

Biodiversity Survey and Inventory of  
Parasites of Aquatic Ectotherms in the Big Thicket  
The Next Stage

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## Introduction

Parasitism is probably the least understood way of life on the planet, but ironically it is also the most common, represented in about 70% of the world's taxa. Biodiversity studies focused on vertebrates effectively catalog less than half of the animal diversity in an area if the parasites are ignored. The discovery of a parasite necessarily requires the documentation of the organism in which it resides (its host), and parasitologists are frequently good taxonomists of their host groups. Thus, inventories that focus on parasites can return twice the information on biodiversity in an area.

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. I plan now to fundamentally alter the collecting and processing strategy of my TWIG's work in two ways: 1) dedicate extensive time to the project over 2010 through an extended stay in Texas; and 2) refocus the sampling plan to maximize species recovery and identification.

Collections in 2007 and 2008 were conducted using traditional parasitological survey techniques, in which large collections of multiple host species are collected from individual sites and then processed to recover their parasites. The strategy was successful in three regards: 1) large numbers of parasite species were recovered; 2) large numbers of host species were surveyed; and 3) two species of parasites unknown to science were found and described (see CV). Unfortunately, this approach results in the processing of very few individuals of each host species, and therefore the recovery of very few individual parasites of each parasite species. Because identification of parasites requires relatively large samples (>20 individuals in most cases), most of the species collected in 2007 and 2008 are currently unidentified and will remain so until additional collections are made.

A more effective strategy was implemented in 2009: fewer host species were targeted for collection to the exclusion of any by-catch (other species collected incidentally were not processed for parasites). By focusing on fewer host species, more individuals of each host species were collected, and a larger sample of each parasite species was recovered. This has resulted in identification of most of the parasite species in a much shorter time frame than previously required just to process the parasites that could not be identified, i.e., unproductive time and effort was eliminated and the accumulation of correctly identified parasites is proceeding at a much higher pace than previously.

I propose to implement this new strategy intensively in the summer of 2010 by dedicating nearly 100% of my research time to the project over a 14 week period. In addition, I am applying for sabbatical leave for the fall semester, 2010 to work on the ATBI, and if awarded, would spend an additional 3-4 months in Texas working on this project. Implementing this new strategy over a long period such as described above should result in a substantially higher return-on-investment and substantial progress towards accomplishing the TWIG goals.

## Background

Freshwater ectotherms (fishes, most amphibians, many turtles) comprise the bulk of the diversity of inland vertebrate animals on the planet. In North America, there are about 950 species of fishes, 90 species of amphibians, and 40 species of turtles, of which 140 (100 fishes, 28 amphibians, 12 turtles) have been reported from the Primitive Big Thicket Area of Texas (Freeman, 1976). Aquatic ectotherms harbor thousands of species of parasites, representing no fewer than 10 phyla of protists and animals (Sarcocystophora; Ciliophora; Apicomplexa; Acanthocephala; Platyhelminthes; Nematoda; Pentastomida; Annelida; Mollusca; Arthropoda).

Given this host and parasite taxic diversity, the Big Thicket is a potential gold-mine of parasite species. For example, sixteen species of fishes in the waters of the Big Thicket have *never* been surveyed for parasites at all; an additional 12 species occur that have fewer than 5 species of parasites recorded from them across their range (Hoffman, 1999). In addition, a large number of parasites, including new

taxa, are likely to be discovered in well-studied hosts. For example, even though over 150 species of parasites are recorded from bluegills across the eastern half of the U.S., most of these parasites have restricted ranges: the necropsy of commonly studied hosts in un-surveyed areas consistently uncovers new species of parasites.

In three years of collection, nearly 80 species of parasites have been collected. Because no survey of parasites has been conducted in the Big Thicket previously, each of these species represents a new record for the National Preserve. Due to the problems described above, fewer than 20% of these are identified or can be identified with the material that is now available. However, the records of these species (in which hosts and locales) in my data books facilitate collection during 2010. Three new species have been discovered (2 of which have been described; see CV), and several species are likely to be the subject of taxonomic revision at some level.

## **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 a 14 week period in the summer of 2010 (an additional 3-4 months during the fall if my application for sabbatical leave is awarded from my home institution);
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 30 species of fishes and 20 species of herpetiles in 2010 using the new collecting strategy. Briefly, 2-5 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.

Because of the extended time for research involved, I can now target a larger fraction of the Big Thicket Units for collection. Previous work has been confined to the Big Sandy Creek Unit, the Menard Creek Unit, and the Turkey Creek Unit. In addition to continued sampling in these areas, collections in 2010 will include the Lance Rosier Unit, the Neches Bottom and Jack Gore Baygall Unit, the Upper Neches Corridor Unit, the Beech Creek Unit, the Village Creek Corridor Unit, and the Lower Neches River Corridor Unit. Some of these locales require additional logistical support (boat rentals, etc.) because of their physical size, and the extended period of my time in Texas will allow these larger habitats to be surveyed.

***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 from the 2<sup>nd</sup> week in May to the 2<sup>nd</sup> week in August. Most 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 1 round-trip 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, 2010 and the winter and spring, 2011. 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 2010.

**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 2010. However, I feel it would be inappropriate for them to be burdened by these costs for such an extended period of time, and therefore ask for modest compensation to support the costs incurred for my lodging and meals.

**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.
- Freeman, R. 1976. National Park Service, Final Environmental Statement, Establishment of the Big Thicket National Preserve, Texas. United States Department of the Interior, FES 76-40.
- Hoffman, G. L. 1999. *Parasites of North American Freshwater Fishes* (2<sup>nd</sup> Edition). Comstock Publishing Associates, Ithaca, New York.
- 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 \$5,390.00. 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 miles roundtrip), local field travel (25 round-trips averaging 210 miles/trip; total: 5,250

miles), and periodic travel to Sam Houston State University to perform laboratory work (20 trips @ 40 miles/trip; total: 800 miles). I will drive my personal vehicle (NE rate: \$0.35/mile). *Total cost: \$2,660.00.*

***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. However, I request \$25/week to defray costs to Drs. Tamara and Jerry Cook, who will host me at their private residence during this period. *Total cost: \$350.00.*

***Collecting.*** Most collecting equipment (seines, minnow traps, gill nets, etc.) are in-hand. However, collecting during this period will be intense, and I request funds to defray the cost of replacing degraded and destroyed equipment (100 feet of seines @ ~\$4.00 per net-foot, \$400.00; 100 feet of gill net @ 4.00 per net-foot, \$400.00; 4 turtle traps @ \$70 each, \$280.00). In addition, I request funds for boat rentals necessary in large bodies of water (~\$80.00/day; 10 days; \$800.00). *Total cost: \$1,880.00.*

***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: \$500.00.*

***Total Request: \$5,390.<sup>00</sup>.***

***Sabbatical leave.*** I have applied for sabbatical leave to take effect August, 2010 and extend through December, 2010. My sabbatical application focuses entirely on the ATBI work in the Big Thicket. If it is awarded, I will then be able to spend the bulk of the fall semester, 2010 (from mid-August to the end of November) in Texas continuing my work on the parasites of the Big Thicket aquatic ectotherms. Sabbatical awards are not announced until the mid-point of the spring semester (2010, in this case). If awarded, I will then seek funding through the PSC faculty development fund to support the additional time I will spend in Texas. To the extent these funds do not fully compensate for the planned work, I will seek funds through the ToD ATBI. By the time of the sabbatical, I will be in a position to focus primarily on species identification and description. Thus, although collecting would continue, its intensity and frequency would diminish, as would the costs associated with collecting (travel, equipment, etc.).

## 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>Carpionodes 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 buchanani</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.**

<i>Chelydra serpentina</i>
<i>Kinosternon subrubrum</i>
<i>Sternotherus odoratus</i>
<i>Pseudemys concinna</i>
<i>Trachemys scripta</i>
<i>Graptemys reticularia</i>
<i>Trionyx spiniferus</i>

## Michael A. Barger, Ph.D.

### Professional Preparation

University of Nebraska—Lincoln  
University of Nebraska—Lincoln  
Wake Forest University

Biological Sciences  
Parasitology  
Parasitology/Ecology

B.S., 1994  
M.S., 1997  
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**: *in press*.
- Barger, M.A. 2010. 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**: *in press*.
- 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.
- Barger, M.A., and G.W. Esch. 1999. *Allopodocotyle chiliticum* n. sp. (Digenea: Opecoelidae) from redlip shiners, *Notropis chiliticus*, in Basin Creek, North Carolina. *Journal of Parasitology* **85**: 891-892.

*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).