range of 1-10% FBS did not affect swimming of cercariae and FBS improved transfer of cercariae between vessels compared to transfers using water only. We investigated the ability of cercariae (suspended either in 5% FBS or water) to infect mice via the tail in comparison to conventional abdominal exposure. Mouse tails were immersed in glass tubes containing water with and without 5% FBS. Cercariae were transferred from water or 5% FBS to respective tubes with fine-tipped Pasteur pipettes. We show here that cercariae suspended either in 5% FBS or water effectively penetrate the tail (>90% efficacy). The mean adult worm yield per mouse was different among the groups, F (2, 24), p=0.00201. Mean worm yield from the 5% FBS group (16.9) was significantly greater compared to the water group (6.40, p=0.00431) and the abdomen-exposed group (9.44, p=0.0254). Abdominal infection yielded significantly more worms than the water/tail immersion group (p=0.0393). These data indicate that a tail immersion method is effective for *S.japonicum* mouse infections and adding a dilute amount of FBS improves infection. This finding will significantly improve our *S. japonicum* life cycle production through reproducibility and avoiding the need for abdominal exposure. Importantly, it will allow determination of penetration rates and assist in troubleshooting experiments. Future studies will investigate different parameters from the three

treatment groups, including egg burdens, *S. japonicum*-infectivity rates of *Oncomelania hupensis* ssp. snails, sporocyst development, and cercariae production.

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CYTOSKELETAL REARRANGEMENT IN HUMAN RED BLOOD CELLS INDUCED BY THE *PLASMODIUM FALCIPARUM* PROTEIN EBA-175

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Morphological studies have described the general steps involved in the invasion of human red blood cells (RBCs) by the *Plasmodium* parasite. However, little is known about the molecular mechanism involved in RBC invasion by the parasite. In our study we used a recombinant EBA-175 protein as a model to simulate parasite invasion *in vitro*. We are testing the hypothesis that the binding of EBA-175 to Glycophorin A, on the surface of red blood cells, leads to a cytoskeletal rearrangement in the red blood cells that aids in the invasion process. Using confocal microscopy we labeled the cytoskeletal anchor protein Ankyrin and follow its location in the red blood cells before and after incubation with EBA-175. We found that the location of Ankyrin changes in the presence of EBA-175 and accumulates at the site of EBA-175 binding to Glycophorin A. We tested the phosphorylation state of Ankyrin using anti phosphoserine antibody and found that Ankyrin is hyper-phosphorylated in the presence of EBA-175. The kinase associated with the hyper-phosphorylation of Ankyrin is still under investigation. Characterizing the molecular mechanism of RBC invasion by the parasite will allow us to find ways to prevent this crucial step in the disease and will improve our ability to treat and control malaria.

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DIFFERENTIAL IMPACTS OF MATERNAL NEMATODE INFECTION AND PROTEIN DEFICIENCY ON HORMONE, CYTOKINE AND ANTIBODY COMMUNICATION AT THE MATERNAL-FETAL INTERFACE IN MICE

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Gastrointestinal (GI) nematode infections and protein deficiency (PD) commonly co-occur in developing countries, particularly during pregnancy. We have previously observed that PD impairs immunity to the murine GI nematode *Heligmosomoides bakeri*, and in the pregnant mouse, combined PD and *H. bakeri* infection impairs linear growth of pups. In addition, *H. bakeri*-associated cytokine patterns in amniotic fluid do not follow those seen in maternal serum, suggesting that fetal responses to maternal stress may be more dynamic than simply reflecting those observed in the dam. Our objective was to explore the maternal-fetal interface in response to single or combined stresses of GI infection and PD during pregnancy. Pregnant CD-1 mice were given a trickle infection every 5 days with either 0 (sham) or 100 *H. bakeri* infective L3-stage larvae and fed protein sufficient (PS; 24%) or PD (6%) isocaloric diets beginning on day (d) 5 of pregnancy. Fetuses were removed by C-section on d 18 of pregnancy, amniotic fluid and placentas were collected and organs were weighed. Corticosterone, insulin-like growth factor 1 (IGF-1), parasite-specific IgG1 antibodies and a range of cytokines were measured and compared between maternal and fetal serum and amniotic fluid to determine whether perturbations to the maternal system were reflected in the pup at birth through correlation and regression analysis. We found that fetus mass was significantly lower in the maternal PD group ($P = 0.0461$) but that fetal growth was not affected by maternal infection. In contrast, placental mass was increased ($P = 0.0048$) and placental efficiency (fetus mass to placental mass ratio) decreased in infected dams ($P = 0.0296$). These data suggest that the fetus may be protected from infection-related growth effects through modifications in the placenta. Findings on

the relationships between maternal, fetal and amniotic fluid levels of stress hormones, cytokines and growth factors in relation to PD and infection will also be discussed.

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ABSENCE OF APOLIPOPROTEIN E PROTECTS MICE FROM CEREBRAL MALARIA

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Cerebral malaria claims the life of millions of children each year worldwide. From our previous work where we utilized proteomic approach and sera from malaria-infected individuals, we have identified unique malaria biomarkers, which include Apolipoprotein E (ApoE). Due to its implication in cerebral diseases and as the major Apolipoprotein in the brain, and we sought to investigate the role of ApoE in cerebral malaria. We here report the first finding that the complete absence of ApoE protects mice from cerebral malaria. While C57BL/6 mice die of cerebral malaria within 7-9 days of infection with Plasmodium berghei ANKA, mice lacking ApoE significantly survived up to the third week of infection. Overall, we have observed 75-80% survival of $ApoE^{-/-}$ compared to 0% for the WT. Interestingly, the $ApoE^{-/-}$ exhibited comparable levels of parasitemia to that of the WT during the earlier period of infection. Whereas the WT mice display the clinical manifestation of cerebral malaria such as paralysis of the limbs, convulsion and comma, the surviving $ApoE^{-/-}$ were devoid of these symptoms. We also investigated whether this survival benefit is due to brain-ApoE receptors. Mice lacking the major brain ApoE receptors ($LDLr^{-/-}$, $VLDLr^{-/-}$, $LRP1^{-/-}$) were used to assess the role of the receptors and none showed a significant survival. Since $ApoE^{-/-}$ mice also exhibit alterations in lipid profile, we utilized the ApoA1-/- mice, which have similar lipid profile to the $ApoE^{-/-}$ mice. Nevertheless, our results show that $ApoA1^{-/-}$ mice are susceptible to cerebral malaria. These experiments clearly showed that it is ApoE glycoprotein per se as opposed to the involvement of brain ApoE receptors or the alteration of lipid profiles responsible for the observed effect. Overall, here we report for the first time that the lack of ApoE renders resistance to mice cerebral malaria infections and this could be used as a potential therapeutic target in combating the deadly cerebral malaria disease.

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TRYPANOSOMA CRUZI INFECTION CAUSES THE TRUNCATION OF APOLIPOPROTEIN A1 IN HOST HIGH DENSITY LIPOPROTEINS (HDL)

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Chagas disease (CD) is caused by the protozoan parasite, *Trypanosoma cruzi*. Endemic in Central and South America where ~10 million persons are infected, latent infections can persist for decades, causing terminal cardiomyopathy in ~30% of subjects. According to previous observations, CD patients, even those who die from cardiac complications have a lower incidence rate of atherosclerosis. However, levels of high density lipoprotein (HDL) and Apolipoprotein A1 (ApoA1) in CD patients are normal. Our laboratory discovered several novel biomarkers for CD using mass spectrometry. We have identified intact Apo A1 as a negative biomarker for CD and several truncated forms of Apo A1 as positive biomarkers for CD. Apo A1 is the principle protein found in HDL. We investigated the cause of these Apo A1 truncations

and concluded that cruzipain, the major cysteine protease of *T. cruzi* is responsible through *in vitro* experiments. We demonstrated that cruzipain is capable of cleaving Apo A1 in lipid-poor protein form or lipid-rich HDL complex at acidic pH. We obtained the exact molecular weight and the relative quantity of these fragments using surface-enhanced laser desorption/ionization-mass spectrometry (SELDI-MS) and confirmed their identity being Apo A1 using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS/MS). Adipocytes have recently come under the spotlight of CD research as the reservoir of *T. cruzi* during chronic infections. We differentiated human primary adipocytes and demonstrated that *T. cruzi* was able to establish a constant infection. We imaged the infected adipocytes using two-photon confocal microscopy technique. More importantly, when HDL was added to *T. cruzi* infected adipocytes, we were able to detect the biomarker Apo A1 fragments. This finding suggested that cruzipain is able to cleave Apo A1 even when it is intact with the parasite. It has also provided further confirmation that the Apo A1 fragments we observed in the human infected sera were caused by *T. cruzi* infection. Furthermore, we incubated HDL with *T.cruzi* cell culture supernatant, parasitic intracellular soluble components and parasite membrane fractions. The full truncation of Apo A1 proteins were only obtained when HDL was incubated with *T.cruzi* membranes at the pH 5.5. When these cell culture components were blotted using anti-cruzipain antibody, the membrane fraction had the highest level of cruzipain presence. This not only confirmed once again that the Apo A1 fragments were produced by cruzipain, but also shed some light on the potential location and mechanism of the truncation. Biomarkers are useful for the development of new and improved diagnostic tools. A number of groups have published their biomarker findings for a broad spectrum of diseases. However, we are the first group to investigate the cause of these biomarkers. Our findings demonstrated that these biomarkers can be used in the development of valid and improved diagnostic tests and also as biomarkers of cure for *T. cruzi* infection.

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LEISHMANIA EXOSOMES AND THEIR INFLUENCE IN THE DEVELOPMENT OF LEISHMANIASIS

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Exosomes are small membrane vesicles (50-100nm) secreted by a variety of eukaryotic cell types from multivesicular endosomes, playing many roles in intercellular cross-talk. Their content carries information in the form of stimulatory molecules (mainly RNAs and proteins) from donor to receptor cells, triggering numerous cellular responses. Despite the abundant knowledge obtained by *in vitro* experiments on the transfer of molecules between cells through exosomes, the function of these vesicles *in vivo* remains unclear. *Leishmania* parasites - considered as early divergent eukaryotes – also release exosome vesicles as a general mechanism of protein secretion. In the past few years, our group has shown that *Leishmania* exosomes are capable of reaching host macrophages *in vitro* and their content is proficient in activating protein tyrosine phosphatases (PTPs), central tools used by the parasites to subvert macrophage functions in their benefit. Corroborating these findings, proteomic analysis pointed to GP63, the major virulence factor of *Leishmania* responsible for activating host PTPs, as the most abundant protein present in exosomes. In addition, comparative proteomics of exosomes secreted by GP63-knockout parasites and by wild type parasites revealed major differences in their overall protein content, suggesting a role for GP63 in the protein sorting to exosomes. Herein we further compare the release, content and ultrastructure of exosome vesicles secreted by several *Leishmania* species and strains, submitted to different environmental conditions *in vivo* and *in vitro*. Proteomic analysis showed that their content can vary, potentially affecting their impact on host infections. Of utmost interest, we will present the first report regarding the formation and release of *Leishmania* exosomes within its vector, the sand fly. Analysis of guts from *Leishmania*-infected sand flies by transmission electron microscopy

(TEM) and proteomic analysis of exosomes isolated from sand fly gut lavages showed that promastigotes release exosomes within the insect host, and these vesicles seem to be similar to the ones obtained from *in vitro Leishmania* cultures. Our data suggests that in addition to transmitting *Leishmania* promastigotes during its blood meal, the sandfly can also transfer exosomes to the host, potentially modulating phagocyte function during early events of the infection. Collectively, our findings contribute to a better understanding of the function of exosomes in the *Leishmania* pathology, opening new possibilities to better design therapeutic and prophylactic drugs against leishmaniasis.

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LEISHMANIA INHIBITS THE PHAGOSOMAL RECRUITMENT OF SEC22B

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Soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins mediate vesicle fusion and play an important role in macrophages during phagocytosis of microbes. Recently, we showed that VAMP8 is cleaved upon infection with *Leishmania* through the surface zinc-metalloprotease GP63. Using cells from VAMP8-deficient mice, we found that this SNARE was required for antigen crosspresentation. The endoplasmic reticulum-associated SNARE Sec22b was recently shown to regulate antigen crosspresentation. Upon infection of bone marrow-derived macrophages with *Leishmania* promastigotes, we observed a significantly reduced Sec22b recruitment to phagosomes containing *Leishmania*. Using mutants defective in either LPG or GP63, we found that this inhibtion was GP63-dependent. In contrast to other SNAREs, we did not observe cleavage of Sec22b, suggesting that the inhibition of Sec22b recruitment to *Leishmania*-phagosomes was indirect, probably due to the cleavage of a cognate SNARE required for the recruitment of Sec22b. To test this possibiltity, we knocked down Syntaxin4 and SNAP23, which are cleaved by GP63 and are involved in microbicidal activity of phagosomes. However, confocal microscopy analysis revealed that absence of either Syntaxin4 or SNAP23 had no effect on the recruitment of Sec22b to phagosomes. We are currently investigating the possible role of Syntaxin 5, which we found is cleaved in a GP63-dependent manner. We assume that, by finding the intermediate protein in fusion with Sec22b, we can uncovered the novel mechanism used by *Leishmania* to evade recognition by the immune system, whereby parasite impair crosspresentation by degrading key regulators of vesicular trafficking. *Supported by a grant from the CIHR*

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THIS DE-WORMED WORLD?

ASP PRESIDENTIAL ADDRESS

E.S. Loker, University of New Mexico

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TAXONOMIC COMPOSITION, ENDEMISM AND PATTERNS OF DISTRIBUTION OF THE HELMINTH PARASITES OF FRESHWATER FISHES OF MEXICO

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In order to draw patterns in helminth parasite composition and species richness in Mexican freshwater fishes we analysed a presence-absence matrix representing every species of adult helminth parasites of freshwater fishes from 23 Mexican hydrological basins. We examined the taxonomic composition and endemism of adult helminth parasites of freshwater fishes of Mexico. Using multidimensional scaling, we examined the distributional patterns of the helminth parasites with regard to the main hydrological basins of the country. We found that the helminth fauna of freshwater fishes of Mexico can characterise hydrological basins the same way as fish families do. The helminth fauna of freshwater fishes of Mexico consists of a large group of Central American Neotropical species (S=119) and another set, less rich of Nearctic species (S=48), which are distributed along with the families of its fish hosts. This fauna is composed predominantly by nematodes, trematodes, and monogeneans, which together contributed 86 % of the total species recorded; cestodes and acanthocephalans being the taxa with the least species recorded. Current data indicates a 22 % (37/170) endemism amongst helminths of freshwater fishes of Mexico. Data suggests that the isolation of bodies of water in the Mexican territory, mostly in the Neotropical areas of southeastern Mexico and in the central Mexican Highland Plateau, with well delimited basins separated by orographic features, provided peculiar conditions that have been conducive to the diversification of a the helminth fauna. It also shows distance decay of similarity and a linkage between host and parasite distributions.

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CERCARIAL EMERGENCE OF *TRICHOBILHARZIA* SPP. (DIGENEA: SCHISTOSOMATIDAE) FROM TWO LYMNAEID SNAIL HOSTS UNDER DIFFERENT LABORATORY CONDITIONS

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The free-living trematode larvae, the cercariae, are essential components of food webs subsuming a substantial fraction of biomass in the freshwater ecosystems. The cercariae of the bird schistosomes of the genus *Trichobilharzia* parasitizing freshwater snails and waterfowl are the most common and important agents of swimmer's itch in humans. Although information about various aspects of the biology of *Trichobilharzia* spp. has been gathered in past decades, data on the patterns of cercarial emission are still limited. Cercarial emergence of *Trichobilharzia szidati* and *Trichobilharzia franki* from 13 naturally infected *Lymnaea stagnalis* and 3 *Radix auricularia*, respectively, was investigated in different laboratory conditions, i.e. natural photoperiodic regime and 12:12 light-dark cycle under standard conditions. Series of laboratory experiments were performed over 24-hour periods for 3 consecutive days for each experiment in June and September 2012 to determine (i) daily output rates (ii) peaks in cercarial emission, and (iii) output variation among conditions and experiments. Cercarial output, independent of snail size, was circadian with high levels in the light period, however variable for individual replicates. The mean daily emergence rate per snail of *T. szidati* was 1,143 cercariae snail^{-1}day^{-1} in June with a maximum of 4,560 cercariae day^{-1}. There were significant differences in *T. szidati* emergence rates between 4 experiments performed in June and 1 experiment in September with higher cercarial emission rates in September (mean 10,140 snail^{-1}day^{-1}; maximum of 29,560 cercariae day^{-1}). Emission rates of *T. franki* were on average 1,057 snail^{-1}day^{-1}, reaching a maximum of 3,050 cercariae day^{-1}. The experiments confirmed a peak in emission in the morning hours but revealed complex intraspecific variation

depending on experimental conditions. This study provides insights into the patterns in cercarial emergence of the two causative agents of swimmer's itch. The ecological and epidemiological consequences are discussed.

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HELMINTH INFECTIONS IN JUVENILE ROUND GOBY *NEOGOBIUS MELANOSTOMUS* IN THE ST. LAWRENCE RIVER, CANADA

J.J. Forest, Concordia University
D.J. Marcogliese, Environment Canada
J.D. McLaughlin, Concordia University

The round goby, *Neogobius melanostomus*, is a freshwater fish native to Europe that has become established as an invasive species in the St. Lawrence River, Canada. Over the past six years, the relative abundance of gobies within specific areas of the St. Lawrence has increased from zero to the point where it is currently estimated to represent up to 90% of the fish caught. In its native range, the goby is often one of the most highly parasitized hosts in diversity and abundance. In the St. Lawrence River however, gobies are infected with generalist parasites but their diversity is less than that found in native hosts. Gobies spawn continuously through the season but to date, there is no information on how soon they acquire their infections in the St. Lawrence River. We sampled gobies, targeting young fish, monthly from June through November, 2012 from two sites in the St. Lawrence River to determine when they acquired their infections. Preliminary results indicate that infections do not occur in recently hatched fish, but do occur in fish by the time they are a year old.

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OXYSPIRURA PETROWI IN NORTHERN BOBWHITES FROM SOUTH TEXAS AND THE ROLLING PLAINS

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Oxyspirura petrowi is an indirect lifecycle nematode found under the nictitating membrane and within the conjunctival sacs and nasolacrimal ducts of various gallinaceous birds. Recent observations suggest that *O. petrowi* infections in the Northern Bobwhite (*Colinus virginianus*) may negatively affect vision, supporting the hypothesis that this helminth parasite may reduce individual fitness within bobwhites. Historically, much of what is known about *O. petrowi* infections in bobwhites within Texas comes from the Rolling Plains, whereas little is known about this species in bobwhites from South Texas. The objective was to determine prevalence, intensity, and abundance of *O. petrowi* in bobwhites from South Texas and the Rolling Plains of Texas and western Oklahoma using hunter-shot samples collected in the winter 2012–2013. Findings are discussed in this presentation. Our research provides additional information about *O. petrowi* in Northern Bobwhites occurring in South Texas and the Rolling Plains ecoregion.

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OPERATION IDIOPATHIC DECLINE: SURVEY AND ASSESMENT OF PARASITIC INFECTIONS OF NORTHERN BOBWHITES IN THE ROLLING PLAINS ECOREGION

A. Bruno and **A.M. Fedynich**, Texas A&M University-Kingsville
D. Rollins, Texas A&M University

Parasites and diseases are often overlooked or disregarded as inconsequential factors in population declines of the northern bobwhite (*Colinus virginianus*). To learn more about disease agents, a multi-year survey is being conducted on bobwhites from the Rolling Plains ecoregion of Texas and western Oklahoma. The objectives of this study are to survey for the presence of *Trichomonas gallinae,* assess helminth prevalence, intensity, and abundance by host age, sex, body weight, and year of collection, and identify potential pathological responses caused by the cecal worm *Aulonocephalus pennula* and the eyeworm *Oxyspirura petrowi*. Northern bobwhites were trapped and euthanized on 31 ranches in August and October of 2011 (n = 43) and 2012 (n = 59) and examined for helminths. Samples for *T. gallinae* were taken from 194 live bobwhites during the same two-year period and tested using DNA Polymerase Chain Reaction (PCR) and Gel Electrophoresis. Seven species of helminths were found with *A. pennula*, *Oxyspirura petrowi*, and *Tetrameres* sp. representing the most frequently occurring species. Details on the data collection, results, and analysis from the first two years of this study are discussed further in this presentation. This research provides additional information about parasites infecting northern bobwhites in the Rolling Plains ecoregion.

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ENVIRONMENTAL DNA: AN EFFECTIVE METHOD TO DETECT PARASITE PRESENCE IN WATER BODIES

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Detecting the presence of most parasites in the environment is challenging. This is most often accomplished through collections and necropsies of known hosts but is time-consuming and can be difficult logistically. We present a method of detecting the presence of *Ribeiroia ondatrae*, a pathogenic trematode causing death, delayed development, and various malformations in larval amphibians. Owing to its devastating effects on many amphibians, it is critical to understand the extent of this parasite's range in order to predict its possible future impacts on amphibian populations and species. Environmental DNA (eDNA) has been used to assess the presence of various organisms but has not previously been employed in this context. We developed a PCR method using primers specific to *R. ondatrae* that showed no cross-reaction with other common trematodes and is capable of detecting as little as 1/2500000th of a single cercaria under optimal conditions. A water filtration unit was also developed in order to conduct field sampling from sites known to have *R. ondatrae* present or not based on multiple years of host examination. The PCR method was very accurate with no false positives but use of quantitative PCR resulted in even greater accuracy. Controlled tests indicated a high resilience of the parasite's DNA over times and temperatures associated with a field season. Use of parasite eDNA can thus be further developed as an effective tool for detecting specific species in sites of interest.

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HOST BEHAVIOURAL MANIPULATION OF THE SPOTTED LADY BEETLE BY A PARASITIC WASP

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Parasites and parasitoids have evolved various strategies to exploit hosts to their own advantage. Bodyguard manipulation is one of those and consists of usurping the behaviour of the host to confer some protection to the parasite and/or its offspring. The parasitic wasp *Dinocampus coccinellae* is a solitary endoparasitoid of the spotted lady beetle *Coleomegilla maculata*. Following larval development within the host body cavity, a single parasitoid larva egresses from the host abdomen, spins a cocoon between the ladybird's legs and initiates pupation. Unlike most parasitoids, *D. coccinellae* does not kill its host during development, but keeps the coccinellid partially paralyzed on top of the cocoon, where it acts as a bodyguard against natural enemies. Another remarkable fact is that some ladybirds are able to survive from this parasitism and to recover their motility, their foraging behaviour and even can reproduce. Through this presentation, we will provide a brief overview of different works carried out to date in order to better understand the nature and the consequences, for both the parasite and the host, of such an amazing behavioural manipulation.

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REDUCED PARASITISM AND DIET BREADTH IN A NATIVE PREDATOR (*LEPOMIS GIBBOSUS*) FEEDING ON INTRODUCED PREY (*DREISSENA POLYMORPHA*)

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D.J. Marcogliese, St. Lawrence Centre, Environment Canada
M.R. Forbes, Department of Biology, Carleton University

In novel environments, invasive host species tend to have fewer parasites than their native counterparts. A 'release' from native parasites could be part of the reason why invasive species often rapidly become highly abundant, but it also has unstudied implications for native predators feeding on exotic prey. In particular, shifts to non-indigenous prey should result in lower levels of trophically transmitted parasites. We tested this hypothesis in populations of native pumpkinseed sunfish (*Lepomis gibbosus*) (total n=99) and introduced zebra mussels (*Dreissena polymorpha*). We studied fish in Lake Opinicon, where extensive data on the stomach contents of pumpkinseed are available from the early 1970s, prior to the appearance of zebra mussels in the mid-1990s. The stomach contents of modern day pumpkinseeds were dominated by zebra mussels, and stable isotopes of carbon and nitrogen confirmed they represent a major proportion of long-term diets. Because historical parasite data are not available in Lake Opinicon, we surveyed stomach contents and parasites in pumpkinseed in both Lake Opinicon and an ecologically similar, neighbouring lake where zebra mussels were absent. The stomach contents of pumpkinseed in the companion lake did not differ from those in pre-invasion fish from Lake Opinicon, but were more diverse than in fish from post-invasion Lake Opinicon. The companion lake is therefore used as a surrogate "pre-invasion" reference to estimate the effects of consuming zebra mussels on parasites. Trophically transmitted parasites were markedly less diverse and abundant in fish that feed on zebra mussels. Both isotopes and parasites suggest that predation on zebra mussels contributes to a novel trophic coupling between littoral and pelagic food webs in Lake Opinicon, with native pumpkinseeds experiencing a form of "parasite release" by feeding on these introduced prey.

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EXPANSION OF THE INVASIVE ASIAN FISH TAPEWORM IN THE LOWER GREAT LAKES AND ST. LAWRENCE RIVER

D.J. Marcogliese and **A.D. Gendron**, St. Lawrence Centre Environment Canada
J.J. Forest and **J.D. McLaughlin**, Concordia Univerity

A single specimen of the Asian fish tapeworm, *Bothriocephalus acheilognathi*, was found in a bluntnose minow (*Pimephales notatus*) collected from Grosse Isle in the Detroit River in 2002. To evaluate the potential spread of the parasite in the Great Lakes, emerald shiners (*Notropis atherinoides*) were collected from 15 sites in Lake Erie, Lake Ontario, the St. Clair River, the Detroit River, the Niagara River and the St. Lawrence River as far east as Cornwall in Lake St. Francis in 2009-10. The parasite has increased in abundance and spread across lakes Erie and Ontario. Prevalence of infection reached 43%, with mean intensities surpassing 5 parasites per fish at certain sites. In 2012, the parasite was found for the first time in the St. Lawrence River, in bluntnose minnows from Lake St. Louis, a fluvial lake west of Montreal. Parasites were also found in baitfish obtained from suppliers along the Lower Great Lakes, implicating this industry in the spread of the invasive cestode. In pooled samples of emerald shiners, intensity was negatively correlated with condition factor, suggesting that the parasite negatively affects the health of native minnows.

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ESTIMATION OF THE EFFECTIVE POPULATION SIZE (NE) AS A GENETIC EPIDEMIOLOGICAL TOOL TO MONITOR METAZOAN PARASITE POPULATION AND TRANSMISSION DYNAMICS

C.D. Criscione, Texas A&M University

Effective parasite control requires data on transmission and population dynamics. Yet, direct observation of parasite population dynamics is often precluded. As an alternative, population genetics analyses with multilocus genotypic data can provide an indirect means to infer transmission and population growth patterns for parasites. For example, landscape genetics methods revealed focal transmission centered on households and that people reacquired infections of *Ascaris lumbricoides* from the same source pool over time in Jiri, Nepal (Criscione et al. 2010. PLOS NTD 4:e665). Also, studies have begun to use parasite genetic diversity as a means to monitor the impact of parasite control programs. These studies largely rely upon statistics such as allelic richness or expected heterozygosity. While it is useful to report these statistics, they have caveats and lack predictive utility. Here, I present a novel integration of the effective population size (N_e) parameter into population monitoring and epidemiological studies of metazoan parasites. N_e is the size of an ideal population that has the same rate of genetic drift as the population of interest; it predicts gene diversity within a population and is needed to assess the relative importance of the other evolutionary mechanisms: mutation, gene flow, and selection. Recent developments of single-sample, contemporary genetic estimators of N_e hold great promise for epidemiological, ecological, or microevolutionary studies of parasites. Using the *Ascaris* microsatellite data from Jiri, I demonstrate the utility of these estimators and offer epidemiologically related questions that can be addressed with N_e. I also discuss how life history and sampled parasite stage (e.g., adult vs. larva) affect interpretation of the estimated effective size. I stress the significance of the latter in relation to the idiosyncrasies of *Ascaris* biology and for parasites where some stages cannot be sampled (e.g., adult schistosomes from humans).

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RELATIVE BODY CONDITION OF THE GUPPY, *POECILIA RETICULATA*, AND FOOD AVAILABILITY INFLUENCE *GYRODACTYLUS TURNBULLI* (MONOGENEA) EPIDEMIC DYNAMICS

C.P. Tadiri, **F. Dargent** and **M.E. Scott**, McGill University

Understanding disease transmission is important to species management and human health. Host body condition, nutrition and disease susceptibility interact in a complex manner, and while the individual effects of these variables are well known, our understanding of how they interact and translate to population dynamics is limited. Our objective was to determine whether host relative body condition influences epidemic dynamics of a fish ectoparasite, and how this relationship is affected by food availability. 210 *Poecilia reticulata* (guppies) of roughly similar size were selected and assembled randomly into populations of 10 guppies assigned to 3 different food availability treatments. The weight, length and the relative condition index (Kn) of each fish were calculated. We infected 1 guppy per population ('source' fish) with 2 *Gyrodactyus turnbulli* and counted parasites on each fish every other day for 10 days. Epidemic parameters for each population were calculated and compared among food availability treatments and host Kn using generalized linear models. High host Kn—particularly that of the 'source' fish—exerted a positive effect on incidence, peak parasite burden, and the degree of parasite aggregation. Low food availability increased the strength of the associations with peak burden and aggregation. Our findings suggest that host Kn and food availability interact to influence epidemic dynamics, and that the condition of the individual that brings the parasite into the host population has a profound impact on the spread of infection.

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SEASONALITY OF GREGARINE (EUGREGARIONORIDA) INFECTIONS OF AMPHIPODS (*GAMMARUS*) IN THE NORTH BRANCH OF THE RARITAN RIVER

R.L. Grunberg and **M.V. Sukhdeo**, Rutgers University

Gregarines, unicellular protozoan parasites, are an extremely diverse group of organisms infecting most invertebrate hosts. In this study we examine 2 gregarine species that parasitize amphipods (*Gammarus* sp.) in the Raritan River, NJ. The tentative identification of the gregarines are *Heliospora* sp. and *Rotundula* sp., both have which been described in *Gammarus pulex* from other parts of the world. Prevalence and intensity of gregarine infections were monitored over a year with bi-monthly collections using a surbur sampler. The population density of amphipods was highest in June, with the majority of the individuals belonging to the smallest size class. By October, the overall population size significantly decreased and was comprised mostly of the larger adult size class. Prevalence was high in the summer among the small size classes of amphipods with intensity increasing with increasing size and age. This supports a connection between amphipod population demographics and the seasonality of gregarine infections. Detailed descriptions of the 2 species based on oocyst and gregarine morphology will be used to clarify their taxonomic identities.

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TEMPORAL VARIATION OF *ARGULUS YUCATANUS* IN THE MAYAN CICHLID *CICHLASOMA UROPTHALMUS* IN CELESTUN, YUCATAN, MEXICO

A. May-Tec, CINVESTAV, México

Little is known about the temporal variability of helminth parasites of aquatic organisms in tropical regions. This is currently an issue in the tropics due to the potential effect of Global Climate Change (GCC) on parasite dynamics. Indeed, there is a lack of information on key environmental factors affecting parasite abundance through time in these regions. Rainfall has been recently suggested as a key factor because both aquatic hosts and their parasites experience numerical oscillations due to annual patterns of precipitation and hydrodynamic disturbances such as tropical storms and hurricanes. A further complication in the tropics is the influence of natural disturbances occurring at time scales longer than a year (e.g. El Niño Southern Oscillation (ENSO) 3-5 years), for which it is necessary to carry out long-term observations. The aim of our study was to determine the potential influence of physicochemical factors in the long-term fluctuations of the prevalence and mean abundance of *Argulus yucatanus* in it host *Cichlasoma urophthalmus* from a coastal lagoon in Yucatan over a period of 10 years. Variability in physicochemical factors and monthly infection parameters of *A. yucatanus* were analyzed using time series and wavelets to determine potential recurrent patterns. The infection parameters of *A. yucatanus* showed peaks every 6, 12 and 24 months with a peak of maximum variability in 60 months. The peaks of maximum variability were related with fluctuations of salinity, rainfall and temperature suggesting the influence of ENSO. The present work showed that rainfall is not the only key factor, but also a synergism among different physicochemical factors and processes acting at a long temporal scale which in turn affect this host parasite system. The temporal variability of infection parameters of *A. yucatanus* expanded over periods longer than a year, suggesting the need for long term data sets in studying temporal variability in infection levels in the context of GCC.

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HELMINTHS OF *MICAELAMYS NAMAQUENSIS* AND *AETHOMYS CHRYSOPHILUS* (RODENTIA: MURIDAE) FROM NORTHWESTERN BOTSWANA

L.E. Reid, **T.J. Cook** and **M.L. Thies**, Sam Houston State University

Two species of Botswanan rodents, *Micaelamys namaquensis* (Namaqua rock rat) and *Aethomys chrysophilus* (veld rat), were collected from the Koanaka Hills region of Ngamiland Province in northwestern Botswana in June 2008 and July 2009. The gastrointestinal tracts of 40 specimens (20 *M. namaquensis* and 20 *A. chrysophilus*) collected from 3 of 6 microhabitats identified as part of a biotic inventory project investigating the effects of wildfire were examined for helminths. Prevalence and intensity were calculated to potentially reveal correlations between parasite prevalence and host sex, habitat range, and environmental factors. Gastrointestinal tracts were removed in the field, preserved in 95% ethanol, and returned to SHSU for subsequent analyses. Helminths were removed and stored in 70% ethanol for preservation. Cestodes were stained with hematoxylin and eosin and nematodes were viewed under wet mounts of glycerin and ethanol. Helminths were examined with an Olympus BX51 microscope and digital images of all specimens were captured with an Olympus DP 72 digital camera. Nematodes had the highest prevalence of infection in *A. chrysophilus* at 70%, with a lower prevalence in *M. namaquensis* at 65%. Prevalence of cestodes in *A. chrysophilus* was 10%, while the prevalence of cestode infection in *M. namaquensis* was 5%.

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A MULTIVARIATE ANALYSIS OF SOME DIGENEAN SPECIES COLLECTED FROM SEVERAL RED SEA FISHES IN SAUDI ARABIA

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A group of ten digenean species needs a considerable interest to analyze and discuss their phylogeny and classification. Phylogenies and classification of these trematodes are poorly discussed in Saudi Arabia. The present study aimed to better understand the phenetic relationship between the genera and species of ten digenean parasites obtained from some commercial fishes of the Red Sea at Jeddah city, Saudi Arabia, within their families. Three types of analysis were performed with statistical V7 software, Cluster, Principal Component Analysis (PCA) and non parametric Multi-Dimensional Analysis (MDA). These analyses were based on the ten species of Digenea (Operational Taxonomic units, henceforth OTUs) described by Thirty-two morphological characters. The result showed that the position of the present species in the phenogram is identical to their taxonomic relationships, and the different Digenea studied are distinguished into four subgroups recording families, Lepocreadiidae, Hemiuridae, Cryptogonimidae, Angiodictyidae, Acanthocolpidae and Fellodistomidae. PCA explains 62.4% of the total observed variations. The percentages of the morphological variations within the components recorded were 24.9%, 19.4% and 18.3% for the first, second and third axes, respectively. The main characters utilized for the separation (characters with high loading factor >0.7) are those pertaining to the following, location of oral sucker, shape of pharynx, location of ovary, distance between anterior testis and ovary, uterus, shape of ventral sucker, ventral sucker location, testes location, egg size and shape of ovary. MDA confirms the separation of ten digenean species into four subgroups representing six families which is conforming with that of Cluster and PCA analyses. These results stressed the importance of some morphological features (Highest loading factor < 0.7) as an indicator of the relationships.

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HISTORICAL BIOGEOGRAPHY OF *RHABDOCHONA* SPECIES

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Historical biogeography of species of freshwater fish nematode parasites of the genus *Rhabdochona* is presented. Area hypotheses were generated with published fish and parasite area phylogenies of proteocephalids, *Spinitectus* spp., fossil teleosts (both freshwater and marine), and Cyprinidontiformes. BPA2 and PACT 2.0. were used to generate single area cladograms for all taxa. A single area cladogram was recovered from the PACT analysis of the area cladograms of *Rhabdochona* spp. and Cyprinodontiformes. The PACT tree of Cyprinodontiformes and *Rhabdochona* spp. recovers a series of common postdispersal speciation events for the nematode species within African, South and Central Asian, and Eastern American sister clades. This pattern was superimposed on a 140 mya map. From this analysis it can be seen that most of the neighboring areas inhabited by sister species of *Rhabdochona* spp. are reticulated and presumably represent coastal marine tracks of southern, western and northern Tethys. These clades seem to have diversified before Africa and South America separated. A reticulated biogeographic pattern of fossil fishes distribution might indicate that freshwater and marine dispersion throughout Western Tethys took hold once Laurasia and Gondwana drifted apart as inferred from marine fossil fish. *Rhabdochona* spp. present distribution seems to have been influenced by similar wide-ranging dispersal routes that included modern South America, Cuba, North America and eastern Asia (northern Tethys sea and freshwater drainages flowing into it). Therefore, it seems that *Rhabdochona* was originally

a freshwater parasite that has undergone widespread marine dispersion. Additionally, the original hosts of *Rhabdochona* species were not cyprinids, but some other group of teleosts, probably silurids.

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PHYLOGENETIC RELATIONSHIPS AND GENEALOGY OF A BLOOD PARASITE *HEMOLIVIA MAURITANICA*

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While many new species of coccidia are being continuously described, their host specificity and precise phylogenetic positions often remain unclear. The phylogenetic uncertainty stems largely from highly incomplete sampling of the available species-lineages. Similar problems prevent evolutionary analyses of geography and host specificity patterns at intraspecific genealogical level. The situation is even more complicated in "lower coccidia" (suborder Adeleorina) that are less studied than Eimeriorina. Here we present the evolutionary relationships of a blood parasite *Hemolivia mauritanica*, infecting *Testudo graeca* tortoises. *H. mauritanica*, belonging to "haemogregarines sensu lato", is a heteroxenous parasite transmitted by *Hyalomma aegyptium* ticks, the definitive host. Tortoises of the genus *Testudo*, serving as its intermediate hosts, can be infected locally with prevalence reaching almost 90 %. Interestingly, young tortoises up to 4 years are always *Hemolivia*-negative, perhaps due to an unknown trait in transmission routes from ticks. During our field studies, we managed to obtain *Hemolivia* blood-positive samples from *Testudo* tortoises from 9 countries (Algeria, Bulgaria, Greece, Iran, Iraq, Lebanon, Romania, Syria and Turkey). Phylogenetic analyses of nuclear 18S rRNA gene repeatedly revealed that *H. mauritanica* is most closely related to *Hepatozoon* parasites. However, this gene is quite conserved and does not allow for further resolution of intraspecific relationships. For that purpose, we sequenced mitochondrial gene for cytochrome *b*. Preliminary results indicate that *H. mauritanica* splits into several lineages and haplotypes. Phylogenetic position of the other *Hemolivia* species, *H. mariae* from Australian lizards, will be also discussed. This project represents the first study of "lower coccidia" on the population level. *Study is supported by the grant GAČR P506/11/1738 of the Czech Science Foundation.*

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ECOLOGY, TAXONOMY, AND EVOLUTION OF THE *CHAETOGASTER LIMNAEI* (OLIGOCHAETA, ANNELIDA) SPECIES COMPLEX

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Within Oligochaeta, *Chaetogaster limnaei* is unusual in exhibiting a parasitic relationship with freshwater pulmonate snails. Taxonomic confusion has been caused by differences in what have been considered two subspecies of this worm: *C. limnaei limnaei* is an ectosymbiont and is found just inside the shell or mantle cavity of the snail, whereas *C. limnaei vaughini* is endoparasitic and lives in the nephridium of the snail. Little research has been conducted on the ecology, population structure and species status of these worms. Morphological evidence has previously noted differences between the two subspecies, including the number and size of chaetae. This study aimed to explore the distribution of these annelids in Central New York and use mitochondrial DNA sequence data from the COI locus to examine the relationship and species status of the ectosymbiotic and endoparasitic forms of *C. limnaei*. Snails (*Physa gyrina*) were

collected from seven locations in central New York streams and lakes. One hundred forty snails were dissected, and at least one form of *Chaetogaster* was found in eighty-eight specimens, a prevalence of 62.9%. COI sequence data from New York worms, combined with data from Massachusetts, did not reveal separate ectosymbiotic and endoparasitic lineages. Instead, all endoparasitic forms were part of a mixed clade including ectosymbiotic and endoparasitic forms. This mixed clade was nested within clades of ectoparasitic forms, suggesting that a lineage of *C. limnaei* able to be both endoparasites and ectosymbionts evolved from ectoparasitic ancestors.

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MOLECULAR PHYLOGENY OF SPECIES OF *LIGOPHORUS* (MONOGENEA: DACTYLOGYRIDAE) AND THEIR AFFINITIES WITHIN THE DACTYLOGYRIDAE

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We evaluated the taxonomic framework of *Ligophorus*, monogenean specialists of the gills of grey mullets (Mugilidae), and assessed its interspecific relationships using molecular data. The position of *Ligophorus* within the paraphyletic Ancyrocephalinae was re-assessed based on newly sequenced species. Furthermore, we evaluated the relationship between morphometric and genetic interspecific similarities. We analysed partial 28S and complete ITS1 rDNA sequences from representatives of 14 of the 16 nominal species of *Ligophorus* from the Mediterranean, Black and Azov Seas together with published sequences of members of the Dactylogyridae. The phylogenetic analyses of the Dactylogyridae (i) confirmed the position of *Ligophorus* within the marine Ancyrocephalinae; (ii) revealed a sister relationship between *Ergenstrema* and *Ligophorus*, whose species are all exclusive parasites of grey mullets; and (iii) substantiated the affinities of *Ergenstrema* with the marine Ancyrocephalinae. The phylogenetic analysis restricted to *Ligophorus* confirmed the distinct status of the included species. The ITS1 region provided higher divergence between species (from 0.4 to 22.7%) than the 28S region (from 0.4 to 9.3%). Both the 28S and ITS1 phylograms revealed two main clades. One included species from hosts with Mediterranean and NE Atlantic distribution and another was formed by species parasitising several *Liza* spp., including *Lz. haematocheilus* from the Northwestern Pacific, and *Mugil cephalus*, which suggests an origin outside the Mediterranean for the latter clade. The phylogenetic evidence presented herein indicated that a combination of host-switching and lineage duplication events accounted for the diversification of this genus in the Mediterranean basin. The correlation between the phenetic matrix and each of the genetic matrices was significant (r=0.47, P=0.003 for ITS1, and r=0.31, P=0.02 for 28S), which supports the validity of morphometric characters used for species identification in *Ligophorus*.

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RELAXED HOST SPECIFICITY IN A NEW CESTODE GENUS FROM *DASYATIS* AND *HIMANTURA*

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Survey work on elasmobranchs and their parasites in Borneo and elsewhere has revealed the presence of multiple new genera of cestodes. One new genus of rhinebothriidean cestode, referred to by Healy et al., 2009 as Rhinebothriinae new genus 3, is distinguished from other rhinebothriidean genera by its possession of tear drop-shaped bothridia that possess a posterior row of loculi that are longer than wide. To date, survey work has resulted in discovery of specimens of Rhinebothriinae new genus 3 in >25

species of batoid elasmobranchs from the Indo-Pacific and the eastern Atlantic. The initial goal of this project was to describe a new species of this new cestode genus from the stingray *Dasyatis* cf. *zugei* from Borneo. Preliminary light- and scanning electron microscope examination of cestodes from other stingray species, however, suggests that the species of Rhinebothriinae new genus 3 initially observed from *D.* cf. *zugei* may occur in multiple species of stingrays. In this study, cestodes from seventeen stingray species from Vietnam and Borneo, Indonesia were examined. Of the 17 species, four species, *Dasyatis zugei, Himantura uarnak* 3*, Himantura pastinacoides* and *Himantura undulata,* were infected with specimens of Rhinebothriinae new genus 3 that appear to be conspecific with those specimens originally observed from *D.* cf. *zugei*, based on characteristics of the bothridia and proglottids. Specimens from each of the aforementioned stingray species possess bothridia with the same orientation and number of anterior and posterior loculi. In addition, the left- and rightmost longitudinal septa in the posterior region of the bothridia adjoin three short incomplete septa in each of the specimens examined. Cestodes from each of the five stingray species also shared sexual organ characteristics including cirrus sac size, shape, and extent, and distribution of the vitellaria. These preliminary morphological data suggest that host specificity is relaxed in this new species of Rhinebothriinae new genus 3.

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SCHISTOSOMIASIS IN NEPAL

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Events that occurred in central Asia are pivotal to understanding the evolutionary history and diversification of a medically important group of parasites, the schistosomes that are responsible for causing schistosomiasis in people and animals throughout the world. Because Nepal has been unstudied with respect to its schistosome fauna, we have been sampling freshwater snails and animal dung samples from southern Nepal for schistosomes. We have recorded host usage, standard morphological features and sequenced DNA from the large ribosomal subunit (28S) and mitochondrial (*cox*1) sequence to facilitate identification and phylogenetic relationships. We have thus far found all three of the known members of the *Schistosoma indicum* group (*S. indicum*, *S. nasale*, and *S. spindale*) to be present in Nepal: all have been recovered from the planorbid snail, *Indoplanorbis exustus*. We have also recovered *Bivitellobilharzia nairi* from dung samples of the Indian elephant, *Elaphus maximus*, and surprisingly, from dung samples of the Asian great one-horned rhinoceros, *Rhinoceros unicornis*. Our work suggests elephants and rhinos share *B. nairi* in Chitwan National Park in Nepal, even though they belong to unrelated mammalian families. In addition, we have found at least two lineages of schistosome cercariae from Nepalese lymnaeid snails. These either cluster with mammalian schistosomes such as *Schistosoma (*formerly *Orientobilharzia) turkestanicum,* or with the avian schistosome genus *Trichobilharzia*. From *I. exustus*, a snail previously associated only with mammalian schistosomes, we have found an avian schistosome. This is the largest schistosome cercaria recovered to date (average 1.18mm in length, including body, tail stem and furcal length). This species clusters phylogenetically with species of *Macrobilharzia*, a schistosome genus thus far known only as adult worms from anhingas and cormorants. Our studies indicate Nepal has a diverse schistosome fauna, and that it will provide new insights into understanding the evolution, host use, and transmission biology of schistosomes.

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ARE SCHISTOSOMES A SPECIAL CASE OR TYPICAL STORY OF PARASITE DIVERSIFICATION?

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The evolutionary impact of host switching has been great and is considered the major driver for the diversification of parasites. Identifying mechanisms for species diversification and characteristics of those processes remain at the core of evolutionary studies. Centuries of discoveries continue to reveal that digenetic trematodes represent one of the most spectacular radiations of helminthes. One of the most distinctive, biologically intriguing and medically significant families of digeneans is the Schistosomatidae. What can the schistosomes tell us about the features important in their diversification and are these features unique to this family or perhaps indicative of more general trends in parasite diversification? Here schistosome diversity will be explored within a phylogenetic framework to summarize important features that seem to be correlated with the observed patterns and biology. Globally, schistosomes are significant pathogens of birds and mammals, primarily associated with freshwater habitats and aquatic snails of several families serve as intermediate hosts. In fact, 13 families of marine and freshwater snails are used by members of this relatively small family (~100 described species), a diversity of snail hosts, as far as we know, has not been observed in any other trematode family, even among some of the more speciose families. Furthermore, the diversity of definitive host use is paradoxically both broad and narrow, depending on the lineage. There are two basic morphologies and associated reproductive strategies that might also contribute: large robust males and long females, when mated stay together unless she is laying eggs and the long, small and thin worms who probably only mate once, after which the female goes to egg laying sites and does not return. We will discuss how particular features of host use, morphology and reproductive strategy may have all played a role in schistosome diversification and how that compares to what we know about other digenetic trematode families. This study supported by grants NSF DEB 1021427 to SVB and NIH to ESL P20RR18754 and R01 AI101438

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PHYLOGENETIC ANALYSIS AND RECONFIGURATION OF THE GENERA IN THE CESTODE ORDER DIPHYLLIDEA

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The generic boundaries of the cestode order Diphyllidea were reassessed based on phylogenetic analyses of 28S rDNA, 18S rDNA, and COI sequence data for 31 species representing morphological variation across the order. These analyses yielded a number of well-supported clades each substantiated by unique morphological features mandating generic revision of the order and erection of several new genera. Species originally assigned to *Echinobothrium* van Beneden, 1849 but bearing a corona of spines on the region of the scolex posterior to the apical organ armature, and that generally parasitize triakid sharks, are transferred to New genus 1. Species originally assigned to *Echinobothrium* that possess lateral hooklets arranged in continuous bands, rather than in 2 distinct clusters, and that parasitize batoids are transferred to New genus 2. Our analyses support transfer to *Echinobothrium* of the 5 species lacking cephalic peduncle spines that were originally assigned to *Macrobothridium* Khalil and Abdul-Salam, 1989. As a result, *Echinobothrium sensu stricto* includes species both with and without cephalic peduncle spines, but all members of which possess lateral hooklets arranged in 2 distinct clusters on either side of

the dorsal and ventral apical hooks. Most of the species that remain in *Echinobothrium* parasitize skates of the family Rajidae, guitarfish of the family Rhinobatidae, and stingrays of the dasyatid genera *Taeniura* Müller and Henle, *Dasyatis* Rafinesque, and *Himantura* Müller and Henle. A clade of diphyllideans parasitizing catsharks was also recovered. This consisted of *Ahamulina* Marques, Jensen and Caira, 2012, unique in possessing apical hooks but lacking lateral hooklets and cephalic peduncle spines, and *Ditrachybothridium* Rees, 1959, unique in lacking scolex armature entirely. However, also found within this catshark-hosted clade was a species parasitizing the skate *Leucoraja wallacei*, which is assigned to New genus 3. Although the majority of diphyllideans parasitize batoids, taxa parasitizing sharks and catsharks in particular, remain problematic. Additional collections from these carcharhiniform hosts are likely to be particularly illuminating.

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NOVELTY AND HOST SPECIFICITY OF PARUTERINID CESTODES FROM SOUTH AMERICAN BIRDS

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Newly collected specimens of paruterinid cestodes from passerine birds in Brazil and Chile were examined using light and scanning electron microscopy. In combination with museum specimens from Paraguay and Bolivia, these specimens included a new paruterind genus and species in tyrant flycatchers (Tyrannidae), a new species of *Anonchotaenia* also in tyrannids, and facilitated the redescription of *Anonchotaenia brasiliensis* and *Anonchotaenia macrocephala*. Light microscopy revealed variation in structures not traditionally relied upon for species delimitation within *Anonchotaenia*, despite their use in other paruterinid genera. Scanning electron microscopy of both known *Anonchotaenia* spp., the first use of this technology on paruterinids, revealed less microthrix variation across the scolex surfaces of both species than has been reported for other cyclophyllideans. Molecular data were generated for nuclear 18S rDNA and 28S rDNA and mitochondrial 16S rDNA and COI for *A. brasiliensis*, *A. macrocephala*, and the new genus and species. Results of Maximum Likelihood and Bayesian Inference analyses of these data supported *A. macrocephala* as distinct from *A. brasiliensis*, but revealed cryptic diversity among nominal *A. brasiliensis* specimens collected from hosts of different families. Newly collected and museum specimens expanded the range of host associations of these *Anonchotaenia* spp. and prompted a formal assessment of host specificity. Specificity of *A. macrocephala* and the new species of *Anonchotaenia* was found to be metastenoxenous (HS_S=>3–<5), while specificity of *A. brasiliensis* was found to be euryxenous (HS_S=>5). *Anonchotaenia macrocephala* parasitizes only members of Hirundinidae, while the new species of *Anonchotaenia* and *A. brasiliensis* parasitize a number of host species belonging to different passerine families known to participate in mixed-species foraging flocks in the Atlantic Forest. A diversity of bird species of different families join these foraging flocks and are among the enormous number of passerine species in South America that have yet to be examined for cestodes.

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LINEAGE DIVERSITY, MORPHOLOGICAL VARIABILITY AND HOST SPECIFICITY WITHIN *POTAMOTRYGONOCESTUS* PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS

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Members of the genus *Potamotrygonocestus* (Tetraphyllidea: Onchobothriidae) are parasites that exclusively infect stingrays of the family Potamotrygonidae, which are endemic to the Neotropics. To date, seven species are recognized in this genus (*P. magdalenensis*, *P. travassosi*, *P. amazonensis*, *P. maurae*, *P. fitzgeraldae*, *P. chaoi* and *P. marajoara*), and two undescribed lineages were mentioned in the most recent taxonomic revision published. The taxonomy of these parasites has traditionally been based on morphometric characters and therefore relies on soft tissue structures whose observed morphology is highly dependent on the fixation method used. This, in addition to the use of a restricted number of specimens from distant localities on taxonomic studies, has led researchers to believe the genus has a low level of morphological variation. In this study, 1753 *Potamotrygonocestus* specimens were examined from almost all of the South American basins. The traditional morphometric characters for the generic taxonomy were not useful for species diagnosis, but the discrete characters from the hook morphology were shown to be informative. The hooks are sclerotized structures, the shapes of which do not dependent on the method of specimen fixation. Based mainly on the morphology of this structure, we recognize 11 putative species for *Potamotrygonocestus*. We also found that these recognized lineages possess greater morphological variability than previously documented for tetraphylideans, leading us to suggest that extensive sampling is very important to understand the diversity of this system. Finally, we found that most species of potamotrygonids exhibit lower host specificity in comparison to what has been documented previously for marine tetraphyllideans.

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MOLECULAR DIVERSITY AMONG LINEAGES OF *POTAMOTRYGONOCESTUS* PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS: TESTING MORPHOLOGY-BASED SPECIES BOUNDARIES

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Here we present the results of our preliminary efforts to document the molecular diversity of lineages within *Potamotrygonocestus*, an endemic genus of tetraphyllidean cestodes inhabiting Neotropical freshwater stingrays. This is the first attempt to include molecular data in the systematics of this diverse group of tapeworms, and it was done to test morphological concepts of species and/or reveal cryptic lineages. Our results are based on the cladistic analysis of 62 representatives of this genus for which we obtained nucleotide sequences for partial regions of the markers cytochrome *c* oxidase subunit I mDNA and 28S rDNA. Our results corroborate the monophyly of *Potamotrygonocestus*, for which we recognize two major clades, each of which possesses a set of diagnostic morphological characters and also recovered by previous phylogenies based on morphological data. We found molecular support for most of the species recently recognized within the genus based on morphological data. However, we found that there is no molecular support to justify the recognition of *P. marajoara* as a valid species. Re-evaluation of morphological data and host specificity data suggest that *P. marajoara* should be considered a junior synonym of *P. chaoi*. However, the status of these two nominal species is also associated with the recognition of *P. travassosi* as a valid species, for which our results are inconclusive at this stage. Finally, we discuss the patterns of host association and distribution found thus far.

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PARASITISM BY *HYMENOLEPIS DIMINUTA* INCREASES EGG CANNIBALISM BY *TRIBOLIUM CONFUSUM*

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Egg cannibalism is widespread in insects and particularly common among beetles (Coleoptera). In members of the genus *Tribolium*, egg cannibalism is a potent population regulatory mechanism. Previous studies report that infection with the cestode *Hymenolepis diminuta* increases conspecific egg cannibalism by *T. castaneum* but not by *T. confusum,* and suggest that parasitism might put *T. confusum* at a competitive advantage. Those studies used groups of infected beetles with low overall infection intensity, and therefore could not quantify the contribution of beetles with different levels of infection to overall cannibalism by the group. We modified a published cannibalism assay that uses dye-marked eggs to estimate egg cannibalism rates by individual *T. confusum* and compare that with the intensity of infection in each beetle. Uninfected virgin female beetles had a cannibalism rate (% eggs eaten per beetle per day) of about 30% and uninfected virgin males had a cannibalism rate about 10%. Infected beetles harbored 1 to 30 parasites, and had higher cannibalism rates than uninfected beetles. These results suggest that a previous conclusion – the absence of parasite effect on egg cannibalism by *T. confusum* – may have been due to the small number of parasites infecting those beetles, and that *H. diminuta* does affect egg cannibalism by *T. confusum*.

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THE EFFECT OF THE BOPYRID ISOPOD PARASITE *PROBOPYRUS PANDALICOLA* ON THE BEHAVIOR OF *PALAEMONETES PUGIO* AND THE PREDATION PREFERENCES OF *FUNDULUS HETEROCLITUS*

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The daggerblade grass shrimp *Palaemonetes pugio* plays a crucial role in estuarine communities on the Atlantic and Gulf coasts. Parasitization by the isopod *Probopyrus pandalicola* affects some aspects of shrimp physiology, but its effect on behavior is unclear. The purpose of this study was to determine how the parasite affected shrimp behavior and the predation preferences of the mummichog *Fundulus heteroclitus*. Each laboratory aquarium contained 2 fish along with 1 parasitized and 1 unparasitized shrimp. Prey behavior was recorded immediately prior to predation by *F. heteroclitus*. The 4 types of shrimp behavior in order of perceived activity level were: motionless, walking, swimming, and backward thrusting. Significantly more parasitized shrimp (51) were selected than unparasitized shrimp (34). Mummichogs preferentially selected shrimp that were swimming (44.7%) and backward thrusting (41.2%) regardless of parasitization. Overall, parasitized shrimp were less likely to backward-thrust than unparasitized shrimp. The major findings of this study were that *Probopyrus pandalicola* affected both the behavior of its host and the predation preferences of *Fundulus heteroclitus*. Furthermore, *P. pugio* that were more active were consumed more frequently. An unexpected benefit of parasitization may be a resulting reduction in host activity level, and thus decreased potential visibility to a predator.

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PLASMODIUM FALCIPARUM: CHARACTERIZATION AND CELLULAR LOCALIZATION OF ABCG PROTEIN

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Plasmodium falciparum (*P.falciparum*) is the causative agent of malaria. For decades, the high incidence of malaria and drug-resistance poses a major problem in malaria control. One of the mechanisms underlying drug-resistance involves ATP-binding cassette transporters (ABC transporters). Two membrane transport proteins, PfMDR1 (*Plasmodium falciparum* multidrug resistance 1) and PfMRP1 (*P. falciparum* multidrug resistance protein 1), have been associated with the resistance of *P. falciparum* to several antimalarial drugs as their mammalian homologues which have been shown to be causative of drug resistance to anti-cancer drugs. The *P. falciparum* genome encodes 16 members of ABC transporters with one member of the ABCG superfamily. In humans members of the ABCG subfamily have been shown to mediate the transport of normal cell metabolites (heme, uric acid and GSH) and anti-cancer drug as in the case of hABCG2 (human ABCG2); while other members of the family (hABCG1, G4,G5 and G8) appear to mediate the transport of sterols, including cholesterol. Analysis of PfABCG shows significant but equal sequence identity to hABCG1 and hABCG2. In this study, we show that PfABCG migrates as a 68KDa polypeptide on SDS-PAGE using N-terminal directed antibody against a recombinant fragment of PfABCG. Interestingly, PfABCG appears to migrate lower than its predicted molecular mass. Analysis of total protein extracts from the different asexual stages of the parasite shows PfABCG to be expressed in all stages, with lower expression levels in the ring stage of the parasite. Immunofluorescence localization of PfABCG in the asexual stages shows a staining at the plasma membrane of the parasite. Efforts are ongoing to further characterize the function of PfABCG in the parasite and its role in drug transport or the transport of normal cell metabolites.

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INFECTION PREFERENCE OF AN INVASIVE TREMATODE, *CENTROCESTUS FORMOSANUS*, TO AN INVASIVE CICHLID, *CICHLA OCELLARIS*, IN THE PANAMA CANAL

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The global spread of introduced species can impact ecological and evolutionary processes by reshuffling species distributions and facilitating biological interactions in novel habitats among species that do or do not share a common evolutionary history. Host-parasite interactions provide excellent systems with which to test alternative predictions on the processes that govern biotic interactions in invaded systems, either niche conservatism or adaptive evolution, This is because parasites have an intimate and sustained relationship with their host, yet vary in their compatibility to a particular host species, such that they can interact with many species or are limited in their host range through physiological and/or behavioral host defenses, or have adapted specificity to a particular host species. For parasites with broad host ranges, one prediction is that natural selection will favor adaptation to a particular host that increases its transmission to a definitive host. While invasive species are widely recognized as important drivers for diseases through density mediated effects on host density, the extent to which parasites may actively prefer an invasive host, and why, is not well understood. Here, we test the extent to which an invasive parasite prefers the infection to a novel host that is also invasive, and speculate on the ecological and evolutionary implications of this emerging host-parasite interaction. Trematodes parasites have complex life cycles with varying degrees of host-specificity, such that they are less specific to second intermediate hosts than they are to first intermediate snail hosts. Specifically, we investigate how a widespread invasive parasite,

Centrocestus formosanus, interacts with a novel community of native and introduced cichlid fish in the Panama Canal that can serve as potential second intermediate hosts. We report that in natural assays of prevalence and in preference experiments in the laboratory, infection of *Cichla ocellaris* was significantly higher than other co-occurring cichlids. Single species experiments also demonstrate a higher infection rate for this fish than other available hosts. We speculate on the ecological and evolutionary drivers that could explain this pattern of infection preference for a locally invasive and aqua-cultured host in a human dominated landscape.

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PATTERNS OF HOST USE OF PARAMPHISTOMOID FLUKES FROM KENYA, WITH IMPLICATIONS FOR *SCHISTOSOMA MANSONI* TRANSMISSION

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Infectious diseases exist in complex ecological settings, in which the diversity of host species present may mitigate transmission to target species. One possibility is that multiple parasite species may exploit the same necessary host, such that transmission of a particular parasite to a target species might be lessened or enhanced as a result of parasite-parasite interactions. We are interested in exploring such possibilities in stream environments in west Kenya, where transmission of the human parasite, *Schistosoma mansoni* is supported by the snail, *Biomphalaria pfeifferi*. Other trematode species also exploit *B. pfeifferi* as their first intermediate host, including paramphistomoids (amphistomes).We are particularly interested in amphistomes because they are common and produce rediae, and thus may prey upon *S. mansoni* sporocysts in snails. To pursue this study, we need background information on the species composition and host usage patterns of the amphistomes present in west Kenya. We have sampled amphistome cercariae derived from *B. pfeifferi* and other local snails. Adult amphistome flukes were recovered from local abattoirs. Combining anatomical studies of adults with results obtained from sequencing target sequences (ITS2), we have learned the amphistome fauna of west Kenya is complex. It appears *Bulinus* and *Biomphalaria* each support at least three lineages of amphistomes, and *Ceratophallus* snails support two lineages. Thus the very snails that transmit mammalian schistosomes in Kenya are also exploited by a diversity of amphistomes. It is yet unclear if particular amphistome lineages can be transmitted by multiple snail genera, but closely-related amphistomes can be recovered from multiple ruminant species. We are in the process of developing techniques for harvesting amphistome eggs to be used in future experimental snail infections with schistosomes, to learn if associations occur among amphistomes and *S. mansoni,* which may have implications on the transmission of human schistosomiasis. This study was supported by NIH grants P20RR18754 and R01 AI101438.

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GORDIID (PHYLUM NEMATOMORPHA) CYST DEVELOPMENT AND SURVIVAL TO DRYING IN PARATENIC HOSTS

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Freshwater gordiids are free-living aquatic worms that parasitize terrestrial arthropods. After emerging from their arthropod host, worms mate and females produce egg strings that develop and hatch into larvae. The larvae reside on the bottom of streams where they are ingested by aquatic invertebrates. Once ingested by aquatic invertebrates, gordiid larvae develop into cysts. Some of these infected invertebrates act as paratenic hosts by carrying cysts to land where they are somehow consumed by omnivorous or predatory arthropods. However, one part of the life cycle that has not been examined in detail is the cyst

formation and survival process. In this study, we first examined cyst formation of the gordiid, *Paragordius varius*, by exposing laboratory reared snails to *P. varius* larvae and examining these snails for cyst formation. After exposure snails were fixed every few days and processed using standard histological techniques. Snails infected with cysts were stained and examined for morphological changes in the larval pseudointestine an organ thought to be responsible for cyst formation. Second, we tested the ability of cysts of *P. varius* and *Gordius* cf. *robustus* to transfer from snail to snail host, by feeding infected snails with cysts to uninfected snails. Finally, we tested the ability of gordiid cysts in their paratenic hosts to survive drying for long periods of time. Our study indicated that as soon as gordiid larvae penetrate snail hosts morphological changes occur in the pseudointestine. Larvae emptied a portion of their pseudointestine during penetration however, no other morphological changes occurred in the pseudointestine during cyst formation. Approximately 40% of gordiid cysts from infected snails were transferred over to uninfected snails. Additionally, we show that gordiid cysts have the ability to survive drying for up to 3 months suggesting a mechanism of how terrestrial arthropod hosts become infected with gordiids in nature. Our study suggests that the function of the pseudointestine may be important in host infection but not cyst formation.

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ADVANCES IN APPLIED MARINE PARASITOLOGY STUDIES IN SOUTH AFRICA

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The South African purse-seine fishery for small pelagic species (anchovy - *Engraulis encrasicolus*, sardine *Sardinops sagax*) boasted average landings of 380 000 tons per annum from 1950 - 2005 and over 500 000 tons from 2005 - 2009. Despite its historic existence and long-term economic value, very few studies have focused on parasites and diseases that may influence this fishery, the largest in South Africa. Some 'in house' Fishing Industry Research Institute (FIRI) reports written during the late 1980s and early 1990s concerning the effect of the myxozoan parasite *Kudoa thyrsites* on the quality of sardine fillets used for canning exist, but virtually no work has been conducted since. 2010 saw the initiation of a focused attempt to understand relationships between small pelagic species and their parasites. A survey of parasites infecting sardine was conducted, recording seven different parasite species. Subsequent research includes an assessment of one of those parasites (digenean "tetracotyle" metacercariae in the eyes) as a biological tag for examining sardine stock structure, and how the prevalence and infection intensity of this parasite varies both spatially and seasonally. In addition, the occurrence and physiological effects of another parasite species, the testicular coccidian *Eimeria sardinae*, is also underway, that parasite having been documented as causing "parasitic castration" in another sardine species. Early in 2012 a new study on parasites of Cape horse mackerel (*Trachurus capensis*) was initiated, previous work in the mid-1970s indicated a high prevalence of nematodes (*Ascaris* sp.) infecting horse mackerel gonads. Results of these studies will be described in the presentation. Sardine and horse mackerel are but two of many economically important marine fish species living off South Africa, and we believe that research on parasites of other economically fish can contribute to the sustainable management of these important natural resources.

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OVERCOMING OBSTACLES TOWARDS THE IN VITRO ANALYSES OF HEPATOCYTE INVASION AND EXO-ERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM*

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The primary aim of our research is to elucidate the protein repertoire deployed by the exo-erythrocytic (EEF) liver-stages of *Plasmodium* and to understand how these proteins manipulate the host cell. We are especially interested in the determinants of cellular invasion and the modulation of liver cell apoptosis, as the ability to block host cell death has been linked with parasite survival. Here we address several basic methodological obstacles and questions of cellular biology regarding a human cell line, HC-04, which is capable of supporting *Plasmodium falciparum* and *P. vivax* EEF development at low intensities. Using a variety of cell culture techniques, focused Click-chemistry glycoproteomics, targeted transcriptomics and confocal microscopy, we have begun to answer fundamental questions regarding (**i**) the optimization of in vitro *P. falciparum* invasion and development, **(ii)** HC-04 transcriptomic and proteomic profiles in the context of apoptosis vs. proliferation, and (**iii**) the comparative cell surface glycoproteomic analysis of HC-04 relative to human hepatocyte lines that do not support the development of human malaria EEF stages. We believe that this paves the way towards a better understanding of how *Plasmodium* invades and manipulates host cells. Ongoing experiments regarding infection of HC-04 with *P. falciparum* will also be discussed.

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EXPOSURE OF *BIOMPHALARIA HAVANENSIS* TO SCHISTOSOMA MANSONI: EXPLORATION OF THE BASIS OF INCOMPATIBILITY IN THIS HOST-PARASITE SYSTEM

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E.S. Loker, University Of New Mexico

Most parasite species exhibit some degree of host specificity. In general, our mechanistic understanding of why a particular parasite can infect some host species but fails to infect others is poor. We address this question using the digenean *Schistosoma mansoni* as our model parasite. The larval development of *S. mansoni* occurs in certain species of snails of the genus *Biomphalaria*, but not in others. A series of laboratory experiments was undertaken using *Biomphalaria havanensis* from Texas, Louisiana, and Mississippi to explore the underlying reasons for its lack of compatibility with *S. mansoni*. Histological sections of snails exposed to *S. mansoni* revealed that miracidia penetrated *B. havanensis,* and by 8 days post-exposure, sporocysts were surrounded by an encapsulation response. Next *B. havanensis* were exposed to the parasite *Echinostoma paraensei* that has been shown to interfere with hemocyte responses of *B. glabrata* to *S. mansoni*. We showed that 43% of *B. havanensis* were capable of supporting cercariae-producing infections of this South American echinostome. To avoid the complication of having viable predatory rediae of *E. paraensei* present in *B. havanensis,* yet still accruing this parasite's immunosuppressive benefits, we exposed *B. havanensis* to irradiated *E. paraensei* miracidia. These snails were then challenged four days later with viable *S. mansoni* miracidia. None of the snails became infected with either parasite. A microarray analysis was conducted comparing *S. mansoni*-exposed and unexposed *B. havanensis* snails to see if there is a significant change in transcription of known or suspected immune factors. Microarray data showed a slight decrease in gene expression in snails challenged with *S. mansoni* compared to control *B. havanensis*. When compared to *B. glabrata* (M-line and BS-90 strains), *B.*

havanensis shows an overall limited transcriptional response to infection with *S. mansoni*. To date, no exposed *B. havanensis* snails have shed *S. mansoni* cercariae.

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GOING IT ALONE? CHARACTERIZING THE RESPONSE OF A PARASITE UPON IMMUNE CHALLENGE OF THE HOST-PARASITE UNIT

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Host-parasite symbionts involve the complex interaction of two interconnected biological systems with a net flow of energy and nutrients. Once the symbiosis establishes, the host and parasite can be viewed as a unit, often acting and appearing different than each of the symbionts individually. Many facets of the host-parasite unit have been studied; however, we lack a clear understanding of how the unit responds to immunological threats. Do the symbionts of the unit react separately, as they would as non-symbionts? Does the parasite rely on the host's immune system, as a tapeworm relies on the host for digestion? Does the parasite need its own highly honed immune system to survive in an immune-compromised host? Or, does the parasite produce immune factors helpful to the host as has been documented in several bacterial-vertebrate mutualisms. To gain a better understanding of how a host-parasite unit responds to threats, we investigated how the parasite reacts to bacterial challenge of the host. The parasite *Paragordius varius* (Nematomorpha: Gordiida) causes severe damage to the host, especially its immunologically-active fat body, while relying on the host to survive until its maturation; the parasite depends on the host for transportation to water. Using 454 Next Generation Sequencing (NGS), we captured a snapshot of the immune factor expression of *P. varius* worms in hosts exposed to the insect pathogen *Serratia marcescens* (G-), or to a sham injection. NGS produced 790,000 reads for the control group and 728,000 reads for the experimental group (average length of reads was 437bp and 426bp respectively). The data were analyzed to measure relative expression rates of individual transcripts, and to provide an overview of the immune repertoire of this parasite.

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ROLE OF INNATE IMMUNITY IN THE DEVELOPMENT OF MALARIA RELATED PATHOLOGIES

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Individuals infected with the protozoan parasite *Plasmodium* spp. are known to develop severe symptoms that are the consequence of intense inflammatory response triggered by the release of the merozoite form of the parasite into blood circulation. Of interest, hemozoin (HZ), a crystalline and brown pigment formed in the digestive vacuole of Plasmodium as a catabolism product of hemoglobin (Hb), is also simultaneously released. In the past, HZ was considered as a metabolic waste of the parasite, solely the result of heme detoxification. However, the fact that this molecule has been shown to be actively engulfed by phagocytes and to modulate MØ functions, as well as to be trapped in various organs, suggests that HZ can potentially contribute to the development of malaria immunopathogenesis. Following its release from ruptured Plasmodium-infected RBC, monocytes/macrophages rapidly engulf HZ. Furthermore, in human and murine malaria, a large number of circulating phagocytes are loaded with HZ, as well as those in lymphoid organs and the brain, where their presence seems to correlate with disease severity. It has been demonstrated that human monocytes and murine MØ stimulated with HZ purified from various species of *Plasmodium* or synthetically generated as β-hematin, produce large amounts of cytokines, inflammatory molecules, MIF erythropoietic inhibitor and adhesion molecules. In accordance with these observations, we published the first report that in vivo inoculation of synthetic HZ rapidly induces the generation of various pro-inflammatory mediators including myeloid-related proteins, chemokines and

cytokines, strongly suggesting that HZ per se may have an important role to play in the development of malaria-related pathologies. Additionally, we revealed that HZ significantly enhanced IFNγ-induced MØ NO generation, an important inflammatory event that could favor cerebral malaria development. Thereafter, we found that HZ-induced MØ chemokine expression were regulated by oxidative stress-dependent and –independent mechanisms involving PTP inhibition and kinases activation. Recently, we, and 2 other groups, have clearly demonstrated that induction of MØ pro-inflammatory cytokines (e.g. IL-1β) by HZ is TLR-independent but fully dependent on the activation of the NLRP3/Inflammasome complex, and that the up-stream Src kinase Lyn is pivotal for IL-1β secretion. Furthermore, we reported that HZ size influences MØ activation in vitro and provided in vivo demonstration that malarial DNA never interacts with HZ within *Plasmodium*-infected erythrocytes. Finally, having recently identified human inflammatory biomarkers (i.e. ApoE, SAA, LBP) from malaria patients that interact with the malarial HZ. We became interested to determine the impact of these sera biomarkers on the macrophage's innate immune response triggered by HZ. Interestingly, we found that those adhering biomarkers differently but effectively influence HZ recognition by MØ, modifying not only IL-1β production, but in addition ROS generation and phagocytosis. Using ko mice for some of those host biomarkers, we found that their absence can strongly influence the development of cerebral malaria caused by P. berghei ANKA. Collectively, our previous and present studies provide important clues about the impact of inflammatory mediators induced during malaria to modify, on one hand, the impact of HZ in its interaction with MØ, and on the other hand, to show that systemic release of those inflammatory biomarkers could play a critical role in the development of malaria-related pathologies. Findings stemming from our studies could lead to the development of new therapy to tame-down innate inflammatory response and potentially reduce the death rate cause by cerebral malaria.

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ABORTIVE FOLLICULAR HELPER DIFFERENTIATION IS ASSOCIATED WITH DEFECTIVE HUMORAL RESPONSE IN *LEISHMANIA INFANTUM*-INFECTED RHESUS MACAQUES

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Leishmania infantum is responsible for a chronic and potentially fatal infectious disease named visceral leishmaniasis (VL). Technical and ethical issues preclude an in-depth analysis of the immune events associated with parasite establishment and chronicity in human patients. In this study, rhesus macaques were infected with *L. infantum* and the T helper and B cell immunological profiles characterized in the blood and lymphoid organs during acute and chronic phases of infection. Parasite detection in visceral compartments during the acute phase was associated with differentiation of effector memory CD4 T cells and increased levels of Th1 transcripts. At the chronic phase, parasites colonized novel lymphoid niches concomitant with increased expression of *IL10*. Despite the occurrence of hypergammaglobulinemia, the production of parasite-specific IgG was poor being confined to the acute phase and positively correlated with the frequency of an activated memory splenic B cell population. Additionally, the expansion of a splenic CD4 T cell population expressing CXCR5 during acute infection was associated with the differentiation of the activated memory B cell population and production of specific IgG. However, at chronic infection these populations contracted impacting the production of parasite-specific IgG. Our study provides new insights into the immune events taking place during VL in a physiological relevant host and a mechanistic basis for the inefficient humoral response during VL.

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CHLOROQUINE'S MODE OF ACTION IS NOT RESTRICTED TO THE *PLASMODIUM FALCIPARUM* DIGESTIVE VACUOLE

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Chloroquine (CQ) belongs to one of the most effective and low-cost antimalarial drugs made to date. No other antimalarial has been as efficient as CQ, and unlike more recently approved treatments, resistance to this drug has taken decades to evolve. Chloroquine is known to interact with heme and inhibit its detoxification. Resistance to CQ in *Plasmodium falciparum* is thought to rely on the K76T mutation in the *P. falciparum* chloroquine resistance transporter (PfCRT). This mutation causes the expulsion of CQ from the parasite's digestive vacuole (DV), where heme gets biocrystallised to hemozoin. However, new findings indicate that there must be an additional mechanism involved in chloroquine resistance since the accumulation of CQ in the DV does not proportionally correlate with drug resistance. Using live cell imaging, we show that CQ-sensitive parasites have hemozoin crystals within small round compartments found in the parasite cytosol as well as the digestive vacuole. Treatment with jasplankinolide, which inhibits fusion of hemoglobin-laden vesicles with the DV, also resulted in the hemozoin crystals seen in small compartments found in the parasite's cytosol in both CQS and CQR parasites. This data suggests that CQ may be involved in impaired vesicle fusion in CQ sensitive parasites. In contrast, CQR parasites did not show any evidence for the formation of cytosolic hemozoin crystals even when the CQ levels were raised to 30fold their IC_{50} concentration, suggesting that they found a way to maintain vesicle fusion under CQ pressure. We propose a model that incorporates multiple mechanisms how CQ resistant parasite strains control cell viability after CQ exposure.

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CRYPTOSPORIDIUM PARVUM SURFACE PROTEINS AS CANDIDATES FOR A CRYPTOSPORIDIOSIS VACCINE TO PROTECT NEWBORN CALVES

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Cryptosporidium parvum can cause both human and bovine cryptosporidiosis and has been responsible for many outbreaks in the past decade. Immunocompromised humans and newborn calves who become infected may suffer from severe diarrhea and dehydration which can be life threatening. In the province of Quebec, bovine cryptosporidiosis is of concern for both dairy and beef industries and is responsible for important economic losses. Newborn calves can be infected soon after birth and can present severe clinical symptoms. Calves may even die from this disease before they can develop a competent immune system. Our hypotheses are that a vaccine composed of multiple *C. parvum* surface proteins can induce the production of protective antibodies in the colostrum of cows immunized before calving and that neutralizing antibodies will block sporozoite adhesion to host cells, effectively preventing cell invasion and infection. Our first objective is to express and purify four *C. parvum* surface proteins and produce antibodies specific to these recombinant proteins. Four recombinant surface proteins (p23, CP2, gp45 and gp900) have been expressed and purified. Our second objective is to test the efficacy of antibodies against p23, CP2, gp45 and/or gp900, alone and in combination, to inhibit *C. parvum* adhesion to and invasion of host cells *in vitro* using Madin-Darby bovine kidney cells. Immunofluorescence will be used to evaluate antibody-dependent inhibition of sporozoite adhesion to host cells. Different combinations of specific antibodies will be tested to identify if combinations are more promising than single-target antibodies.

Preliminary results on the *in vitro* efficacy of these antibodies will be presented. Promising results would constitute the first step in the production of a vaccine against bovine cryptosporidiosis.

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LIVE IMMUNIZATION AGAINST VISCERAL LEISHMANIASIS USING A NATURALLY ATTENUATED CUTANEOUS *LEISHMANIA DONOVANI* ISOLATE FROM SRI LANKA

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Leishmaniasis, caused by *Leishmania* protozoa, is associated with a spectrum of clinical manifestations, from self-resolving cutaneous lesions to the much more severe visceral leishmaniasis. Disease phenotype is determined in part by the infecting species, and *Leishmania donovani* is the major causative agent of visceral leishmaniasis in Southeast Asia. However, in Sri Lanka, there have been over 2000 cases of cutaneous leishmaniasis caused by *L. donovani* while only four cases of visceral leishmaniasis have been reported in the same period. We hypothesized that prior infection with the more prevalent cutaneous form of *L. donovani* protects against visceral leishmaniasis in Sri Lanka. Two clinical isolates were obtained from Sri Lanka, one from a cutaneous lesion (SL-CL) and one from a visceral leishmaniasis patient (SL-VL). BALB/c mice were immunized subcutaneously with 10^3 to 10^6 SL-CL promastigotes, or with PBS as a negative control. Seven weeks following immunization, mice were challenged intravenously with SL-VL. Significant protection in the liver was observed for mice immunized with SL-CL doses of 10^4 and higher. Protection was associated with a mixed Th1/Th2 response prior to challenge, with higher IFNg and IL4 production in protected groups and significantly increased total IgG levels in mice immunized with 10^6 SL-CL promastigotes. No antigen-specific IL10 production was observed prior to challenge. Post-challenge, no difference in IFNg production was observed between the different groups while IL4, IL10 and total IgG levels were elevated in mice that had been immunized with 10^4-10^6 SL-CL. Liver protection was durable since protection was maintained when mice were challenged three months post-immunization. Overall, these results provide a possible rationale for the scarcity of visceral leishmaniasis in Sri Lanka and could guide leishmaniasis vaccine development efforts.

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CHARACTERIZATION OF A NOVEL *PLASMODIUM FALCIPARUM* CALCIUM-BINDING EXPORTED PROTEIN

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After invading a host erythrocyte, *Plasmodium falciparum* induces modification of the host erythrocyte, a cell lacking organelles and protein trafficking machinery. These modifications allow the parasite access to nutrients and alter the host to provide a more favorable environment for the parasite. Here, we have identified and characterized PfEXP-250, a unique *P. falciparum*-exported protein containing a PEXEL motif located at the carboxyl terminus of the protein. Antisera to PfEXP-250 was generated and used in immunofluorescence assays. Co-precipitation studies and solubility analyses were performed to determine the protein-protein interactions and membrane solubility of PfEXP-250. Our data show that PfEXP-250 is expressed at all stages of the intraerythrocytic cycle and is exported to the host erythrocyte. We have also found that is membrane-associated PfEXP-250 binds to calcium. These data indicate that PfEXP-250 is part of the *P. falciparum* exportome despite not being identified as a PEXEL-positive protein and therefore may be suggestive of an alternative export pathway

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FUNCTIONAL DIVERSIFICATION OF LEVAMISOLE RECEPTORS IN THE TRICHOSTRONGYLID NEMATODE *HAEMONCHUS CONTORTUS*

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Pentameric cys-loop ligand-gated ion channels (pLGIC) are key mediators of fast ionotropic neurotransmission and are invaluable as drug targets in parasitic nematodes. The function of a particular channel depends critically on the subunit composition of its pentameric structure. The anthelmintic levamisole paralyses nematodes by binding to an acetylcholine-gated pLGIC, L-AChR, in the model free-living worm *Caenorhabditis elegans*. The *C. elegans* L-AChR is composed of five different pLGIC subunits, *unc-63*, *unc-38*, *lev-8*, *lev-1* and *unc-29*. The parasitic nematode of small ruminants, Haemonchus contortus shows significant changes in the genes encoding the L-AChR despite its close evolutionary relationship with *C. elegans*. The *lev-8* gene appears to be absent, the *lev-1* subunit gene lacks a signal peptide and does not contribute to the receptor and three additional copies of the *unc-29* gene are present in the parasite genome. All four *unc-29* copies are shared by other trichostrongylid parasites suggesting a common difference with the *C. elegans* model. Channels were reconstituted by microinjection of cRNA into *Xenopus* oocytes and identified by two-electrode voltage clamp electrophysiology. The role of each *unc-29* copy was evaluated by sequential replacement of UNC-29.1 in the *H. contortus* L-AChR-1. The pharmacological profile of each channel identified was produced using a panel of known agonists and antagonists. Two new channels were identified, produced with *Hco-unc-29.3* and *Hco-unc-29.4*. Dose-response assays revealed a high but similar affinity of both receptors to acetylcholine with respective EC_{50s} of 2.94 ± 1.03 μM and 2.90 ± 1.01 μM as well as to levamisole with EC_{50s} of 0.83 ± 1.02 μM and 1.63 ± 1.05 μM. A saturating concentration of pyrantel, nicotine or bephenium produced weak responses of both receptors compared to acetylcholine and levamisole. Acetylcholine currents of these two receptors were entirely inhibited by d-tubocurarine and only by mecamylamine using *Hco-unc-29.4*. Dihydro-β-erythroidine did not affect signals in all cases. No receptor could be produced using *Hco-unc-29.2*, despite a similar level of sequence divergence to the other copies. It may be that an as yet unidentified channel containing UNC-29.2 and other subunit partners exists. These results confirm functional divergence among the four *Hco-unc-29* paralogs and highlight the potential role of these genes in levamisole resistance as well as their potential as novel parasite specific anthelmintics targets.

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INSERTION OF THE *LEISHMANIA DONOVANI* PEROXIN 5 INTO GLYCOSOMAL MEMBRANES

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Leishmania is the causative agent of a spectrum of devastating diseases, collectively termed leishmaniasis. *Leishmania donovani* parasites contain a novel organelle called a glycosome, evolutionarily related to peroxisomes of higher eukaryotes, which compartmentalizes multiple metabolic and biosynthetic pathways. Correct targeting of this enzymatic machinery to the glycosome is essential for parasite viability. Proteins destined for glycosomal import contain either a C-terminal tripeptide PTS1 (peroxisomal targeting signal 1) or an N-terminal nonapeptide PTS2 (peroxisomal targeting signal 2) signal sequence. The PTS1 and PTS2 sequences are rapidly bound by the *Leishmania* trafficking receptors peroxin-5 (*Ld*PEX5) and peroxin-7 (*Ld*PEX7), respectively. Glycosomal import is initiated by docking of the cargo loaded receptors to peroxin 14 (*Ld*PEX14), a peripheral membrane protein anchored to the cytosolic face of the glycosomal membrane, a process that mediates the translocation of the cargo proteins into the glycosomal lumen by a yet undefined mechanism. We have demonstrated that an interaction

between *Ld*PEX5 and *Ld*PEX14 causes *Ld*PEX5 to undergo a biophysical change from a soluble to an integral membrane protein that is resistant to alkaline carbonate extraction. To this end, *in vitro* studies are underway to identify the mechanism and domain(s) of *Ld*PEX5 required for membrane insertion using both full length and truncated *Ld*PEX5 in liposome membranes containing *Ld*PEX14. To further characterize *Ld*PEX5-*Ld*PEX14 formed in the lipid bilayer we are using recombinant *Ld*PEX5 and *Ld*PEX14 labelled with the fluorescent dyes Oregon green and Texas Red, respectively. Preliminary results indicate that a domain situated in the N-terminal region of *Ld*PEX5 is involved in mediating the *Ld*PEX5-membrane interaction. Further studies will examine the role of this *Ld*PEX5 domain in translocation of PTS1 proteins into the lumen of *L. donovani* glycosomes.

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A UNIQUE MODE OF REGULATION OF THE *LEISHMANIA* EUKARYOTIC INITIATION FACTOR EIF2ALPHA SUBUNIT VIA POSTTRANSLATIONAL PROCESSING IN RESPONSE TO BIOLOGICAL STRESS DURING THE PARASITE'S DEVELOPMENT

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Phosphorylation of the eukaryotic initiation factor eIF2alpha plays a central role in determining the rate of global protein synthesis under cellular stress conditions. We have shown recently that phosphorylation of the *Leishmania* eIF2alpha at Thr 166 is critical for the adaptation of the parasite within mammalian macrophages and its inhibition markedly delays the *Leishmania* promastigote to amastigote differentiation. Here we show that in contrast to its eukaryotic counterparts, the *Leishmania* eIF2alpha homolog has a 94 aa N-terminal extension, which seems to play an important role in eIF2alpha regulation throughout the life cycle of the parasite. While amastigotes produce the full-lenght 47kDa eIF2alpha product, additional peptide fragments of ~41, 35, and 25 kDa are made in promastigotes. Mutagenesis of ^{1}Met or ^{95}Met and deletions within the eIF2alpha N-terminal extension showed that at least the 35 kDa product in promastigotes was not due to alternative translation initiation. We provide experimental evidence that the stage-regulated eIF2alpha processing is initiated by the excision of the N-terminal methionine (NME) through the action of aminopeptidases (MetAPs). MetAPs are involved in N-terminal methionine excision and cleave substrates with small side chain residues at the penultimate position. Replacement of the *Leishmania* eIF2alpha Ala at P1' position by a bulky residue (e.g. Arg) completely blocked eIF2alpha processing in promastigotes, consistent with previous reports on NME. Our data support that developmental regulation of eIF2alpha in *Leishmania* is most likely initiated by NME followed by additional proteolytic events. The reason for the stage-regulated eIF2alpha processing is not well understood but our data support a model where the cleaved eIF2alpha fragments could compete with the full-length eIF2alpha protein for phosphorylation, allowing thus higher levels of translation for the highly replicating promastigote form of the parasite.

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REGULATION OF RNA METABOLISM BY ARE-BINDING PROTEINS IN *TRYPANOSOMA BRUCEI*

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In trypanosomes, gene expression is regulated mainly through differential mRNA decay or other post-transcriptional mechanisms, mediated by a poorly characterized network of *cis*- and *trans*-acting

elements that encompasses interaction of RNA-binding proteins (RBPs) with particular sequence elements of their target mRNAs. Our computational studies have identified highly conserved adenosine-uridine (AU)-rich elements (AREs) in the 3' untranslated regions (UTRs) of a large number of *T. brucei* mRNAs. These ARE-containing transcripts are up-regulated in the stationary-phase of *in vitro*-cultured procyclic form cells and down-regulated in stumpy and slender bloodstream form cells, suggesting a role for AREs in the regulation of parasite differentiation. Through sequence analysis of *T. brucei* RBPs, we have identified three potential remote homologs of ELAV (Embryonic Lethal, Abnormal Vision) proteins. In higher eukaryotes, proteins of the ELAV-like family regulate gene expression by stabilizing or promoting degradation of ARE-containing transcripts, suggesting a similar role of these proteins in regulating trypanosomatid ARE-containing mRNAs. Using *in vitro* electrophoretic mobility shift assays (EMSA) and RNA immunoprecipitation followed by deep sequencing (RIP-Seq), we confirmed that the *T. brucei* ELAV-like proteins bind specifically to AREs. Furthermore, microarray analysis of over-expression and RNAi knockdown cell lines suggests that the three identified ELAV-like proteins specifically regulate expression of ARE-containing transcripts. In addition, tandem affinity purification (TAP) followed by mass spectrometry revealed association of ELAV-like proteins with a number of other RBPs that may be involved in ARE-mediated mRNA turnover. Together, these results suggest a widespread role of ELAV-like proteins in genome-wide regulation of trypanosomatid mRNAs, and warrant further studies to examine the role of these proteins in *T. brucei* life cycle progression and infection.

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ALBA DOMAIN PROTEINS IN *LEISHMANIA*: DIFFERENTIAL PROTEIN LOCALIZATION DURING AMASTIGOTE DIFFERENTIATION AND THEIR ROLE IN AMASTIN MRNA DEVELOPMENTAL REGULATION

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Amastins belong to a multigenic surface protein family of potential virulence factors that are specifically expressed in the amastigote lifestage of *Leishmania*. Regulation of gene expression in these parasites takes place almost exclusively at the post-transcriptional level. However, mechanisms regulating mRNA stability and translation efficiency are for the most part poorly understood in *Leishmania*. To better understand the mechanism of developmental regulation of amastin transcripts, we carried out RNA affinity chromatography combined to mass spectrometry using a regulatory U-rich region within the amastin 3'UTR as bait. We have identified an Alba domain protein (*Li*Alba20) as an amastin RNA binding partner. Alba domain proteins have multiple functions in other organisms from DNA binding and nucleosome constituent in *Archaea*, translation inhibition in other protozoans to RNAse P/MRP activity in higher eukaryotes. Genomic inactivation of the *LiAlba20* gene decreases amastin mRNA stage-specific accumulation by 2-fold, further supporting a role of *Li*Alba20 in amastin gene regulation. *Li*Alba13, an interacting partner of *Li*Alba20, seems to act similarly on amastin transcripts. Imaging Alba proteins in parasites differentiating from promastigotes to amastigotes shows differential localization of the protein from the cytoplasm to flagellum and the nucleolus. Flagellum (or primary cilium) and nucleolus are two distinct organelles described as sensors and stress responsive elements in eukaryotes. Phenotypic analysis of the *Li*Alba20(-|-) and *Li*Alba13(-|-) mutants underlines the importance of *Li*Alba20 in both life stages of the parasite, whereas *Li*Alba13 seems to be required specifically for amastigote growth. Overall, our work suggests a possible role of Alba proteins in early stress response events during the parasite differentiation, resulting in differential expression of target mRNAs, such amastins.

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IDENTIFICATION AND CHARACTERIZATION OF POST-TRANSCRIPTIONAL REGULATORS IN *LEISHMANIA BRAZILIENSIS*

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Leishmaniasis is a disease caused by an intracellular, protozoan parasite of the species *Leishmania*. Worldwide 88 countries are affected by this parasite and estimates indicate there are 2 million new infections per year. During its life cycle, *Leishmania braziliensis* alternates between the sandfly and mammalian host. It has evolved to reproduce and survive within both hosts. During its life cycle, the parasite encounters environmental changes such as temperature and pH and must adapt to these two distinct environments. During this process, the parasite also changes morphologically. The change this parasite undergoes is a result of its gene expression. It is known that *L. braziliensis* gene expression is regulated post-transcriptionally. microRNAs which are posttranscriptional modifiers may play a significant role in the gene expression of *L.braziliensis*. From our bioinformatic searches, a miRNA with significant homology to mmu-miR-466i-5p was discovered. As a follow-up, we are characterizing the first miRNA in *Leishmania*, Lb-miR-1. Here we show our progress in cloning and expressing Lb-miR – 1 in *L. braziliensis* and our work on finding novel small RNAs using Deep Sequencing.

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HABITAT ELEMENTS IMPACT THE PREVALENCE AND HOST SPECIFICITY OF AVIAN HAEMOSPORIDIAN PARASITES IN TROPICAL ECOSYSTEMS

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Human domination of the planet has rapidly led to increases in the relative fractions of deforested and agricultural land on the Earth's surface. Here we give a cross-continental perspective on how land use changes, especially deforestation and agricultural use can affect the prevalence and lineage diversity of avian blood parasites. Work from Sub-Saharan Africa demonstrates that anthropogenic habitat change can affect host-parasite systems and result in opposing trends in prevalence of haemosporidian parasites in wild bird populations. Additionally, habitat impacts the degree of host specificity of parasites and their hosts. With further work in Costa Rica, we quantify how avian malaria in an abundant sedentary bird species responds to landscape features at fine scales. Debate over balancing agricultural production and biodiversity conservation has generated two opposing strategies: a "land sparing" approach involving large-scale nature reserves, versus a "land sharing" approach where agricultural areas support wildlife through fine-scale conservation. This debate however has largely ignored the effects of the different strategies on parasite dynamics. Data revealed no significant differences in habitat usage or home range size associated with infection status. However, we simulated "land sparing" and "land sharing" landscapes and modeled malaria prevalence to find that land sharing mitigates malaria prevalence more effectively at all agricultural scales. We emphasize that influences of land use changes on parasite prevalence are complex, and will require the detailed study of the vector ecology, and the further quantification of fine-scale habitat effects.

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SKY ISLAND HAIRWORMS: BIODIVERSITY OF FRESHWATER NEMATOMORPHS FROM ISOLATED MOUNTAIN TOPS IN SOUTHEASTERN ARIZONA.

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Freshwater hairworms (Nematomorpha: Gordiida), or Gordian worms, are parasites of terrestrial insects including orthopterans, beetles, cockroaches, and mantids. Recent estimates suggest that only 15% of hairworm species have been described globally. In addition, our knowledge of hairworms is patchy; they are well-studied in some regions, and virtually unknown from others. One area from which we know little about hairworms is the Madrean Sky Islands. The Madrean Sky Islands are a complex of high altitude Madrean pine-oak woodlands surrounded by lower elevation Sonoran and Chihuahuan deserts, found in northern Mexico and southwestern United States. The rugged mountainous terrain, high relief, and deep canyons of the Madrean pine-oak woodlands have led to a highly diverse set of unique ecosystems and microhabitats, containing almost 4,000 endemic plant species. Due to their unique nature, these Sky islands have been named one of the world's biodiversity hotspots. Over 4 years, we collected free-living adult hairworms in several Sky island locations in southern Arizona: Santa Rita Mountains, Huachuca Mountains, and Chiricahua Mountains. Worms were collected by hand, and parts of each worm were fixed in 100% ethanol for DNA analysis and 70% ethanol for scanning electron microscopy (SEM). Molecular barcoding and microscopy revealed the presence of a comparatively high diversity of Gordian worms. We collected 4 previously described species, *Paragordius varius*, *Chordodes morgani*, *Neochordodes occidentalis*, and *Gordius difficilis*, and 1 new species, *Gordius* n. sp. All species, with the exception of *P. varius*, are new state records for the state of Arizona. Since our collections began, all sites have been consumed by wildfire during two of the most active fire seasons in Arizona history, 2011 and 2012. The impact of these fires on the life cycle and survival of these parasite species will be discussed.

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NEW PARASITES AND PREDATORS FOLLOW THE INTRODUCTION OF TWO FISH SPECIES TO A SUBARCTIC LAKE: IMPLICATIONS FOR FOOD-WEB STRUCTURE AND FUNCTIONING

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Introduced species can alter the topology of food webs. For instance, an introduction can aid the arrival of free-living consumers using the new species as a resource, while new parasites may also arrive with the introduced species. Food-web responses to species additions can thus be far more complex than anticipated. In a subarctic pelagic food web with free-living and parasitic species, two fish species (arctic charr Salvelinus alpinus and three-spined stickleback Gasterosteus aculeatus) have known histories as deliberate introductions. The effects of these introductions on the food web were explored by comparing the current pelagic web with a heuristic reconstruction of the pre-introduction web. Extinctions caused by these introductions could not be evaluated by this approach. The introduced fish species have become important hubs in the trophic network, interacting with numerous parasites, predators and prey. In particular, five parasite species and four predatory bird species depend on the two introduced species as obligate trophic resources in the pelagic web and could therefore not have been present in the pre-introduction network. The presence of the two introduced fish species and the arrival of their associated parasites and predators increased biodiversity, mean trophic level, linkage density, and nestedness;

altering both the network structure and functioning of the pelagic web. Parasites, in particular trophically transmitted species, had a prominent role in the network alterations that followed the introductions.

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VARIATION IN HOST ECOLOGY CAN LEAD TO DISCORDANT POPULATION DEMOGRAPHICS AMONG PARASITES WITHIN A *TRICHOBILHARZIA* SPECIES COMPLEX

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Ecological and life history variation can cause demographic discordance between congeneric species, altering microevolutionary processes. *Trichobilharzia* is a globally distributed genus of avian schistosome infecting migratory waterfowl and freshwater pulmonate snails. “Clade Q” a species complex within *Trichobilharzia* has undergone at least two intermediate host-switching events from an ancestral lymnaeid to a physid snail host. In addition, there is notable variation in definitive host specificity and ecology, among species. We hypothesized that both intermediate and definitive host use influence the contemporary and historical demographic patterns of *Trichobilharzia* spp., to test this we analyzed the *cox*1 mtDNA gene region, of three *Trichobilharzia* species, *T. querquedulae, T. physellae* and *Trichobilharzia* sp., a species yet to be formally described. *Trichobilharzia querquedulae* and *T. physellae* are unique within the genus infecting physid snails as intermediate hosts in contrast to *Trichobilharzia* sp., which is transmitted, by the ancestral lymanid host. We estimated the population genetic parameters and the demographic history of the three species using coalescent methods. Physid infecting species have significantly larger contemporary effective population sizes than the lymnaeid transmitted *Trichobilharzia* sp., possibly due to the ubiquity of *Physa sp*. throughout North America. Demographic analyses reveal that *T. querquedulae* has undergone significant population growth relative to *T. physellae* and *Trichobilharzia* sp., with effective population sizes approximately 4X larger than *T. physellae*, likely due to differing habitat use among definitive hosts. This study is the first of its kind to show that differences in host use within avian schistosomes creates variation in parasite demography, ultimately affecting the transmission dynamics and evolutionary ecology of this important group of parasites.

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ADDING PARASITES TO FOOD WEBS

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Of the more than 200 published food webs only nine include parasites in a systematic and standardized fashion. This neglect of parasites reduces food web resolution and constrains our understanding of the complex systems that food webs describe. Here, we present a methodology for augmenting published foods webs by incorporating parasitological information into them. As all parasite additions are based on published host-parasite records this methodology does not require novel empirical work. To test the accuracy of our methodology we extracted the free-living versions of three of the nine published food webs for which we have empirical data on parasites. We then added parasites to the free-living webs using our methodology. We compare the augmented webs to their empirical counterparts using 14 commonly reported measures of network structure.

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LONG-TERM DYNAMICS OF THE METAZOAN PARASITE SPECIES RICHNESS AND DIVERSITY OF *CICHLASOMA UROPHTHALMUS* FROM CELESTúN, YUCATáN, MEXICO

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A monthly data base from 2003 to 2012 of the metazoan parasites of *Cichlasoma urophthalmus* from the coastal lagoon of Celestún, Yucatán was examined to determine changes in parasite diversity, species richness and species composition over time. A total of 70 metazoan parasites were recovered along this time period. Most of them were larval stages of digeneans (37%), nematodes (17), cestodes (5%) and acantocephalans (5). Adult worms were represented only by 7% of digeneans, 4% of nematodes and copepods each, and 3% of monogeneans and acanthocephalans. Other groups such as branchiurids, leeches and pentastomids had just one species each. There was an average of 17 ± 4.5 parasite species per month at component community level and 7.8 ± 30 species per individual host at infracommunity level. At component community level, an increase in species richness was observed throughout time, with almost twice the number of parasite species recovered in recent years (2007-2012) in comparison with 2003-2005. Wavelet analysis was used to identify temporal patterns in species richness and diversity along the 10 years. The results showed patterns at different temporal scales. There were recurrent peaks of species richness and diversity occurring every 24 months. These peaks could be possibly related to pulses of helminths species travelling with their hosts into the lagoon on a regular basis.

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THE FOOD WEB OF OTSEGO LAKE, NEW YORK FROM A PARASITOLOGICAL PERSPECTIVE

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This study is part of a survey of the intestinal parasites of fishes of Otsego Lake and its tributaries (Cooperstown, New York) from 2008 to 2012. In total, 430 individual fish were collected by hook and line, seine, gill net, or ElectroFisher, and subsequently examined for intestinal parasites, and in many cases, for parasites in other fish organs. Helminths encountered in the alimentary canal were prepared as whole mounts using conventional methods and subsequently examined with light microscopy. The survey included a total of 27 fish species, consisting of six centrarchid species, one ictalurid species, eleven cyprinid species, three percid species, three salmonid species, one catostomid species, one clupeid species, and one esocid species. Thirteen of the 27 fish species examined were infected with parasitic worms in the alimentary canal, including four species of acanthocephalans, five species of cestodes, two species of digenetic trematodes, and at least six species of nematodes. Among the intestinal parasitic worms in fish in Otsego Lake, the most prevalent and least host specific is the undescribed acanthocephalan, known as Leptorhynchoides thecatus 'Large form' (see Steinauer 2004). Additional species of helminths were encountered as larvae in the body cavity, or the viscera, in at least 18 of the 27 fish species examined. Information on the life cycles of each of the parasitic worms that were encountered and identified to species is compiled with reference to current and historic data for the invertebrate and vertebrate fauna of Otsego Lake. These survey data highlight which fish species serve the role as definitive host, intermediate host, or both, in the life cycles of the parasitic worms encountered.

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THE EFFECT OF PH AND OTHER ENVIRONMENTAL VARIABLES ON THE SPATIAL DISTRIBUTION OF FLATFISHES AND THEIR HELMINTH PARASITES IN THE GULF OF MEXICO

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Over the last 200 years, the world's oceans have sequestered more than a half of the CO_2 released from burning fossil hydrocarbons. The excess of CO_2 in the sea releases H^+ ions, which decreases ocean pH affecting marine organisms, mostly those with calciferous skeletons. Our goal was to determine the potential association between pH and other environmental variables in sea floor sediments and the abundance of helminth parasites of 3 flatfish species in Campeche Sound, southern Gulf of Mexico. We also used redundancy analysis (RDA) and MaxEnt to generate potential geographical distributions for both, helminths and their hosts using pH changes suggested by the Intergovernmental Panel of Climate Change (IPCC) between 2020 and 2100. We sampled 204 localities in the Gulf of Mexico, collecting 563 flatfishes (156 *Symphurus plagiusa*, 240 *Cyclopsetta chittendeni* and 167 *Syacium guntheri*) and encountered 21, 816 individual helminths belonging to 23 species. The statistical analysis was restricted to the most frequent and abundant species: *Stephanostomum* sp. (Digenea), *Prochristianella* sp. (Cestoda), *Hysterothylacium* sp. (Nematoda) and *Acanthocephaloides plagiusae* (Acanthocephala). The RDA showed significant statistical negative associations among digeneans and pH in combination with salinity, amonium, oxygen concentration and silicates. The MaxEnt model suggested a reduced geographical distribution of both the host fishes and digenean parasites. In contrast, distributional ranges increased for cestode and acanthocephalan and nematodes did not change. The results suggest a differential effect of the ocean acidification on the various helminth parasites and a strong negative effect on digeneans, potentially due to an indirect effect on their mollusc first intermediate hosts.

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REDUCED PARASITISM OF A MARINE WHELK, *KELLETIA KELLETII*, IN ITS EXPANDED GEOGRAPHIC RANGE

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If invasive species tend to escape parasitism, what happens to species that have recently expanded their geographic range margins? Do populations in recently expanded parts of ranges also tend to have a lower abundance and diversity of parasites than populations in historic ranges? The answer to this question clearly bears on the basic ecology of range expansions and may also shed light on the factors that set geographic range boundaries. Research on the ecology of expanded-range populations is also particularly relevant because climate change appears to be driving the pole-ward expansion of many species' range margins. However, we are aware of only one study that has examined parasitism of expanded- versus historical-range populations, and this was for a terrestrial insect and its parasitoid wasps. Here, we study a large marine whelk, the buccinid, *Kelletia kelletii* (Kellet's whelk), which has not been subject of any reported parasitological surveys. Previously found only south of Point Conception in southern California and Baja California, this whelk has recently expanded its range north along the central California coast to Monterey Bay. A lack of contact between expanded- and historic-range populations and the probable lack, in the expanded-range, of other hosts required for complex life cycle parasites led us to predict a decrease in parasitism in expanded-range whelk populations. We quantified the prevalence, mean intensity, and

diversity of parasites of 199 whelks in total from 25 sub-tidal reef populations (20 in the historical range and 5 in the expanded range). Our parasitological examinations revealed trophically transmitted stages of one nematode species (Gnathostomidae) and six tapeworm species in three orders (Tetraphyllidea, Diphyllidea and Trypanorhyncha). Further, we recovered adults of a rhabdocoelan graffilid flatworm parasite, a very common shell-boring mytilid bivalve, and a (putatively) commensal Acotylean flatworm. Concerning overall levels of parasitism, infection prevalence was ~80% lower, mean intensity was ~90% lower, and parasite species diversity was ~75% lower in the expanded- range than in the historic-range. These findings are not explained by host density, a general latitudinal gradient in parasite diversity or abundance, or sampling effort. Hence the results support the specific hypothesis that Kellet's whelk in the expanded range has been released from parasitic natural enemies, and the general hypothesis that enemy release may play a role in the ecology of geographic range margin extensions, with interesting similarities and differences with invasive species.

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LONG-TERM SURVEILLANCE OF A SALMONID PARASITE BY RIVER WATER SAMPLING AND QPCR

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Monitoring pathogen levels and predicting related mortality in wild fish populations is difficult. A practical alternative to host sampling is the direct measurement of waterborne parasite stages. *Ceratomyxa shasta* (Myxozoa) causes enteronecrosis in juvenile salmon and trout in the Pacific Northwest and is limiting their recovery in the Klamath River. *C. shasta* has two waterborne stages: actinospores released from freshwater polychaete worms and myxospores from salmonid fishes. In response to high prevalence and severity of *C. shasta* infection in Klamath River salmonids, we developed a parasite monitoring program that included detection of parasite DNA by qPCR of river water samples. We established 5 index sites in the mainstem and 4 sites in tributaries. Weekly, automatic samplers collected and pooled 1L of river water every 2h for 24h. Replicate 1L samples from the pool were filtered and DNA extracted. *C. shasta* was quantified using a TaqMan qPCR which targeted the ssrRNA gene. ITS-1 genotypes were determined using a SYTO9 qPCR and sequencing. We assayed >5000 samples over 7 years. We genotyped a subset of 278 samples, which comprised weekly samples for 1 index site from 2006-2011, and all samples available for the other 4 index sites in 2 years of high total parasite density. We identified spatial and temporal patterns of parasite density and genetic diversity across high-impact and low-impact years. *C. shasta* was detected at all mainstem sites, but levels differed among sites. Levels were low in the tributaries. Typically, parasite density increased in early spring (when salmonids were migrating) and peaked in late spring/early summer. Levels then decreased, but increased again to a lower second peak in late summer/early autumn. Parasite genotypes varied among sites and years. We are now exploring relationships among parasite occurrence, invertebrate and vertebrate host life histories, and water temperature and flow. These data influence management practices and inform epidemiological models and risk assessments.

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THE ROLE OF MORPHOMETRIC DISCONTINUITIES IN TAXONOMIC PRACTICE: ARE WE MEASURING ENOUGH WORMS?

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Here I present a critique to the use of morphometric discontinuities in taxonomy using tetraphyllidean cestodes as a model system. I will argue that we should increase our sample sizes far beyond what has

been reported in recent taxonomic literature if we seek sound statements about morphometric discontinuities between and among hypothesized lineages. My arguments are based on data gathered from selected groups of freshwater tetraphyllidean parasites of potamotrygonids, a historically unique clade of marine-derived stingrays endemic to South American river systems. Based on a probability theory and empirical molecular and morphological data, I will address the following questions: What does it take to get a reasonable idea of morphometric variation? How is morphometric variability correlated with phylogenetic patterns in freshwater tetraphyllidean lineages? What are the implications of the observed patterns in freshwater tetraphyllideans for taxonomic practice in general? Should we favor a reduced number of variables for a large number of individuals? The main contentions of this presentation are that a careful assessment of morphological variation is required before using morphometric discontinuities in taxonomy, and that this assessment can only be achieved with considerable sampling. Finally, I call the attention of parasitologists to a set of procedures that would provide a better documentation of cestode variability than what is currently being used.

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THE PHYLOGENETIC POSITION OF UNARMED CESTODES PARASITES OF NEOTROPICAL FRESHWATER STINGRAYS

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Here we address for the first time the phylogenetic positions of particular tetraphylidean and rhinebothriidean lineages (Cestoda), which parasitize Neotropical freshwater stingrays (Potamotrygonidae). Our intent is to detect closely related marine lineages that might help test hypotheses for existing associations (biogeographic and/or co-phylogenetic), which are related to the origin of the marine-derived potamotrygonids (the Neotropical freshwater stingrays). Our preliminary results are based on a phylogenetic analysis of two nuclear loci (partial SSU and LSU) from 149 terminals under parsimony with direct optimization. This set of terminals included members of , Lithobothriidea (2, outgroups), Cyclophyllidea (7), Lecanicephalidea (4) , Nippotaeniidea (3), Proteocephalidea (7), Rhinebothriidea (88), Tetrabothriidea (3), and Tetraphyllidea (35). Among these 149 terminals, 54 represented freshwater cestodes. Collectively, these terminals also represent all major marine bodies of water and a large spectrum of host clades. We recovered four independent lineages of non-hooked cestodes of Neotropical freshwater stingrays. Our results suggested that *Anindobothrium anacolum* is a member of Rhinebothriidea and sister to all other members of the order. *Rhinebothroides* was recovered as monophyletic, but its taxonomic status is dependent on the revision of *Rhinebothrium*, which is a polyphyletic cosmopolitan genus. *Nandocestus guariticus* nested as sister to *Paraorygmatobothrium*. Contrary to the latest phylogenetic hypothesis for cestodes, we recovered the clade Tetrahyllidea+Proteocephalidae as sister to Rhibebothriidea. The inclusion of cestodes from the amphi-American species *Himantura schmardae*, a representative to the putative sister group of potamotrygonids, unveiled a putative single event of co-phylogenetic association, depicted in the *Anindobothrium* clade. No clear correlation between biogeography and phylogeny was recovered at this stage, highlighting the need for better sampling from the hypothesized ancestral area of potamotrygonids, the Caribbean and Eastern Pacific.

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A NOVEL GENUS AND TWO NEW SPECIES OF DIPHYLLIIDEAN CESTODES FROM THE YELLOWSPOTTED SKATE, *LEUCORAJA WALLACEI*, FROM SOUTH AFRICA

L.M. Abbott and **J.N. Caira**, University of Connecticut

Two morphologically disparate novel species of diphyllidean cestodes from the Yellowspotted skate Leucoraja wallacei off the coast of South Africa were included in a recent molecular phylogenetic study aimed at a revision of the Diphyllidea. In that study these two species grouped well away from one another, one robustly among species of Echinobothrium sensu stricto, the other among primarily shark-hosted genera, most of which entirely lack or exhibit reduced scolex armature. Given the second species exhibits full, rather than reduced, scolex armature this result suggests that erection of a novel genus may be warranted. The present study aimed to formerly describe both species and to explore the existence of one or more distinct morphological features to support erection of a novel genus for the latter diphyllidean taxon. Material of both species was examined using light microscopy of whole mounts and histological sections, and scanning electron microscopy. Close examination revealed that among the valid Echinobothrium species the first species most closely resembles Echinobothrium joshuai, an affinity supported by the molecular analysis. This work also resulted in identification of a unique characteristic for the second species unlike other members of the order, it was found to exhibit lateral hooklets arranged in anterior and posterior rows, rather than a single row, supporting its placement in a novel genus. In combination, these results indicate that the two diphyllideans parasitizing L. wallacei are not each others' closest relatives. They further suggest that the association of the potential novel genus with a skate, rather than a shark, represents a host-switching event.

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THE OTHER SIDE OF THE WORLD: ASSESSMENT OF THE DIVERSITY OF ECHENEIBOTHRIINAE

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The molecular analyses resulting in the erection of the order Rhinebothriidea yielded a number of intriguing results; conspicuous among them was the nesting of members of the subfamily Echeneibothriinae among rhinebothriideans, rather than tetraphyllideans. This result was unexpected given that unlike most rhinebothriideans these taxa parasitize skates and possess, rather than lack, an apical myzorhynchus on their scolex. The subfamily has been poorly studied and is in urgent need of attention. At present it is fairly diverse including at least 3 valid genera: *Notomegarhynchus*, *Pseudanthobothrium* and *Echeneibothrium*, the latter being by far the most speciose. The 30 known species appear to be highly host specific and parasitize a total of 29 species of skates. Interestingly, most of this diversity comes from hosts in Europe and North America, with only 9 species of echeneibothriines having been described from only 6 species of skates in the Southern Hemisphere. Given that ~50% of the 257 known skate species occur primarily in the Southern Hemisphere, and the pattern of host specificity in this group, this discrepancy suggests that skates from this region not previously examined for cestodes will be found to host a substantial diversity of novel echeneibothriines. In order to test this hypothesis, 10 species of skates collected from New Zealand, South Africa and Chile were examined for echeneibothriines for the first time. Cestodes were removed from the spiral intestine and fixed in either 4% formaline or 95% ethanol, allowing for both morphological and molecular analyses to be carried out. The former specimens were examined with light and electron microscopy. Molecular analysis, which included sequencing of partial 28S rDNA (D1-D3) were conducted on the latter specimens. A subset of the skate species from each locality was found to host at least one echeneibothriine species. In most cases,

more than one morphotype was found in each host species. This study makes it clear that the echeneibothriine fauna of skates inhabiting the Southern Hemisphere have been thus far underestimated. Future efforts should be placed on detailed examination of these hosts and a much needed revision of the Echeneibothriinae.

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SITE SPECIFICITY OF TAPEWORMS OF THE GENUS *CALLIOBOTHRIUM* IN THE SPIRAL INTESTINE OF SMOOTHHOUND SHARKS (CARCHARHINIFORMES: TRIAKIDAE)

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The cestode genus *Calliobothrium* contains two groups of morphologically disparate and molecularly distinct species. Species in the first group are large and laciniate while those in the other group are small and nonlaciniate. Data published for 4 species of *Mustelus* suggest that the two morphotypes differ in their site of attachment within the spiral intestine of their smoothhound shark hosts. In each case, the small species was found to attach in the anterior 1–3 chambers and the larger species was found to attach with a normal distribution around the middle chambers of the spiral intestine. Given the two morphotypes are now known to belong two separates clades, these results suggest there is an underlying phylogenetic component to site specificity in these taxa. The present study was undertaken to more formally test this hypothesis using *Calliobothrium* species parasitizing *Mustelus palumbes* from South Africa. *Calliobothrium* species exhibiting each of the two morphotypes were found parasitizing *M. palumbes*; phylogenetic analysis confirmed the new species represent both clades of *Calliobothrium*. All specimens of the small species were found in the anterior 3 chambers while the specimens of the large species were found around the middle chambers of the spiral intestine. Furthermore, the two morphotypes differed in their mode of attachment to the intestinal surface. Preliminary work on *Calliobothrium* species parasitizing *Mustelus antarcticus* suggests a similar pattern of site specificity. Not only do these differences in site specificity suggest ecological divergence in the two clades of *Calliobothrium*, but they also support the existence of a phylogenetic component to site specificity.

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A REVISED LOOK AT THE GENUS *CAULOBOTHRIUM* (CESTODA: TETRAPHYLLIDEA): NOVEL SPECIES AND THEIR INTRIGUING HOST ASSOCIATIONS

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Although the nature of the tetraphyllidean tapeworm genus *Caulobothrium* has not been completely elucidated, considerable work has been undertaken to circumscribe the identity of this genus. A valuable molecular study, published in 2009, supported the monophyly of the group but included data from five undescribed species. In an effort to formally describe the species included in the study, four of the undescribed species, in addition to two novel species not included in the molecular study and two existing, but poorly understood, members of the genus, were examined using light microscopy, scanning electron microscopy, and histological sectioning. The examination of these and other known species of *Caulobothrium* has resulted in an emendation of the generic diagnosis, to include the presence of a previously overlooked apical sucker. Three of the novel species examined here were of particular interest as they were collected from stingrays of the family Dasayatidae, found in Australia, Borneo, and the Solomon Islands, thereby expanding both the geographic and host range of *Caulobothrium* to include a novel family. Analysis of the host associations of the genus revealed that *Caulobothrium* species parasitizing disparate hosts in the same locality are more closely related to one another than they are to *Caulobothrium* species in phylogenetically similar hosts from different localities. This leads to the

surprising conclusion that in this genus, geography, rather than host affinities, is a better predictor of parasite relationships.

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CESTODES FROM DEEP-WATER SQUALIFORM SHARKS SURVEYED OFF AZOREAN SEAMOUNT REVEAL ADDITIONAL DIVERSITY OF ADULT AND LARVAL FORMS

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The majority of our knowledge on marine cestodes is limited to those worms that are relatively easy to obtain (i.e., those that parasitize shallower water taxa). The invitation to participate in a deep-water research survey off the Condor Seamount in the Azores offered the opportunity to gain information regarding those less often studied meso- and bathypelagic shark hosts and their parasites. This survey resulted in the examination of 114 shark specimens from 12 species of squaliform sharks representing 4 different families from depths ranging between 450 and 1290 m. Host identifications were verified using the elasmobranch barcoding gene, NADH2. Cestodes were recovered in the spiral intestines from 10 of the 12 shark species: *Deania calcea*, *D. profundorum*, *Etmopterus princeps*, *E. pusillus*, *E. spinax*, *Centroscyllium fabricii*, *Centroscymnus coelolepis*, *C. owstonii*, *Centroselachus crepidater*, and *Dalatias licha*. No adult cestodes were found from *Deania* cf. *profundorum* or *Centrophorus squamosus*. Light microscopy, scanning electron microscopy, and molecular methods were used to examine the cestodes in more detail. These methods reveal that at least several novel trypanorhynch species and a single novel tetraphyllidean species remain to be formally described. Aporhynchid and gilquiniid trypanorhynchs dominated the adult cestode fauna of *Etmopterus* and *Deania* host species, respectively, while larval phyllobothriids were found across several host genera, including, *Deania*, *Centroscyllium*, and *Centroselachus*. Molecular data allow us to investigate the relationships of these larval forms with one another, as well as compare them to sequences available in GenBank of adult cestodes from other localities, providing implications of transmission dynamics.

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THE METAZOAN PARASITES OF OPOSSUMS IN BOLIVIA: AN INVENTORY OF 25% OF MARSUPIAL DIVERSITY

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The diversity of marsupials in Bolivia includes 27 species from the orders Didelphimorphia and Paucituberculata that have been recorded from almost all biomes of the country. This number of species roughly represents a quarter of the marsupial diversity in the New World. Herein, we present the helminthological records for 16 of these species, which include 21 species of nematodes, 3 species of tapeworms and 2 species of trematodes. Our results show that species of didelphimorph marsupials or opossums share up to 60% of their parasites and that these parasites occur over vast areas of the country. With the exception of two species, most parasites are known to infect only marsupials and correspond to taxa that appear to be specific to these mammals. However, the phylogenetic reconstructions of Viannaiidae, Oxyuridae, and Aspidoderidae reveal that the sister taxa for these groups occur in caviomorph rodents, armadillos, and even monkeys. These phylogenies indicate that the diversity of these parasites resulted from events of host switching and subsequent adaptive diversification.

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MOLECULAR EVIDENCE SUGGESTS THAT TWO *ISOSPORA* SPECIES CAN CAUSE VISCERAL ISOSPOROSIS ('ATOXOPLASMOSIS') CONCURRENTLY IN CAPTIVE-BRED SUPERB GLOSSY STARLINGS (*LAMPROTORNIS SUPERBUS*)

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A number of Superb Glossy Starling (*Lamprotornis superbus)* nestlings and fledglings died in a captive breeding program undertaken at the Metro Toronto Zoo, Ontario, Canada. Post-mortem and histological observations confirmed that extraintestinal coccidiosis ('visceral isosporosis' or 'atoxoplasmosis') were associated with these deaths. DNA was obtained from sporulated oocysts and tissues sampled during necropsy (liver, lung or spleen) and then PCR amplified using primer pairs specific for coccidial mitochondrial COI (cytochrome c oxidase subunit I) or nuclear 18S rDNA. Resulting PCR products were gel-purified and sequenced completely in both directions. Neither the COI nor 18S rDNA sequences had been previously submitted to GenBank. The oocysts recovered from the faecal samples were spherical to subspherical, with a double-layered, smooth, colorless oocyst wall, 17.47 ´ 17.31 µm (17-18 ´ 18-20; n=30) with 2 to 4 rice grain-shaped polar bodies. Sporocysts were ovoid, 13.45 ´ 9.34 µm (13-20 ´ 9-12; n=30), each with a small Stieda body, indistinct sub-Stieda body and prominent sporocyst residuum. Sporozoites were vermiform and each had a large refractile body at their posterior end. With the one exception, all birds had molecular evidence of the presence of two *Isospora* species that varied in their relative abundance. In one bird and the associated fecal sample, two distinct COI sequences and two distinct18S rDNA sequences were found at almost the same abundance; in other specimens, one of the 18S and one of COI sequences were less abundant than the other and in the tissues of a single bird only a single COI and single 18S sequence were present. In all cases, the same pair of 18S rDNA and COI sequences fluctuated in abundance in parallel suggesting that there were two distinct species present rather than one species with more than one COI and/or 18S locus. We have demonstrated that 2 *Isospora* spp. were involved in simultaneous enteric and extra-intestinal infections in captive Superb Glossy Starlings.

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EIMERIA SPECIES OF TURKEYS: LINKING MOLECULES AND MORPHOMETRICS

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Biological and molecular data on *Eimeria* spp. of turkeys is lacking compared to *Eimeria* spp. of chickens. To address this, both molecular (nuclear 18S rDNA sequences and mitochondrial cytochrome c oxidase subunit I [COI] sequences obtained using PCR and sequencing) and biological features (shape and size of oocysts, endogenous parasite development obtained via experimental infections *in vivo*) of *Eimeria* spp. of turkeys were examined. Multiple single oocyst-derived lines from 5 *Eimeria* species affecting turkeys were selected and propagated in specific parasite free poults. Both mitochondrial COI and nuclear 18S rDNA sequences were generated from *E. meleagridis*, *E. meleagrimitis*, *E. dispersa* and 2 different strains of *E. adenoeides*. Phylogenetic analysis of the COI sequences from each species formed well supported monophyletic groups with markedly lower intraspecific genetic distances compared to the 18S rDNA sequences. Divergent, paralogous 18S rDNA copies were discovered within the nuclear genome of *E. meleagrimitis*. This confirms the utility of the COI locus as a species-level genetic marker for *Eimeria* species; 18S rDNA sequences may be better suited for inferring deeper evolutionary relationships. Mono-specific lines of parasites (i.e. single-oocyst derived lines that share the same COI sequence and are

putatively the same species) all possessed consistent oocyst dimensions and features. While oocyst dimensions overlapped among some species, other morphological features such as presence of a polar granules or details of oocyst wall structure could be used to differentiate these similarly sized parasites. Histological examination of the endogenous development of these molecularly characterized turkey *Eimeria* spp. identified previously unrecognized details regarding the location and number of stages during endogenous development of some turkey *Eimeria* spp. These observations call into question the assumption that different strains of a single *Eimeria* spp. infecting turkeys can possess widely variable oocysts sizes and still be considered a single species.

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USING EXISTING DRUGS AS LEADS TO COMBAT NEGLECTED DISEASES

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Parasitic nematode infection is a large global health and economic problem, infecting around 2 billion people and costing $100 billion in crops and livestock. Nematodes are becoming resistant to currently available anthelminthics and existing pesticides have deleterious effects on food production and a negative economic impact worldwide, thereby creating an urgent need to develop new drugs to combat these parasites. We use nematode genomics, transcriptomics, functional genomics, proteomics, interactome etc (systems biology) which when coupled with innovative bioinformatic approaches provide supportive information essential for developing of novel diagnostics, vaccines and anthelmintics/pesticides. Our recent focus has been on combining a variety of bioinformatics and cheminformatics approaches, along with laboratory screening on *C. elegans* and parasitic nematodes, to learn more about metabolic chokepoints as drug targets in several nematodes spanning the phylum Nematoda. The chokepoint was defined as either produced or consumed by a single reaction and elucidated the chokepoint enzyme that drives the reaction. If the enzyme that catalyzes that reaction is blocked, a toxic build-up of a compound or lack of compound necessary for subsequent reaction will occur, potentially causing adverse affects to the parasite organism. Chokepoint enzymes were identified in proteomes of 10 nematode species, and the intersection and union of all chokepoint enzymes were found. Chemogenomic screening was performed on drug-like compounds from public drug databases to find existing drugs that target homologs of nematode chokepoints. The compounds were prioritized based on chemical properties frequently found in successful drugs and were experimentally tested using *Caenorhabditis elegans* and 2 parasitic nematodes with different mode of parasitism, a blood-feeding and a filarial nematode. Several compounds showed efficacy in *C. elegans* and one compound showed efficacy in all three species and yielded expected physiological effects, indicating this drug-like compound may have efficacy on a pan-phylum level through the predicted mode of action. The methodology to find and prioritize metabolic chokepoint targets and prioritize compounds could be applied to other parasites. The prioritized compounds could be a good leads for developing anthelminthics and pesticides, and have potential to provide accessible treatment to people in developing countries, as well as improving the health of livestock and boosting food production globally.

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RELEASE FROM A KEY PARASITE (*GYRODACTYLUS* SPP) IN THE WILD LEADS TO REPEATABLE SEXUALLY ASYMMETRIC EVOLUTION OF RESISTANCE

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Although males and females of a population tend to be under the same spatial and temporal constraints, they can experience significantly different selective pressures. These different selective pressures are often used to explain sexual dimorphism in the ability of individuals to reduce their parasite load (resistance). Even though theoretical and empirical research has shown that populations that experience different levels of parasitism (i.e. different selection pressures) tend to show divergence in the expression of resistance, whether this divergence is consistent between males and females is less studied. To assess the evolution of resistance in a species that shows sexual dimorphism in defence against parasites we tested guppies (*Poecilia reticulata*) that were released from selection by two species of a key Monogenean ectoparasite (*Gyrodactylus turnbulli* and *Gyrodactylus bullatarudis*) in four replicate translocations in the wild. After four and eight generations, guppies from the translocated populations, and from the source population which remained exposed to *Gyrodactylus*, were sampled and bred to second generation under common garden conditions in the laboratory. We exposed these descendants to individual infections with *G. turnbulli* and monitored parasite numbers on isolated guppies over a period of 24 days. Resistance was quantified using parasite numbers at a series of days, parasite peak burden and estimates of parasite intrinsic rate of increase. The release of wild fish from *Gyrodactylus* led to asymmetric evolution of resistance in the sexes: females derived from three of four translocated populations showed increased resistance relative to the source population, whereas males showed no change in the expression of resistance compared to the source population. Our results confirm the possibility of divergent natural selection among the sexes under identical environmental conditions. It appears that the same selective environment can impose differential selection pressures on the male and female members of a population.

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CHARACTERIZATION OF THREE ORPHAN LIGAND-GATED ION CHANNEL SUBUNITS IN *DROSOPHILA MELANOGASTER* – POTENTIAL PESTICIDE TARGETS?

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Cys-loop ligand gated ion channels (LGIC) are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their large diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive molecular targets for various pesticides. Despite the widespread use of such pesticides, issues regarding drug specificity and resistance continue to pose serious problems in regions that rely on pesticides for crop protection and prevention against disease. We are characterizing three orphan Cys-loop LGIC subunits; CG7589, CG6927 and CG11340 in *Drosophila melanogaster*. These genes are of particular interest because they are specific to arthropods and do not possess any orthologs in vertebrate systems (Dent, 2006). Consequently, pesticides that target channels formed by these genes are predicted to be safe and have low risk for cross-resistance with existing pesticides. We generated putative knockouts of all three genes via P-element excision mutagenesis or FRT mediated recombination. Genetic data suggest that mutations in CG7589 and CG11340 are semi-lethal. CG7589 and CG11340 are expressed in the midgut and Malpighian tubules; tissues involved in ion regulation and renal function, while CG6927 appears to be expressed in the salivary glands. Consistent with expression in secretory epithelia, CG11340 and CG7589 mutants exhibit deficits in osmoregulation. Furthermore, electrophysiological tests indicate that CG11340 can form a functional homomeric channel while CG7589 and CG6927 form a heteromeric channel. CG7589 also forms a constitutively open homomeric channel. Based on the relative divergence of these genes from other Cys-loop LGIC subunits as well as the lethal phenotypes associated with the corresponding mutants, these channel subunits may provide a promising molecular target for the development of a novel class of highly selective and efficient pesticides.

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PUTATIVE CATION-SELECTIVE NICOTINIC ACETYLCHOLINE RECEPTORS OF *SCHISTOSOMA MANSONI*

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Schistosoma mansoni is the major causative agent of schistosomiasis, a socioeconomically devastating human disease, second only to malaria among all parasitic infections. Nicotinic acetylcholine receptors (nAChRs) are proven anthelmintic drug targets, as this family of proteins form pentameric ion channels which are directly linked to motility of the worm. Schistosomes have a rich diversity of putative nAChRs encoded within the genome, both chloride- and cation-selective channels, but they have not been characterized at the molecular level. Here, we take a first look at putative cation-selective nAChRs of this parasite, which are predicted to have important excitatory neuromuscular activity in the parasite. First, we investigated the tissue localization of predicted nAChR subunits by confocal immunofluorescence, using specific peptide antibodies. The confocal pictures showed high-level expression throughout the central and peripheral nervous systems of the parasite, including regions of the nervous system that innervate the body wall muscles. In subsequent studies we knocked down gene expression of four predicted nAChR subunits by treatment of S. mansoni larvae with small interfering RNA (siRNA) and looked for effects on movement compared to control groups. The results identified two types of motor phenotypes, either hypoactivity or hyperactivity, depending on the subunit targeted. Further analysis revealed that RNAi-suppressed animals were resistant to treatment with cholinergic drugs but the response varied among the subunits. Together, the results suggest that cation-selective nAChR subunits are associated with at least two different types of channels, which have different pharmacological properties and different effects on worm motility. The four subunits have been cloned from *S. mansoni* and verified by DNA sequencing. Studies are under way to express the protein in Xenopus oocytes for functional analyses and to verify the identity of the channels.

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ANTI-SCHISTOSOMAL MODE OF ACTION OF OXAMNIQUINE

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Drug resistance to oxamniquine evolved in the human blood fluke (*Schistosoma mansoni*) in Brazil in the 1970s. It has also been experimentally selected in the laboratory. We exploited the genome sequence, genetic map and experimental tractability of this parasite, to identify the gene responsible for this trait. To do this, we staged a cross between parental parasites that differed by ~500 fold in oxamniquine resistance, determined drug sensitivity in clonally-derived F2 parasites, and identified a single Quantitative Trait Locus (QTL) with a LOD score of 30 on the upper arm of chromosome 6. The causative gene, a sulfotransferase (named SmSULT), was identified using both RNAi knockdown and biochemical complementation assays. By identifying the gene for drug resistance, we were able to demonstrate the mechanism of action. Oxamniquine is taken up by adult schistosomes. A schistosome sulfotransferase sulfonates oxamniquine by catalyzing the transfer of a sulfuryl group (SO_3) from the active sulfate donor such as 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to oxamniquine to activate the drug to bind to schistosome macromolecules like DNA to interfere with DNA synthesis and transcription. This is cidal for the adult worms.

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HEME PROTEINS OF *GIARDIA LAMBLIA*

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The *Giardia lamblia* genome encodes heme proteins despite lacking heme biosynthetic capacity. One is a flavohemoglobin that counteracts nitrosative stress, but Giardia also encodes three members of the cytochrome b_5 family. While these act as electron shuttles in known pathways of other species, clues about their roles in Giardia are scant. We describe here our ongoing work in defining the structures and functions of the Giardia cytochromes b_5 (gCYTB5s). *Structural characterization:* We previously reported that expression of gCYTB5-I in *E. coli* yields holoprotein. More recently we found that recombinant gCYTB5-II and III are also obtained as heme-bound proteins. All are ~17.5 kDa monomers that are amenable to structural studies by NMR, and we have optimized conditions for growth on minimal media that are necessary for expression of ^{15}N and ^{13}C-labelled protein required for such studies. *Functional studies:* Cytochromes b_5 in other species are members of redox chains that mediate electron transfer between electron donors and acceptors. However, only one candidate partner of the gCYTB5s has been identified from the Giardia genome. This is a Giardia cytochrome P450 reductase (gCP450R) homolog; in other species these flavoenzymes transfer electrons from NADPH to cytochrome P450 as well as to cytochrome b_5. We have begun recombinant expression studies on gCP450R and intend to measure its ability to catalyze the NAD(P)H-dependent reduction of gCYTB5s. We are also screening Giardia cell lysates directly for such activity; interestingly the presence of recombinant gCYTB5-I stimulates NAD(P)H consumption of Giardia lysates by 70%. These experiments are a prelude to fractionation of Giardia lysates to identify the electron donors to the cytochromes. Finally, we will use an antibody against gCYTB5-I to detect the presence and location of this protein within Giardia extracts and whole cells. We will also use this antibody to monitor changes in the expression level of gCYTB5-I in response to heme, which the protist presumably obtains from the host by an unknown mechanism.

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THE M1 ALANYL AMINOPEPTIDASES OF *PLASMODIUM FALCIPARUM* (PFM1AAP) MALARIA: BIOCHEMICAL AND MUTATIONAL ANALYSIS

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The *P. falciparum* alanyl aminopeptidase (*Pf*M1AAP) plays a role in the terminal stages of hemoglobin digestion and is a potential anti-malarial drug target. The molecular structure of *Pf*M1AAP complexed with aminopeptidase inhibitors bestatin and phosphonate dipeptides has been determined and revealed that 16 active site residues are involved in the interaction between the enzyme and these inhibitors. When the sequence of *Pf*M1AA*P* is aligned with homologs of other malaria parasites we found that all these 16 residues are highly conserved. However, when we align the sequence of *Pf*M1AAP with homologs from other apicomplexan parasites (*T. gondii*, *C. parvum*, *C. hominis*, *T. parva*, *B. bovis*) several different alterations appear at specific positions. In this study, we substituted the residues of *Pf*M1AAP to corresponding residues of these other apicomplexan parasite homologs. We found that substitutions corresponding to residues observed in *Cryptosporidium parvum*, *Cryptosporidium hominis* and *Babesia* enhanced the catalytic efficiency (K_{cat}/K_m) of the enzyme while changes that corresponded to *Toxoplasma gondii* homologs and *Theileria* had detrimental effect on the catalytic efficiency of the enzyme. Based on these results we identified positions that were crucial for the activity of the enzyme, and for inhibitor

binding. In order to confirm these observations, we generated additional mutants of *Pf*M1AAP with changes at these significant positions. Interestingly, we found that subtle changes in the active site residues either increased or lowered the catalytic efficiency of the enzyme. The inhibitor constant, K_i of the enzyme against 4 inhibitors bestatin, compound 4, tosedostat and H-Leu-chloromethylketone was obtained. Collectively, we have identified critical residue in substrate and inhibitor binding that could be important to understanding the potential drug resistance to aminopeptidase inhibitor

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INTERACTION OF *LEISHMANIA DONOVANI* PEX14 WITH GLYCOSOMAL MEMBRANE

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Leishmania is the causative agent of leishmaniasis, a group of devastating diseases that are deemed among the top ten neglected tropical diseases by the World Health Organization. *Leishmania* contain a unique glycosomal organelle that compartmentalizes a variety of vital metabolic pathways. The glycosomes are related to peroxisomes of higher eukaryotes, lack a genome and post-translational machinery. Therefore, glycosomal proteins are synthesized in the cytosol and post-translationally translocated across the glycosomal membrane via a C-terminal PTS1 or N-terminal PTS2 topogenic signal sequence. Proteins with a PTS1 or PTS2 signal are bound by PEX5 and PEX7, respectively, and bind to the glycosomal membrane associated protein PEX14. PEX 14 is essential for the correct targeting of protein to the glycosome and viability of kinetoplastid parasites. The *Leishmania* PEX14, is peripheral membrane protein that is anchored to the cytosolic face of the glycosomal membrane, and contain a variety of functional and structural domains that include binding sites for the receptor proteins PEX5 and PEX7, a leucine zipper thought to be involved in PEX14-PEX14 interactions, and a hydrophobic region conjectured to mediate association with the glycosomal membrane. Previous studies demonstrated that the hydrophobic region spanning residues 120-200 were essential for the binding of PEX14 to liposomes mimicking the phospholipid composition of the *L. donovani* glycosomal membrane. Moreover, using dye leakage assays it was shown that a fragment spanning this region, pex14(120-200), was capable of forming a pore in the liposome bilayer. To further investigate this protein-membrane interaction we generated a panel of mutants in which a tryptophan residue was inserted at various positions of the hydrophobic region. Intrinsic fluorescence studies performed using the quenching agents potassium iodide and 10-doxylnonadecane were carried out to examine the topology of this peptide in the lipid bilayer and to assess the depth to which the tryptophan inserted into the hydrophobic core of the membrane. Our studies support the notion that this hydrophobic region which favors adopting an amphipathic helical conformation is critical not only for anchoring PEX14 to the glycosome membrane, but also in the transport of protein cargo into the glycosome.

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IN VITRO SCREENING OF COMPOUNDS IDENTIFIES RNA EDITING INHIBITORS

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RNA editing in kinetoplastid parasites is an essential process for the expression of most mitochondrial proteins. It is carried out by a multi-protein complex, called the editosome. With more than 20 proteins, the editosome is unique to the kinetoplastids and a potential drug target. We previously developed an *in vitro* fluorescence-based reporter assay to monitor RNA editing. Here, we used this assay to screen a collection of 1280 annotated Library of Pharmacologically Active Compounds at 20 μM concentrations.

The screen led to the identification of compounds that showed inhibitory effects on RNA editing. To validate the specificity of inhibition we performed a comprehensive set of *in vitro* RNA editing assays, including a variation of fluorescence-based reporter assay that bypasses the initial rate-limiting endonucleolytic cleavage step. The characteristics of the alterations indicate which step of the editosome function is affected by the compounds.

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CHARACTERIZATION OF AN NEW PUTATIVE RHOPTRY PROTEIN IN *PLASMODIUM FALCIPARUM*

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The malaria parasite is the most important member of the Apicomplexa, a large and highly successful phylum of intracellular parasites. Invasion of a red blood cell by *Plasmodium falciparum* merozoites is an essential step in the malaria lifecycle. Several of the proteins involved in this process are stored in the apical complex of the merozoite, a structure containing secretory organelles called dense granules, micronemes and rhoptries, that are released at different times during invasion. Our laboratory is focussed on identifying and characterizing the molecular players involved in erythrocyte invasion since these represent key targets for both therapeutic and vaccine-based strategies to block parasite development. We present here our initial characterization of *P. falciparum* PF13_0116, a putative rhoptry protein. To investigate a potential role of PF13_0116 in invasion we generated a parasite strain where the endogenous PF13_0116 was tagged with GFP. Western blot analysis showed that the protein is expressed throughout the erythrocytic cycle and immunofluorescence assays demonstrated that it was potentially associated with the Golgi apparatus in rings and trophozoites but that it partially colocalized with RAP1, a marker of the rhoptry, in schizonts. Efforts are currently underway to narrow down the subcellular localization by immuno-electron microscopy and to identify interacting partners by mass spectrometry. The studies will allow us to determine if PF13_0116 plays a role in the process of merozoite invasion.

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VENOM ALLERGEN-LIKE (VAL) PROTEINS IN EARLY INTRAMOLLUSCAN LARVAL STAGES OF *SCHISTOSOMA MANSONI*

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Schistosome venom allergen-like (SmVAL) proteins belong to a large gene superfamily structurally defined by the presence of the Sperm-coating protein/tpx-1/Ag5/PR-1/Sc7 (SCP/TAPS) domain. These proteins have been implicated in a diversity of functions including insect envenomization, sperm/testis development, imuune regulation and host invasion by nematode parasites. Recently we have shown that VAL proteins representing at least 7 family members (SmVAL2, SmVAL3/23, SmVAL9, SmVAL15, SmVAL5/15, SmVAL26/28, SmVAL27) are released during *in vitro* miracidium-to-sporocyst transformation. In order to gain further insights into the possible role of these proteins during snail infection, we generated antibodies to recombinant (r)VAL9 and investigated the distribution of this

protein during *S. mansoni* larval development. Using immunocytochemistry and Western blotting immunoreactive VAL9 appeared to be highly expressed in miracidia, but significantly reduced in subpopulations of culture-derived primary sporocysts 24-hr post-transformation. Sporocysts continued to produce low levels VAL9 protein upon extended cultivation up to 10 days. Larval transformation proteins (LTP) released into medium upon initial larval cultivation contained VAL9, confirming its presence in LTP during early miracidial development. VAL9 was localized by confocal microscopy in the ciliated epidermal plates, apical papillae, parenchyma, and presumptive germinal cells of miracidia, while sporocysts displayed variable immunoreactivity, mainly in parenchymal and germinal cells. With the finding of miracidium-derived VAL9 in LTP, we are currently investigating the direct effects of rVAL9 on expression of immune-related transcripts/proteins in hemocytes from susceptible and resistant *Biomphalaria glabrata* snails.

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MORPHOLOGICAL ALTERATIONS OF FEMALE BRUGIA MALAYI FOLLOWING EXPOSURE TO FLUBENDAZOLE: EFFECTS OF VARIATION IN THE EXPOSURE PROFILE

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Human onchocerciasis and lymphatic filariasis (LF) are currently controlled using microfilaricidal drugs. Unfortunately, these drugs have little effect on adult worms. This necessitates prolonged yearly dosing, and given that LF-associated pathology is caused by adult worms residing in lymph nodes, there is an urgent need for a macrofilaricidal drug. Flubendazole (FLBZ), a benzimidazole anthelmintic, is an appealing candidate macrofilaricide. FLBZ has demonstrated profound and potent macrofilaricidal effects in a number of experimental filarial rodent models. This study was designed to characterize effects of FLBZ and its reduced metabolite (FLBZ-R) on filarial nematodes following *in vitro* exposure, and to determine the exposure profile which results in the greatest macrofilaricidal effect. Adult *Brugia malayi* were exposed to varying concentration of FLBZ or FLBZ-R (10 nM - 10 μM) *in vitro* for up to five days, after which worms were fixed for histology and TEM. Morphological damage following exposure to FLBZ was observed prominently in the intestine, female gonads and developing embryos at concentrations as low as 10 nM. From these data, exposure profiles were developed. Adult *B. malayi* were exposed to: 1.) high concentration for a short duration (100 nM – 32 μM; 24 hours); 2.) low concentration continuously (1 nM – 100 nM; 21 days); or 3.) high concentration pulse (10 μM). Worms were fixed on day 21. Damage was assessed using motility scores and histological techniques. This study provides preliminary information concerning the exposure profile required for FLBZ to have detrimental effects on tissues required for parasite development and survival.

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THE EFFECT OF QUINOLINE DRUGS ON INTRACELLULAR PH IN *PLASMODIUM FALCIPARUM*

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The digestive vacuole (DV) of *Plasmodium falciparum* parasites is the site of action of several antimalarial drugs and its membrane contains transporters that are implicated in the regulation of drug resistance. To date, the mechanism of action of quinoline drugs such as chloroquine (CQ), mefloquine (MQ) and quinine (QN) is still unclear. Despite the importance of low pH in DV functions, including hemoglobin degradation and heme detoxification, the possible alteration of DV pH in response to

quinoline drugs has remained controversial. Five *P. falciparum* strains with differential response to CQ, QN and MQ were selected for quantitative intracellular pH measurements: HB3, 3D7, K1, FCB and Dd2. Using confocal microscopy of single live *P. falciparum*–infected erythrocytes, steady-state cytosolic pH values were determined with the ratiometric pH-sensitive dye SNARF-4F (3D7: 7.29, Dd2: 7.12, HB3: 7.06, FCB: 7.13, K1: 7.13). CQ-resistant Dd2 and CQ-sensitive 3D7 parasites were transfected with a DNA construct encoding the ratiometric pH-sensitive green fluorescent protein pHluorin alone or pHluorin with a targeting peptide that mediates trafficking of the fluorescent protein to the DV. Cytosolic pH measurements using pHluorin yielded similar results (3D7: 7.32, Dd2: 7.21). In the DV, steady-state pH values of 5.29 (3D7) and 5.19 (Dd2) were determined. Upon addition of CQ, QN and MQ, alkalinization of the DV lumen and acidification of the cytosol was observed, which might be due to H^+-coupled drug export from the DV into the parasite cytosol mediated by *P. falciparum* chloroquine resistance transporter (PfCRT). To elucidate the mechanism of intracellular pH modulation by quinoline drugs, the pH effects of PfCRT inhibitor verapamil, *P. falciparum* multidrug resistance protein 1 (PfMDR1) inhibitor tariquidar and the V-ATPase inhibitors concanamycin A and bafilomycin A1 were tested in combination with the antimalarials.

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DNA AMPLIFICATION, LOCUS DELETION AND A POINT MUTATION CONTRIBUTE TO ANTIMONY RESISTANCE IN *LEISHMANIA (VIANNIA) GUYANENSIS*

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Leishmania (*Viannia*) *guyanensis* is the etiological agent of mucocutaneous leishmaniasis, one of the most severe, disfiguring and difficult to treat form of the disease. Antimony(Sb)-based chemotherapy frequently fails and resistant or relapsed cases have been reported. Here we investigate the mechanisms of Sb resistance in four independent laboratory-selected *L.* (*V.*) *guyanensis* resistant mutants. One of the major accepted mechanisms in Sb-resistant Leishmania is the formation of circular extrachromosomal DNA containing the Multidrug Resistance associated Protein A (MRPA) encoding gene, which allows the drug sequestration into intracellular vesicles. However, gene amplification is rare in *Viannia* subgenus species. In this study we demonstrate the presence of an extrachromosomal MRPA circular DNA in 3 out of 4 mutants. The amplicon is generated through homologous recombination between conserved repeated nucleotide binding domain sequences present in MRPA and in a nearby related gene MRPB. A real-time PCR assay confirmed an increase in transcript levels for the genes present in the circular amplicon. Transport assays suggested a defect in transport in the four mutants. The route of entry of Sb is mediated by the aquaglyceroporin AQP1. The expression level of AQP1 was decreased by >400 fold in three independent mutants. This decrase in expression was due to a partial deletion of the chromosome 31 where the gene is located. In the last mutant with reduced transport, the expression level of AQP1 was normal but sequencing of the gene revealed a mutation in the mutant leading to a single amino acid mutation G133D. Functional work indicated that AQP1 with the G133D mutation does not transport antimony in cells. Our molecular work has revealed a multiplicity of resistance mechanisms in the *Viannia* subgenus and for the first time has shown that either a gene deletion or a point mutation can lead to high level antimony resistance.

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NOVEL COMPOUND AGAINST *CRYPTOSPORIDIUM PARVUM* INFECTIONS IN BOTH IN VITRO AND IN VIVO

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Cryptosporidium parvum is protozoan parasite that can cause life-threatening illnesses in humans. As it is globally distributed, *C. parvum* has the capacity of infecting the whole human populace. This is one reason this organism is categorized as a Class B bioterrorist weapon. Despite the threats these parasites pose, no significant improvements have been made in managing the infection. Current treatments, have failed to show consistent activity. Given the need to find need treatments we tested the *in vitro* and *in vivo* activity of compound 1 against *C. parvum*. We also looked at the toxic effects of the drug. *In vitro,* compound 1 had an IC_{50} of 25nM. Toxicity was evaluated using human ileocecal adenocarcinoma cells (HCT-8 cells) and was not significant at concentrations below 100μM (TC_{50}=123 μM). The ratio between the TC_{50} and the IC_{50} also calculated to be $5x10^3$. *In vivo* experiments confirmed the activity of compound 1 previously seen *in vitro*. Treating C57BL/6 IFNγR-KO mice with 40mg/kg of compound 1 for ten days resulted in a complete absence of parasites in 75% of the cases and a significant increase in survival rate. Using real-time PCR, these successfully mice showed no detectable levels of parasitic DNA in their intestines 30 days post infection. These results were confirmed by the histological analysis of sections of the ileum which were absent of *C. parvum* oocysts. Non-infected mice treated with 40mg/kg were monitored for visible behavioral changes and weight loss as signs of drug toxicity. However, even after ten days of treatment, no signs of toxicity were seen in these mice. Overall, compound 1 demonstrated clear activity against *C. parvum* without exhibiting any signs of severe or permanent side effects. Therefore, compound 1 is a novel candidate for the treatment of cryptosporidiosis and should be considered for further evaluation.

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PREVALENCE OF *PLASMODIUM VIVAX* AND MALARIAE IN HIV-INFECTED PATIENTS IN BENIN CITY, EDO, NIGERIA

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Malaria and HIV are endemic to tropical and subtropical areas. Due to similar geographical distribution, co-infection of malaria and HIV can occur in individuals. Previous research has shown that individuals infected with malaria and HIV who are taking anti-retroviral and anti-malarial drugs still have the *Plasmodium* parasite in their bloodstream. Malaria is caused by the protozoan parasite *Plasmodium* and transmitted by the *Anopheles* mosquito. There are five known species of *Plasmodium* that infect humans*: falciparum, ovale, vivax, malariae* and *knowlesi*. HIV is a viral infection transmitted sexually or parentally. It compromises the immune system. Blood samples have been collected from patients co-infected with malaria and HIV native from the Edo district of Nigeria in Benin City. Our collaborator has shown a prevalence of 28.7% of *P. falciparum* infections in this study group. We will focus on *P. vivax* and *P. malariae*. The results from our studies will establish the prevalence of *P. vivax* in HIV patients and will pave the way to determining whether antiretroviral drugs interfere with antimalarial drugs.

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ZOONOTIC AND HUMAN RELATIONSHIPS OF LYME DISEASE IN VIRGINIA

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Exposure to the *Borrelia* bacterium during Lyme Disease (LD) possibly causes a long-lived and damaging inflammatory response, a form of *Borrelia burgdorferi* - induced autoimmune disease. Lyme disease manifests itself as a multisystem inflammatory disease that affects the skin in its early localized stage, and then spreads to the joints, nervous system, heart and other organ systems. Chronic symptoms may develop from an autoimmune reaction, explaining why some symptoms persist even after spirochetes elimination. This response may be due to a form of molecular mimicry, where *Borrelia* avoids being killed by the immune system by resembling normal body tissues. Most of the mortality associated with patients infected with LD may be the result neurologic and rheumatic complications caused by the co-infection with other tick-borne pathogens such as *Babesia microti* and several species of *Ehrlichia*. This case involves a 50-year-old woman from Pulaski, Virginia who presented with headaches, fever, generalized joint pain, excessive thirst and fluid intake and a progressing rash on her back. She recalled a walk in the woods three weeks prior but denied specifically finding a tick on her body. Three days before her visit to the clinic (Day 1) the patient noticed an initial small raised lesion resembling a tick or spider bite. On Day 2, the patient's fever reached 39.3°C with increased burning sensation at the site of the tick bite. On admission, her temperature was 40.2°C. On physical examination, she had an overly large circular red rash on her back with bull's eye appearance, 16 cm x 18 cm in diameter. By Day 4, the patient felt worse with 10/10 joint pain, high fever and excessive thirst and fluid intake. The lesion on her back progressed in size and intensity. Clinically, the lesion resembled an autoimmune hyper-response representative of LD. Serologic tests confirmed a diagnosis of Lyme disease. In Virginia LD is spread by the bite of vector Ixodid ticks and are responsible for transmitting the spirochetes from mammals to humans. Ixodid ticks normally feeds on the white-footed mouse, the white-tailed deer, and certain other mammals. Nymph stages are responsible for 90% of human disease and are rarely noticed because of their small size (< 2 mm). The incidence of LD in Virginia has increased over fourfold, from 1995 to 2011. This increase is the result of multiple factors, especially increased diagnosis and reporting by clinicians. Other factors include the progressive geographic spread of zoonotic hosts, especially rodents and white-tailed deer, and the geographic spread and increased activity of the Ixodid ticks southward and westward from northern Virginia. This increase coincides with the overall expansion of LD throughout the eastern United States. From 1995 to 2011 the incidence of LD cases/100,000 In Virginia has increased fourfold. This increase is the result of increased diagnosis and reporting by clinicians. In the early 1990s, most of the cases of LD were from counties along the Eastern shore. Presently, the areas of high incidence now include counties to the south and west of Northern Virginia. During 2011 there were 1,023 confirmed or probable cases of LD officially reported in Virginia from 59 of the 95 counties.

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EGG SHAPE DIVERSITY IN ACANTHOCEPHALANS: EFFECTS OF HABITAT TYPE

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Acanthocephalan parasites are distributed worldwide and occur in both diverse habitats and hosts. Associated with this diversity, there is considerable variation in morphology of both larvae and adult acanthocephalans. However, little is known about the variation in morphology of eggs. We have examined the relationship between egg morphology (egg shape) and habitat type (terrestrial, aquatic) to determine whether habitat type could have influenced the evolution of egg morphology. To examine this relationship we focused on members of the genus *Acanthocephalus* and compared egg shape between habitat types for

16 different species. We found that egg shape differed between habitat types. Eggs that are dispersed in aquatic habitats have a smaller width to length ratio than eggs that are dispersed in terrestrial habitats. We will discuss the potential relevance of this variation to acanthocephalan dispersal and transmission.

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TREMATODE COMMUNITIES IN FRESHWATER SNAILS FROM THE RUHR AREA IN GERMANY WITH A FOCUS ON BIRD SCHISTOSOMES

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This study reports the results of an intensive sampling of multiple snail populations in a reservoir system in the Ruhr area in Germany. The aim of this study was the assessment of the composition and structure of trematode communities in the most abundant freshwater snail hosts with a focus on bird schistosomes causing swimmer's itch in humans. Snail populations were sampled in five reservoirs (Baldeneysee, Hengsteysee, Sorpe-, Verse- and Hennetalsperre) of the River Ruhr and examined for patent and pre-patent trematode infections in the laboratory. A total of 3,599 planorbid and lymnaeid snails were examined between May and September 2012: 1,106 *Gyraulus albus*, 142 *Segmentina nitida* (Planorbidae), 128 *Lymnaea stagnalis*, 1,339 *Radix auricularia*, 310 *Radix peregra* and 574 *Stagnicola palustris* (Lymnaeidae). A total of 31 trematodes was identified, 21 to the species level, 8 to the generic and a further 2 to the familial level. Component community richness and overall prevalence varied among snail species: *G. albus* (1-5 spp., 14.3 %), *S. nitida* (1 sp., 1.4 %), *L. stagnalis* (1 sp., 13.3 %), *R. auricularia* (1-14 spp., 26.6 %), *R. peregra* (1-4 spp. 6.1 %) and *S. palustris* (1-4 spp., 4.2 %). The most prevalent trematode species recorded were parasites completing their life-cycles in birds: *Australapatemon burti* in *G. albus*, *Diplostomum pseudospathaceum* in *L. stagnalis* and *S. palustris* and *Echinoparyphium recurvatum* in *R. auricularia* and *R. peregra*. Four bird schistosome species were found in three reservoirs: *Trichobilharzia franki* from *R. auricularia* (0.9 %), *Bilharziella polonica* from *G. albus* (0.27%) and *S. nitida* (0.7%), plus two schistosomatid species from *G. albus* (0.09 % each). Although bird schistosomes constitute only a fraction of the diverse trematode communities in the studied aquatic ecosystems, their infective stages, the cercariae, can still pose a considerable risk of swimmer's itch due to the high number of cercariae emitted from infected snails and the possibility of local infection hot spots.

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CHANGES IN A HIGH ELEVATION MOLLUSCAN-TREMATODE COMMUNITY OVER THE LAST 50 YEARS IN CRESTED BUTTE, CO

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Around the world, trematodiases such as fascioliasis, schistosomiasis, and cercarial dermatitis have seemed to be on the rise. Many studies indicate climate change as a major driver of these recent changes through direct (increases in growing season) and indirect (changes in bird migration) effects. Residential development and eutrophication can reduce community biodiversity as well as increase infection prevalence and intensity through increases in snail forage quantity and quality. There is a dearth of field studies conducted over suitable timescales (30+ years) to investigate the effects of anthropogenic drivers (such as climate change) on trematode diversity and prevalence. Cases of cercarial dermatitis caused by avian schistosomes were reported in swimmers at Grant Lake in Crested Butte, CO during the summers of

1956 and 1958. During the summers of 1957, 1958, and 1959 infection surveys were carried out in Grant Lake and several surrounding ponds documenting larval trematode morphotypes and prevalence. Between 1959 and 2010 cercarial dermatitis was reported sporadically from some of these ponds, with some years producing intense transmission. Ponds were resurveyed during the summers of 2010, 2011, and 2012 using the same methods used in 1957-1959. The goal of this study was to determine if trematode morphotype diversity and prevalence has changed over a 50+ year period from 1957-1959 to 2010-2012. Also, a focused assessment has been conducted to determine changes in the interannual species diversity of schistosomes at these ponds. Water from each pond was sampled for dissolved nutrients and snails. Snails were collected by hand and dissected and larval trematodes were identified to morphotype. All schistosome specimens were collected, preserved in 75% ethanol, and amplified and sequenced. Climate data were obtained from both the Rocky Mountain Biological Laboratory (RMBL) and the National Oceanic and Atmospheric Administration. From 1957-1959 to 2010-2012 snail diversity decreased at those sites closest to new residential developments in the town of Crested Butte while staying the same or increasing at sites within national forest and RMBL lands. Trematode morphotype diversity was more variable with some decreases close to residential areas and constant diversity values within national forest and RMBL lands. Documented schistosome species diversity was 4 times higher from 2010-2012 than 1957-1959. While trematodes were relatively insensitive to snow melt date from 1957-1959, prevalence values decreased drastically during 2011 in response to later snow melt dates and increased 5 times in 2011 in response to earlier snow melt dates. Climate has been changing in the Crested Butte area from 1957 to 2012 with an overall shift towards earlier snow melt dates. From 1957 to 2012 the difference between temperatures at lower elevations (data from Albuquerque, NM 1,490m) and higher elevations (data from Crested Butte, CO 2,708m) has been increasing (R^2=0.27, $p<0.05$). Arrival dates for some migrant birds who cue largely on temperature have been advancing in response (American Robin R^2=0.24, $p<0.05$; Fox Sparrow R^2=0.12, $p<0.05$). Earlier spring migrant arrival coupled with longer growing seasons and more frequent early melt dates may increase the risk of cercarial dermatitis at these ponds in the future.

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FIRST REPORT ON AVIAN SCHISTOSOMATIDS FROM BIRDS AND MOLLUSKS IN NORTH DAKOTA

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Avian schistosomatid digeneans inhabit the blood circulatory system of birds. Although adults are specific to birds, cercariae of several species may penetrate human skin and cause cercarial dermatitis also known as "swimmer's itch". Avian schistosomatids are known from birds belonging to several orders, but are most common and prevalent in water birds, particularly Anseriformes and some Charadriiformes. North Dakota is an important breeding region for a large number of water birds. Several areas in the state, especially the prairie pothole region, serve as important stopover sites for migratory aquatic birds. To the best of our knowledge, no research has been done on avian schistosomatids in North Dakota in either bird definitive hosts or intermediate mollusk hosts. During 2008-2012 we have examined a variety of birds in the eastern North Dakota for the presence of the blood flukes. We have also screened a large number of snails belonging to 6 species, from various types of water bodies for the presence of schistosomatid cercariae. Obtained adult specimens were studied morphologically as total mounts on slides. Sequences of ITS and 28S regions of nuclear ribosomal RNA and/or mitochondrial cox1 gene were obtained from all samples of adult worms and cercariae. Cercariae were identified by matching their DNA sequences with previously published and our own sequences of adult digeneans. As a result, schistosomatids belonging to the genera *Trichobilharzia*, *Gigantobilharzia*, *Dendritobilharzia* and *Austrobilharzia* have been found in several bird species in the region. *Austrobilharzia variglandis* has a marine life cycle, therefore its presence in a marbled godwit in landlocked North Dakota is obviously a result of infection during a seasonal migration. Besides avian schistosomatids, cercariae and sporocysts of a mammalian schistosomatid *Schistosomatium douthitti* were also found in snails. Diversity and prevalence of

schistosomatids in birds and snails are presented and discussed. This study was supported by the grant DEB 1021431 from the National Science Foundation.

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THE *GIARDIA INTESTINALIS* CELL CYCLE

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Giardia intestinalis is among the most commonly reported intestinal protozoa in the world with infections reported in humans and over 40 species of animals. The lifecycle of *Giardia* alternates between the motile trophozoite and the infectious cyst. The proliferation of *Giardia* trophozoites during an active infection, and the restriction point for the differentiation of trophozoite to cyst are dependent on the tight regulation of the cell cycle. The drugs aphidicolin and nocodazole have been successfully used in the cell cycle synchronization of *Giardia* trophozoites cultures, but these drugs are also associated with an increase in the level of phosphorylated histone H2Ax within the treated cells—which is an indicator of double-stranded DNA breaks. Hence, we developed counterflow centrifugal elutriation (CCE), a size based separation technique, to obtain fractions of *Giardia* cultures enriched in defined stages of the cell cycle, and determined that the cells in these fractions do not have an increase in phosphorylated histone H2AX compared to control cells. The RNA levels of 12 genes suspected to be involved in *Giardia* cell cycle progression were analyzed in the CCE fractions by using quantitative RT-PCR. Several of these genes, including the core histone and the mitotic cyclin genes, were similarly expressed in *Giardia* and in more recently evolved model eukaryotes, indicating that these genes may be part of the core cell cycle regulatory process. We also observed a small percentage of cells in *Giardia* trophozoites cultures that have a 16C DNA content, which is twice the DNA content of G2 cells. The 16C cells are induced by the addition of drugs that perturb the cell cycle and by nutrient deprivation. Our hypothesis is that the 16C cells represent a pre-encystation stage of *Giardia* that is induced by cellular stress. The number of nuclei and the distribution of DNA among the nuclei in these cells will be examined by fluorescent microscopy. We will also examine the expression of genes that are expected to be induced by stress or by encystation in these samples.

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IDENTIFICATION OF ANISAKIS SPECIES (NEMATODA: ANISAKIDAE) IN MARINE FISH HOSTS FROM PAPUA NEW GUINEA

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The third-stage larvae of several genera of anisakid nematodes are important etiological agents for zoonotic human anisakiasis. The present study investigated the prevalence of potentially zoonotic anisakid larvae in fish collected on the coastal shelves off Madang and Rabaul in Papua New Guinea (PNG) where fish represents a major component of the diet. Nematodes were found in seven fish species including *Decapterus macarellus*, *Gerres oblongus*, *Pinjalo lewisi*, *Pinjalo pinjalo*, *Selar crumenophthalmus*, *Scomberomorus maculatus* and *Thunnus albacares*. They were identified by both light and scanning electron microscopy as Anisakis Type I larvae. Sequencing and phylogenetic analysis of the ribosomal internal transcribed spacer (ITS) and the mitochondrial cytochrome C oxidase subunit II

(cox2) gene identified all nematodes as Anisakis typica. This study represents the first in-depth characterisation of Anisakis larvae from seven new fish hosts in PNG. The overall prevalence of larvae was low (7.6%) and no recognised zoonotic Anisakis species were identified, suggesting a very low threat of anisakiasis in PNG.

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THE ROLE OF AN ARGONAUTE-LIKE PIWI PROTEIN HOMOLOG IN *LEISHMANIA*

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Argonaute proteins are evolutionarily conserved and can be phylogenetically classified into the AGO and the PIWI subfamily proteins. Argonautes function in RNA interference and micro-RNA pathways, whereas PIWIs bind to PIWI-interacting RNAs and regulate germ line development, stem cell maintenance, epigenetic regulation, and transposition. The Old World *Leishmania* species such as *L. infantum* and *L. major* lack a functional RNAi pathway. However, their genome codes for an Argonaute-like protein with a PIWI domain lacking the PAZ domain found in Argonautes from RNAi proficient organisms. Using sub-cellular fractionation and confocal fluorescence microscopy, we showed that unlike other eukaryotes, the PIWI-like protein resides mainly in the single mitochondrion in *Leishmania*. To address the role of this protein, we have generated a PIWI (-/-) mutant by double-targeted gene replacement both in *L. infantum* and *L. major*. Depletion of PIWI has no effect on the viability of insect promastigote forms but leads to an important growth defect of the mammalian amastigote lifestage in vitro and significantly delays disease pathology in mice, consistent with a higher expression of the *PIWI* transcript in amastigotes. RNA–seq analysis revealed that loss of *Lin*PIWI in *L. infantum* amastigotes affects mostly the expression of specific subsets of developmentally regulated genes and mitochondrial proteins. Several transcripts encoding surface and membrane-bound proteins, RNA binding proteins, RNA helicases and nucleases were found downregulated in the *Lin*PIWI (-/-) mutant supporting the possibility that PIWI plays a direct or indirect role in the stability of these transcripts. Although our data suggest that PIWI is not involved in the biogenesis or the stability of small noncoding RNAs, additional studies are underway to assess whether PIWI gene inactivation affects RNA regulation and amastigote development.

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IN SITU EXPRESSION PATTERNS OF *BRUGIA MALAYI* CYS-LOOP LIGAND-GATED ION CHANNEL GENES CONFIRM A ROLE IN REPRODUCTION

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The largest selling group of anthelmintic drugs is the macrocyclic lactones (MLs) and the nicotinic agonists; both target members of the cys-loop ligand-gated ion channel (CLGIC) gene family. The MLs and nicotinic agonists, which include drugs such as ivermectin (IVM) acting on glutamate chloride-gated channels (GluCls) and levamisole acting on nicotinic acetycholine receptors (nAChR), are effective for the treatment of filarial infections such as *Onchocerca volvulus* due to their antimicrofilarial activity. Effects on fecundity have also been observed in other species, implying that the GluCl and nAChR may have a role in reproduction, but the nature of that role is poorly understood. The distribution of the known GluCl subunits *in H. contortus* and *C. elegans* seems to correlate well with the observed actions of these drugs; however, the effects of the anthelmintics on fecundity are unexplained, because there is no direct evidence for any involvement of the GluCls and /or nAChR in the reproductive system. Previously, we have found

that gene expression patterns correlate well with their biological function. We have investigated mRNA expression patterns of 7 *B. malayi* CLGIC genes including genes from GluCls and nAChR gene family by *in situ* hybridization. Majority of genes were strongly expressed in the early developing embryos such as morula and folded-pretzel stages in uterus and spermatogonia in testes, weakly expressed in later stage of embryos and not expressed in stretched microfilaria and sperm. Interestingly, most of these genes were strongly expressed in the wall of the uterus and vas deferens with stretched microfilaria or sperms, respectively; some of them were expressed in the body wall of female or male worms as well. The unique expression patterns of genes encoding for IVM sensitive channel subunits (AVR-14A and -14B) correlate the observed effects of IVM on embryogenesis in filarial nematodes after repeated IVM treatments**.** The strong presences of CLGLC genes in developing embryos and the walls of the uterus and/or vas deferens with mature offspring confirm a role of these genes in reproduction of microfilaris in *B. malayi*. The findings provide additional insights on the mechanism of antifilarial actions of MLs in vivo and that could be valuable for drug and vaccine development**.** The gene expression patterns we have observed suggest that these receptors are essential for embryo development and microfilaria production. These results may help to explain effects of certain anthelmintics on fecundity.

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MOLECULAR DISCRIMINATION OF *ECHINOCOCCUS GRANULOSUS* AND *ECHINOCOCCUS MULTILOCULARIS* BY SEQUENCING AND A NEW PCR - RFLP METHOD WITH THE POTENTIAL USE FOR OTHER *ECHINOCOCCUS* SPECIES

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In this study, we aimed that develope a PCR-RFLP protocol using a new genomic marker sequence and a novel set of restriction enzymes in order to detect and discriminate two Echinococcus species, *E. granulosus* and *E. multilocularis*, found in FFPE human tissues. Eleven formalin fixed paraffin embedded human tissue samples positive for cystic Echinococcus or alveolar Echinococcus were used for DNA isolation. A novel primer pair was designed to amplify and to sequence a genomic marker region for the discrimination of *E. multilocularis* and *E. granulosus*, additionally, a new PCR-RFLP protocol was developed for the detection and discrimination of *E. granulosus* and *E. multilocularis* using of a novel set of restriction enzymes including *AccI*, *MboI*, *MboII*, and *TsoI***.:** A pair of novel primers was designed to amplify a selected mitochondrial genomic region of both *E. granulosus* (9171-10042 bp) and *E. multilocularis* (11585-12461 bp). The PCR fragment was digested by *MboI* (788 bp and 89 bp), *MboII* (690 bp and 187 bp), *AccI* (660 bp and 217 bp), *TsoI* (digestion didin't result) for *E. multilocularis*. The PCR fragment was digested by *MboI, MboII*, *AccI* and the digestion didn't result any digested DNA fragments excepted to *TsoI* (362 bp and 510 bp) for *E. granulosus.* In this PCR-RFLP protocol, use of any single restriction enzyme is enough for the discrimination of *E. granulosus* and *E. multilocularis.* Additionally, the new PCR-RFLP protocol may be used potentially for the detection and discrimination of other *Echinococcus* species.

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DEVELOPING OF ELISA BASED ON RECOMBINANT CATHEPSIN L1 PROTEIN FOR DIAGNOSIS OF *FASCIOLA HEPATICA*

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In this study, we aimed to produce cathepsin L protein which has an important act in *Fasciola hepatica* pathogenesis using recombinant DNA technology and to develop an ELISA kit for diagnostic purpose. We utilized recombinant DNA technology to obtain cathepsin L protein from adult stages of *Fasciola hepatica* and prepared in-house ELISA test from that purified protein in our laboratory. By using commercial kits for routine testing in our laboratory we evaluated 4 patient's sera as 2 positives and 2 negatives and the results was compared with the results obtained using in house ELISA kits. Total RNA isolation from adult stages of *F. hepatica* was performed, cDNA was produced by reverse transcription and the target gene region of 981 bp length was amplified using Cathepsin L1 gene specific primers. Then cathepsin L1 gene was cloned into pET TOPO vector which was transformed to competent *E. coli* cells and plasmid DNA was obtained using miniprep method. Presence of Cathepsin L1 gene was confirmed by DNA sequence analysis. Recombinant protein was expressed by transforming plasmid DNA into BL21 *E. coli* cells and this recombinant protein was purified. *An* in-house ELISA kit was designed utilizing the purified recombinant protein. Results obtained from the commercial ELISA kit and *a*n in-house ELISA kit was compared. The results were found to be compatible. Protein cathepsin L was obtained by recombinant DNA technology. In this study, we demonstrated that this protein could be utilized in an ELISA kit for the diagnostic purpose in a serological test. The experiment needs to be repeated with a higher number of samples.

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FURUNCULAR MYIASIS OF THE FOOT CAUSED BY THE TUMBY FLY, *CORDYLOBIA ANTHROPOPHAGA*

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Most cases of cutaneous myiasis are acquired when traveling to tropical areas of Africa, Central America or South America. Symptoms of cutaneous myiasis are not specific and are often confused with other diseases causing cutaneous lesions. Furuncular myiasis in humans presents as a small raised erythematous bite-like or boil-like lesion that progress to an enlarging pruritic tender nodule from which there can be both a sensation of movement and a lancinating pain. This case report involves a 26-year-old male medical student who visited Ntagatcha Tanzania on a medical mission trip. Three weeks following his return to the United States he develops a furuncular lesion on the side of the 5th digit on his right foot. Following his return to the United States he noticed a scab developing on the side of his little toe on the right foot. The student dismissed the scab as a simple corn or callus which he thought was a result of his close fitting shoes and soccer cleats. The student later reported pain with activity and the scab became raised throughout the month. The student decided to remove the top layer of the scab and instead an entire plug of dead flesh came neatly out of the side of his foot leaving a well circumscribed crater. There was neither bleeding nor pus in the crater but there was what seemed like pus at the bottom layer of the plug. The different layers of healing and destruction could be seen on the plug as the layers got deeper. On closer inspection of the plug the student noticed that in the lower pus-like layer there seemed to be something moving. The layer was moving rhythmically as if breathing. Suspecting a larva, he mechanically removed the maggot and placed the plug in 70% alcohol to preserve it. Two days later he took the specimen to Edward Via College of Osteopathic Medicine infectious diseases laboratory where the formal identification was made. On examination, the larva was about 12 mm in length, yellow-brown with two longitudinal stripes on the thorax, and transverse black bands on the abdomen. The oral area contained a pair of toothed spade-like hooklets. There were fleshy processes proceeding from the eighth abdominal segment posteriorly. The posterior segment contained the characteristic spiracles which lacked a chitinous rim. The size and morphology of the larva is consistent with those reported for the Tumbu fly, *Cordylobia anthropophaga*. The public health aspects of furuncular myiasis caused by *Cordylobia anthropophaga* involves the use of simple measures such as washing and thoroughly drying clothes and by improvements in personal sanitation and hygiene and the use of insecticides to eliminate the flies from living and work areas. With the increase in travel to areas where furuncular myiasis is endemic, it is important that returning travelers become familiarized with the clinical presentation and treatment of

furuncular myiasis especially if being treated by physicians in non-tropical countries. Ultimately, this may avoid a delay in diagnosis, misdiagnosis or being treated unnecessarily with antibiotics.

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DETERMINING THE BURDEN OF INTESTINAL PARASITES IN PATIENTS WITH GASTROINTESTINAL SYMPTOMS IN VERóN, THE DOMINICAN REPUBLIC

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Intestinal parasites including *Giardia* sp., *Trichuris* sp., and *Ascaris* sp. are estimated to be prevalent in the Dominican Republic (DR), however, there are few studies to determine which parasitic infections are present and to what extent they affect the population. The purpose of this study is to determine the prevalence of several intestinal parasites in patients that present to Clínica Rural de Verón in the DR with gastrointestinal (GI) symptoms. Another goal of this study is to determine the utility of lactoferrin detection in evaluating the disease burden associated with intestinal parasitic infections. Determining the prevalence and disease burden of intestinal protozoa in the DR helps better guide physicians in their care of patients in this specific population. This study was conducted at Clínica Rural de Verón from November 2012 to December 2012. We first determined the number of patients presenting with GI symptoms that had a positive test for a parasitic infection. Fecal samples were tested for the presence of lactoferrin. Eighty nine participants were selected after presenting to the clinic with symptoms related to the GI system such as diarrhea, nausea, vomiting, constipation or general abdominal pain. Fecal samples from these patients were examined microscopically for the presence of parasite ova after preparation using a flotation technique. A commercial kit was used to detect *Giardia* sp. or *Cryptosporidium* sp. cysts. Another commercial kit was used to test for the presence of lactoferrin. Survey analysis was used to associate symptoms to our findings. Ova were identified in 8/89 (8.9%) samples consisting of *Blastocystis* sp. (4/8), *Trichuris* sp. (3/8), *Ascaris* sp. (2/8), and *Hymenophilus* sp. (1/8). Diarrhea was the most common symptom associated with patients having a positive microscopy result. Pain, vomiting, fever and constipation were also reported among these patients. 6/89 (6.7%) of the samples that had a positive result for *Giardia* sp. and 1/89 (1.1%) tested positive for *Cryptosporidium* sp. using the commercial test kit. 45/89 (50.5%) tested positive for lactoferrin. Diarrhea (18/45) was the most common symptom associated with the presence of lactoferrin. Positive lactoferrin results were also associated with *Giardia* sp., *Trichurius* sp., *Ascaris* sp., *Blastocystis* sp., and *Hymenophilus* sp. Our results demonstrate that several different parasites are present in this population which needs to be considered when choosing treatment methods for patients that have symptoms related to the GI system. Lactoferrin was associated with invasive and non-invasive infections. Further studies are needed with increased diagnostic testing ability in order to detect other infections such as *Entamoeba histolytica*. These findings help us gain a better understanding of which organisms are present in this population and will guide us in future research.

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GIARDIASIS REVISITED: GLOBAL SOCIOECONOMIC IMPACT AS A REEMERGING ZOONOTIC DISEASE

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Presently, the world population exceeds 7 billion. Globally, municipalities are more crowded than ever and basic human needs, such as nutritious healthy foods and clean drinking water, are becoming less attainable to many people. With the increase in globalization and the breakdown of international barriers

through travel and trade, there is an increase in many of the common parasitic diseases. Many of those diseases are reemerging, often in zoonotic contexts, and are often underdiagnosed or misdiagnosed by healthcare providers who lack sufficient knowledge to include parasitic infections in their differential diagnosis. Both the Centers for Disease Control and Prevention and the World Health Organization report the prevalence of giardiasis to range from 2-7% in developed countries and upwards of 30% in developing countries. In the United States giardiasis has been recognized as one of the most common waterborne or foodborne parasitic disease in humans and in companion animals such as dogs, cats, rabbits, and horses. Cases are most likely undiagnosed or misdiagnosed because of a lack of access to proper healthcare in rural or underserved areas or due to inadequately trained healthcare providers. The epidemiology of giardiasis has changed dramatically over the past decade due to the presence of different *Giardia* species, strains, and genotypes and their wide and variable host ranges. Understanding the epidemiology of giardiasis is particularly important in determining the zoonotic potential of infected domestic animals and in determining the human disease burden. There are many synonyms for *G duodenalis* in the literature. The taxonomy of *Giardia* is complicated with seven known species sub divided into eight assemblages. The species include *G. intestinalis, G. lamblia, Cercomonas intestinalis, Lamblia intestinalis*, and *Megastoma enterica*. *G. duodenalis* of nonhuman host origin can become infectious to humans. Many common farm animals, such as cattle, pigs, goats, and sheep, can be infected with *G duodenalis*. In addition, many wild animals, including gorillas, reindeer, dolphins, coyotes, harbor seals, and herring gulls, can be infected, making giardiasis one of the most important zoonotic diseases. *G duodenalis* is divided into 8 assemblages, suggesting that *G duodenalis* may represent a multispecies complex. The assemblages are based on phylogenetic analyses of nucleotide sequence of the small-subunit rRNA. The 8 assemblages include the following: assemblage A (humans, nonhuman primates, and many mammals); assemblage B (humans, nonhuman primates, canines, and cattle); assemblages C and D (canines); assemblage E (domestic ruminants and pigs); assemblage F (cats); assemblage G (rodents); and assemblage H (seals). Giardiasis can be difficult to diagnose, and for this reason, fecal immunoassays should be used for identifying the organism in conjunction with more classical techniques when possible. The use of molecular diagnostic tools has significantly changed our present understanding of the epidemiology of giardiasis including its zoonotic impact and global distribution.

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ORAL MYIASIS ASSOCIATED WITH TERTIARY SYPHILIS-INDUCED BRAIN STEM HEMORRHAGE

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Myiasis is caused by fly larvae parasitism of living or necrotic human or other vertebrate tissue. Oral myiasis is a rare pathological occurrence in humans that is associated with poor oral hygiene. It is most commonly caused by exposure of the oral cavity to the external environment for a prolonged period of time. Individuals are usually mentally or physically incapacitated during infestation. Syphilis is a sexually transmitted disease caused by *Treponema pallidum* infection. Tertiary syphilis may present as gummatous syphilis, cardiovascular syphilis, meningovascular syphilis, general paresis, or *tabes dorsalis*. Here, we report the case of oral myiasis caused by *Musca sorbens* in a 64-year-old man with tertiary syphilis-induced brain stem hemorrhage and review the current literature on this infestation in humans. The patient was successfully treated with a large, long-term dose of intravenous penicillin G to treat his syphilis, along with supportive therapy for the brain stem hemorrhage, mechanical removal of the larvae, and systemic treatment with ivermectin for the myiasis. The present case and its consequences demonstrate that educational measures, basic sanitation, and medical care close to home should be

implemented to improve quality of life, particularly in developing countries. Unfortunately, in developing countries, many people live in low socioeconomic conditions, predisposing them to the occurrence of insect infestations. Further, oral hygiene is important for coma patients, as these types of patients are at high risk for myiasis. Medical personnel caring for elderly, debilitated, and/or shock patients should be aware of the possibility of myiasis development, and to be able to make a prompt diagnosis and implement relevant interventions to prevent extensive tissue destruction.

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GALECTIN-3 FACILITATES NEUTROPHIL RECRUITMENT AS AN INNATE IMMUNE RESPONSE TO A PARASITIC PROTOZOA *LEISHMANIA MAJOR* CUTANEOUS INFECTION

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When infection occurs, neutrophils rapidly migrate to the affected site. While the neutrophils neutralize microorganisms, they can also cause tissue damage or render invasion pathways to pathogens. Thus, the migration could be either beneficial or unfavorable in the initial control of infection. Studies on neutrophil recruitment revealed its complexity, especially in terms of the regulation of its initiation. Galectin-3 (*Gal-3*) is a member of the host galectin family that has an affinity for β-galactoside containing glycoconjugates. In this study, we investigated the role of *Gal-3* in neutrophil migration and the biological significance of the rapid migration of neutrophils in an experimental parasitic cutaneous infection with *Leishmania major*. When the substrain of *L. major*, LV39, was used to infect mice, lack of *Gal-3* (C57BL/6 background) impaired neutrophil recruitment in the footpads and the draining lymph nodes one day following infection. Reduced number of recruited neutrophils inversely correlated with local parasite burden. A significant difference in the lesions was also observed between *Gal-3*KO and wild type (WT) mice with *Gal*-3KO mice developing larger aggravated lesions. In contrast, neutrophil migration, induced by the other *L. major* substrain, Friedlin, was unaffected and the initial parasite burden remained similar in *Gal-3*KO mice as compared to WT mice. Similar to neutrophil migration and parasite burden, there was no significant difference between *Gal-3*KO and WT mice in *L. major* Fredelin-induced leishmaniasis. In addition, temporal depletion of neutrophils (by anti-Ly6G antibody) increased the initial *Leishmania* burden at the footpads. These data suggest the significant role of early neutrophil migration in the initial control of parasitic load at the entry port. Infection with *L. major* substrain LV39, but not Friedlin, induced higher levels of extracellular release of *Gal-3*. Further, *Gal-3* alone was able to initiate neutrophil migration *in vivo* even though *Gal-3* is not a chemoattractant for neutrophils. Thus, our data suggest that once extracellularly released, *Gal-3* can act as a DAMP, damage-associated molecular pattern, which could signal the presence of infection to innate immune system to facilitate early neutrophil migration, which is beneficial in the initial control of the *Leishmania* infection.

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IDENTIFICATION OF EXCRETED-SECRETED PROTEINS OF FILARIAL NEMATODES AS NEW DIAGNOSTIC REAGENTS

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Effective diagnostic tests for filarial parasite infections are currently suboptimal. Identification of major excreted-secreted (ES) proteins of filarial nematodes as potential diagnostic reagents is important for the development of new methods to determine level of infection in the host, especially for human filariae. *Dirofilaria immitis*, the canine heartworm, is a widespread and important veterinary pathogen. An analysis of the adult *D. immitis* secretome *in vitro* is available (Geary et al., 2012). This investigation seeks to identify *D. immitis* ES proteins found *in vivo* to validate the *in vitro* secretome and to investigate them as potential diagnostic reagents. *D. immitis* adults collected from infected dogs were cultured for 72 hr in medium that was changed at 24 hour intervals. Proteins were isolated from the collected media by standard methods. These proteins were then passed through a Protein-A column containing purified IgG antibodies from heartworm-infected dogs. Following extensive washing, heartworm proteins that were recognized by the antibodies were eluted from these columns and submitted for analysis by liquid chromatography–mass spectrometry. The goal is to identify the most abundant ES proteins present in the serum of infected hosts that offer a rational approach to the development of new diagnostic assays that may be applicable across the Filaroidia.

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IMPACT OF *LEISHMANIA*-NEUTROPHIL-INTERACTIONS ON MACROPHAGE SIGNALING

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Leishmania parasites belong to the genus of Trypanosomatid protozoa and are the causative agents of Leishmaniasis – a vector-borne disease, which afflicts more than 12 million people in more than 80 countries. Within their vertebrate hosts the parasites prefer to reside in long-lived macrophages, subverting their signaling and secretory profile to their own benefit. Nevertheless, in recent years it has been indicated that a preceding *Leishmania* infection of neutrophils is of importance. Although models, which aim at the description of the role of neutrophil infection by *Leishmania* exist, it is still an area of ongoing research. In this project we focused on the analysis and characterization of a possible myeloid-related-proteins (MRPs)-mediated effect on *Leishmania* infection. MRPs, which are members of the S100 alarmins, form up to 40% of the cytosolic protein of neutrophils and have been shown to be rapidly released due to the actions of pathogens. Data indicates putative roles for this group of proteins in chemotaxis as well as in inflammatory processes. Moreover, MRPs are thought to bind to *Leishmania* in skin lesions. Here we confirm that this is true for recombinant MRP-14 protein and *L. major* promastigotes. Experiments also suggest that MRPs of murine neutrophils behave similarly to a certain extent. In addition using luciferase reporter gene assays, our data indicates that MRP-14 binding does not affect promastigote internalization and parasite elimination *in vitro*. To further characterize the consequences of MRP-14 binding to *Leishmania* we examined parasite-dependent signaling changes in murine macrophages (B10R cells). Hereby we focused on the functionality of cellular phosphorylation events. Thus, we examined global serine and tyrosine phosphorylation as well as alterations mediated by the pathogenicity factor gp63, especially with respect to phosphatases (SHP-1, PTP1B) and NF-κB signaling. In addition, we began to study the consequences of *Leishmania*-MRP-interaction in an *in vivo* context using the air pouch model for *Leishmania* infections. Furthermore, we started to address the question of whether contact of parasites and neutrophils leads to the binding of other cellular proteins. Therefore, we started to identify candidates using a mass-spectrometry approach. In summary there is still no final conclusion on how the contact of neutrophils, a key player in the front line defense against pathogens, affects the *Leishmania* parasite. However, we present evidence that neutrophil protein can attach to *Leishmania*. In addition, our data indicates that myeloid-related proteins can bind to parasites without altering gp63-mediated host-parasite interactions.

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DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST *SCHISTOSOMA MANSONI* INFECTION USING CATHEPSIN B AND PEROXIREDOXIN 1 ANTIGENS

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Schistosomiasis is a fresh-water-borne parasitic disease caused by trematode worms of the genus *Schistosoma*. Due to its morbidity and mortality, schistosomiasis is the most important helminth infection. The pathology of the disease is due to egg deposition, by the female worm, which will trigger an immune reaction and consequently cause progressive damage to the organs. The lack of therapeutic drugs and preventative measures, as well as the high disease burden caused by the infection are justifications for developing a vaccine against schistosomiasis. The development of a recombinant protein vaccine against this parasitic disease has the potential to contribute a long-lasting decrease in disease spectrum and transmission. Furthermore, it would relieve some of the concern surrounding the potential emerging resistance to praziquantel; the drug which is solely being used to treat the infection. Our group has chosen to focus on the *Schistosoma mansoni* antigens Cathepsin B and Peroxiredoxin 1 (Prx1) as vaccine candidates. Our research goals are to determine the safety, immunogenicity, and protective potential of these candidate *S. mansoni* antigens. Upon cloning, expressing, and purifying the proteins of interest, mice were firstly immunized with recombinant Cathepsin B or Prx1 in the presence of either synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides or Montanide ISA 720 VG. The mice received two booster injections following the first immunization. The vaccine formulations were not toxic, and all of the mice survived until the end of the study (9 weeks). The vaccine elicited pronounced production of *S. mansoni* Cathepsin B and Prx1 specific antibodies whereas no antigen-specific antibodies were found in the control animals. Using the Montanide adjuvant generated robust antibody titers in mice immunized with Cathepsin B and Prx1 (endpoint titers = 204,800 and endpoint titers = 89,600 respectively). Moreover, levels of the different IgG isotypes were analyzed. IgG1/IgG2c ratios, which are indicators of Th1 or Th2 bias responses, were calculated. Immunizations with CpG generated strong Th1 responses, whereas those with Montanide generated mixed Th1/Th2 responses. Splenocytes proliferated in response to both antigens and produced elevated levels of Th1, Th17, and inflammatory cytokines. However, greater increases in cytokine secretion were observed in the animals vaccinated with Cathepsin B. These results highlight the potential of *S. mansoni* Cathepsin B and Prx1 as vaccine candidates.

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ANALYSIS OF *ASCARIS SUUM* FLUID COMPARTMENTS USING A PROTEOMICS AND BIOINFORMATICS APPROACH

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Ascaris lumbricoides infects at least 10% of the world's population and is a public health issue in most low-to-middle income countries. Survival of this parasite in its host is thought to be mediated at least in part by materials exported to the host in secretions. Although very little is known about the composition of these secretions, defining their contents and functions could shed some light on host-parasite interactions. In this study, *Ascaris suum*, which infects pigs, was used as a model organism because its

genome is known and it is closely related to *A. lumbricoides*. Excretory/secretory products (ESP), uterine fluid (UF) and perienteric fluid (PE) were collected from *A. suum*. Proteins isolated from these compartments were subjected to LC-MS/MS and analyzed using bioinformatic tools. A comparative analysis between ESP and UF was then performed using relative abundance to determine dominant proteins followed by assignment of gene ontology (GO) terms to characterize them on the basis of predicted functions. We found that ESP *per se* has a different protein composition than PE or UF, which are similar to each other. We conclude from these data that proteins exported through the secretory apparatus have distinct patterns of biological function and that UF proteins are likely derived from PE.

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COMPUTATIONAL RECOGNITION OF CIS-REGULATORY ELEMENTS IN TRYPANOSOMATIDS

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In Trypanosomatids, unlike other eukaryotes, regulation of gene expression occurs mainly at the post-transcriptional level. In this process, *cis*- and *trans*-acting elements play important roles in post-transcriptional gene regulation by affecting mRNA maturation, stability and translation rate. *Cis*-acting elements are usually within the 3´-UTRs, however, systematic identification of these elements are in early stages. Most methods find short conserved sequences or structural patterns based on commonality in a set of related sequences, or conservation across species. In many instances, however, RNA binding proteins (RBPs) (i.e. the trans-acting elements) show preferences for binding to some specific transcripts (bound transcripts) while they do not bind to other transcripts containing the same short sequence motifs (unbound transcripts). In this study, our hypothesis is that special patterns near the conserved motifs facilitate the binding of RBPs. To test this hypothesis, we used a training set of 7 to 100-mers bound and unbound transcripts without considering the nucleotides in each position to fit structural patterns which we then assessed using a held-out test set. We then employed an iterative motif refinement procedure that reduces degeneracy a single base at a time. At each iteration, the motif with the largest mutual information based on its presence at bound transcripts and absence in unbound transcripts was selected. Application of this novel approach to the genome sequence of *T. brucei* will be presented and show how it can predict gene regulatory networks in this organism.

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INSIGHTS INTO THE ARCHITECTURE AND PROTEIN INTERACTION NETWORK OF RNA EDITING ASSOCIATED COMPLEXES IN *TRYPANOSOMA BRUCEI*

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More than 60 proteins co-purify with mitochondrial RNA processing enzymes that include editosomes, the multi-protein complexes responsible for catalyzing mitochondrial RNA editing in *Trypanosoma brucei*. However, the exact roles and organization of these complexes have yet to be clarified. We hypothesized that characterization of the interaction network of proteins will lead to understanding the essential process of RNA editing in trypanosomatid pathogens and regulatory networks that are connected to editosome and modulate RNA editing and/or processes that depend on RNA editing. Based on available microarray data, our own microarray and high-throughput MS/MS experiments, we reconstructed a high confidence posttranscriptional regulatory network for *T. brucei*. One of the pathways that we were highly successful to predict was RNA editing; we were able to find several uncharacterized genes that may be involved in RNA editing process and/or mitochondrial RNA processing in *T. brucei* and contained accessory proteins with known distinct functions such as mitochondrial RNA binding protein

1and 2. To validate the role of some of predicted proteins in RNA editing process, we created transgenic *T. brucei* cells lines which express tagged proteins. We are currently using these cell lines to purify the tagged candidate proteins to identify the proteins they associate with. Moreover, we used RNAi mediated knockdown and found alterations in their target gRNA/pre-mRNAs. These predictions include assignment of uncharacterized genes to several essential biological processes and pathways that leads us to a better understanding of the biology of trypanosomatids, and also provides new potential targets for treatment of their respective diseases.

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IDENTIFICATION OF CANDIDATE SERUM BIOMARKERS FOR *S. MANSONI* INFECTED MICE USING MULTIPLE PROTEOMIC PLATFORMS

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Schistosomiasis is a public health problem that is being given more attention. In order to properly treat the patient; a quick, sensitive diagnostic test is needed to improve the detection of infection in early stages of the disease or when parasitaemic burden is low. The standard diagnostic test for schistosomiasis is microscopic detection of the eggs in stool or urine; however, this method becomes insufficient during the chronic stage of infection as well as when passage of eggs is low. Therefore, the diagnosis of chronic infection or low parasite burden might be undetectable. In order to identify biomarkers of early infection, twenty-six female, C57Bl/6, six- week old mice were used to study serum protein expression patterns. Four groups of mice (n=5) were infected with different numbers of *Schistosoma mansoni* cercariae (eg: 50, 100, 150 and 200) by tail penetration. A fifth group (n=6) was left unmanipulated and uninfected as a control group. Sera collected before infection and at 3, 6 and 12 weeks post infection were analyzed by a range of mass spectrometric (MS) approaches to identify candidate biomarkers. Using SELDI TOF MS to compare uninfected and schistosomiasis-infected samples, 88 candidate biomarkers were obtained. Used in various combinations, these biomarkers could 1) reliably diagnose early-stage disease, 2) distinguish between acute and chronic infection and 3) differentiate between animals with lower vs higher parasite burdens. The most important contributors to these diagnostic algorithms were peaks that were able to differentiate between control & early stage (before egg production (3 weeks)), and between acute (6 weeks) & chronic stage (12weeks) of the infection. Employing sample fractionation and differential gel electrophoresis, we analyzed gel slices either by MALDI-TOF MS or Velos Orbitrap MS. Ten differentially expressed host proteins were identified in the serum at various disease stages using MALDI TOF MS and ~200 parasite-origin proteins were identified during the acute stage using Velos Orbitap. The large number of parasite-origin proteins in acute serum was surprising, particularly the presence of proteins not associated with the parasite tegument (eg: , Thioredoxin peroxidase 3, Fatty acid binding protein). Orbitrap data suggested the presence of schistosome (25 kDa) GST which was confirmed by western blotting with a rabbit polyclonal antibody against schistosome GST. Our approach of using multiple proteomic platforms in parallel revealed that serum protein profiles differ extensively in infected and uninfected mice, offering an extensive repertoire of biomarkers.

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IDENTIFICATION AND INITIAL EXPRESSION STUDIES OF A MANNOSE BINDING LECTIN (MBL)-LIKE PROTEIN IN SUSCEPTIBLE AND RESISTANT STRAINS OF *BIOMPHALARIA GLABRATA* UPON CHALLENGE WITH *SCHISTOSOMA MANSONI*

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Mannose-binding lectins (MBLs) are key players in innate immunity. Soluble MBL is a pattern recognition receptor, able to bind carbohydrate moieties common to invading microorganisms (bacteria, viruses, and parasites). In some organisms MBL can also activate the lectin complement pathway, resulting in the triggering of immune effector functions such as phagocytosis and cell lysis. The snail *Biomphalaria glabrata* serves as an intermediary host for the bloodfluke *Schistosoma mansoni*, and although it is an essential part in the transmission cycle to humans, immunological interactions between the snail host and parasite are still not fully understood. These include possible genetic determinants for differences in parasite susceptibility. The present study focuses on the identification and analysis of MBL proteins in *B. glabrata* and their potential role in the detection mechanisms towards *S. mansoni*. Using primers designed for a related sequence in another mollusc (squid *Euprymna scolopes*), homogenates from *B. glabrata* headfoot region were tested for the presence of MBL transcripts. Preliminary results revealed a transcript sequence of 726 bp containing an open reading frame (ORF) of 242 amino acids (AA) with homology to known lectins. The translated ORF contains a 110-AA segment corresponding to a C-type carbohydrate-recognition domain (CRD) with putative mannose/galactose binding affinity. Atypical in other known MBL proteins, this *B. glabrata* lectin also contains a 45-AA segment corresponding to a thrombospondin type-1 repeat (TSP1) known to be present in various complement proteins (C6, C7, C8A, C8B, C9). Comparative analysis showed that this *B. glabrata* protein shared 79.5% amino acid identity to a previously identified *E. scolopes* MBL-like sequence (unpublished). Currently we are employing qRT-PCR methods to confirm expression of *B. glabrata*'s MBL-like gene in both susceptible (NMRI) and resistant strains (BS90) under control (non-infected) conditions and when challenged with *S. mansoni*. Expression and protein activity of pattern recognition receptors, such as lectins, may be one of the factors dictating *B. glabrata* susceptibility to *S. mansoni* infection, thus allowing the development of the parasite in the snail and potentially contributing to human disease.

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ATTENUATION OF CEREBRAL MALARIA PATHOLOGY BY PARENTERAL IRON OVERLOAD

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Malaria is a major cause of morbidity and mortality in the developing world, resulting in approximately 216 million cases and nearly one million deaths each year. Malaria is caused by parasites of the *Plasmodium* genus, primarily *P. falciparum* and *P. vivax*, with severe malaria being caused almost exclusively by *P. falciparum*. The manifestation of severe malaria depends upon age group and transmission intensity, and includes severe anemia, multi-organ failure and cerebral malaria. Cerebral malaria is an acute encephalopathy that involves pathophysiology of the blood-brain barrier and accounts for the majority of malaria-related deaths. The most commonly used model to study cerebral malaria involves infecting susceptible strains of mice with *P. berghei* ANKA, which then develop many of the same histopathological features observed in human cases. It has been previously shown that hepcidin levels are regulated during blood-stage malaria and that overexpression of hepcidin improved the outcome of *P. berghei ANKA* infection in mice. Hepcidin is a key regulator of iron homeostasis, and its expression is controlled by many factors, including erythropoiesis, inflammation and systemic iron levels. This project aims to examine the mechanistic relationship between body iron levels and cerebral malaria in detail. Our

data indicates that administration of iron dextran before and at early stages of infection with *P. berghei* ANKA prevented the development of cerebral malaria in 90% of C57Bl/6 mice (n=10). The iron-treated mice had similar levels of parasitemia compared to the control mice, suggesting that the protective phenotype observed was not due to an effect on parasite growth. Furthermore, the decreased cerebral malaria pathology, correlated with a significant reduction in blood-brain barrier disruption and parasite load, as measured by the presence of Evans blue and luminescence in the brain, respectively. We have started to examine the expression of proteins that have been shown to play essential roles in iron homeostasis and cerebral malaria pathology (e.g., BMP6, hepcidin, CXCL9 and CXCL10), and the recruitment of leukocytes and macrophages, in relation to this iron-mediated protection. In conclusion, our results suggest for the first time a protective role of iron dextran in the context of cerebral malaria, showing protection even when administered during early stages of the infection. Therefore, an in depth analysis of the underlying mechanisms will afford a better understanding of the possible ramifications of iron supplementation in malaria-endemic areas.

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MOLECULAR VARIABILITY OF *PERKINSUS MARINUS* IN OYSTERS *CRASSOSTREA VIRGINICA* FROM GULF OF MEXICO

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The protozoan *Perkinsus marinus* has been related to mortality of oysters *Crassostrea virginica* in the Gulf of Mexico. The infection parameters and transmission of protozoan parasite are associated with environmental conditions, pathogenic strains and the host genotype. Therefore our aim were determinate the prevalence, infection intensity and *P. marinus* strain from Gulf of Mexico. We collected from 75 to 300 oysters in different coastal lagoons from the Gulf of Mexico: Terminos, Campeche; Carmen-Machona, Tabasco; Mandinga, Veracruz and la Pesca, Tamaulipas. We determinate the intensity infection and weighted prevalence (WP) by Ray´s Fluid of Tioglicolate Medium (RFTM) and the prevalences and *P. marinus* strains by PCR. The sequences obtain were compared with databases from the GenBank™ and aligned with ClustalX (2.0.12). The divergent sequences were estimated using the Maximum Likelihood from the MEGA 5 software. We found moderate infection in the Carmen-Machona lagoon (4.7%) but in the rest of the lagoons the intensity infection and WP were light. The highest prevalence was in Carmen-Machona lagoon (72.4%) with decreased in Terminos (48.3%), Mandinga (46.7%), and la Pesca (34.1%). Additionally, we obtained 8 sequences which showed 96-100% of similitude with 4 sequences obtained from the GenBank™ that correspond to the NTS region of *P. marinus* (JN676160, EU617394.1, AF497479.1 and S78416.1). The sequences aligned showed variation in ~26 nucleotides (307±1pb). The highest similitude was detected between the sequence of Carmen-Machona lagoons, *P. marinus* sequence (EU617394.1) that infect to *Crassostrea corteziensis* from Nayarit and *P. marinus* sequence (JN676160) that infect to *Saccostrea palmula* from Sinaloa. Additionally, these 3 sequences are in the same clade of the phylogenetic tree. Our results, contribute to the knowledge of the spatial distribution and the presence of phylogeny strains of *P. marinus* in Mexican waters. Likewise, the presence of *P. marinus* in the Mexican Pacific Coast could be due to introduction of infected oysters from the Gulf of Mexico, particularity from the Carmen-Machona lagoons.

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LEISHMANOLYSIN-MEDIATED DEGRADATION OF SYNAPTOTAGMIN XI LEADS TO DEREGULATED CYTOKINE SECRETION

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Synaptotagmins (Syts) are type-I membrane proteins that regulate vesicle docking and fusion in processes such as exocytosis and phagocytosis. All Syts possess a single transmembrane domain, and two conserved tandem Ca^{2+}-binding C2 domains. However, Syt XI contains a conserved serine in its C2A domain that precludes this Syt from binding Ca^{2+} and phospholipids, and from mediating vesicle fusion. We recently discovered that Syt XI is a recycling endosome- and lysosome-associated protein that negatively regulates the secretion of tumour necrosis factor (TNF) and interleukin 6 (IL-6). Moreover, Syt XI controls the microbicidal activity of the phagosome by mediating the recruitment of phagosome maturation markers $gp91^{phox}$ and the lysosome-associated membrane protein 1. Leishmanolysin (GP63) is a major zinc metalloprotase that allows the parasite to alter multiple signaling pathways within macrophages. In infected macrophages, GP63 action ensues in defective transcription, translation, and antigen presentation. Here, we demonstrate that Syt XI is excluded from *Leishmania* parasitophorous vacuoles in a GP63- and lypophosphoglycan-dependent manner. Remarkably, we found that the *L. major* GP63 degrades Syt XI directly: recombinant Syt XI is degraded by live parasites and by parasite lysates. Chelation of zinc also abolishes Syt XI degradation. On the other hand, infected macrophages release TNF and IL-6 in a GP63-dependent manner. To demonstrate that cytokine release is dependent on GP63-mediated degradation of Syt XI, siRNA-mediated knockdown of Syt XI before infection revealed that the effects of siRNA knockdown and GP63 degradation were not cumulative. Altogether, our findings unravel a mechanism in which *L. major* induces the secretion of proinflammatory cytokines through Syt XI degradation. Enhanced knowledge of *Leishmania*-induced inflammation will further our understanding of how the parasite modulates the immune response to achieve reproductive success. *Supported by a grant from the CIHR.*

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THE EFFECTS OF TWO PARASITES ON SWIMMING PERFORMANCE IN THE SPOTTED SEATROUT (*CYNOSCION NEBULOSUS*)

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Parasites are often associated with detrimental impacts on host physiology, but very few studies have examined the impact of parasites on the swimming performance of fish. In this study, we aimed to determine the impacts of two parasite species, *Cardicola laruei* (Aporocotylidae) and *Kudoa inornata* (Myxosporea), on the swimming performance of spotted seatrout, *Cynoscion nebulosus*. We measured burst (anaerobic) and endurance (aerobic) swimming performance of 18 fish using a swimming flume. Many of the fish (72%) were infected with *C. laruei*, and a significant positive relationship was found between infection density and endurance swimming performance. All of the fish (100%) were infected with *K. inornata* and a significant positive relationship was found between infection density and burst swimming performance. These results suggest that these parasites are aiding, rather than impeding, swimming performance.

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RNA INTERFERENCE (RNAI) SCREEN OF PUTATIVE NEUROMUSCULAR RECEPTORS OF *SCHISTOSOMA MANSONI*

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Schistosoma mansoni are parasitic blood flukes that cause schistosomiasis, a helminthic disease of humans in many tropical and subtropical countries. Neuromuscular signaling in these parasites is mediated by a variety of neurotransmitters, both small molecules (“classical”) transmitters and neuropeptides. Biogenic amines (BA) constitute the largest subset of classical neurotransmitters and play

several key roles in the control of schistosome muscle function and movement. There are several putative BA receptors identified in the *S. mansoni* genome, the majority of which are Class A G Protein-Coupled receptors (GPCRs). Recently, we showed that one of these putative receptors is activated by serotonin, a biogenic amine that has strong motor and metabolic effects in schistosomes. To characterize the receptor further, we first determined the expression pattern of the *S.mansoni* serotonin receptor (Sm5HTR) along with other predicted BA receptors at the RNA level in different developmental stages of parasite using RT-qPCR. Next we performed the RNAi on (Sm5HTR) to temporarily silence the expression of receptor by transfecting the *S.mansoni* larvae with small interfering RNA (siRNA) and analyzed for effects on motor activity by comparing with the control groups. The results identified a hypoactive phenotype with significant reduction in movement. Studies are underway to analyze the effect of serotonin in the RNAi suppressed parasites and we are also screening other BA receptors for motor phenotypes.

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ANALYSIS OF *NAVICULA PELLICULOSA* GROWTH AND *NAVICULA PELLICULOSA*-FED *ONCOMELANIA HUPENSIS* SSP. SNAILS UNDER EXPERIMENTAL CONDITIONS

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The freshwater diatom *Navicula* (*Fistulifera*) *pelliculosa* is a ubiquitous alga with global distribution. It has been used in the culturing of freshwater *Oncomelania hupensis* ssp. snails. These snails are known for their capacity to serve as intermediate hosts for the larval stages of *Schistosoma japonicum*, an infectious parasitic disease of people living in tropical areas of the world. In order to more effectively maintain *Oncomelania* snails in the laboratory, we investigated different types of culture media to cultivate *Navicula pelliculosa*. The following media for growth were tested: filtered tap water, filtered mud-water, mud plates used for conventional growth, and a modified diatom growth medium of Cohn and Pickett-Heaps (1988) and Cohn et al. (2003). Cultures containing fixed volumes of media were inoculated with a specified number of diatoms suspended in solution. Diatom growth was quantified at 5 and 10 days post-inoculation. Mean diatom grown differed in the populations by day 5, $F(2,6)=44.9$, $p=0.00024$. Of the four media types tested, the diatom growth medium produced the greatest increase in the number of diatoms at day 5 (a 7-fold change), followed by the filtered mud-water (4-fold), and filtered tap water (3-fold). Data were unable to be recorded for diatoms cultured on mud plates because difficulties were encountered with isolating diatoms from the surrounding mud. Comparisons across groups found statistically significant differences between media and water groups ($p=0.0005$) and media and mud-water groups ($p=0.0072$). There was not a significant difference between growth in the water and mud-water groups ($p=0.070$). By day 10, all populations were in decline most likely due to limited resources and no statistically significant growth differences were detected. An additional experiment was conducted to test the effects of combining different media types with diatoms (conventional mud plate, filtered tap water, diatom growth media) on *Oncomelania hupensis hupensis* growth rates. Ten *Oncomelania* snails each ranging in size from 0.5-1.75 mm were placed into petri dishes containing equal amounts of diatoms. No media type adversely affected snail growth and preliminary studies did not detect a change in growth rates. Future studies will investigate fecundity and *S. japonicum*-infectivity rates of snails from the different groups as well as development of sporocysts and cercarial yield.

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IL-10 LIMITS CD8+ T CELL DURING EXPERIMENTAL *LEISMANIA DONOVANI* INFECTION

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Visceral Leishmaniasis is a disease caused by the protozoan parasites *Leishmania donovani* and

Leishmania infantum\chagasi. Experimental infection with *L. donovani* results in chronic infection in the spleen and in immune suppression. Thus far, the only cells that are known to be able to reduce the splenic parasite burden following immunotherapy are antigen-specific $CD8^+$ T-cells. Yet, *L. donovani* appears to be able to evade this defense by limiting the expansion and effector functions of parasite-specific $CD8^+$ T-cell responses. Since strong clonal expansion is needed to control infection, we are particularly interested in dissecting the mechanisms by which *L. donovani* interferes with the expansion of parasite-specific $CD8^+$ T-cells. In order to characterize antigen-specific CD8+ T cell responses during *L. donovani* infection, we designed an adoptive transfer system that employs OVA-transgenic parasites and TCR-transgenic OTI CD8 + T cells. Here, we show that *L. donovani* not only limits expansion of CD8+ T cells, but also induces mainly memory precursors rather than end-differentiated effector cells. Furthermore, blocking inhibitory molecules such as B7H1 and LAG3, or TGFβ blockade failed to increase expansion of antigen-specific CD8+ T cells. In contrast, IL10R blockade resulted in increased expansion and greater cytotoxic capacity of parasite-specific CD8+ T-cells.

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MACROPHAGE TRANSLATIONAL CONTROL BY *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE

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The Gram-negative bacterial endotoxin lipopolysaccharide (LPS) triggers important microbicidal and inflammatory responses in immune cells, including macrophages. However, these events can cause death by endotoxic shock. Macrophage activation by *Escherichia coli* (*E. coli*) LPS constitutes an excellent model to understand the molecular mechanisms underlying transcriptional regulation. LPS activates multiple signaling cascades that lead to the expression cytokine, chemokine and microbicidal genes. In addition to enhanced transcription, activation of mRNA translation contributes to a rapid cell response against pathogen invasion; however, the role of LPS in macrophage translational control remains largely unexplored. Previous studies indicated that LPS increases macrophage protein synthesis and activated the PI3K/mammalian target of rapamycin (mTORC1) pathway. Moreover, blockage of this signaling cascade inhibited nitric oxide (NO) production and interleukin-6 (IL-6) secretion in LPS-stimulated macrophages. Despite these findings, a direct link between mTOR activation by LPS and translational control of macrophage mRNAs had not been established. We found that LPS induces a stronger inflammatory response in macrophages deficient in eIF4E-binding proteins 1 and 2 (4E-BP1/2), the main repressors of translation initiation. Similarly, in absence of 4E-BP1/2, macrophages produced more NO and IL-12 than the wild-type (WT) cells following LPS treatment. To confirm and extend these data, we stimulated several cell lines of human and mouse macrophages as well as primary macrophages with *E. coli* LPS. An increase in global protein synthesis in LPS-stimulated versus control cells was detected by metabolic cell labelling with ^{35}S-Methione and measurement of radioactivity incorporation. Next, to determine whether modulation of macrophage protein synthesis by LPS occurred at the initiation step of translation, polysome profile analyses were performed in stimulated and control cells. We observed an increase in mRNAs associated with polysomes (i.e. actively translated mRNAs) in response to LPS. Given that translation initiation is largely dependent on the activation of the PI3K/mTORC1 pathway, we investigated its role in LPS-inducible macrophage translational activity. At first, macrophages were stimulated with LPS alone or in combination with specific inhibitors of the mTORC1 pathway, and changes in translation initiation were monitored by polysome profile analyses. As a complementary approach, we isolated macrophages from WT and knock-out (KO) mice in the main downstream targets of mTORC1 (S6 kinase 1 and 2, 4E-BP1/2). After WT and KO macrophage stimulation with LPS, samples were processed as described above. Our data indicate that LPS activates macrophage translation initiation through mTORC1-dependent and -independent pathways. We are currently identifying the macrophage mRNAs linked to the inflammatory and immune response whose translation is modulated by LPS. To this end, we will perform polysome profile assays of LPS-stimulated versus control macrophages and the fractionated mRNAs will be submitted to microarray analysis. The identified targets will be validated by RT-PCR and western blot. Altogether, our study will contribute to a better understanding of the molecular

mechanisms involved in macrophage activation by LPS and to establish the contribution of translational control to the inflammatory and microbicidal effects mediated by LPS.

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COMPUTATIONAL RECOGNITION OF CIS-REGULATORY ELEMENTS IN TRYPANOSOMATIDS

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In Trypanosomatids, unlike other eukaryotes, regulation of gene expression occurs mainly at the post-transcriptional level. In this process, *cis*- and *trans*-acting elements play important roles in post-transcriptional gene regulation by affecting mRNA maturation, stability and translation rate. *Cis*-acting elements are usually within the 3´-UTRs, however, systematic identification of these elements are in early stages. Most methods find short conserved sequences or structural patterns based on commonality in a set of related sequences, or conservation across species. In many instances, however, RNA binding proteins (RBPs) (i.e. the trans-acting elements) show preferences for binding to some specific transcripts (bound transcripts) while they do not bind to other transcripts containing the same short sequence motifs (unbound transcripts). In this study, our hypothesis is that special patterns near the conserved motifs facilitate the binding of RBPs. To test this hypothesis, we used a training set of 7 to 100-mers bound and unbound transcripts without considering the nucleotides in each position to fit structural patterns which we then assessed using a held-out test set. We then employed an iterative motif refinement procedure that reduces degeneracy a single base at a time. At each iteration, the motif with the largest mutual information based on its presence at bound transcripts and absence in unbound transcripts was selected. Application of this novel approach to the genome sequence of *T. brucei* will be presented and show how it can predict gene regulatory networks in this organism.

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CATHELICIDIN-LIKE HELMINTH DEFENSE MOLECULES (HDMS): ABSENCE OF CYTOTOXIC, ANTI-MICROBIAL AND ANTI-PROTOZOAN ACTIVITIES IMPLY A SPECIFIC ADAPTATION TO IMMUNE MODULATION

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Host defence peptides (HDPs) are expressed throughout the animal and plant kingdoms. They have multifunctional roles in the defence against infectious agents of mammals, possessing both bactericidal and immune-modulatory activities. We have identified a novel family of molecules secreted by helminth parasites (helmintn defence molecules; HDMs) that exhibit similar structural and biochemical characteristics to the HDPs. Here, we have analyzed the functional activities of four HDMs derived from Schistosoma mansoni and Fasciola hepatica and compared them to human, mouse, bovine and sheep HDPs. Unlike the mammalian HDPs the helminth-derived HDMs show no antimicrobial activity and are non-cytotoxic to mammalian cells (macrophages and red blood cells). However, both the mammalian- and helminth-derived peptides suppress the activation of macrophages by microbial stimuli and alter the response of B cells to cytokine stimulation. Therefore, we hypothesise that HDMs represent a novel family of HDPs that evolved to regulate the immune responses of their mammalian hosts by retaining potent immune modulatory properties without causing deleterious cytotoxic effects.

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IMPACT OF *LEISHMANIA* METALLOPROTEASE GP63 ON NLRP3 INFLAMMASOME NETWORK

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Parasites of the *Leishmania* genus infect and survive within macrophages by inhibiting several microbicidal molecules, such as nitric oxide and by taming down some pro-inflammatory cytokines. For instance, various species of *Leishmania* have been reported to inhibit or significantly reduced the production of IL-1b both *in vitro* and *in vivo*. However, the mechanism whereby *Leishmania* parasite can influence IL-1b production by macrophages is still not fully understood. Recent reports have been proposed that IL-1b production is importantly regulated by the NLRP3 inflammasome complex. NLRP3 senses danger molecules such as the inorganic crystals MSU, asbestos, silica and malarial hemozoin. In the present work we investigated whether *Leishmania* parasites could modulate inflammasome activation. Using PMA-differentiated THP-1 cells we demonstrated that *Leishmania* infection effectively inhibit macrophage IL-1b production, and to involve the expression of the zinc metalloprotease GP63, a critical virulence factor produced by all infectious *Leishmania* sps. As GP63 can be secreted or membrane bound to the surface of the parasite, as well as to be released with *Leishmania* exosomes vesicles, we have isolated GP63-containing exosomes from *L. mexicana* and evaluate its effect on IL-1b production. Both *L. mexicana* purified GP63 and GP63-containing exosomes inhibited macrophage IL-1b production and caspase-1 cleavage, suggesting their effect upstream of caspase-1. Some known mechanisms to regulate the inflammasome activation involve ROS production, cathepsin B release and signaling pathway. Indeed ROS production is inhibited by *Leishmania* infection and could confer in part the explanation whereby *Leishmania* can interfere with NLRP3 complex. Additionally, we have monitored whether GP63-mediated cleavage of various NLRP3 complex molecules and PTPs activation concurred to this inhibition. Collectively, we report herein the first observation that a pathogen -and for instance the protozoan parasite *Leishmania* and its surface metalloprotease GP63- can downregulate NLRP3 inflammasome complex concurring to tame-down IL-1b production.

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A *POLYPOCEPHALUS* METACESTODE FROM BAY SCALLOPS LIVES AS AN ADULT IN COWNOSE RAYS

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Twenty species of bivalves were collected from the northern Gulf of Mexico over the course of several years and examined for metacestodes. DNA was extracted from the cestodes after they were photographed. Small subunit, large subunit, and (when possible) internal transcribed spacer regions of the ribosomal RNA genes were amplified and sequenced. One of the species found, a morphologically unusual *Polypocephalus* species (flattened, with laterally extended sides), was earlier reported by Edwin Cake in his much more extensive survey of bivalve metacestodes in the same area conducted in the 1970s. He, like us, found it only in *Argopecten irradians*. We later found adult specimens of *Polypocephalus* having the same rRNA sequence in a cownose ray, *Rhinoptera bonasus*, caught near Cat Island, Mississippi. The adults are not as distinctive as the metacestodes, conforming to the general appearance of *Polypocephalus* adults. Sixteen tentacles can be protruded from a cavity in the scolex, which bears four acetabula. The worms are up to about 1.5 mm in size and have three to five proglottids. Cownose rays, the hosts for the adults, are known to feed on scallops as well as other bivalves.

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STRIPED BASS, *MORONE SAXATILIS*: A NEW HOST FOR A PARASITE OF PUBLIC HEALTH CONCERN IN SOUTH CAROLINA

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Live metacercariae of *Phagicola nana* (Heterophyidae) were identified in the striped bass, *Morone saxatilis* (Moronidae), in South Carolina. Infections by *P. nana* metacercariae have been documented in Cichlidae in South America and in Centrarchidae in North America but never in members of the family Moronidae. Specimens for morphological and molecular comparisons were obtained from both striped bass (N= 24) and largemouth bass, *Micropterus salmoides* (a known centrarchid host; N=7) collected from the Ashley and Cooper River systems. Metacercariae were extracted from the somatic musculature and measurements were taken of encysted and manually excysted specimens either fresh or following heat killing and AFA fixation. As part of this work the 18S rDNA gene of *P. nana* was sequenced for the first time. None of the seven young of the year striped bass observed were infected, but 94% of the one year or older fish were infected. Densities of metacercariae in the striped bass were calculated via the collection of a total of 27 biopsies for each fish from the epaxial and hypaxial white musculature as well as from the red muscle in the midline (N= 13). The maximum density determined was 49 metacercariae per gram of tissue and there was no significant statistical difference in the distribution of metacercarie among the three muscular areas sampled. Striped bass is a popular recreational fish in South Carolina and, when consumed without proper cooking, infected fish may be a potential health risk to humans who can serve as definitive hosts and develop cardiac heterophyidiasis.

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HAEMOGREGARINE PARASITE OF GOPHER TORTOISES (*GOPHERUS POLYPHEMUS*): SEARCH FOR A VECTOR

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Intraerythrocyte hemogregarines parasites are common in amphibians and reptiles, especially aquatic turtles. To date, only a few species have been reported from tortoises and little is known about their life cycles. Recently, an undescribed haemogregarine was reported from gopher tortoises (*Gopherus polyphemus*). We conducted the current study to better transmission and host-parasite interactions of this parasite. In the current project, we are concentrating on a translocated population of gopher tortoises (on St. Catherine's Island, Georgia (USA). Testing to date indicates that 86% of 22 sampled tortoises were positive for haemogregarines at the time of introduction on the island in 1994. At translocations, 100% of tortoises were infested with *Amblyomma tuberculatum*, the gopher tortoise tick. All ticks were removed and tortoises treated with acaricides. Examination of blood smears from the translocated tortoises in subsequent years, some as late as 2009, indicated that the tortoises were still infected; however, parasitemias had decreased. When analyzing parasitemias over time, from 1994 to 2009 (n=18), 11 (61%)

decreased, 4 (22%) were stable, 1 (0.06%) increased, and 2 (0.11%) remained negative for haemagregines. Surveillance for the tick on other gopher tortoises on the island, indicate that the tick is rare with only 5% of captured tortoises having been infested with low numbers of *A. tuberculatum* (17 from 1994-1997 and only eight from 2001-2010). Continued detection of ticks could be due to persistence of low numbers on uncaptured tortoises or due to the translocation of low numbers of tortoises to the island that may not be thoroughly examined for ticks. Interestingly, only a single tortoise that previously tested negative in 1994 became positive in 2005 and external examination revealed that tortoise was infested with *A. tuberculatum*. To date, none of the tortoises born on the island (n=11) have been positive for haemogregarines. These data suggest that gopher tortoises maintain long-term infections with this haemogregarine and that *A. tuberculatum* is a possible vector. Future studies include serial testing of tortoises introduced to the island, testing of tortoises from additional sites with and without ticks, and testing of ticks for haemogregarine developmental stages.

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LOW HOST SPECIFICITY IN HAEMOGREGARINES FROM AFRICAN TERRAPINS OF THE FAMILY PELOMEDUSIDAE

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More than 300 species of the genus *Haemogregarina* have been isolated and described from various, mostly freshwater, vertebrates. Most of the descriptions were based on the gamonts morphology in peripheral blood and the presumed strict host specificity. However, the latter preposition has been repeatedly questioned. To investigate the relevance of host specificity in phylogenetic/taxonomic framework, we examined haemogregarines from 89 recently imported terrapins belonging to 10 species; namely 9 *Pelomedusa subrufa*, 25 *Pelusios nanus*, 14 *P. upembae*, 12 *P. subniger*, 9 *P. marani*, 6 *P. rhodesianus*, 6 *P. gabonensis*, 6 *P. williamsi* and one individual of each *P. sinuatus* and *P. castaneus*. The animals were imported from Angola, Central African Republic, Democratic Republic of the Congo, Gabon, Kenya and Mozambique. Prevalence, parasitaemia and morphology of haemogregarines were studied using light microscopy of Giemsa-stained blood smears. We detected presence of various morphotypes of blood parasites belonging to the genus *Haemogregarina* in 36 terrapins (40 %). All specimens were also examined by PCR-based methods, further connected with phylogenetic analyses of 1500 bp long sequences of 18S rDNA. Our results confirmed a low host specificity and considerable conspecificity of isolates from different host species showing shallow branching pattern of the obtained trees. Most of the isolated parasites showed significant similarity to the genus *Haemogregarina*, two isolates were similar to the genus *Hepatozoon*. PCR diagnosis and morphological examination proved to be of the same sensitivity and accuracy. *This work was supported by the grant IGA VFU (11/2012/FVHE) and by the Grant Agency of the Czech Republic (P506/11/1738).*

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DIGENETIC TREMATODES OF THE FISHES OF OTSEGO LAKE, NEW YORK

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This study of digenetic trematodes is part of a survey of the intestinal parasites of fishes of Otsego Lake and its tributaries (Cooperstown, New York) from 2008 to 2012. In total, 430 individual fish were collected by hook and line, seine, gill net, or ElectroFisher, and subsequently examined for intestinal

parasites, and in many cases, for parasites in other fish organs. The survey included a total of 27 fish species, consisting of six centrarchid species, one ictalurid species, eleven cyprinid species, three percid species, three salmonid species, one catostomid species, one clupeid species, and one esocid species. Digenetic trematodes were examined with light microscopy following preparation of whole mounts. Among the fish examined, seven fish species were infected with adult digenetic trematode species in the alimentary canal, whereas 18 fish species were infected with metacercaria in other organs. Adult trematodes included *Azygia longa* in five host fish species: *Esox niger, Perca flavescens*, *Ambloplites rupestris*, *Lepomis macrochirus*, and *Lepomis gibbosus*; and a species of *Cryptogonimus* in two fish species: *A. rupestris* and *Micropterus dolomieu*. There are ongoing efforts to characterize the species through histology, scanning electron microscopy and possibly DNA sequence data. Attempts are being made to obtain species identification of metacercaria using the DNA barcodes in a different study. In addition to identifying parasites to species, we hope to better define the species characteristics and clarify conflicting information in the literature.

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NEMATODES OF THE FISHES OF OTSEGO LAKE, NEW YORK

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This nematode study is part of a survey of the intestinal parasites of fishes of Otsego Lake and its tributaries (Cooperstown, New York) from 2008 to 2012. In total, 430 individual fish were collected by hook and line, seine, gill net, or ElectroFisher, and subsequently examined for intestinal parasites, and in many cases, for parasites in other fish organs. The survey included a total of 27 fish species, consisting of six centrarchid species, one ictalurid species, eleven cyprinid species, three percid species, three salmonid species, one catostomid species, one clupeid species, and one esocid species. Intestinal nematodes were studied by light microscope examination of specimens that were whole-mounted using conventional methods. A minimum of five species of nematodes were encountered in the alimentary canal of fishes examined. These include *Spinitectus gracilis* in *Ambloplites rupestris, Micropterus dolomieui*, and *Lepomis auritis*; *Spinitectus carolini* in *M. dolomieu* and *A. rupestris*; *Spinitectus micracanthus* in *Lepomis macrochirus, Lepomis gibbosus, L. auritis, A. rupestris, Perca flavescens,* and *M. dolomieu*; and *Dichelyne cotylophora* in *P. flavescens*. There is an ongoing attempt to further identify additional nematode species that were found using light microscopy, scanning electron microscopy and histology. In addition, there was a diversity of nematodes found outside of the alimentary canal. For example, *Eustrongyldes tubifex* was found in the body cavity of *L. gibbosus*, *L. macrochirus*, *L. auritis, Esox niger*, and *P. flavescens*. Through research on the nematodes, difficulties were found on the overall identification of several species owing to limited information in the literature. An update on morphology through re-description of selected species may be beneficial to the further understanding of these nematodes.

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SINUOLINEA INFECTIONS IN THE URINARY SYSTEM OF *CYNOSCION* SPECIES (SCIAENIDAE) AND PHYLOGENETIC POSITION OF THE TYPE SPECIES OF *SINUOLINEA* DAVIS, 1917 (MYXOZOA: MYXOSPOREA)

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I. Fiala, **W.A. Roumillat**

The fishes, *Cynoscion nebulosus* and *C. regalis* (Sciaenidae), collected in the estuarine systems of South Carolina, USA, were found to be commonly infected in the urinary tract by the myxosporean, *Sinuolinea dimorpha* (Davis, 1916). The parasite was identified based on the morphology of both spores and plasmodial stages. As the result of the SSU rDNA sequences generated in this study from type host material, this species of *Sinuolinea* Davis, 1917 has found its place in the current phylogenetic reconstruction of Myxozoa and has enlarged the limited number of myxosporean genera represented in phylogenetic analyses using sequences of type species. The sequences of SSU rDNA of *S. dimorpha* from *Cynoscion* host species formed two clusters, irrespective of their host species, and also revealed differences within each cluster. These findings contribute to the recognition of myxosporean cryptic species diversity, an important topic that emphasizes the general necessity of species delimitation and of the need for continued efforts to improve our knowledge of Myxosporea based on both the morphology of spores and molecular data.

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PACO: A NOVEL PROCRUSTES APPLICATION TO COPHYLOGENETIC ANALYSIS OF HOSTS AND PARASITES

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We present Procrustean Approach to Cophylogeny (PACo), a novel global-fit method to test for congruence between phylogenetic trees, or between phylogenetic distance matrices, of hosts and parasites. Unlike previous global-fit tests, PACo evaluates the dependence of the parasite phylogeny upon the host one. This makes it especially appropriate to test the classical coevolutionary model that assumes that parasites track the phylogeny of their hosts. The new method does not require fully resolved phylogenies and allows for multiple host-parasite associations. PACo produces a Procrustes superimposition plot enabling a graphical assessment of the fit of the parasite phylogeny onto the host phylogeny, and a goodness-of-fit statistic, whose significance is established by randomization of the host-parasite association data. The contribution of each individual host-parasite association to the global fit is measured by means of jackknife estimation of their respective squared residuals and confidence intervals. We carried out different simulations to evaluate the performance of PACo in terms of Type I and Type II errors with respect to two similar published tests. In most instances, PACo performed at least as well as the other tests and showed higher overall statistical power. In addition, the jackknife estimation of squared residuals enabled more elaborate validations about the nature of individual links than the ParaLink1 test of the program ParaFit. To promote usability, we provide a script written in the public-domain statistical software R and a user guide with a worked-out example.

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CESTODES OF THE FISHES OF OTSEGO LAKE, NEW YORK

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This study is part of a survey of the intestinal parasites of fishes of Otsego Lake and its tributaries (Cooperstown, New York) from 2008 to 2012. In total, 430 individual fish were collected by hook and line, seine, gill net, or ElectroFisher, and subsequently examined for intestinal parasites, and in many cases, for parasites in other fish organs. The survey included a total of 27 fish species, consisting of six centrarchid species, one ictalurid species, eleven cyprinid species, three percid species, three salmonid

species, one catostomid species, one clupeid species, and one esocid species. Collected cestodes were examined with light microscopy following preparation of whole mount slides. Among the fish examined, six fish species were infected with adult cestodes in the intestine. The adult cestodes encountered represented three genera in three respective cestode orders. The caryophyllid cestode *Glaridacris catostomi* occurred in *Catostomus commersoni*. The bothriocephalid cestode *Bothriocephalus cuspidatus* occurred in *Perca flavescens*. Finally, the game fish species *Esox niger*, *P. flavescens*, *Micropterus salmoides*, *Micropterus dolomieu*, *Salvelinus nemaycush*, and *Coregonus clupeaformis* were host to ~four species of the proteocephalidean cestode *Proteocephalus*. Each *Proteocephalus* species had distinctive scolex and bothrial features in combination with features of the strobila, but preliminary morphological examinations did not enable species identification due to limitations of the taxonomy of *Proteocephalus* in North America. Adult cestode species encountered during this survey were less prevalent than most species of the digenetic trematodes, nematodes, and acanthocephalans that were encountered.

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MORPHOLOGICAL DIVERSITY OF RHINEBOTHRIINAE NEW GENUS 3 (CESTODA: RHINEBOTHRIIDEA)

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Recent survey work in Borneo, Australia, Senegal and Vietnam on elasmobranchs and their parasites has revealed the presence of multiple new genera of cestodes. One new genus of rhinebothriidean cestode, referred to as Rhinebothriinae new genus 3 by Healy et al., 2009, has been found in 28 species of batoid elasmobranchs. This new genus is distinguished from other rhinebothriidean genera by its possession of tear drop-shaped bothridia that possess a posterior row of loculi that are longer than wide. Based on preliminary examination with light- and scanning electron microscopy, it is evident that the specimens of Rhinebothriinae new genus 3 collected from the 28 species of batoid elasmobranchs represent multiple species. In order to guide ongoing taxonomic studies of specimens of this new genus, morphological features that vary intra-generically were identified. Bothridial features that vary within the genus include whether the bothridia is wider than long or longer than wide; whether the bothridia is constantly constricted; whether the anterior loculus overhangs the remainder of the bothridium; number of complete transverse septa in the anterior region; presence or absence of incomplete transverse septa in the anterior region; presence or absence of complete longitudinal septa in the posterior region; and presence or absence of lateral posterior subloculi. Proglottid features that vary within the genus include the presence or absence of an external seminal vesicle; a thick- or thin-walled cirrus sac; presence or absence of a seminal receptacle; and a vagina that does or does not recurve anteriorly. Combinations of these features have aided in recognizing several potential morpho-types or species within the genus. Although cestodes of this genus are known to infect nearly 30 batoid elasmobranch species, they have conserved characteristics of the bothridia, and only a few key morphological features can be used to concretely separate one species from another.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF HEMATOZOAN PARASITES OF AMERICAN BLACK DUCKS (*ANAS RUBRIPES*) IN THE EASTERN UNITED STATES

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Avian hematozoan parasites have a near worldwide distribution, and although are frequently identified in dabbling ducks, molecular characterization of parasites from ducks is limited. For example, prevalence rates in the American black duck (*Anas rubripes*) is high (>20%), but no hematozoa have been genetically characterized. In the current study, we aim to investigate the prevalence and genetic diversity of hematozoa in American Black Ducks by combining molecular data from partial cytochrome b (*cyt b*) sequences with morphological data from thin blood smear analysis. We collected blood samples from 106 black ducks from January to March 2010 in four states, Connecticut (n=20), Maine (n=38), Maryland (n=43), and Rhode Island (n=5). Two PCR assays were run for three hematozoan genera of interest. A total of 69 (65%) and 66 (62%) were positive for *Haemoproteus*/*Plasmodium* and *Leucocytozoon*, respectively. A subset of positive samples was sequenced and phylogenetic analysis of 41 *Haemoproteus*, 31 *Leucocytozoon*, and one *Plasmodium* revealed two monophyletic groups of *Haemoproteus* and extensive genetic variation (~15% differences) among the *Leucocytozoon*. One black duck *Haemoproteus* group, morphologically identified as *H. nettionis*, included the majority of black duck sequences (n=38) and two other *Haemoproteus* spp. from waterfowl while the other group, morphologically identified as *H. greineri*, included three black duck sequences and a *Haemoproteus* from a Galapagos penguin. Despite extensive variation among the *Leucocytozoon* sequences, all grouped into three monophyletic clades of other *Leucocytozoon* associated with waterfowl and were morphologically similar to *L. simondi*. However, because of high genetic variation, careful morphological examination may reveal that *L. simondi* is a species-complex.

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UTILITY OF TESTING BLOOD-FED AND QUESTING TICKS FOR PIROPLASMS FOR IDENTIFICATION OF NOVEL VERTEBRATE HOSTS OR VECTORS

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The piroplasms are tick-transmitted protozoan parasites in the genera *Babesia*, *Theileria*, and *Cytauxzoon*. Worldwide, the prevalence and diversity of piroplasms in humans and wild and domestic animals is increasing. Recently, a novel *Babesia* sp. was detected in a man from TN and several novel *Babesia* spp. have been reported in domestic dogs. For many piroplasms, no vector is known but it is often presumed to be an Ixodid tick. This study was conducted to characterize the prevalence and diversity of piroplasms in Ixodid ticks from several states in the United States. A piroplasm-specific PCR was used to screen 1,632 Ixodid ticks from Georgia (n=486), Kentucky (n=103), Tennessee (n=626) and Texas (n=416) that were questing (n=42), collected from animals (n=627), or collected from humans (n=963). The study focused on two common species, *Dermacentor variabilis* (n=702) and *Amblyomma americanum* (n=743), but when available, other tick species were tested including *A. maculatum* (n=16), *A. cajennense* (n=89), *Ixodes scapularis* (n=4), *I. woodi* (n=1) and unspeciated *Amblyomma* (n=77). Based on PCR testing, few ticks were positive for piroplasms with 37 (2.3%), 34 (2.1%), and 9 (0.6%) ticks positive for *Theileria*, *Babesia*, or *Cytauxzoon,* respectively. Of the 34 *Babesia*-positive ticks, 14 (41%)

were detected from *A. americanum*, 19 (56%) were from *D. variabilis* and one (3%) *was from I. scapularis*. Importantly, eight of the 34 *Babesia*-positive ticks were removed from humans from Kentucky (n=1), Georgia (n=2), Texas (n=4) and Pennsylvania (n=1). Of note was that three *Babesia*-positive ticks were questing *A. americanum* which represents the first report of *Babesia* infection of a questing *Amblyomma* species in the United States. Also, six of the *Babesia*-infected *A. americanum* (one from a dog, two from feral hogs, and three from humans) were positive for *Babesia* sp. Coco, a *Babesia* sp. previously only detected in immunocompromised dogs and currently no vector or reservoir is known for *Babesia* sp. Coco. These data highlight the usefulness of screening both questing and partially fed ticks for the detection of piroplasms as long as data interpretation includes the potential that host-blood meals can be present in non-questing ticks. Finally, these data suggest that *A. americanum* might be a vector of *Babesia* sp. Coco, although experimental transmission studies are needed to confirm the vectoral capacity of *A. americanum*.

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HISTORICAL PERSPECTIVES AND "ANOTHER NEW COCCIDIUM, SO WHAT!"

R. BARCLAY MCGHEE MEMORIAL LECTURE

D. Duszynski, University of New Mexico

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STANDING ON THE SHOULDERS OF BENEVOLENT GIANTS

H. B. WARD MEDAL LECTURE

S.A. Nadler, University of California - Davis

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GENOMICS OF DRUG RESISTANCE IN *LEISHMANIA*

M. Ouellette, Université Laval

Resistance in *Leishmania* is often due to gene copy number variations (CNVs) and to point mutations. Next generation sequencing (NGS) allows the detection of both CNVs and point mutations. We have sequenced different *Leishmania* cells selected for resistance to antimonials, miltefosine, amphotericin B, and paromomycin. NGS of resistant parasites has allowed the detection of a plethora of resistance mechanisms. Chromosome aneuploidy, gene amplification or deletion of specific loci and point mutations were found in the various resistant cells. Amplification/deletion happens at the level of repeated sequences that are widespread throughout the *Leishmania* genome. We obtained evidences that the *Leishmania* genome is continuously being rearranged at the level of these repeated sequences, which serve as a functional platform for constitutive and stochastic amplification (and deletion) of genomic segments in the population. This process is adaptive as the copy number of the extrachromosomal elements increase upon selection and also reversible since the copy number decreases to baseline levels when selection is removed. Our data also revealed that resistance at the population level is highly heterogeneous both at the genotype and phenotype level, that the same gene can be mutated or deleted, and mutations in a common gene are found in cells resistant to different drugs. Finally new resistance markers were found that were validated by gene transfection.

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ROLE OF P-GLYCOPROTEIN IN THE MACROCYCLIC LACTONE RESISTANCE MECHANISM IN *DIROFILARIA IMMITIS*

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Heartworm, a major disease of dogs that can also affect cats, wild canids and ferrets is caused by a filarial parasite, *Dirofilaria immitis*. This nematode parasite, transmitted by mosquito vectors, occurs commonly in tropical, sub-tropical and temperate regions of the world, including North and South America. Movement of dogs from US after periods of hurricanes has led to a recent increase in the number of cases in Canada, particularly in southern regions of Ontario, Manitoba and Quebec. Macrocyclic lactone (ML) anthelmintics are used for chemoprophylaxis for heartworm. Among the MLs, the Avermectins (ivermectin, selamectin) and Milbemycins (milbemycin oxime, moxidectin) are used to kill developing L_3/L_4 stages of the parasite and microfilariae. These MLs acts on glutamate gated chloride channels (GluCl) leading to paralysis and finally death of the parasite. There are recent reports of Loss of Efficacy (LOE) of MLs, particularly in the Mississippi Delta region of USA. In a series of studies by Bourguinat et al., it was found that the *D. immitis* genome is heterogenous, with loss of heterozygosity in low responders, a phenomenon seen during the selection process towards resistance. They also found a strong negative correlation between a GG-GG genotype of a P-glycoprotein (*Dim-Pgp-12*) with *in vitro* response of microfilariae (mf) to ivermectin, with the frequency of this genotype increasing in mfs from dogs in which ML resistance was apparent. This membrane protein P-gp, a member of the ATP binding cassette (ABC) superfamily, is believed to be overexpressed during multidrug resistance, effluxing drug out of the cell so that less drug reaches its target. In order to study the interaction of this membrane protein P-gp with the 4 MLs, the full length P-gp was identified, cloned and stably transfected into a mammalian cell line, LLC-PK1. Clones of cells with maximum expression of *Dim-Pgp-12* were selected and used for functional studies at the cellular and subcellular levels. We are also studying the effect of this GG-GG genotype on the functioning of the expressed protein in terms of the interaction with the MLs. By this study, the effect of single nucleotide polymorphisms (SNPs) on P-gp function and the involvement of the P-gp on the transport profile, substrate specificity, structure-activity relationship between avermectins and milbemycins, etc., will be elucidated, in addition to field evidence of P-gp involvement with ML resistance in *D. immitis*.

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PLASMODIUM FALCIPARUM CHLOROQUINE RESISTANCE TRANSPORTER (PFCRT) INTERACTING PROTEINS

F. Baakdah and **E. Georges**, McGill University

Malaria is a major disease in the developing and tropical countries with more than 200 million infections and ~1 million preventable deaths yearly. Of the five different *Plasmodium* species that infect humans, *Plasmodium falciparum* is the most lethal accounting for ~75% of all malaria infections. Malaria infection has been successfully treated with several quinoline-based drugs, including quinine, quinidine, and chloroquine. Indeed, chloroquine (CQ), a diaphoretic weak base, has been one of the most effective antimlalarials until the raise and spread of chloroquine resistant *P. falciparum*. Research over the past two decades has shown that resistance to CQ is primarily due to the mutation in a trans-membrane protein localising to the digestive vacuole (DV) of the parasite known as PfCRT (*Plasmodium falciparum* CQ Resistance Transporter). Unlike wild type PfCRT, the lysine substitution with threonine at position 76 de-trap`s CQ in the DV, thus conferring a resistance phenotype. Indeed, PfCRT and the digestive vacuole

continue to be attractive drug targets for the development of novel anti-malarial drugs, however little is known about the normal function/substrate nor is its know how PfCRT transport functions are regulated. It is currently believed that PfCRT is a member of the drug/metabolite transporter superfamily. Furthermore, it was recently suggested that PfCRT may mediate the transport of glutathione into the digestive vacuole and/or is responsible for the transport of dipeptides into the parasite cytosol from the digestive vacuole. In this study, we have focused on identifying PfCRT interacting proteins using several approaches. Using Far Western and in-situ chemical crosslinking, several proteins (~17 kDa to 120 kDa) have been seen to interact with the N-terminal domains of PfCRT. Work is ongoing to identify and characterize these interacting proteins.

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NEW PROGRESS ON THE MODE OF ACTION OF MILTEFOSINE IN *L. INFANTUM* USING METABOLOMICS

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S. Weidt, University of Glasgow
L. Rivas, Consejo Superior de Investigaciones Científicas
K. Burgess, University of Glasgow
M. Ouellette, Universite Laval

Miltefosine is the only orally available leishmanicide and has been licensed for more than 10 years for the treatment of visceral and cutaneous leishmaniasis, even though its mode of action (MOA) is unknown. Contradictory theories as to the MOA may indicate the presence of multiple targets or may be due to the different experimental designs and analyses carried out. The three main theories for the MOA in *Leishmania* include alterations to the membrane lipid content, induction of apoptosis and modulation of macrophage responses. Here we perform untargeted metabolomics to elucidate the metabolic changes involved in miltefosine action. Nearly 900 metabolites were detected, 10 % of which were significantly altered after 3.75 hours. Many of the changes related to an increase in lipid breakdown leading alkane fragments and sugar release. Fragment release is synchronised with reactive oxygen species (ROS) production, which presumably causes lipid peroxidation leading to the breakdown of native phospholipids. Signs of DNA damage were also detected as were changes to the levels of some thiols and polyamines. After five hours of miltefosine treatment the cells showed depleted levels of most metabolites, which would indicate that the cells' outer membranes integrity had become compromised and internal metabolites were escaping. In miltefosine resistant cells, the drug was not internalised and the changes to the internal metabolite levels were not seen. Cells resistant to SbIII had slightly depleted levels of miltefosine uptake and a corresponding depletion in the levels of internal metabolite changes. This work will be important do inform the design of combination therapies to combat leishmaniasis, something that the research community should be prioritising in the coming years.

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HAEMONCHUS CONTORTUS: TUBULINS AND IVERMECTIN SELECTION

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Haemonchus contortus (*H. contortus*) is an important parasitic nematode of livestock causing substantial loss to animal industries. *Ivermectin (IVM)* has been successfully used to treat haemonchosis for almost three decades. However, since its introduction many studies have shown resistance to IVM in this parasite. Previous reports have demonstrated that repeated IVM treatment results in selection on β-

tubulin in *H. contortus*. We have conducted further analysis to investigate a possible direct relationship between IVM and tubulin/microtubules. Recombinant *H. contortus* β-tubulin isotype 1 and α-tubulin were expressed and purified. Following polymerization, the structure of microtubules was confirmed by electron microscopy and functional analysis was performed by polymerization assay. Differences in the degree of polymerization were observed when β-tubulin isotype 1 was polymerized with α-tubulin in the presence and absence of IVM. Furthermore, it was found that IVM prevented limited proteolysis of β-tubulin isotype 1. These results suggest that IVM may have a direct effect on stabilizing the microtubules and hence alter tubulin dynamics in the parasite. Further investigations are being conducted to characterize IVM binding to the nematode tubulin/microtubules. Supported by NSERC and the FQRNT Centre for Host-Parasite Interactions

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MRE11 INVOLVEMENT IN DNA REPAIR AND DRUG RESISTANCE IN *LEISHMANIA*

M.C. Laffitte, **A. Mukherjee**, **D. Légaré** and **M. Ouellette**, CRI Université Laval

The protozoan parasite *Leishmania* is known to modulate the copy number of its genes (and thus their expression levels) through the formation of extrachromosomal DNA amplicons as a mechanism of drug resistance. During the gene amplification process, rearrangements occurring between direct or inverted DNA repeated sequences (RS) lead to the formation of circular and/or linear amplicons containing genes implicated in resistance. We have shown that circular amplifications are dependent on RAD51 but not linear amplicons (manuscript in preparation). We hypothesize that the linear amplification involves the formation of a DNA break nearby the RS, followed by its repair by homologous recombination after annealing between the RS. Here we investigated the role of the gene *MRE11*, which codes for a putative DNA-binding protein (part of a complex with RAD50 and NBS1), in DNA break repair through homologous recombination. Our hypothesis is that MRE11 should have a role in the formation of linear amplicons, and hence in drug resistance. We generated *Leishmania infantum MRE11* null mutants by gene replacement using hygromycin and neomycin as selecting markers. We observed a growth delay in the *MRE11*$^{-/-}$ strain, compared to the wild-type cells. This phenotype was partially reverted when the *MRE11* null mutants were complemented with an episomal *MRE11* construct. The role of MRE11 in DNA-damage repair was further studied by inducing double strand breaks in our panel of strains using the DNA-damaging agents methylmethane sulfonate and hydroxyurea. Interestingly, the *MRE11*$^{-/-}$ parasites were more sensitive to the damaging agents than both the wild-type and the genetically-complemented strains, suggesting the involvement of MRE11 in DNA repair in *Leishmania infantum*. To confirm the role of MRE11 in homologous recombination, we carried-out a quantitative recombination assay by measuring the integration efficiency of a reporter construct targeting the *GSH1* gene encoding for the gamma-glutamylcysteine synthetase, the rate-limiting enzyme in the biosynthesis of glutathione and of trypanothione in *Leishmania*. Unexpectedly, the *MRE11* null mutants seemed to have higher integration efficiency than the wild-type cells, which suggests the presence of other mechanisms putatively compensating for the lack of MRE11. To further confirm the role of MRE11 in the formation of linear DNA amplicons relevant to drug-resistance, we compared the generation of *PTR1* linear amplicons in wild-type, *MRE11*$^{-/-}$ and complemented strains resistant to methotrexate, a model antifolate drug known to induce the amplification of the *PTR1* gene. Consistent with our hypothesis, the *MRE11*$^{-/-}$ parasites exhibited much less *PTR1* linear amplification than the wild-type strain, suggesting a significant involvement of MRE11 in the formation of linear amplificons.The thorough characterization of MRE11 could help in increasing our understanding of the mechanisms underlying the formation of linear amplicons in this parasite. Globally, targeting amplification events and their key players may represent a good strategy to circumvent or limit the development of resistance in *Leishmania*.

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1942 No meeting
1943 No meeting
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2014 New Orleans, LA

* With American Society of Tropical Medicine; since 1952, American Society of Tropical Medicine and Hygiene
† With American Institute of Biological Sciences
‡ With Helminthological Society of Washington
§ With American Microscopical Society
¶ With the International Congress of Parasitology; 1970 (ICOPA-II), 1982 (ICOPA-V), 2002 (ICOPA-X), 2006 (ICOPA-XI)
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** With American Association of Veterinary Parasitologists
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‡‡ With Society of Nematologists
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¶¶ With Parasitology Section, Canadian Society of Zoologists
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