

Biodiversity Survey and Inventory of
Parasites of Aquatic Ectotherms in the Big Thicket
Continued Survey

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Background

The present proposal outlines a continuing study of the parasites of fishes and aquatic herpetiles from the Primitive Big Thicket Area of Texas. A Taxonomic Working Group (TWIG) was established in 2007 that focuses on the aquatic ectotherms and their parasites for this ATBI. Two week-long collecting trips in 2007 provided the preliminary data necessary to make predictions regarding future sampling; a 3-week collecting trip in 2008 and a 2-week collecting trip in 2009 provided data for the first empirical estimates of species diversity of parasites in the Big Thicket (see previous reports and applications for funding) and the necessary experience in the field to plan for successful completion of the project.

In 2010, I spent 7 months on sabbatical in Texas working on the project, and collected approximately 85 species of parasites from 421 fishes representing 39 species. Many of these have been identified and deposited in the Harold W. Manter Laboratory of Parasitology. Others are new and are being described currently; 2 have been described already (Barger 2010a,b) and 1 has been redescribed (Barger, 2011). In 2011, no collections were conducted.

I propose to continue the strategy implemented in summer of 2010 during collections in the spring and late summer in 2012.

Objectives

Project objective. The project continues the main-line work of the TWIG's survey by implementing a new collection and processing strategy. Fishes, amphibians, and turtles will be surveyed from multiple units in the Big Thicket Preserve, as well as sites within the historical Big Thicket (but outside the preserve itself).

Project goals.

1. Sample and necropsy fishes, amphibians, and reptiles from multiple units within in the Big Thicket over 2 week-long trips in 2012 (spring and late summer);
2. Identify recovered species of parasites, describe new species, and upload information to the database;
3. Distribute project results via the WWW, through peer-reviewed publication, and through presentations at scientific meetings.

Research Plan

Sampling and field protocol. Over ninety species of fishes and 40 species of herpetiles have been reported from the 18-county area of the Primitive Big Thicket and its immediate surroundings. A complete inventory of these taxa and their parasites is a goal that will require multiple years to complete. In any one year, only a fraction of the fishes, amphibian, and turtle species can be recovered and processed. A full list of species that might be encountered is included as an appendix. No more than 10 individuals per site will be collected per fish species; no more than 5 individuals per site will be collected per amphibian and turtle species. My goal is to sample 10 species of fishes and 5 species of herpetiles in 2012. using the new collecting strategy. Briefly, 5-7 host species will be targeted each week, and collecting will proceed until sufficient sample sizes have been obtained. If a targeted host species cannot be collected in sufficient numbers, it will be abandoned in favor of a species that can be collected.

Collections will be focused in areas not yet sampled adequately, particularly the Neches River and Village Creek systems.

Estimates of species recovery. Preliminary work has resulted in the recovery of approximately 2.5 species of parasite for every species of fish surveyed and 0.5 species of parasite for every *individual* fish surveyed. These necropsies were not always comprehensive, so these numbers probably underestimate the diversity of parasites in these Big Thicket vertebrates. Thus, I expect to recover at least 125 species of parasites, and probably closer to 175. Of these, approximately 10% (12-18) will be new to science.

Specimen preparation and museum curation. Specimens for species identification and description are prepared according to standard protocols (Pritchard and Kruse, 1982) as modified by Barger and Nickol (2004) and Snyder and Clopton (2005). Briefly, specimens are relaxed, fixed chemically, and stored in ethanol. Trematodes, cestodes, acanthocephalans, leeches, and crustaceans are stained and mounted in permanent media on glass slides. Monogeneans and nematodes are examined using temporary

mounts and remain stored in ethanol. Blood smears are made in the field, fixed in methanol, and stained with Giemsa stain. All preparation and storage techniques produce specimens that become part of the permanent, public specimen base. The Harold W. Manter Laboratory of Parasitology (Lincoln, Nebraska) will serve as the place of final disposition once specimens are identified and new taxa are described. In addition, subsamples of helminths are stored in ethanol for future biochemical (DNA) analyses.

Voucher specimens of hosts will be preserved according to standard protocols used for fishes, turtles, and amphibians at the Natural History Collection at Sam Houston State University. A small subsample (1-2 specimens) of each species from each site will be processed in this manner and deposited.

Project Management Plan

Travel and sampling. I will travel from Nebraska to the Big Thicket and work in the area 1 week in March and 1 week in late July. All of the work can be conducted independently, and Sam Houston State University has offered the assistance of undergraduate student volunteers for field collection. I will have the use of my personal vehicle (Chevy Silverado) and request funds for 2 round-trips from Auburn, NE to Huntsville, TX, as well as weekly round-trip collections to each locale targeted. Examination of parasite specimens, preparation of permanent mounts, and identification/species description will proceed through the summer and fall, 2012 and the winter and spring, 2013. In the past, I have based my research out of the facilities provided by Drs. Tamara and Jerry Cook and their labs at Sam Houston State University. These facilities are excellent, and I plan to continue using them in 2012.

Lodging and meals. In the past, Drs. Tamara and Jerry Cook have provided free lodging and essentially expense-free board while on my collecting trips. They have offered to do the same for the planned work in 2012.

Data management. Each necropsied host and host voucher receives a unique identification number (e.g., MAB2007-1) that serves as the key to organizing, managing, and retrieving all other data. Locality information (GPS coordinates, date of collection, etc.) and host-related characters (size, sex, etc.) are recorded along with the identification number in bound notebooks. Parasite information is then attached to each host number, with each parasite within a host receiving a unique number (e.g., MAB2007-1-1). This system produces a dataset that can be input easily into both spreadsheet and database software for eventual coordination with other data sets and dissemination of results via the WWW. These data are eventually uploaded into the ATBI database.

Literature Cited

- Barger, M. A., and B. B. Nickol. 2004. A key to the species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from turtles. *Comparative Parasitology* 71: 4-8.
- Barger, M.A. 2010a. A new species of *Caecincola* (Trematoda: Cryptogonimidae) from spotted bass (*Micropterus punctulatus*) in the Big Thicket National Preserve, Texas, U.S.A. *Comparative Parasitology* 77: 6-8.
- Barger, M.A. 2010b. A new species of *Lissorchis* (Trematoda: Lissorchiidae) from creek chubsuckers (*Erimyzon oblongus*) in the Big Thicket National Preserve, Texas, U.S.A. *Comparative Parasitology* 77: 1-5.
- Barger, M.A. 2012. Redescription of *Polylekithum catahoulensis* (Trematoda) and anatomical differentiation from *Polylekithum ictaluri*. *Comparative Parasitology* 79: 143-146.
- Pritchard, M. H., and G. O. Kruse. 1982. *The Collection and Preservation of Animal Parasites*. University of Nebraska Press, Lincoln, Nebraska.
- Snyder, S. D., and R. E. Clopton. 2005. New methods for the collection and preservation of spirorchiid trematodes and polystomatid monogeneans from turtles. *Comparative Parasitology* 72: 102-107.

Budget and Budget Justification

Total funds requested to support the research in this proposal are \$2,103.00. I am requesting funds to defray the costs of travel, lodging, and to purchase field and laboratory supplies used during necropsy and processing of host and parasite specimens.

Travel. Funds are requested to defray the costs of travel to and from Huntsville, Texas from Auburn, Nebraska (1,550 x 2 trips = 3,100 miles total), local field travel (4 collections averaging 210 miles each x 2 trips = 1,680 miles). I will drive my personal vehicle (NE rate: \$0.35/mile). *Total cost: \$1,603.00.*

Lodging and food. I will be on regular salary during summer, 2010 and can be expected to meet the costs of food that I would normally incur.

Collecting. Most collecting equipment (seines, minnow traps, gill nets, etc.) are in-hand. However, I request funds to defray the cost of replacing degraded and destroyed equipment (50 feet of seines @ ~\$4.00 per net-foot, \$200.00; 50 feet of gill net @ 4.00 per net-foot, \$200.00; \$140.00). *Total cost: \$400.00.*

Lab supplies. Preparation of parasite specimens for identification requires a number of chemicals and various supplies (slides, coverslips, etc.). Some of the use of these materials from my laboratory will be incidental to normal use. However, funds are requested to purchase glass slides and coverslips; chemicals for fixation, storage, staining, and preparation of permanent mounts of specimens; and vials for storage of specimens. *Total cost: \$100.00.*

Total Request: \$2,103.⁰⁰.

Appendix

Based on Freeman, 1976.

List of fish species for sampling.

<i>Lepisosteus osseus</i>	<i>Phenacobius mirabilis</i>	<i>Lepomis auritus</i>
<i>Amia calva</i>	<i>Pimephales vigilax</i>	<i>Lepomis cyanellus</i>
<i>Alosa chrysochloris</i>	<i>Semotilus atromaculatus</i>	<i>Lepomis humilis</i>
<i>Dorosoma cepedianum</i>	<i>Carpionotus carpio</i>	<i>Lepomis macrochirus</i>
<i>Dorosoma petenense</i>	<i>Erimyzon oblongus</i>	<i>Lepomis marginatus</i>
<i>Esox americanus</i>	<i>Ictiobus bubalus</i>	<i>Lepomis megalotis</i>
<i>Cyprinus carpio</i>	<i>Minytrema melanops</i>	<i>Lepomis microlophus</i>
<i>Hybognathus nuchalis</i>	<i>Moxystoma poecilurum</i>	<i>Lepomis punctatus</i>
<i>Hybopsis aestivalis</i>	<i>Ictalurus furcatus</i>	<i>Lepomis symmetricus</i>
<i>Notemigonus chrysoleucas</i>	<i>Ictalurus melas</i>	<i>Micropterus punctulatus</i>
<i>Notropis amnis</i>	<i>Ictalurus punctatus</i>	<i>Micropterus salmoides</i>
<i>Notropis atherinoides</i>	<i>Noturus nocturnus</i>	<i>Pomoxis annularis</i>
<i>Notropis atrocaudalis</i>	<i>Noturus gyrinus</i>	<i>Pomoxis nigromaculatus</i>
<i>Notropis buechanani</i>	<i>Pylodictus olivaris</i>	<i>Etheostoma asprigene</i>
<i>Notropis fumeus</i>	<i>Aphredoderus sayanus</i>	<i>Etheostoma chlorosomum</i>
<i>Notropis lutrensis</i>	<i>Fundulus chrysotus</i>	<i>Etheostoma gracile</i>
<i>Notropis potteri</i>	<i>Fundulus notatus</i>	<i>Etheostoma histrio</i>
<i>Notropis sabinae</i>	<i>Fundulus olivaceus</i>	<i>Etheostoma parvipinnae</i>
<i>Notropis shumardi</i>	<i>Gambusia affinis</i>	<i>Etheostoma proeliare</i>
<i>Notropis texanus</i>	<i>Morone chrysops</i>	<i>Etheostoma whipplei</i>
<i>Notropis umbratilis</i>	<i>Morone mississippiensis</i>	<i>Ammocrypta vivix</i>
<i>Cyprinella venusta</i>	<i>Elassoma zonatum</i>	<i>Aplodinotus grunniens</i>
<i>Notropis volucellus</i>	<i>Centrarchus macropterus</i>	
<i>Opsopoeodus emiliae</i>	<i>Chaenobryttus gulosus</i>	

List of amphibian species for sampling.

<i>Siren intermedia</i>	<i>Necturus beyeri</i>	<i>Bufo valliceps</i>
<i>Ambystoma opacum</i>	<i>Acris crepitans</i>	<i>Bufo woodhousei</i>
<i>Ambystoma talpoideum</i>	<i>Hyla chrysoscelis</i>	<i>Rana areolata</i>
<i>Ambystoma texanum</i>	<i>Hyla cinera</i>	<i>Rana catesbiana</i>
<i>Notophthalmus viridescens</i>	<i>Hyla crucifer</i>	<i>Rana clamitans</i>
<i>Amphiuma tridactylum</i>	<i>Hyla squirella</i>	<i>Rana palustris</i>
<i>Desmognathus auriculatus</i>	<i>Pseudacris strecheri</i>	<i>Rana sphenoccephala</i>
<i>Eurycea quadridigitata</i>	<i>Pseudacris triseriata</i>	

List of turtle species for sampling.

Chelydra serpentina
Kinosternon subrubrum
Sternotherus odoratus
Pseudemys concinna
Trachemys scripta
Graptemys reticularia
Trionyx spiniferus

Michael A. Barger, Ph.D.

Professional Preparation

University of Nebraska—Lincoln	Biological Sciences	B.S., 1994
University of Nebraska—Lincoln	Parasitology	M.S., 1997
Wake Forest University	Parasitology/Ecology	Ph.D., 2001

Appointments

2008-present	Director, Honors Program, Peru State College.
2006-present	Associate Professor of Biology, Peru State College.
2001-2006	Assistant Professor of Biology, Peru State College.

Publications

Publications most closely related to proposed project:

- Barger, M.A. 2010. A new species of *Caecincola* (Trematoda: Cryptogonimidae) from spotted bass (*Micropterus punctulatus*) in the Big Thicket National Preserve, Texas, U.S.A. *Comparative Parasitology* **77**: 6-8.
- Barger, M.A. 2010. A new species of *Lissorchis* (Trematoda: Lissorchiidae) from creek chubsuckers (*Erismyzon oblongus*) in the Big Thicket National Preserve, Texas, U.S.A. *Comparative Parasitology* **77**: 1-5.
- Barger, M.A. 2012. Redescription of *Polylekithum catahouleensis* (Trematoda) and anatomical differentiation from *Polylekithum ictaluri*. *Comparative Parasitology* **79**: 143-146.
- Barger, M.A. 2005. A new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from turtles in Florida. *Comparative Parasitology* **72**: 6-9.
- Barger, M.A., V.E. Thatcher, and B. B. Nickol. 2004. A new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from a turtle in Mexico. *Comparative Parasitology* **71**: 1-3.

Other significant publications:

- Barger, M.A. 2006. Spatial heterogeneity in the parasite communities of creek chub (*Semotilus atromaculatus*) in southeastern Nebraska. *Journal of Parasitology* **92**: 230-235.
- Richardson, D.J., and M.A. Barger. 2006. Redescription of *Oligacanthorhynchus major* (Machado Filho, 1963) Schmidt, 1972 (Acanthocephala: Oligacanthorhynchidae) from the white-lipped peccary (*Tayassu pecari*) in Bolivia. *Comparative Parasitology* **73**: 157-160.
- Barger, M.A. 2004. The *Neoechinorhynchus* of turtles: distribution, host use, and specimen base. *Comparative Parasitology* **71**: 118-129.
- Barger, M.A., and G.W. Esch. 2002. Host specificity and the distribution-abundance relationship in a community of parasites infecting fishes in streams of North Carolina. *Journal of Parasitology* **88**: 446-453.
- Barger, M.A., and G.W. Esch. 2000. *Plagioporus sinitsini*: A 1-host trematode life cycle. *Journal of Parasitology* **86**: 150-153.

Skills and experience relevant to proposed project

- 17 years experience collecting, identifying and studying fishes, herpetiles and their parasites in 12 states and 2 countries.
- 3 large-scale, multi-year survey projects (1 in NE, 1 in NC, 1 in TX) of fishes and their parasites from streams, each involving the collection and identification of dozens of species of fishes and parasites.

Description of 2 new species of parasites from turtles, 3 new species of parasite from fishes, and redescription of 1 species of parasite from mammals.

Publication of dichotomous key to species of acanthocephalan parasites in turtles in North America.

Biodiversity inventory of fishes and macroinvertebrates from western Nebraska streams for Cedar Point Biological Station, University of Nebraska—Lincoln.

2 National Science Foundation grant proposals (1 standard grant; 1 CAREER) for support of research on the parasites of fishes and turtles.

Collection and necropsy of turtles in Texas as part of ongoing continental survey of acanthocephalan parasites of aquatic turtles.

Synergistic Activities

I developed, wrote, implemented, teach within, and am director of a new Honors Program at Peru State College.

I developed and authored the following laboratory manuals currently in use at Peru State College: *Introductory Zoology, Ecology, Biology of the Ectotherms, Wildlife Management Techniques, Limnology.*

I have held 11 offices in regional, national, and international societies and journals.

I have mentored 8 undergraduate students conducting independent research in my laboratory.

I contributed to the comprehensive curriculum revision for the Department of Natural Science at Peru State College, 2001-2002.

Collaborators and Other Affiliations

Collaborators and co-editors:

Esch, G.W., Department of Biology, Wake Forest University.

Lindeman, P.V., Department of Biology and Health Services, Edinboro University.

Nickol, B.B., Department of Biological Sciences, University of Nebraska—Lincoln.

Richardson, D.R., Department of Biology, Quinnipiac University.

John Hnida, Department of Natural Science, Peru State College.

Graduate advisors:

M.S.: Brent B. Nickol, Department of Biological Sciences, University of Nebraska—Lincoln.

Ph.D.: Gerald W. Esch, Department of Biology, Wake Forest University.

Thesis advisor and postgraduate sponsor:

None; graduate committee, Ms. Heather Robinson, Sam Houston State University (M.S. student).