

BASELINE INVENTORY OF FREELIVING AND PLANT-PARASITIC NEMATODES OF THE BIG THICKET NATIONAL PRESERVE

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Project Narrative

Description

Free-living and plant-parasitic nematodes will be surveyed in 2013 at selected sites within each of the ten terrestrial units in Big Thicket National Preserve (BITH) including the Beaumont, Beech Creek, Big Sandy Creek, Canyonlands, Hickory Creek Savannah, Houseman Tract, Lance Rosier, Loblolly, Neches Bottom and Jack Gore Baygall and Turkey Creek Units. Each unit will be sampled by establishing 2-3 temporary 40x40m² plots within each unit to provide an estimate of nematode community changes with changing plant communities. A comprehensive species list will be prepared as an Excel spreadsheet or Access Database with complete collection and taxonomic data. Voucher specimens will be deposited in the BITH reference collection or other repository designated by BITH. Digital and DNA vouchers will be maintained and accessible through the University of Nebraska. Specimen web pages that include high-resolution images of the diagnostic characteristics of the nematode together with ecological and habitat information, will be added to the Plant and Insect Parasitic Nematode web site (<http://nematode.unl.edu>). DNA analysis of a select group of plant parasitic species will be generated to establish genetic estimates of biodiversity. These diversity estimates will contribute to a continent-wide survey of North American ecosystems.

Justification

Nematodes are routinely considered among the most abundant and diverse animals on earth (Groombridge, 1992; Campbell et al., 2006). Surprisingly, little is known about how that diversity is distributed on the planet. Many people, scientists and general public alike, are unaware of the complex and highly structured nematode communities that exist in every square meter of soil. E.O. Wilson has remarked "We live in a nematode world" (Seely, 2006). Nathan Cobb famously commented on a dimly recognizable world if all matter except nematodes were removed (Cobb, 1915). Both men could have gone further, presenting an image of a global veneer of nematodes that in its species composition and genetic makeup reflects the movement

of continents over hundreds of millions of years as well as the comparatively recent postglacial recolonization that followed the Ice Ages (Pielou, 1991; Hewitt, 2004).

Through the application of DNA-based approaches, the structural complexity of nematode communities across the landscape are beginning to be revealed (Powers et al., 2009). Enhanced knowledge of species composition in native nematode communities can facilitate the application of nematodes as environmental indicators (Ficus and Neher, 2002; Freckman and Ettema, 1993; Neher, 2000; Neher 2001), indicators of climate change (Anderson et al. 2004; Neher et al., 2004; Ruess et al., 1999), and reflect the belowground impact of invasive species (Rebetz and Dobbertin, 2004; Reinhart and Callaway, 2006). Recent studies of remnant tallgrass prairies in the Great Plains have recovered endemic nematode species which now exist within disjunct grassland islands widely scattered across the region (Powers, 2010; Todd et al., 2006). The nematode communities within these remnants bear no resemblance to the nematode communities within the monocot agroecosystem monoculture that now surrounds the prairie. Characterization of these prairie nematode communities provides a baseline for restoration studies and documents the long-term impact of soil disturbance. Similar results have been demonstrated between forest and agricultural nematode communities (Bernard, 1992), and even among endemic plant communities in Hawaii (Bernard & Schmitt, 2005).

The long history of human activity and disturbance in the Big Thicket likely has had similar significant effects on nematode community composition, but the duration of these effects once activity has ceased is unknown. In other words, is rebound of nematode communities to a pre-disturbance state a matter of months, years, or centuries? For instance, while little old-growth forest remains in the area, some significant, many-decades regrowth has occurred. These sites may have maintained the below-ground diversity and species composition characteristic of old-growth sites. Our previous observations from restored prairies in Nebraska and reforested lowland rainforest in Costa Rica indicate that soil nematode community reestablishment lags decades behind the reestablishment of the vegetation. Nematode recolonization of land exposed by retreat of the Columbia Glacier begins to occur within a few years of exposure, but the communities for many decades are depauperate and unrepresentative of surrounding taiga (Bernard et al., 1995). Properly conducted surveys will provide valuable baseline data for comparison with the effects of future reclamation efforts, such as restoration of land impacted by oil extraction or the reestablishment of grassland nematode communities after removal of loblolly pines. Close cooperation with BITH personnel will insure that we sample preserve areas where monitoring efforts are likely to occur in the near future.

The primary goal of this proposed project is to intensively collect free-living and plant-parasitic nematodes in a statistically valid way for the purpose of defining their diversity in BITH and providing an authoritative list of the species present. Numerous new records and new species can be expected, along with an expanded understanding of the impact of biotic and abiotic disturbance in soils. The data derived from this project will be suitable for comparisons with ongoing ecoregion surveys of natural areas (Ricketts et al., 1999) conducted by the first PI in prairies and with a survey of nematodes in Great Smoky Mountains National Park ATBI sites conducted by both PIs.

Study Area

The nematode inventory will be conducted in each of the 10 terrestrial units of the BITH, Beaumont, Beech Creek, Big Sandy Creek, Canyonlands, Hickory Creek Savannah, Houseman tract, Lance Rosier Unit, Neches Bottom and Jack Gore Baygall, Loblolly and Turkey Creek Unit. Within each of the 10 terrestrial BITH units, 40x40m² sampling sites will be selected after consultation with BITH biologists and land managers to reflect areas of high plant diversity, endemism, or varying levels of disturbance. We are interested in sampling each of the major plant communities found in BITH: longleaf pine uplands, Beech-magnolia-loblolly pine, arid sandyland, upper floodplain/lower floodplain, cypress-tupelo swamps, palmetto hardwood flats, acid bog/baygall, and pine savannah wetlands.

Methods

Nematode collection and extraction: At each sampling site a single 40x40m² plot will be established with the corners marked by GPS (UTM) coordinates. At a predetermined point along one border, the plot will be entered and traversed by the collector with a hand-held Oakfield tube soil corer of 2cm diameter. Every 6 meters a core will be extracted to a depth of approximately 20 cm, until 500-1000cc of soil is accumulated. The soil is then placed into a plastic collection bag and stored in a cooler until transportation to the laboratory for analysis. Images of the study site and notes on the plant community will be recorded. Each site will be sampled at least once, preferably in the spring or fall depending on root growth. In some cases, nematode analysis may suggest a second sampling of the site will be productive. The discovery of new species or the observation of unusual nematode assemblages or stages could initiate a second sampling session. In some cases, depending on topography and dimensions of the sampling area, sampling may be modified to include closer intervals and more samples. All samples will be returned to the PI laboratories for extraction, identification, and preservation.

In the laboratory nematodes will be isolated from the soil sample by a process of floatation and sieving followed by sugar centrifugation. Members of the suborder Criconematina will be measured, photographed and then stored for PCR/DNA sequencing analysis. A critical component of this analysis is the linkage of nematode morphology from individual specimens with their corresponding DNA haplotype. All images and DNA sequences will be deposited in public access data repositories. Formalin-fixed nematodes stored in vials and permanent slides will be deposited at the Nebraska State Museum in the Manter Parasitology Collection, in the Nematology Collection at the University of Tennessee, or in the National USDA Collection in Beltsville, MD. A representative microscope slide set of glycerine infiltrated nematodes mounted on glass slides will be provided to BITH.

Nematode identification: For nematode identification, specimens are typically examined by DIC light microscopy. For criconematid species, a group known to be differentially affected by soil disturbance and host association, microscopic identifications will be supplemented by a set of diagnostic PCR primers which amplify both mitochondrial and nuclear genetic markers (Bernard, 1982; Powers et al., 2010; 2011). New species, cryptic species, or species not yet assessed by molecular methods will necessitate a species delimitation step. All molecular analyses will be conducted on individual specimens that have been digitally photographed, measured, and removed from temporary glass microscope slides. Both laboratories will be involved in identification and photomicrography of collected nematodes. The PIs will jointly

prepare any papers that describe new species or community composition. We expect to identify to species level well over 200 nematode taxa, and the number could be much higher. A typical temperate deciduous forest sample may contain 60 or more species, so a survey across several plant community types and soil formations should yield several times that.

Deliverables

Data: All identifications will be entered into a spreadsheet that provides complete collection information, including GPS (UTM) coordinates. Numbers collected from extractions will be extrapolated to numbers per surface m² to facilitate comparisons with results from other studies. Photomicrograph files will be provided to BITH with annotation, as well as being posted on the web site indicated below.

Specimens: In addition to the PI institutions, voucher specimens will be deposited with BITH and (or) in other depositories as designated by BITH.

Web based: For the last 17 years the UNL Nematology Laboratory has been managing and constantly updating an extensive web-based collection of taxonomic, morphological, and diagnostic information of plant parasitic nematodes (<http://nematode.unl.edu>). Included at this site is a set of pages dedicated to the analysis of nematode communities at specific localities. For example several native prairie sites are profiled in the prairie ecosystem section (<http://nematode.unl.edu/prairiesurveys.htm>). We propose to design a section that will similarly describe the nematode communities found in the BITH. Also, criconematid species found during the survey will be described in detail as part of the Criconematina project site (<http://nematode.unl.edu/CriconematidProject.htm>). This page provides navigation points to access species descriptions, GenBank accessions and all data pertaining to specimens. These include photo galleries of both light and scanning micrographs of specimens, their placement on phylogenetic trees, distribution maps and information regarding the eco-regions and specific habitats where specimens have been collected.

Budget

| Item | PI Powers | PI Bernard |
|------------------------|------------------|-------------------|
| Travel, lodging, meals | 2,000 | 1,000 |
| Technical assistance | 4,000 | 500 |
| Supplies | 2,000 | 500 |
| Total | 8000 | 2000 |

Budget Justification

Travel, lodging, meals – Powers group: \$2,000: includes 2000 RT miles for one trip by UNL lab from Nebraska (with 3 undergraduate students, UNL Lab personnel) includes transportation of samples and meals and housing at the BITH Field Station while sampling. Bernard group: \$1800 1600RT miles for Dr. Bernard (and graduate student). A general introduction to the biodiversity of terrestrial nematodes (workshop) for interested personnel from BITH, Thicket of Diversity and the general public can be presented at the Field Research Station by Dr. Powers.

Technical assistance – Powers: \$4,000 for technical assistance in nematode extraction, DIC light and scanning microscopy, data entry, collection maintenance, nematode fixation, preservation, mounting, identification assistance, web design and construction.

Bernard: Technical assistance in nematode extraction, data entry, collection maintenance.

Supplies and Equipment – Powers: \$2,000 for DNA extraction, amplifications, DNA sequencing, sequence analysis and expenses associated with slide preparation, light and scanning microscopy, including software for image analysis. Bernard: Supplies for imaging, nematode fixation, preservation, mounting.

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