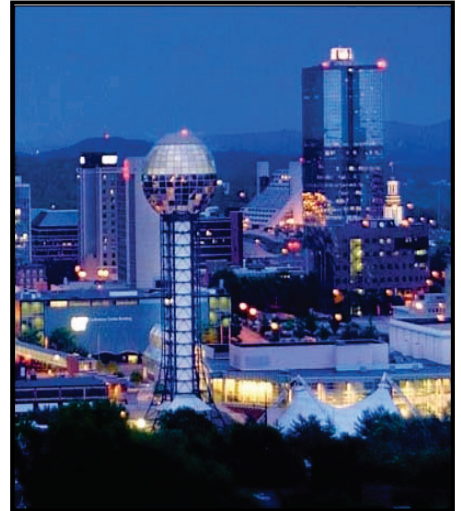
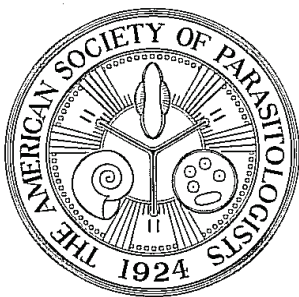


The 84th Annual Meeting of the American Society of Parasitologists

Crowne Plaza – Knoxville
Knoxville, Tennessee
August 14 – 17, 2009



Museum of Appalachia



Downtown Knoxville



BBQ-Bluegrass Cruise on the Tennessee River

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 putting together this year's annual meeting:

The ASP Local Arrangements Committee

Drs. Sharon Patton and Charles Faulkner

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Knowledge • Compassion • Discovery

*A letter of Welcome to the ASP from
John J. Duncan, Jr. - US Congress, Tennessee 2nd District*



HOUSE OF REPRESENTATIVES
WASHINGTON, D. C. 20515

JOHN J. DUNCAN, JR.
SECOND CONGRESSIONAL DISTRICT
TENNESSEE

August 13, 2009

Knoxville Tourism & Sports Corp.
301 South Gay Street
Knoxville, Tennessee 37902

Dear Friends:

I am glad to have this opportunity to send my greetings to the members of the American Society of Parasitologists at their 84th Annual Meeting.

It is an honor to have such a diverse group of extraordinary scientists from industry, government, and academia visiting our city.

I send my best wishes for a successful and productive meeting and if I can ever be of service to you in the future, please do not hesitate to let me know.

With kindest regards, I am

Yours truly,

A handwritten signature in black ink that reads "John J. Duncan, Jr." in a cursive style.

JOHN J. DUNCAN, JR.
Member of Congress

84th Annual Meeting

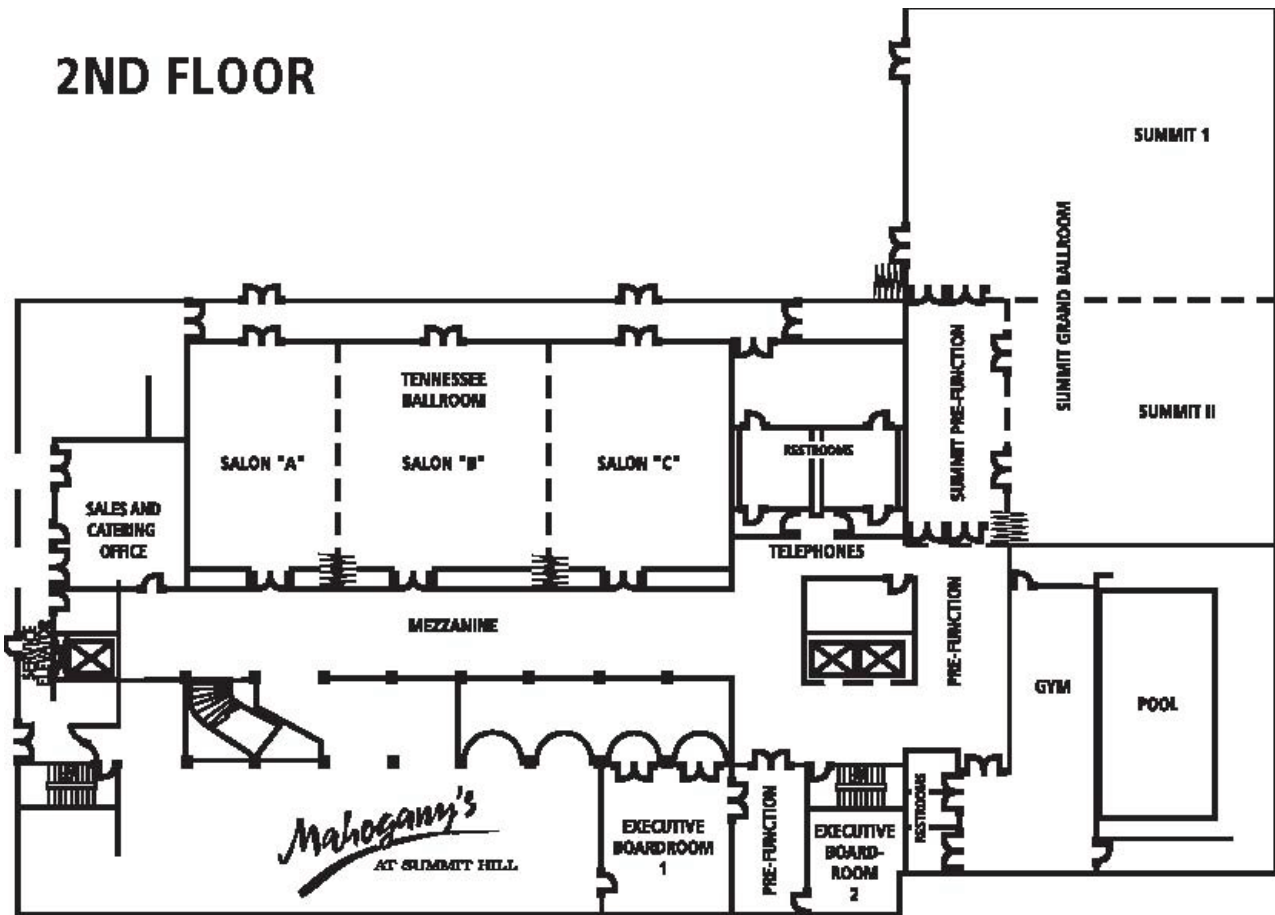
<u>Day/Times</u>	<u>Activity/Function</u>	<u>Room/Space</u>
<u>August 14 (Friday)</u>		
8:00 a.m.-Noon	ASP Council	Summit 2
8 am to 5 pm	Speaker Ready room	Exec Boardroom 1
1:00 -5:00 p.m.	Student Paper Competition I	Salon B
1:00-3:00 p.m.	Life Cycles, Epidemiology I	Salon C
3:00-3:15 p.m.	Coffee Break	
3:15-4:45 p.m.	Vector Biology, Chemotherapy, Physiology	Salon C
7:00 – 10:00 p.m.	Welcome Reception	Summit Ballroom
<u>August 15 (Saturday)</u>		
7:00-8:30 a.m.	Editorial Board Breakfast	Private Dining Room
8:30-10:30 a.m.	ASP President's Symposium	Tennessee Ballroom*
10:30-11:00 a.m.	Coffee Break	
11:00-Noon	R. Barclay McGhee Lecture	Tennessee Ballroom
1:00-3:00 p.m.	Student Paper Competition II	Salon B
1:00-3:15 p.m.	Genetics, Molecular Biology, Immunology	Salon C
3:15-3:30 p.m.	Coffee Break	
3:30-5:30 p.m.	ASP Students' Symposium	Salon B/C
3:00-6:00 p.m.	Auction Set Up	Summit Ballroom
5:30-6:30	Student Social	Private Dining Room
6:00-7:00 p.m.	Auction Preview	Summit Ballroom
7:00-9:00 p.m.	20 th Annual ASP Student Auction	Summit Ballroom
<u>August 16 (Sunday)</u>		
8:00-10:00 a.m.	Associate Editors Symposium	Salon B
8:30-11:15 a.m.	Ecology	Salon C
10:00-10:15 a.m.	Coffee Break	
10:15-12:15 p.m.	41 st Coccidiosis Conference	Salon B
1:00-2:00 p.m.	ASP President's Address	Tennessee Ballroom
2:00-2:15 a.m.	Coffee Break	
2:15-4:45 p.m.	CAPC Symposium	Salon A
2:15-5:00 p.m.	Host Parasite Interactions	Salon B
2:15-5:45 p.m.	Taxonomy, Systematics, Phylogeny	Salon C
3:30-3:45	Coffee Break	
3:00-5:00 p.m.	Poster Boards delivered, authors may set up posters	Summit 2
6:30 to 9:00 pm	Knoxville Riverboat Dinner Cruise	
<u>August 17 (Monday)</u>		
8:30-10:15 a.m.	Life Cycles, Epidemiology II	Salon B-C
8:30-10:30 a.m.	Authors complete poster set up	
10:30-Noon	Poster Session, coffee, snacks	Summit Ballroom
1:00-1:50 p.m.	H.B. Ward Lecture	Tennessee Ballroom
1:50-2:40 p.m.	Eminent Parasitologist Lecture	Tennessee Ballroom
2:40-3:00 p.m.	Coffee Break	
3:00-4:30 p.m.	ASP Awards and Business Meeting	Tennessee Ballroom
8:00 a.m.-5:00 p.m. (daily)	Slide Preview Room	Board Room
Noon to 5pm (daily)	Registration	Mezzanine

***Tennessee Ballroom is Salon A, B, and C combined**

Floor Plan

Crowne Plaza Knoxville
Knoxville, Tennessee

2ND FLOOR



Friday Morning, August 14, 2009

8:00 – Noon **ASP COUNCIL MEETING, Summit 2**

Presiding: B. Conn, Berry College, Mount Berry, GA

Friday Afternoon, August 14, 2009

1:00 – 5:00 **STUDENT PAPER COMPETITION – I, Salon B**

Presiding: J. Camp, Purdue University, West Lafayette, IN
B. Rosenthal, ARS, USDA, Beltsville, MD

<u>Time</u>	<u>Paper No.</u>	
1:00	1	INVESTIGATING THE ROLE OF <i>ANOPHELES</i> SGS PROTEINS IN <i>PLASMODIUM</i> -MOSQUITO INTERACTIONS AND IN IMMUNITY. J.G. King and J.F. Hillyer.
1:15	2	ORAL TRANSMISSION OF <i>TRYPANOSOMA CRUZI</i> WITH OPPOSING EVIDENCE FOR THE ROLE OF CARNIVORY IN HORIZONTAL TRANSMISSION. D.M. Roellig , A.E. Ellis, M.J. Yabsley, and D.B. Warnell.
1:30	3	ASSESSING PREDICTABILITY IN PARASITE COMMUNITIES: A MULTI-YEAR, MULTI-SITE STUDY OF <i>DACTYLOGYRUS</i> SPP. ON A NATIVE NORTH AMERICAN CYPRINID. A.K. Knipes and J. Janovy, Jr.
1:45	4	CO-OCCURRENCE OF <i>HAEMATOLOECHUS COMPLEXUS</i> (TREMATODA: HAEMATOLOECHIDAE) AND <i>RHABDIAS JOAQUINENSIS</i> (NEMATODA: RHABDIASIDAE) IN THE PLAINS LEOPARD FROG (<i>RANA BLAIRI</i>). M.S. Vhora , G. Langford, and J. Janovy Jr.
2:00	5	MOLECULAR PHYLOGENY OF <i>RHABDIAS</i> SPP.: IMPLICATIONS FOR LUNGWORM LIFE CYCLES AND HOST SPECIFICITY. G.J. Langford and J. Janovy Jr.
2:15	6	POPULATION GENETICS AND HOST SPECIFICITY OF <i>SPIRORCHIS SCRIPTA</i> (DIGENEA: SPIRORCHIIDAE) IN EMYDID TURTLES. D.S. Freyre , A.S. Freyre, V.V. Tkach, and S.D. Snyder.
2:30	7	<i>TRYPANOSOMA</i> OF AUSTRALIAN FRESHWATER TURTLES: OCCURRENCE AND DIVERSITY. A.S. Freyre , D.S. Freyre, V.V. Tkach, and S.D. Snyder.
2:45	8	TAXONOMY AND EVOLUTION OF THE SYMBIOTIC <i>CHAETOGASTER LIMNAEI</i> COMPLEX (ANNELIDA). A.L. Patti , R. Hochberg, and M. Litvaitis.

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3:00 -3:15 COFFEE BREAK

- 3:15 9 CHARACTERIZATION OF THE CESTODE FAUNA OF A DEEP WATER SQUALIFORM SHARK FROM MADEIRA. **M. Pickering** and J. Caira.
- 3:30 10 EFFECTS OF THE ACANTHOCEPHALAN PARASITE, *PROFILICOLLIS ALTMANI*, ON TISSUE CAROTENOID CONCENTRATIONS AND EGG MASS OF THE PACIFIC MOLE CRAB, *EMERITA ANALOGA*. **J.E. Anderson**, W.E. Melroy, A.R. Richins, L.F. Constancio, M.S. Rice, G.R. Kolluru, and L.K. Vredevoe.
- 3:45 11 EFFECTS OF *PROFILICOLLIS ALTMANI* (ACANTHOCEPHALA: POLYMORPHIDAE) ON TISSUE CAROTENOID PROFILES OF THE PACIFIC MOLE CRAB, *EMERITA ANALOGA*. **L.F. Constancio**, J.E. Anderson, M.S. Rice, G.R. Kolluru, and L.K. Vredevoe.
- 4:00 12 THE TAXONOMIC STATUS OF THE ENIGMATIC ELASMOBRANCH TAPEWORM *TENIA NARINARI* AND AN INVESTIGATION OF ITS MODE OF ATTACHMENT. **S.L. Russell** and K. Jensen.
- 4:15 13 A RECOMBINANT BP1 ANTIGEN-BASED ENZYME LINKED IMMUNOSORBENT ASSAY FOR DIAGNOSIS OF *BAYLISASCARIS PROCYONIS* LARVA MIGRANS IN HUMANS. **S. Dangoudoubiyam**, R. Vemulapalli, M. Ndao, and K.R. Kazacos.
- 4:30 14 EXPLOITATION OF ASYMMETRIC PREDATOR-PREY INTERACTIONS BY TROPHICALLY TRANSMITTED PARASITES. **W. Rossiter** and M.V.K. Sukhdeo.
- 4:45 15 ABIOTIC VERSUS BIOTIC HIERARCHIES IN THE ASSEMBLY OF PARASITE COMMUNITIES. **T.K. Anderson** and M.V.K. Sukhdeo.

1:00 – 3:00 LIFE CYCLES, EPIDEMIOLOGY I, Salon C

Presiding: I. de Buron, College of Charleston, Charleston, SC
R. Fayer, ARS, USDA, Beltsville, MD

- | <u>Time</u> | <u>Paper</u>
<u>No.</u> | |
|-------------|----------------------------|--|
| 1:00 | 16 | THE ESTABLISHMENT OF A PURE STRAIN OF <i>PLAGIORCHIS ELEGANS</i> (RUD.1802) AND AN ESTIMATE OF THE PARASITE'S BIOTIC POTENTIAL ORIGINATING FROM A MONOMIRACIDIAL INFECTION. G. Gelder . |
| 1:15 | 17 | IDENTITY OF RRNA SEQUENCES FROM METACESTODES IN SHRIMP NERVE CORDS AND <i>POLYPOCEPHALUS</i> ADULTS FROM RAYS. J.T. Payne and J. Gunderson. |

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- 1:30 **18** ON THE CURRENT STATUS OF HUMAN INFECTIONS WITH THE BROAD FISH TAPEWORM (*DIPHYLLOBOTHRIUM*). **K. Roman**, T. Scholz, and B. Wicht.
- 1:45 **19** HIGH PREVALENCE OF *BAYLISASCARIS PROCYONIS* IN RACCOONS TRAPPED IN SOUTH CENTRAL KENTUCKY. **C.D. Davis**, C. Groce, T. Holaday, and C. Walker.
- 2:00 **20** *TRYPANOSOMA CRUZI* INFECTION IN RACCOONS AND OPOSSUMS TRAPPED IN SOUTH CENTRAL KENTUCKY. **C. Groce**, C. Moss, M. James, and C.D. Davis.
- 2:15 **21** MOLECULAR ANALYSIS OF *TRYPANOSOMA CRUZI* ISOLATES OBTAINED FROM RACCOONS TRAPPED IN SOUTH CENTRAL KENTUCKY. **L. Bi**, S. Varikuti, C. Groce, and C.D. Davis.
- 2:30 **22** DEVELOPMENT OF A NEW PCR PROTOCOL TO DETECT AND SUBTYPE *BLASTOCYSTIS* SPP. FROM HUMANS AND ANIMALS. **M. Santín**, M.T. Gómez-Muñoz, and R. Fayer.
- 2:45 **23** SURVEY OF *BITHYNIA TENTACULATA* (CLASS: GASTROPODA) COLLECTED FROM RATTLESNAKE RESERVOIR AND GEORGETOWN LAKE, MONTANA FOR THE PRESENCE OF *SPHAERIDIOTREMA GLOBULUS* AND *CYATHOCOTYLE BUSHIENSIS* (CLASS: TREMATODA). **R. Cole**, K. Schuler, R. Jaffe, and J. Hansen.

3:00 – 3:15 **COFFEE BREAK**

3:15 – 4:45 **VECTOR BIOLOGY, CHEMOTHERAPY, and PHYSIOLOGY, Salon C**

Presiding: J.F. Hillyer, Vanderbilt University, Nashville, TN
M.V.K. Sukhdeo, Rutgers University, New Brunswick, NJ

- | <u>Time</u> | <u>Paper No.</u> | |
|-------------|------------------|---|
| 3:15 | 24 | THE EFFECT OF FRACTIONS OF HUMAN SEMEN ON STIMULATION AND INHIBITION ON THE GROWTH OF <i>TRICHOMONAS VAGINALIS</i> . J.J. Daly Sr. and J.K. Sherman. |
| 3:30 | 25 | THE NEUROTRANSMITTER DOPAMINE STIMULATES PROLIFERATION OF <i>TOXOPLASMA GONDII</i> TACHYZOITES IN HUMAN FIBROBLAST CELL CULTURE. D. Goodwin , J. Strobl, T. Hrubec, B. Klein, A. Zajac, and D.S. Lindsay. |
| 3:45 | 26 | THE EFFECT OF THE METAM SODIUM ON VIABILITY AND INFECTIVITY OF <i>EIMERIA</i> OOCYSTS. R.H. Fetterer , M.C. Jenkins, K.B. Miska, and G.D. Cain. |
| 4:00 | 27 | EFFICACY OF NATURAL OR SYNTHETIC IMMUNOSTIMULATORS AGAINST <i>TRICHINELLA SPIRALIS</i> IN IMMUNOCOMPROMISED MICE. M.A. Shalaby , W.E. Melroy, A.R. Richins, L.F. Constancio, M.S. Rice, G.R. Kolluru, and L.K. Vredevoe. |

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- 4:15 **28** FUNCTIONAL CHARACTERIZATION OF THE MOSQUITO HEART AND ITS IMPLICATION IN MALARIA SPOOROZOITE TRANSPORT. **J.F. Hillyer**, J.G. King, and J.D. Glenn.
- 4:30 **29** EVIDENCE OF *HEPATOZOON CANIS* IN MISSISSIPPI DOGS. **A.S. Varela-Stokes**, C. Panuska, K. Lednum, B. Baughman, E. Chenney, and J. Cooley.

Friday Evening, August 14, 2009

7:00 pm – 10:00 pm WELCOME RECEPTION – Summit Ballroom

Saturday Morning, August 15, 2009

7:00 – 8:30am Editorial Board Breakfast, Private Dining Room

8:30 – 10:30am ASP PRESIDENT'S SYMPOSIUM, Tennessee Ballroom

Presiding: J. Caira, University of Connecticut, Storrs, CT

Theme: *Parasites on a Shrinking Planet.*

Time Paper
 No.

8:30 Introduction. **J. Caira.**

8:35 Welcoming Comment: **Senator Tim Burchett**, Tennessee, (District 7, Knoxville).

8:40 **30** STRANGE BEDFELLOWS: INVASIVE WILDLIFE AND INVASIVE PARASITES ON A SHRINKING PLANET. **W.F. Font** and M. Sukhdeo.

9:15 **31** OUR PARASITES ON A SHRINKING PLANET - IT' STILL A BIG WORLD FOR HOOKWORMS. **J.M. Hawdon** and M. Sukhdeo.

9:50 **32** DENGUE ON A SHRINKING PLANET: PRESENT AND FUTURE CHALLENGES FOR UNDERSTANDING AND CONTROLLING AN EMERGING MOSQUITO-BORNE INFECTION. **L.C. Harrington.**

10:25 Closing comments and questions. **J. Caira.**

10:30 – 11:00 **COFFEE BREAK**

11:00 – Noon **R. BARCLAY MCGHEE LECTURE**

84th Annual Meeting

Presiding: C.T. Atkinson, USGS, Chair, R. Barclay McGhee Committee

Time Paper
 No.

- 11:00 Introduction of Dr. G. Valkiunas, Center for Tropical Research,
 Institute of Ecology, Vilnius University, Vilnius Lithuania and
 Expert-member of the Lithuanian Academy of Sciences
- 11:10 **33** Observations on Blood Parasites of Birds in Costa Rica, **G.**
 Valkiunas.



Saturday Afternoon, August 15, 2009

1:00 – 3:00 STUDENT PAPER COMPETITION II, Salon B

Presiding: B. Fredensborg, University of Texas-Pan American, Edinburg, TX
 T. Ruhnke, West Virginia State University, Institute, WV

Time Paper
 No.

- 1:00 **34** IDENTIFYING THE BINDING SITES, RESPONSE ELEMENTS AND TARGET GENES OF HOOKWORM
 DAF-16. **X. Gao**, M.S. Zavada, and C.T. Faulkner.
- 1:15 **35** UNEXPECTED DIVERSITY AND SPECIFICITY IN FRESHWATER FISH PARASITES: EFFECTS OF
 PHYLOGENY, ECOLOGY AND PARASITE HABITAT. **S.A. Locke**, D.J. McLaughlin, and D.J.
 Marcogliese.
- 1:30 **36** MAKING LIFE EASIER: WHEN DOES A TREMATODE SKIP A HOST? **K. Herrmann** and R. Poulin.
- 1:45 **37** CRICKETS GROOM TO AVOID PARASITIZATION BY THE PARAITOID FLY *ORMIA OCHRACEA*. **C.**
 Vincent, G. Langford, and J. Janovy Jr.
- 2:00 **38** SPATIO-TEMPORAL VARIATION IN PARASITE BIOVOLUME AND ITS RELATIONSHIP TO NUMERICAL
 ABUNDANCE OF PARASITES IN A HOST POPULATION. **M.S. Sokolowski**, A.D.M. Dove, and S.B.
 Munch.
- 2:15 **39** PREVALENCE OF THE METACERCARIAE ASSOCIATED WITH WATERBIRD MORTALITY IN OPEN
 WATER SITES ON POOL 7 OF THE UPPER MISSISSIPPI RIVER WILDLIFE AND FISH REFUGE. **E.**
 Koppel and R.E. Sorensen.

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2:30 **40** ENVIRONMENTAL FACTORS AND THEIR IMPACT ON PARASITE INTENSITY AND DIVERSITY IN BALINESE LONG-TAILED MACAQUES (*MACACA FASCICULARIS*). **K.E. Lane**, W.E. Melroy, A.R. Richins, L.F. Constancio, M.S. Rice, G.R. Kolluru, and L.K. Vredevoe.

2:45 **41** PENTHOUSE PARASITES: POSITIONAL PREFERENCE OF AN ENDOPARASITIC COPEPOD WITHIN ITS NUDIBRANCH HOST. **M. Wolf**.

1:00-3:00 GENETICS, MOLECULAR BIOLOGY, IMMUNOLOGY – Salon C

Presiding: M. Belosevic, University of Alberta, Edmonton, AB, Canada
R. Kuhn, Wake Forest University, Winston-Salem, NC

Time Paper
 No.

1:00 **42** GENOTYPING AND EVIDENCE OF GENETIC EXCHANGE IN US ISOLATES OF *TRYPANOSOMA CRUZI*. **D.M. Roellig**, W.A. Fujita, M.Y. Savage, E.L. Brown, M.L. Yabsley, and D.B. Warnell.

1:15 **43** GAMMA IRRADIATION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS AFFECTS INTRACELLULAR LEVELS OF THE VIRAL SYMBIONT CPV. **M.C. Jenkins**, C. O'Brien, B. Rosenthal, and R. Fayer.

1:30 **44** GENETIC LINKAGE MAP OF THE HUMAN BLOOD FLUKE *SCHISTOSOMA MANSONI*. **C.D. Criscione**, C. Valentim, H. Hirai, P. LoVerde, and T.J.C. Anderson.

1:45 **45** MOLECULAR BIOLOGY AND EVOLUTION OF CANDIDATE IMMUNE RECEPTORS IN *DROSOPHILA*. **E.S. Keebaugh** and T.A. Schlenke.

2:00 **46** MICROSATELLITE ANALYSIS INDICATES THAT SOME STRAINS OF THE ESTUARINE PARASITE *PERKINSUS MARINUS* ARE ENDEMIC WHILE OTHERS ARE SHARED BETWEEN POPULATIONS. **P.C. Thompson**, B.M. Rosenthal, and M.P. Hare.

2:15 **47** BLACKHEAD DISEASE (HISTOMONIASIS): STRAIN VARIATION AS A FACTOR IN VIRULENCE. **L.R. McDougald**, L. Lollis, R.B. Beckstead, R.W. Gerhold and L.R. McDougald.

2:30 **48** ANTIBACTERIAL ACTIVITY OF NITRIC OXIDE IN THE MOSQUITO HEMOCOEL. **J.F. Hillyer** and T. Estévez-Lao.

2:45 **49** CHARACTERIZING THE TRANSCRIPTIONAL PROFILE OF *BIOMPHALARIA GLABRATA* AFTER IMMUNOLOGICAL CHALLENGE. **P.C. Hanington**, C.M. Lun, C.M. Adema, and E.S. Loker.

3:00 **50** *NEOSPORA CANINUM* PROFILIN-LIKE PROTEIN IS EXPRESSED BY TACHYZOITES AND REGULATES HOST CYTOKINE PRODUCTION. **W. Tuo**, X. Feng, L. Cao, R. Fetterer, and M. Jenkins.

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3:15 **144** EFFICACY OF A TSA-1 ENCODING DNA VACCINE AGAINST TRYPANOSOMA CRUIZI IN CONTROLLING VERTICAL TRANSMISSION IN ICR MICE. **W. Bullard**, S. Posey, C. Champion, N. Acuff, E. Dumonteil, C.A. Hall

3:30 – 3:45 **COFFEE BREAK**

3:45 – 5:40 **ASP STUDENTS' SYMPOSIUM, Salon B/C**

Presiding: J. Detwiler, Purdue University, West Lafayette, IN

Theme: *Parasites in Conservation: A riddle wrapped up in a mystery, inside an enigma.*

Time Paper
 No.

3:45 Introduction. **J. Detwiler.**

4:00 **51** PARASITES AND CONSERVATION: LESSONS FROM THE STUDY OF GLOBAL AMPHIBIAN POPULATION DECLINES. **L.K. Belden.**

4:25 **52** CONSERVATION MEDICINE IN THE GALAPAGOS ISLANDS. **P. Parker.**

4:50 **53** ROLE OF PARASITES IN BIOLOGICAL INVASIONS. **M. Torchin.**

5:15 Audience questions for the panel of guest lecturers.

5:30 Summary and closing remarks, **J. Detwiler.**

3:00 – 6:00 Auction Set Up, Summit Ballroom

Saturday Evening, August 15, 2009

6:00 – 7:00 Auction Preview

7:00 – 9:00pm **20th ANNUAL ASP STUDENT AUCTION**, Summit Ballroom

Sunday Morning, August 16, 2009

8:00 – 10:00 ASSOCIATE EDITORS SYMPOSIUM, Salon B

Presiding: M.V.K. Sukhdeo, Rutgers University, New Brunswick, NJ

<u>Time</u>	<u>Paper No.</u>	
8:00	54	HISTORIC PERSPECTIVES ON DISCOVERY AND DOGMA IN PARASITE IMMUNITY, THERAPEUTICS, AND SYSTEMATICS. G.W. Esch and M.V.K. Sukhdeo.
8:20	55	DRUG DISCOVERY FOR SCHISTOSOMIASIS: HIT AND LEAD COMPOUNDS IDENTIFIED IN A LIBRARY OF KNOWN DRUGS BY MEDIUM-THROUGHPUT PHENOTYPIC SCREENING. C.R. Caffrey , D.S. Ruelas, M.H. Abdulla, F. Xu, S. Štefanić, KC. Lim, J.H. McKerrow, B. Wolff, J. Snedecor, A.R. Renslo, J. Williams, S. Chen, M. Arkin, and R. Singh.
8:50	56	THE TANGLED TREES OF MALARIA PARASITES: PROBLEMS OF THE PAST, PRESENT PITFALL, AND PROSPECTS FOR PROGRESS. S.L. Perkins and M.V.K. Sukhdeo.
9:20	57	INCREASED SUSCEPTIBILITY TO IMMUNODEFICIENCY VIRUS INFECTIONS IN RHESUS MACAQUES WITH ACUTE SCHISTOSOMIASIS. W.E. Secor and M.V.K. Sukhdeo.
9:50		Audience question for the panel of guest lecturers, summary and closing remarks, G.W. Esch .

8:30-11:30 ECOLOGY – Salon C

Presiding: N. Negovetich, St. Jude Children’s Research Hospital, Memphis, TN
H. R. Yoder, Lamar University, Beaumont, TX

<u>Time</u>	<u>Paper No.</u>	
8:30	58	INTERACTIONS BETWEEN PARASITES AND POLLUTANTS IN YELLOW PERCH (<i>PERCA FLAVESCENS</i>) IN THE ST. LAWRENCE RIVER, QUEBEC, CANADA. D.J. Marcogliese , A.D. Gendron, C. Dautremepuits, and M. Fournier.

84th Annual Meeting

- 8:45 **59** LARVAL *ANISAKIS* SPP. IN SUBYEARLING CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*): AN INDICATION OF CHANGING OCEAN CONDITIONS IN THE NORTHERN CALIFORNIA CURRENT SYSTEM. **K.C. Jacobson**, R.E. Baldwin, and A.T. Claxton, J.P.Losee, M.B. Rew, and D. Teel.
- 9:00 **60** PARASITE SPECIES RICHNESS AS A METRIC TO ASSESS THE TROPHIC INTERACTIONS AND HABITAT QUALITY OF PACIFIC SALMON IN THE FRESHWATER AND MARINE ENVIRONMENT. **J.P. Losee**, K.C. Jacobson, and R.E. Baldwin.
- 9:15 **61** CERCARIAL BEHAVIOR PATTERNS AND HOST SPECIFICITY OF *GLYPHTELMINS* SPP. IN TADPOLES AND METAMORPHOSED ANURANS. **M.G. Bolek** and H.R. Tracy.
- 9:30 **62** PARASITISM AND HABITAT USE OF JUVENILE CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) IN THE COLUMBIA RIVER ESTUARY. **A.T. Claxton**, M. Bhuthimethee, and K.C. Jacobson.
- 9:45 **63** IMPACT OF COAL MINE EFFLUENT ON FISH PARASITE ASSEMBLAGES IN SOUTHERN ILLINOIS STREAMS. **A.T. Claxton** and J.R. Laursen.
- 10:00 – 10:15 COFFEE BREAK**
- 10:15 **64** TWO ADULT PARASITES FROM THE SAME FISH HOST OCCUPY DIFFERENT TROPHIC LEVELS. **S.E. Lettini** M.V.K. Sukhdeo.
- 10:45 **65** COMBINED IMPACT OF PARASITES AND PREDATORS ON WOOD FROG TADPOLES. **L.K. Belden** and J.M. Wojdak.
- 11:00 **66** GENETIC DIFFERENTIATION IN LARGE TURKEY LOUSE (*CHELOPISTES MELEAGRIDIS*) POPULATIONS REVEALS LIMITED MOVEMENT OF TURKEYS ACROSS THE MISSISSIPPI RIVER. **K.G. LeCompte** and S. Meagher.
- 11:15 **67** COMMUNITY AND GENETIC ANALYSES OF MACROPARASITES FROM PACIFIC SARDINE (*SARDINOPS SAGAX*) CAUGHT IN THE CALIFORNIA CURRENT SYSTEM. **R.E. Baldwin**, K.C. Jacobson, M.L. Johansson, and M.A. Banks.

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10:15 – 12:15 **41st COCCIDIOSIS CONFERENCE, Salon B**

Presiding: R.S. Seville, University of Wyoming, Laramie, WY

Time Paper
 No.

10:15 Introduction: **R.S. Seville**

10:25 **68** ESPECIALLY GREAT PHYLOGENETIC DIVERSITY CHARACTERIZES THE COCCIDIA INFECTING FISH.
B.M. Rosenthal, K. Molnar, G. Ostoros, and D.B. Dunams

10:55 **69** ECOLOGY AND PHYLOGENY OF AMPHIBIAN COCCIDIA: A REVIEW. **M.G. Bolek** and C. Whipps

11:25 **70** COCCIDIA FROM MAMMALS: INTERESTING INSIGHTS ON PHYLOGENETIC RELATIONSHIPS, HOST
SPECIFICITY AND MORPHOLOGY. **J. Kvicerova**

11:55 Roundtable Discussion with all participants and audience members.

Notes:

Sunday Afternoon, August 16, 2009

1:00 – 2:00 ASP PRESIDENTIAL ADDRESS, Tennessee Ballroom

Presiding: T.K. Graczyk, Johns Hopkins University, Baltimore, MD

1:00 Introduction of **Dr. BRUCE CONN**
Professor of Biology and Dean, School of Mathematics and
Natural Science, Berry College

1:10 **71** PARASITES ON A SHRINKING PLANET. **B. Conn.**



Bruce Conn,
ASP President

2:00 – 2:15 COFFEE BREAK

2:15 – 4:45 COMPANION ANIMAL PARASITIC COUNCIL SYMPOSIUM, Salon A

Presiding: M.A. Paul, Executive Director of the Companion Animal Parasite Council (CAPC), Anguilla, British West Indies

Time Paper
 No.

2:00 Introduction: **M.A. Paul.**

2:15 **72** PREVALENCE OF INTERNAL PARASITES OF DOGS AND CATS IN THE UNITED STATES: UPDATE OF A STUDY IN PROGRESS. **B. Blagburn** J.M. Butler, T. Land, J.D. Mount, J.C. Wright, S.E. Little, B. Griffin, W. Kelch, and J. Hostetler.

2:45 **73** *CRYPTOSPORIDIUM* AND *GIARDIA*: ZONOTIC IMPLICATIONS OF THOSE FOUND IN DOGS AND CATS. **D.D. Bowman.**

3:15 **74** *WOLBACHIA*: RATIONALE AND EVIDENCE FOR INCORPORATING DOXYCYCLINE INTO TREATMENT PROTOCOLS FOR *DIROFILARIA IMMITIS*. **C.T. Nelson.**

3:45 **75** SURVEY OF HEARTWORM PREVENTION PRACTICES AMONG DOG OWNERS AND TRAINERS IN NORTH AMERICA. **S. Patton**, A. Odoi, and B.W. Rohrbach.

4:00 **76** SURVEY OF RISK FACTORS FOR FAILURE OF HEARTWORM PROPHYLAXIS IN DOGS. **B.W. Rohrbach** A. Odoi, and S. Patton.

4:15 Questions, Summary, and Conclusions. **S. Patton.**

2:15 – 5:15 HOST PARASITE INTERACTIONS, Salon B

Presiding: V.A. Connors, University of South Carolina Upstate, Spartanburg, SC
 S.G. Kayes, University of South Alabama, Mobile, AL

<u>Time</u>	<u>Paper</u> <u>No.</u>	
2:15	77	MANIPULATION OF HOST TEMPERATURE AND DIET: EXPERIMENTS ON THREE SPECIES OF FLOUR BEETLES AND THEIR GREGARINE PARASITES. Z. Baumfalk , G.J. Langford, and J. Janovy Jr.
2:30	78	<i>HYMENOLEPIS DIMINUTA</i> INFECTION AND LIFE HISTORY TRADE-OFFS BY THE INTERMEDIATE HOST <i>TRIBOLIUM CONFUSUM</i> . A.W. Shostak , C. Roderick, J. Lawrence, S. Mortensen.
2:45	79	<i>TRYPANOSOMA CARASSII</i> HSP70 UP-REGULATES EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES IN GOLDFISH MACROPHAGES. A. Oladiran and M. Belosevic.
3:00	80	PATHOLOGY ASSOCIATED WITH ADULT AND MICROFILARIAE OF <i>DIROFILARIAEFORMA PULMONI</i> IN DELMARVA FOX SQUIRRELS (<i>SCIRUS NIGER CINEREUS</i>). M.C. Sterner III , D.E. Green, C. Meteyer, N.J.Thomas, and R.A. Cole.
3:15	81	PREVALENCE OF <i>PROFILICOLLIS ALTMANI</i> CYSTACANTHS (ACANTHOCEPHALA: POLYMORPHIDAE) IN PACIFIC MOLE CRABS, <i>EMERITA ANALOGA</i> , ON THE CENTRAL CALIFORNIA COAST. L.K. Vredevoe , L.F. Constancio, K.L. Stingely, L.M. Martini, A.L. Zopfi, and A.A. Schaffner.
3:30 – 3:45		COFFEE BREAK
3:45	82	PARASITE-RELATED MATING SUPPRESSION IN THE INTERMEDIATE HOST <i>CAECIDOTEA INTERMEDIUS</i> (ISOPODA): EFFECTS OF ANTIPREDATORY BEHAVIOR, ENERGY RESERVES AND NEUROMODULATION. T. Sparkes , E. Korkofigas, D. Kopp, S. Bierbower, and A. Murphy.
4:00	83	EXPERIMENTAL EVIDENCE FOR EVOLVED RESISTANCE TO AVIAN POX VIRUS AND MALARIA (<i>PLASMODIUM RELICTUM</i>) IN LOW ELEVATION HAWAII <i>AMAKIHI</i> . C.T. Atkinson , K. Schletz, L. Patch-Highfill, and D.Triglia, and S.I. Jarvi.
4:15	84	IDENTIFICATION OF ENDOPARASITIC HELMINTH EGGS IN THE STOMACH CONTENTS OF AN ARTICULATED MIOCENE TAPIR (<i>TAPIRUS POLKENSIS</i>) FROM THE GRAY FOSSIL SITE, NORTHEAST TENNESSEE. S.M. McConnell , M.S. Zavada, and C.T. Faulkner.
4:30	85	CHARACTERIZATION OF PARTIALLY PURIFIED EXCRETORY/SECRETARY (ES) ANTIGENS OF <i>GIGANTOCOTYLE EXPLANATUM</i> , A LIVER INFECTING AMPHISTOME PARASITE OF INDIAN WATER BUFFALOES <i>BUBALUS BUBALIS</i> M.K. Saifullah , A. Gul, and M.A. Sayed.

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4:45 **86** DIFFERENTIAL GLYCOTOPE EXPRESSION DURING THE MIRACIDIUM-TO-SPOROCTYST TRANSFORMATION OF *SCHISTOSOMA MANSONI*. **N.A. Peterson**, C.H. Hokkeeb, A.M. Deelderb, and T.P. Yoshino.

2:15 – 5:45 **TAXONOMY, SYSTEMATICS, PHYLOGENY, Salon C**

Presiding: R.S. Seville, University of Wyoming, Laramie, WY
 V.V. Tkach, University of North Dakota, Grand Forks, ND

Time Paper
 No.

2:15 **87** A REPORT ON A MONOGRAPH OF THE PHYLLOBOTHRIIDAE. **T. Ruhnke**, R. Vemulapalli, M. Ndao, and K.R. Kazacos.

2:30 **88** SPECIES LEVEL INVESTIGATIONS OF *PARAORYGMATOBOTHRIUM* USING ND1 MTDNA SEQUENCE. **K. Cappellari** and T. Ruhnke.

2:45 **89** SYSTEMATIC INVESTIGATIONS OF TWO NEW PHYLLOBOTHRIID GENERA FROM SHARKS. **R. Workman**, and T. Ruhnke, and J. Greenwood.

3:00 **90** ANATOMICAL VARIABILITY IN THE ACANTHOCEPHALA. **O.M. Amin** and M. Sukhdeo.

3:15 **91** CURIOSITIES IN THE ACANTHOCEPHALA. **O.M. Amin**, G. Langford, and J. Janovy Jr.

3:30 – 3:45 **COFFEE BREAK**

4:00 **92** ISOLATES OF THE ZOONOTIC PARASITE *TRICHINELLA SPIRALIS* POSSESS PREVIOUSLY UNRECOGNIZED VARIATION IN THEIR MITOCHONDRIAL GENOMES. **K.M. Webb** and B.M. Rosenthal.

4:15 **93** DELIMITING HOOKWORN SPECIES PARASITIZING PINNIPED HOSTS USING GENE TREES: PHYLOGENETIC EVIDENCE FOR HOST-SHARING AND SWITCHING. **S.A. Nadler**, K. Beckmen, C. Bell, B. Berón-Vera, A. Castinel, K. Burek Huntington, E.T. Lyons, D. Morgades, R. Norman, C. Pagan, and T.R. Spraker.

4:30 **94** MONOZOIC TAPEWORMS (EUCESTODA: CARYOPHYLLIDEA) PARASITIC IN NORTH AMERICAN SUCKERS: A CLOSED CHAPTER OR A BIG CHALLENGE? **T. Scholz** and J.S. Mackiewicz.

4:45 **95** A NEW SCHISTOSOME (DIGENEA: SCHISTOSOMATIDAE) FROM MURID RODENTS IN THE LAKE VICTORIA BASIN, KENYA AND ITS PHYLOGENETIC POSITION WITHIN THE *SCHISTOSOMA HAEMATOBIIUM* SPECIES GROUP. **B. Hanelt**, S.V. Brant, M.L. Steinauer, G.M. Maina, J.M. Kinuthia, L.E. Agola, I.N. Mwangi, B.N. Mungai, M.W. Mutuku, G.M. Mkoji and E.S. Loker.

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- 5:00 **96** A NEW SPECIES OF MEDICINAL LEECH FROM PERU SHINES LIGHT ON THE LEECHES OF MEXICO. **A.J. Phillips** and M.E. Siddall.
- 5:15 **97** PHYLOGENETIC ANALYSIS OF PARASITIC CEPHALOBOIDEA (NEMATODA). **L.E. Camp**, C.A. Pagan, O. Holovachov, and S.A. Nadler.
- 5:30 **98** BIODIVERSITY AND SYSTEMATIC INTERRELATIONSHIPS OF FISH BLOOD FLUKES (DIGenea: APOROCOTYLIDAE) INFECTING THE BODY CAVITY AND HEART OF SOUTH AMERICAN FRESHWATER CATFISHES (OSTARIOPHYSI: SILURIFORMES). **S.A. Bullard**, S.S. Curran, and M. Sabaj-Perez.
- 3:00 – 5:00 Poster Boards delivered, authors may set up posters, Summit 2

Sunday Evening, August 16, 2009

6:30 – 9:00 **Riverboat Dinner Cruise**

Monday Morning, August 17, 2009

8:30 – 10:30 **Authors complete poster setup**

8:30 – 10:15 **LIFE CYCLES, EPIDEMIOLOGY II, Salon B-C**

Presiding: L. S. Roberts, Homestead, FL
A.L. Shostak, University of Alberta, Edmonton, AB, Canada

<u>Time</u>	<u>Paper</u>	<u>No.</u>	
8:30	99		CONCURRENT INFECTIONS WITH <i>CRYPTOSPORIDIUM</i> SPP., <i>GIARDIA DUODENALIS</i> , <i>ENTEROCYTOZON BIENEUSI</i> , AND <i>BLASTOCYSTIS</i> SPP. IN NATURALLY INFECTED DAIRY CATTLE FROM BIRTH TO TWO YEARS OF AGE. R. Fayer M.T. Gómez-Muñoz, J.M. Trout, and M. Santín.
8:45	100		WESTWARD MOVEMENT OF <i>BITHYNIA TENTACULATA</i> AND 3 TREMATODES <i>CYATHOCOTYLE BUSHIENSIS</i> , <i>SPHAERIDIOTREMA GLOBULUS</i> AND <i>LEYOGONIMUS POLYOON</i> TO LAKE WINNIBIGOSHISH, MINNESOTA. R. Cole , C. Roderick, J. Lawrence, and S. Mortensen
9:00	101		EFFECTS OF AGE AND SEX ON PINWORM INFECTIONS IN AUSTRALIAN COCKROACHES. S. Meagher , K. Winters, and K. McCravy.
9:15	102		SITE DENSITY DISTRIBUTION OF YELLOW GRUB (<i>CLINOSTOMUM COMPLANATUM</i> SYN. <i>MARGINATUM</i>) IN POND-RAISED CHANNEL CATFISH (<i>ICTALURUS PUNCTATUS</i>). J. Singleton , J.J. Daly Sr., and R.J. Keller

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- 9:30 **103** OCCURRENCE OF *SARCOPTES SCABIEI* ON A VIRGINIA OPOSSUM. **R.D. McKown**
- 9:45 **104** PREVALENCE OF IGG ANTIBODIES TO *TOXOPLASMA GONDII* AND *ENCEPHALITOZOOM CUNICULI* IN CATS EXAMINED AT THE TEACHING HOSPITAL OF THE VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE. **V. Hsu** D.C. Grant, and D.S. Lindsay.
- 10:00 **105** PREVALENCE OF *CRYPTOSPORIDIUM* SPP., *GIARDIA* SPP., AND *TROGLODYTELLA* SPP. IN CHIMPANZEES (*PAN TROGLODYTES SCHWEINFURTHII*) FROM MAHALE MOUNTAINS NATIONAL PARK IN WESTERN TANZANIA. **D.S. Lindsay**, T. Kaur, and J. Singh

10:30 – Noon **POSTERS, COFFEE, SNACKS, Summit 2**

All authors please stand with you posters from 10:30 – Noon.

BIOCHEMISTRY, PHYSIOLOGY

- 106** COMPARATIVE CHEMICAL ANALYSIS USING ENERGY DISPERSIVE X-RAY ANALYSIS (EDXA) OF THREE SPECIES OF ACANTHOCEPHALA. **N.A. Radwan**, R.A. Heckmann, O.M. Amin, M.D. Standing, and D.L. Egget.
- 107** FINE STRUCTURE AND ENERGY DISPERSIVE X-RAY ANALYSIS (EDXA) OF THE PROBOSCIS HOOKS OF *RHADINORHYNCHUS ORNATUS* (RHADINORHYNCHIDAE: ACANTHOCEPHALA). **N.A. Radwan**, R.A. Heckmann, O.M. Amin, and M.D. Standing.

CELL BIOLOGY

- 108** ANALYSIS OF THE *NEOSPORA CANINUM* ARGONAUTE PROTEIN IN TACHYZOITES. **L. Cao**, X. Feng, J.P. Dubey, and M. Jenkins, and X. Zhang.
- 109** EFFICACY OF DNA VACCINATION AGAINST SCHISTOSOMIASIS INFECTION USING *SCHISTOSOMA MANSONI* ALDOLASE GENE THROUGH DIFFERENT ROUTES OF INJECTION. **T.M. Diab**, M.A. Saber, O.M. Hammam, A. Karim, A. Medhat, M.S. Khella, and E. El-Dabaa.
- 110** *FASCIOLA GIGANTICA*: PARASITOLOGICAL AND SCANNING ELECTRON MICROSCOPY STUDY OF THE *IN VITRO* EFFECTS OF IVERMECTIN AND/OR ARTEMETHER. **T.M. Diab**, S.S. Mahmoud, and H. Mansour.

ECOLOGY

- 111** INTERACTIONS BETWEEN A SUITE OF TREMATODES INFECTING THE HORN SNAIL, *CERITHIDEA PLICULOSA* IN A NORTHERN GULF OF MEXICO SALT MARSH. **J.J. O'Brien**, R.E. Nowlin, M.S. Pruitt, and J.M. Dean.

- 112 DISTRIBUTION AND ABUNDANCE OF AN ECHINOSTOMATID TREMATODE IN OYSTERS FROM SOUTHERN TEXAS. **M. Rodriguez** and B.L. Fredensborg.
- 113 GENETICS MEETS ECOLOGY: MOLECULAR AND POPULATION DATA SUPPORT THE PRESENCE OF FOUR SPECIES OF PHILOMETRIDS IN THE SOUTHERN FLOUNDER, *PARALICHTHYS LETHOSTIGMA*. **I. de Buron**, S.C. France, V.A. Connors, and W.A. Roumillat.
- 114 POPULATION AND INFECTION DYNAMICS OF *DAUBAYLIA POTOMACA* (NEMATODA: RHABDITIDA). **M.R. Zimmermann, K.E. Luth, L.E. Camp, J. Rodriguez, and G.W. Esch.**

GENETICS & MOLECULAR BIOLOGY

- 115 GENE REGULATION IN THE SNAIL, *BIOMPHALARIA GLABRATA*. **J.E. Humphries**, J.W. Antony, and N.R. Grattan.
- 116 *SCHISTOSOMA MANSONI* CAMP-DEPENDENT PROTEIN KINASE (PKA): A POTENTIAL NEW DRUG TARGET. **B.E. Swierczewski and S.J. Davies.**
- 117 REAL-TIME PCR/RFLP ASSAY TO DETECT GIARDIA INTESTINALIS GENOTYPES IN HUMAN ISOLATES WITH DIARRHEA IN EGYPT **M. Helmy.**

HOST PARASITE INTERACTIONS

- 118 INFECTIONS WITH GEOGRAPHICALLY AND GENETICALLY DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI* IN TWO NORTH AMERICAN RESERVOIR HOSTS INDUCE DISSIMILAR INFECTION DYNAMICS. **D.M. Roellig**, A.E. Ellis, M.J. Yabsley, and D.B. Warnell.
- 119 EXPERIMENTAL INFECTION OF TWO SOUTH AMERICAN ANIMAL RESERVOIRS WITH DISTINCT STRAINS OF *TRYPANOSOMA CRUZI*. **D.M. Roellig**, K. McMillan, A.E. Ellis, M.J. Yabsley, and D.B. Warnell.
- 120 *KUDOIA INORNATA*, A MYXOSPOREAN INFECTING SKELETAL MUSCLES OF THE SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*: TAXONOMY AND PATHOGENICITY. **I. de Buron**, I. Dykova, I. Fiala, and W.A. Roumillat.
- 121 HUMORAL IMMUNE RESPONSE OF CREOLE AND MIXED BREED HORSES AGAINST NATURAL INFECTIONS WITH *TRYPANOSOMA EVANSI* IN THE VENEZUELAN PLAINS. **R.D. Meléndez**, M.D. Forlano, and J.L. Canelón.
- 122 IDENTIFICATION OF PARASITE LIGANDS BY SCREENING A *CRYPTOSPORIDIUM PARVUM* CDNA T7 PHAGE DISPLAY LIBRARY. **J. Yin**, A. Guo, Y. Su, J. Song, Y. Zhang, and Q. Chen.

- 123** TRANSMISSION TO THE FREE-LIVING STAGE IN *ACANTHOCEPHALUS DIRUS* (ACANTHOCEPHALA): ARE EGGS DISPERSED BY DEFINITIVE HOSTS OR MATURE FEMALES? **T. Sparkes, D. Kopp, D. Elke,** and **L. Rodriguez.**
- 124** VISCERAL LEISHMANIASIS IN *CERDOCYON THOUS*. **W.A. Starke-Buzetti,** M. da Silva Tenório, K.I. Tasca, and N.M Gual Pimenta Queiroz, L. de Oliveira Sousa, C.M. Nunes, and J. Assis.
- 125** NORTH AMERICAN AND AFRICAN NEMATOMORPH HOST SPECIFICITY IN SIX SPECIES OF AQUATIC SNAILS. **M.G. Bolek** and A. Kubat.
- 126** ENDOPARASITES OF GROUND SQUIRRELS (*SPERMOPHILUS CITELLUS*) FROM THE CZECH REPUBLIC AND SLOVAKIA. **J. Kvicerova,** M. Jan, H. Stepanka, N. Petra, and U. Jitka.
- 127** ENDOPARASITES OF THE GENUS *APODEMUS* (RODENTIA: MURIDAE) FROM THE SLOVAK REPUBLIC. **J. Kvicerova,** J. Fricova, L. Mosansky, and M. Stanko.
- 128** GASTROINTESTINAL PARASITES OF THE PELAGIC STINGRAY (*PTEROPLATYTRYGON VIOLACEA*) FROM THE WESTERN NORTH ATLANTIC OCEAN. **M. Taylor,** H. Laubach, and D. Kerstetter.
- 129** LIFE HISTORY AND HISTOPATHOLOGY OF *PHYLLODISTOMUM* SP. (DIGenea: GORGODERIDAE) IN THE YELLOW SANDSHELL, *LAMPSILIS TERES* (RAFINESQUE, 1820) (BIVALVIA: UNIONIDAE) IN LINE CREEK, ALABAMA, USA. **M.B. Ferrell** and S.A. Bullard.

IMMUNOLOGY

- 130** EVALUATION OF A RAPID IMMUNOCHROMATOGRAPHIC ASSAY FOR DETECTION OF *TRYPANOSOMA CRUZI* ANTIBODIES IN WILDLIFE RESERVOIRS. **D.M. Roellig,** M.J. Yabsley, and E.L. Brown.
- 131** CYTOKINE PRODUCTION NAÏVE MURINE DENDRITIC AND SPLEEN CELLS IN RESPONSE TO *NEOSPORA CANINUM* STIMULATION. **X. Feng,** N. Zhang, and W. Tuo.

LIFE CYCLES & EPIDEMIOLOGY

- 132** SURVEY OF ACARICIDE RESISTANT AND MANAGEMENT FACTORS THAT INDUCE RESISTANCE TO ACARICIDES IN *RHIPICEPHALUS MICROPLUS* IN TAMAULIPAS, MEXICO. **C.A. Cantú** and G. Zeferino.
- 133** VITAL RATES OF THE LIFE HISTORY STAGES OF THE PARASITIC DINOFLAGELLATE *AMYLOODINIUM OCELLATUM*. **R.B. Blaylock,** I. Masson, and J.M. Lotz.

- 134** SPATIAL DISTRIBUTION OF YELLOW GRUB (*CLINOSTOMUM COMPLANATUM*) IN SMALLMOUTH BASS (*MICROPTERUS DOLIMIEUI*) FROM CROOKED CREEK (AR) AS DETERMINED BY METACERCARIAL CYST COUNTS IN THE GILL-MOUTH SITES ONLY. **J.J. Daly Sr.**

TAXONOMY, SYSTEMATICS, PYLOGENY

- 135** THE LECANICEPHALIDEAN FAUNA OF THREE SPECIES OF EAGLE RAYS OF THE GENUS *AETOMYLAEUS* (MYLIOBATIFORMES: MYLIOBATIDAE). **K.R. Koch** and K. Jensen.
- 136** BIOTIC CONVERGENCE OF THE GENUS *RHABDIAS* (NEMATODA) IN MEXICO. **E.A. Martínez-Salazar**, G. Pérez-Ponce de León, O. A. Flores-Villela, and V. León-Règagnon.
- 137** MORPHOLOGY OF FREE-LIVING ADULTS OF THREE PARASITIC NEMATODE *RHABDIAS*. **E.A. Martínez-Salazar** and A. Pires da Silva.
- 138** DISCERNABLE BUT LIMITED INTROGRESSION HAS OCCURRED WHERE *TRICHINELLA NATIVA* AND THE T6 GENOTYPE OCCUR IN SYMPATRY. **D.B. Dunams**, D. Zarlenga, M. Reichard, L. Torretti, E. Pozio, and B. Rosenthal.
- 139** RELATIONSHIPS OF *EIMERIA CALLOSPERMOPHILI* (APICOMPLEXA: EIMERIIDAE) PARASITES FROM SCIURID HOSTS BASED ON ITS1 AND ITS2 RDNA. **R.S. Seville** and D. Motriuk-Smith.

VECTOR BIOLOGY

- 140** DISTRIBUTION OF SPOTTED FEVER-GROUP RICKETTSIAE IN CANINES FROM TENNESSEE. **M.E. Rowland** J. Maloney, J. Huang, J.R. Dunn, R. Carpenter, T.F. Jones, and A.C. Moncayo.

Notes:

Monday Afternoon, August 17, 2009

1:00 – 1:50 H.B. WARD LECTURE, Tennessee Ballroom

Presiding: C.D. Criscione, Texas A&M University, College Station, TX
A. Kuris, University of California, Santa Barbara



Kevin Lafferty, 2009 recipient of H.B. Ward Medal

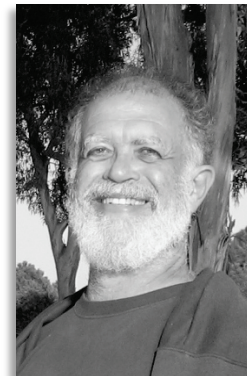
Time Paper
 No.

1:00 Introduction of Dr. KEVIN LAFFERTY
 US Geological Survey

1:10 **141** ACCEPTANCE OF THE 2009 HENRY BALDWIN WARD MEDAL, **K.D. Lafferty.**

1:50 – 2:40 Eminent Parasitologist Lecture, Tennessee Ballroom

Presiding: R. Hechinger
Marine Science Institute, University of California, Santa Barbara



Armand Kuris
Eminent Parasitologist 2009

Time Paper
 No.

1:50 Introduction of Dr. ARMAND KURIS. Marine Science Institute,
 University of California, Santa Barbara.

2:00 **142** Parasites approach the Darwinian Demon: adaptations, evolutionary
 wormholes and their ecosystem visibility, **A. Kuris.**

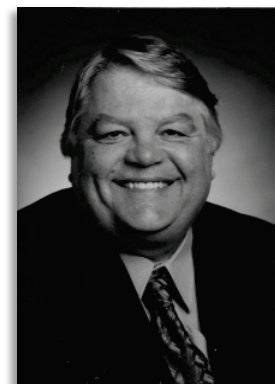
2:40 – 3:00 COFFEE BREAK

3:00 – 4:30 ASP AWARDS and BUSINESS MEETING

ASP AWARDS

CLARK P. READ MENTOR AWARD LECTURE, TN Ballroom

Presiding: P.C. Hanington
University of New Mexico, Albuquerque, NM



Mike Belosovic,
2009 C.P. Read Mentor
Award Winner

84th Annual Meeting

<u>Time</u>	<u>Paper</u>	<u>No.</u>
3:00	Introduction of Dr. MIKE BELOSOVIC, FRSC, Distinguished University Professor, Biological Sciences, University of Alberta, Edmonton, Canada.	
3:10	143	Mentor and mentee: A lasting relationship, M. Belosovic.

ASHTON CUCKLER NEW INVESTIGATOR AWARD

Presiding: K. Lafferty, US Geological Survey

The recipient of the 2009 New Investigator Award is Dr.
RYAN F. HECHINGER
Marine Science Institute, University of California, Santa Barbara



Ryan Hechinger,
2009 New Investigator Award

WILLIS A. REID, JR. STUDENT RESEARCH GRANT AWARDS

Presiding: L. Couch, University of New Mexico, Albuquerque, NM

BEST STUDENT PRESENTATIONS AND MARC DRESDEN TRAVEL GRANT AWARDS

Presiding: R.B. Blaylock, The University of Southern Mississippi, Ocean Springs, MS

ASP BUSINESS MEETING

Presiding: B. Conn, Berry College, Mount Berry, GA

Thank you for attending this year's ASP meeting and have a safe trip home.

See you June 22-25, 2010 at our next meeting in Colorado Springs, CO!

1

INVESTIGATING THE ROLE OF ANOPHELES SGS PROTEINS IN PLASMODIUM-MOSQUITO INTERACTIONS AND IN IMMUNITY.

J.G. King and J.F. Hillyer, Vanderbilt University.

Malaria remains one of the most serious problems facing mankind. The invasion of the mosquito salivary glands is a key *Plasmodium*-mosquito interaction that is necessary for transmission to vertebrates. A salivary gland specific protein named AaSGS1 was recently shown to be important during the invasion of *Aedes aegypti* salivary glands by *Plasmodium gallinaceum* sporozoites. Four homologs were identified bioinformatically in the *Anopheles gambiae* genome and named AgSGS2, 3, 4 and 5. The SGSs are large proteins (~380 kDa), predicted to be membrane-bound, and only share sequence homology with *Wolbachia* (Proteobacteria) proteins and the viral/bacterial YD-repeat protein family. Recent work from others suggests that horizontal gene transfer from *Wolbachia* led to the origin of mosquito SGS. Despite advances, the function of SGS and its involvement in the human malaria parasite's life cycle remains unknown. In the current study, RT-PCR and western blot analyses showed that the expression of AgSGS4 and AgSGS5 is salivary gland specific and occurs only in female mosquitoes. Immunohistochemistry showed that AgSGS4 and 5 localize primarily on the apical and basal surfaces of the distal-lateral lobes of the salivary glands, the regions sporozoites bind before invading. Interestingly, Western analyses showed that AgSGS4 and 5 are secreted into the saliva and are also released into the hemolymph following injury and blood feeding. Salivary gland and hemolymph AgSGS differ in mass and their respective masses correspond to predicted serine protease cleavage sites. We hypothesize that a prophenoloxidase-activating protease cleaves AgSGS, implicating these proteins in insect wound healing and immune responses. Possible SGS functions will be outlined, along with ongoing experiments examining AgSGS involvement in salivary gland invasion and insect immunity.

2

ORAL TRANSMISSION OF *TRYPANOSOMA CRUZI* WITH OPPOSING EVIDENCE FOR THE ROLE OF CARNIVORY IN HORIZONTAL TRANSMISSION.

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Trypanosoma cruzi, the causative agent of Chagas disease in humans, has become a parasite of increasing interest in the United States following the diagnosis of two human autochthonous cases within the last year. Maintenance of *T. cruzi* in native US wildlife populations increases the potential for zoonotic transmission in North America. While considerable research has been conducted on the molecular evolutionary ecology of *T. cruzi* in recent years, transmission studies pertaining to the sylvatic cycle are limited. In the southeastern US, only two vectors (*Triatoma* spp.) are present; however, the prevalence of *T. cruzi* in raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) can be high. To investigate an alternative non-vector-based transmission method, we tested the hypothesis that raccoons scavenging infected hosts can result in infection. Macerated tissue from selected organs infected with the amastigote stage of *T. cruzi* was orally administered to experimental groups of raccoons (n=2/group) at 2, 12, or 24 hours after collection of the tissue samples. Additionally, raccoons in control groups were inoculated intravenously (n=2) or per os (n=1) with trypomastigotes. To further elucidate transmission routes of *T. cruzi* to raccoons, infected *Rhodnius prolixus* were fed to raccoons (n=2). Attempts to detect minicircle kDNA from blood and tissue, seroconversion and parasitemias revealed that no raccoons became infected after ingestion of amastigote-infected tissues collected from parasitemic animals. However, per os transmission can occur by ingestion of the infective trypomastigote stage or infected reduviid bugs. We can conclude from these findings that oral transmission of *T. cruzi* may be a route of infection for wildlife in sylvatic cycles, but the scavenging behavior of animals is not a factor.

 3

 ASSESSING PREDICTABILITY IN PARASITE COMMUNITIES: A MULTI-YEAR, MULTI-SITE STUDY OF *DACTYLOGYRUS* SPP. ON A NATIVE NORTH AMERICAN CYPRINID.

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A high degree of host specificity, characteristic of *Dactylogyrus* spp., reveals opportunities for, and suggests mechanisms of, parasite dispersal because the parasite's movement is restricted by that of its particular host. This study examined the ways in which a community of congeneric parasite species distributed themselves on a population of *Pimephales promelas* (fathead minnow), at 3 distinct but geographically proximate streams in southeastern Nebraska, to assess ways in which populations of parasites respond to abiotic factors and distribute themselves in nature. The study also explored how, despite seasonal and yearly population fluctuations, species are maintained in the environment. Over a period of three years, the study determined that at these three sites: (1) are not sharing *Dactylogyrus* species with other cyprinids; (2) fish size and sex are not predictive of *Dactylogyrus* infection; (3) *Dactylogyrus* spp. populations respond differently to abiotic and biotic factors; (4) *Dactylogyrus* spp. vary (not always predictably) in their seasonal occurrence, and (5) the order of *Dactylogyrus* spp. abundance is independent of environment. This study of seasonal dynamics constitutes the first multi-

year, multi-site study of a complex community of *Dactylogyrus* spp. on a Native North American cyprinid species.

4

CO-OCCURRENCE OF HAEMATOLOECHUS COMPLEXUS (TREMATODA: HAEMATOLOECHIDAE) AND *RHABDIAS JOAQUINENSIS* (NEMATODA: RHABDIASIDAE) IN THE PLAINS LEOPARD FROG (*RANA BLAIRI*).

M.S. Vhora, G. Langford and J. Janovy Jr., School of Biological Sciences, University of Nebraska-Lincoln.

Haematoloechus spp. and *Rhabdias* spp. are common parasites that infect the lungs of anurans. These parasites are often reported from the same host species, however no published studies have addressed the co-occurrence of these parasites in single hosts. In this study we attempted to determine if *Haematoloechus complexus* and *Rhabdias joaquinensis* co-occurred in the plains leopard frog (*Rana blairi*) sampled from eastern Nebraska. In October 2008, we sampled 42 plains leopard frogs using minnow traps and hand capture (at night) from Elk Creek, Lancaster County, Nebraska. The trachea and lungs were removed and examined for *Haematoloechus* spp. and *Rhabdias* spp. using standard dissecting techniques, worms were designated whether they were found in the left or right lung. The parasites collected were preserved and identified as *H. complexus* and *R. joaquinensis*. We found a high prevalence and mean intensity of both *H. complexus* and *R. joaquinensis*, and our results suggest these lung parasites co-occur in this frog population. Given that worms co-occur it seems unlikely competitive exclusion is acting on this system, however more subtle types of competition cannot be ruled out.

5

Molecular Phylogeny of *Rhabdias* spp.: Implications for Lungworm Life Cycles and Host Specificity.

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Members of the nematode genus *Rhabdias* are among the most commonly encountered helminths of amphibians and reptiles around the world. In recent years lungworms have increased in popularity as a model system for the study of host-parasite interactions. Unfortunately, lungworms can be difficult to identify due to a lack of unique morphological characters in many species and the unknown potential for species complexes. Mis-identification can lead to problems when describing the life cycle and host specificity of morphologically similar species. The goal of this study was to elucidate the relationships of several species of lungworms using a molecular phylogeny and discuss implications for lungworm host specificity and life cycles. PCR was used to amplify approximately 2,100 base pairs of rDNA that included

the 39 end of 18S gene, the ITS region (ITS1, 5.8S, ITS2), and the 59 end of the 28S gene. Maximum parsimony and maximum likelihood trees were constructed using PAUP, and a Bayesian inference tree was constructed with Mr. Bayes. Host specificity and life history data were collected using both experimental infections and samples from anurans and snakes collected in nature. Our results suggest that host specificity varies widely within lungworms and is constrained by ecological factors in some species. The phylogeny suggests anuran lungworms form a tight clade that is distantly related to snake lungworms, life history data support this division. However, sequences from related genera (e.g. *Entomelas*) are needed before the relationship between snake and anuran lungworms can be resolved.

6

Population Genetics and Host Specificity of *Spirorchis scripta* (Digenea: Spirorchiidae) in Emydid Turtles.

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Spirorchis scripta is a common turtle blood fluke distributed across North America, making it an ideal species for studies of population genetics. Specimens were obtained from freshwater turtles collected east of the Rocky Mountains and partial COX1 mtDNA was sequenced for 58 worms. Maximum parsimony analysis was performed on the resulting 848 bp COX1 fragments and revealed two strongly supported clades: a *Trachemys* clade and an emydid clade. The *Trachemys* clade consisted of 22 worms taken only from the slider turtle, *Trachemys scripta*, whereas the emydid clade was comprised of 36 worms from *T. scripta*, *Chrysemys picta*, *Graptemys ouachitensis*, and *Pseudemys concinna*. A 95% statistical parsimony network mirrored the maximum parsimony analysis. These results reveal the existence of two *S. scripta* lineages, one a *T. scripta* specialist and the other an emydid generalist. These two lineages, while morphologically indistinguishable, are distinct in their abilities to parasitize different definitive host species, even when collected syntopically. Nineteen worms were removed from *T. scripta* and *C. picta* collected from a small ditch bordering Reelfoot Lake, Tennessee. Fifteen of these worms belonged to the emydid clade while the remaining four worms belonged to the *Trachemys* clade. In this ditch worms in the *Trachemys* clade parasitized only *T. scripta*, strongly suggesting that worms from this lineage cannot develop in other emydid turtles, even though cercariae must surely come into contact with *C. picta*. Intriguingly, both lineages of *S. scripta* were found to exist in a single *T. scripta*, suggesting that the lineages are evolutionarily stable and do not sexually reproduce with one another. This work is supported by NSF grant 0515460.

Trypanosoma of Australian Freshwater Turtles: Occurrence and Diversity.

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The genus *Trypanosoma* is comprised of approximately 500 species of obligate haemoflagellate parasites found globally in all classes of vertebrates. Only three species of trypanosomes have been reported from freshwater turtles, suggesting that our understanding of species diversity among these parasites is lacking. The present study sought to elucidate trypanosome diversity and prevalence in 194 freshwater turtles representing 13 species and three genera (*Chelodina*, *Emydura*, and *Elseya*) collected from seven geographical regions across Australia. *Trypanosoma* prevalence was determined using microscopy and polymerase chain reaction (PCR). Light microscopy was found to underestimate prevalence; only 39.1% of turtles were positive using this technique compared to 83.2% using PCR. Trypanosome diversity was assessed through the examination of 18S and ITS2 rDNA sequence data. Phylogenetic analysis of the 31 ITS2 sequences produced two strongly supported clades: a *Chelodina* clade that consisted of 20 trypanosome sequences taken from seven *Chelodina* species and *Elseya latisternum* and an *Emydura* clade comprised of 11 trypanosome sequences taken from four *Emydura* species and *El. latisternum*. The resulting *Trypanosoma* phylogeny showed congruence with Australian chelid turtle phylogeny, reflecting the evolutionary divergence of *Chelodina* and *Emydura*. The parallel phylogenies suggest that host and parasite lineages evolved in synchrony, however, examination of 18S rDNA and morphological data do not provide convincing evidence that these lineages represent distinct species. Thus, the first continent-wide survey of turtle trypanosomes suggests that *Trypanosoma chelodinae* is the only trypanosome species in the freshwater turtles of Australia. This work is supported by NSF grant 0515460.

Taxonomy and Evolution of the Symbiotic *Chaetogaster limnaei* Complex (Annelida).

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Chaetogaster limnaei is a well-known oligochaete that infects freshwater molluscs worldwide. To date, two subspecies are recognized from pulmonate gastropods: *C. l. limnaei*, an ectosymbiont that lives in the snail's mantle cavity, and *C. l. vaghini*, an endoparasite that lives in the snail's nephridium. The two forms differ in morphological characteristics and reproductive timing, but have yet to be recognized as

separate species. Furthermore, the origins of the commensal and parasitic forms are unknown, i.e., if each form represents an independent invasion of the molluscan host, or if the commensal form gave rise to the parasitic form, or vice versa. To date, I have confirmed the morphological differences between the subspecies using scanning electron microscopy, and I have used molecular sequence data to reveal differences in the cytochrome oxidase I subunit (cox-I) of both symbionts. Molecular results suggest that sequence differences range from 13.2-15.2%, which is comparable to the differences measured between the symbionts and free-living species (15-19%) and between two different free-living species (14-15%). A neighbor joining tree reveals that the symbionts are sister taxa, using free-living species as outgroups. In total, these results suggest that the subspecies of *Chaetogaster* are separate biological species, but further data from other populations is required before making an assessment on the origins of the symbiotic lifestyle.

9

Characterization of the Cestode Fauna of a Deep Water Squaliform Shark from Madeira.

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While recent attention has focused on parasites of elasmobranchs in tropical regions, many of the deep, colder water squaliform shark species have never been examined for cestodes. Based on records that do exist, it appears that the cestode faunas of squaliforms from deeper waters are relatively depauperate. A collecting trip in January 2009, to the Portuguese Island of Madeira resulted in the examination of 31 specimens of the deep water species *Centrophorus squamosus*, the Leafscale Gulper Shark. The spiral intestines, and in a few cases the stomachs, were examined for metazoan parasites. Parasites were removed and fixed in ethanol or formalin. Surprisingly, no adult cestodes or adult nematodes were recovered; the fauna consisted entirely of larval forms. Light microscopy, histology, scanning electron microscopy, and molecular methods were used to identify the cestodes. Only 35.5% of the sharks were found to be infected with cestodes. The fauna of the spiral intestine consisted predominantly of trypanorhynch larva encysted in the intestinal wall. These appear to represent 2 superfamilies of trypanorhynchs. The intensity of infection of the most prevalent species ranged from 1 to 6 worms per host. One shark hosted 2 trypanorhynch larvae in its stomach wall. Only 4 sharks hosted tetraphyllideans. Three of these hosted 1 to 3 unencysted larvae, consistent with the poorly known Bilucularia, in their spiral intestine. One shark hosted 3 larvae of a trilobulated form, which may represent a new taxon in its stomach. Larval nematodes were also found in the spiral intestines of 22.6% of the sharks; however, none of these sharks also hosted cestodes. These data are consistent with the notion that squaliform sharks exhibit depauperate cestode faunas relative to other elasmobranch orders.

10

Effects of the Acanthocephalan Parasite, *Profilocollis altmani*, on Tissue Carotenoid Concentrations and Egg Mass of the Pacific Mole Crab, *Emerita analoga*.

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Profilocollis altmani commonly parasitizes *Emerita analoga* along the Pacific coast of North and South America, but the impact of parasitism on the crab intermediate host is poorly understood. *Emerita* are nearshore filter feeders that obtain carotenoids from their plankton diet. At least eight carotenoid pigments have been identified in ovaries, carapace, eggs, and hemolymph of *E. analoga*. It may be that *P. altmani* alters the allocation of dietary carotenoids to *E. analoga* tissues and eggs, possibly impacting host health and fecundity. The parasite may also acquire pigments normally allocated to the host. We investigated the relationship between crab parasite load and the carotenoid content of individual crab ovaries, esophagus, carapace and eggs. Additionally, the egg masses between parasitized and unparasitized females were compared. Crabs were collected between 2008-2009 from Pismo Beach, California. Carotenoids extracted from each tissue type were analyzed by UV-vis spectrophotometry. Preliminary results indicate the relationship between carotenoid content and parasite load differed between tissue types, season, and crab size classes. In particular, female crabs in small vs. large size classes may allocate carotenoids to their eggs and carapace differently in response to parasite load. However, no significant effects of infection on overall egg mass were apparent in *E. analoga*. Host carotenoid profile patterns differed between tissues and some with respect to parasite loads. Cystacanths infecting crabs contain carotenoids, but their UV-vis profile appears to differ from that of host tissues. This suggests that the parasites may modify host-acquired carotenoids.

11

Effects of *Profilicollis altmani* (Acanthocephala: Polymorphidae) on Tissue Carotenoid Profiles of the Pacific Mole Crab, *Emerita analoga*.

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Emerita analoga serves as an intermediate host for the acanthocephalan parasite *Profilicollis altmani* along the Pacific coast of North and South America. Acanthocephalan eggs are ingested by crabs during filter feeding and terminate their development in this host as cystacanths, which typically accumulate in the crab hemocoel near the digestive gland and female ovarian tissue. *Emerita* are nearshore filter feeders that obtain carotenoids from their plankton diet. At least eight carotenoid pigments have been identified in ovaries, carapace, eggs, and hemolymph of *E. analoga*. We investigated whether *P. altmani* cystacanths 1) modify host allocation of carotenoids to the carapace and eggs and 2) acquire host carotenoids. Crabs were collected between 2008- 2009 from Pismo Beach, California and pooled into similar size, sex, and parasite load classes for analysis. To examine the relationship between parasite load and host carotenoid content, pooled tissue carotenoid concentrations were analyzed by reverse-phase HPLC. We confirmed that the primary and secondary carotenoids present in egg tissue were beta carotene and astaxanthin, respectively. Additionally, preliminary results revealed that the crab egg concentration of astaxanthin, but not beta carotene, decreases as parasite load increases. Moreover, the carotenoid profile of cystacanths differs from that of host tissues, with an astaxanthin-like compound predominating. This suggests that the parasites may acquire and modify astaxanthin from the host.

12

The Taxonomic Status of the Enigmatic Elasmobranch Tapeworm *Tenia narinari* and an Investigation of Its Mode of Attachment.

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In 1917, MacCallum described a new tapeworm species, *Tenia narinari*, with a highly unusual scolex morphology, but did not formally place it into a higher taxon. *Tenia narinari* was described from the spotted eagle ray, *Aetobatus narinari*, and has not been reported since its original description. Recent collections of lecanicephalidean tapeworms from the stingray species *Himantura undulata*, *H. pastinacoides*, and *H. uarnacoides* from Borneo and *H. cf. uarnak* (reticulated form) from Australia

revealed the presence of tapeworms consistent with the description of *Tenia narinari*. Specimens found were prepared for light microscopy as whole mounts and histological sections and for scanning electron microscopy. Specimens from the 4 host species revealed morphological differences in scolex size, apical organ structure, and overall length that suggest each ray species hosts its own species of the *Tenia narinari* type. Owing to its unique scolex morphology, a new lecanicephalidean genus will be erected to house *Tenia narinari* and the new species. Members of this new genus possess a multi-tiered and eversible apical modification of the scolex proper and apical organ, that are more than 2 times the length of the scolex proper and can be completely retracted into the scolex proper. Internally, 2 distinct glandular regions are visible. The various regions of the scolex were characterized to facilitate comparison between species and different states of eversion within species. The unusual morphology of the scolex, especially the presence of the 2 distinct glandular regions, warranted investigation into the mode of attachment of these worms to the intestinal epithelium of their hosts. Histological sections of scolices were stained using the periodic acid-Schiff reaction and traditional staining methods. Worms were found to possess regions within the apical organ that likely contain glycogen or mucosubstances, which suggest their use in the adhesion of the scolex to the intestinal epithelium of their host.

13

A Recombinant Bp1 Antigen-Based Enzyme Linked Immunosorbent Assay for Diagnosis of *Baylisascaris procyonis* Larva Migrans in Humans.

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Larva migrans caused by the raccoon roundworm, *Baylisascaris procyonis*, is an emerging public health concern in America, Europe and parts of Asia. To date, at least 25 cases of *Baylisascaris* neural larva migrans have been identified in children. The outcome of the disease in most cases is permanent neurological damage. Currently, a *B. procyonis* larval excretory–secretory (ES) antigen based enzyme linked immunosorbent assay (ELISA) performed in our laboratory is being used to help diagnose this infection in children. However, cross-reactivity is a drawback in the use of this ES antigen-based ELISA for diagnosis. Therefore, there is a need to identify potential diagnostic antigens that could be generated as recombinant proteins for use in immunoassays. In order to identify the genes encoding ES antigens, a *B. procyonis* third-stage larvae cDNA expression library was constructed in Lambda ZAP® II vector. Immunoscreening of this library resulted in the identification of an ES antigen, designated Bp1, with promising diagnostic potential. The Bp1 protein was expressed as a recombinant protein (recBp1) in BL-21(DE3)pLysS *E. coli* host cells and purified under denaturing conditions using Talon™ superflow metal affinity resin. The purified recBp1 protein was used in ELISA to assess the diagnostic potential of this protein. Serum from rabbits infected with *Baylisascaris* spp. and related ascarids such as *Toxocara canis* and *Ascaris suum* were used in the recBp1 based ELISA. This protein demonstrated increased

specificity with anti-B. procyonis serum and a lower degree or no cross-reactivity with antiserum to *T. canis* and *A. suum*. In addition, when serum samples from humans with *Toxocara larva migrans* and other parasitic diseases were tested in ELISA, the recBp1 antigen showed dramatically less cross-reactivity than the ES antigen. Based on these results, the identified Bp1 protein appears to be an excellent diagnostic antigen for serological detection of *Baylisascaris larva migrans*.

14

Exploitation of Asymmetric Predator-Prey Interactions by Trophically Transmitted Parasites.

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Classic studies of parasite-host interactions suggest that strong interactions between predator-prey hosts will favor the trophic transmission of parasites. Most trophic interactions within ecological networks are asymmetric, and the transmission of parasites is contingent upon the frequency and success of predation by the downstream host. Thus the exploitation of asymmetric predator-prey interactions by trophically transmitted parasites can act to increase transmission efficiency. This would occur if trophically transmitted parasites are ‘funneled’ from intermediate hosts that have relatively few predators towards downstream hosts that feed on many prey species. To test this, we decomposed three published food webs that contain parasites, to quantify and compare the degree of mismatch between predator-prey interactions that contain trophically transmitted parasites and those that do not. Trophically transmitted parasites were found in predator-prey interactions that were more asymmetric than predicted by random chance alone (0.7673 compared to 0.2863, Fisher’s $p = 0.0296$). Additionally, predator-prey interactions containing trophically transmitted parasites were more asymmetric than those interactions free of parasites (0.7673 compared to 0.2099, $p = 0.0563$). These findings suggest that the establishment of life cycles with trophic transmission occurs through the nonrandom exploitation of intermediate and definitive hosts.

15

Abiotic Versus Biotic Hierarchies in the Assembly of Parasite Communities.

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The presence or absence of parasites within individual hosts or within host populations is the result of a complex of factors, some biotic and others abiotic. This study uses a non-parametric classification and

regression tree approach to evaluate the key abiotic and biotic drivers controlling the presence/absence of parasites with complex life cycles in sentinel killifish *Fundulus heteroclitus*. Parasite communities were classified from four distinct salt marsh areas reflecting a gradient in host species diversity and time after restoration (untouched, 0, 10 and 20 years) from 480 individuals representing 15 fish per site in each of four consecutive seasons between 2006-07. Abiotic parameters for each marsh were recorded at continuous water monitoring stations located in each of the four marshes. Eleven taxa of metazoan parasites were identified: seventy percent of all fish had helminth infracommunities that were dominated by four species, a two-host nematode, a three-host digenean and two directly-transmitted monogenean species. Classification trees identified benthic invertebrate species (*Gammarus* sp., a copepod and *Littorina* sp.) as the most important variables in determining the presence parasites: secondary splitters were dominated by abiotic variables such as conductance, pH and temperature. Seventy percent of hosts were successfully classified into the appropriate category (infected/uninfected) based on only these two criteria. These data suggest that the more important determinant of parasite community assembly in this system is the availability of diverse communities of benthic invertebrates, and not the presence of competent definitive hosts.

16

The Establishment of a Pure Strain of *Plagiorchis elegans* (Rud.1802) and an Estimate of the Parasite's Biotic Potential Originating from a Monomiracidial Infection.

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A pure strain of *Plagiorchis elegans* (Rud. 1802) was initially established by infecting laboratory reared *Lymnaea stagnalis* with eggs teased from a single worm. Cercariae released from the infected snails penetrated and encysted in chironomid larvae. Metacercarial cysts obtained from the insect larvae were fed by gavage to laboratory reared LACA mice. Adult worms harvested from the mouse small intestine provided the eggs used to establish monomiracidial infections. Six snails were each infected with a single egg. Two were sacrificed and from each of them approximately 650 daughter sporocysts were recovered. Cercarial counts were performed during the course of the infection in the other four specimens. It is estimated that up to 700,000 xiphidiocercariae were released from one snail. The fecundity of the adults was determined using the McMaster method. Egg counts were performed after mice had been fed known numbers of metacercarial cysts by gavage. Based upon the number of worms present in the gut when the hosts were sacrificed, it is estimated that individual worms released between 200 – 700 eggs per day during the patent period. *Plagiorchis elegans* does not survive long in the laboratory mouse. Typically the patent period is 10 to 14 days. Thus from a single egg and after only one generation, in the unlikely event that all offspring survived, $1.4 - 4.9 \times 10^9$ eggs could be deposited.

17

Identity of rRNA Sequences from Metacestodes in Shrimp Nerve Cords and *Polypocephalus* Adults from Rays.

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Bait shrimp from the Gulf of Mexico belonging to the species *Farfantepenaeus duorarum* often harbor small metacestodes in their ventral nerve cord. The larvae tend to occur in pairs in the ganglia. They cannot be identified morphologically, so we decided to identify them through their ribosomal RNA (rRNA) sequences. It proved difficult to separate them from host tissue, so PCR primers specific for cestode rRNA were used for amplifying their rRNA genes. Metacestodes together with a small bit of host tissue were frozen individually in small aliquots of filtered sea water, and the DNA was later extracted from individual specimens. We amplified a segment of DNA containing the small subunit (SSU) rRNA gene, the internal transcribed spacer I, the 5.8S gene, the internal transcribed spacer II, and a section from the 5' end of the large subunit (LSU) rRNA gene, using either FidelityTaq (USB Corp.) or Phusion (New England Biolabs) DNA polymerases. We amplified the entire rRNA coding region from a series of possible adult stages collected from rays. It was amplified by using a general forward primer complementary to the 5' end of eukaryotic SSU rRNA genes, and a general reverse primer complementary to the 3' end of eukaryotic LSU genes. A single terminal proglottid from each adult specimen was frozen and processed in the same way as the larvae for DNA amplification, except in the case of *Polypocephalus caribbensis* (syn. *Discobothrium caribbensis*) from *Urobatis jamaicensis* (Yellow Stingray), which was so small that DNA was extracted from entire worms. The remainder of each specimen was fixed, stained, and mounted on slides. The larval sequence matched the sequence from one of the *Polypocephalus* species found as adults in *Dasyatis americana* (Southern Stingray).

18

On the Current Status of Human Infections with the Broad Fish Tapeworm (*Diphyllobothrium*).

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Tapeworms (Cestoda) continue to be an important cause of morbidity in humans globally. Diphyllbothriosis, a human disease caused by a species of *Diphyllobothrium* (and *Diplogonoporus*), is the most important fish-borne zoonosis caused by cestodes. Up to 20 million humans are estimated to be infected worldwide. Besides man, definitive hosts include piscivorous birds and mammals, which

represent a significant zoonotic reservoir. The second intermediate hosts include freshwater and marine fish, especially anadromous species such as salmonids. The zoonosis is most common in countries in which the consumption of raw or marinated fish is a frequent practice. Due to the increasing popularity of dishes such as “ceviche”, “sushi”, “sashimi” or “carpaccio”, numerous cases of human infections have recently appeared, even in North America, Europe and Japan. To date, 14 of the >50 valid *Diphyllobothrium* species have been reported from humans. *D. latum* and *D. nihonkaiense* are the most important pathogens. However, the following species have been reported as adults from humans in North America: *D. latum* and *D. dendriticum* (freshwater), *D. dalliae* (freshwater, Alaska only), *D. lanceolatum* (marine), *D. ursi* and *D. alascense* (anadromous, Alaska only), and, just recently, *D. nihonkaiense* (anadromous). *D. pacificum* is the marine species most important on the Pacific coast of South America. Salmonids are likely the most common sources of human infection (mostly *D. nihonkaiense*), but infections may also be transmitted by whitefish, trout, pike (mostly *D. latum* and *D. dendriticum*), among other species. Although morphology-based diagnostics are cheap and relatively easy, many samples of *D. nihonkaiense* and other taxa are commonly misidentified as *D. latum*. The reliable identification of human-infecting species with molecular tools (e.g., sequence data from mitochondrial genes), as well as the necessity of epidemiological studies aimed at determining the sources of the infections, are discussed.

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High Prevalence of *Baylisascaris procyonis* in Raccoons Trapped in South Central Kentucky.

C.D. Davis, Department of Biology, Western Kentucky University, C. Groce, Auburn University, T. Holaday and C. Walker, Transylvania University.

The large raccoon roundworm, *Baylisascaris procyonis*, has recently emerged as a potential zoonotic pathogen of humans. The incidence of human infection with this dangerous parasite is expected to increase as raccoon populations continue to expand into peri-domestic habitats. The purpose of our study was to determine the prevalence of *B. procyonis* infection in raccoons trapped from Warren and Barren counties of Kentucky. Raccoons were live-trapped between June 2007 and January 2008. Following inhalant anesthesia with isoflurane, fresh fecal samples were removed and placed into specimen bags. An overdose of isoflurane was then given to ensure death, and intestines were removed and examined for the presence of intestinal parasites. Helminths were removed with forceps and placed into vials containing 70% ethanol. In the laboratory, parasite eggs were separated from fecal matter using a sodium nitrate fecal flotation/centrifugation method. Parasite eggs were observed, identified, and photographed using an Olympus BX51 microscope. The overall prevalence of *B. procyonis* infection in raccoons was 36%. Prevalence was highest in Barren County, with nearly 50% of raccoons positive for

the parasite. NIH Grant 2 P20 RR-16481 from the National Center for Research Resources is gratefully acknowledged.

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Trypanosoma cruzi Infection in Raccoons and Opossums Trapped in South Central Kentucky.

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Trypanosoma cruzi has been isolated from a wide variety of mammals indigenous to the southern U.S., but it has not been reported from the state of Kentucky. The principal goal of the present study was to determine if the sylvatic cycle of *T. cruzi* infection occurs in the state, and if present, to assess the prevalence of infection in Warren and Barren counties. Raccoons and opossums were live-trapped between June and December, 2007. Animals were anesthetized using an inhalant anesthesia, and blood samples were collected using a vacutainer system. Sera were frozen at -80C for subsequent analysis, and whole blood samples were inoculated in duplicate into liver infusion tryptose (LIT) medium and cultured at 27C. Eighteen *T. cruzi* isolates from raccoons were positively identified by hemoculture. Raccoon blood samples were also determined to be positive for *T. cruzi* by PCR analysis. No opossum blood samples yielded positive hemoculture results, however, opossum blood samples were determined to be *T. cruzi* positive by PCR amplification. The partial support of NIH Grant Number 2 P20 RR-16481 from the National Center for Research Resources is gratefully acknowledged.

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Molecular Analysis of *Trypanosoma cruzi* Isolates Obtained from Raccoons Trapped in South Central Kentucky.

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Although *T. cruzi* has been isolated from a variety of wild and domestic mammals, particularly in the southern United States, it has only recently been identified in raccoons and opossums in the state of Kentucky. Eighteen isolates of *T. cruzi* were successfully obtained from raccoon blood samples by hemoculture in LIT medium supplemented with 10% newborn calf serum and penicillin/streptomycin. The purpose of the present study was to use a previously published molecular typing approach to determine the genotypes (type I, or types IIa-IIe) of 15 of the 18 isolates. DNA samples were prepared

from each isolate using a Qiagen mini kit, and PCR amplification was performed using published primers for the 24S rRNA sequence (D71 and D72), the non-transcribed spacer of the mini-exon genes (TC, TC1, and TC2), the 18S rRNA sequence (V1 and V2), and the TCZ1 and TCZ2 primers that amplify a 188-base pair segment of the repetitive 195-bp nuclear DNA sequence of *T. cruzi*. Based upon the results of this analysis, all 15 isolates were positively confirmed as *T. cruzi*, and all 15 isolates showed identical PCR amplification results with all 4 sets of *T. cruzi*-specific primers. The support of NIH Grant Number 2 P20 RR-16481 from the National Center for Research Resources is gratefully acknowledged.

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Development of a New PCR Protocol to Detect and Subtype *Blastocystis* spp. from Humans and Animals.

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Blastocystis spp. has been found in several other species of vertebrates and it is one of the most prevalent parasites found in the feces of humans worldwide. Infection with *Blastocystis* in humans has been reported as asymptomatic, acute symptomatic, infection, and chronic symptomatic. The range of responses to infection with *Blastocystis* spp. could be related to the genetic diversity of morphologically indistinguishable specimens obtained from infected hosts. The former name *Blastocystis hominis* is now reported as *Blastocystis* spp. because it has become apparent that different species or subtypes exist but have not been fully characterized. Genetic diversity among *Blastocystis* spp. isolates has been studied by molecular biology approaches. Because *Blastocystis* spp. has been identified in feces from several animal species a potential zoonotic role has been proposed for organisms in this genus. In this study, a PCR and sequencing protocol was developed using primers that were complementary to conserved regions of published nucleotide SSU rDNA sequences of *Blastocystis* downloaded from GenBank from all *Blastocystis* subtypes. This PCR protocol that amplifies a fragment of SSU rDNA of around 500 bp was found to be highly sensitive compared with previously published primers. The SSU rDNA gene fragment amplified by this PCR contained high variable regions that allow phylogenetic analysis of *Blastocystis*. These primers were able to detect and subtype *Blastocystis* spp. isolated from naturally infected cattle, pigs, humans, primates, ostrich, and chicken. Application of this method can elucidate the complexity of this heterogeneous genus and its role in human or animal diseases as well as its zoonotic potential.

Survey of *Bithynia tentaculata* (Class: Gastropoda) Collected from Rattlesnake Reservoir and Georgetown Lake, Montana for the Presence of *Sphaeridiotrema globulus* and *Cyathocotyle bushiensis* (Class: Trematoda).

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The faucet snail, *Bithynia tentaculata*, a non-indigenous aquatic snail from Eurasia, was introduced into Lake Michigan in 1871 and spread to the mid-Atlantic states, Great Lakes region, Montana, and the Mississippi River (2002) (Pools 7-13). The snail was most recently (2008) found to be in Lake Winnibigoshish, Minnesota. The faucet snail serves as intermediate host for three trematodes that have been responsible for several large scale mortality events among water birds, primarily lesser scaup (*Aythya affinis*) and American coot (*Fulica americana*), in the Great Lakes region, Montana, and Minnesota. The trematodes *Cyathocotyle bushiensis*, *Sphaeridiotrema globulus* and *Leyogonimus polyoon* may have been introduced into the United States as early as the 1870's when the snail was introduced. In Montana, 3 water bodies - Georgetown and Smith Lakes and Rattlesnake Reservoir have recently reported mortalities due to trematodiasis in water birds. Large-scale mortality events that have sporadically occurred since the early 1990's were most recently reported in 2006 and 2007. These mortality events involved primarily American coot and smaller numbers of other species of waterbirds including lesser scaup. Birds were found to be infected with *C. bushiensis* and *S. globulus* suggesting that *B. tentaculata* had colonized the three water bodies. Sampling of snails was undertaken in August, 2008 at Rattlesnake Reservoir and Georgetown Lake. Both sites had faucet snails infected with larval stages of *C. bushiensis* and *S. globulus*. Rattlesnake Reservoir had 5 collection sites where prevalence of infected snails ranged from 58-97%. Specifically, prevalence of *C. bushiensis* metacercariae ranged from 28-90% and *S. globulus* metacercariae ranged from 35-97% which was similar to sites on the Mississippi River (Lake Onalaska, Wisconsin and Lake Winnibigoshish, Minnesota). Only 2 sites (N=8) at Georgetown Lake had similar prevalences with 90% and 80% of the snails infected. At one site, 86% of the snails had metacercariae of *C. bushiensis* while 66% had metacercariae of *S. globulus*.

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The Effect of Fractions of Human Semen on Stimulation and Inhibition on the Growth of *Trichomonas vaginalis*.

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Trichomonas vaginalis cannot only survive in human semen but can grow through several generations (Daly et al., 1989. Genitourin. Med. 6:106). In order to determine its growth or inhibitory components, semen was separated into fractions which were tested for their effects on *T. vaginalis*. Semen was separated by low speed centrifugation into a pellet containing spermatozoa and a fluid component, seminal plasma. The latter was further centrifuged through a Centricon 30 protein filter system giving a filtrate and a retentate. These fractions were added to a 0.4-0.6 ml microassay for growth containing a minimal growth amount (25%) of TYM medium and a small inoculum of *T. vaginalis*. Samples were incubated at 37 C for 24 hrs and then cells were counted with a hemocytometer. The standard for growth or inhibition was the difference between the sample and 25% TYM (100 % TYM was the control for maximum growth). The pellet was found to have a growth stimulating effect whilst seminal plasma was inhibitory. The retentate inhibited growth and the filtrate had little or no effect. Boiling the retentate erased the inhibition indicating the involvement of a high molecular weight protein. Boiling did not affect the activity of the other fractions. The activity of the fractions was affected by dilution and pH. Surprisingly, pellets from vasectomized donors showed as much growth stimulation as pellets from other donors, indicating a role apart from the presence of spermatozoa.

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The Neurotransmitter Dopamine Stimulates Proliferation of *Toxoplasma gondii* Tachyzoites in Human Fibroblast Cell Culture.

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Toxoplasma gondii is an obligate intracellular parasite. *T. gondii* is capable of infecting every warm-blooded animal. Infection with *T. gondii* in the intermediate host has two phases, the acute phase of infection where it disseminates throughout the body and the chronic phase when it encysts in all tissues with neural tissue of the brain being a frequent location. Traditional thought is there are no adverse side effects of chronic *T. gondii* infection because there is little to no reaction around tissue cysts. Research

has shown a correlation between prevalence of antibody titers to *T. gondii* and psychological illness in humans. Recent research has shown a correlation between people with psychotic disorders, schizophrenia, bipolar disease and *T. gondii* infection. These disorders have been associated with changes in the dopamine neurotransmitter system. Dopamine in the brain may play a role in proliferation/chemotraction of *T. gondii*. The link between mental illness and *T. gondii* infection is not 100%, however, there is a strong correlation between the two, indicating *T. gondii* infection could be an environmental factor for some mental disorders whose effect is mediated through changes in the dopamine system. Research in rodents with toxoplasmosis has indicated alterations in cognitive learning, fear response, and overall open field activity. Some of these behavior changes can be related to altered neurotransmitter levels, in specific dopamine. In an *in vitro* cell culture assay dopamine was tested against developing tachyzoites. To address the hypothesized effects of dopamine in *T. gondii* infection, dopamine was tested at 2 concentrations, 100 nM and 250 nM. An increase of tachyzoite proliferation and increased destruction in cell monolayer was observed at both concentrations of dopamine. The highest concentration of dopamine, 250 nM, yielded the greatest increase in tachyzoite proliferation.

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The Effect of the Metam Sodium on Viability and Infectivity of *Eimeria* Oocysts.

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Metam sodium (MS, sodium N-methyldithiocarbamate) is a widely used soil fumigant. Fumigation or chemical sterilization of poultry litter containing the infectious oocysts could be an effective strategy to block the transmission of avian coccidia. In the current study the effect of MS on the viability and infectivity of *Eimeria* oocysts was investigated. The development of isolated unsporulated *E. tenella* oocysts was inhibited in a dose related manner (IC₅₀ = 30 µg/ml, IC₉₀ = 300 µg/ml) by exposure to aqueous MS. Most treated oocysts failed to develop beyond early stages of sporulation. To determine the effect of MS on infectivity, isolated oocysts of *E. tenella*, *E. acervulina* and *E. maxima* were exposed for 24 hr to aqueous concentrations of MS ranging from 0 to 1000 µg/ml. Treated oocysts were inoculated into chickens and parameters of coccidiosis infection were compared to chickens inoculated with equal numbers of untreated oocysts. MS significantly reduced the infectivity of oocysts in a dose related manner with maximum effect observed at a dose of 300 µg/ml. When oocysts of the three coccidian species were exposed to 300 µg/ml MS from 0 to 24 hrs infectivity of oocysts was significantly reduced after a minimum of 12 hrs of exposure. Treatment of aqueous slurries of litter samples obtained from commercial poultry houses with 300 µg/ml MS for 24 hrs prevented the sporulation of *Eimeria* oocysts in the litter samples relative to untreated controls.

Efficacy of Natural or Synthetic Immunostimulators Against *Trichinella spiralis* in Immunocompromised Mice.

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Trichinosis is a cosmopolitan disease with serious health effects that may be lethal. Therefore, proper diagnosis and treatment of early infections is key in the prevention of morbidity and mortality. In the present study, the drugs Ivermectin, Nigella sativa and Levamisole were tried as the individual agent and in different combinations in the treatment of trichinosis in 140 immunocompetent and immunosuppressed mice. Albendazole was chosen as a control drug. Assessment of the agents was done by larval count 45 days post infection (DPI) and serodiagnosis (IFAT). Ivermectin was administered either alone or combined with Nigella sativa at 3 DPI. In immunocompetent mice, the number of larval showed a significant reduction (47%, 48% and 84% respectively) compared to the corresponding control. While in immunocompromised mice the percentage of larval reduction was 38%, 48% and 87% respectively. On the other hand, when ivermectin was given at 20 DPI, the percentage of larval reduction was 32%, 26% and 29% respectively in the immunocompetent groups. While in the immunocompromised mice, the percentage of larval reduction was 25%, 40% and 50% respectively. When Nigella sativa was given alone at 3 DPI, the percentage of larval reduction was 29% and 33% in the immunocompetent and immunocompromised groups respectively. When Nigella Sativa was administered at 20 DPI, the percentage of larval reduction was 26% in the immunocompetent group and 30% in the immunocompromised group. Treatment with Levamisole alone at 3 DPI resulted in a percentage of larval reduction that was 96% and 51% for the immunocompetent and immunocompromised groups, respectively. When Levamisole was administered at 20 DPI, the percentage of larval reduction was 32% and 29% respectively. IFAT was specific and sensitive for *Trichinella* diagnosis and thus evaluation of therapeutic agents. In conclusion, it seems that the synergistic effect of Ivermectin and either Nigella sativa or Levamisole is promising, especially in immunocompromised infected mice.

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Functional Characterization of the Mosquito Heart and Its Implication in Malaria Sporozoite Transport.

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Completion of the malaria life cycle requires two hosts: a vertebrate and a mosquito. Inside the mosquito, the sporozoite stage of *Plasmodium* must migrate from the midgut to the salivary glands. The mechanism for this migration is not well understood, but we have previously shown that sporozoites enter the mosquito heart and are shuttled to the head region, where they are deposited in the vicinity of the salivary glands. Here we will present data that structurally and functionally characterizes the heart of the mosquito *Anopheles gambiae* and highlights the implications of its function in pathogen dissemination within the mosquito host. Light and fluorescence microscopy showed that the heart is a dynamic organ that contracts an average of 82 times per minute and switches contraction direction approximately 5 times per minute. Approximately 70% of contractions occur in the anterograde direction (toward the head) and 30% of contractions occur in the retrograde direction (toward the tip of the abdomen). Hemolymph enters the heart through paired ostia located in each abdominal segment and propulsion is accomplished by the peristaltic contraction of muscles that spirally wrap the heart and are stabilized by alary muscles. Binucleate pericardial cells flank the heart, and although their function is not yet clear, fluorescence labeling of acidic organelles suggests that they may be involved in immune surveillance. When *Plasmodium*-infected mosquitoes were examined, sporozoites entered the dorsal vessel through the ostia and flowed to the head at speeds much greater than can be accounted for by active motility alone. Overall, these data support the hypothesis that the mosquito heart facilitates the transport of malaria parasites from the midgut to the salivary glands.

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Evidence of *Hepatozoon canis* in Mississippi Dogs.

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Canine hepatozoonosis is a tick-borne infection with either *Hepatozoon canis* or *H. americanum*, apicomplexan protozoan parasites similar to the *Plasmodium* species. *Hepatozoon americanum* was first recognized as a distinct species from *H. canis* in 1997, and until recently, was considered the only species of *Hepatozoon* in the United States. *Hepatozoon canis* is present in Africa, Asia, and Europe.

Disease differs depending on the species infecting dogs. *Hepatozoon americanum* is considerably more pathogenic than *H. canis*, which is apparently more adapted to its hosts. Recent evidence has shown *H. canis* or *H. canis*-like organisms infecting dogs in Oklahoma; however, 18S rRNA gene sequences from those organisms were less than 100% identical to *H. canis*. In this study, we demonstrate two cases of dogs in Mississippi infected with an organism that shares 100% identity with *H. canis* based on 18S rRNA sequences using the same primers as used in the Oklahoma study. The dogs had no history of travel and had mild or no disease. Interestingly, they were co-housed with other dogs that had been euthanized after a diagnosis of *H. americanum*, based on characteristic lesions on pathology and clinical signs typical for *H. americanum*. Clinical signs of the euthanized dogs included muscle atrophy and bony proliferations; pathologic findings included myocarditis and myositis with intralesional protozoal cysts. Tissues from one of the euthanized dogs had molecular evidence of *H. americanum* by sequencing. Ticks from the location were sampled and tested for the presence of *Hepatozoon* species by PCR of the 18S rRNA gene. One adult *Rhipicephalus sanguineus* was positive for *H. canis*. Our results lend support to the occurrence of *H. canis* in the United States; both species appear to be circulating in dogs and ticks in Mississippi. Further work to determine the natural history of this protozoan, including its tick vector, in the U.S. is warranted.

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Strange Bedfellows: Invasive Wildlife and Invasive Parasites on a Shrinking Planet.

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The global spread of wildlife and the dispersal of parasites to new geographic areas threaten native species. Human activities play an increasingly greater role in altering host-parasite relationships when compared with natural colonization events. Anthropogenic introductions may be accidental byproducts of trade and commerce, but many species are deliberately introduced by humans for various reasons such as agriculture, aquaculture, and recreational hunting or fishing. Introduced hosts may be the sources of alien parasites that have the potential to infect native hosts, producing diseases that threaten their survival. Conversely, introduced host species often reach new geographic areas with few of the parasite species that affect them in their native ranges. These alien hosts, relatively free of parasites, may gain a competitive advantage over native hosts that must contend with their entire community of co-evolved parasites. Oceanic islands, often called natural laboratories for the study of evolution, are also ideal locations for the study of invasive species. Because of constraints imposed by extreme geographic isolation and recent geological origins of oceanic islands like the Hawaiian archipelago, natural colonization of wildlife and parasites is minimal when compared with anthropogenic introductions. Biological characteristics of parasites that have good colonization potential are more

readily discernible on oceanic islands. Hawaiian honeycreepers represent a well known example of the vulnerability of oceanic residents to human induced alterations of ecosystems. Mosquitoes and avian diseases brought to Hawai'i by man act synergistically with other human induced changes including habitat destruction, and have devastated the native Hawaiian avifauna. In Hawaiian streams, the 5 native species of gobioid fishes are threatened by 3 species of invasive helminths, a leech, a tapeworm, and a roundworm. These exotic parasites are much more abundant than native fish helminths and present disease problems for native fishes.

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Our Parasites on a Shrinking Planet - It' Still a Big World for Hookworms.

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Pathogens of humans have always traveled with their hosts, and the movement of humans has spread many our diseases to naïve populations, with devastating effects. Notable examples include pandemics of plague in Europe and Asia in the 14-17th centuries, smallpox and other diseases brought to the New World in the 16th century by Spanish explorers, and influenza in the early 20th century. Improved travel technology has only increased the speed of microparasite travel. For example, HIV infection first appeared in humans in the early 20th century, but did not reach pandemic status until the 1980's when intercontinental travel became more commonplace. In 2002-2003, SARS traveled around the world in a period of months, but thankfully never reached endemicity, and the world anxiously waits for the arrival and spread of avian influenza. In fact, diseases and potential epidemics are only a jet flight away. However, the macroparasites (trematodes and nematodes) are far less mobile due to specific life history requirements, and in the case of hookworms in China, the world for *Necator americanus* actually appears to be incredibly large. Our evidence indicates that even with a highly mobile migrant labor pool, *N. americanus* populations remain highly isolated in parts of southern China. Possible reasons for this isolation and predictions for the future of hookworm disease in China will be discussed.

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Dengue on a shrinking planet: present and future challenges for understanding and controlling an emerging mosquito-borne infection.

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Mosquito-borne arboviruses are undergoing rapid range expansion as the pace of international travel and commerce accelerates and as major urban centers experience unplanned growth and expansion. Dengue, also known as “break bone fever”, is a viral infection primarily transmitted the *Aedes aegypti* mosquito. Factors responsible for the dangerous global rise in dengue infections will be discussed and studies on novel targets for understanding and breaking the cycle of transmission will be presented.

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Observations on Blood Parasites.

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No abstract submitted.

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Identifying the Binding Sites, Response Elements and Target Genes of Hookworm DAF-16.

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Hookworm infection remains an important infectious disease, mainly in developing countries. People become infected when they contact the developmentally arrested, third stage infective larva (L3) in the environment. During infection, host-specific signals re-activate arrested developmental programs, culminating in development to the adult stage. An appropriate response to the signal and execution of these developmental programs are the critical steps for hookworm to establish a successful parasitic relationship. Resumption of development during infection is mediated by insulin-like signaling (ILS), which terminates on a forkhead transcription factor homologous to DAF-16 of the non-parasitic model nematode *Caenorhabditis elegans*. DAF-16 controls genes involved in development, metabolism and

longevity in this organism. Previously we have identified and characterized a DAF-16 homolog from the model hookworm *Ancylostoma caninum*. We hypothesize that the gene network downstream of the hookworm DAF-16 controls the resumption of developmental pathways during infection, and therefore presents potential targets for therapeutic intervention. The current study identifies DAF-16 binding elements (DBE) and target genes by genomic selection, and investigates the regulatory elements of hookworm genes. DAF-16 DNA binding domain was expressed and purified from *E. coli* cells. It selectively binds to canonical DBE and DBE-like sequence, but not random oligo DNA sequences. The long-term goal is to define DAF-16 target gene network and better understand the critical molecular events required for hookworm infection and its adaptation to a parasitic life cycle in the host.

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Unexpected Diversity and Specificity in Freshwater Fish Parasites: Effects of Phylogeny, Ecology and Parasite Habitat.

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The parasites of freshwater fishes have been models in the study of community and evolutionary ecology of parasites for decades. However, most studies have dealt only with adult parasites in these hosts, even though larval stages often dominate. Another problem is that studies generally include replicates of either host species or sampling localities, but not of both, and few control for host age. We examined larval and adult parasites in >700 one-year-old fishes in six species collected from six localities in the St. Lawrence River. Sequences of rDNA and, principally, of the barcode region of the CO1 gene were used to distinguish species of >800 metacercariae in the Diplostomoidea (Platyhelminthes: Trematoda). Diplostomoids are among the most common parasites of freshwater fishes in the world, and many are thought to be generalists capable of infecting a wide variety of hosts. Unexpectedly high numbers of species were found within *Ornithodiplostomum*, *Posthodiplostomum* and tetracotyle larvae, with most showing surprising host specificity. *Diplostomum* was also unexpectedly diverse, and *Diplostomum* species that infect the lenses of fishes emerged as generalists, while those colonizing other tissues were host specific. This indicates that the immune potential of a parasite's infection site within fish hosts can limit host spectrum. We examined how infection levels of parasites varied across hosts and found, in contrast to recent work, that more closely related hosts shared quantitatively similar parasite communities. We also found that parasite communities from proximate host populations tended to be similar, and that the relationship between parasite community similarity and geographic distance was strong for allogenic and absent among autogenic parasites. Finally, we assessed parasite communities as attributes of host taxa and of habitats, and found that, within our system, host phylogeny was much more important in structuring parasite communities than spatial scale or habitat effects.

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Making Life Easier: When Does a Trematode Skip a Host?

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Most trematodes have a typical three-host life cycle. However, some species can abbreviate their life cycle via progenesis, which is the precocious maturation of the parasite in the second-intermediate host resulting in the production of eggs through self-fertilization within the metacercarial cyst. Adoption of the progenetic life cycle may be a conditional strategy in response to different environmental cues related to low probability of transmission to the definitive host. Thus cues associated with life span and density of the second-intermediate host and presence of the definitive host may trigger one of the alternative strategies. Our research examined how ecological factors, pertaining to the perception of the probability of transmission, influence facultative truncation of the typical three-host life cycle by progenesis in *Stegodexamene anguillae* (Trematoda: Lepocreadiidae). Numerous small fish species are used as second-intermediate hosts, while eels (*Anguilla* spp.) serve as the definitive host. We tested the effect of body condition and life span of the fish second-intermediate host (*Gobiomorphus cotidianus*, common bully) on the probability of progenesis. A factorial experimental design utilizing variations in water temperature and diet, two factors that affect condition and life span of freshwater fish, was run for 5 ½ weeks. All fish were then euthanized, measured and frozen until examination for parasites. Cysts of *S. anguillae* were opened, and parasite development, size, encystment site and number of eggs were recorded. Results from the different treatments will be discussed in relation to the effects of environmental cues, like body condition and life span of the second-intermediate host, which may trigger the parasite to adjust its developmental strategy accordingly.

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Crickets Groom to Avoid Parasitization by the Paratoid Fly *Ormia ochracea*.

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Organisms that are subject to ectoparasites that attach externally prior to migrating into the organism's body, should incur the costs associated with grooming in an effort to dislodge the parasite, rather than the cost of being parasitized. Calling males of the Texas field cricket, *Gryllus texensis*, are acoustically stalked and parasitized by gravid females of the parasitoid fly *Ormia ochracea*, which lay live 1st-instar larvae, or planidia, on and around the cricket. Larvae then attach and burrow inside the cricket and proceed to feed and grow. Crickets infected with *O. ochracea* larvae die within seven to ten days. There

should be strong selection for crickets to avoid the lethal effects of being parasitized. We investigated whether field caught *G. texensis* groom to avoid parasitism by *O. ochracea*. We quantified the grooming behavior of crickets when in the same area as *O. ochracea* adults or larvae (proximity avoidance) and following contact between the cricket and *O. ochracea* adults or larvae (contact avoidance). Crickets did not adjust grooming behavior when in close proximity to adult gravid female *O. ochracea*, nor did they avoid planidia-laden grass. Furthermore, crickets that groomed more were less likely to succumb to parasitoid infection, compared to crickets that groomed less, suggesting that grooming in *G. texensis* might function as a defense against panidia of *O. ochracea*.

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Spatio-Temporal Variation in Parasite Biovolume and Its Relationship to Numerical Abundance of Parasites in a Host Population.

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Spatio-temporal variation in the numerical abundance of parasites has been shown in many species. A common seasonal pattern is an increase in abundance when infections are likely and a decrease in abundance throughout the year as parasites and/or host die until the next re-infection period. How the biovolume of the parasites change over this same period is unknown. Information on the spatial and temporal variation of parasite biovolume and numerical abundance may provide insight into the dynamics of parasite growth within hosts. The endoparasitic helminthes of several fish species from the south shore of Long Island, NY were collected from three different seasons and three different locations in order to study the changes of parasite biovolume through space and time and its relationship to their numerical abundance.

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Prevalence of the Metacercariae Associated with Waterbird Mortality in Open Water Sites on Pool 7 of the Upper Mississippi River Wildlife and Fish Refuge.

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Waterbirds have been dying of trematodiasis on Pool 7 of the Upper Mississippi River Wildlife and Fish Refuge since 2002. Two trematodes, *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*, have been

implicated in the mortality of the waterbirds. These trematodes are transmitted to waterbirds when the birds consume infected exotic *Bithynia tentaculata* snails. These snails were previously found in high densities near man-made islands in Pool 7. Waterbirds that experience the heaviest mortality generally feed in open water rather than rocky shallows like those found around the islands; therefore, I sampled open water areas of Pool 7 to identify where birds could encounter infected snails. Pool 7 was sampled from May to October in 2007 and 2008. *Bithynia tentaculata* occurred at 33.5% of the collection sites and were most densely clustered on muddy clay substrate at depths of 1.5-1.9 meters. A total of 528 live specimens were dissected and examined for larval trematodes. Over half of these snails were infected with metacercariae of either *C. bushiensis* (64.5%) or *S. globulus* (62.2%). Patterns of metacercariae abundance suggest that while *S. globulus* metacercariae are generally more abundant overall, snails in the deepest water (2.0-2.5 meters) are significantly more heavily infected with *C. bushiensis* than *S. globulus* ($p < 0.001$). These findings suggest that waterbirds may be encountering heavily infected *B. tentaculata* while diving to forage in deep water away from the islands.

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Environmental Factors and Their Impact on Parasite Intensity and Diversity in Balinese Long-Tailed Macaques (*Macaca fascicularis*).

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The distribution of wildlife parasites in a landscape is intimately tied to the spacial distributions of the host, limited as that may be by habitat and resource specificity, human tolerance, and host population dynamics. In extremely successful host species, such as humans and macaques, which thrive across a wide variety of habitat types and qualities, the dynamics of the parasite are driven largely by geophysical components of the environment directly. Here, we examine the influence of environmental and anthropogenic components of the landscape on the spatial distribution, intensity and species diversity of gastrointestinal parasites across 15 populations of long-tailed macaques on the island of Bali, Indonesia. In examining nineteen enteric parasites across host populations and habitats, we uncovered patterns of environmental influence on parasite abundance and intensity but not on parasite diversity. While one environmental factor may be a significant force in parasite dynamics locally, or for one parasite species, the significance of this force can be lessened or eliminated entirely when compared across multiple populations and multiple parasites. Further, we identified elevation as the only environmental factor significantly influencing parasite species diversity, suggesting isolation plays an important role in increasing parasite diversity. These results are relevant to future studies of wild parasite-host systems, especially among primate systems. In primates and other endangered species, it

is critical to develop a system-wide approach for understanding host-parasite interactions in order to create successful conservation management programs.

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Penthouse Parasites: Positional Preference of an Endoparasitic Copepod
Within Its Nudibranch Host.

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Site specificity within a host is common in parasites. Parasitic castrators often reside in the host's gonad, a non-vital organ. Members of the genus *Ismaila* (Splanchnotrophidae, Copepoda), are endoparasites of opisthobranch molluscs, inhabiting a large area in the host's main body cavity and/or cerata. On the Oregon coast, *Ismaila belciki* can infect over 60% of the population of their obligate host, the nudibranch *Janolus fuscus*. In this study, we examine whether *I. belciki* prefers a particular location or nutritional source within the host as well as their impact on gonad production. *Janollus fuscus* were collected from two sites in Coos Bay, OR from 2007 to 2009. Nudibranchs were externally examined for infection, noting the position of *I. belciki* under the host's transparent mantle. Periodically, uninfected and infected *J. fuscus* were preserved for dissection to more closely examine the positioning of the parasite. Host body and gonad mass were measured, as well as the sex, mass, and location and orientation of each copepod in infected nudibranchs. Female *I. belciki* inhabit the anterior position more frequently than any other, with secondary female infectors beside the large anterior female or in a posterior position. This site preference is surprising, as the host's posterior has a greater concentration of gonad than the anterior. Male copepods reside in close proximity to the female, often within the embrace of her legs and lateral processes. Male and female copepods were found with their cephalic appendages in contact with the host gonad, suggesting *I. belciki* may partially castrate their host by consuming gonadal tissue. Potential benefits of site preferences in females include access to nutritional sources and for males, access to mates.

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Genotyping and Evidence of Genetic Exchange in US Isolates of
Trypanosoma cruzi.

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and M.L. Yabsley, Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine and D.B. Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, GA.

Trypanosoma cruzi, the causative agent of Chagas disease, has a broad host and geographic range, leading to many questions concerning its epizootiology. In particular, it is imperative to understand the association between the genetic type, virulence, and primary reservoir hosts of the parasite. While molecular characterization of South American isolates of *T. cruzi* has demonstrated homologous recombination and nuclear hybridization, as well as the presence of two phylogenetic lineages (Type I and II), few studies have extensively investigated such exchange events and genetic diversity in North American isolates. In the current study, we genetically characterized over 100 US isolates from wildlife reservoirs (raccoons, opossums, armadillos, skunks, woodrats), dogs, humans, nonhuman primates, and reduviid vectors. All molecular analysis was performed using culture-derived parasites. To determine genotype, a classical typing scheme was carried out using three gene targets, mini-exon, divergent domain of 24s alpha rRNA, and 18s rRNA. Genetic exchange was determined by comparing sequence phylogenies of nuclear and mitochondrial gene targets, dihydrofolate reductase-thymidylate synthase and the NADH dehydrogenase subunit I-cytochrome oxidase subunit II region, respectively. Genotyping of isolates from ten states (TX, CA, OK, AL, SC, FL, GA, MD, LA, TN) showed support for the existence of two genotypes (I and IIa) and distinct genotypes that preferentially infect one host species or a group of hosts. Further, confirming previous evidence of a single genetic exchange event in a Florida isolate, we have observed genetic exchange in several US isolates as demonstrated by incongruent mitochondrial and nuclear genes phylogenies.

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Gamma Irradiation of *Cryptosporidium parvum* Oocysts Affects Intracellular Levels of the Viral Symbiont CPV.

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Previous studies have shown a dose-dependent effect of gamma irradiation on *Cryptosporidium parvum* development in neonatal mice and newborn calves. In mice, *C. parvum* oocysts exposed to 200 Gy showed nearly complete inability to develop as measured by *C. parvum*-specific quantitative PCR of ileal tissue. In calves, higher irradiation doses (400 Gy) were required to prevent parasite development as measured by oocyst shedding. The present study was conducted to determine the extent of development of *C. parvum* and its viral symbiont (CPV) after exposure to irradiation, using a cell culture model. *Cryptosporidium parvum* oocysts were exposed to 0-, 200-, or 400 Gy gamma irradiation, excysted *in vitro*, and inoculated into HCT-8 cell culture. RNA was harvested from cells and culture medium at 2, 24, and 48 hr post-inoculation, and analyzed by standard and real-time RT-PCR to estimate

parasite and CPV abundance at various times post-inoculation. Preliminary results indicate that CPV- and *C. parvum*-specific RNA were present in both cells and culture medium, suggesting that both virus and parasite were released from host cells during development. Also, irradiation doses of 400Gy resulted in lower CPV levels compared to 200 Gy and non-irradiated oocysts at 24 and 48 hr post-infection. Studies are underway to evaluate the effects of higher doses of gamma irradiation on the parasite and its symbiont.

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Genetic Linkage Map of the Human Blood Fluke *Schistosoma mansoni*.

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Schistosoma mansoni is a blood fluke that infects ~90 million people. The complete life cycle can be maintained in the laboratory making this one of the few experimentally tractable human helminth infections and a rich literature reveals heritable variation in important biomedical traits such as virulence, host-specificity, transmission and drug resistance. Yet, *S. mansoni* remains a neglected pathogen due, in part, to a lack of tools needed to study molecular, quantitative, and population genetics. Furthermore, the 300Mb genome is peppered with transposable elements making sequence assembly a challenge. We genotyped grandparents, parents and 88 progeny to construct a 5.6 cM linkage map containing 243 microsatellites positioned on 203 of the largest scaffolds in the genome sequence. The map allows 70% of the genome sequence to be ordered on chromosomes, and highlights where scaffolds have been incorrectly assembled. The markers fall into 8 main linkage groups, consistent with 7 pairs of autosomes and one pair of sex chromosomes, and we were able to anchor linkage groups to chromosomes using fluorescent in situ hybridization (FISH). The genome measures 1228.6 cM (1cM=227-244kb). Marker segregation reveals higher female recombination, confirms ZW inheritance patterns, and identifies recombination hotspots and regions of segregation distortion. The linkage map provides the critical tool necessary for quantitative genetic analysis, aids genome assembly, and furnishes a framework for comparative flatworm genomics and field-based molecular epidemiological studies.

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Molecular Biology and Evolution of Candidate Immune Receptors in
Drosophila.

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A common method by which arthropod hosts kill their macroparasites is melanotic encapsulation, a process whereby host hemocytes (blood cells) form a multi-layered capsule around the parasite and release free radicals inside the capsule to kill the entrapped parasite. As a first step in the encapsulation response, the host must be able to recognize the parasite as foreign. We are interested in identifying the immune receptors *Drosophila* use to identify parasitic wasp eggs as foreign. Microarray analysis of *Drosophila* larvae post-wasp attack identified several promising candidate immune receptors. Using an RNAi-based approach, we are investigating the encapsulation potential of *Drosophila melanogaster* strains expressing knock-down levels of these candidate genes. Furthermore, we are conducting population genetic analyses of these genes to identify the types of selection pressures these genes have been subjected to in the past. Results from this study may lead to a more thorough understanding of the molecular biology and evolution of key genetic elements involved in the process of melanotic encapsulation.

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Microsatellite Analysis Indicates that Some Strains of the Estuarine
Parasite *Perkinsus marinus* are Endemic While Others are Shared Between
Populations.

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The protistan estuarine parasite *Perkinsus marinus* is found continuously along the east coast of North America from Mexico to southern parts of Canada. Previous study indicates that genetically similar populations comprise three regions: the Gulf of Mexico, the southeastern United States, and populations north of Chesapeake Bay. Here, we sampled multiple individuals from 15 locations across the parasite range in order to understand the connectivity of parasite populations within and between regions. Seven novel microsatellite loci were used to genotype *P. marinus* strains directly from infected oysters. Genotypes were analyzed using population genetic methodologies to better understand local and regional population structure. Estimates of F_{st} and R_{st} indicated that most populations were genetically distinct and did not show regional patterns as previously described. Furthermore, the genetic distance between populations did not increase with increasing geographic distance as observed in other

estuarine species. Rather, cluster analysis of individual multilocus genotypes revealed a distribution of two categories of parasite lineage; endemic strains, limited to only one or two locations, and cosmopolitan strains, occupying the entire parasite range. Interestingly, certain populations were composed almost entirely of endemic strains while other populations contained only cosmopolitan strains. Analyses conducted after removal of endemic genotypes reaffirmed that populations have significantly different allelic and genotypic distributions and that differentiation was not greatest between locations that are most geographically distant. Taken altogether, local populations of *P. marinus* were highly variable and consisted of both endemic and cosmopolitan strains. We conclude that contemporary population dynamics are driven by local conditions while evolutionary gene flow is frequent enough to spread *P. marinus* strains among distant geographic populations.

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Blackhead Disease (Histomoniasis): Strain Variation as a Factor in Virulence.

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Histomoniasis (blackhead disease) remains a serious problem in poultry, often causing high mortality in turkeys and morbidity in broiler breeder pullets, resulting in significant economic loss. Mortality in infected turkeys often exceeds 50%. There is considerable variation in the severity of outbreaks in various parts of the country. However, no one has studied strain variation as a possible cause of this observation. We have developed a method for DNA extraction from histologic samples (paraffin blocks) and amplification by PCR, using primers against the ITS1, 5.85S, and ITS2 rRNA regions. Once amplified, DNA samples are cut from the gel and sequenced. The resulting sequences are compared with published gene sequences for known protozoa. Twenty-four tested samples came from outbreaks of blackhead in chickens, diagnosed at the Oakwood GA Diagnostic Lab. These tests revealed considerable variation in the amplified region. Phylogenetic analysis strongly suggested two distinct clades within *Histomonas meleagridis*. We expect that the results could be used to trace the origin of outbreaks and to locate reservoirs of infection. We are in the process of obtaining samples from turkeys, gamebirds and other poultry, from cooperators in California, Pennsylvania, and Arkansas for comparison.

Antibacterial Activity of Nitric Oxide in the Mosquito Hemocoel.

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Nitric oxide is a signaling and immune effector molecule synthesized by the enzyme nitric oxide synthase. In the mosquito midgut, nitric oxide is a parasite antagonist that kills *Plasmodium* ookinetes via lysis. However, nothing is known about the role nitric oxide plays in immunity in the hemocoel (body cavity). Here, we report studies on the characterization of the *Anopheles gambiae* nitric oxide synthase gene and the role of nitric oxide in the antibacterial response in the mosquito hemocoel. Quantitative PCR and Western blot analyses showed that nitric oxide synthase is expressed in hemocytes (immune blood cells) and fat body, as well as the midgut. It is transcriptionally upregulated following infection with *Escherichia coli* and *Micrococcus luteus*, and protein levels peak at six days post-infection. Diaphorase staining and immunofluorescence showed that nitric oxide synthase is present in the granulocyte subpopulation of hemocytes, and both the staining intensity and the percentage of cells that stain for nitric oxide synthase significantly increase after a bacterial challenge. When nitric oxide production was inhibited using the chemical L-NAME, the mosquito's ability to kill *E. coli* was drastically reduced. The inert stereoisomer D-NAME had no effect in the antibacterial response, while the precursor of nitric oxide, arginine, enhanced bacterial killing. Together, these data show that nitric oxide is an important component of the antibacterial immune response in the mosquito hemocoel.

Characterizing the Transcriptional Profile of *Biomphalaria glabrata* After Immunological Challenge.

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Biomphalaria glabrata is a freshwater snail that serves as an intermediate host for the medically important trematode, *Schistosoma mansoni*. Using an oligo-based microarray consisting of 1152 features emphasizing immune/stress responses, we monitored the transcriptional profiles of *B. glabrata* after exposure to bacterial and trematode pathogens. It has been demonstrated that *B. glabrata*'s ability to resist infection by a parasite is determined by the immunological strategies employed by the host, as well as the evasion/immuno-suppression strategies used by the parasite. With this in mind, we targeted array studies to address questions relating to: the specificity of the *B. glabrata* immune response; the long-term relationship between the trematode parasites *S. mansoni* and *Echinostoma paraensei* and the host snail; and the possibility of *B. glabrata* acquiring resistance to infection by prior sensitization to *E. paraensei*. From these array experiments we demonstrated that the acute immune response of *B.*

glabrata is capable of discriminating a variety of pathogen challenges (gram-positive or -negative bacteria, and *E. paraensei* or *S. mansoni*). The responses elicited by each of these stimuli resulted in a distinct profile of transcripts being up or down-regulated in *B. glabrata* after only a 12 hour exposure. When infections using *E. paraensei* or *S. mansoni* were analyzed at 1, 2, 4, 8, 16 or 32 days post infection, a long-term profile of transcriptional changes emerged that detailed an initial increase in immune-related transcripts in *B. glabrata*. This early increase was followed by a sharp decline in the expression of many of the same transcripts, and a significant down-regulation of transcripts involved in immune and physiological processes. These transcription profiles match closely with earlier experimental studies that both parasites evade immune detection by suppressing the host response. Interestingly, the final array study demonstrates that *B. glabrata* is capable of defending itself against *E. paraensei* infection (the parasite thought to employ a strong immuno-suppressive strategy), if it is first sensitized by exposure to irradiated *E. paraensei* miracidia. This acquired resistance has been shown to last at least 8 days after sensitization and is associated with the up-regulation of a number of unique immune transcripts that are being further investigated for their possible role in parasite resistance. Supported by NIH 1P2ORR18754, R01 24340 [ESL], R01 52363 [CMA] and a NSERC PDF Fellowship [PCH].

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Neospora caninum Profilin-Like Protein is Expressed by Tachyzoites and Regulates Host Cytokine Production.

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Apicomplexan parasites interact with their host species using sophisticated mechanisms that involve up-regulation rather than suppression of host immune responses. *Toxoplasma gondii* profilin was shown to bind to the toll-like receptor 11 and trigger interleukin-12 (IL-12) production, facilitating a typical type 1 immune response. The *N. caninum* profilin (NcPRF) has an open reading frame of 492 bp, encoding a protein of 163 amino acids, which is 96% similar to the *Toxoplasma* homologue. With the goal of studying cytokine responses induced by *N. caninum*, a cDNA coding for NcPRF was cloned from tachyzoites of the NC1 isolate and expressed as a polyHis recombinant protein in *E. coli*. rNcPRF was purified by NiNTA affinity chromatography and was used to raise rabbit antisera specific for NcPRF. The immunoregulatory function of NcPRF was tested using mouse enriched dendritic cells (DCs) and splenocytes. The presence of NcPRF was confirmed by Western blotting in tachyzoite lysate and abundantly localized in *Neospora* tachyzoites by the indirect immunofluorescence staining and immunoelectromicroscopy. Purified rNcPRF induced high levels of IL-12 and tumor necrosis factor-alpha by DCs and interferon-gamma by splenocytes. The present study demonstrated the expression of NcPRF

in tachyzoites and a role for a profilin-like protein in mediating host inflammatory responses to *N. caninum* infection.

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Parasites and Conservation: Lessons From the Study of Global Amphibian Population Declines.

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As a part of the ongoing global loss in biodiversity, many amphibian populations have declined or experienced range reductions over the last several decades. A multitude of factors appear to be responsible for these amphibian losses, including habitat loss, the introduction of exotic species, chemical pollution, global environmental changes, and disease. While parasites have not been directly linked to amphibian population declines, there is recent evidence that the trematode *Ribeiroia ondatrae* can cause limb malformations and large-scale mortality in larval amphibians. Therefore, considering the role of parasites in amphibian conservation is certainly relevant. In addition, the history of studies addressing amphibian population declines can provide some interesting insight into how we might generally approach the question of parasites and species conservation in a wide array of systems.

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Conservation Medicine in the Galapagos Islands

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Since 2001, we have worked in a four-way partnership between US and Ecuadorian institutions, to describe the pathogens and parasites present in the endemic avifauna of the Galapagos Islands. The US partners include a university with strength in evolutionary ecology and tropical biology, and a zoo with strength in veterinary medicine; the Ecuadorian partners include the governing agency overseeing the Park, and an international group of science advisors residing on the islands. Our work is motivated by the high endemism and absence of extinctions of Galapagos bird species in light of significant species losses to diseases in other Pacific archipelagoes. Working through university graduate students, zoo veterinarians, and local partners, we have screened more than 7000 individual birds representing 31 species of seabirds and terrestrial birds on 16 islands. Working with ectoparasites, blood parasites, and serological data for viruses and bacteria, we have described a suite of pathogens and parasites in Galapagos birds that fall into three main categories: (1) Those that arrived with the colonists of the current endemic lineages and evolved alongside their hosts; (2) Those that arrived on one species and jumped to another; and (3) Those that are more recent arrivals associated with human colonization.

Using a genetic phylogeographic approach, we examine the history of colonizations and host switches of parasites of Galapagos birds. Pathogens of particular concern are haemosporidian blood parasites and avipox viruses, and we are examining the hypothesis that the host lineages that have longer histories on the islands are more negatively impacted by the arrival of new pathogens. We are working with local agencies to develop management plans and programs to prevent future introductions.

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Role of Parasites in Biological Invasions

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Biological invasions cause billions of dollars in economic damage each year and are a serious threat to native biodiversity. Introduced species often escape most of the parasite and pathogen species from their native range. While they do accumulate novel parasites from their new location, this is generally only a fraction of the number lost. Introduced populations are also generally less frequently infected (prevalence - % individuals infected) compared to native conspecific populations. I discuss patterns of parasite release across a broad range of aquatic and terrestrial species and give some specific examples of how this may influence invasions in marine systems. In conjunction with other biological and physical factors, release from parasites can explain the increased demographic performance of invasive species, thereby accounting for much of the damage they cause.

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Historic Perspectives on Discovery and Dogma in Parasite Immunity, Therapeutics, and Systematics.

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The Symposium will deal with historic perspectives on discovery and dogma in parasite immunology, drug therapy, and systematics. In all three sub-disciplines of parasitology, there is a long tradition of innovation, breakthrough, and change, extending back nearly five thousand years. The parasite groups under focus in the Symposium will include those that cause schistosomiasis, malaria, and HIV-AIDS. The three presenters are all Associate Editors for the Journal and include Conor Caffrey, Susan Perkins, and Evan Secor. A question and answer period will follow.

Drug Discovery for Schistosomiasis: Hit and Lead Compounds Identified in a Library of Known Drugs by Medium-Throughput Phenotypic Screening.

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Praziquantel (PZQ) is the mainstay drug for treatment and control of the helminthic tropical disease, schistosomiasis. Given this reliance on a single drug and the recent political momentum to increase its availability, PZQ-treatment failure that becomes both clinically relevant and widespread is a worrying prospect. Therefore, the UCSF Sandler Center and Small Molecule Discover Center have designed a phenotypic (whole organism) screen workflow of higher throughput to identify compounds with anti-schistosomal activity from in-house collections of small molecule libraries. The screen workflow is formatted to 96-well plates and commences with schistosomula (immature worms) harvested in their tens of thousands from the snail vector. 'Hit' compounds arising are then tested against the more limiting adult worms in the second stage of the workflow. Finally, hit compounds are prosecuted in an animal model of disease for parasitological efficacy and decreased disease pathology. The screen workflow incorporates two GO/NO GO filters to prioritize compounds with respect to a target product profile that is based on PZQ. The screen was inaugurated with a collection of over 2,000 compounds that includes drugs approved for human use, some of which are off-patent. Our intention is to 're-position' existing drugs for which substantial preclinical and clinical data exist thereby accelerating drug development while keeping costs down. Whereas death is the desired phenotype in screens of single-celled parasites, the schistosome worm can display a variety of phenotypes, which we have categorized into groups based on visual assessment. A number of hit compounds, across diverse chemistries and drug classes have been identified and these are being tested in the disease animal model. The results generated from all stages of the screen workflow are placed on public databases in the hope of spurring further discovery. Our research is supported by the Sandler Foundation.

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The Tangled Trees of Malaria Parasites: Problems of the Past, Present Pitfall, and Prospects for Progress.

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Malaria remains one of the most important diseases of humankind, yet the handful of species that are responsible for this scourge are just a tiny fraction of the diversity of the genus *Plasmodium*. Past classification has relied on host information, basic life history characteristics and morphometric data, however molecular data have recently been at odds with these delineations. I will trace the history of the molecular systematics of the malaria parasites, illustrating why the strange genomes of these organisms have stymied many efforts to both collect and analyze the data. I will also present new strategies and new markers that are beginning to allow for more substantial progress toward unraveling the phylogeny of *Plasmodium* and related genera. Combining these markers with new taxa (sometimes from very unexpected sources!) is helping to better illuminate the history of these diverse and important parasites.

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Increased Susceptibility to Immunodeficiency Virus Infections in Rhesus Macaques with Acute Schistosomiasis.

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Studies of parasite ecology are often used to address how environmental changes affect populations of hosts and parasites within a given ecosystem. But parasites can similarly alter the “environment” within a host, thereby impacting the susceptibility of the host to other infectious agents. We have been investigating how host responses to *Schistosoma mansoni* infections of rhesus macaques may alter infection of the monkeys with immunodeficiency viruses to model the possible impact of parasitic helminth infections on HIV/AIDS in sub-Saharan Africa. Presence of schistosomes increases viral replication whether the monkey is infected with schistosomes first or with virus first, coinciding with a parasite induced Th2-type immunologic shift. Furthermore, the AID₅₀ in animals exposed rectally to immunodeficiency virus is 17-fold lower in animals with schistosomiasis than it is in animals not exposed to *S. mansoni*. Thus, schistosomes greatly increase experimental host susceptibility to immunodeficiency

virus infection, possibly affecting the epidemiology of HIV/AIDS in developing countries endemic for schistosomiasis.

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Interactions Between Parasites and Pollutants in Yellow Perch (*Perca flavescens*) in the St. Lawrence River, Quebec, Canada.

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Oxidative stress enzymes and their metabolites are considered good biomarkers of general animal health. A previous pilot study demonstrated that oxidative stress in yellow perch (*Perca flavescens*) was affected by both pollution and infections with certain parasites. To further examine the potential combined effects of contaminants and parasitism on fish health, we measured protein content, thiols, and activities of glutathione-S-transferase, glutathione reductase, catalase, ceruloplasmin, and lysozyme in yellow perch from 2 polluted and 2 reference localities in the St. Lawrence River, Quebec, Canada. A total of 21-30 yellow perch (age 1+) were collected from each locality in June 2004. Gills and head kidneys were immediately removed for biomarker measurements, and the remainder of the fish was frozen for subsequent parasitological analyses. Two-way ANOVAs revealed interactions between infection levels of *Apophallus brevis*, *Diplostomum* spp., and *Ichthyocotylurus* spp. and locality class (polluted and reference) for certain biomarkers. Furthermore, various biomarkers were affected by the infection levels of *A. brevis* and *Diplostomum* spp. Activity of glutathione reductase in gill tissue decreased with increasing numbers of *A. brevis*, but only at the 2 most polluted localities. Catalase activity in kidney increased with numbers of *Diplostomum* spp. at the 2 polluted localities, but not at the 2 least contaminated sites. Results suggest that parasites may affect expression of biomarkers of pollution and that the pathogenicity of parasites may be enhanced under polluted conditions. Contamination appears to reduce tolerance, but not resistance, to parasites in yellow perch in this system.

Larval *Anisakis* spp. in Subyearling Chinook Salmon (*Oncorhynchus tshawytscha*): An Indication of Changing Ocean Conditions in the Northern California Current System.

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Early ocean residence is a critical period for survival of Pacific salmon. A major focus of our research is directed toward measuring the physical and biological conditions in the Northern California Current that affect growth and survival of juvenile salmonids during their first months in the ocean. Our program samples juvenile salmon, associated fish assemblages, zooplankton, etc., and physical ocean variables off of the Oregon and Washington coasts during May, June and September annually. Determining linkages between ocean conditions and juvenile salmon ecology can be difficult. As part of this study, we have collected larval *Anisakis* from body cavities of ocean-caught juvenile salmon. Since 2001 prevalences have been consistently low in yearling coho salmon (*Oncorhynchus kisutch*) and Chinook salmon (*Oncorhynchus tshawytscha*), ranging from 0.7 to 2.9% annually in coho, and 0 to 2.9% annually in Chinook salmon. Mean intensities were also low, ranging from 1.0 to 2.0 annually. In contrast, subyearling Chinook salmon, which typically enter the ocean later in the summer than yearling salmon, had higher annual prevalences than yearlings, and higher interannual variability, ranging from 2% to 17%. Mean intensities, however, were not higher in subyearlings than yearlings. Prevalences of infection were highest among subyearlings caught in September; from 2.5% to 22.8%. This prevalence has been highest the past four years, reaching 22.8% in Sept of 2008. Genetic analysis of the salmon suggests that the observed recent increase in the prevalence of larval worms from the body cavity of subyearling Chinook salmon occurred in all three of the most dominant stock groups. This observed increase in prevalence could be an indication of changing ocean conditions in mid- to late-summer and evidence of the linkages between subyearling Chinook salmon and their ocean habitat. We are currently examining other biological variables and physical ocean conditions that may be associated with the observed increased prevalence of larval *Anisakis* spp.

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Parasite Species Richness as a Metric to Assess the Trophic Interactions and Habitat Quality of Pacific Salmon in the Freshwater and Marine Environment.

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Macroparasites have been an increasingly important area of study in regards to trophic interactions, stock identification and habitat use of Pacific salmon. However, parasite communities have not been used to directly monitor the trophic success and survival of Pacific salmon upon their first year of ocean entry when mortality rates are the highest. Because salmon are fast growing, short lived, anadromous fish they benefit from opportunistic feeding strategies in different habitat types during their seaward migration. Currently we are examining the macroparasite communities of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) acquired through diet, to measure the importance of various habitat types of juvenile salmonids as they approach and enter the northern California Current. In June and September of 2002 and 2004 fish were collected from eight transects located from Northern Washington (La Push) to southern Oregon (Newport) with the Columbia River as a central transect. A total of 19 macroparasite species were recovered from the stomachs, intestines and body cavities of 920 salmon. Four trematodes, *Deropegus aspina*, *Lecithaster gibbosus*, *Parahemiurus merus*, and *Plagioporus shawi* were the most common parasites recovered. In 2002 and 2004 parasite species richness was positively correlated with coho salmon ocean growth. Southern Oregon and Northern Washington salmon showed the highest levels of parasite prevalence, intensities and species richness, suggesting decreased trophic interactions and/or less favorable habitat in and around the Columbia River estuary and plume. Parasite Species richness, may then be a useful tool as a metric of trophic success and habitat quality for juvenile salmon.

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Cercarial Behavior Patterns and Host Specificity of *Glythelmins* spp. in Tadpoles and Metamorphosed Anurans.

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Glythelmins pennsylvaniensis and *Glythelmins quieta* are 2 common anuran intestinal trematodes with a 2 host life cycle. After emerging from the snail first intermediate host, cercariae of *G. pennsylvaniensis* infect the tadpole stage of anurans, whereas the cercariae of *G. quieta* infect the frog stage of anurans. Field studies also indicate that *G. pennsylvaniensis* is restricted to treefrogs in the

genus *Pseudacris*, whereas *G. quieta* is restricted to true frogs in the genus *Rana*. In order to investigate the mechanism of this host specificity we exposed 4 species of adult frogs and their tadpoles (*Pseudacris maculata*, *Bufo woodhousii*, *Rana blairi*, and *R. catesbeiana*) to laboratory reared cercariae of *G. pennsylvaniensis*. Our results indicate that cercarial behavior pattern dictated stadial host specificity in *G. pennsylvaniensis* and no cercariae attached to any adult frogs, whereas *G. pennsylvaniensis* cercariae attached to all 4 species of tadpoles and species specific innate immunity in tadpoles dictated metacercarial development resulting in only tadpoles of *Pseudacris maculata* becoming infected. These alternative mechanisms for host specificity in *Glythelmins* spp. suggest different selective pressures on different trematode life cycle stages (cercariae and metacercariae), and we discuss the evolutionary implications of these selective pressures on amphibian trematode life cycle evolution.

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Parasitism and Habitat Use of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in the Columbia River Estuary.

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This study was part of a larger project that examined the importance of the Columbia River estuary and the extent that juvenile salmonids use it for growth and rearing. We examined the relationship between habitat use and the parasite communities of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in three different habitat types in the Columbia River estuary. Salmon were collected from April through July at: 3 main stem sites in tidal freshwater areas 53 km into the estuary and 4 main stem sites in the marine/estuarine mixing zone near the mouth of the Columbia River and peripheral wetland habitats in the mid-estuary not on the main stem of the Columbia River. Multidimensional scaling suggests differences in parasite communities of juvenile salmon collected at different sites in 2004. There were significant differences between sites in the prevalence of *Hysterothylacium* sp ($p < 0.01$), *Echinorhynchus lageniformis* ($p < 0.01$), and *Deropegus* sp ($p < 0.01$). All of these parasites were most prevalent in the mid-estuary wetland sites: *Hysterothylacium* sp (34%), *E. lageniformis* (27%), and *Deropegus* sp (25%). Greater levels of parasitism in this area may reflect increased or different feeding opportunities which provides better habitat for rearing than in the main stem sites. The freshwater and estuarine mixing zone had low prevalence of these parasites which indicates that these salmon in these areas were either not using the habitat for rearing or the habitats did not have the intermediate hosts. Differences in parasite communities between habitat types suggests that some salmon use the estuary for feeding and growth which results in increased prevalence, whereas, others use the estuary as a corridor to ocean entry which results in decreased prevalence of parasites.

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Impact of Coal Mine Effluent on Fish Parasite Assemblages in Southern Illinois Streams.

A.T. Claxton, Cooperative Institute of Marine Resource Studies, Oregon State University, Hatfield Marine Science Center, Newport OR and J.R. Laursen, Department of Biological Sciences, Eastern Illinois University, Charleston IL.

This study examined the impact of coal mine effluent on helminth community structure in fish from the Saline River Basin in Illinois. Sunfish (*Lepomis* spp.) were collected from three sites upstream, and three sites downstream of a mine effluent point source, below which the Illinois EPA has documented a “dead zone” extending for several kilometers. Distributions of both fish host species and parasites varied in relation to coal mine effluent. Bluegills (*L. macrochirus*) and Green sunfish (*L. cyanellus*) were found at all sites. Twelve genera of adult helminths were recovered: 3 Acanthocephalans (*Neoechinorhynchus*, *Eocollis*, *Acanthocephalus*); 5 Nematodes (*Spinitectus*, *Camallanus*, *Capillaria*, *Spiroxys*, *Contracaecum*); 2 Cestodes (*Bothriocephalus*, *Proteocephalus*); and 2 Trematodes (*Pisciamphistoma*, *Crepidostomum*). Multidimensional scaling was used to determine differences in parasite assemblages between sites. Community similarity between the six sites ranged from 59% to 85%. The two sites with the least similarity were the two most proximate spatially with the coalmine situated between them. Individual parasite taxa also responded to coal mine impact. *Spinitectus* spp., which uses mayflies as intermediate hosts, were significantly more common upstream and may be useful as bio-indicators of quality habitats. *Eocollis* spp., which uses crustacean intermediates, were more common downstream and may be indicative of stressful habitats. *Camallanus* spp., which uses copepod intermediate hosts, did not follow any clear trend related to coal mine effluent. These differences may be due to changes in intermediate host assemblages above and below the point source, diet shifts associated with intermediate host prey availability or fish gape size limits due to growth retardation, or the effect of physiological stress on host fish.

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Two Adult Parasites From the Same Fish Host Occupy Different Trophic Levels.

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In food web studies, it is frequently not clear to which trophic level parasites be assigned. The objective of this study was to determine the trophic positions of two internal parasites infecting the same fish

host (*Aphredoderus sayanus*). The acanthocephalan parasite (*Acanthocephalus tehlequahensis*) lives in the intestine and feeds on host food by absorption. The trematode parasite (*Phyllodistomum* sp.) lives in the ureters and feed directly on host blood and tissue. Stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was performed on representative species from all trophic levels in the natural stream habitat of this fish host and these parasites. *Phyllodistomum* sp. was significantly enriched in ^{15}N compared to *A. tehlequahensis* suggesting that the two parasites occupy different trophic levels. There were significant differences between the isotopic signatures of adult acanthocephalan parasites and their pirate perch host, indicating that their food source was not identical and thus acanthocephalan resides at a lower trophic position than its fish host. Whereas trematode tissues were significantly enriched in $\delta^{15}\text{N}$ when compared to its fish host, suggesting that trematodes feed on fish tissue and therefore occupy a trophic level above fish.

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Combined Impact of Parasites and Predators on Wood Frog Tadpoles.

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Predators can have important impacts on host-parasite dynamics in natural systems. For many directly-transmitted parasites, predators can reduce transmission by removing the most heavily infected individuals from the population. Less is known about how predators might influence parasite dynamics in systems where the parasite relies on vectors or numerous different host species to complete their life-cycles. Digenetic trematodes are a diverse group of parasitic flatworms with complex life-cycles typically involving three host species. They can infect all vertebrate classes, and are common parasites in freshwater systems containing aquatic snails, which serve as obligate first intermediate hosts. In this study, we examined the impact of two trematode species (*Echinostoma trivolvis* and *Ribeiroia ondatrae*) and predatory larval salamanders (*Ambystoma jeffersonianum*) on short-term survival of larval wood frogs (*Rana sylvatica*). Both parasites and predators significantly reduced tadpole survival in experimental outdoor pools. After six days, tadpole survival was reduced from 100% in control pools to a mean of 46% in pools containing infected snails and a mean 49% in pools containing predators. In pools containing both infected snails and predators, tadpole survival was further reduced to a mean of just 5%. These dramatic results suggest that non-host tadpole predators could potentially limit transmission of these parasites in systems where there is substantial predation pressure. In addition, the parasites themselves may cause substantial mortality to second intermediate hosts which could also negatively influence the probability of transmission to the next host.

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Genetic Differentiation in Large Turkey Louse (*Chelopistes meleagridis*) Populations Reveals Limited Movement of Turkeys Across the Mississippi River.

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Molecular genetic data from specialist parasites can reveal information about the population genetic structure of their hosts. In this study, mitochondrial cytochrome oxidase I DNA sequences of the highly host specific large turkey louse (*Chelopistes meleagridis*) were investigated to determine wild Turkey (*Meleagris gallopavo*) movement patterns within the Midwest. DNA was extracted from 190 lice found on 26 turkeys sampled from Iowa, Missouri, Nebraska, and Illinois. A 356-base-pair sequence was amplified via PCR, aligned with Clustal W, and analyzed with DnaSP v4.20 and GenAlEx v6.1 software. Eighteen haplotypes were found, distinguished by 35 polymorphic sites. Two distinct lineages (“West” and “East” clades), indicating a population split across the Mississippi River, were revealed by both a neighbor-joining tree and a haplotype network. One might expect significant isolation by distance between populations of a non-migratory species. However, a Mantel test revealed no correlation between genetic distance and geographic distance. Furthermore, AMOVA analysis supported the West and East clade split across the Mississippi River, with twice as much variation partitioned across the river, as within either side of the river ($\Phi_{RT}=0.34$, $p=0.001$). Our data indicate that there is little gene flow between *C. meleagridis* populations on opposite sides of the river, suggesting that Turkey movement across the river is either limited or absent. Furthermore, the absence of isolation by distance on either side of the river could reflect translocation events between distant sites within states. Thus, it appears that *C. meleagridis* may be a good parasite proxy for the management and conservation of wild Turkey populations across the Midwest.

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Community and Genetic Analyses of Macroparasites from Pacific Sardine (*Sardinops sagax*) Caught in the California Current System.

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A Pacific sardine (*Sardinops sagax*) fishery has resumed in the Pacific Northwest (PNW) more than 50 years after the fishery collapsed in 1948. Tagging studies of Pacific sardine during the peak of the fishery in 1930s and 1940s suggested larger individuals migrated north from southern California to feedings

grounds off the Columbia River and into Canadian waters after spawning off of southern California in April. It is believed these large sardines remain in the PNW until the onset of winter storms before returning to southern California to overwinter. To assess the current behavior and potential population structure of Pacific sardine within the California Current System we are examining the community structure of macroparasites acquired through diet. Approximately 1000 sardines collected from 2005 to 2007 have been examined to date, and five parasite species have been identified as potential biological tags. *Lecithaster gibbosus* and *Pseudopentagramma petrovi* were common only off of Vancouver Island, while *Anisakis* spp. was common from Vancouver Island to northern California. *Parahemiurus merus* and *Myosaccium ecaude* were found throughout the study area but were most prevalent off of southern California. Recently, we began to assess if the genetic structure of a nematode species could provide insight into the population structure of sardines. Fifty-five unique haplotypes of the mitochondrial gene *COX2* were identified in 66 individual *Anisakis* spp., and individual nematodes with identical haplotypes were observed from southern Washington to northern California. Overall nucleotide diversity was 4.7%, and more individual *Anisakis* spp. are currently being sequenced. With four different macroparasite communities currently identified, our data suggest that Pacific sardine are not migrating throughout the entire study region, and that the historical paradigm of one sardine population migrating between southern California to southern British Columbia may no longer be accurate.

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Especially Great Phylogenetic Diversity Characterizes the Coccidia
Infecting Fish.

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Coccidian parasites are ubiquitous among vertebrate hosts, yet we understand little concerning those coccidia infecting the oldest and most phylogenetically diverse group of vertebrates: fish. We were motivated to better understand the evolutionary origins and diversity of the coccidia by beginning to redress this historical oversight. To do so, we coupled diagnoses based upon morphological characters (which are often fragile in such aquatic species) with phylogenetic reconstructions based upon variation in ribosomal DNA. Our work to date, although only beginning to address diversity among these parasites, establishes that: 1) coccidia of fish, variously attributed to the genera *Goussia* and *Eimeria*, are extraordinarily diverse, 2) some represent early branches of *Eimeria*, 3) others derive from one of several independent evolutionary lineages, and that the first vertebrates parasitized by coccidia were fish. These findings gain practical importance when considering the possibility that people may contract coccidian infections when consuming uncooked fish.

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Ecology and Phylogeny of Amphibian Coccidia: A Review.

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Amphibian coccidia are the least studied group of coccidians of tetrapod vertebrates. Currently 32 species of coccidia are known (19 *Eimeria*, 9 *Isospora*, 3 *Goussia*, and 1 *Hyaloklossia* species) which infect anurans, salamanders and caecilians. Recent studies on the ecology and host specificity of amphibian coccidia in European and North American anurans and salamanders indicate complex ecological and transmission strategies that differ among genera of coccidia as well as the hosts (salamanders or anurans) that they infect. We present a review of these ecological transmission strategies along with the first molecular phylogenetic hypothesis based on the small subunit ribosomal DNA (SSU) of selected North American and European anuran coccidian.

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Coccidia from Mammals: Interesting Insights on Phylogenetic Relationships, Host Specificity and Morphology.

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In addition to standard morphologic descriptions, molecular characterization is becoming an important and inevitable component for the identification, diagnosis and taxonomy of coccidian parasites. Based on nuclear 18S rDNA sequences, the most sequenced DNA region, coccidia are divided into two lineages, Eimeriidae and Sarcocystidae. This trait clearly corresponds with the type of excystation structures. The genus *Eimeria* splits into well-formed lineages according to the host species, such as fowl-specific or rabbit-specific lineages. However, this pattern is not universal. For example, rodent-associated *Eimeria* species cluster into two lineages independent of the rodent families. While each of these lineages themselves are monophyletic, their relationships are paraphyletic or even polyphyletic. Another interesting feature, the oocyst residuum, seems to represent an important structure in phylogeny of Eimeriidae. The clade of rodent-specific and rabbit-specific *Eimeria* species each divide into two lineages corresponding to the presence/absence of the oocyst residuum. Other morphological or biological traits do not explicitly correlate with the phylogeny of *Eimeria*. Extended analyses of 18S rDNA and ORF470 of several recently sequenced coccidian species from interesting mammals will be presented. The pattern regarding the oocyst residuum remains, and other peculiarities and observations will be discussed.

However, the 18S rRNA gene is usually not sufficient to infer relationships among closely related *Eimeria* species. Therefore, in the future other suitable genes such as the plastid ORF470, plastid 23S rDNA or mitochondrial COI will need to be sequenced to learn more about coccidia, their relationships and evolutionary patterns.

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ASP Presidential Address.

B. Conn, Professor of Biology and Dean, School of Mathematics and Natural Science, Berry College.

No abstract.

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Prevalence of Internal Parasites of Dogs and Cats in the United States:
Update of a Study in Progress.

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Determining the prevalence of internal parasites of dogs and cats is important for monitoring effects of parasiticides, assessing potential for pet-associated zoonotic diseases in humans, and monitoring changes in parasite distribution. In 1996, Blagburn *et al.*, (*Comp Cont Ed Pract Vet* 18: 483-509) published the first comprehensive survey of parasites of shelter dogs in the United States (US). Reported prevalences in 6,458 dogs were as follows: *Ancylostoma caninum*: 19.2%, *Toxocara canis*; 14.5%, *Trichuris vulpis*: 14.3% and *Cystoisospora (Isospora) spp*: 4.8%. The present study was undertaken to obtain prevalence data in shelter dogs and cats in 2008-2009 and to compare current prevalences to those reported in the 1996 survey. Methods employed in this survey were similar to those used in the 1996 survey. Fecal specimens were acquired from shelter dogs and cats and shipped via overnight courier to Auburn University. Specimens (2-5 grams) were examined using centrifugal sucrose flotation (SG 1.26). At this time, 4,909 fecal specimens (3,364 canine/1,545 feline) have been processed. Current prevalences of selected parasites are: *A. caninum* (AC), 32.3%; *A. tubaeforme* (AT). 8.7%; *T. canis* (TC), 13.8%; *T. cati* (TCt), 21.4%; *T. vulpis* (TV), 19.1%; *C. (Isospora) felis* (CF), 10%; *C. rivolta* (CR), 7.3%; *C. canis* (CC), 4.9% and *C. ohioensis*-like (CO), 8.5%. Prevalences in the southeastern US were: AC, 54.5%; AT, 22.9%; TC, 17.6%; Tct, 20.3%; TV, 28.1%; CC, 6.1%; CO, 9.6%; CF, 11.3% and CR, 9.2%. It appears,

based on current data, that selected endoparasite prevalences have not decreased in the 13 years since the 1996 survey. The authors gratefully acknowledge the support of Bayer HealthCare, Shawnee Mission, KS.

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Cryptosporidium and *Giardia*: Zoonotic Implications of Those Found in Dogs and Cats.

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Dogs and cats are common hosts of *Giardia*. At one time, *G. felis*, *G. canis*, and *G. lamblia* were accepted as the species of cats, dogs, and humans, respectively; but they are currently not viewed as valid. Cryptosporidial infections are fairly uncommon in companion animals, and recently recognized species of *Cryptosporidium* are *C. felis* in cats, *C. canis* in dogs, and *C. hominis* in people. However, with both *Giardia* and *Cryptosporidium*, the question remains: Are they zoonotic? Fifteen years ago, this was a fact; now, like the old specific names of *Giardia*, these facts seem ethereal. *Giardia* is currently divided into molecularly identified Assemblages and subtypes: Assemblages C & D in dogs, Assemblage F in cats, and Assemblages A or B in people. The trouble is that the subtype A1 can show up in animals, and B has been reported on rare occasions from dogs and cats. *C. felis* and *C. canis* are restricted almost exclusively to cats and dogs, but if people are immunocompromised, they can be infected with these parasites, and in areas with poor sanitation, children have been found infected with *C. canis*. It may be possible to infect dogs with human Assemblage *Giardia*, but *Cryptosporidium parvum* (the zoonotic form in calves) does not infect dogs and cats, and it is likely that *C. hominis* does not infect canine and feline hosts. The zoonotic potential of these agents is not an esoteric problem: people want asymptomatic pets treated, pet stores have been closed because their puppies have *Giardia*, and state laws have been interpreted to preclude the adoption of a pet shedding *Giardia* cysts in its stool. Also, treatment in pets is not always simple. *Giardia* regularly becomes a post-treatment Cheshire Cat that reappears between multiple rounds of therapy. Fortunately, because the drugs may or may not be efficacious, *Cryptosporidium* in cats and dogs is usually asymptomatic. The need remains for veterinarians to inform clients of the minimal risks associated with these infections in cast and dogs and the need to appropriately clean up after their pet.

Wolbachia: Rationale and Evidence For Incorporating Doxycycline Into Treatment Protocols for *Dirofilaria immitis*.

C.T. Nelson, Animal Medical Centers.

Human and animal parasitic filarial nematodes, often the cause of severe disease, harbour intracellular bacteria of the genus *Wolbachia* (Rickettsiales). It is thought that these bacteria play an important role in the pathogenesis and immune response to filarial infection. There are 14 filarial nematodes which harbor a species of *Wolbachia*. These organisms are necessary for these filarial nematodes to reproduce and thrive. They also produce metabolites which have shown contribute to the disease process. Doxycycline is now being used in the treatment of several human filarial diseases and has been the subject of several studies in heartworm treatment. One such study showed that heartworm positive dogs pretreated with ivermectin and doxycycline prior to receiving melarsomine injections had less pulmonary pathology associated with the death of the heartworms. The same study also showed a 78% reduction in heartworm numbers using ivermectin and doxycycline only after 36 weeks of therapy. Doxycycline administered at 10mg/kg BID for 4 weeks has been shown to eliminate over 90% of the *Wolbachia* organisms and the levels remain low for 3 to 4 months. Clinical experience with heartworm treatment protocols incorporating doxycycline have resulted in decreased adverse reactions and decreased mortality.

Survey of Heartworm Prevention Practices Among Dog Owners and Trainers in North America.

S. Patton, A, Odoi, and B.W. Rohrbach.

The FDA generated a safety signal for lack of effectiveness of macrolide heartworm chemoprophylactic agents in 2004/05 due to an increasing number of reports of prophylaxis failure. Successful prophylaxis requires drugs to be administered at the appropriate dose and time for the duration of the period of exposure to infection. Owners and trainers of dogs that belonged to an international club for hunting and retrievers were surveyed to obtain information on their knowledge and practice of administration of heartworm prophylaxis. Responses were compared with recommendations of the American Heartworm Society (AHS). Of 708 respondents the median years caring for dogs was 19, interquartile range (IR 10, 28) and 65% currently cared for 2-5 dogs. A majority of respondents reported that heartworm prophylaxis was administered year round, one or more means to control exposure to

mosquitoes were used and dogs were outdoors at some time during the hours from dusk to dawn during the mosquito season, one or more heartworm tests were conducted annually and dogs were tested for heartworm prior to entry to their home or kennel. Respondents to our survey were computer literate and due to their association with a dog club that provides access to educational material, are likely to be more knowledgeable about heartworm prevention than the average dog owner. A substantial number of respondents did not test dogs for heartworm annually or test dogs prior to introduction into the kennel, and of those residing south of the 37th parallel did not administer heartworm preventive year round in contrast to the recommendation of the Companion Animal Parasite Council.

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Survey of Risk Factors for Failure of Heartworm Prophylaxis in Dogs.

B.W. Rohrbach, A. Odoi, and S. Patton.

The FDA generated a safety signal for lack of effectiveness of macrolide heartworm chemoprophylactic agents in 2004/05 due to an increasing number of reports of prophylaxis failure. Macrocylic lactones are considered highly effective and there are no published reports to suggest resistance of L3 and L4 stage heartworm larvae to this drug class. A survey of heartworm prevention practices used by 708 dog owners and trainers was conducted during the summer and fall of 2008. Risk factors for failure of prophylaxis were compared among respondents reporting failure in the past 12 months with those reporting successful prophylaxis. Factors associated with failure in the univariate analyses were; being a dog owner versus a trainer or commercial kennel operator, being responsible for the care of a single dog and dogs kept outdoors for fewer hours during the months when mosquitoes are active. Testing for heartworm at least once a year was associated with successful prophylaxis. Efforts to reduce exposure of dogs to mosquitoes, and factors that favor the presence of mosquito breeding sites in proximity to kennel or residence, were similar among the comparison groups. These data suggest that the risk of prophylaxis failure may be related to the number of dogs cared for and whether there is a commercial association with dogs and is not related to exposure to mosquitoes. Commercial association with dogs and increasing number of dogs under respondents care may be associated with increased knowledge, financial investment, and therefore, motivation to comply with current recommendations to prevent heartworm infection. In a multivariable model, respondents that administered heartworm prophylaxis only during months mosquito activity was observed were more likely to experience failure of prophylaxis. Failure to recognize mosquito activity, particularly at the end of the transmission season, increases the risk of heartworm infection.

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Manipulation of Host Temperature and Diet: Experiments on Three Species of Flour Beetles and Their Gregarine Parasites.

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The purpose of this experiment was to clear flour beetles of their gregarine parasites through host starvation and temperature manipulation. Three flour beetle larvae were examined in this study: *Tenebrio molitor*, *Tribolium destructor* and *Tribolium freemani*. To clear gregarine infections beetles were placed in 37°C incubators with no food for 48 hours. Following exposure we found that *Tenebrio molitor* were virtually cleared of their gregarine parasites. *Tribolium destructor* also displayed a similar (yet incomplete) reduction in prevalence and mean intensity, indicating varying levels of resilience to heat and starvation in gregarine parasites. *Tribolium freemani*'s established infection showed a positive response under environmental stimuli. Prevalence remained relatively the same, while mean intensity sky rocketed. A wide array of ecological questions arose during these experiments. Specifically, in *Tenebrio molitor* we wondered (1) if the host could be re-infected, (2) if temperature played a role in re-infection, and (3) if re-infection was not observed, then how was the host-parasite relationship altered to prevent reestablishment of parasites. To determine if hosts could be re-infected and if host temperature played a role in re-infection, *T. molitor* larvae were simultaneously re-fed sterilized food and placed into 4 treatments: 27° C + frass, 27° C + no frass, 33° C + frass, or 33° C + no frass. Only larvae exposed to the 27° C + frass treatment reestablished infections. We noted that re-infected larvae exhibited significantly greater mean intensities than time-0 and time-t controls. Further experiments are planned to explore the increase in mean intensity following re-exposure, and determine what stage of the gregarine life cycle is arrested during host re-exposure to frass in a high heat environment.

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Hymenolepis diminuta Infection and Life History Trade-Offs by the Intermediate Host *Tribolium confusum*.

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Previous studies have clearly shown that infection with the cestode *Hymenolepis diminuta* reduces fecundity, yolk volume and hatchability of eggs of the mealworm *Tenebrio molitor*. Infection of the much smaller flour beetle *Tribolium confusum*, in which energetic demands by the parasite should be

proportionately greater, also reduces fecundity but effects on other aspects of host life history have not been studied. This study examined effects of infection on fecundity and egg size of *T. confusum*. Intrinsic variation among host individuals was high. Fecundity or egg length in uninfected females did not vary relative to female mass, but egg length varied inversely with fecundity. Fecundity was reduced to 50% of pre-exposure levels during the first week post-exposure but this was due solely to the pre-exposure fast. Thereafter infected beetles exhibited intensity-dependent reduction in fecundity. The reduction in fecundity following fasting was associated with a small (3%) but transient increase in egg length, but subsequent fecundity reduction due to parasitism had little or no effect on egg size. Thus, the intrinsic trade-off between egg size and fecundity in *T. confusum* seems plastic with respect to nutritional stress caused by fasting, but not the nutritional stress associated with parasitism.

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Trypanosoma carassii hsp70 Up-Regulates Expression of Pro-Inflammatory Cytokines and Chemokines in Goldfish Macrophages.

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Trypanosomes are known for their remarkable immune modulation and evasion strategies, which are critical for their survival in the vertebrate hosts. After entering the fish host, *T. carassii* encounters stressful conditions such as changes in pH, temperature and immune responses of the host. To survive inside the host the parasite must activate several immune evasion strategies, that include the synthesis of heat shock proteins (hsp). We cloned and characterized parasite hsp70 that was initially identified in membrane fraction and excreted/secreted fraction of *in vitro* grown parasites using 2-dimensional gel electrophoresis and mass spectroscopy. Although a high degree of similarity exists between *T. carassii* and fish hsp70, parasite hsp70 was recognized by immune sera from infected fish suggesting a directed immune response to the non-homologous regions of *T. carassii* hsp70. We expressed *T. carassii* hsp70 using a prokaryotic expression system and found that it induced immune responses of the host. Treatment of goldfish macrophages with known amounts of recombinant parasite hsp70 induced up-regulation of pro-inflammatory cytokine expression and nitric oxide response of activated macrophages. These effects are believed to be due to interaction of parasite hsp70 with specific receptors on goldfish macrophages since pre-treatment of macrophages with protease abrogated hsp70-induced cytokine expression. [Supported by NSERC, CANADA]

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Pathology Associated with Adult and Microfilariae of *Dirofilariaeforma pulmoni* in Delmarva Fox Squirrels (*Sciurus niger cinereus*).

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A total of 63 endangered Delmarva Fox Squirrels (*Sciurus niger cinereus*), a subspecies of the Fox Squirrel (*Sciurus niger*), have been examined at the National Wildlife Health Center over a period of 31 years. The squirrels were collected in Maryland (18), Delaware (24) and Virginia (20). Nematodes were the only helminths recovered from the 63 squirrels representing 6 genera infecting heart, lung, pulmonary artery, intestine and ceca. *Dirofilariaeforma pulmoni* adults or microfilariae were recovered from 11 squirrels. The only parasite to cause disease was *D. pulmoni*. This is the first report of *D. pulmoni* from Delmarva Fox Squirrels. The only reports of *D. pulmoni* in wild animals were from three gray squirrels, two from Dorchester County Maryland and one from Trigg County Kentucky. Pathology associated with *D. pulmoni* has only been documented in one gray squirrel from Dorchester County, Maryland, where large numbers of adults occluded two thirds of the pulmonary artery forming a thrombus with adhesions to the arterial wall. Adult *D. pulmoni* in the pulmonary artery of the Delmarva Fox Squirrels occluded one third to one half of the artery restricting blood flow to the lungs. There were no thrombi nor adhesions noted. Histological sections from our 11 squirrels revealed microfilariae in most major organs. Nothing has been reported in the literature concerning the microfilariae or the pathology they may cause. Our examinations revealed that microfilariae were associated with pulmonary edema, and proliferative glomerulonephritis. It is not conclusive whether the microfilariae are the direct cause of the renal pathology previously mentioned or merely a contributing factor.

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Prevalence of *Profilocollis altmani* Cystacanths (Acanthocephala: Polymorphidae) in Pacific Mole Crabs, *Emerita analoga*, On the Central California Coast.

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Profilocollis altmani utilizes Pacific mole crabs, *Emerita analoga*, as intermediate hosts along the Pacific coast of North and South America. Acanthocephalan eggs are ingested by *E. analoga* during filter feeding, developing and accumulating in the hemocoel as cystacanths. Various shore and sea birds serve

as normal definitive hosts via ingestion of infected crabs. We investigated the spatial and temporal distribution of profilicollid cystacanth infections from 1,875 *E. analoga* collected from six beaches in San Luis Obispo County, CA during 2007-2008. A subset of cystacanths collected from crabs was identified by use of a taxonomic key. All were identified as *P. altmani*. Parasite loads increased with crab carapace length and between southern to northern beaches. Females and females with eggs had the largest parasite loads overall. However, independent of size, males appear to acquire parasites at a faster rate than females during growth. Differences in overall parasite loads between beaches may be primarily driven by abiotic factors and bird host activity patterns, but at a finer scale, male crabs may acquire parasites at a faster rate during growth than females due to immunological, hormonal, and/or behavioral factors.

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Parasite-Related Mating Suppression in the Intermediate Host *Caecidotea intermedius* (Isopoda): Effects of Antipredatory Behavior, Energy Reserves and Neuromodulation.

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The aquatic isopod, *Caecidotea intermedius*, is an intermediate host for the acanthocephalan, *Acanthocephalus dirus*. Development of *A. dirus* into the cystacanth stage correlates with suppression of mating behavior in male *C. intermedius*. Using a combination of field-based and lab-based approaches, we examined variation in physiology, neurobiology and antipredatory behavior of *C. intermedius* to identify potential mechanisms underlying parasite-related mating suppression. We used field-based surveys to examine the relationship between infection status and behavior and found that infected males were less likely to initiate mating attempts with females. Males were also more likely to be located in the open than uninfected males. We used a lab-based experiment to examine whether this microhabitat shift correlated with increased feeding activity, which could potentially compensate for increased energy demands associated with infection. We found that infected males did not feed more than uninfected males but instead wandered around in the open where they would be exposed to visually-hunting predators. Biochemical assays of energy content (glycogen, lipid) also revealed that infected males contained more energy than uninfected males. These results are consistent with the interpretation that mating suppression is not due to an increase in energy demands but rather may be part of a strategy of parasitic manipulation that increases exposure to definitive hosts. Finally, we examined the relationship between parasite infection, mating suppression, antipredatory behavior and neurochemical levels to determine whether parasite-related changes in behavior could be modulated by serotonin (5-HT) or dopamine (DA). Using HPLC, we measured variation in 5-HT and DA in the CNS of infected and uninfected males. We found that infected males had lower levels of 5-HT and DA than

uninfected males indicating that parasite-related neuromodulation could potentially underlie mating suppression in *C. intermedius*.

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Experimental Evidence for Evolved Resistance to Avian Pox Virus and Malaria (*Plasmodium relictum*) in Low Elevation Hawaii *Amakihi*.

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Introduced avian pox (*Avipoxvirus* sp.) and malaria (*Plasmodium relictum*) have had a profound effect on the geographic and altitudinal distribution of native Hawaiian forest birds, restricting most species to elevations above 1200 m. Transmission of both diseases varies across an altitudinal gradient of temperature and rainfall on the main Hawaiian Islands, creating a range of selection pressures between sea level and tree line. We challenged Hawaii *Amakihi* from both disease free high elevation habitats and from a rapidly expanding low elevation population from the island of Hawaii to *P. relictum* and concomitant infections with both *Avipoxvirus* and *Plasmodium*. In the first experiment, low and high elevation *Amakihi* were challenged with *P. relictum* with single infective mosquito bites. Low elevation *Amakihi* were just as susceptible to infection with *Plasmodium*, but had lower mortality and were more successful at controlling parasitemia than high elevation birds. In the second experiment, we challenged high and low elevation *Amakihi* with chronic malarial infections with one of two genetically defined variants of avian pox virus that have been isolated from wild forest birds. Mortality in high elevation *Amakihi* was 100% following challenge with pox virus and associated with recrudescing malarial infections. By contrast, low elevation *Amakihi* were able to control parasitemia after infection with pox and mortality was only 20%. This relatively recent host-parasite association is a good model for investigating mechanisms of disease resistance in wild avian hosts.

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Identification of Endoparasitic Helminth Eggs in the Stomach Contents of an Articulated Miocene Tapir (*Tapirus polkensis*) from the Gray Fossil Site, Northeast Tennessee.

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Stomach contents of an articulated fossil tapir from the Late Miocene (4-7 million bp) Gray Fossil Site were examined to reconstruct the diet and look for evidence of parasitic infection. Sediment samples were processed to extract fossil pollen acquired either by inhalation or ingestion, and the diagnostic products of endoparasitic helminths and protozoan species infecting the tapirs. Eggs of endoparasitic helminths identified to date are morphologically comparable to *Capillaria* sp. (50-60um X 24-35um) and *Ascaris* sp. (38-52um X 34-46um). Neither of these parasitic genera have been reported from extant wild tapir populations according to a brief survey of the scientific literature. Ongoing efforts are directed at examination of fecal samples from *Tapirus bardi* in Costa Rica for comparative evaluation of the parasitic fauna in modern and extinct host populations.

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Characterization of partially purified Excretory/Secretory (ES) antigens of *Gigantocotyle explanatum*, a liver infecting amphistome parasite of Indian water buffaloes *Bubalus bubalis*

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In Indian subcontinent paramphistomes constitute most abundant group of digenetic trematodes infecting the livestock. More than 70 species of paramphistomes have been described in cattle and approximately 40 species of paramphistomes have been reported from India. The disease "paramphistomosis" caused by immature migrating flukes is responsible for acute gastroenteritis and diarrhea with high morbidity and mortality in young stocks while the adult *G. explanatum* cause haemorrhage, periductal fibrosis and other hyperplastic changes. In some localities the prevalence of infection with paramphistomes is 80-90%. In India, livestock contributes 32% of the total energy requirements of rural areas. Considering the importance of cattle in our economy it is important to control the paramphistome infection. Recognizing the importance of immuno-diagnosis present study was undertaken to identify the immunodominant antigens of the amphistome *G. explanatum*, which

may be useful for immunodiagnosis of liver paramphistomosis. Sephadex G-200 purified fractions of *G. explanatum* were characterized by Enzyme Linked Immunosorbent assay (ELISA), SDS-PAGE and Western Blot analysis. A total of 3 fractions (F1, F2 and F3) were obtained by gel filtration. Antibody titer using hyperimmune rabbit sera against ES proteins of *G. explanatum* was checked by ELISA which revealed that F1 was highly antigenic (detecting IgG titer up to 1:12800 dilution) while F2 and F3 were low antigenic fractions (detecting IgG titer up to 1:400 dilution). The Western blot of ES fractions (F1, F2, and F3) detected 7, 4 and 6 antigenic polypeptides respectively. Taken together, this preliminary study could be helpful for the immunodiagnosis of paramphistomosis. Further studies are required to ascertain the persistence of the specific diagnostic candidate antigens during the different phase of life cycle.

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Differential Glycotope Expression During the Miracidium-To-Sporocyst Transformation of *Schistosoma mansoni*.

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Fucosylated carbohydrate epitopes (glycotopes) expressed by larval and adult schistosomes are thought to modulate the host immune response and possibly mediate parasite evasion in intermediate and definitive hosts. While previous studies showed glycotope expression is developmentally and stage-specifically regulated, relatively little is known regarding their occurrence in miracidia and primary sporocysts. In this study, previously defined monoclonal antibodies were used in confocal laser scanning microscopy, standard epifluorescence microscopy, and Western analyses to investigate the developmental expression of the following glycotopes in miracidia and primary sporocysts of *Schistosoma mansoni*: GalNAc β 1-4GlcNAc (LDN), GalNAc β 1-4(Fuca α 1-3)GlcNAc (LDN-F), Fuca α 1-3GalNAc β 1-4GlcNAc (F-LDN), Fuca α 1-3GalNAc β 1-4(Fuca α 1-3)GlcNAc (F-LDN-F), GalNAc β 1-4(Fuca α 1-2Fuca α 1-3)GlcNAc (LDN-DF), Fuca α 1-2Fuca α 1-3GalNAc β 1-4(Fuca α 1-2Fuca α 1-3)GlcNAc (DF-LDN-DF), Gal β 1-4(Fuca α 1-3)GlcNAc (Lewis X), and Man α 1-3(Man α 1-6)Man β 1-4GlcNAc β 1-4GlcNAc β 1-Asn (TriMan). All but Lewis X were variously expressed by miracidia and sporocysts of *S. mansoni*. Most notably, α 3-fucosylated LDN (F-LDN, F-LDN-F, LDN-F) was prominently expressed on the larval surface and amongst glycoproteins released during larval transformation and early sporocyst development, possibly implying a role for these glycotopes in snail-schistosome interactions. Interestingly, Fuca α 2Fuca α 3-substituted LDN (LDN-DF, DF-LDN-DF) and LDN-F, were heterogeneously surface-expressed on individuals of a given larval population, particularly amongst miracidia. In contrast, LDN and TriMan primarily localized in

internal somatic tissues and exhibited only minor surface expression. Immunoblots indicate that glycotopes occur on overlapping but distinct protein sets in both larval stages, further demonstrating the underlying complexity of schistosome glycosylation. Additionally, sharing of specific larval glycotopes with *Biomphalaria glabrata* suggests an evolutionary convergence of carbohydrate expression between schistosomes and their snail host.

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A Report on a Monograph of the Phyllobothriidae.

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A monograph of the Phyllobothriidae has been completed. A taxonomic history of the family is provided. Of the 74 genera historically associated with the family, only *Phyllobothrium* is an unambiguous member of the family. The genera *Bibursibothrium*, *Calyptrobothrium*, *Cardiobothrium*, *Ceratobothrium*, *Clistobothrium*, *Crossobothrium*, *Dinobothrium*, *Flexibothrium*, *Gastrolecithus*, *Marsupiobothrium*, *Monorygma*, *Nandocestus*, *Orectolobicestus*, *Orygmatobothrium*, *Paraorygmatobothrium*, *Ruhnkecestus*, *Scyphophyllidium* and *Thysanocephalum* are considered provisional members of the family. Sixteen genera are considered valid or provisional members of the recently erected order Rhinebothriidea. Two phyllobothriid genera have been allocated to the family Serendipidae. A set of criteria were consistently used to determine the taxonomic status of the remaining 37 problematic phyllobothriid genera. Of these remaining genera, five are considered *nomina dubia*, 11 are considered *genera inquirendae*, and eight are synonyms of other genera. Thirteen other valid genera are considered of uncertain familial placement with respect to the Phyllobothriidae. Full treatment of the 3 valid species of the type genus, *Phyllobothrium*, in addition to the 41 valid species of *Clistobothrium*, *Crossobothrium*, *Marsupiobothrium*, *Monorygma*, *Nandocestus*, *Orectolobicestus*, *Orygmatobothrium*, *Paraorygmatobothrium*, *Ruhnkecestus* and *Scyphophyllidium* were completed. The taxonomic status of problematic species within these 11 genera was also addressed. A better understanding of the phylogenetic relationships of the phyllobothriid genera is needed before the taxonomy of the family can be fully revised. Research supported by NSF-PEET (DEB Nos. 9521943 & 0118882).

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Species Level Investigations of *Paraorygmatobothrium* Using ND1 mtDNA Sequence.

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Paraorygmatobothrium is a genus of tetraphyllidean tapeworm that infects sharks, and currently houses 11 species. However, collections of carcharhiniform sharks from a variety of localities indicate that *Paraorygmatobothrium* may be much more speciose. Turner (2006) revealed substantial DNA sequence variability between 47 geographically diverse samples of *Paraorygmatobothrium* using a fragment of the cytochrome c oxidase subunit 1. She found 5 distinct clusters of samples from blacktip sharks alone, and three of these clusters contained samples of *Paraorygmatobothrium* from other shark species. The present study continues investigation into the species level systematics of *Paraorygmatobothrium* using a fragment of the NADH dehydrogenase subunit 1 (ND1). The study concentrated on samples collected from sharks of Northern Australia and Malaysian Borneo. Thus far, a 423 base pair fragment of ND1 has been sequenced for 23 samples of *Paraorygmatobothrium* collected from 12 shark species. Only three of the samples were of described species. Fifty-four percent (230/423) of sites varied in the alignment, and the average genetic distance between samples was 19%, ranging from total genetic identity to 26% divergence. This variability compares to that found for ND1 sequences of nine species of *Taenia* (see Gasser et al., 1999). Analysis of the sequence via neighbor joining produced 12 principle branches containing samples of *Paraorygmatobothrium* with a genetic distance greater than 10%. Seven of these branches contained samples of *Paraorygmatobothrium* collected from sharks of Northern Australia and Malaysian Borneo. Two putatively new species are in the process of being described. One of these is from the Blacktip shark, *Carcharhinus limbatus*, and the other is from the Australian blacktip shark, *Carcharhinus tilstoni*. Research supported by NSF-PEET (DEB No. 0118882), NSF-BS&I (DEB Nos. 0103640 & 9300796) and the WV NASA Space Grant Consortium.

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Systematic Investigations of Two New Phyllobothriid Genera from Sharks.

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Tetraphyllidean cestodes collected from sharks of Australia, the Marshall Islands in the Pacific Ocean, and the Bahama Islands in the Atlantic Ocean, included three new phyllobothriid species. Cestodes taken from the Snaggletooth shark *Hemipristis elongata*, collected from the Northern Territories,

Australia, represent one new genus (NewPhy1). Cestodes collected from the Sicklefins lemon shark *Negaprion acutidens*, taken from Pacific waters, and cestodes collected from the Lemon shark, *Negaprion brevirostris*, taken from Atlantic waters, represent a second new genus (NewPhy2). Both new genera have bothridia that are uniloculate, and bear an apical sucker. As such, these cestodes are consistent in morphology with genera considered provisional members of the Phyllobothriidae *sensu* Ruhnke. The scolex morphology of these species is similar to species in *Paraorygmatobothrium*, as well as other phyllobothriid species from sharks. The two new genera can be distinguished from known genera, as well as each other, using characteristics of their scolices and proglottids. Scanning electron microscopy of specimens revealed that NewPhy1 possesses serrated spinitriches and filitriches on its bothridial surfaces, whereas specimens of NewPhy2 possess aciculate spinitriches and filitriches on its bothridial surfaces. Phylogenetic analyses of *lsrDNA* revealed that NewPhy1 always appeared as the sister taxon to species of *Paraorygmatobothrium* and an undescribed phyllobothriid species from *Alopias pelagicus*. The phylogenetic position of NewPhy2 was affected by the type of data partition analyzed for *lsrDNA*. Ongoing work focuses on the sequencing of the entire *ssrDNA* and the D1-D3 region of *lsrDNA* for NewPhy1 and 2, in addition to *Paraorygmatobothrium janineae* and *P. kirstenae*. These sequences will be analyzed within the context of a broader taxonomic sampling of tetraphyllidean cestodes. Research supported by NSF-PEET (DEB Nos. 9521943 & 0118882), and the WV NASA Space Grant Consortium.

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Anatomical Variability in the Acanthocephala.

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in 2 parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds, are also featured.

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Curiosities in the Acanthocephala.

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This treatment of variability in the Acanthocephala is in 3 parts: (1) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in *Eoacanthocephala* and worm reproductive cycle. (2) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (3) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here.

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Isolates of the Zoonotic Parasite *Trichinella spiralis* Possess Previously Unrecognized Variation in Their Mitochondrial Genomes.

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Trichinella spiralis is a parasitic roundworm that infects domestic swine, rats and humans. Ingestion of infected pork by humans can lead to the potentially fatal disease trichinosis. The phylogeny and historical dispersal of *Trichinella* spp. have been studied, in part, by sequencing portions of the mitochondrial genome. Such studies rely on two untested beliefs: that variation in a portion is representative of the entire mitochondrial genome, and that each isolate is characterized by only one mitochondrial haplotype. We have used next generation DNA sequencing technology to obtain a second complete mitochondrial genome of a *T. spiralis* isolate and have aligned it to the previously sequenced genome. We sought to know whether the exceptionally deep sequencing coverage provided by such an approach could detect misassembled regions in previously published data and/or identify nucleotide positions which vary within an isolate. We found no major disagreement in terms of gene order and sequence assembly for all protein coding regions. However, in the highly repetitive non-coding region, alignment to the previously published genome sequence proved difficult. Such discrepancies may represent true biological variation, or may rather result from methodological or algorithmic sources. Within the protein-coding region, 5 polymorphisms were found in the 13,899 bases evaluated. One of these polymorphisms alters an amino acid sequence, two were silent, and two were found to vary within our isolate. In the non-coding region, 3 additional polymorphic sites were identified across 2,807

bases, one varying within our isolate. Comparing only two isolates of *T. spiralis* has enabled discovery previously unrecognized variation within the species. Characterizing diversity within, and among, the mitochondrial genomes of additional species of *Trichinella* would undoubtedly yield further insights into the diversification history of the genus. Our study affirms that next generation DNA sequencing technology can reliably characterize complete mitochondrial genomes.

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Delimiting Hookworm Species Parasitizing Pinniped Hosts Using Gene Trees: Phylogenetic Evidence for Host-Sharing and Switching.

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Uncinaria hookworms have been widely reported from juvenile pinniped hosts, however, investigations of their systematics have been limited, with only 2 species formally described: *Uncinaria lucasi* Stiles from the northern fur seal (*Callorhinus ursinus*) and *Uncinaria hamiltoni* Baylis from the South American sea lion (*Otaria flavescens*). *Uncinaria* hookworms were sampled from these two hosts and six additional species including Steller sea lions (*Eumetopias jubatus*), California sea lions (*Zalophus californianus*), South American fur seals (*Arctocephalus australis*), Australian fur seals (*Arctocephalus pusillus*), New Zealand sea lions (*Phocarctos hookeri*) and southern elephant seals (*Mirounga leonina*). Approximately 200 individual hookworms were sequenced for 5 genes representing 2 loci (mtDNA and nuclear rDNA). Intra-individual sequence polymorphism was extremely rare. Phylogenetic analysis of these data, both as separate loci (mtDNA versus nuclear rDNA) and combined data sets, yielded strong evidence for 6 independent evolutionary lineages or species. Both of the described species each matured in 2 different host species: *U. lucasi* parasitized *C. ursinus* and *E. jubatus*, and *U. hamiltoni* parasitized *O. flavescens* and *A. australis*. The other 4 undescribed species were each associated with unique host species. Patterns of *Uncinaria* host sharing and phylogenetic relationships of species inferred from gene sequences revealed a strong geographic component to host-sharing, and in one case the potential directionality of the host-switching event.

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Monozoic Tapeworms (Eucestoda: Caryophyllidea) Parasitic in North American Suckers: A Closed Chapter or a Big Challenge?

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Caryophyllideans are unique among “true” tapeworms (Eucestoda) in that they possess an unsegmented, monozoic body containing only one set of genital organs. They have been assumed by some to represent the most basal eucestodes, but molecular data has not provided any convincing support for this assumption. The highest number of caryophyllidean genera has been described from North America, where most species parasitize catostomid fishes (suckers) and some endemic cyprinids. Despite fairly intensive research on these cestodes carried out by North American parasitologists, such as Hunter, Mackiewicz, Calentine, Ulmer, and Williams, among others, there are still many unresolved problems related to their species composition, systematics, phylogenetic relationships, cytogenetics and geographical distribution. The current knowledge of the group is briefly reviewed and the most challenging problems are listed to stimulate future research. Due to a almost complete absence of molecular data, new material of caryophyllideans from North America is highly demanded for taxonomic and phylogenetic studies.

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A New Schistosome (Digenea: Schistosomatidae) from Murid Rodents in the Lake Victoria Basin, Kenya and Its Phylogenetic Position Within the *Schistosoma haematobium* Species Group.

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A new species of schistosome is described based on 6 adult male and 2 adult females collected from the circulatory system of 3 murid rodent species, *Pelomys isseli*, *Mastomys natalensis*, and *Dasymys incomtus*. Specimens were collected from a single location, Nyabera Swamp, in Kisumu, Kenya in the Lake Victoria Basin. This new species is morphologically similar to members of the *Schistosoma haematobium* group, currently represented by 8 species parasitizing artiodactyls and primates, including humans. The new species differs from these species by producing relatively small *Schistosoma intercalatum*-like eggs (135.2 x 52.9 µm) with a relatively small length to width ratio (2.55). Comparison

of approximately 3,000-base-pair sequences of nuclear rDNA (partial 28S) and mtDNA (partial *cox1*, *nad6*, *12S*) strongly supports the status of these worms as a new species and as a sister species of *S. intercalatum*. The *cox1* genetic distance between these two species (6.3%) is comparable to other pairwise comparisons within the *S. haematobium* group. Separation of the Congo River and Lake Victoria drainage basins is discussed as a possible factor favoring the origin of this species.

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A New Species of Medicinal Leech from Peru Shines Light on the Leeches of Mexico.

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A recent molecular analysis of the medicinal leeches, once thought to be monophyletic, has shown the Hirudinidae to actually be composed of two families: the Hirudinidae *sensu stricto* and the Semiscollecidae. The Semiscollecidae contains mostly New World taxa within three clades, two of which in the past have been subfamilies; the Macrobdellinae for New World bloodfeeders, and the Semiscollecinae for South American non-bloodfeeders. The third clade within this family, which has not been recognized in previous taxonomic revisions, contains the Mexican genera *Limnibdella* and *Pintobdella* and the Old World genus *Limnatis*, although this clade cannot be named its own family due to the lack of a morphological synapomorphy. Interestingly, a new species from Peru with a unique jaw morphology among leeches places at the base of this unnamed clade. Phylogenetic analyses of four genes (nuclear 18S rDNA and 28S rDNA and mitochondrial 12S rDNA and cytochrome c oxidase subunit I) were paired with morphological investigations to assess these relationships. Sister to the new taxon is a species previously described within the Mexican genus *Pintobdella*, making the Peruvian species not only a species new to science, but also elevating these sister species to the level of a new genus.

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Phylogenetic Analysis of Parasitic Cephaloboidea (Nematoda).

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Phylogenetic trees demonstrate that parasitism has evolved numerous times within Nematoda, and these parasites exploit diverse host groups, including plants, invertebrates and vertebrates. The

repeated evolution of parasitism from free-living progenitors in combination with their range of lifestyles and host types makes nematodes a good group for investigating changes associated with the evolution of parasitism. Phylogenetic analyses are key to identifying the free-living sister taxa of parasitic lineages for comparative analyses. Members of the nematode suborder Cephalobina include taxa that appear particularly useful for studying the evolution of parasitism. Specifically, although most species in this suborder are free-living microbivores, two genera of invertebrate parasites are proposed to belong to Cephaloidea. Phylogenetic analyses of nuclear large- and small-subunit ribosomal DNA were used to infer trees for Cephaloidea and to develop hypotheses for relationships of representative parasitic taxa, *Daubaylia potomaca* and *Dicelis* spp. Phylogenetic hypotheses based on maximum parsimony and maximum likelihood methods confirm that *Daubaylia* and *Dicelis* are nested within Cephalobidae with strong support. Within Cephalobidae, *Daubaylia potomaca* was sister to *Pseudacrobeles* sp. with moderate support whereas two *Dicelis* spp. were commonly resolved as sister to *Zeldia punctata* but support for this clade was generally low. The present study is designed to test relationships within Cephalobidae.

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Biodiversity and Systematic Interrelationships of Fish Blood Flukes
(Digenea: Aporocotylidae) Infecting the Body Cavity and Heart of South
American Freshwater Catfishes (Ostariophysi: Siluriformes).

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South American fishes exhibit a high degree of endemism and may comprise the most species-rich assemblage of fishes: 85% of them are ostariophysans, which collectively total ~75% of the world's freshwater fish fauna and >25% of all nominal fish species. Despite this extraordinary host diversity, only 3 nominal fish blood flukes (Digenea: Aporocotylidae) in 2 genera have been reported from the continent of South America, and only an additional 3 species are known from all catfishes (Siluriformes). During two month-long expeditions to the Peruvian Amazon we surveyed a diversity of catfishes for their metazoan parasites. The results of that survey indicated that the biodiversity of catfish blood flukes has been underestimated. The collections yielded blood flukes representing a new genus and species from the heart plus another new species from the body cavity and belonging to a long-ignored but accepted genus, *Plehnella* Szidat, 1955. Species of *Plehnella* are distinguished ecologically among other freshwater aporocotylids because they mature in the body cavity of catfishes rather than the heart, branchial vessels, or mesenteric vessels. A provisional phylogeny of the Aporocotylidae indicates that the catfish blood flukes are monophyletic and sister to other freshwater fish blood flukes ranging on

other continents. The presence of several related blood flukes infecting the body cavity of African catfishes suggests that the ancestor of these flukes infected siluriformes in Gondwanaland.

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Concurrent Infections with *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Blastocystis* spp. in Naturally Infected Dairy Cattle from Birth to Two Years of Age.

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Fecal specimens were collected directly at weekly and then monthly intervals from each of 30 dairy calves from birth to 24 months to determine the prevalence and age distribution of *Cryptosporidium* spp., *Giardia duodenalis* assemblages, *Enterocytozoon bieneusi* genotypes, and *Blastocystis* spp. subtypes. After sieving and density gradient centrifugation to remove fecal debris and concentrate parasites, all 990 specimens were examined by polymerase chain reaction (PCR) for each of the four parasites. A fragment of the SSU-rDNA gene of *Giardia*, *Cryptosporidium*, and *Blastocystis* and a fragment of the internal transcriber spacer (ITS) region of the rDNA gene of *E. bieneusi* were amplified by PCR, a total of 3960 PCRs. All positive PCR products (~1400) were sequenced to determine species and genotype identity. All 30 calves shed *Cryptosporidium*, *Giardia*, *E. bieneusi*, and *Blastocystis* at some time during the study, each exhibiting unique age-related patterns but all being found concurrently in some animals. Four species of *Cryptosporidium* were detected: *C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni* with cumulative prevalences of 100, 80, 60, and 3.3%, respectively. Two assemblages of *Giardia* were detected: A (zoonotic) and E with cumulative prevalences of 70 and 100%, respectively. *E. bieneusi* was detected in all calves including genotypes J, I and BEB4 that appear to be cattle-specific. A unique genotype of *Blastocystis*, not previously reported, was detected in all calves. This long term study of cattle documents for the first time concurrent infections with these four genera of pathogens, and recognizes both the potential impact of animal health as well as potential for zoonotic transmission.

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Westward Movement of *Bithynia tentaculata* and 3 Trematodes
Cyathocotyle bushiensis, *Sphaeridiotrema globulus* and *Leyogonimus*
polyoon to Lake Winnibigoshish, Minnesota.

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The faucet snail, *Bithynia tentaculata*, a non-indigenous aquatic snail from Eurasia, was introduced into Lake Michigan in 1871 and has spread to the mid-Atlantic states, Great Lakes region, Montana, the Mississippi River (2002) (Pools 7-13) and was recently (2008) found to be in Lake Winnibigoshish, Minnesota (NWHC, unpublished data). The faucet snail serves as intermediate host for three trematodes responsible for several large scale mortality events among water birds, primarily lesser scaup (*Aythya affinis*) and American coot (*Fulica americana*), in the Great Lakes region, Montana, and Minnesota. The trematodes *Cyathocotyle bushiensis*, *Sphaeridiotrema globulus* and *Leyogonimus polyoon* may have been introduced into the United States as early as the 1870's when the snail was introduced. The trematodes infect the small intestine (*S. globulus* and *L. polyoon*) or the ceca (*C. bushiensis*) and feed on blood and tissue. At Lake Winnibigoshish approximately 7,000 lesser scaup and a few hundred coots died due to trematodiasis during the fall 2007. Those losses prompted this study to evaluate snails and parasites in the lake. Protected shallow sites (<2ft) which were often at the edge of wild rice (*Zizania aquatica*), bulrushes (*Schoenoplectus fluviatilis*) and/or cattails (*Typha spp*) had a high percentage (93%) of snails infected. Rocky shallow areas were also sites with moderate to high percentages of infected snails. *L. polyoon* had the most restricted distribution in snails, being found at only 3 sites with the highest prevalence of 15%. Snails collected at trawl sites sampled at 3-7ft in depth had low prevalence of infection and were infected only with metacercariae of *C. bushiensis*. This survey documents new geographical locations for *B. tentaculata* and the 3 trematodes.

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Effects of Age and Sex on Pinworm Infections in Australian Cockroaches.

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Parasites infect all species, and it is important to know why some hosts are more prone to infection. Pinworms are small roundworms that live in the digestive tract of most animals; including cockroaches.

We studied whether age or sex affect infection levels of pinworms in the Australian cockroach (*Periplaneta australasiae*). 356 cockroaches were trapped in the Western Illinois University Biological Sciences green house over a 1-year period. They were killed, and their hind femur lengths were measured. Cockroaches were then dissected, and pinworms were collected. 2146 pinworms were found, and 142 pinworms have been positively identified as *Leidynema appendiculata*. Pinworms showed a clumped distribution (variance: mean ratio = 8.88). Most cockroaches had 5 or fewer pinworms, while a few cockroaches had many pinworms. Prevalence was high in both adults and juveniles (mean = 86.6%). It did not differ between age classes ($G = -0.011$, $df = 1$, $p = 0.92$). Intensity increased with age (femur length) among juveniles ($p = 0.506$, $df = 203$, $p < 0.0001$), but not among adults ($p = 0.014$, $df = 105$, $p = 0.89$). Intensity was significantly higher in adults (mean \pm SEM = 9.66 ± 1.06), than juveniles (mean \pm SEM = 5.58 ± 0.37), ($t = 4.58$, $df = 306$, $p < 0.001$). Among adults, there was a significant effect of sex on prevalence ($G = 4.00$, $df = 1$, $p = 0.045$). Prevalence was higher in females (91.4%) than males (83.3%). Intensity was also higher in females than males (female mean \pm SEM = 10.82 ± 1.37 , male mean \pm SEM = 6.12 ± 0.72), but this difference was not significant (1-tail $t = 1.59$, $df = 66.5$, $p = 0.06$). These results differ from other pinworm-cockroach associations. Here, the pinworm distribution was clumped, and prevalence and intensity were high. Other associations show uniform distributions, low prevalences and intensities. More work is necessary to understand the causes of these differences.

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Site Density Distribution of “Yellow Grub” (*Clinostomum complanatum* syn. *marginatum*) in Pond-Raised Channel Catfish (*Ictalurus punctatus*).

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Fifty-four channel catfish (*Ictalurus punctatus*) from a commercial pond in Northwest Arkansas were examined for “yellow grub” (*Clinostomum complanatum*) densities in various anatomical sites of the host. Overall mean abundance and its standard deviation were 31.7 and 21.2, respectively. The site distribution of these cysts and their relative percentages were: Mouth 46, gill 20, fins 20, muscle and subdermal cysts 14. Daly et al. 2007 (J. Ark. Acad. Sci. 61:37) found that the ratio of grubs in the gills and mouth of smallmouth bass correlated significantly with the total worm burden in a population of host fish and gave good estimates of the population parameters of prevalence, mean abundance, maximum abundance, and mean intensity. To determine if site density in catfish could also predict the total number of metacercarial cysts, the number of cysts in the different sites was regressed against the total number in the rest of the host body. For all three visible sites combined the coefficients were: $r = 0.97$

and $p = <0.001$. Since the largest number of parasites were found in both the gills and the mouth, that combination was also regressed against the total body cysts, with $r = 0.90$ and $p = <0.001$. Gills, by themselves were least correlative with $r = 0.62$. Regressions were also done between the visible sites and the non-visible site. Fins were found to be the best predictor of cysts in the muscle and subdermally. The large number of parasites in the gill and mouth areas can be used by catfish farmers or by fisheries biologists to assess the total yellow grub load in their fish populations through visible sites without harming the hosts. Also, the site distribution results did not agree with site density results reported for wild-caught smallmouth bass and South American catfish. The reason for this is probably habitat differences of those two hosts and the pond environment of the catfish in this study.

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Occurrence of *Sarcoptes scabiei* on a Virginia Opossum.

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A Virginia opossum (*Didelphis virginiana* Kerr, 1792) found dead on a road near College Station, Texas was examined for external parasites. A single nymphal tick (*Ixodes texanus* Banks, 1909), several listiphorid mites, and several other mites identified as *Sarcoptes scabiei* (L. 1758) Latereille, 1802 (Acaridida: Sarcoptidae) were collected. This represents the first reported occurrence of *Sarcoptes scabiei* on a wild marsupial in North America.

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Prevalence of IgG Antibodies to *Toxoplasma gondii* and *Encephalitozoon cuniculi* in Cats Examined at the Teaching Hospital of the Virginia-Maryland Regional College of Veterinary Medicine.

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Renal disease is common in cats. Two zoonotic parasites *Toxoplasma gondii* and *Encephalitozoon cuniculi* can potentially develop in this organ and cause disease. We are interested in examining the prevalence of *T. gondii* and *E. cuniculi* in cats with renal disease compared to cats with no renal disease. This is important due to the fact that renal disease is a critical condition in cats and renal transplants in these animals are increasing as veterinarians become familiar with the procedure. Cat plasma was obtained from 81 feline patients at the teaching hospital of VMRCVM. Samples were examined blinded to presenting clinical signs in an indirect immunofluorescent antibody test at a dilution of 1:25 for

antibodies to tachyzoites of the RH strain of *T. gondii* and a dilution of 1:10 for antibodies to spores of a dog strain of *E. cuniculi*. Of the 81 samples, 11 (16%) were from cats diagnosed with renal disease. Antibodies to *T. gondii* were found in 23 (28%) of the 81 samples and 5 (46%) of the 11 cats with renal disease were positive. Antibodies to *E. cuniculi* were found in 2 (4%) of the 81 samples and none of the 11 cats with renal disease were positive. Plasma from no cat was positive for antibodies to both parasites. The 28% prevalence of *T. gondii* antibodies is slightly lower than in previous reports on owned cats from the United States. There was a low prevalence 4% of *E. cuniculi* in cats examined in the present study. We were unable to find any published prevalence surveys of *E. cuniculi* in cats from the United States. Additional samples need to be examined to determine the importance of *T. gondii* or *E. cuniculi* as a factor in the development of renal disease in cats.

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Prevalence of *Cryptosporidium* spp., *Giardia* spp., and *Troglodytella* spp. in Chimpanzees (*Pan troglodytes schweinfurthii*) from Mahale Mountains National Park in Western Tanzania.

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We examined feces from a group of chimpanzees (*Pan troglodytes schweinfurthii*) from Mahale Mountains National Park in western Tanzania for *Cryptosporidium* spp., *Giardia* spp., and *Troglodytella* spp. Beginning in March 1966, habituation efforts were initiated by researchers from Japan to attract chimpanzees from one unit-group, referred to as the M-Group, allowing human observers to approach them in their natural habitat. Over the past 15 years, individuals in the M-Group have been observed having intermittent loose to runny stools. In our study, fresh fecal samples from identified individuals were collected immediately after defecation and fixed in 10% formalin solution. None of 49 samples were positive for oocysts of *Cryptosporidium* spp. or *Giardia* spp. cysts when examined using a direct fluorescent antibody test on feces. Trophozoites of *Troglodytella* spp. (abrassarti?) were observed in wet smears from 48 (92%) of 52 chimpanzees. The prevalence of nematodes in these chimpanzees is currently being determined by sugar flotation. The high prevalence of *Troglodytella* spp. in these animals is consistent with previous reports of this organism in primates. Because they are potentially zoonotic the prevalence of *Cryptosporidium* spp. and *Giardia* spp. needs further study. Funded in part by NSF grant #0238069 to TK (aka TJS). Any opinions, findings and conclusions or recommendations expressed in this materials are those of the author(s) and do not necessarily reflect the views of NSF.

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Comparative Chemical Analysis using Energy Dispersive X-ray Analysis (EDXA) of Three Species of Acanthocephala.

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Three species of Acanthocephala: *Rhadinorhynchus ornatus*, *Acanthocephalus dirus* and *Neoechinorhynchus idahoensis* were evaluated with energy dispersive X-ray microanalysis (EDXA) for chemical elements. Acanthocephala were collected from fish inhabiting marine and freshwater habitats. For *R. ornatus* and *A. dirus*, both males and females were examined, while only females were available for *N. idahoensis*. EDXA scans were completed in triplicate on 3 individual specimens for each species for three regions of the proboscis (tip, middle and base), mid body and posterior body, and the weight-percent and peak height were registered. Data were statistically analyzed using a SAS computer program. Elements characteristic of living cells (C,H,N,O) were recorded as well as sulphur (S), calcium (Ca) and phosphorus (P). Due to their unique nature, the later three were stressed in this study. The proboscis has the highest concentrations of S, Ca and P followed by the posterior body. The lowest concentrations were detected in the mid body. Female Acanthocephala have a statistically higher concentration of the examined elements in the body regions compared with males. The level of S is high at the proboscis base and gradually decreased towards the tip where both Ca and P, having the same distribution pattern, reach their highest levels. Sulfur ions are probably polymerized as complex polypeptides using disulphide bonds represented by the amino acids cystine and cysteine.

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Fine Structure and Energy Dispersive X-Ray Analysis (EDXA) of the Proboscis Hooks of *Rhadinorhynchus ornatus* (Rhadinorhynchidae: Acanthocephala).

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The proboscis hooks of *Rhadinorhynchus ornatus* were examined using EDXA (X-ray microanalysis) for three chemical elements: sulfur, calcium and phosphorus. Statistically significant differences for the three chemical elements in the hooks were recorded in relation to position on the proboscis, as well as different regions of the individual hook (base, middle and tip). Hooks at the base of the proboscis had higher levels of sulphur than those at the tip, where calcium and phosphorus reached their highest concentrations. The fine structure depicted a multi-layered hook structure with some similarities to mammalian teeth. Thickness of both the outer cortical and inner medullary layers of the proboscis hooks reached its highest in the hook tip where sulphur was in highest amounts. The chemical composition of the hook resembles to some extent that of mammalian hair. The high sulphur content of the hooks can be related to disulphide bonds within the amino acids cystine and cysteine in the polypeptide molecules.

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Analysis of the Neospora caninum Argonaute Protein in Tachyzoites.

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The Argonaute (AGO) protein plays a pivotal role in regulating gene activities and functions in various species. AGO protein has not been characterized in *N. caninum*. A putative gene encoding the 73 kDa *Neospora* AGO (NcAGO) was identified in the gene database (www.genedb.org) with RGG repeats at the N-terminus and a centrally located PAZ domain, but lacking the polyQ domains at the N-terminus and the Piwi domain at the C-terminus. In comparison, the *Toxoplasma gondii* AGO (TgAGO) only contains the Piwi domain, but is missing other functional motifs. Western blot analysis using an NcAGO-specific antibody revealed the presence of a 73 kDa protein in *Neospora* tachyzoites. Further analysis demonstrated that NcAGO was only present in the insoluble fraction of *Neospora* tachyzoite lysate. Immunoreactive NcAGO was also identified by immunohistochemistry (IHC) in tachyzoites of *N. caninum* in the mouse lung tissue. The presence of NcAGO in *Neospora* tachyzoites was further confirmed by indirect immunofluorescence staining. NcAGO was intensely localized in the cytoplasm of tachyzoites, and may be associated with cytoplasmic bodies where mRNA is known to undergo RNAi-directed degradation. The present studies demonstrate that an AGO homologue is present in the *Neospora* tachyzoites and it may function to regulate the transcriptional and post-transcriptional gene silencing in this organism.

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Efficacy of DNA Vaccination Against Schistosomiasis Infection Using *Schistosoma mansoni* Aldolase Gene Through Different Routes of Injection.

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The objective of this study was to evaluate different routes of injection for genetic vaccination against *S. mansoni* infection using fructose 1, 6-bis phosphate aldolase (SMALDO) gene. Methods included the cloning of SMALDO into the eukaryotic expression vector pcDNA3.1/V5-His TOPO-TA. Swiss Albino mice were injected with the vaccine. Different routes of injection were examined: intramuscular (IM), intraperitoneal (IP) or subcutaneous (SC) injections with 50 of pcDNA3.1/SMALDO. Mice vaccinated with non-recombinant pcDNA3.1 served as controls. Each group was immunized four times at week 0, 2, 4 and 6. Two weeks after the last booster dose, all mice groups were challenged with 80 *S. mansoni* cercariae via tail immersion. Eight weeks post-challenge, all mice groups were sacrificed for subsequent assessment of parasitological and immunological parameters. Both IP and SC vaccinated groups showed a significant reduction in worm count (45.96% and 29.06%, respectively). This was accompanied by a significant reduction in ova count in both the liver and intestine (41.7% and 40.2%) of the IP group only. The percent of dead ova was significantly increased in both IP and IM groups ($p < 0.01$ and $p < 0.001$, respectively). High anti-SMALDO IgG levels were detected in the sera of all vaccinated groups ($p < 0.01$) while anti-SMALDO IgM showed a slight insignificant increase when compared with the control group. Also, no significant change in granuloma diameter between the three injection groups and control group was detected. In conclusion, DNA vaccination using the *S. mansoni* aldolase gene via the IP route could be a candidate vaccine against schistosomiasis.

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***Fasciola gigantica*: Parasitological and Scanning Electron Microscopy Study of the *In Vitro* Effects of Ivermectin and/or Artemether.**

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The objective of this study was to evaluate the *in vitro* effects of different concentrations of ivermectin and/or artemether on *Fasciola gigantica* worms and to study the parasitological changes and

tegumental alterations using scanning electron microscopy (SEM). *F. gigantica* worms were incubated *in vitro* for 24 and 48 hrs with 3 concentrations of either ivermectin or artemether (10, 20 and 50 µg/ml) or both in half the concentration of each drug (5, 10 and 25 µg/ml). Exposure of *Fasciola* worms to 25+25 µg/ml of combined drug regimens or to 50 µg/ml of either ivermectin or artemether for 48 hrs led to 100%, 33.3% and 66.7% worm killing which were accompanied by a significant reduction in egg laying capacity and significant increase in dead eggs which were maximally recorded in combined drug regimens. SEM of the flukes incubated for 48 hrs with combined drug regimens showed maximal tegumental disruption with swelling of the worm body, roughness, blebbing, sloughing and complete loss of spines. Disruption to the tegument of the flukes induced by artemether was more than that of ivermectin. In conclusion, artemether alone or combined with ivermectin in half doses had potent fasciocidal activities. In addition, half doses of combined drug regimens had higher ovicidal effects than each drug alone. *In vivo* studies are recommended to further explore the efficacy of combined regimens against *Fasciola* infection.

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Interactions Between a Suite of Trematodes Infecting the Horn Snail,
Cerithidea pliculosa in a Northern Gulf of Mexico Salt Marsh.

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Collections of *Cerithidea pliculosa* were taken intermittently from a host population of the horn snail in Airport Marsh on Dauphin Island, AL. Although dissections of hosts were conducted, the most common assay used to determine presence/absence of parasites was shedding or non-shedding of cercariae. Two species, *Probolocoryphe lanceolata* (Holliman 1961) and *Parorchis acanthus* (Nicoll 1906) were the most commonly encountered trematodes. Definitive hosts of these trematodes are shore birds. Prevalence of infection typically increased with host size, with 100% infection in the largest host size-classes (25-35 mm) especially during the summer months. Egg-laying by female snails was only observed in intermediate (10-15 mm) size-classes, which could be an adaptive, evolutionary response to parasite castration induced by the trematodes. Efforts to determine effects of parasites on growth of individual snails were attempted by mark-and-recapture as well as tethering.

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Distribution and Abundance of an Echinostomatid Trematode in Oysters
from Southern Texas.

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Parasites of oysters (*Crassostrea virginica*) have been studied extensively due to the economic impact that they may have on the oyster industry. A more than 40 year-old research note published in the Journal of Parasitology described the infection of oysters in the Laguna Madre, South Texas by the echinostomatid, *Acanthoparyphium spinulosum*. This digenean is of particular importance because it not only affects oyster fitness but it also is a potential zoonotic in humans eating raw oysters. In nature *Acanthoparyphium* primarily use birds as definitive hosts, but this parasite has also been successfully raised into adults in rats suggesting that humans may be suitable hosts. The aim of our study was to 1) verify the infection of *A. spinulosum* in *C. virginica* from the southernmost part of the U.S.A, 2) to determine the abundance of metacercariae of *A. spinulosum* as well as other macroparasites in *C. virginica* from Lower Laguna Madre, TX, and 3) to identify and quantify the density of infected first intermediate snail hosts acting as a resource of infections of *A. spinulosum* to oysters. We sampled a large number of *C. virginica* and three possible first intermediate snail hosts from two sites in the Lower Laguna Madre. Oysters from one of the two sites harbored metacercariae morphologically identical to *A. spinulosum*. The prevalence of *A. spinulosum* in oysters was 80 % and the mean abundance 28.3 per host of which most were located in the mantle tissue. Specimens of an unidentified gymnophallid, and an unidentified nematode were also found in the oysters. Cercariae and rediae similar to *A. spinulosum* were identified from the plicate horn snail, *Cerithidea pliculosa* (prevalence = 2.8%, density of infected snails = 1.15 m⁻²). This snail is most abundant on mudflats in connection with stands of the black mangrove, *Avicennia germinans*. Future studies will experimentally address transmission patterns of *A. spinulosum* cercariae to oysters and the impact of infection on oyster fitness.

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Genetics Meets Ecology: Molecular and Population Data Support the
Presence of Four Species of Philometrids in the Southern Flounder,
Paralichthys lethostigma.

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The southern flounder *Paralichthys lethostigma* is host to two species of philometrid nematode: *Philometra overstreeti* and *Philometroides paralichthydis*. Individuals of *P. overstreeti* are located both

between the teeth and inside the bony part of the branchial arches of the fish whereas individuals of *P. paralichthydis* are associated with the bones of the buccal cavity and among muscles that control the dorsal and anal fin rays. Sequencing of part of the COI gene indicated the splitting of each currently recognized species into two distinct genetic clades. Because of the high sequence variation between the resulting four clades and because each clade corresponded exactly to each location of the parasite in the host, we hypothesized that each clade represents a separate species. To test this hypothesis, population level parameters of the clades comprising each currently recognized species were compared in order to determine if the parasites making up each clade exhibited distinct life history characteristics. For each currently recognized species the presence of worms from one clade was found to be negatively correlated with the presence of worms from the other clade of that species and very few hosts were infected concurrently with worms from both clades. Results also indicated significant differences between the clades in prevalence, intensity, and abundance relative to host size, salinity level at capture site, and time of year. As such, these results clearly indicate major differences in the ecology of the philometrids constituting each clade. Taken as a whole, these molecular and ecological data support the contention that the four genetic clades are likely four distinct species.

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Population and Infection Dynamics of *Daubaylia potomaca* (Nematoda: Rhabditida).

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Daubaylia potomaca is an unusual parasite for a number of reasons. Specifically, it has a direct life cycle in which it uses a planorbid snail, *Helisoma anceps*, as the definitive host. Additionally, females have been shown to be both the infective stage, and the only stage documented to be shed from a live, infected host. Finally, adults, juveniles, and eggs have been observed in all tissues and blood spaces of the host, suggesting that the parasite consumes and actively migrates through host tissue. The current study seeks to examine the population and infection dynamics of *D. potomaca* in Mallard Lake, a 4.9 ha public access pond in the Piedmont Region of North Carolina. In particular, this study will determine the role of seasonality and substratum type on the prevalence and intensity of infection in the snail host. Data collected from August, 2008 to present suggest that prevalence and intensity are inversely related in the spring and fall. Specifically, prevalence peaks at 46.9% in March (2009) while intensity (3.1 ± 0.3 worms/infected host) is low, and intensity peaks at 52.4 ± 8.9 worms/infected host in September (2008) while prevalence (27.1%) is relatively low. These trends differ slightly from those observed in a previous study conducted during the 2006-07 collecting season. Attention was then focused on the southeast corner of the pond, where prevalence peaked at 59.7 and 59.4% in April and July, respectively, and

intensity peaked at 48.1 ± 5.5 worms/infected host in May. The current study includes data collected from sites throughout the pond. Areas of mud/clay cover (those with no leaf-litter) possess snails with higher prevalence than areas of mixed (leaf and mud/clay) and leaf-litter substrata. Mud/clay cover and mixed cover differ significantly from leaf-litter ($P < 0.002$ and $P < 0.001$, respectively), and also from one another ($P < 0.025$). Additionally, co-infections between *D. potomaca* and trematodes, and an oligochaete (*Chaetogaster* sp.), or both, were not uncommon.

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Gene Regulation in the Snail, *Biomphalaria glabrata*.

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The snail *Biomphalaria glabrata* is studied primarily due to its role as the intermediate host of the trematode parasite, *Schistosoma mansoni*. Interestingly, the susceptibility of snails to infection is varied; some strains are capable of killing *S. mansoni* relatively soon after it has penetrated the host while infection in others is chronic. This difference has prompted researchers to investigate the underlying differences between resistant and susceptible snails. In addition to other variables, these snail strains have been reported to vary in their gene expression profiles, suggesting that the mechanisms regulating gene transcription may be partially responsible for differences between resistant and susceptible strains. Despite this key observation, no transcription factors have been described in *B. glabrata*. In addition, information regarding *B. glabrata* promoter sequences is lacking. Therefore, the focus of this project has been twofold: 1) identification and characterization of *B. glabrata* promoter sequences, and 2) cloning and analysis of *B. glabrata* B proteins, a family of transcription factors involved in immune responses in other organisms.

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Schistosoma mansoni cAMP-dependent Protein Kinase (PKA): A Potential New Drug Target.

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Protein kinases represent novel drug targets for the treatment of diseases caused by eukaryotic pathogens such as helminth parasites. We therefore explored the anti-parasite potential of targeting cAMP-dependent protein kinase (PKA) enzymes in *Schistosoma mansoni*. Examination of the *S. mansoni* genomic sequence database identified sequences of four putative PKA genes. Using reverse

transcriptase-PCR and RACE, transcripts from two distinct PKA genes were identified in adult *S. mansoni* cDNA, one of which is expressed as two distinct splice variants that utilize different exons at the 5' end (SmPKA-C). Western blot analysis of adult *S. mansoni* proteins, using a polyclonal antibody directed against conserved sequences of the PKA alpha catalytic subunit, identified several protein species with expected molecular weights of PKAs. PKA activity was detectable in adult *S. mansoni* lysates at various nanogram concentrations, confirming that *S. mansoni* worms express active PKAs. Further, schistosome PKA activity was significantly inhibited and activated by commercially available PKA inhibitors and agonists. Three PKA inhibitors were shown to have schistosomocidal effects on adult worms *in vitro* at various micromolar concentrations within four hours to six days. RNA interference experiments using SmPKA-C dsRNA significantly decreased transcription and produced lethality in 50% of treated adult worms while decreasing overall SmPKA activity by 50% in surviving worms. Further studies revealed that *S. haematobium* and *S. japonicum* cDNA express nearly identical PKA C homologues to those of *S. mansoni*. These data suggest that inhibitors of PKA have potential as novel chemotherapeutics for the treatment of schistosomiasis and other helminth infections.

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Real-time PCR/RFLP Assay to Detect *Giardia Intestinalis* Genotypes in Human Isolates with Diarrhea in Egypt

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Two major genotypic assemblages of *Giardia intestinalis* infect humans; the nested real-time polymerase chain reaction (PCR) was used for targeting the triose phosphate isomerase (*tpi*) gene to detect and genotype *G. intestinalis* in human feces in Egypt. Among 97 fecal samples, 30 (31%) were diagnosed as giardiasis by saline wet mount microscopy after staining with Lugol's iodine. The *tpi* gene was amplified from 41 (42.3%) fecal samples, of which 11 were microscopy-negative specimens. Of the total samples, 24 (58.5%) contained assemblage A group I, and 7 (17.1%) were assemblage A group II from the group of patients complaining of intermittent diarrhea. Eight (19.5%) samples contained assemblage B from patients with persistent diarrhea. Two (5%) samples had a mixture of assemblage A group II and assemblage B. The technique was able to detect as few as 20 trophozoites per PCR on fecal DNA-isolated, microscopy-negative, and quantitative (q)PCR-positive specimens; there was a higher average cycle threshold value than microscopy-positive and qPCR-positive specimens, suggesting that they represented true, low-burden infections. In conclusion, we could genotype *G. intestinalis* from fresh stool samples in Egypt; in infections commonly presented with intermittent diarrhea, the most prevalent genotype was assemblage A group I. The most vulnerable age group included 10- to 20-yr-old individuals.

Infections with Geographically and Genetically Different Strains of *Trypanosoma cruzi* in Two North American Reservoir Hosts Induce Dissimilar Infection Dynamics.

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Reservoir hosts induce dissimilar infection dynamics. *Trypanosoma cruzi*, etiologic agent of Chagas disease, is capable of infecting a variety of mammalian hosts within a wide geographic range in the Americas. In addition, *T. cruzi* is genetically and biologically diverse with molecular associations occurring between strain genotype and host origin. The objective of the present study was to determine the dynamics of *T. cruzi* infection in *Didelphis virginiana* and *Procyon lotor* and to provide experimental evidence for an observed host species-parasite strain dichotomy. Based on previous molecular typing and hemoculture evidence from wild-trapped animals, we hypothesized that raccoons would have a longer patent period than opossums, and raccoons would be more competent reservoirs for all genotypes of *T. cruzi* compared with opossums. Individuals (n=2 or 3) of each species were intraperitoneally or intravenously inoculated with 1×10^6 culture-derived *T. cruzi* trypomastigotes of Type IIa (North America-raccoon), Type I (NA- opossum), Type IIb (South America-human), or both Type I and IIa. One animal in each group was euthanized during acute (1 and/or 2 months) and chronic stages (4 months) and tissues collected for PCR and histopathology. Opossums had a more gradual increase in parasitemia, peaking around 35 DPI, and a rapid decline by week six. Raccoons quickly reached peak parasitemia at 18-21DPI and maintained relatively high parasitemia for 5 weeks. Additionally, raccoons became infected with all *T. cruzi* strains, while infection was not detected by PCR, serology, or hemoculture in opossums inoculated with Type IIa. Opossums inoculated with *T. cruzi* IIb had detectable infections by PCR and cleared the infection at approximately 10 DPI, as was evident by seroconversion and the absence of *T. cruzi* DNA by PCR after this day. Serology as determined by IFA demonstrated raccoons seroconverted sooner (3-7 DPI) than opossums (10 DPI).

Experimental Infection of Two South American Animal Reservoirs with Distinct Strains of *Trypanosoma cruzi*.

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Trypanosoma cruzi, causative agent of Chagas disease, is a diverse species with two primary genotypes, Type I and Type II, with Type II further subdivided into five subtypes (IIa-e). Isolates are highly variable and differ in numerous biological characteristics including host range and virulence. This study evaluated infection dynamics of four genetically and geographically diverse *T. cruzi* strains in *Octodon degus* and *Monodelphis domestica*. We hypothesized that *O. degus* (placental) would more readily become infected with *T. cruzi* II than *M. domestica* (marsupial) which would be a more competent reservoir for *T. cruzi* I. Individuals (n= 3) of each species were intraperitoneally inoculated with 1×10^6 culture-derived *T. cruzi* trypomastigotes of Type IIa (North America-raccoon), Type I (NA-Virginia opossum), Type IIb (South America-human), Type IId (SA-triatome), or both Type I and IIa. Infection dynamic differences between species were dramatic; *O. degus* parasitemias peaked earlier (7-14 DPI) with a nearly 4-fold higher parasitemia than *M. domestica*. Infections established in both species when inoculated with any strain, except Type IIa, which was not infective to *M. domestica*. Seroconversion as determined by indirect IFA occurred by 14 DPI in all animals. One animal in each group was euthanized at 1, 2, and 4 months and major organs were examined for *T. cruzi* and histopathologic lesions. Lesions associated with *T. cruzi* I were more severe with *O. degus* than *M. domestica*. These results indicate that both South American reservoirs support infections with parasites from North and South America; however, infection dynamics differed with host and parasite strain.

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Kudoa inornata, a Myxosporean Infecting Skeletal Muscles of the Spotted Seatrout, *Cynoscion nebulosus*: Taxonomy and Pathogenicity.

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A new myxosporean species, *Kudoa inornata* (Myxosporea: Multivalvulida) was described from skeletal muscles of the spotted seatrout *Cynoscion nebulosus* (Cuvier, 1830), collected in estuarine waters along the coast of South Carolina, USA. Light microscopic and ultrastructural characters rank this species to the group of *Kudoa* species with simple-shaped spores. The uniqueness of the SSU and LSU rDNA sequences justifies its status as a new species, phylogenetically closely related to *Kudoa paniformis*. The high prevalence and pathogenicity of *K. inornata* recognized in this study should motivate further screening for infections in its host, which is considered a commercially important game fish with a wide distribution in the western North Atlantic.

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Humoral Immune Response of Creole and Mixed Breed Horses against Natural Infections with *Trypanosoma evansi* in the Venezuelan Plains.

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Trypanosoma evansi is a hemoparasite of horses, dogs, and laboratory rodents which has a worldwide distribution. Outbreaks of equine trypanosomiasis are often reported either from wild horses or from those used on cattle raising farms of the Venezuelan Plains. Thus, the main objective of this work was to assess the humoral immune response of Creole Venezuelan horses and from mixed breeds using an enzymatic immune assay (ELISA) and an indirect fluorescent test (IFAT). A total of 304 sera samples were collected from horses located in the Apure, Portuguesa and Trujillo States. In addition, data about age and sex of each horse were recorded. The total seroprevalence to *T. evansi* by IFAT was 40.1% (124 positive horses out of 304), whereas by ELISA the seroprevalence was 27% (82 positives out of 304). Horses between the ages of 1 and 3 years showed higher seropositivity to *T. evansi* compared with horses less than 1 year and over 3 years of age. Female horses, mares, showed higher seropositivity to *T.*

evansi, compared with male horses, regardless of the age group. The study concluded that herds of Venezuelan Creole horses showed a rather low seropositivity to *T. evansi*; nonetheless, these animals live in an enzootic area, which seems to indicate that these breeds of wild horses have some degree of resistance or tolerance to equine trypanosomiasis.

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Identification of Parasite Ligands by Screening a *Cryptosporidium parvum* cDNA T7 Phage Display Library.

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The protozoan parasite, *Cryptosporidium parvum*, is a causative agent of human gastrointestinal disease worldwide. Infection is usually self-limited, but in immunocompromised hosts it may result in severe diarrhea and wasting for which no effective therapy is currently available. Knowledge of the molecular basis of host-parasite interactions is crucial for understanding the pathogenic mechanisms underlying infection and designing strategies to combat cryptosporidiosis. In our study, a *C. parvum* cDNA T7 phage display library was constructed and screened with Caco-2 cells and specific polyclonal antibodies. After several rounds of panning, specific clones were enriched and subjected to sequencing. BLAST search in Genbank identified 5 specific gene fragments, one of which encoded the CP2 protein. CP2 has previously been identified as a surface molecule of sporozoites and involved in parasite invasion of host cells. The others are proteins whose functions were unknown. Bioinformatic analysis showed two proteins were mucin-like glycoproteins which are believed to mediate host-parasite interactions in other protozoan parasites. Two proteins were identified by a Caco-2 cells-binding assay and were recognized by specific antibodies. All of these proteins contain signal peptides indicating that they are either secreted out or anchored on the parasite membrane. The results suggest that these proteins are important molecules related to parasite development in the host and are potential candidate antigens for vaccine development.

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Transmission to the Free-Living Stage in *Acanthocephalus dirus* (Acanthocephala): Are Eggs Dispersed by Definitive Hosts or Mature Females?

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The acanthocephalan parasite, *Acanthocephalus dirus*, infects two hosts during its life cycle. Transmission to the intermediate host, the freshwater isopod *Caecidotea intermedius*, occurs when isopods consume *A. dirus* eggs along with their food. Transmission to the definitive host (creek chub, sunfish), occurs when these predators consume infected isopods. Transmission to the free-living stage occurs when eggs are released into the stream. These eggs could potentially be released with the feces of the fish if the females release the eggs into the intestine. Alternatively, mature females could pass out of the intestine of the fish and the eggs could be released into the stream when the body of the female degrades. We used a combination of field-based and lab-based approaches to distinguish between these alternative mechanisms. Using field collections of creek chub and sunfish combined with laboratory-based dissections, we found that *A. dirus* eggs were found almost exclusively inside the bodies of females rather than in the intestines of the fish. Thus, female *A. dirus* did not appear to be releasing the eggs into the intestines. We then collected creek chub from nature, transferred them to lab and observed them daily to determine if female *A. dirus*, containing eggs, were released intact from the fish. We found that females were released intact and they contained eggs. Finally, we exposed juvenile isopods to *A. dirus* eggs obtained from these females to determine if these eggs were viable. We found that the eggs contained in females were viable and were able to establish in the isopod intermediate hosts. We propose that egg dispersal in *A. dirus* occurs when the mature female degrades in the stream. This pattern differs from results obtained in other acanthocephalans and could provide insights into the evolution of alternative transmission strategies in these parasites.

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 Visceral Leishmaniasis in *Cerdocyon thous*.

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The fox *Cerdocyon thous* is well known as a wild reservoir of many zoonotical diseases, particularly visceral leishmaniasis (VL). The aim of this study was to report the presence of *Leishmania* sp. amastigotes in different organs of one *C. thous* found dead in the Zoo of Ilha Solteira, SP, Brazil. This animal was positive by IFAT (indirect fluorescence antibody test) and had many clinical signs of VL. After the natural death, this animal was autopsied and tissue samples from many organs were collected and examined by a direct parasitological examination and PCR. Intact amastigote forms of *Leishmania* sp. were seen inside the neutrophils and macrophages in sample tissues from the skin, lymph nodes (popliteal, submandibular, prescapular and mesenteric), spleen, liver, kidneys and lungs. Higher numbers of infected neutrophils with *Leishmania* amastigotes in comparison with macrophages were

seen in submandibular and prescapular lymph nodes. However, in other organs (spleen, liver and mesenteric lymph nodes) the numbers of infected macrophages were superior to neutrophils. In addition, PCR demonstrated extensive distribution of *Leishmania* sp. DNA in *C. thous* tissues from many organs. Intact *Leishmania* sp. inside neutrophils and macrophages and DNA of this parasite in many organs characterized the presence of VL in *C. thous* in Brazil.

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North American and African Nematomorph Host Specificity in Six Species of Aquatic Snails.

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Freshwater snails are commonly infected with horsehair worms cysts (Nematomorpha). However, it is unclear if all snail species are equally susceptible to nematomorph infections. In this study we tested host specificity of 6 species of North American (NA), South American (SA) and African snails (AF) from 3 different families to infections with a North American horsehair worm, *Paragordius varius*, and an undescribed African horsehair worm, *Paragordius* sp. Two groups of 6 species (N = 10 per group/species) of laboratory reared snails including *Physa gyrina* (Physidae, NA), *Biomphalaria sudanica* (Planorbidae, AF), *Biomphalaria glabrata* (Planorbidae, SA), *Planorbella trivolvis* (Planorbidae, NA), *Gyraulus parvus* (Planorbidae, NA), and *Stagnicola elodes* (Lymnaeidae, NA) were each exposed to approximately 500 laboratory reared larvae of *Paragordius* sp. and *P. varius*. After exposure, nematomorph larvae were allowed to develop to the cyst stage for 5 days, and each snail was crushed and examined for the presence and intensities of nematomorph cysts. Our results indicate, that independent of horsehair worm continent of origin, based on prevalence, mean abundance and cyst development, *Physa gyrina* (NA), *Biomphalaria sudanica* (AF), and *Biomphalaria glabrata* (SA) were classified as suitable hosts for nematomorphs with high prevalence (90-100%) and/or high mean abundance (3-114), whereas *Gyraulus parvus* (NA), *Stagnicola elodes* (NA), and *Planorbella trivolvis* (NA) were classified as not suitable or less suitable hosts for nematomorphs with low prevalence (0-50%) and low mean abundance (0-4). Our study is the first to document host specificity of snails to any horsehair worm species.

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Endoparasites of Ground Squirrels (*Spermophilus citellus*) from the Czech Republic and Slovakia.

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During 2006-2008, fresh faecal samples of ground squirrels (*Spermophilus citellus*) from all available localities in the Czech Republic and several localities in Slovakia were collected and preserved in 2.5% K₂Cr₂O₇ solution. Faeces were examined for the presence of endoparasites. Oocysts of the genus *Eimeria* (Apicomplexa: Eimeriorina: Eimeriidae) were found in all examined samples (prevalence 100%). Three different *Eimeria* species were recorded. Mixed infections with these species often occurred. Furthermore, *Trichuris* eggs (Nematoda: Enoplida) and oocysts of pseudoparasites of the genus *Adelina* were found in some samples. Stains for *Cryptosporidium* were negative in all samples.

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Endoparasites of the Genus *Apodemus* (Rodentia: Muridae) from the Slovak Republic.

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During summer and autumn 2008, faecal samples of three species of the genus *Apodemus* (Rodentia: Muridae) from three climatically different areas from eastern, southern and western Slovakia were examined for endoparasites. Oocysts of the genus *Eimeria* (Apicomplexa: Eimeriidae) were found in approximately 40 % of all *Apodemus* species. This is the first report of *Eimeria* in *Apodemus microps*.

Cysts of *Giardia* sp. (Metamonada: Diplomonadida) were detected in a few samples. From the helminths, strongylid eggs (Nematoda: Strongylida), eggs of *Capillaria* sp. (Nematoda: Enoplida), eggs of *Hymenolepis nana*, *Hymenolepis diminuta* and tetrathyridia of *Mesocestoides* sp. (Cestoda: Cyclophyllidea) were detected.

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Gastrointestinal Parasites of the Pelagic Stingray (*Pteroplatytrygon violacea*) from the Western North Atlantic Ocean.

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Concerns regarding the conservation status of many elasmobranchs have prompted investigations into less obvious sources of population declines. Endoparasite loads in the elasmobranch spiral valve may be a source of mortality and morbidity in the host by both inhibiting nutrient uptake and stimulating an inflammatory response within the gastrointestinal tract. The pelagic stingray (*P. violacea*) is a poorly understood elasmobranch found worldwide in oligotrophic waters. Unlike many other stingrays, very little is known of the endoparasite fauna found in this species. Over 100 pelagic stingrays were collected from the western North Atlantic Ocean during 2008 and 2009. Spiral valves were dissected and initially preserved in a 90:10 seawater to buffered formalin solution. Parasites were manually extracted from preserved spiral valves and individually stained for identification to the lowest taxonomic level. Microscopic samples were identified with the aid of a scanning electron microscope (SEM). The majority of extracted parasites identified to date are cestodes, although species found to date also include trematodes. Total spiral valve parasite loads are compared against the size (length and weight) of the host stingray, showing a weak relationship. Potential transmission vectors are being explored.

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Life History and Histopathology of *Phyllodistomum* sp. (Digenea: Gorgoderidae) in the Yellow Sandshell, *Lampsilis teres* (Rafinesque, 1820) (Bivalvia: Unionidae) in Line Creek, AL.

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Although well-documented that larval digeneans (sporocyst, redia) castrate their molluscan first intermediate host, details about how such larvae affect specific tissues of freshwater mussels

(Unionidae) is largely undocumented in the primary literature. In specific, we know of no detailed histopathological report that documents the host-parasite relationship between a digenean and a unionid in the southeastern United States. We herein document the histopathological effects and life history traits of a trematode in its intermediate host the yellow sandshell, *Lampsilis teres*. Infected yellow sandshells (5 of 65, 0.08) harbored both cercariae in sporocysts plus encysted metacercariae that we tentatively identified as *Phyllodistomum* sp. based on a suite of shared morphological characters. Infected yellow sandshells had fewer sperm compared to non-infected yellow sandshells but none were castrated and all demonstrated active gametogenesis. Pathological changes to the visceral mass, including digestive tubules and gonad, were limited or difficult to delineate consistently. The presence of both active cercariae and encysted, well-developed metacercariae of the putative *Phyllodistomum* sp. suggests that this gorgoderid exhibits a truncated life cycle, using the yellow sandshell as both the first and second intermediate host and perhaps maturing in the urinary bladder of a stream-dwelling vertebrate.

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Evaluation of a Rapid Immunochromatographic Assay for Detection of
Trypanosoma cruzi Antibodies in Wildlife Reservoirs.

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An immunochromatographic assay (Chagas Stat-Pak™) was evaluated for the detection of *Trypanosoma cruzi* antibodies in four species of wildlife reservoirs. Antibodies to *T. cruzi* were detected in both raccoons (*Procyon lotor*) (wild and experimental) and degus (*Octodon degu*) using the Chagas Stat-Pak™. In naturally-exposed wild raccoons, the Chagas Stat-Pak™ had a sensitivity and specificity of 66.7-80% and 96.3%, respectively, for raccoons. Seroconversion by Chagas Stat-Pak™ compared with IFA seroconversion was delayed for experimentally-infected raccoons, but occurred sooner in experimentally-infected degus. The Chagas Stat-Pak™ did not detect antibodies in wild or experimentally-infected Virginia opossum (*Didelphis virginiana*) nor in experimentally-infected short-tailed opossums (*Monodelphis domestica*). These data suggest that the Chagas Stat-Pak™ might be useful in field studies of raccoons and degus when samples would not be available for more conventional serologic assays. Because this assay did not work on either species of marsupial, the applicability of the assay should be examined before it is used in other wild species.

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Cytokine Production Naïve Murine Dendritic and Spleen Cells in Response to *Neospora caninum* Stimulation.

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The intracellular parasite *Neospora caninum* has been known as a major cause of reproductive failure in cattle. A biased type 1 immune response characterized by production of pro-inflammatory or inflammatory cytokines is often observed in pregnant animals following *N. caninum* infection. This type of immune response is favorable to controlling the intercellular multiplication of this parasite, but is likely to cause immunopathology of the placenta/fetus as well. Early events in innate immune responses, especially the cytokine production, are able to prime and define a type 1 or type 2 immune response following infection. The goal of this study was to evaluate the profile of cytokine production in murine naïve dendritic cells and spleen cells stimulated by whole *Neospora* tachyzoites (live, heat-killed, frozen-killed) or whole-tachyzoite cell lysate in the form of total (NcAg), insoluble (isNcAg) and soluble antigen (sNcAg). Our results showed that whole *Neospora* tachyzoites and antigen preparations can elicit high levels of interleukin (IL)-12, tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma. Whole *Neospora* tachyzoites showed an overall higher stimulating capability than antigen preparations. Furthermore, the heat-killed tachyzoites induced less ($p < 0.05$) IFN-gamma and IL-10 but more IL-4 in comparison with live and frozen-killed tachyzoites. sNcAg induced a moderate level of IL-12 and very low ($p < 0.05$) levels of IFN-gamma and TNF-alpha. Therefore, heat-killed tachyzoites and soluble antigen preparations may not be ideal vaccine candidates against neosporosis. This study provides further understanding of the immune responses elicited by *N. caninum* and may facilitate the development of vaccines against neosporosis.

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Survey of Acaricide Resistant and Management Factors that Induce Resistance to Acaricides in *Rhipicephalus microplus* in Tamaulipas, Mexico.

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The increase of high populations of *Rhipicephalus (Boophilus)* ticks affects the cattle production. Tick control is carried out mainly by the use of different acaricide families in Mexico. Therefore, resistance to acaricides is becoming a serious problem associated with high costs for tick control. The presence of acaricide resistance is also a barrier for cattle exportation to the US. In 1981, resistance of *R. microplus* to organophosphates was first reported in Mexico, specifically in tropical areas along the Gulf of Mexico and the Yucatan Peninsula. In 1993, resistance to pyrethroid was documented in *Rhipicephalus* ticks, in

Tamaulipas and Tabasco, Mexico. Since 1998, the phenomenon of acaricide resistance had been reported in 13 states of Mexico. In 2002, multi-resistance to organophosphates, pyrethroid and amitraz, was reported in Tabasco. There is one report of multi-resistance in Tamaulipas. This objective of this study was to determine the distribution of acaricide resistance in *R. microplus* in Tamaulipas and to determine the risk factors associated with acaricide resistance in the different geographic zones from Tamaulipas state. Tick samples were collected from 57 ranches and tested by discriminating dose to the three main acaricides used in Mexico. A questionnaire was given to ranchers at each of the collection sites to obtain information regarding vector control at the ranches. Of the 57 ranches sampled in six municipalities, 59% contained amitraz resistant *R. microplus*, 9% included organophosphates-pyrethroid-amitraz multiresistant *R. microplus*, 44% harbored amitraz-pyrethroid resistant *R. microplus*, 2% amitraz-organophosphate resistance, 34% simple resistance to amitraz and 3% resistance to pyrethroid. Only 7% showed susceptibility to the three families of ixodicides. Areas with the greatest levels of resistance are located in the south. Simple resistance to amitraz occurs throughout the state. Organophosphates are still effective across 80% of the state, a good option for tick control. Risk factors like treatment frequency every 14 days, and spraying had a strong association with the presence of resistant ticks ($\chi^2 P < 0.01$). In conclusion, a high prevalence of resistant populations of *Boophilus* ticks exists in Tamaulipas, Mexico. The generation of new methods of integrated control make help to reduce the cattle lose and the indiscriminate use of ixodicides; thus, increasing the benefits in conjunction with costs.

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Vital Rates of the Life History Stages of the Parasitic Dinoflagellate
Amyloodinium ocellatum.

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I. Masson and J.M. Lotz.

Amyloodinium ocellatum is a parasitic dinoflagellate that infects the skin and gills of a variety of warm water marine bony fishes and causes extensive mortalities in aquaculture settings. The life cycle of *A. ocellatum* consists of 3 stages: a parasitic feeding stage (trophont) which produces a detached reproductive stage (tomont), each of which divides to produce up to 256 free-swimming infective stages (dinospores). The life cycle has been studied extensively, but little research has been done on the population dynamics of the parasite. We are developing a stage-structured population model for the life cycle of *A. ocellatum* infecting spotted seatrout *Cynoscion nebulosus* in a closed culture system and estimating experimentally all the vital rates associated with the model. In this poster, we present estimates of the vital rates for the three life history stages. Trophonts obtained from spotted seatrout were incubated individually in the dark at 25C° and 33ppt in 96-well culture plates and their survival and

development into dinospores were monitored daily, accounting for the number of cell divisions, the time to sporulation and the number of dinospores released. Regression analysis was used to obtain the equations from which the vital rates were estimated based on the mean size of the trophonts at detachment ($71.55 \pm 1.34 \mu\text{m}$, mean \pm SE) determined in a separate study. The mortality rate of the trophont stage was 0.0037 trophonts/day whereas the mortality rate for the tomont stage was 0.0100 tomonts/day. The mean number of dinospores produced by tomonts was 82 dinospores/tomont with a mean time to sporulation of 4.6 days. The mortality rate of the dinospore was 0.3787 dinospores/day. None of the observed dinospores lived beyond day 8 post-sporulation. The development of a population model for *A. ocellatum* and sensitivity analyses on the effects of changes in the magnitude of the vital rates on the parasite's overall population growth may identify critical control points in the life cycle at which management and control strategies can be directed.

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Spatial Distribution of Yellow Grub (*Clinostomum complanatum*) in Smallmouth Bass (*Micropterus dolimieu*) from Crooked Creek (AR) as Determined by Metacercarial Cyst Counts in the Gill-Mouth Sites Only.

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Metacercarial numbers in the gill-mouth area can be used to estimate total population parameters of yellow grub in smallmouth bass (Daly et al., 2007). In 1991, 614 smallmouth bass (*Micropterus dolomieu*) from Crooked Creek in North Central Arkansas were examined for yellow grub (*Clinostomum marginatum*) infection by counting only the number of metacercarial cysts in the gill-mouth anatomical site. Bass were captured by angling from Huzzah Creek to the stream's confluence with the White River. Bass were collected in a linear spatial fashion by moving down river and releasing fish after examination. Data from the gill-mouth area only was compared to data from a study in 1988-90 done with total necropsy, to see if the distribution of yellow grub along the stream was similar. The 1988-90 study, unlike the present one, was done by collecting fish at selected locales along the stream. Both studies showed that upstream the infection was light, and then became heavy, and then dropped to a moderate intensity, and finally at the White River there is a super-heavily infested area. Use of gill-mouth counts and collect-and-release for smallmouth bass, saves necropsy time, conserves a valuable and important game fish but still provides a detailed profile of yellow grub infection not necessarily found with selection of individual collection locales.

The Lecanicephalidean Fauna of Three Species of Eagle Rays of the Genus *Aetomylaeus* (Myliobatiformes: Myliobatidae).

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The eagle ray genus *Aetomylaeus* comprises 3 species: *A. vespertilio*, *A. maculatus* and *A. niehofii*. In general, members of this genus inhabit the waters of the Indo-West Pacific. Recent collecting efforts in this region resulted in specimens of the 3 *Aetomylaeus* species available for study. While tapeworms have been reported from *A. niehofii* and *A. maculatus*, no tapeworm data exist for *A. vespertilio*. Moreover, no lecanicephalideans have been described from any of these 3 eagle ray species. As part of a survey of parasites of elasmobranchs from Borneo and Northern Australia, 5 specimens of *A. vespertilio* (from Northern Australia), 5 of *A. maculatus* (from Borneo) and 17 of *A. niehofii* (10 from Borneo and 7 from Northern Australia) were collected. While each species hosted an assemblage of cestode species representing up to 4 of the 7 orders of tapeworms known to parasitize elasmobranchs, the greatest diversity was seen among lecanicephalideans. The lecanicephalideans encountered were identified as belonging to the genera *Tylocephalum*, *Polypocephalus* and *Aberrapex*, as well as 3 genera new to science. One of these new genera is unique among all elasmobranch tapeworms in possessing conspicuous dorsally and ventrally positioned glandular tissue along the length of the proglottids. This genus is represented by 1 species in *A. maculatus* and 2 in *A. niehofii*. *Aetomylaeus vespertilio*, *A. maculatus* and *A. niehofii* hosted, at a minimum, 10, 10 and 7 lecanicephalidean species, respectively. All are unique and appear to be new to science. At the generic level, the lecanicephalidean faunas of *A. maculatus* and *A. niehofii* were more similar to one another than either was to that of *A. vespertilio*. In *A. niehofii*, only 2 lecanicephalidean species collected from rays from Borneo overlapped with those collected from Northern Australia.

Biotic Convergence of the Genus *Rhabdias* (Nematoda) in Mexico.

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There are around 70 species of *Rhabdias* (Stiles and Hassall, 1905) distributed in the New and Old World, occurring in the lungs of amphibians and reptiles. An overview of the life history of these parasites and their possible biogeographical history in Mexico were conducted. A preliminary biogeographical analysis of *Rhabdias* was based on the phylogenetic analysis of intra- and interspecific levels using partial sequences of COX1 of the mtDNA. Three monophyletic groups were analyzed and the areas of endemism were based exclusively on the information contained in the selected cladograms. The general area cladograms were produced by four methods: primary Brooks parsimony analysis (BPA), secondary BPA analysis, reconciled trees, and analysis of paralogy-free subtrees. The biogeographic scenario is complex and as a result several interrelated causes may obscure the results. Redundant areas are treated differently in the cladistic biogeographical methods due to the fact that sampling efforts are biased. The results suggest that the areas of the Balsas Basin and Sierra Madre del Sur could represent biotic convergence zones for this parasite group in Mexico. The necessity to carry out other biogeographic studies to provide information about the history of the group is proposed. Additional information on the geographical distribution of *Rhabdias* in the central and northern parts of Mexico is needed to clearly establish the relationship between the areas and the limits of biotic convergence.

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Morphology of Free-Living Adults of Three Parasitic Nematode *Rhabdias*.

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The lungworm *Rhabdias* is an important amphibian and reptile parasite of cosmopolitan distribution. Very little is known about the morphology and development of free-living forms of these lungworms. The comparative morphology and development study of the adults of free-living forms of three *Rhabdias* species from three amphibian genera (*Polypedastes*, *Bufo* and *Taricha*) was conducted. Parasitic adults of rhabdiasids from these amphibians were the source of eggs for culturing the free-living forms (larval stages and adults). These stages were maintained in the laboratory in agar plates around two weeks until they generated the next generation. The cultures were kept at several temperatures (4°C to 25°C) and observed daily. Images and measurements were made with a Leica DM LB2 and QCapture-Pro 5.1 Program. Drawings were made from photographs. Measurements are given in micrometers unless otherwise stated. In preparation for scanning electron microscopy (SEM), specimens were stored in 4% formalin, dehydrated in a series of gradual alcohol, and critical point dried. Specimens were coated with a gold-palladium mixture and examined using a Hitachi S-2460N scanning electron microscope. There are differences among the general morphology of free-living adult forms of these species of *Rhabdias* from amphibians. This new taxonomic information provides details on the biology of this group, as well as our data were compared with the information presented in the literature. The number of lips is considered a homology in this group in the adult free-living forms; however, the

position of these are slightly different compared with the parasitic adult forms. The morphology of males is presented. The development is slow at 4°C, we do not obtain adults of free-living forms at this temperature. (Thanks to Berenit Mendoza for assisting in the processing of samples for SEM (IB-UNAM), Lenor Price, Carl Franklin and Eric Smith for their invaluable technical assistance.

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Discernable but Limited Introgression has Occurred Where *Trichinella nativa* and the T6 Genotype Occur in Sympatry.

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The genetic diversity within and among parasite populations provide clues to their evolutionary history. We recently concluded, from an analysis of mitochondrial and microsatellite variation, that *Trichinella spiralis* was introduced to the Americas from Europe. Here, we sought to determine whether such data could be used to evaluate the extent of differentiation and gene flow among parasites attributed to *Trichinella nativa*, a freeze-resistant taxon endemic to the Arctic, and the T6 genotype, believed to occupy a more southerly range. To investigate the extent of historical reproductive isolation among these taxa, we compared representatives of each taxon to 41 new isolates derived from the diaphragms of 41 wolverines (*Gulo gulo*) inhabiting an arctic region of Nunavut, Canada where both *T. nativa* and T6 have been diagnosed. Interestingly, conflict was identified between diagnostic assays of mitochondrial DNA (which resembled representatives of *T. nativa*) and assays of the expansion subunit of nuclear rDNA (which resembled representatives of T6). To adjudicate among these conflicting diagnoses, we characterized each isolate according to alleles at several microsatellite loci, and compared these to isolates of T6 and *T. nativa*. Employing an assignment test (Structure 2.2) that made no a priori assumption concerning the composition or identity of population subdivisions, we established that most of these isolates unequivocally resemble representatives of T6, and that a few unequivocally resemble representatives of *T. nativa*. Evidently, the sampled population shares maternal ancestry with those attributed to *T. nativa*, but their nuclear genomes are more generally characteristic of individuals denoted as T6. Although that conclusion implies that introgression has occurred among these taxa (a possibility previously examined by means of experimental crosses), the unambiguous bifurcation of microsatellite variation into T6 and *T. nativa* partitions suggests that such interbreeding has been infrequent, enabling clear genetic subdivisions to be maintained. The disappearance of glacial barriers

that once separated such populations may account for historically differentiated populations that have experienced limited introgression.

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Relationships of *Eimeria callospermophili* (Apicomplexa: Eimeriidae)
Parasites from Sciurid Hosts Based on ITS1 and ITS2 rDNA.

R.S. Seville, Department of Zoology and Physiology, University of Wyoming, Laramie, WY and D. Motriuk-Smith.

The taxonomy of the coccidia has historically been morphologically based. The purpose of this study was to establish if conspecificity of isolates of *Eimeria callospermophili* from four ground-dwelling squirrel hosts (Rodentia: Sciuridae) is supported by comparison of rDNA sequence data and to examine how this species relates to other eimerian species. *Eimeria callospermophili* was isolated from four hosts: *Urocitellus elegans*, *Cynomys leucurus* (collected in Wyoming, Natrona County), *Marmota flaviventris* (Wyoming, Teton County), and *Cynomys ludovicianus* (Colorado, Larimer County). The ITS1 and ITS2 genomic rDNA sequences were PCR generated, sequenced and analyzed. The highest intraspecific pairwise distance values of 6% in ITS1 and 6.7% in ITS2 were observed in *C. leucurus*. *E. callospermophili* ITS1 and ITS2 genomic rDNA sequences were compared to *Eimeria lancasterensis* from *Sciurus niger* and *Sciurus niger cinereus*, and *Eimeria ontarioensis* from *S. niger*. Resulting Neighbor Joining and Maximum Parsimony trees supported the conspecificity of *E. callospermophili*. *E. callospermophili* ITS1 and ITS2 sequences formed a single well supported clade.

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Distribution of Spotted Fever-Group Rickettsiae in Canines from
Tennessee.

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Rocky Mountain spotted fever (RMSF) is an important tick-borne disease throughout the southeastern United States and the most common tick-borne infection in Tennessee. RMSF is caused by *Rickettsia rickettsii*, a member of the spotted fever-group (SFG), and transmitted by *Dermacentor variabilis* ticks. Since 1990, RMSF incidence in Tennessee has increased making Tennessee one of the highest reporting states. In Tennessee a gradient is seen with increasing incidence from east to west. The reasons for this

disease gradient remain elusive at this point. Domestic canines may be used as sentinels to assess geographic foci of RMSF. Additionally, dogs may play an important role in human RMSF as potential carriers of rickettsiae-infected ticks. This study seeks to assess the prevalence of SFGR among dogs and relate canine prevalence to human RMSF cases. A survey was conducted to assess the seroprevalence of antibodies to spotted fever-group rickettsiae (SFGR) among canines throughout the state of Tennessee. Serum samples were collected from 860 dogs and antibodies to SFGR were detected using enzyme immunoassays (EIA). Samples were initially screened to identify *Rickettsia* and subsequently tested for antibodies to *R. rickettsii*, *R. montana*, and *R. ambloymii*. Preliminary data suggest that exposure to *Rickettsia* at the county level is 3–64%. Similar to RMSF human cases, the Ridge and Valley ecoregion in east Tennessee has the lowest exposure to *Rickettsia* in canines. Additionally, Cheatham, Henderson, Henry counties were found to be “hot spots” for canine exposure as they are for human RMSF incidence. Our data indicate that *Rickettsia* exposure in domestic canines is wide-spread throughout the state of Tennessee.

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Acceptance of the 2009 Henry Baldwin Ward Medal.

K.D. Lafferty, US Geological Survey

No abstract submitted.

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Parasites Approach the Darwinian Demon: Adaptations, Evolutionary
Wormholes, and Their Ecosystem Visibility.

A. Kuris, Marine Science Institute, University of California, Santa Barbara, Dept. of Ecology,
Evolution and Marine Biology.

No abstract submitted.

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Mentor and Mentee: A Lasting Relationship.

M. Belosovic, FRSC, Distinguished University Professor, Biological Sciences, University of Alberta, Edmonton, Canada.

No abstract submitted.

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Efficacy of a TSA-1 encoding DNA vaccine against *Trypanosoma cruzi* in controlling vertical transmission in ICR mice.

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Trypanosoma cruzi is a highly zoonotic, protozoan pathogen, and the etiologic agent of Chagas Disease in humans. Although transmission is considered largely vector driven, vertical transmission from mother to fetus has been increasingly recognized as an important route for infection. Previous studies have reported the development of significant levels of protection in ICR mice treated with a recombinant plasmid encoding immuno-dominant epitopes of the trypomastigote surface antigen 1 (TSA-1) protein. We have tested whether the TSA-1 DNA vaccine is effective in reducing the incidence of vertical transmission when administered to breeding female ICR mice. Female ICR mice were infected with the Brazil strain of *T. cruzi* and subsequently vaccinated with the TSA-1 encoding plasmid, the empty plasmid alone, or saline. Parasitemias were evaluated weekly until approximately 10 weeks post-infection. Males were introduced at 4-weeks post-infection and breeding success measured over nine months. To assess whether vertical transfer of the parasite had occurred, pups were sacrificed at two-weeks of age and tissues harvested for PCR analysis. The rate of vertical transmission measured over the course of the study was similar for all treatment groups. When the data was separated by acute and chronic stages,

lower rates of transmission were measured in all groups during the chronic phase, with the largest decline evident in the TSA-1 treated group. Interestingly, breeding females from both the empty plasmid and saline control groups tested positive by PCR when DNA was extracted from pooled heart, spleen, and skeletal muscle tissues, but mice in the TSA-1 treated group were largely negative. When uterine tissue served as the DNA source, the TSA-1 treated female mice were conformed positive. This suggests that the vaccine was effective in mitigating infection in these non-immune privileged tissues, but that the immunologically privileged uterus may be refractive to these protective mechanisms. This highlights both the advantages to the parasite of adopting a strategy of vertical transmission, as well as the challenges of developing effective vaccines to block it.

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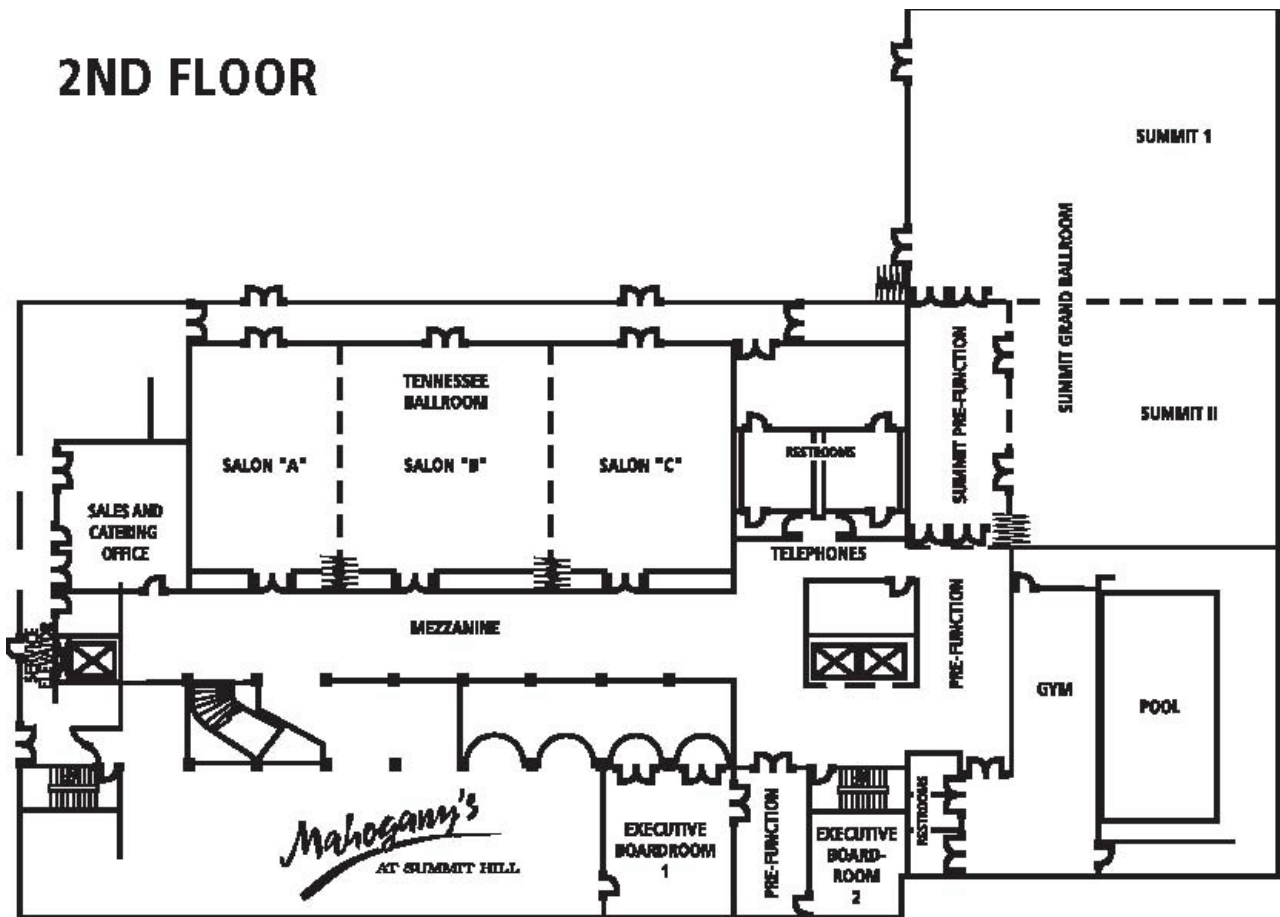
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Floor Plan

Crowne Plaza Knoxville

2ND FLOOR



Knoxville, Tennessee

ASP Meeting History

1925 Kansas City MO	1957 Philadelphia PA *	1986 Denver CO *
1925 Philadelphia PA	1958 Bloomington IN †	1987 Lincoln NE #
1927 Nashville TN	1959 University Park PA †	1988 Winston-Salem NC
1928 New York NY	1960 Los Angeles CA *	1989 Vancouver, BC, Canada
1928 Des Moines IA	1961 Lafayette IN †	1990 East Lansing MI
1930 Cleveland OH *	1962 Washington DC ‡	1991 Madison WI
1931 New Orleans LA	1963 Chicago IL *	1992 Philadelphia PA
1932 Atlantic City NJ	1964 Boulder CO †	1993 Atlanta GA *
1933 Boston MA	1965 Atlanta GA	1994 Ft. Collins CO
1934 Pittsburgh PA	1966 San Juan PR *	1995 Pittsburgh PA **
1935 St Louis MO	1967 Tucson AZ §	1996 Tucson AZ ††
1936 Atlantic City NJ	1968 Madison WI †	1997 Nashville TN
1937 Indianapolis IN	1969 Washington DC *	1998 Kona HI
1938 Richmond VA	1970 Washington DC ¶	1999 Monterey CA ‡‡
1939 Columbus OH	1971 Los Angeles CA	2000 San Juan PR ††
1940 Philadelphia PA	1972 Miami Beach FL *	2001 Albuquerque NM
1941 Dallas TX	1973 Toronto, ON, Canada	2002 Vancouver, BC, Canada ¶§§
1942 No meeting	1974 Kansas City MO	2003 Halifax, NS, Canada
1043 No meeting	1975 New Orleans LA *	2004 Philadelphia PA **
1944 Cleveland OH	1976 San Antonio TX	2005 Mobile AL
1945 St. Louis MO	1977 Las Vegas NV	2006 Glasgow, Scotland ¶
1946 Boston MA	1978 Chicago IL *	2007 Merida, Yucatan, Mexico §§¶¶
1947 Chicago IL	1979 Minneapolis MN	2008 Arlington TX
1948 New Orleans LA *	1980 Berkeley CA	2009 Knoxville TN
1949 New York NY	1981 Montreal, QB, Canada	2010 Colorado Springs CO
1950 Cleveland OH	1982 Toronto, ON, Canada ¶	(tentative)
1951 Chicago IL *	1983 San Antonio TX *	
1952 Ithaca NY †	1984 Snowbird UT	
1953 Madison WI †	1985 Athens GA	
1954 Memphis TN *		
1955 Atlanta GA		
1956 Storrs CT †		

* 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)

With Wildlife Disease Association

** With American Association of Veterinary Parasitologists

†† With Society of Protozoologists

‡‡ With Society of Nematologists

§§ With Sociedad Mexicana de Parasitología

¶¶ With Parasitology Section, Canadian Society of Zoologists