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

Dr. Michael A. Barger

Department of Natural Science
Peru State College
Peru, Nebraska 68421-0010
402-872-2326
mbarger@peru.edu
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 an 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. 2012. Redescription of *Polylekithum catahoulensis* (Trematoda) and anatomical differentiation from *Polylekithum ictaluri*. Comparative Parasitology 79: 143-146.


**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.

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**Appendix**

Based on Freeman, 1976.

**List of fish species for sampling.**

Lepisosteus osseus  
Amia calva  
Alosa chrysochloris  
Dorosoma cepedianum  
Dorosoma petenense  
Esox americanus  
Cyprinus carpio  
Hybognathus nuchalis  
Hybopsis aestivalis  
Notemigonus chrysoleucas  
Notropis amnis  
Notropis atherinoides  
Notropis atrocaudalis  
Notropis buchanani  
Notropis fumeus  
Notropis lutrensis  
Notropis potteri  
Notropis sabinae  
Notropis shumardi  
Notropis texanalis  
Notropis umbratilis  
Cyprinella venusta  
Notropis volucellus  
Opsopoeus emiliae  
Lepisosteus mirabilis  
Pimephales vigilax  
Semotilus atromaculatus  
Carpiodes carpio  
Erinynzoon oblongus  
Ictiobus bubalus  
Minytrema melanops  
Moxystoma poecilurum  
Ictalurus furcatus  
Ictalurus melas  
Ictalurus punctatus  
Noturus nocturnus  
Noturus gyrrinus  
Pylodictus olivaris  
Aphredoderus sayanus  
Fundulus chrysotus  
Fundulus notatus  
Fundulus oliveaceus  
Gambusia affinis  
Morone chrysops  
Morone mississippiensis  
Etheostoma aspynii  
Etheostoma chlorosum  
Etheostoma gracile  
Etheostoma histrio  
Etheostoma parvipinniae  
Etheostoma proliare  
Etheostoma whipplei  
Ammocrypta vivix  
Aplodonus grunniens

**List of amphibian species for sampling.**

Siren intermedia  
Ambystoma opacum  
Ambystoma talpoideum  
Ambystoma texanum  
Notophthalmus viridescens  
Amphiura tridactylum  
Desmognathus auriculatus  
Eurycea quadridigitata  
Necturus beyeri  
Acris crepitans  
Hyla chrysoscelis  
Hyla cinera  
Hyla crucifer  
Hyla squirella  
Pseudacris streicheri  
Pseudacris triseriata  
Bufo valliceps  
Bufo woodhousei  
Rana areolata  
Rana catesbiena  
Rana clamitans  
Rana palustris  
Rana sphenolphala
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. 2012. Redescription of Polylekithum catahoulensis (Trematoda) and anatomical differentiation from Polylekithum ictaluri. Comparative Parasitology 79: 143-146.

Other significant publications:

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).