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Collaborative Research: Gymnosperms on the Tree of Life: Resolving the Phylogeny of Seed Plants

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Final Report for Period: 10/2010 - 09/2011**Submitted on:** 10/04/2011**Principal Investigator:** Campbell, Christopher S.**Award ID:** 0629713**Organization:** University of Maine**Submitted By:**

Campbell, Christopher - Principal Investigator

Title:

Collaborative Research: Gymnosperms on the Tree of Life: Resolving the Phylogeny of Seed Plants

Project Participants**Senior Personnel****Name:** Campbell, Christopher**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Graduate Student****Name:** Connolly, Margaret**Worked for more than 160 Hours:** Yes**Contribution to Project:**

I have worked closely with Dr. Christopher Campbell at the University of Maine in an effort to collect, extract, amplify, and analyze DNA of members of the Pinaceae. I amplified and analyzed 5 Variable Plastid Coding Regions in members of Cedrus, Abies, Tsuga, Picea, Nothotsuga, Pseudolarix, and Keteleeria in contribution to a larger dataset assembled by Dr. Linda Raubeson. I have completed multiple collecting trips to the Arnold Arboretum, have extracted DNA from over 25 species of Abies, have amplified the matK gene through PCR, and have analyzed the resulting DNA sequences. Recently I have been involved in training an undergraduate student, Kristen Paul, in the various laboratory techniques involved in this project. In addition, I developed a Gymnosperm Phylogeny curriculum to teach high school students about the field of phylogenetics and current hypotheses of gymnosperm evolution.

Name: Burgess, Michael**Worked for more than 160 Hours:** Yes**Contribution to Project:**

[Chris Campbell is responding for Michael Burgess.] Margo Connolly, who worked on this project in year 1, decided that her interests were primarily in education and switched to a Masters program in education in Fall 2007. Michael Burgess worked in Fall 2007 and Spring 2008 continuing activities that Margo Connolly had been working on, namely extracting DNA from conifer leaf samples and carrying out PCR and DNA sequence analyses on these samples. Michael also worked extensively on PCR amplification of the nuclear gene PHYP.

Name: Holman, Garth**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Garth Holman was the research assistant for this entire year. He performed laboratory work, helped supervise the undergraduate students in the lab, participated in a workshop on conifer evolution for high-school teachers in Lewiston Maine, and is presenting a poster on his work on this project at the annual meeting of the Botanical Society of America/American Society of Plant Taxonomists in August of this year.

Undergraduate Student**Name:** Paul, Kristen**Worked for more than 160 Hours:** Yes

Contribution to Project:

[C. S. Campbell is responding for Kristen.]

Kristen joined the lab about three weeks ago, and is involved in all phases of our work on the phylogeny of seven genera of Pinaceae. She went on one of our collecting trips to the Arnold Arboretum, extracted DNA, performed PCR, and is learning to edit sequences and analyze them. She is also learning how to clone genes in preparation for work on the PHYP gene.

Name: Lyons, Shannon

Worked for more than 160 Hours: Yes

Contribution to Project:

Shannon Lyons performed basic laboratory work on this project, including DNA extractions; polymerase chain reactions (PCR); agarose gel preparation, loading, and photography; DNA cloning; dish-washing; and other routine chores. She is a biology major.

Name: Hendershot, Sara

Worked for more than 160 Hours: Yes

Contribution to Project:

Sara performed basic lab techniques, including DNA extraction, assisting with PCR and cloning, and general tasks. She also worked on various databases.

Technician, Programmer**Other Participant****Research Experience for Undergraduates****Organizational Partners****Arnold Arboretum**

From the Arnold Arboretum's extensive collection of living trees, we obtained leaf material, pollen cones, seed cones, and branches of species of Pinaceae that are integral to our project. Without this facility, costs of obtaining this material would have been much greater. The staff of the Arnold Arboretum has been extremely helpful in locating trees of interest that were healthy and ensuring that a reference collection has been made for the herbarium. The Arboretum staff has helped graduate student Garth Holman locate and make voucher specimens of many of the species that we are working on.

Graduate student Garth Holman and PI Campbell are collaborating with Peter Del Tredici, Senior Research Scientist at the Arnold Arboretum, on the relationships of the hemlock on Ullung Island (between Japan and Korea).

Royal Botanic Garden Edinburgh

As a result of PI Chris Campbell's trip to the Royal Botanic Garden in Edinburgh (RBGE), Scotland, leaf material of about a dozen species of conifers has been obtained. Reference collections of specimens from which material was collected have been made. Obtaining these materials without this facility would have been far more costly.

Graduate student Garth Holman and PI Campbell have continued collaboration with Sabina Knees, a graduate student at RBGE, with a co-authored presentation at the 2009 annual meeting of the Botanical Society of America/American Society of Plant Systematists and one or more future publications.

Montgomery Botanical Center, Miami FL

(via Patrick Griffith, Director, and Chad Husby, Botanist). Griffith made a presentation at our annual project meeting on cycad phenology in the phylogenetic and biogeographic context. Two of his co-authors were AToL PI, Dennis Stevenson, and senior personnel Damon Little. Husby hosted the Mathews lab to collect Podocarpaceae and Araucariaceae from their collections.

University of Maine

The University of Maine provided facilities for our pilot outreach workshop on gymnosperm phylogeny. Graduate student Garth Holman and PI Campbell are working with Prof. William Livingston (School of Forest Resources) to present a workshop on conifer evolution for Lewiston,

Maine high-school teachers as part of a larger effort to encourage these teachers to incorporate material about trees and forests into their curricula.

Other Collaborators or Contacts

This is a collaborative project to start with, and the other organizations are specified in the proposal.

Collaboration on the phylogeny of *Abies* was established with Sabina Knees at the Royal Botanic Garden Edinburgh (RBGE) and Keith Rushforth who has extensive experience with the genus and coauthored its most recent classification. Graduate student Garth Holman has been collaborating with Graduate student Markus Ruhsam of the RBGE on gymnosperm plastome sequencing. Markus works on the genus *Araucaria*, Garth helped Markus obtain plastome sequences in this genus, and Markus has kindly sent us a plastome sequence as an outgroup for a plastome phylogenetic tree of the Pinaceae that Garth and others in this project are assembling.

Graduate student Garth Holman and PI Campbell are collaborating with Peter Del Tredici, Senior Research Scientist at the Arnold Arboretum, on the relationships of one of a curious hemlocks on Ullung Island (between Japan and Korea). This collaboration is explained more fully in the Activities section.

Graduate student Garth Holman and PI Campbell are collaborating with Suzanne Renner and Aleksic Jelena on a molecular phylogeny of *Picea*.

Activities and Findings

Research and Education Activities:

The primary goals of PI Campbell, along with a graduate student working on this project, in this collaborative research are to (1) obtain DNA sequence data for about 100 species in seven genera of the pine family, including firs (*Abies*), spruces (*Picea*), hemlocks (*Tsuga*), true cedars (*Cedrus*), and three smaller genera; (2) obtain full sequences of the chloroplast genome for 11 core taxa representing these seven genera; and (3) assemble morphological data for the same core taxa representing these seven genera of Pinaceae; and sequence the full chloroplast genome for these same core taxa. Phylogenetic relationships of most of the species in the two largest of these genera, firs (approximately 50 species) and spruces (35 species), are poorly known.

DNA sequence data being obtained for phylogenetic analyses of gymnosperms includes the nuclear locus PHYP plus the chloroplast locus *matK* for all gymnosperm species as well as the entire plastome for core taxa.

Findings:

In addition to contributing sequences from PHYP, *matK*, and whole chloroplast genomes as well as adding to the gymnosperm morphological matrix, we have a particular interest in relationships within the Pinaceae. It is clear from considerable data that there are two large groups within the Pinaceae that are mostly referred to as subfamilies Abietoideae and Pinoideae. In addition to these two groups, there is the genus *Cedrus*, the true cedars, with 2-4 species ranging from the Mediterranean to south-central Asia. The relationships of *Cedrus* are not clear. Some data and some analytical approaches put *Cedrus* as sister to the Abietoideae, whereas other data place it as sister to the remainder of the family. Unfortunately, Pinaceae are so distant from all other gymnosperms that it is difficult to assess the outgroup character states for much of the data. Graduate student Garth Holman, along with many collaborators from this project, gave a presentation on this topic at the 2010 annual meeting of the Botanical Society of America and American Society of Plant Taxonomists dealing with this matter. This paper explored relationships within the Pinaceae, in particular the phylogenetic position of *Cedrus* and the relationships of *Pseudolarix*, an endemic genus of eastern Asia. Molecular data included plastome, PHYP, and PHYN sequences, and morphological data consists of a small number of characters that are potential synapomorphies for *Cedrus* plus Abietoideae and *Cedrus* plus Pinoideae.

Since then, the monotypic southeast Asian genus *Cathaya* has been added to this project in order to attempt to clarify relationships between this genus and *Picea* and *Pinus*. We expect to submit this paper for publication in the winter of 2011-2012.

We have sequenced all taxa for which we are responsible for *matK*, except for a few, very rare taxa from eastern Asia. Similarly, we have completed our sequencing of PHYP, with multiple representatives of all clades within the genera for which we are responsible.

Our contribution to the plastome sequencing effort has been completed; Graduate student Garth Holman has obtained full-genome sequences

for all the core taxa for which we are responsible.

We have completed our contribution to the morphological matrix, except for some of the taxa for which it is difficult to obtain good material. For example, we have not been able to get good material of the seed cones of the Asian endemic *Nothotsuga longibracteata*. Most of our additions to the matrix have come from the literature, but a small portion comes from direct observation of plants. Here at the University of Maine, we found a series of microscope slides with high-quality sections of cones of two of our core taxa, eastern hemlock and white spruce. An undergraduate student, Andrew Fuller, did a senior thesis on these slides. Garth Holman has seeds of some taxa that he germinated in order to study the anatomy of the root.

We continue our collaboration with Peter Del Tredici, Senior Research Scientist at the Arnold Arboretum, on the relationships of the hemlock on Ulleung Island (Korea). While hemlocks on this island have long been identified as *Tsuga sieboldii* from Japan, molecular data indicate a closer relationship between the hemlock on Ulleung Island and the other Japanese hemlock, *T. diversifolia*, than with *T. sieboldii*. Both chloroplast and nuclear DNA sequences support this relationship (see publication on which PI Campbell is a coauthor in the Publications section). Peter Del Tredici collected material of the Ullung hemlock, and Graduate student Garth Holman and PI Campbell will collect morphological and molecular data to better understand this conflict between morphology and molecular data. Holman is close to completing a manuscript on this hemlock.

PI Campbell and graduate student Garth Holman worked on physical acquisition of plant material genera of Pinaceae for which PI Campbell is responsible (see Publication and Products section, physical collections section).

Training and Development:

Graduate students (Michael Burgess, Margaret Connolly, and Garth Holman) plus undergraduate students (Kristen Paul, Shannon Lyons, and Sara Hendershot) learned basic laboratory techniques in molecular systematics, including DNA extraction, polymerase chain reaction (PCR), cloning, DNA sequence editing, and phylogenetic analysis. These students are learning about the biology of conifers from making collections of these plants and from discussions in the lab. Undergraduate student Andrew Fuller learned about the morphology of Pinaceae while working on his senior thesis in our lab.

The same graduate students have developed their understanding of phylogenetics and molecular systematics as a result of working on this project.

Outreach Activities:

Part of this Tree of Life project is to present workshops on gymnosperms to K-12 teachers in order to increase public understanding of science.

At the University of Maine, 24 August 2007, graduate student Margo Connolly and PI Chris Campbell ran a one-day workshop on Gymnosperm phylogeny for four high-school teachers (one also does 7-8 grade) on 24 August at the University of Maine. A fifth teacher canceled the day before the workshop. Enrollment was much lower than what we wanted, perhaps because we did not start advertising until late June. We considered this to be a test run of a workshop and were very encouraged by the teachers' reactions. Margo prepared a detailed curriculum with lots of background information about gymnosperms and many exercises for teachers to use in their classrooms.

We provided a spiral-bound version of the curriculum, coffee, lunch, a \$100 stipend (one teacher said this was important to her to pay for childcare), and travel cost. Campbell collected branches of 21 species of gymnosperms for the teachers to use in making a key and that they could take home with them to help them get to know these plants.

We did inside activities in the morning, a tour of gymnosperms in the Fay Hyland Botanical Garden after lunch, and a concluding session back inside to end the day. We did not take the time to complete all activities we started. Instead, we got far enough into them to give teachers a sense of what they involved so that they could determine whether they would work for their students.

The teachers were quite enthusiastic, eager to get new material to use. We asked them to complete a written evaluation of the workshop before going home, and they all said the curriculum was appropriate for their students and that they would use parts or all of it in their classrooms. No two teachers liked the same activity most or the same activity least. Similarly, teachers differed in their preferences for the amount of time devoted to phylogeny and identification of gymnosperms versus activities. In response to our question about topics that should be added to the workshop, a prominent one is the importance of gymnosperms. We all concluded as a group that this topic could be good motivation for students to learn about gymnosperms. Campbell wrote a brief piece on the importance of gymnosperms.

Some of the material developed for this curriculum is available on the Gymnosperm AToL site

(<http://www.huh.harvard.edu/research/mathews-lab/atolHtmlSite/>) in the educational resources section.

Overall we consider the workshop to be excellent outreach, and in the summer of 2009, graduate student Garth Holman offered a workshop on conifer evolution for High School teachers in Lewiston, Maine. This workshop provided teachers with general background information about gymnosperms and especially conifers, an overview of how phylogenies are inferred from DNA sequence data, and some exposure to conifers in the Pine Tree State Arboretum in Augusta, Maine. We developed two exercises for the teachers to use with their students, the first of which is making an identification key to conifers. This activity encourages observation and comparison of plants and trains students in the use of identification keys. The second exercise is exploration of an alignment of DNA sequences to look for phylogenetically informative information. A digital copy of the curriculum developed for the first workshop was provided to the teachers along with a list of useful websites and other resources for further study of conifers and their use in understanding evolution will be provided. This workshop was part of a larger commitment by the University of Maine to high-school teachers in Lewiston, Maine, and one of the goals of this commitment is for teachers to incorporate new material about trees and forests into their curricula. PI Campbell has offered to be a resource and answer questions about conifer evolution as teachers develop material for their students.

In the fall of 2009, Garth Holman hosted several teachers from this workshop in the Campbell lab to show them how we extract DNA from conifers.

In May, 2010, Garth Holman participated in a gymnosperm phylogeny workshop at the Fairchild Tropical Garden in Miami. In July, 2011, Holman gave a short workshop on conifers at the Arnold Arboretum, and he is slated to participate in full workshop at the Morton Arboretum in the spring of 2012.

Journal Publications

Havill, N.P.; Campbell, C.S.; Vining, T.F.; LePage, B.; Bayer, R. J. ; Donoghue, M.J., "Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal DNA ITS and chloroplast DNA sequence variation", *Systematic Botany*, p. 478, vol. 33, (2008). Published,

Books or Other One-time Publications

Web/Internet Site

URL(s):

<http://www.huh.harvard.edu/research/mathews-lab/atolHtmlSite/index.html>

Description:

A new project web site went live shortly before our annual meeting. It can be viewed at <http://www.huh.harvard.edu/research/mathews-lab/atolHtmlSite/index.html>. This was a joint effort by Nathalie Nagalingum and Hardeep Rai in the Mathews lab, with content and images provided by various group members. Many PIs provided constructive suggestions for improvement of the site.

Other Specific Products

Product Type:

Physical collection (samples, etc.)

Product Description:

We have assembled DNA samples of about 95 species from seven genera of conifers (*Abies*, *Cedrus*, *Keteleeria*, *Nothotsuga*, *Picea*, *Pseudolarix*, and *Tsuga*).

Sharing Information:

We will make these DNA samples available to any researcher who wants them for legitimate reasons.

Contributions

Contributions within Discipline:

The discipline is taken to be botany, and the primary contributions to this discipline of the entire collaborative project are understanding evolutionary relationships of major groups of seed plants. These plants dominate most of the global terrestrial ecosystems and are the major source, directly or indirectly, of most of human nutrition and many other essential materials. Despite considerable research directed at the evolutionary relationships of seed plants, there remain largely unresolved questions that our research is intended to answer. This research helps us understand some fundamental elements of biodiversity. For my part of this collaborative research, we have developed or are working on phylogenies of important groups of plants. There are no solid, published phylogenies for *Abies* (the firs) and *Cedrus* (the true cedars), and published phylogenies of *Picea* (the spruces) and *Tsuga* (the hemlocks) could be improved. *Nothotsuga* (a relative of hemlocks) and *Pseudolarix* (the golden larches) each have only one species, and, while we will not undertake phylogenetic analyses of these genera, we are interested in their relationships to other genera of conifers. These efforts will build an accurate understanding of the tree of life.

Contributions to Other Disciplines:

Conifers and other gymnosperms are in many cases important timber species and ecological dominants. Understanding the part of the tree of life where these species occur is basic to many disciplines. Ecologists, physiologists, foresters, plant pathologists, and other scientists will benefit from greater knowledge of the relationships of these important trees. Many conifers are endangered or threatened, and the more robust phylogenetic relationships that our work will generate will be helpful to conservation biologists.

Contributions to Human Resource Development:

Our workshops on gymnosperms for K-12 teachers developed by graduate student Margaret Connolly, PI Campbell, and graduate student Garth Holman will expose pre-college teachers and young people to science. These workshops are designed in part to help teachers teach their students about biodiversity and evolution, both of which have been designated as important areas in the Maine Learning Results of the Maine Department of Education. Graduate student Garth Holman has participated in one gymnosperm workshop outside of Maine and may do another later this year.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Conference Proceedings

Categories for which nothing is reported:

Any Book

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering

Any Conference