Examining the marine acanthocephalan *Profilicollis altmani*:
a generalist parasite or cryptic species complex?

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Introduction

The field of parasitology has traditionally been driven primarily by the eradication of human parasites and the parasites of our domestic animals; however it is not often appreciated the large role parasites can play in natural ecosystems by manipulating host behavior and regulating host abundances [1-2]. Understanding the evolutionary and ecological relationships of parasites and their hosts can have many implications for restoring threatened ecosystems and preserving the biodiversity therein [3-5]. Molecular systematic techniques offer an invaluable view into the evolution of parasites and allow us to study the specificity of parasites that may display little or no observable morphological differences [6].

Acanthocephalans or "spiny-headed worms" are a relatively small phylum of obligate endoparasites that infect an assortment of hosts in marine, freshwater and terrestrial habitats. Unlike other parasitic helminths, acanthocephalans are not known to engage in any form of asexual reproduction [7]. This lack of asexual reproduction may have consequences reflected in the genetic structure of different populations. Multiple acanthocephalans can be found within a single host, and, since acanthocephalans must be a product of sexual reproduction, each individual is genetically distinct. Acanthocephalan eggs can endure long time periods in the oceanic environment, therefore increasing the potential for geneflow. All of these unique characteristics make acanthocephalans an interesting system to examine host specificity and look for the presence of cryptic species. Little is known about the levels of cryptic diversity and specificity of acanthocephalans compared to more diverse groups of parasites, such as trematodes, cestodes and nematodes [8-9].

The acanthocephalan parasites found in mole crabs (*Emerita spp.*) are a model representative of marine acanthocephalans due to their abundance in sandy beach food webs over a broad geographic range [10-12]. The taxonomic status of these acanthocephalans remains unsettled; originally, several species were described based on morphological characters (*Profilicollis altmani, P. kenti, P. texensis,* and *P. bullocki)* [13-15] and it was even reported that three species of acanthocephalan co-occur in the mole crab *E. analoga* along the central coast of California [16]. However, due to the intraspecific variability in morphological characters, it has been suggested that morphological descriptions of this parasite may not be reliable indicators of species status[17]. Genetic techniques offer an alternative means of addressing if acanthocephalans from different crab populations have genetically diverged and actually represent different species [6].

Objectives

There are three main objectives of this research. The first objective is to test if genetic analysis supports the previous report of multiple co-occuring acanthocephalan species infecting *Emerita analoga* in central California and examine the haplotype diversity of this parasite between local California populations. The second objective is to create a phylogeny from the parasite genomic sequences to investigate the relatendess of acanthocephalans from geographically isolated crab populations and examine population connectivity. I will use this phylogeny to test the hypothesis that the profilicollid acanthocephalans have genetically diverged between different species of the *Emerita* mole crabs. The third objective is to infer if there are multiple cryptic species of acanthocephalan found among any single species of crab or shared between crab species.

Methods

To address these different research objectives I am obtaining samples of *Emerita* crabs from the five species found around North America and dissecting the crabs to obtain their parasites. I have sampled *E. analoga* from eight locations along the coast of California and *E. talpoida* from two locations on the

east coast of the United States. I currently have one acanthocephalan found in *E. rathbunae* from the west coast of Panama, but I will be obtaining more specimens from Dr. Mark Torchin, Smithsonian Tropical Research Institute. I will also be obtaining a samples of *E.benedicti* from the gulf coast of Texas and *E. portoricensis* from Puerto Rico.

To investigate if the acanthocephalan parasites of mole crabs have genetically diverged I will be examining three genetic loci: 626 bp of the mitochondrial gene cytochrome oxidase I (COI), 570 bp of the nuclear ribosomal internal transcribed spacers (ITS), and 865 bp of the nuclear heat shock protein 82 (hsp82). Two of these loci, COI and ITS, have been previously used to identify cryptic species or strains of acanthocephalans from freshwater hosts [18-21]. The third locus, hsp82, has been used in examining the close relationship between acanthocephalans and rotifers, but has yet to be applied to studying very closely related species of acanthocephalan; this locus was chosen due to the variability it exhibited among other acanthocephalans [22]. Outgroups will include the three species of acanthocephalans which have published sequences for hsp82, COI and ITS. Phylogenetic analysis will be performed on the different haplotypes obtained from the 210 samples for each locus. MrModel test[23] will be used to select the mode of molecular evolution that best fit the data. The programs PAUP [24] will be used to create a maximum likelihood phylogeny of the different haplotypes and the program MrBayes [25] will be used for Bayesian analysis.

Significance

Given the complex life cycle of this acanthocephalan it is currently unknown to what degree this parasite utilizes a generalist or specialist strategy in their infection of hosts. Due to its wide dispersal capabilities I expect that, similar to many marine species with planktonic life histories, gene flow among acanthocephalans could have impeded speciation [26]. However, since this parasite has been found infecting many different species of birds, it is also possible that this parasite could actually be a number of cryptic specialist species, each being restricted to a fewer number or a possibly a singular bird species.

My preliminary results from sequencing the COI and ITS loci acanthocephalan samples have revealed low genetic variation (0-1.8%) among individuals. I have also found shared haplotypes between the US east and west coast populations suggesting that there may be wide dispersal of this parasite. A possible mechanism for transport of the parasite is the seasonal migration of their final hosts; a variety of sea ducks and gulls that release acanthocephalan eggs through their feces. Since I have found no evidence so far of different species of acanthocephalan infecting mole crabs it suggests that, unlike the freshwater acanthocephalans previously studied, this marine acanthocephalan does not represent a group of cryptic species and may have maintained a generalist parasitic strategy. This would be very interesting from an evolutionary perspective since little is known about the specificity of acanthocephalans from marine environments. However, these preliminary results are based only on sequences of acanthocephalans from two species of *Emerita* crab. I will be obtaining the other species of crabs this summer when the crabs are easily sampled from the intertidal. Inclusion of acanthocephalan genomic sequences from additional species of crabs found in the tropical waters of the Gulf of Mexico and Pacific could change these conclusions.

This phylogeographic research is the first to study the genetic divergence of a marine acanthocephalan; previous studies of this phylum have examined species from freshwater and brackish hosts [19-22]. I plan to publish this research in the *Journal of Parasitology* to not only clarify the taxonomy of this group, but also to contribute to our understanding of the evolution and population connectivity of a prevalent marine acanthocephalan.

Schedule

This project constitutes the core of my master's thesis that I have been working on for the last year and a half at San Francisco State University's marine lab, the Romberg Tiburon Center for Environmental Studies. I have currently obtained 151 of the 210 specimens that I will analyze at multiple loci. DNA has been extracted from 150 samples, many of which I have sequenced for the COI locus and the ITS locus. The heat shock protein locus will most likely require some cloning to obtain clear

sequences. Thus far, I have obtained funding for field work but I am applying to the Society of Systematic Biologists for a Graduate Student Research Award to help offset the costs of my genetic analysis. The next few months I will be working on sequencing the hsp82 locus from my current samples. Beginning in summer I will finish my collecting of the different mole crab species and begin sequencing the three genetic loci from the new samples. Sequences will be analyzed in early 2011 and I will submit my results for publication later that year.

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Budget : 150 samples

Nucleospin DNA Extraction ($1.62 per sample * 210 samples)	$340.20
TOPO TA Cloning kit ($462/20 reactions)	$462
PCR- Taq polymerase ($0.12 per sample * 3 loci * 210 samples)	$75.60
PCR cleanup – SAP/Exo ($0.22 per sample * 3 loci * 210 samples)	$138.60
ABI- Big dye ($0.15 per sample * 3 loci * 210 samples)	$94.50
ABI – Hi-Di Formamide ($0.04 per sample * 3 loci * 210 samples)	$25.20
Sequencing polymer ($173/3500ul)	$173
Total	**$1309.10**

Budget Justification

For my research I am requesting $1309.10 to cover the costs of sequencing three loci for 210 individual parasites. The 210 samples span 14 sampling sites across 5 host species, with 15 parasites extracted per site. Each sample will be sequenced for two nuclear loci and one mitochondrial locus, which requires Taq polymerase for PCR reactions, SAP/Exo for PCR cleanup, Big Dye for sequencing reactions, and Hi-Di formamide for sequencing. The TOPO TA cloning kit will only be used for one genetic locus, heat shock protein 82. The sequencing polymer is required to run the ABI 3130 Genetic Analyzer available at the Romberg Tiburon Center. Fieldwork costs are not included in this budget and have been partially covered by a Sigma-Xi Grant in Aid of research I received in 2009.

Tricia Goulding

EDUCATION

M.S., Marine Biology, Romberg Tiburon Center for Environmental Studies.
San Francisco State University, 2008-present

B.A., Biology, College of Creative Studies, Honors, GPA 3.68
University of California, Santa Barbara. June 2008

PROFESSIONAL EXPERIENCE

Teaching Assistant, Marine Ecology Laboratory Course San Francisco State University
August 2009-December 2009

Contributor, Gulf of the Farallones Marine Sanctuary
Provided scientific review of educational curriculum on mole crabs and their parasites
January 2010

Teaching Assistant, NSF Summer Research Experience for Undergraduates
Biological Research in Ecological and Evolutionary Developmental Biology
June 2009-August 2009

RESEARCH EXPERIENCE

Research Experience for Undergraduates (REU) Program, 2007
Shannon Point Marine Center, Western Washington University.

Volunteer Research Assistant, Partnership for Interdisciplinary Studies of Coastal Oceans
Enumeration and identification of juvenile marine invertebrates
April 2007- June 2007

Volunteer Research Assistant, Hofmann Lab, University of California, Santa Barbara
Extracted proteins and DNA from *Mytilus* mussels, aided in PCR and electrophoresis
January 2007- March 2007

AWARDS

Achievement Rewards for College Scientists (ARCS) Scholarship, 2009 ($10,000)
Sigma-Xi Grants in aid of Research, 2009 ($560)
San Francisco Bay Scholarship 2008, 2009 ($500 each)

PRESENTATIONS

Goulding, T., and Cohen, S. Examining genetic variation of the acanthocephalan *Profilicollis altmani* parasitizing mole crabs (*Emerita* spp.) in North America. Oral presentation. The Society for Integrative and Comparative Biology 2010 Meeting. Seattle, WA. January 3-7, 2010.

Goulding, T., Miner, B.G., and Donovan, D.A. Phenotypic changes in the dogwinkle *Nucella lamellosa* in response to chemical cues of crab and sea star predators. Poster presentation. Western Society of Naturalists 88th Annual Meeting. Ventura, CA. November 11-14, 2007.

Miner, B.G., Donovan, D.A., and **Goulding, T**. Life history plasticity in embryos of *Nucella lamellosa*.Western Society of Naturalists 88th Annual Meeting. Ventura, CA. November 11-14, 2007.

ACTIVITIES

RTC Student Association Vice President, 2009-2010

MEMBERSHIPS

Society for Integrative and Comparative Biology, Society of Systematic Biologists